The Conservation Genetics of Ecologically and Commercially Important Coral Reef Species

A thesis submitted to the University of Manchester for the degree of PhD in the Faculty of Life Sciences

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**Abstract:**
Identifying the extent to which coral reef species are connected by dispersal is a fundamental challenge for developing marine conservation strategies. Many coral reef species are relatively sedentary as adults, yet have a pelagic larval phase where larvae can potentially be widely dispersed by ocean currents. This thesis focuses on the role of ocean currents in driving spatially explicit patterns of population connectivity among ecologically and commercially important coral reef species by combining research tools from population genetics, oceanography, and biophysical modeling. Despite the substantial differences among the life histories of each coral reef species in this thesis, some similarities in connectivity patterns were found among all species. The results of the kinship and genetic outlier analyses consistently found high levels of connectivity among distant populations separated by hundreds to thousands of kilometers. Despite the high levels of connectivity among distant populations, there was substantial variation in geneflow among the populations of each species. The findings of this thesis highlight the importance of international cooperation for the sustainable management of ecologically and commercially important coral reef species in the Caribbean. In conclusion, the
findings of this thesis suggest that marine conservation strategies should conservatively plan for uncertainty, particularly since the many of ecological and physical drivers of connectivity among coral reef species in the Caribbean remain uncertain.

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Chapter 1

Thesis Introduction

Marine ecosystems provide essential ecosystem goods and services such as food resources, flood control, and detoxification of waste for billions of people (Worm et al. 2006). Despite the fundamental value of these ecosystem services, many marine ecosystems have become degraded by human activities over enormous spatial scales (Halpern et al. 2008). The rapid decline of coral reef ecosystems in recent decades is an unprecedented challenge for marine conservation and management agencies (Mumby & Steneck 2008). Management strategies that consider the entire ecosystem and integrate natural and social science perspectives, termed “ecosystem-based management”, have become important tools for conservation efforts to restore coral reef ecosystems and the services they provide to humanity (Crowder & Norse 2008).

Marine protected areas (MPAs) that regulate human activities have become a central tool for ecosystem-based management strategies in the Caribbean (Lester et al. 2009). However, MPAs are often isolated from each other with unknown numbers of individuals of any species moving between MPAs. Because of this potential isolation, spatial and temporal patterns of larval connectivity among marine populations need to be explicitly considered to inform managers and scientists of the ecological effects that MPAs are having on the marine environment (Mumby et al. 2010). Obtaining these data for marine species is a challenging and multidisciplinary task. For instance, identifying the extent to which coral reef
species are connected by dispersal remains a fundamental challenge in marine conservation (Sale et al. 2005). Many coral reef species are relatively sedentary as adults yet have a pelagic larval phase where larvae can potentially be widely dispersed by ocean currents (Roberts 1997). Larvae can conceivably be dispersed hundreds to thousands of kilometers depending on the speeds of ocean currents and the pelagic larval duration of the species (Cowen et al. 2007). As a result, many of the first genetics studies of connectivity suggested that coral reef populations were demographically open, without genetic isolation over ecological and evolutionary timescales (Hellberg 2009). However, accumulating evidence over the past decade strongly suggests otherwise (Hauser & Carvalho 2008; Selkoe et al. 2008; Hellberg 2009). Research on larval dispersal of coral reef species using mark-recapture, chemical tagging, population genetics, and biophysical modeling techniques have all provided support for the hypothesis that larvae may travel far less than their apparent dispersal potential (Jones et al. 2009). Despite the recent progress in marine connectivity science there is currently a lack of spatially explicit connectivity data for many ecologically and commercially important coral reef species. This lack of data is often identified as a critical gap in the scientific knowledge required for the effective ecosystem-level management (Sale et al. 2005). Specifically, ecosystem-level management strategies such as the spatial configuration of networks of MPAs depend on the maintenance of connectivity patterns to support population replenishment and persistence within MPA’s, between MPA’s and in adjacent habitats (Palumbi 2003).
Determining levels of subdivision among marine populations is essential for guiding MPA management strategies to preserve the biological diversity within marine environments and to maintain the ecosystem services they provide (Palumbi 2003; Worm et al. 2006). The most widely used measure of population subdivision are fixation indices, or $F$-statistics, originally developed by Sewall Wright (Wright 1931; 1951). Throughout this thesis I frequently measure $F_{IS}$ and $F_{ST}$, which are two types of fixation indices that form part of the underlying mathematical framework of Wright’s $F$-statistics. The index $F_{IS}$ measures non-random mating within subpopulations. $F_{IS}$ ranges from negative one when all individuals in the subpopulation are heterozygous to one when no heterozygotes are present in the subpopulation. The index $F_{ST}$ measures allele frequency divergence among subpopulations. $F_{ST}$ ranges from zero when all local subpopulations have the same allele frequencies to one when all local subpopulations are fixed for unique alleles.

Wright’s $F$-statistics were originally designed for loci containing only 2 alleles. Nei’s $G$-statistics (e.g. $G_{IS}$ and $G_{ST}$) expanded upon Wright’s $F$-statistics by incorporating loci with 3 or more alleles (Nei 1973). It should be noted that $G$-statistics are often referred to as $F$-statistics in the literature and the two are used interchangeably. Despite these advances interpreting the values of $F_{ST}$ or $G_{ST}$ among subpopulations with high levels of genetic diversity is not always straightforward (reviewed by (Meirmans & Hedrick 2010). For instance, the maximum possible value of $F_{ST}$ or $G_{ST}$ is not necessarily equal to one when using multiallelic markers (*i.e.* containing $> 2$ alleles per locus) such as microsatellites, but instead is determined by the amount of diversity within subpopulations. Therefore, it is not
uncommon for microsatellite studies of genetically diverse marine subpopulations to report $F_{ST}$ values of 0.05 even when no alleles are shared between subpopulations (Hellberg 2009). More recent metrics of population differentiation such as Jost’s $D$ address this issue by producing more initiative values of population subdivision (Jost 2008). Since the maximum values for Jost’s $D$ are not limited the diversity within subpopulations values can truly range from zero when there is no differentiation to one when complete differentiation exists. Despite the limitations of Wright’s $F$-statistics they remain the most commonly used type of statistic to measure population subdivision primarily due to their familiarity and long history of use (Allendorf et al. 2012). Therefore, in this thesis I report Wright’s $F$-statistics alongside Jost’s $D$ and Nei’s $G$-statistics to allow for multiple comparisons of population subdivision.

This thesis focuses on the role of ocean currents in driving spatially explicit patterns of both population subdivision and population connectivity among ecologically and commercially important coral reef species by combining research tools from population genetics, oceanography, and biophysical modeling. Prior to data collection for this thesis a collaborative consultation process among marine conservation NGOs, MPA managers and scientists identified a substantial lack of connectivity data for of two species of Caribbean spiny lobsters ($Panulirus argus$ and $Panulirus guttatus$) and yellowtail snapper ($Ocyurus chrysurus$). Population genetics data for these ecologically and commercially important coral reef species were considered priorities for spatial management of MPAs among Central American nations in the Caribbean. My collaborators provided access to the most
comprehensive sample collection of spiny lobsters ever made in the Caribbean (Moss et al. 2013). These samples were used to perform a study of genetic connectivity and population subdivision in *Panulirus argus* specifically related to oceanographic conditions in the Caribbean Sea. Additionally, this thesis provides the first scientific studies of genetic connectivity for the rarely studied species of spiny lobster, *Panulirus guttatus* and for yellowtail snapper, *Ocyurus chrysurus*, populations specifically from the southern region of the Mesoamerican Barrier Reef System (MBRS).

This thesis attempts to address several interrelated questions relevant to the spatial management of spiny lobsters and yellowtail snapper: Firstly, *what is the appropriate scale of spatial management for these species?* Secondly, *is there evidence of limited connectivity or genetically unique subpopulations?* Thirdly, *is there evidence of self-recruitment?* Finally, *are there any site-specific correlations between genetic differentiation or genetic diversity and oceanographic conditions?*

In order to address these questions several chapters of this thesis focus on the interaction between vertical migratory behaviors of marine larvae and the oceanographic environment since these factors are widely believed to shape spatial and temporal patterns of population structure in many marine species (reviewed by (Pineda et al. 2007; Cowen & Sponaugle 2009). Diel vertical migration is one of the most common types of vertical migratory behavior, whereby larvae swim upwards at night to food rich surface waters and return to the depths during the day (reviewed by Ringelberg 2010). This type of behavior can be quite flexible and may change over the course of development in many marine species (Leis 2006).
Ontogenetic vertical migration is a type of vertical migratory behavior where individuals spend different stages of their larval development at different depths (Butler et al. 2011). Since ocean currents tend to differ in direction or speed with increasing depth, the vertical migratory behavior of larvae needs to be taken into account when investigating spatial patterns of population connectivity (Paris et al. 2007). For instance, larval vertical migratory behaviors were suggested to significantly limit the dispersal potential of Caribbean spiny lobster and several species of coral reef fish and these effects can be particularly strong within retentive oceanographic environments (Cowen et al. 2006; Butler et al. 2011).

All of the species that were studied in this thesis have relatively long larval durations, where large-scale and more permanent types of ocean currents may play an important role in shaping patterns of population subdivision (White et al. 2010). For example, an ocean gyre is a large system of rotating ocean currents that have a circular pattern of flow. Gyres are a well-described type of physical mechanism that retains larvae and are common features in the Caribbean seascape (Andrade & Barton 2000; Cowen 2000) that tend to occur in where the Caribbean current becomes constrained by landmasses (Figure 1A). Coastal topography, particularly large shallow banks, may also create regions of reduced flow where larval retention is also likely.
Figure 1. Advective and retentive Caribbean ocean currents visualized using satellite data from the NASA ECCO2 model provided courtesy of the NASA/GSFC Scientific Visualization Studio (Panel A). The long white winding arrow indicates the direction of flow for advective Caribbean and Gulf Stream currents. The several circular shaped white arrows highlight several retentive gyres in the Caribbean. The locations of sampling sites throughout the Caribbean are coded by color (Panel B). Red = Caribbean spiny lobster, *Panulirus argus*. Yellow = Caribbean spiny lobster and yellowtail snapper, *Ocyurus chrysurus*. Green = Caribbean spotted lobster, *Panulirus guttatus*. Blue = Caribbean spiny lobster and Caribbean spotted lobster.

A retentive oceanographic environment is a region where larval retention and self-recruitment is likely due to gyres or reduced flow (Butler et al. 2011). Self-recruitment is the return of larvae to their natal environment (Cowen et al. 2007). In contrast, an advective oceanographic environment is a region where larval retention is unlikely due to the strong flow of surface currents. Boundary currents, formed by flow of energy from the tropics to the poles, are an excellent example of an advective oceanographic environment (Pidwirny 2006). The Caribbean and Gulf Stream currents are well-studied types of boundary currents with surface flows ranging from 40 to 120 km/day (Pidwirny 2006). These high velocity surface flows are sufficient to transport marine larvae of spiny lobsters and coral reef fish 100s to 1000s of km (Cowen et al. 2006; Kough et al. 2013). Thus, in order to improve the interpretation of spatial patterns of population subdivision in marine species environmental, physical, and behavioral parameters need to be explicitly integrated into population genetics analyses (Foster et al. 2012). This approach termed ‘seascape genetics’ has been applied throughout the chapters of this thesis (reviewed by Selkoe 2006).

This thesis is presented as a collection of nine individual papers, each paper taking up a single chapter of the thesis. The individual papers of the thesis are
already published, submitted for publication, or prepared for submission to a specific peer-reviewed scientific journal. The locations of all the sampling sites and species studied in this thesis are displayed in Figure 1B. The first three papers are methods papers that specifically address the development of species-specific genetic markers that will be used in the following papers to investigate population structure and levels of connectivity in spiny lobsters and yellowtail snapper. The fourth, fifth, and sixth papers are case studies designed to test the statistical power of genetic markers to detect spatial and temporal signals of genetic population structure in both species spiny lobsters. After the utility of the genetic markers was validated, the seventh paper conducts a thorough population genetics study of the spiny lobster (*Panulirus argus*) among several advective and retentive oceanographic environments throughout the Caribbean. The eighth paper focuses on patterns of connectivity among spiny lobsters (*P. argus*) residing in MPAs in Central America. The final paper of this thesis examines levels of connectivity between yellowtail snapper populations from the Miskito Cayes region of Honduras and southern MBRS. In the last chapter of the thesis I provide a brief summary of the major findings and discuss how this information can be used to support international cooperation among fisheries management and marine conservation agencies in the Caribbean.

**References**


Chapter 2

Isolation and characterization of eight polymorphic microsatellites for the spotted spiny lobster, *Panulirus guttatus*

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Contributions: NKT, RFP, DB, and MB designed the study. NKT, DB, and MB collected the samples. NKT conducted the laboratory work. NKT and RFP analyzed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

Microsatellite sequences were isolated from enriched genomic libraries of the spotted spiny lobster, *Panulirus guttatus*. Twenty-nine previously developed polymerase chain reaction primer pairs of *Panulirus argus* microsatellite loci were also tested for cross-species amplification in *Panulirus guttatus*. In total, eight consistently amplifying, and polymorphic loci were characterized for 74 individuals collected in the Florida Keys and Bermuda. The number of alleles per locus ranged from eight to 15 and observed heterozygosities ranged from 0.45 to 0.95. Significant deviations from Hardy-Weinberg equilibrium were found in five loci from Florida and seven loci from Bermuda, suggesting the presence of null alleles. Quality control testing indicated that all loci were easy to score, highly polymorphic, did not deviate significantly from genotypic equilibrium, and had low to moderate null allele frequencies (3% to 21%). These eight microsatellites should provide sufficient statistical power for detecting fine scale genetic structure for future population genetics studies of *P. guttatus*. 
1. Introduction

The spotted spiny lobster *Panulirus guttatus* is a coral reef dwelling species that occurs from Bermuda to Suriname and throughout the Caribbean Sea (Sharp et al. 1997). The larger and more common Caribbean spiny lobster, *Panulirus argus*, co-occurs with *P. guttatus* on Caribbean coral reefs, but their life histories vary in several key respects (Lozano-Alvarez et al. 2007). Both species have long pelagic larval durations, but while *P. guttatus* occupies the same coral reef habitat through all of its benthic stages (Sharp et al. 1997); *P. argus* uses hard-bottom, seagrass, or mangrove as juvenile nursery habitat (Acosta and IV 1997; Behringer et al. 2009) and typically migrates to feeding grounds each night (Acosta and Robertson 2003). The growth and reproductive dynamics also vary with *P. guttatus* maturing at a much smaller size (females 32 mm carapace length (CL), males 36-37 mm CL) and attaining a smaller maximum size (Robertson and Butler 2013; Robertson and Butler 2003). The larger size and greater abundance of *P. argus* have allowed it to support the most important fishery in the Caribbean with annual landings near 1B USD (FAO 2010). Consequently, the vast majority of scientific research and fisheries management in the Caribbean has focused primarily on *P. argus* (Fanning et al. 2011).

Despite research and management efforts, *P. argus* fisheries have declined in many regions of the Caribbean (Fanning et al. 2011) leading to increased fishing pressure on *P. guttatus* (Wynne and Côté 2007). Fishery regulations for *P. guttatus* are either extremely limited (e.g., Bermuda and Martinique) or non-existent, and fisheries are emerging in the British West Indies and several other Caribbean
nations to satisfy the demand for luxury seafood (Acosta and Robertson 2003; Wynne and Côté 2007). Management is hindered by a lack of basic life history, ecology, and population information – all of which would be facilitated by the development of species-specific genetic tools.

This study aims to enable future genetic studies on *P. guttatus* by characterizing new microsatellites for the species and testing all polymerase chain reaction (PCR) primer pairs of nuclear-encoded microsatellites previously developed for *P. argus* (Diniz et al. 2005; Diniz et al. 2004; Tringali et al. 2008) for cross-reactivity in *P. guttatus*. These microsatellite primers will allow researchers to identify genetically unique subpopulations, determine levels of genetic diversity, and measure levels of connectivity among subpopulations of *P. guttatus*.

### 2. Methods and Results

Total genomic DNA was isolated from muscle tissue in 49 individuals from Long Key Florida (24°44'46.28"N, 80°46'58.46"W) and 50 individuals from Bermuda (North Rock: 32°28'25.26"N, 64°47'9.60"W, East Blue Cut: 32°23'31.93"N, 64°52'44.54"W) using the Wizard SV-96 Genomic DNA extraction kit (Promega). Genomic DNA from 25 individuals from Long Key Florida was sent to GenoScreen, France (www.genoscreen.fr) for microsatellite development. The DNA from the remaining individuals was used to test the polymorphism of the microsatellite primers developed by GenoScreen. The DNA quantity was assessed using the Picogreen assay (Invitrogen). To improve polymorphism detection the DNA from 12 individuals were pooled equimolarly.
Microsatellite libraries were developed using 1 µg of pooled DNA and 454 GS-FLX Titanium pyrosequencing of the enriched DNA (Malausa et al. 2011). Briefly, total DNA was enriched for microsatellite loci using 8 probes (AG, AC, AAC, AAG, AGG, ACG, ACAT and ATCT) and subsequently amplified. The PCR products were purified, quantified, and GsFLX libraries were developed following the manufacturer’s protocols (Roche Diagnostics) and sequenced on a GsFLX-PTP. This technique allowed the identification of 12676 potential microsatellite primers. The bioinformatics program QDD was used (Meglécz et al. 2010) to identify sequences that were optimal for primer design and validated 737 pairs of primers. Tri-repeats and tetra-repeats were favored in order to minimize stutter bands and increase the probability of accurate allele scoring. Twenty-four validated sets of *P. guttatus* primers and 29 sets of previously designed microsatellite primers for *P. argus* (Diniz et al. 2004; Diniz et al. 2005; Tringali et al. 2008) were tested for amplification. Primer sets were discarded if they failed to amplify or lead to > 2 fragments. Finally, 13 microsatellites developed by Genoscreen and 2 microsatellites (Tringali et al. 2008) previously developed for *P. argus* were tested for polymorphism in *P. guttatus*.

Each PCR reaction was performed in a total volume of 5 µl with a Veriti thermal cycler (Applied Biosystems). Our protocol followed the manufacturer’s recommendations (Qiagen Microsatellite Multiplex PCR Kit), but the total volume of the PCR reaction was scaled down from 25 µl to 5 µl whilst keeping the concentrations of all PCR reagents the same. The PCR reaction mix consisted of 0.5 µl of the 10X primer mix (1µM primer + 1µM fluorescent primer), 2.5 µl of Type-it
Multiplex PCR Master Mix (Qiagen), 1 µl of molecular grade water and 1 µl of (10-20 ng/µl) genomic DNA. The PCR conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 26 cycles at 95 °C for 30 s, 57 °C for 120 s, and 72 °C for 30 s. This was followed by final extension at 60 °C for 30 min. To facilitate the fragment analysis, PCR products were diluted 1:1 with 5 µl MQ water. From the diluted product, 0.5 µl was mixed with 9.5 µl of a mix consisting of Hi-Di Formamide® (Applied Biosystem) and GeneScan – 500 LIZ Size Standard (37:1) in a 96 well PCR plate. Fragment analysis was performed on an ABI 3730xl automatic DNA sequencer (Applied Biosystems, USA) at the University of Manchester DNA Sequencing Facility. Microsatellite alleles were scored using the GeneMapper® v3.7 software package (Applied Biosystems). Binning of microsatellite alleles and error checking were preformed using the R package MsatAllele version 1.02 (Alberto 2009) and R statistical software v2.15.1 (Ihaka and Gentleman 1996). The entire data set was checked for variability and departures from Hardy-Weinberg equilibrium (HWE) and the fixation index (FIS) was calculated using the software package Genodive v2.0b23 (Meirmans 2012; Meirmans and van Tienderen 2004). Linkage disequilibrium (LD) between loci was tested using Genepop on the Web v4.2 (Raymond and Rousset 1995; Rousset 2008). Markov chain parameters for were set to the following: dememorization number 10K, number of batches 1K, and number of iterations per batch 10K. The genetics software program FreeNA (Chapuis and Estoup 2007) was used to calculate null allele frequencies for each locus and population (following the expectation maximization algorithm of (Dempster et al. 1977)).
**Table 1** Characterization of eight microsatellite loci for *Panulirus guttatus* with Na (number of alleles), Ho (observed heterozygosity), He (expected heterozygosity), Fis (fixation index) and P (test for deviation from Hardy-Weinberg equilibrium). Significant values are in bold.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequence (5’ to 3’)</th>
<th>Genbank accession number</th>
<th>Repeat motif</th>
<th>Range size (bp)</th>
<th>Florida (N = 24)</th>
<th>Bermuda (N = 50)</th>
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<tr>
<td></td>
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<td>Ho</td>
<td>He</td>
<td>Fis</td>
<td>P</td>
<td>Na</td>
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<td>Pgut-3</td>
<td>GCTGGAGAGGGAGGAACTGTCCCTCCTCATCTTTCCTTCGCCCTTTCATTTTCGTCACTAAGCTGTTCAATGCTGCC</td>
<td>KC800822</td>
<td>gag</td>
<td>95-131</td>
<td>11</td>
<td>0.792</td>
<td>0.875</td>
<td>0.116</td>
<td>0.101</td>
<td>12</td>
</tr>
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<td>GCTGGAGAGGGAGGAACTGTCCCTCCTCATCTTTCCTTCGCCCTTTCATTTTCGTCACTAAGCTGTTCAATGCTGCC</td>
<td>KC800823</td>
<td>atc</td>
<td>140-165</td>
<td>8</td>
<td>0.667</td>
<td>0.832</td>
<td>0.220</td>
<td>0.016</td>
<td>10</td>
</tr>
<tr>
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<td>KC800824</td>
<td>tgt</td>
<td>78-119</td>
<td>13</td>
<td>0.958</td>
<td>0.835</td>
<td>0.127</td>
<td>0.082</td>
<td>16</td>
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<td>Pgut-15</td>
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<td>KC800825</td>
<td>gata</td>
<td>133-178</td>
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<td>0.875</td>
<td>0.832</td>
<td>0.031</td>
<td>0.493</td>
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<tr>
<td>Pgut-21</td>
<td>GCTGGAGAGGGAGGAACTGTCCCTCCTCATCTTTCCTTCGCCCTTTCATTTTCGTCACTAAGCTGTTCAATGCTGCC</td>
<td>KC800826</td>
<td>tcta</td>
<td>167-224</td>
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<td>0.425</td>
<td>&lt;0.001</td>
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<td>KC800827</td>
<td>atgt</td>
<td>74-115</td>
<td>10</td>
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<td>&lt;0.001</td>
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<td>0.019</td>
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<td>Par-Fwo5</td>
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<td>EF620542</td>
<td>ca</td>
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<td>0.837</td>
<td>0.191</td>
<td>0.021</td>
<td>21</td>
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</table>
Figure 1 Allele sizes (in base pairs) and frequency of occurrence of the eight microsatellite markers characterized for *Panulirus guttatus*.
Six out of 13 microsatellites developed by Genoscreen were found to be either monomorphic or too difficult to score and were removed from the analysis. Twenty-seven out of 29 *P. argus* microsatellites failed to produce PCR products. One out of the two *P. argus* microsatellites that did produce a PCR product was too difficult to score and was removed from the analysis. Table 1 summarizes the characteristics of the eight primer pairs of polymorphic and easy to score microsatellite loci developed for the spotted spiny lobster *P. guttatus*. Figure 1 summarizes the scoring and binning of all alleles for each microsatellite locus. Samples from Long Key Florida and Bermuda were genotyped using the eight developed primers. For the 74 samples (24 in Florida, 50 in Bermuda) genotyped, the number of alleles ranged from eight to 15 per locus. Five of the eight loci failed to meet Hardy-Weinberg Equilibrium (HWE) in Florida whilst seven of the eight loci failed to meet HWE in Bermuda. All the deviations from HWE in our study were due to heterozygote deficiencies. These deficiencies could be due to null alleles or the Wahlund effect (Johnson and Black 1984). The latter is possible considering the potential for extensive geneflow in this species. However, null alleles are a common characteristic of the microsatellites of many marine invertebrates, so could also be responsible for the deviations from HWE (Dailianis et al. 2011). All loci that deviated from HWE were tested for the presence of null alleles (Table S1). Null allele frequencies were low at loci Fwc5, Pg3, Pg6, Pg15, and Pg3 (ranging from 3% to 10%). Null allele frequencies were moderate at loci Pg21 and Pg22 (ranging from 18% to 21%). Although null alleles have been found to inflate levels of population structure, they do not create population structure
where it does not already exist (Carlsson 2008; Chapuis and Estoup 2007). Therefore, these eight primers would be useful in genetic studies on *P. guttatus* and could be useful in conservation or fishery management of the species.

**Acknowledgements**

We thank Dr. Tammy Trott from the Bermuda Fisheries Department for providing samples for this study and Josh Anderson, Jason Spadero, and Mike Dixon for helping to collect samples in the Florida Keys. We are grateful to Antoine Destombes at Genoscreen for his help with this project. NKT is supported by postgraduate fellowships from the Sustainable Consumption Institute and the Faculty of Life Sciences at the University of Manchester. This work was funded in part by NSF grant OCE0929086 to MJB and DCB.

**References**


**Supplementary information**

**Table S1.** Estimates of null allele frequency using the methodology of Dempster et al. (1977) in the software package FreeNA.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Site</th>
<th>Null Allele Frequency</th>
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</thead>
<tbody>
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<td>PG9</td>
<td>Bermuda</td>
<td>0</td>
</tr>
<tr>
<td>PG15</td>
<td>Bermuda</td>
<td>0.0339</td>
</tr>
<tr>
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<td>Bermuda</td>
<td>0.05146</td>
</tr>
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<td>Bermuda</td>
<td>0.05618</td>
</tr>
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<td>Bermuda</td>
<td>0.07017</td>
</tr>
<tr>
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<td>Bermuda</td>
<td>0.0829</td>
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<td>Bermuda</td>
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<td>PG22</td>
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</tr>
<tr>
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<td>Florida</td>
<td>0</td>
</tr>
<tr>
<td>PG15</td>
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<td>0</td>
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<td>Florida</td>
<td>0.04942</td>
</tr>
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<td>0.06623</td>
</tr>
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<td>PG23</td>
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<td>0.07858</td>
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<tr>
<td>PG6</td>
<td>Florida</td>
<td>0.08429</td>
</tr>
<tr>
<td>PG21</td>
<td>Florida</td>
<td>0.18163</td>
</tr>
<tr>
<td>PG22</td>
<td>Florida</td>
<td>0.21173</td>
</tr>
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</table>
Chapter 3

Characterization of two microsatellite PCR multiplexes for high throughput genotyping of the Caribbean spiny lobster, *Panulirus argus*

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\(^2\)University of Florida, Fisheries and Aquatic Sciences, Gainesville, Florida 32653, USA
\(^3\)Old Dominion University, Department of Biological Sciences, Norfolk, Virginia 23529, USA

Running Title: Microsatellite multiplexes for *Panulirus argus*

Key Words: Connectivity, Conservation, Population, Genetics, Parentage, Kinship, Analysis

*Prepared for submission to Conservation Genetics Resources*

Contributions: NKT, RFP, DB, and MB designed the study. NKT, DB, and MB collected the samples. NKT conducted the laboratory work. NKT and RFP analyzed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

The spiny lobster *Panulirus argus* supports one of the most economically important commercial fisheries in the Caribbean, yet its sustainable management is problematic due to uncertainty regarding levels of population connectivity among Caribbean nations. We developed two microsatellite multiplex panels for *P. argus* to assist in future conservation genetics research studies of this important Caribbean species. Significant deviations from Hardy–Weinberg equilibrium were observed at locus Par7 in multiplex 1 and loci Fwc08 and Fwc17 in multiplex 2. No evidence of linkage disequilibrium was observed. All 12 loci were used in both microsatellite multiplexes were polymorphic, with an average of 12 alleles per locus (ranging from 3 to 29 alleles per locus) and $H_O$ ranging from 0.368 to 0.921. These two microsatellite multiplexes will be a valuable resource for ongoing and future studies of conservation genetics in the Caribbean spiny lobster, *Panulirus argus*. 
1. Introduction

The spiny lobster *Panulirus argus* supports one of the most economically important commercial fisheries in the Caribbean, yet its sustainable management is problematic because of its widespread larval dispersal and, consequently, unknown patterns in population connectivity among Caribbean nations (Kough et al. 2013). Polymorphic microsatellite loci with high information content are of great utility for population genetics and connectivity studies. Microsatellite loci have previously been characterized for *P. argus* (Diniz et al. 2004; Tringali et al. 2008), but studies of *P. argus* genetics would benefit from a microsatellite multiplex methodology because it decreases the cost and time required for genotyping individuals while increasing throughput. Our objective was to develop novel microsatellite multiplex panels for *P. argus* to assist in future conservation genetics research studies of this important Caribbean species.

2. Methods

Total genomic DNA was isolated from leg muscle tissue from 56 individuals collected from Caye Caulker, Belize using the Wizard SV-96 Genomic DNA extraction kit following the manufacturer’s protocol (Promega). Previously characterized microsatellite primers were combined in a multiplex polymerase chain reactions (PCR) based upon the fragment lengths of the PCR products and the annealing temperatures of each primer pair (Table 1; Diniz et al. 2004; Tringali et al. 2008). The PCRs were performed in a separate run for each multiplex (Table 1). Each PCR reaction was performed in a total volume of 5 µl using a Veriti thermal
cycler (Applied Biosystems). Our methods followed the manufacturer’s recommendations (Qiagen Microsatellite Multiplex PCR Kit), however, the total volume of each PCR reaction was scaled down from 25 µl to 5 µl whilst keeping the concentrations of all PCR reagents the same. The final PCR reaction mix consisted of 0.5 µl of the 10X primer mix (1µM primer + 1µM fluorescent primer), 2.5 µl of Type-it Multiplex PCR Master Mix (Qiagen), 1 µl of molecular grade water and 1µl of (10-20 ng/µl) genomic DNA. The PCR parameters consisted of an initial denaturation at 95 °C for 5 min, followed by 26 cycles at 95 °C for 30 s, 57 °C for 120 s, and 72 °C for 30 s. This was followed by final extension at 60 °C for 30 min. The PCR products were detected on an ABI 3730xl Sequencer (Applied Biosystems) at the University of Manchester DNA sequencing facility. The resulting microsatellite fragments were examined using GENEMAPPER 3.7 (Applied Biosystems) and peaks were scored manually. Any primer pairs that failed to amplify or were difficult to score due to excessive stuttering or split peaks were discarded and not used in further analyses. Microsatellite alleles were binned and error checking was performed using the R package MsatAllele (Alberto 2009). The R-package POPGENREPORTS was used to estimate observed (Hₒ) and expected (Hₑ) heterozygosity, number of alleles (Nₐ), and deviations from Hardy–Weinberg equilibrium. Bonferroni corrections were applied in POPGENREPORTS when multiple statistical tests were conducted. The program MICROCHECKER (van Oosterhout et al. 2004) was used to check for null alleles and scoring errors caused by excessive stuttering or large allele dropout. Deviations from linkage equilibrium were tested in GENEPOP (Rousset 2008).
<table>
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<tr>
<th>Locus</th>
<th>Primer Sequences (5’-3’)</th>
<th>Repeat Structure</th>
<th>N&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Size Range</th>
<th>H&lt;sub&gt;o&lt;/sub&gt;</th>
<th>H&lt;sub&gt;e&lt;/sub&gt;</th>
<th>P&lt;sub&gt;HWE&lt;/sub&gt;</th>
<th>Publication</th>
<th>GenBank Accession No.</th>
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<td>F: GACGGACAGAAATAGATGGATAGA-6FAM</td>
<td>AGAT(14)</td>
<td>17</td>
<td>80-178</td>
<td>0.698</td>
<td>0.877</td>
<td>0.0550</td>
<td>Diniz et al. 2004</td>
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<td></td>
<td>R: ACGAAAATAGGCGGACCAAGAA</td>
<td>TCTA(7)</td>
<td>6</td>
<td>152-176</td>
<td>0.66</td>
<td>0.774</td>
<td>0.4290</td>
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<td>12</td>
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<td>0.771</td>
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<td>0.716</td>
<td>0.5220</td>
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<td>7</td>
<td>90-114</td>
<td>0.714</td>
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<td>0.5220</td>
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<td>R: GTCCGTTGTTGTCGATATTTC</td>
<td>TCTG(5)</td>
<td>4</td>
<td>86-104</td>
<td>0.696</td>
<td>0.58</td>
<td>0.6080</td>
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<td>0.716</td>
<td>0.5220</td>
<td>Diniz et al. 2004</td>
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<td>R: GTCCGTTGTTGTCGATATTTC</td>
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<td>4</td>
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<td>0.58</td>
<td>0.6080</td>
<td>Diniz et al. 2004</td>
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<td>R: GCAACAGTGGACCGAGAGA</td>
<td>CA(33)</td>
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<td>244-294</td>
<td>0.804</td>
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<td>TCTG(12)</td>
<td>12</td>
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<td>0.435</td>
<td>0.869</td>
<td>0.0000</td>
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<td>18</td>
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<td>244-294</td>
<td>0.804</td>
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<td>174-200</td>
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<td>29</td>
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<td>0.518</td>
<td>0.515</td>
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<td>R: CAGACAGACACACGAGTCTTGGT</td>
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<td>3</td>
<td>113-119</td>
<td>0.518</td>
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<td>0.804</td>
<td>0.905</td>
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<td>Fwc18</td>
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<td>TAG(9)/TAG(2)/TAG(3)</td>
<td>8</td>
<td>102-132</td>
<td>0.821</td>
<td>0.766</td>
<td>0.9300</td>
<td>Tringali et al. 2008</td>
<td>EF620540</td>
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<tr>
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<td>TAG(9)/TAG(2)/TAG(3)</td>
<td>8</td>
<td>102-132</td>
<td>0.821</td>
<td>0.766</td>
<td>0.9300</td>
<td>Tringali et al. 2008</td>
<td>EF620540</td>
</tr>
</tbody>
</table>

Number of alleles (N<sub>a</sub>), range of allele sizes, observed (H<sub>o</sub>) and expected (H<sub>e</sub>) heterozygosity, Hardy-Weinberg Equilibrium P-values (P<sub>HWE</sub>) are based on 56 individuals. Publication refers to the source of the originally published microsatellite primers. The types of fluorescent labels used on forward primers are indicated (6-FAM, NED, PET, VIC). Multiplexes, fluorescent labels, and significant deviations from HWE after using the Bonferroni correction for multiple comparisons are indicated in **bold**.
3. Results

All 12 loci were used in both microsatellite multiplexes were polymorphic, with an average of 12 alleles per locus (ranging from 3 to 29 alleles per locus) and $H_O$ ranging from 0.368 to 0.921. Significant deviations from Hardy–Weinberg equilibrium were observed at locus Par7 in multiplex 1 and loci Fwc08 and Fwc17 in multiplex 2. No evidence of linkage disequilibrium was observed. MICROCHECKER detected evidence for null alleles only for locus Par7 and no evidence of scoring errors due to stutter or large allele dropout were detected. Therefore, these two microsatellite multiplexes will be a valuable resource for ongoing and future studies of conservation genetics in the Caribbean spiny lobster, *Panulirus argus*.

Acknowledgements

We thank James Azueta and Isaias Majil at the Bermuda Fisheries Department for helping to collect samples in the Belize. NKT is supported by postgraduate fellowships from the Sustainable Consumption Institute and the Faculty of Life Sciences at the University of Manchester. This work was funded in part by NSF grant OCE0929086 to MJB and DCB.

References


Chapter 4

Characterization of two microsatellite multiplex PCR protocols the yellowtail snapper, *Ocyurus chrysurus*

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Running Title: Microsatellite multiplexes for *Ocyurus chrysurus*

Key Words: Connectivity, Conservation, Population Genetics, Parentage and Kinship Analyses

Prepared for submission to Conservation Genetics Resources

Contributions: NKT, RFP, SB, and SC designed the study. SB and SC collected the samples. NKT conducted the laboratory work. NKT an RFP analyzed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

Management of fisheries in the Caribbean has been limited by a lack of information regarding levels of genetic diversity and population connectivity for many coral reef species. Thirteen microsatellite loci for the yellowtail snapper, *Ocyurus chrysurus*, were successfully assigned into two multiplex panels to assist in future conservation genetics research studies. These multiplex panels were characterized in 46 *Ocyurus chrysurus* individuals from Belize. All loci were polymorphic. The number of alleles per locus ranged from 4 to 20. Observed heterozygosity (*H*<sub>O</sub>) varied from 0.269 to 0.920. Three loci deviated significantly from Hardy–Weinberg equilibrium and no pairs of loci showed evidence of significant linkage disequilibrium. These two microsatellite multiplexes will be a useful resource for future studies of conservation genetics and population connectivity in the yellowtail snapper, *Ocyurus chrysurus*. 
1. Introduction

The sustainable management of coral reef fisheries in the Caribbean has been limited by a lack of information regarding levels of genetic diversity and population connectivity for individual species. The yellowtail snapper, *Ocyurus chrysurus*, is a coral reef associated fish that supports commercial and recreational fisheries throughout the western Atlantic ranging from the southeastern USA, Gulf of Mexico and Caribbean to Brazil. A total of 24 microsatellite markers have previously been characterized for yellowtail snapper (Renshaw et al. 2007). A recent population genetics study that used these microsatellite markers identified four unique populations of yellowtail snapper occurring in the Florida Keys, the west coast of Puerto Rico, between the east coast of Puerto Rico and St. Thomas, and offshore of St. Croix (Saillant et al. 2012). The management of yellowtail snapper fisheries among other nations in the Caribbean would benefit from a simple and easy to use microsatellite multiplex that significantly reduces the cost and time required for future population genetics studies. The objective of this study was to develop two novel microsatellite multiplex panels for the yellowtail snapper *O. chrysurus* to assist in conservation genetics research for this species.

2. Methods

Muscle and fin tissue was collected from 46 individuals obtained from the yellowtail snapper fishery in Caye Caulker, Belize. Total genomic DNA was isolated from tissue samples using the Wizard SV-96 Genomic DNA extraction kit following the manufacturer’s protocol (Promega). The PCRs were performed in a
separate run for each yellowtail snapper multiplex (Table 1) using the Qiagen Microsatellite Multiplex PCR Kit. We followed the Qiagen Microsatellite Multiplex PCR Kit protocol, however, the total volume of each PCR reaction was scaled down from 25 µl to 5 µl whilst keeping the concentrations of all other PCR reagents the same. The PCR mixtures contained: 0.5 µl of the 10X primer mix (1µM primer + 1µM fluorescent primer), 2.5 µl of Type-it Multiplex PCR Master Mix (Qiagen), 1 µl of molecular grade water and 1µl of (10-20 ng/µl) genomic DNA. The final concentration of all fluorescently labeled forward primers (6-FAM, PET, VIC; Table 1) used in both multiplexes was 0.2 µM. The PCR parameters were: 95 °C for 5 min, followed by 26 cycles at 95 °C for 30 s, 57 °C for 120 s, and 72 °C for 30 s. The final extension step was 60 °C for 30 min. The fluorescently labeled PCR products were detected on an ABI 3730xl Sequencer (Applied Biosystems) at the University of Manchester DNA sequencing facility. The resulting microsatellite data were examined using GENEMAPPER 3.7 (Applied Biosystems) and peaks were scored manually. Any microsatellite loci that failed to amplify or were difficult to score were not used in further analyses. The alleles for all microsatellite loci were binned using the R-package MsatAllele (Alberto 2009). Observed (H₀) and expected (Hₑ) heterozygosity, number of alleles (Nₐ), and detect deviations from Hardy–Weinberg equilibrium (HWE) were calculated using the R-package POPGENREPORTS. The Bonferroni correction was applied to correct for the multiple statistical tests used to detect deviation from HWE. The presence of null alleles or scoring errors caused by excessive stuttering or large allele dropout was
Table 1 Characteristics of two microsatellite multiplexes for the yellowtail snapper *Ocyurus chrysurus*. Number of alleles ($N_A$), range of allele sizes, observed ($H_O$) and expected ($H_E$) heterozygosity, Hardy–Weinberg Equilibrium $P$-values ($P_{HWE}$) are based on 46 individuals. The three types of fluorescent labels used on forward primers are indicated (6-FAM, PET, VIC). The multiplexes, fluorescent labels, and the Bonferroni corrected significant deviations from HWE are indicated in **bold**.

<table>
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<tr>
<th>Locus</th>
<th>Primer Sequences (5' - 3')</th>
<th>Repeat Structure</th>
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<th>Size Range</th>
<th>$H_O$</th>
<th>$H_E$</th>
<th>$P_{HWE}$</th>
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<td>11</td>
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<td>&lt;b&gt;0.001&lt;/b&gt;</td>
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**Table 1 Continued.** Number of alleles (N<sub>A</sub>), range of allele sizes, observed (H<sub>O</sub>) and expected (H<sub>E</sub>) heterozygosity, Hardy–Weinberg Equilibrium P-values (pHWE) are based on 46 individuals. The three types of fluorescent labels used on forward primers are indicated (6-FAM, PET, VIC). The multiplexes, fluorescent labels, and the Bonferroni corrected significant deviations from HWE are indicated in **bold.**
examined with MICROCHECKER (Van Oosterhout et al. 2004). Tests for linkage disequilibrium were run in GENEPOP (Rousset 2008).

3. Results

All 13 loci tested using these two microsatellite multiplexes were polymorphic, with an average of 12 alleles per locus (ranging from 4 to 20 alleles per locus) and $H_O$ ranging from 0.269 to 0.920. Significant deviations from Hardy–Weinberg equilibrium were observed at locus Och2 in multiplex 1 and loci Lsy11 and Och14 in multiplex 2. Linkage disequilibrium was not observed among any loci. MICROCHECKER detected evidence for null alleles at loci Lsy11, Och2, and Och14. No evidence of scoring errors due to stutter or large allele dropout was detected. These two microsatellite multiplexes have the potential to be a valuable resource for future studies of conservation genetics and population connectivity in the yellowtail snapper, *Ocyurus chrysurus*.

Acknowledgements

We thank James Azueta and Isaias Majil at the Bermuda Fisheries Department for helping to collect samples in the Belize. NKT is supported by postgraduate fellowships from the Sustainable Consumption Institute and the Faculty of Life Sciences at the University of Manchester.
References


Chapter 5

Genetic Connectivity of Caribbean Spiny Lobster (Panulirus argus) in Belize

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Contributions: NKT, KLC, SB, SC, RFP, DB, and MB designed the study. NKT, EBS, SG, and MB collected the samples. NKT, EBS, and SG conducted the laboratory work. NKT and RFP analyzed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

Identifying ecologically relevant patterns of connectivity is an important factor for understanding resilience in coral reef ecosystems, and crucial for managers seeking to build socio-ecological resilience into the management of marine protected areas (MPAs) and fishery resources. We are using neutral genetic microsatellite analyses to test whether spiny lobster populations from MPAs located in regions with high levels of local recruitment are more resilient than those dependent on larvae produced from distant regions. As part of that research, we compared the microsatellite-derived population structure of Caribbean spiny lobster (*Panulirus argus*) in two MPAs in Belize. Despite separation of < 100km, we found limited genetic connectivity between those populations suggesting that larval dispersal may be more limited than expected in regions with complex oceanographic regimes.

KEY WORDS: Spiny lobster, *Panulirus argus*, genetics, microsatellites, marine reserves, connectivity, Belize
Abstract (Spanish Version)

Conectividad Genética de la Langosta Espinosa del Caribe (Panulirus argus) en Belice

Entender la conectividad de los ecosistemas coralinos a través de los patrones ecológicos que lo componen, es básico para poder implementar acciones para mejorar la resiliencia en Áreas Marinas Protegidas. Estamos haciendo un análisis genético (con microsatélites neutrales) para probar si las poblaciones de langosta espinosa del Caribe ubicadas en regiones con altos niveles de auto-reclutamiento son más resistentes que los que dependen de las larvas producidas en regiones distantes. Como parte de esa investigación, se comparó la estructura genética de la población de langosta espinosa del Caribe (Panulirus argus) en dos áreas marinas protegidas en Belice. A pesar de la separación de <100 km, encontramos conectividad genético limitada entre las poblaciones que sugieren que la dispersión de las larvas puede ser más limitado de lo esperado en las regiones con complejos regímenes oceanográficos.

PALABRAS CLAVES: langosta espinosa, Panulirus argus, la genética, microsatélites, las reservas marinas, la conectividad, Belice
Connectivité Génétique dans la Langouste Blanche Del Caribe (*Panulirus argus*) au Belize

Comprendre la connectivité des écosystèmes de récifs coralliens grâce à des modèles écologiques qui la composent, est fondamentale pour mettre en œuvre des actions pour améliorer la résilience dans les aires marines protégées. Nous faisons un test génétique (avec des microsatellites neutres) pour tester si les populations de langouste des Caraïbes situées dans des régions avec des niveaux élevés de l'auto-recrutement sont plus forts que ceux qui dépendent de larves produites dans des régions éloignées. Dans le cadre de cette enquête, nous avons comparé la structure génétique des populations de langouste des Caraïbes (*Panulirus argus*) en deux aires marines protégées au Belize. Malgré la séparation de <100 km, nous avons trouvé la connectivité limitée génétique entre les populations suggèrent que la dispersion des larves peut être plus limité que prévu dans les régions où les régimes complexes océanographiques.

MOTS-CLÉS: langoustes, *Panulirus argus*, la génétique, microsatellites, les réserves marines, la connectivité, le Belize
1. Introduction

The Caribbean spiny lobster, *Panulirus argus*, has one of longest histories of genetic research of any species in the Caribbean. Over the last thirty years numerous studies have attempted to identify genetically unique stocks of *P. argus* and sources of larval recruitment. The uncertainty of the source of newly recruited lobsters (from local or foreign breeding populations) remains a critical missing link in the establishment of sustainable management policies in the Caribbean.

Early genetic investigations (Menzies 1979; and Menzies 1980) used allozyme electrophoresis to test for genetic differentiation among six populations in the Caribbean (Elliot Key, Florida; Key West, Florida; Cancun, Mexico; Jamaica; US Virgin Islands and Trinidad). Despite finding genetic differentiation between sites, their results were difficult to interpret spatially and no temporal replication was conducted. Their results indicated the potential for either long-distance connectivity between some sites and limited connectivity between other sites on smaller spatial scales. For instance, individuals from Trinidad and Florida could not be differentiated, while the lobsters from Jamaica and the Virgin Islands were distinct.

Several allozyme genetic studies tested the hypothesis that local hydrodynamics could be largely responsible for the proposed population structure found by Menzies (1979 and 1980). However, none of these small-scale studies found conclusive evidence of genetic differentiation, despite targeting adult populations within complex oceanographic regimes. Ogawa et al. (1991) found no genetic differences between two Brazilian populations residing in different local
currents (South equatorial and Brazilian). Glaholt and Seeb (1992) found a rare allozyme allele that existed at much higher levels on Glover’s Reef than at Ambergris Caye in Belize. However, high levels of gene flow between sites, small samples sizes (N < 30/site) and few polymorphic loci to choose from (N < 10), made it difficult for them to detect statistically significant genetic signals from the high levels of noise caused by extensive gene flow (see Waples (1998) for a detailed explanation of this phenomenon).

Silberman et al. (1994) conducted the first Pan-Caribbean study of *P. argus* using mtDNA markers, sampling 259 individuals from 9 sites: Los Roques, Venezuela; Martinique; Antigua; Turks and Caicos; Jamaica; San Blas, Panama; Dry Tortugas, Florida; Miami, Florida; and Bermuda. They analyzed levels of genetic differentiation by separating sites based upon 1) isolation by distance, 2) contrasting ocean currents, and 3) continental vs. insular. None of their three models provided evidence of genetic differentiation, lending credence to the widely accepted hypothesis that *P. argus* is a single genetically homogenous population throughout the western tropical Atlantic.

1.1 Biophysical Modelling

The conflicting conclusions of previous genetics studies on *P. argus* led researchers to the use of biophysical models developed specifically to address the dispersal of marine larvae in complex flow fields. A recently developed biophysical model (Butler et al. 2011) has been used to explore the consequences of ontogenetic vertical migration (OVM) and local hydrodynamics on the larval dispersal of *P.*
argus in the Caribbean. Their findings suggest that OVM constrains the dispersal of P. argus larvae and this effect was particularly strong in retentive oceanographic environments.

The regional differences in larval dispersal caused by the interaction among OVM and advective and retentive oceanographic currents could potentially be a driver of spatial genetic patterns in P. argus. However, the previously mentioned genetic studies using allozyme and mtDNA markers failed to detect significant differences in P. argus genetic patterns between advective and retentive oceanographic environments. Why were these previous studies unable to detect any spatial genetic patterns? Is it possible that the high levels of mixing and gene flow were sufficient to mitigate the effect of the oceanographic environments? An alternative explanation is that the previous studies had limited resolution to detect subtle genetic signals due to: 1) small sample sizes (~30 per site), 2) sampling only one site within each oceanographic environment, and 3) the use of genetic markers with too few polymorphic loci.

1.2 Seascape Genetics

The field of seascape genetics has developed a suite of techniques that have demonstrated how subtle, yet ecologically significant genetic patterns can be detected in species whose populations are well connected by high levels of gene flow. A recent seascape genetics study of the spiny lobster Panulirus interruptus found significant levels of genetic differentiation between populations sampled in contrasting oceanographic environments using 7 polymorphic microsatellite
markers and sampling ~70 individuals/site (Selkoe 2010). Detection of spatial genetic patterns increased when habitat variables were integrated into the seascape genetics analysis.

Another recent advancement in seascape genetics was the incorporation of ocean circulation observations directly into isolation-by-distance (IBD) analysis. White and colleagues (2010) used simulated larval dispersal estimates of the subtidal whelk *Kelletia kelletii*, whose planktonic larval duration (PLD) is 40-60 days, to demonstrated that the integration of larval connectivity modelling between advective and retentive oceanographic environments significantly improved the resolution of population genetic structuring. When geographic distances between sites were transformed into relative oceanographic distances and integrated into a genetic IBD framework, nearly 50% of the variance in empirical genetic differences among sites was explained, while conventional IBD analysis found no differences between sites.

**1.3 Study Questions**

The primary goal of this study was to investigate the connectivity of *P. argus* between two MPAs in Belize. To address this question we compared the neutral genetic patterns between *P. argus* from Glover’s Reef and Hol Chan marine reserves.
2. Methods

2.1 Sampling Locations

Glover’s Reef marine reserve (Figure 1) is situated around an isolated coral atoll 45km off the coast of Belize (Walker 2007). The Glover’s Reef atoll is 32km long and 12km wide and the southernmost of Belize’s three offshore atolls. The 35,067 hectare reserve has a no-take zone that is ~¼ of the total area. The Hol Chan reserve is located in northern Belize and has a total area of ~ 1500 ha (Figure 1). Hol Chan reserve is near the town of San Pedro (population ~12,000) and generates more tourism revenue than any of the other marine reserves in Belize, and is thus considered a model for marine ecotourism in the region (Cho 2005).

2.2 Sample collection

Tissue samples were taken from adult lobsters captured by fishermen in the Glover’s Reef marine reserve in July 2009. Samples were collected from Hol Chan in February 2010 by free diving with a tickle stick and net. Muscle tissue was taken from a single leg and stored in 190 proof clear rum purchased from the Travelers Liquor Distillery in Belize City. The samples were stored at room temperature and transported to the University of Manchester where the DNA was extracted from each sample.
Figure 1. Map of marine protected areas in Belize. Samples were collected at Glover’s Reef and Hol Chan reserves (located inside the black circles).

2.3 DNA extraction and Microsatellite amplification

Genomic DNA was isolated from muscle tissue using the ISOLATE Genomic DNA Mini Kit (BIOLINE). DNA quality and quantity was assessed by a NanoDrop 2000 micro-volume spectrophotometer (THERMO SCIENTIFIC). Primers for 5 microsatellite loci (Table 1) were simultaneously amplified by multiplex PCR with a Qiagen Type-it Microsatellite PCR kit. PCR reactions took
### Table 1. Microsatellite primers and allele sizes.

<table>
<thead>
<tr>
<th>Loci</th>
<th>Sequence (5' - 3')</th>
<th>Number of Alleles</th>
<th>Size Range (Base Pairs)</th>
<th>GenBank Accession Number</th>
</tr>
</thead>
</table>
| Par3 | F: TTACCGGTTGACAGGAGAC  
R: GTCCGTTGTTCCGATATTC | 9 | 98-138 | AY526337 |
| Par6 | F: GAAGTTTCCCTAATGTTCGTCT | 4 | 83-95 | AY526340 |
| Par7 | F: TGGGTAACCGTAAGACTATTGA  
R: CAGACAGATGGACCGAGAGA | 11 | 117-157 | AY526341 |
| Par9 | F: CCCTGACCTCTTGTTAAAACCTCG  
R: TCAGTCTATCCATCTATCTAAACCATC | 4 | 155-183 | AY526343 |
| Par10 | F: CAAGCAAAGCACAGAAGCAT  
R: AACCACGTCCAGTCAGTT | 15 | 242-386 | AY526344 |

### Table 2. Hardy-Weinberg equilibrium and $F_{ST}$ for Glover’s Reef and Hol Chan populations.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Hol Chan</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Alleles</td>
<td>$H_O$</td>
<td>$H_E$</td>
<td>$P_{HWE}$</td>
<td>Samples</td>
<td>Alleles</td>
<td>$H_O$</td>
<td>$H_E$</td>
<td>$P_{HWE}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Par3</td>
<td>16</td>
<td>26</td>
<td>1.000</td>
<td>0.824</td>
<td>0.843</td>
<td>41</td>
<td>54</td>
<td>0.925</td>
<td>0.785</td>
<td>0.112</td>
<td>-0.196</td>
<td>0.021</td>
</tr>
<tr>
<td>Par6</td>
<td>16</td>
<td>26</td>
<td>0.692</td>
<td>0.559</td>
<td>0.786</td>
<td>41</td>
<td>44</td>
<td>0.545</td>
<td>0.622</td>
<td>0.494</td>
<td>-0.002</td>
<td>0.019</td>
</tr>
<tr>
<td>Par7</td>
<td>16</td>
<td>12</td>
<td>0.000</td>
<td>0.783</td>
<td>0.001</td>
<td>41</td>
<td>28</td>
<td>0.214</td>
<td>0.814</td>
<td>&lt;0.001</td>
<td>0.822</td>
<td>-0.021</td>
</tr>
<tr>
<td>Par9</td>
<td>16</td>
<td>20</td>
<td>0.100</td>
<td>0.100</td>
<td>&lt;0.001</td>
<td>41</td>
<td>8</td>
<td>0.000</td>
<td>0.425</td>
<td>0.143</td>
<td>0.639</td>
<td>0.787</td>
</tr>
<tr>
<td>Par10</td>
<td>16</td>
<td>28</td>
<td>0.500</td>
<td>0.857</td>
<td>0.003</td>
<td>41</td>
<td>22</td>
<td>0.454</td>
<td>0.809</td>
<td>0.003</td>
<td>0.439</td>
<td>0.018</td>
</tr>
</tbody>
</table>
place in a 25µL reaction volume containing 20-100ng DNA, 1µM forward and reverse primers (5’ end labeled with fluorescent dye, Cyc5/Cyc5.5) in 1x QIAGEN Multiplex PCR Master Mix containing HotStar Taq DNA Polymerase, and 3 mM MgCl₂. Primers were optimized under following conditions: DNA polymerase was activated in an initial activation step (95°C for 5 min), followed by 28 thermocycles of denaturation (95°C for 30 s), annealing (60°C for 90 s), and extension (72°C for 30 s), and a final extension (30 min at 60°C). Floresent- labeled PCR products were size-separated and analyzed in a CEQ 8000 Genetic Analysis System (Beckman Coulter). Allele peak profiles were identified at each locus with alleles designated by their size in base pairs. Binning of allele size was carried out using the CEQ 8000 Genetic Analysis System software. All fragment sizes were pre-analyzed by the software and checked by eye.

2.4 Statistical Analysis

Allelic diversity, heterozygosity, departure from Hardy-Weinberg equilibrium, and F-statistics were calculated using GenePop (Rousset 2007). A population assignment test was carried out using the Bayesian model based software STRUCTURE (Pritchard 2000). The admixture model with standard settings was applied and 100,000 Markov chain Monte Carlo steps was used with a burn-in period of 10,000. Two runs were conducted to test for the number of genetic clusters, K, in the dataset. Each run was repeated three times to test assess convergence. Statistical power analyses were conducted with the software Whichloci (Banks and Eichert 2000).
3. Results and Discussion

3.1 Microsatellite Loci

A total of 16 individual lobsters from Hol Chan marine reserve and 41 lobsters from Glover’s Reef were scored for 5 microsatellite loci to explore levels of gene flow between the marine reserves. Results from the CEQ 8000 Genetic Analysis System software indicated that the multiplex PCR worked well and fragment sizes were similar to those previously described by Diniz et al. (2006; see Table 1). To investigate the potential for null alleles, calculations of observed heterozygosity, expected heterozygosity, and Hardy-Weinberg equilibrium were conducted (Table 2). The number of alleles ranged from 12 - 28 for individuals from Hol Chan marine reserve and 8 - 54 for Glover’s Reef marine reserve. The increased number of alleles present at Glover’s reef is most likely an artifact of increased sample size rather than actual population structure. The low observed heterozygosities and deviation from Hardy-Weinberg equilibrium (i.e., assuming random mating, no mutation, no drift, no migration; P < 0.001) suggests the presence of null alleles (those that fail to amplify during PCR) at Par7 and Par9. The small number of alleles present and 100% non-overlapping allele frequencies at Par9 provided further evidence of null alleles at this locus. As a conservative measure to minimize the effect of fragment scoring error due to null alleles, Par7 and Par9 were excluded from statistical power analyses and Bayesian models of population structure.
Figure 2. Structure assignment test for *Panulirus argus* individuals from Hol Chan (black) and Glover’s Reef (grey) populations. The probability of correct assignment of individuals from Hol Chan was ~90% and > 95% for Glover’s Reef.

Statistical power analyses were conducted to assess how many samples should be collected from each site to achieve a 95% correct population of origin assignment. Power analysis identified Par3 as the most informative locus, followed by Par10, then Par6. Furthermore, a power analysis indicated that collecting samples from 30 individuals from each site was sufficient to achieve 95% correct assignment between populations from Glover’s Reef and Hol Chan marine reserves.

Applying $F$-statistics to the microsatellite data set suggested low levels of population differentiation between Hol Chan and Glover’s Reef populations (Table 2). The overall $F_{ST}$ value among all samples was 0.02. These findings were corroborated by a population assignment test using the program STRUCTURE (Figure 2). All individuals from Hol Chan and Glover’s Reef were correctly assigned to their populations with a probability of > 90%. When Par7 and Par9 were included in the analyses of $F$-statistics, the overall $F_{ST}$ value dramatically increased to 0.279, suggesting strong levels of population differentiation. Similarly, when these two loci were included, the probability of correct population assignment using
STRUCTURE remained high at > 95%. Genotyping of all individuals at Par7 and Par9 should be repeated to confirm if the estimates of population differentiation at these loci are indeed valid, because the presence of null alleles can confound estimates of population differentiation. Finally, even when Par7 and Par9 were excluded from $F$-statistics and spatial analyses in STRUCTURE, the microsatellite loci Par3, Par6, and Par10, in combination with the sampling regime, were sufficiently powerful to detect genetic differentiation between marine reserve populations in Belize.

Population structure in *P. argus* was observed on a small spatial scale between Glover’s Reef and Hol Chan marine reserves using only three microsatellite markers. These results suggest that connectivity may be limited between offshore atolls and barrier reef populations in Belize. The findings of this pilot study provide a glimpse into the connectivity patterns among MPAs in Belize, and although only two MPAs were sampled, a more detailed picture of connectivity will be provided by an ongoing study to genotype several size classes of spiny lobsters from MPAs throughout the region, using 26 microsatellite markers.

### 3.2 Biological Implications

Biophysical modeling should work hand in hand with field and laboratory studies to empirically test model predictions ultimately improving the capabilities of models to test numerous biological hypotheses (Werner 2007). This pilot-study followed that approach by using genetic markers to test the recent findings of Butler et al. (2011). The levels of genetic differentiation found between Glover’s Reef and
Hol Chan suggest that gene flow between *P. argus* populations from the two marine reserves is insufficient to override the effect of genetic drift. These findings support the Butler et al. (2011) biophysical model that suggests northern Belize may be biogeographically different from southern Belize due to localized flow regimes, and are consistent with a growing consensus that larval behavior in combination with local hydrodynamics strongly effect recruitment patterns and genetic population structure (reviewed by Selkoe 2008).

It is an oversimplification to suggest that local hydrodynamics and larval behavior are the only factors responsible for the observed patterns we found. The availability of suitable nursery habitat is crucial for the survival of *P. argus* larvae and may ultimately limit their successful recruitment. Spatial analyses of nursery habitat availability should also be incorporated into future genetic analyses of *P. argus* connectivity. Similarly, marine reserves have been designed throughout the Caribbean to conserve critical nursery and spawning habitats for *P. argus* and the effects of these conservation strategies should be taken into account. Additionally, one must account for the effect that protection from fishing has on *P. argus* genetic structure. Acosta et al. (2003) found a remarkable 20x increase in spiny lobster abundance in un-fished patch reefs after only 5 years of protection in Glover’s Reef marine reserve. Information concerning the increases in lobster abundance in the no-take area of Hol Chan has yet to be published and could potentially provide additional support for these genetic findings.
3.3 Implications for Marine Reserves

The importance of oceanographic current regimes on genetic structure and connectivity is gaining greater recognition in the sustainable management of marine reserves. Improving our understanding of how persistent gyres retain larvae while strong boundary currents sweep them away can be used to assist in the regional management of many organisms, including *P. argus*. For example, Butler et al. (2011) suggested that local management might be more effective in regions with persistent gyres such as Belize, Honduras, and Guatemala, and less so farther north along the Yucatán coast of the Caribbean where locally-derived larvae are swept towards Florida. Future genetic studies are required to improve biophysical models and provide critical insight to fishery managers interested in conserving declining *P. argus* stocks.

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**Literature Cited**


Chapter 6

Microsatellite analysis reveals spatiotemporal genetic differentiation in the Caribbean spotted spiny lobster, *Panulirus guttatus*

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**Running Title:** Spatial and Temporal genetic differentiation in *Panulirus guttatus*

**Key Words:** Spotted spiny lobster, *Panulirus guttatus*, genetics, microsatellites, connectivity, conservation, marine protected areas, fisheries

*Prepared for submission to Conservation Genetics Resources*

**Contributions:** NKT, RFP, DB, and MB designed the study. NKT, DB, and MB collected the samples. NKT conducted the laboratory work. NKT and RFP analyzed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

Fishing pressure on Caribbean spotted spiny lobster *Panulirus guttatus* has begun to increase as fisheries of the Caribbean spiny lobster *Panulirus argus* have been in decline in many countries throughout the Caribbean. Management policies for *P. guttatus* are hindered by a lack of basic population information for this species. This study provides novel data on spatiotemporal patterns of genetic variation in the Caribbean spotted spiny lobster *P. guttatus*. We used eight microsatellite markers to genotype 120 *P. guttatus* individuals from six locations in the Caribbean. Our results using several statistical techniques (\(F_{ST}\), Jost’s D, and, AMOVA) provided evidence of high levels of temporal population structure among size classes within Florida and lower levels of temporal population structure in Bermuda. Higher levels of genetic differentiation in Mexico largely drove spatial patterns of population structure in *P. guttatus*. Finally, this study identified a useful and logistically simple methodology for identifying temporal population dynamics in *P. guttatus* that can be readily applied to other marine species.
1. Introduction

The majority of marine species have a dispersive phase in their life history that connects distant populations in an environment that often lacks obvious barriers to dispersal (Cowen et al. 2007). Over the last decade evidence from a wide variety of studies in marine population connectivity have suggested that many marine populations may be more closed than previously thought (reviewed by (Cowen et al. 2007)). Numerous studies have identified barriers to connectivity on large scales (reviewed by (Hauser and Carvalho 2008)) and the emerging field of seascape genetics has begun to uncover how spatial patterns of environmental heterogeneity are driving patterns of genetic variation in marine populations that were previously believed to be “chaotic” (Selkoe et al. 2010). Despite these advances in our understanding for marine population connectivity, empirical evidence concerning the temporally variation of spatial connectivity patterns is severely lacking (Toonen and Grosberg 2011). For conservation purposes it is critical to understand whether or not connectivity patterns are stable or a simply a “snapshot” in a dynamically changing environment (Toonen and Grosberg 2011).

Detecting temporal patterns of marine connectivity is of particular importance for international cooperation in fisheries management (Kough et al. 2013). For instance, recent biophysical modeling research on the larvae of the Caribbean spiny lobster, Panulirus argus suggests that the dispersal of long-lived larvae of P. argus is driven by temporally unstable hydrodynamics coupled with ontogenetically variable larval behavior (Kough et al. 2013). Kough and colleagues (2013) identified regions in the Caribbean that contributed disproportionately large
amounts of *P. argus* larvae to the wider Caribbean larval pool on a temporally consistent basis. These data strongly suggest that in order to help reverse the declining *P. argus* fishery, management efforts should focus on protecting adult spawning populations in regions that provide the majority larvae to the Pan-Caribbean population (Kough et al. 2013).

Fisheries management and conservation efforts for other species of spiny lobster in the Caribbean could also benefit from spatiotemporal studies of population connectivity. In contrast to *P. argus* the Caribbean spotted spiny lobster *Panulirus guttatus* remains largely neglected by researchers. Until recently, *P. guttatus* has not been targeted by commercial fisheries due to its smaller size, lower abundance, and more reclusive behavior. However, fishing pressure on *P. guttatus* has begun to increase as *P. argus* fisheries have been in decline in many countries throughout the Caribbean (Fanning et al. 2011), (Wynne and Côté 2007). Commercial fishery operations for *P. guttatus* now operate in Bermuda, Mexico, Antigua, and Martinique, and others are on the horizon (Wynne and Côté 2007). Management policies for *P. guttatus* are either extremely limited or non-existent throughout much of their range (Acosta and Robertson 2003), hindered by a lack of basic population information. These data are urgently needed to develop sustainable fisheries management plans for *P. guttatus*, especially as more commercial fishery operations begin to target this species.

Although similar in form, *P. guttatus* has life history adaptations that are quite different from those of *P. argus* (the life history of *P. argus* is explained in further detail in (Butler et al. 2006)). These adaptations may reflect selection for
traits that are advantageous for life within structurally complex coral reef habitats (Briones-Fourzán et al. 2002; Robertson and Butler 2013; Robertson and Butler 2009). Unlike *P. argus*, whose post-larvae settle in shallow hard-bottom, seagrass, or mangrove habitats (Butler et al. 2006), *P. guttatus* post-larvae settle directly on the coral reef (Sharp et al. 1997). The post-larvae of *P. guttatus* are also >50% larger than those of *P. argus*. Their greater size could be an adaptation for predator avoidance and suggests their pelagic larval duration (PLD) may be even longer than that of *P. argus* (PLD ranges from 6-12 months). Mark recapture and behavioral studies have revealed that *P. guttatus* is a reclusive species with a home range limited to small (<100m) sections of reef (Briones-Fourzán et al. 2002; Lozano-Alvarez et al. 1991; Sharp et al. 1997). This extremely limited range is in complete contrast to that of *P. argus*, known for diel and seasonal migrations (Phillips 2008).

Thus far, there have been no attempts to determine connectivity patterns among *P. guttatus* populations and fishery managers have had to assume connectivity patterns for *P. guttatus* resemble those for *P. argus*. Clearly, this assumption is problematic considering the differences in life history and post-larval size between the two species. The recent development of molecular markers specific to *P. guttatus* has opened the possibility to genetic studies of *P. guttatus* population structure and connectivity (Chapter 2).

The objective of our study was to use microsatellite markers to obtain novel data on spatiotemporal patterns of genetic variation in the Caribbean spotted spiny lobster *P. guttatus*. This species is ideal for exploring how temporal variability in larval recruitment dynamics may influence spatiotemporal patterns of genetic
variation, since its larvae are long lived, settle only in coral reef habitat, and individuals have a home range of < 200m after larval settlement, suggesting that patterns of genetic variation will not be obscured by any additional mixing caused by adult migration. We examined temporal patterns of genetic variation in individuals from several different size classes in two distinct geographic locations. Spatial patterns of genetic population structure were investigated by selecting individuals from locations 1) with contrasting types of ocean currents, 2) contrasting types of coral reef habitat and 3) separated by large geographic distances > 1000km. In this study we test the null hypothesis that *P. guttatus* is a panmictic and temporally stable population.

2. Materials and Methods

2.1 Sampling

Tissue samples and carapace length measurements of *P. guttatus* (Figure 1) from North Rock and East Blue Cut in Bermuda were collected by the Bermuda Fisheries Department and taken from adult lobsters captured by the trap fishery in October 2011. Samples were collected from Glover’s Reef and Caye Caulker, Belize in July 2011 by free diving at night with a tickle stick and net. Muscle tissue from all Belize samples were taken from a single leg and stored in 190 proof clear rum purchased from the Travelers Liquor Distillery in Belize City. Our scientific research permit from Belize prohibited us from using SCUBA equipment to collect tissue samples, primarily to avoid any potential conflicts of interest with local fishermen are also prohibited from using SCUBA to fish for lobsters. Due to the
**Figure 1: Study Sites and K-means clustering analysis.** A) Approximate locations of sampling sites in the Caribbean. The colors of the dots represents the mean coordinates of the first two discriminant functions of the K-means clustering analysis that have been recoded as signal intensities of red and green. B) Summary of all individuals assigned to three unique clusters. Each dot represents an individual. The color of the dot corresponds to the cluster that each individual was assigned to (red = cluster 1, yellow = cluster 2, green = cluster 3). C) Subdivision of clusters according to the DAPC method. Dots represent individuals (red = cluster 1, yellow = cluster 2, green = cluster 3) and 95% inertia ellipses are included for each cluster. Visualization of Caribbean ocean currents was provided by the NASA/GSFC Scientific Visualization Studio using flow data from the ECCO2 model.

logistical difficulties of free diving at night we were unable to collect carapace length measurement from individuals from Belize. Samples from Mexico were purchased from a restaurant in Akumal in July 2011. Tissue samples from Florida were collected by SCUBA diving at night at patch reefs near Long Key, Florida in July 2011. Individuals were collected with a net and tickle stick and transported to a live tank on the University of Florida research vessel. All *P. guttatus* individuals were transported to aquaria at the Goshen Marine Lab, in Long Key Florida where their carapace length was measured, and tissue samples were collected from a single leg and stored in 100% molecular grade ethanol. After measurements and tissue samples were collected, all *P. guttatus* individuals were returned to the patch reefs they were originally collected from. All tissue samples were stored at room temperature and transported to the University of Manchester where the original ethanol was replaced with 95% molecular grade ethanol and stored in a cold room at 5 °C until the DNA was extracted from each sample.
2.2 Microsatellite genotyping

Genomic DNA was isolated from muscle tissue using the Wizard SV-96 Genomic DNA extraction kit following the manufacturer’s protocol (Promega). DNA quality and quantity was assessed by a NanoDrop 2000 micro-volume spectrophotometer (THERMO SCIENTIFIC). Based on previous research that characterized microsatellite markers for *P. guttatus*, eight markers (FWC5, PG3, PG6, PG9, PG15, PG21, PG22, PG23) were selected for the study (Chapter 2). Fluorescent-labelled (6-FAM®, NED®, VIC® and PET®) forward primers (Applied Biosystems) and non-labeled reverse primers (Sigma-Aldrich) were used for three PCR multiplex reactions. Each multiplex PCR reaction was performed with a Veriti thermal cycler (Applied Biosystems) in total volume of 5 µl. The PCR multiplex reaction mix consisted of 0.5 µl of the 10x primer mix (2μM of each primer), 2.5 µl of Type-it Multiplex PCR Master Mix (QUIAGEN), 1 µl of molecular grade water and 1µl of (10-20 ng/µl) genomic DNA. The multiplex PCR conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 26 cycles of 95 °C for 30 s, 57 °C for 120 s, and 72 °C for 30 s. This was followed by final extension at 60 °C for 30 min. To facilitate the fragment analysis, PCR products were diluted with 5 µl MQ water. From the diluted product 0.5 µl was mixed with 9.5 µl of a mix consisting Hi-Di Formamide® (Applied Biosystem) and GeneScan – 500 LIZ Size Standard (37:1) in a 96 well PCR plate. Fragment analysis was performed on an ABI 3730xl automatic DNA sequencer (Applied Biosystems, USA) at the University of Manchester DNA Sequencing Facility. Microsatellite alleles were scored using GeneMapper® v3.7 software package (Applied Biosystems). Binning
of microsatellite alleles and error checking were performed by the R package MsatAllele version 1.02 (Alberto 2009) using R statistical software v2.15.1 (Ihaka and Gentleman 1996).

### 2.3 Genetic analyses

Genetic variation and microsatellite metrics including number of alleles (NA), the unbiased heterozygosity (Hₑ), and number of private alleles (PA) were calculated with the software package GenAlEx v6.5 (Peakall and Smouse 2012). The entire data set was checked for variability and departures from Hardy-Weinberg equilibrium (HWE) and the fixation index (Fₛ) was calculated with the software package GENODIVE v2.0b23 (Meirmans and van Tienderen 2004). The analysis was run using the least squares (AMOVA Fₛ) method and was tested with 50K permutations. Linkage disequilibrium (LD) between loci and significance levels of pairwise Fₛ values were tested using Genepop on the Web v4.2 (Raymond and ROUSSET 1995; ROUSSET 2008). Markov chain parameters were set to the following: dememorization number 10K, number of batches 1K, and number of iterations per batch 10K. Microchecker (van Oosterhout et al. 2004) was used to detect allele scoring error and presence of null alleles.

### 2.4 Measures of spatiotemporal genetic differentiation

For analysis of temporal population structuring the individuals within each site were grouped into 5mm size classes based upon growth and size at maturity research of *P. guttatus* from the Florida Keys (Robertson and Butler 2003). Several
comparisons of genetic differentiation (Jost’s $D$, $F_{ST}$, and $G_{ST}$) were conducted among size classes in Blue Cut (Bermuda), North Rock (Bermuda) and the Florida Keys. The genetics software program FREENA (Chapuis and Estoup 2006) was used to calculate, 1) pairwise comparisons of genetic differentiation $F_{ST}$ among all sample sites and all size classes, 2) unbiased estimates of pairwise $F_{ST}$ following the method described in (Chapuis and Estoup 2006) to correct for the presence of null alleles. Exact tests for genic differentiation were used to calculate levels of significance for pairwise comparisons of $F_{ST}$ among size classes in the software GenePop v4.2 (Raymond and Rousset 1995; Rousset 2008). Markov chain parameters in GenePop v4.2 for were set to the default values: dememorization number 10K, number of batches 100, and number of iterations per batch 5K. The R-package DEMETRICS was used to calculate Jost’s $D$ and $G_{ST}$ (Gerlach et al. 2010). DEMETRICS corrects for any loci that deviate from Hardy Weinberg Equilibrium by following the methodology of (Goudet et al. 1996). The levels of significance ($P$-values) for both measures of genetic differentiation were calculated using 10K bootstrap resamplings. Given the high number of tests a correction for multiple comparisons was preformed to avoid type I errors. The Bonferroni correction is often used to correct for multiple comparisons, however it can often be too strict and lead to type II errors. Therefore we applied the Benjamini and Hochberg correction (Benjamini and Hochberg 1995) which controls for the false discovery rate (FDR), the expected proportion of false discoveries amongst the significantly different pairwise comparisons.

An analysis of molecular variance (AMOVA) was used to test for significant differences in genetic variation among individuals and populations among a)
sampling sites, b) size classes in Florida and c) size classes in Bermuda. The AMOVA was run following the methods outlined by (Michalakis and Excoffier 1996) using the software package GENODIVE v2.0b23 (Meirmans 2012; Meirmans and van Tienderen 2004). All microsatellite loci were included in the AMOVA (N = 8). Individuals with missing data had their missing data replaced with randomly drawn alleles based on the overall allele frequencies. An infinite allele model was used thus the reported statistics are equivalent to $F_{ST}$. Significance was tested using 30K permutations.

2.4 Measures of spatial genetic differentiation

For exploration for spatial patterns of genetic variation within our study system we used the population genetics software STRUCTURE was to infer the optimal number of unique genetic clusters (referred to as K) among all sites and within all sites. We followed the recommendations for utilizing and reporting population genetic analyses in the program STRUCTURE published by (Gilbert et al. 2012). For optimizing the parameters for each STRUCTURE run we followed the criteria described by (Evanno et al. 2005) for detecting the number of clusters of individuals using STRUCTURE. Briefly, we ran 100K burn-in iterations and MCMC (Markov chain Monte Carlo) of 200K. We used the admixture model, correlated allele frequencies between populations, and let the degree of admixture alpha be inferred from the data and finally lambda (the parameter of the distribution of allele frequencies) was set to one. For each data set 20 runs were carried out in order to calculate the amount of variation for the likelihood of each K. The Evanno method (Evanno et al. 2005) was
then used to infer $K$. The raw \textsc{structure} data was uploaded into the online version of \textsc{structure harvester} Web v0.693 (Earl and vonHoldt 2011) to calculate Delta $K$ (the inferred number of genetic clusters determined by the Evanno Method).

Several recent studies have also inferred that \textsc{structure} has limited power to detect clusters when levels of geneflow are high and differentiation among populations is low (reviewed by (Kalinowski 2010)). The multivariate statistical method, the discriminant analysis of principle components (DAPC), designed to identify clusters of genetically similar individuals, generally performs better than \textsc{structure} for detecting subtle population subdivision (Jombart et al. 2010). Unlike $F_{ST}$ based analyses DAPC does not rely on any particular population genetics model and is robust to deviations from HWE, null alleles, and linkage disequilibrium (Jombart et al. 2010). For these reasons, we followed a recently described DAPC based methodology for detection of spatial genetic patterns in regions with pronounced biocomplexity (Therkildsen et al. 2013). First, we applied DAPC(Jombart et al. 2010) as implemented in the R-package \texttt{adegenet} (Jombart 2008). Since we did not know \textit{a priori} how many potential populations were present in our study region, we used the \texttt{find.clusters()} function to run $K$-means clustering for $K = 1:10$ and applied the Bayesian Information Criterion (BIC) to identify most likely number of clusters. We applied the \texttt{dapc()} function to describe the genetic relationship among the groups identified by $K$-means clustering. The \texttt{dapc()} function constructs synthetic variables called discriminant functions (DFs) which have been designed to maximize variation between groups whilst minimizing variation within groups. To avoid over-fitting, which could bias our results, we used
the functions xvalDAPC() and optim.a.score() to calculate the optimal number of principle components to retain for the DAPC analysis. Both methods indicated that 15 principle components, representing 54% of the total variation in our dataset were the optimal to retain, to avoid over-fitting. Results were visualized using the scatter () function in ADEGENET.

We then applied a method for visual inspection of genetic differentiation among our sampling sites using color. The method assigns a unique color to each of sampling sites that corresponds to proportion of individuals that belong to each genetically unique cluster that we previously identified with DAPC. Sampling sites that have similar colors are the most similar genetic composition, and as the color signal changes from green to red, so too does the level of genetic differentiation between geographic locations. To create this visualization we calculated the mean sample coordinates of the first two DFs of all individuals from each sampling location and recoded them as signal intensities of red and green using the colorplot () function in ADEGENET. Finally to visualize spatial patterns of genetic differentiation we overlaid the results of the colorplot () function on top of a map of our sampling locations (Figure 1).

2.5 Spatial Outlier Detection

We adapted the protocol developed by (Elphie et al. 2012) for arbitrarily defining a reference population within populations with high levels of mixing and geneflow. The previous methodology used a nonmetric multidimensional scaling procedure to define the reference population. This technique implies that the cloud
of points of the population is well represented in 2 dimensions and also spherical, both of which are unlikely (T. Jombart, personal communication). To overcome these potential limitations we devised a methodology to compare the genetic distances of each individual to all other individuals. This allowed us to separate out the most genetically similar individuals and place them into a reference population for assignment testing in \texttt{GENECLASS2} (Piry 2004). The individuals that were most genetically different for all others were placed into an assignment population.

To identify individuals to place into the reference and assignment populations we began by creating a pairwise matrix of the squared Euclidian distances of the allelic profiles of each individual generated by \texttt{ADEGENET} (located in the \texttt{@tab} slot of the \texttt{genind} object). The mean of all pairwise distances in the matrix was then used to arbitrarily define the reference population for assignment testing in \texttt{GENECLASS2}. Each individual that had a mean pairwise genetic distance < the mean of genetic distances of all individuals was placed into the reference population and all other individuals were placed into the assignment population. We then used \texttt{GENECLASS2} to run assignment tests to identify genetically unique individuals in genetically homogeneous populations, following the methods previously developed by (Elphie et al. 2012). Individuals with a $<5\%$ probability of belonging to the general population after \texttt{GENECLASS2} assignments were considered spatial outliers (Elphie et al. 2012; Hogan et al. 2011). Finally a neighbor-joining tree was constructed using the \texttt{nj()} function in the R-package \texttt{APE} (Paradis et al. 2004) with the squared Euclidian distances of the allelic profiles of each individual. The neighbor-joining tree allows for visual inspection of the relatedness of all spatial
outliers. All individuals that were identified as spatial outliers in GENECLASS2 were identified on the neighbor-joining tree with unique colors for each sampling location.

3. Results

3.1 Data Quality Control and Summary Statistics

Eight microsatellite loci were used to genotype 120 *P. guttatus* individuals from six locations in the Caribbean. Summary statistics of sample sizes, number of alleles, private alleles, observed heterozygosity (H₀), expected heterozygosity (Hₑ), the probability of departure from Hardy-Weinberg equilibrium (HWE) and the average inbreeding coefficient (Fᵢₛ) for each locus within each population are presented in Table S1. Across all loci and populations H₀ was consistently lower than Hₑ, except at locus PG9 (ranging from 0.846 to 1.00). Likewise, the mean H₀ across all loci was < the mean Hₑ across all loci (Table 1). Significant deviations from HWE (P < 0.001) were observed in loci at North Rock (PG3, PG21, and PG22), Florida (PG22), Caye Caulker (PG22), and Glover’s Reef (FWC5, PG22). The inbreeding coefficient Fᵢₛ exhibited mostly positive values, except at locus PG22 at Blue Cut (0.00) and locus FWC5 at Mexico (= -0.017). *Panulirus guttatus* individuals from North Rock had the highest number of private alleles (N = 9), followed by Glover’s Reef (N = 9), Florida (N = 5), Caye Caulker (N = 2), Blue Cut (N = 1), and Mexico (N = 1).

Analysis with MICROCHECKER found no evidence of scoring errors due to large
Table 1. Spatial summary statistics. $N$, number of individuals; $Ho$, mean observed heterozygosity over all loci, $He$, mean expected heterozygosity over all loci; Cluster 1 – Cluster 3, number of individuals assigned by $K$-means clustering in ADEGENET to each cluster with a DAPC posterior probability > 99%; Outliers, number of individuals that had < 5% probability of belonging to the general population after assignment testing in GENECLASS2.

<table>
<thead>
<tr>
<th>Country</th>
<th>Location</th>
<th>$N$</th>
<th>$Ho$</th>
<th>$He$</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Outliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bermuda</td>
<td>Blue Cut</td>
<td>16</td>
<td>0.802</td>
<td>0.819</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Bermuda</td>
<td>North Rock</td>
<td>33</td>
<td>0.719</td>
<td>0.838</td>
<td>8</td>
<td>11</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>USA</td>
<td>Florida Keys</td>
<td>24</td>
<td>0.680</td>
<td>0.831</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Mexico</td>
<td>Akumal</td>
<td>6</td>
<td>0.656</td>
<td>0.778</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Belize</td>
<td>Caye Caulker</td>
<td>15</td>
<td>0.657</td>
<td>0.810</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Belize</td>
<td>Glover's Reef</td>
<td>26</td>
<td>0.680</td>
<td>0.831</td>
<td>10</td>
<td>11</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

allelic dropout or stuttering. MICROCHECKER analysis suggested null alleles were present at locus PG22 for all locations except Blue Cut and Mexico (frequency ranging from 0.18 – 0.37); locus PG3 at North Rock (frequency = 0.14); locus FWC5 at Glover’s Reef (frequency = 0.16); locus PG21 at Florida (frequency = 0.2). The presence of null alleles is not surprising since this phenomenon is commonly observed in a variety of marine invertebrates, particularly species with large population sizes (Ben-Horin et al. 2009; Dailianis et al. 2011). The Wahlund effect, which is caused by grouping multiple genetically differentiated populations into a single population, may also lead to deficiencies in heterozygotes (Johnson and Black 1984a).

Locus PG22 had the highest levels of missing data and estimated null allele frequencies (Table S1) and was initially removed from all statistical tests of population differentiation. These results were then compared to the results of the same analyses with PG22 included. Since there were no differences in the overall trends or in levels of statistical significance we included PG22 in all analyses of
population differentiation. No evidence of linkage disequilibrium was found among any combination of loci.

3.2 Spatial Population Structure

All spatial pairwise comparisons of genetic differentiation using either Jost’s $D, F_{ST}$, $F_{ST}$ corrected for null alleles, or $G_{ST}$ were highly correlated, therefore, for simplicity we will only report uncorrected $F_{ST}$ values (Supplemental Table 2).

Global $F_{ST}$ values using the ENA correction for the presence of null alleles ($F_{ST} = 0.0049$) were slightly higher than uncorrected global $F_{ST}$ values ($F_{ST} = 0.004$). This result suggests that the potential presence of null alleles in our data were not biasing statistical measures of population differentiation.

Mexico consistently had the highest pairwise $F_{ST}$ values among all sites ($F_{ST}$ values ranging from 0.05 to 0.09) and all pairwise combinations were significant after the false discovery rate FDR correction ($P < 0.001$; Figure 2a). Pairwise estimates of $F_{ST}$ among some sites in Belize were significantly different from sites in Bermuda, however, not all pairwise combinations were significantly different. For instance, Caye Caulker had higher levels of differentiation between Blue Cut ($F_{ST} = 0.031; P < 0.03$) than North Rock ($F_{ST} = 0.012; P > 0.05$) and the same trend was observed among comparisons of Glover’s Reef and Blue Cut ($F_{ST} = 0.031; P < 0.02$) and North Rock ($F_{ST} = 0.013; P > 0.05$). Pairwise comparisons of genetic differentiation were not statistically significant between sites in Bermuda; among sites in Bermuda and Florida; among sites in Belize and Florida; or between sites in
**Figure 2: Heatmap of pairwise estimates of genetic differentiation ($F_{ST}$) of *Panulirus guttatus in the Caribbean.* Pairwise estimates of differentiation are color-coded (light green = low values, dark green = high values) and sorted by: A) sampling location; B) Florida size classes; C) Blue Cut, Bermuda size classes; and D) North Rock, Bermuda size classes. Pairwise differentiation values that were significantly different from zero are represented by a bold asterisk (*) after correction for multiple comparisons.

Belize. The AMOVA analysis compared the levels of spatial genetic differentiation among all sites (Table 2) and found significant differences among individuals ($F_{IS} = 0.164, P < 0.001$) and significant differences among populations ($F_{ST} = 0.011, P < 0.001$).

### 3.2.1 Discriminant Analysis of Principle Components

The $K$-means analysis suggested that either two or three clusters of genetically unique individuals were most likely present in the geographic region that we sampled, since these clustering solutions had the lowest BIC values (Fig. S6). Recoding the mean coordinates of the first of the DFs into signal intensity of red and green color two groups were well differentiated using either $K$-means clustering solutions of two or three clusters. Since the $K$-means clustering solution of 3 clusters clearly separated Mexico from all other sites, which was in agreement with the $F_{ST}$ analysis, we proceeded with this clustering solution (Figure 1). Visualization of the geographic distribution of genetically unique clusters suggested that Glover’s Reef and Caye Caulker are comprised of a mixture of individuals from genetically unique clusters that are more similar to Florida and more different from Mexico and both Bermuda sites (Figure 1a). All individuals in our dataset had a posterior probability
Table 2: AMOVA analysis weighted across all eight microsatellite loci in *Panulirus guttatus* for a) all sampling sites, b) among Florida size classes, c) among Blue size classes, and d) among North Rock size classes. Significant *P*-values are in bold.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Variance Component</th>
<th>% Variation</th>
<th>Fixation Indices (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) All Sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Individuals</td>
<td>0.558</td>
<td>0.162</td>
<td><em>F</em>&lt;sub&gt;IS&lt;/sub&gt; = 0.164 (&lt;0.001)</td>
</tr>
<tr>
<td>Among Populations</td>
<td>0.039</td>
<td>0.011</td>
<td><em>F</em>&lt;sub&gt;ST&lt;/sub&gt; = 0.011 (&lt;0.001)</td>
</tr>
<tr>
<td>b) Florida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Individuals</td>
<td>0.54</td>
<td>0.157</td>
<td><em>F</em>&lt;sub&gt;IS&lt;/sub&gt; = 0.164 (&lt;0.001)</td>
</tr>
<tr>
<td>Among Size Classes</td>
<td>0.147</td>
<td>0.043</td>
<td><em>F</em>&lt;sub&gt;ST&lt;/sub&gt; = 0.043 (0.004)</td>
</tr>
<tr>
<td>c) Blue Cut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Individuals</td>
<td>0.2029</td>
<td>0.0598</td>
<td><em>F</em>&lt;sub&gt;IS&lt;/sub&gt; = 0.0604 (0.0656)</td>
</tr>
<tr>
<td>Among Size Classes</td>
<td>0.0321</td>
<td>0.0095</td>
<td><em>F</em>&lt;sub&gt;ST&lt;/sub&gt; = 0.0095 (0.2581)</td>
</tr>
<tr>
<td>d) North Rock</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among Individuals</td>
<td>0.5462</td>
<td>0.1607</td>
<td><em>F</em>&lt;sub&gt;IS&lt;/sub&gt; = 0.1614 (&lt;0.001)</td>
</tr>
<tr>
<td>Among Size Classes</td>
<td>0.0142</td>
<td>0.0042</td>
<td><em>F</em>&lt;sub&gt;ST&lt;/sub&gt; = 0.0042 (0.3111)</td>
</tr>
</tbody>
</table>

> 0.99 to one of the three clusters (Figure 1c). The distributions of each individual assigned to each unique cluster (Figure 1b) suggested that cluster 1 (red) was most common in the southern Belize sites, whilst cluster 3 (green) was most common in the Bermuda sites. Florida was an even mix of all clusters, whilst Mexico had a distribution of clusters more similar to that of Bermuda than that of the sites in Belize. Analysis with *STRUCTURE* and subsequently *STRUCTURE HARVESTER* found similar evidence of multiple genetically unique clusters present within all sites (Figure S1).
3.2.2 Spatial Outlier Detection

The spatial outlier analysis identified the highest number of genetically unique individuals compared to the general population at two locations in the Mesoamerican Barrier Reef. Glover’s Reef and Mexico had two to three times the percentage of outliers compared to sites in Caye Caulker, Florida and Bermuda (Table 1). The neighbor-joining tree separated all individuals into three main branches (Figure 3). The first branch contained all outliers from Mexico (N = 3), no outliers from Blue Cut, Florida or Caye Caulker, and three outliers from Glover’s Reef. The second branch contained two outliers from North Rock, no outliers from Blue Cut, Florida, Mexico, or Caye Caulker, and a single outlier from Glover’s Reef. The third branch contained an outlier from Blue Cut and North, two outliers from Florida, no outliers from Mexico, two outliers from Caye Caulker and two outliers from Glover’s Reef.

3.3 Temporal Population Structure

Analysis of temporal genetic differentiation among size classes was conducted using the same statistical techniques as the comparisons for spatial genetic differentiation except of $K$-means clustering in ADEGENET and assignment tests in GENCLASS2 due to a lack of samples in our size classes to run these analyses (Supplemental Table 3). Pairwise comparisons of genetic differentiation among size classes were only conducted at Bermuda and Florida since these were the only sites where size data was collected. $F_{ST}$ values for pairwise temporal comparisons among size classes were generally higher (ranging from 0.031 to 0.058) than $F_{ST}$ values of
Figure 3: Neighbor-joining tree of genetic ‘outliers’ (see methods) based upon microsatellite allelic profiles. Dots represent individuals with a <5% probability of belonging to the general population after assignment testing and colors represent the geographic location of each genetic outlier (Blue = Blue Cut, Bermuda; Green = North Rock, Bermuda; Red = Florida; Yellow = Caye Caulker, Belize; Purple = Glover’s Reef, Belize; and Pink = Mexico). The red lines indicate the arbitrary cutoff for individuals placed into reference (left of the line) and assignment populations (right of the line) in GENECLASS2. The scale bar located at the bottom left hand corner provides a reference to identify levels of genetic similarity among individuals based upon squared Euclidian distances.

Spatial pairwise comparisons among sampling sites (ranging from 0.0022 to 0.092). The exact G-test analysis found significant differences among all size classes in Florida (Figure 2) both before and after FDR correction (P-values ranging from 0.00022 to 0.00828).

The analysis of genetic differentiation among age classes in Bermuda found no comparisons of among size classes to be statistically significant (Table 2). When the source of genetic variation was compared in Florida the AMOVA analysis found significant differences among individuals (F_{IS} = 0.164, P <0.001) and significant differences among size classes (F_{ST} = 0.043, P = 0.004). The AMOVA analysis in Blue cut found no significant differences among individuals (F_{IS} = 0.06, P = 0.06), or size classes (F_{ST} = 0.009, P = 0.258); whilst at North Rock differences among individuals were significant (F_{IS} = 0.164, P <0.001), however, no significant differences were observed among size classes (F_{ST} = 0.0042, P = 0.311).

4. Discussion

This research reveals the usefulness of collecting size data from every
individual to improve the interpretation of spatial and temporal patterns of genetic differentiation. Temporal variation is rarely analyzed in marine population genetics studies (Berry et al. 2012) and to our knowledge this is the first time it has been reported in a genetics study of spiny lobster. Temporal variation accounted for the highest levels of genetic differentiation observed in our study. This research provides a straightforward methodology that can be easily applied to future research studies of marine connectivity, population genetics, and the design and management of marine protected areas.

4.1 Temporal Patterns of Population Structure

The combined results of several statistical techniques (\(F_{ST}\), Jost’s D, G-test, AMOVA) provided evidence of high levels of temporal population structure among size classes within Florida and lower levels of temporal population structure in Bermuda. The trend of increasing \(F_{ST}\) was apparent in both locations, yet it was only statistically significant in Florida. One potential explanation for these results is that Florida regularly receives new recruits from a wide variety of source populations with high levels of genetic variation, whilst in contrast Bermuda receives recruits from fewer locations with lower levels of genetic variation. A more simple explanation could be that our measurements of size structure were not well correlated with the age of individuals in Bermuda. The individuals we studied in Bermuda were much larger than the individuals from Florida. Accurately aging older individuals is less reliable since the growth rate of \(P.\) guttatus declines in a linear fashion with age (Robertson and Butler 2003). Therefore, it is unlikely that
our age classes in Bermuda were cleanly separated, which would decrease the accuracy of our temporal analysis in Bermuda. Unfortunately, the individuals in the patch reefs we sampled in the Florida Keys tended to smaller than individuals collected from the trap fishery in Bermuda, which tends to select for larger individuals. Therefore, we did not have the opportunity to make comparisons among the same size classes at both locations. Future, studies would benefit from this type of analysis, since it has the potential to uncover an additional signal of temporal genetic variation that is lost when all individuals from a similar geographic region are pooled together.

There are several alternative hypotheses that could be responsible for shaping patterns of temporal variation that we observed in our study, such as: 1) changes in the source populations where larvae originate 2) sweepstakes recruitment 3) self-recruitment of local populations, 4) pre-post settlement natural selection (reviewed by (Planes and Lenfant 2002; Toonen and Grosberg 2011)). Directly testing each one of these hypotheses was beyond the scope our study. (Planes and Lenfant 2002).

The few large-scale studies of temporal genetic variation in marine species that have been conducted suggest that extensive sampling over multiple temporal scales can provide sufficient data to test how each of the multiple hypotheses mentioned in the previous paragraph are driving patterns of connectivity. For example, temporal genetic variation in the marine fish Diplodus sargus was evaluated among: 1) three age classes sampled at the same time (similar to the methodology in our study) and 2) among a single age class sampled three times over
a period of two years (Planes and Lenfant 2002). This additional analysis of temporal genetic variation provided sufficient data to suggest that levels of genetic variation among recruits is largely driven by large variation in reproductive success (supporting the sweepstakes recruitment hypothesis) followed by genetic homogenization through adult movement and selective processes (Planes and Lenfant 2002). Future studies of temporal genetic variation in marine species could benefit from these additional analyses.

### 4.2.1 Spatial Patterns of Population Structure

The combined results of several statistical techniques ($F_{ST}$, Jost’s $D$, $G_{ST}$, AMOVA, DAPC) suggest that spatial population structure in *P. guttatus* was largely driven by differences in Mexico. Pairwise levels of $F_{ST}$ were greater between Mexico and locations in Belize (separated by $< 300$ km) than between locations in Belize and Bermuda (separated by $> 2500$ km). Levels of $F_{ST}$ at Caye Caulker and Glover’s Reef were statistically different from Blue Cut in Bermuda but not from North Rock, located only 12.5 km away. Over large spatial scales no genetic differentiation was observed between sites in Belize and Florida, or between Florida and Bermuda. These counterintuitive patterns of adjacent sites exhibiting higher levels of differentiation than distant sites have become surprisingly common in studies of marine connectivity (Toonen and Grosberg 2011) and have been defined as “chaotic genetic patchiness” (Johnson and Black 1984b).

Microsatellite (Chapter 5) and allozyme analyses (Glaholt and Seeb 1992) in Belize of the Caribbean spiny lobster *Panulirus argus* both provided additional
evidence to the growing consensus that local hydrodynamics may be an important factor in explaining patterns of genetic differentiation on small spatial scales. The recent availability of high resolution biophysical models (Butler MJ et al. 2011; Cowen 2000) have fuelled an interest in linking spatially realistic models of larval dispersal with genetics (Selkoe et al. 2010). When biophysical modeling of Caribbean coral populations were integrated with genetics data there was a significant consensus between modeled estimates of genetic structure and empirical genetics data over large spatial scales (Foster et al. 2012). Over smaller spatial scales in the Mesoamerican Barrier Reef modeled estimates differed from genetics connectivity data, suggesting that larval dispersal may play a more limited role in shaping spatial genetics variation in that region. Relatively few studies have considered how site-based environmental factors influence levels of geneflow and genetic diversity in marine species. Site dependent habitat characteristics could be a particularly important feature that influences levels of genetic variation in *P. guttatus*, since this species is an obligate coral reef dweller; is confined to the same small portion of coral reef throughout its life history; and has very specific sheltering requirements (Sharp et al. 1997). A recent study that incorporated oceanographic modeling with environmental data for the California spiny lobster *Panulirus interruptus* found that kelp forest habitat was a more informative predictor of small-scale spatial patterns of genetic variation than ocean circulation (Selkoe et al. 2010). These findings suggest that future studies of *P. guttatus* should incorporate coral reef specific habitat data in addition to ocean circulation data to improve the explanatory value of genetics results on small spatial scales.
4.2.2 Spatial Outlier Analysis

A previous investigation of the genetic structure of early benthic juveniles of the spiny lobster *Panulirus elephas* suggested that 4.2% of individuals in their study may have originated from a unique source population that had genetically differentiated from the population they were studying (Elphie et al. 2012). Our analysis, which incorporated a neighbor-joining tree, provides additional level of analysis to test the hypothesis that spatial outliers may have originated from a genetically differentiated source population. For example, if all spatial outliers originated from the same branch it would provide evidence to support the previous hypothesis. Our study suggests that spatial outliers of *P. guttatus* since were distributed among all branches of the neighbor-joining tree, implying that the spatial outliers we observed did not originate from a single differentiated source population.

The distribution of spatial outliers within our sampling locations suggested that Glover’s Reef and North Rock had the highest number of spatial outliers, whilst Blue Cut, Florida, Caye Caulker and Mexico all had the lowest number of spatial outliers. Low sample sizes in Mexico, Caye Caulker, and Blue Cut are most likely responsible for the low levels of spatial outliers observed in these locations. However, low sample sizes are unable to account for the observation that Florida (sample size = 24) had fewer spatial outliers (N = 2) than Mexico (sample size = 6; spatial outliers = 3). The ecological and selective process at work creating these genetically unique individuals will clearly require additional research.
5. Conclusion

Our research identified a useful and logistically simple methodology for identifying temporal population dynamics in *P. guttatus* that can be readily applied to other marine species. However, temporal analysis of larger *P. guttatus* (carapace length > 60 mm) may be less reliable due to difficulties in accurately aging these individuals. Long-term temporal sampling of a wide variety of age classes ranging from early benthic juveniles, to sexually mature adults, as well as repeated sampling of the same age classes over several years will be important steps for the maturation of temporal genetics studies. Large sample sizes of multiple age classes will allow for unique spatial analyses of each age classes, which will provide information on the temporally stability of spatial patterns of connectivity. Repeated sampling of the same age class over multiple years, particularly starting with early benthic juveniles, will help to identify how selective processes are shaping temporal levels of genetic variation. These data will be critical for future conservation research projects that target local and regional connectivity patterns, understanding how temporally stable these levels of connectivity are and what potential knock-on effects the decline of one local population will have on the connectivity of other populations in the Caribbean. These data can also be used as a starting point to detect any changes in genetic diversity that may be associated with overfishing or environmental degradation (Hauser 2002) since high levels of connectivity and temporal genetic variation are believed to be largely responsible for the maintaining levels of genetic diversity (Planes and Lenfant 2002; Toonen and Grosberg 2011). This information is urgently needed to help develop sustainable fisheries policies particularly since
fishing pressure is increasing whilst management policies for *P. guttatus* remain nonexistent in many parts of the Caribbean.

**Acknowledgements**

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Supplementary Information

Figure S1. STRUCTURE HARVESTER analysis (top panel) and STRUCTURE analysis (bottom panel) suggesting 4 genetically unique clusters of *Panulirus guttatus* individuals in the Caribbean. STRUCTURE HARVESTER is genetics software used to infer the number of genetically unique clusters of individuals in a population using numerous analyses in the genetics software STRUCTURE. The bottom panel displays STRUCTURE results of 4 genetically unique clusters evenly distributed among all sampling locations.
Table S1. Summary statistics for 8 microsatellite loci FWC5 – PG23. Sample sizes (N), observed number of alleles (Na), observed number of private alleles (Pa), observed heterozygosity (Ho), expected heterozygosity (He), HWE (pHWE) and the average inbreeding coefficient (Fis) for each locus at each population were calculated using Genelex. Null allele frequencies (Fna) were estimated using Microchecker, values of (---) indicate no null alleles were detected. Values in bold are significant after the Benjamini and Hochberg correction for multiple comparisons.

<table>
<thead>
<tr>
<th></th>
<th>FWC5</th>
<th>PG3</th>
<th>PG6</th>
<th>PG15</th>
<th>PG9</th>
<th>PG21</th>
<th>PG22</th>
<th>PG23</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blue Cut (16)</strong></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>16</td>
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<td>16</td>
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| **Mexico (6)** |      |     |     |      |     |      |      |      |
| N              | 6    | 6   | 6   | 5    | 6   | 6    | 4    | 5    |
| Na             | 8    | 6   | 7   | 5    | 8   | 7    | 5    | 4    |
| Ho             | 0.833| 0.333| 0.833| 0.600| 1.000| 0.500| 0.750| 0.400|
| He             | 0.819| 0.778| 0.833| 0.720| 0.819| 0.806| 0.750| 0.700|
| Fis            | -0.017| 0.571| 0.000| 0.167| 0.220| 0.379| 0.000| 0.429|
| pHWE           | 0.363| 0.055| 0.585| 0.744| 0.758| 0.226| 0.586| 0.180|
| Fna            | --- | --- | --- | --- | --- | --- | --- | --- |
| Pa             | 0    | 0   | 0   | 1    | 0   | 0    | 0    | 0    |
Table S2. Pairwise matrix of a) $F_{ST}$ not using the ENA correction for null alleles described by Chapuis and Estoup (2007), b) $F_{ST}$ using the ENA correction, c) Jost’s $D$ without ENA correction, d) $G_{ST}$ without ENA correction, and e) Global $F_{ST}$ (with and without ENA correction) among all sample sites weighted across all eight microsatellite loci for *Panulirus guttatus*. Values in bold are significant after the Benjamini and Hochberg correction for multiple comparisons. Note that the ENA software does not calculate levels of significance for $F_{ST}$.

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Table S3. Pairwise matrix of $F_{ST}$ not using the ENA correction for null alleles, $F_{ST}$ using the ENA correction (described by Chapuis and Estoup, 2007), Jost’s D, and exact $G_{ST}$ (significant p-values after the Benjamini and Hochberg correction for multiple comparisons are in bold) among all age classes for a) Florida, b) Blue Cut, and c) North Rock. Values in each pairwise matrix were weighted across all eight microsatellite loci for *Panulirus guttatus*. Note that the ENA software does not calculate levels of significance for $F_{ST}$.

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*Table S3 Continued*
Chapter 7

Genetic analysis reveals population structure among discrete size classes of Caribbean spiny lobster (Panulirus argus) within marine protected areas in Mexico

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Running Title: Genetic population structure in Panulirus argus size classes

Key Words: microsatellites, connectivity, conservation, marine reserve, fisheries, Banco Chinchorro, Sian Ka’an, biosphere reserve, UNESCO world heritage site

Prepared for submission to Biological Conservation

Contributions: NKT, RFP, KLC, IS, PBF, ELA and BF designed the study. NKT and KLC collected the samples. NKT conducted the laboratory work. NKT and RFP analysed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

Management efforts for improving the sustainability of the Caribbean spiny lobster (*Panulirus argus*) fishery require knowledge of population connectivity. The aim of this study is to investigate population connectivity of *P. argus* at two levels: (1) spatially between two marine protected areas (MPAs) in the Caribbean coast of Mexico, and (2) temporally within MPAs; by genotyping discrete size classes lobsters using microsatellite markers. No evidence of population structure between lobster populations from Banco Chinchorro and Sian Ka’an MPAs were found (*P* = 0.139). In contrast we found significant levels of population structure among discrete size classes of lobsters (*F*$_{ST}$ = 0.0054; *P* = 0.0052). Temporal variation among the genotypes of new larval recruits may explain these results. Future research will be required to directly test the genotypes of new larval recruits in Banco Chinchorro and Sian Ka’an MPAs to confirm this hypothesis.
1. Introduction

The Caribbean spiny lobster, *Panulirus argus* is widely distributed in the Caribbean and Western Atlantic from North Carolina to Rio de Janeiro Brazil (Diniz et al. 2005). This species of spiny lobster is one of the most economically valuable fished single species in the Caribbean (Butler et al. 2011) Ley-Cooper et al 2013). Despite management and conservation efforts to sustain the *P. argus* fisheries, commercial landings have been in decline since the 1990’s (Fanning et al. 2011). Management efforts for improving the sustainability of the *P. argus* fishery requires knowledge of population connectivity among Caribbean nations (Kough et al. 2013). Several studies have used a variety of genetic methods to assess population connectivity in *P. argus* (Sarver et al. 1998; Silberman et al. 1994; Naro-Maciel et al. 2011; Tourinho et al. 2012). Phylogenetic analyses based on mitochondrial (mtDNA) and nuclear sequence markers suggest that Caribbean and Brazilian spiny lobster populations originally attributed to *P. argus* belong to different species (Tourinho et al. 2012). There have been no reports of structuring among subpopulation in the Brazilian subspecies. However, recent studies of population structuring among Caribbean subpopulations using mtDNA markers have provided conflicting results. Diniz et al. (2005) suggested that northern Caribbean subpopulations might be distinct from southern populations, yet Naro-Maciel et al (2011) found no evidence of genetic differentiation among subpopulations in Puerto Rico, Bahamas, and Florida. Polymorphic microsatellite markers (msatDNA) are widely considered more powerful for resolving population structure than mtDNA markers, particularly at small spatial scales (Hellberg 2009; Lukoschek et al. 2008).
For example, a recent preliminary results of spiny lobster genetic structure in Belize based on msatDNA suggested that sub-regional population structure may exist among marine protected areas (MPAs) in the Mesoamerican region (Chapter 5).

The management of many Marine Protected Areas (MPAs) in the Mesoamerican Barrier Reef System (MBRS) often focus on locally based conservation initiatives. For example, preserving important habitats that serve as shelter, foraging grounds or adult movement corridors, as well as protecting local breeding stocks (Goñi, 2010). The implementation of these regulations in the Sian Ka’an and Banco Chinchorro Biosphere Reserves were important criteria for their recent certification by the Marine Stewardship Council. Locally based MPA management of the spiny lobster fishery in Mexico could also benefit from knowledge of sub-regional levels of population therefore, identifying the scale management units for the spiny lobster fishery (Palsboll et al. 2007). The aim of our study is to investigate population genetic structure of *P. argus* at two levels: (1) spatially between MPAs in the Caribbean coast of Mexico, and (2) temporally within MPA; by genotyping individual lobsters using bi-parental inherited microsatellite loci. To explore temporal changes in the levels of population structure we identified cohorts by estimating the age of individuals based up previous research of spiny lobster growth rates in the Sian Ka’an MPA (Lozano-Alvarez et al. 1991). The analysis of population structure among cohorts may provide an additional level of resolution that can be used to improve our understanding of the complex spatiotemporal population dynamics of the Caribbean spiny lobster.
2. Methods

2.1 Sampling

Samples were collected in Mexico from adult lobsters captured by fishermen in Bahía Espíritu Santo located in the Sian Ka’an MPA and Banco Chinchorro MPA between August 23 – 26, 2011 (Figure 1, A and B). The lobster fisheries at Banco Chinchorro and Sian Ka’an MPAs are reviewed in detail by Ley-Cooper et al. (2011 and 2013). The carapace length (CL) of all sampled lobsters were measured to the nearest mm. Tissue samples were obtained from leg muscle and stored in 96% ethanol until DNA was extracted. Genomic DNA was extracted using the Wizard SV-96 Genomic DNA extraction kit following the manufacturer’s protocol (Promega). The quality and quantity of purified DNA was assessed using a NanoDrop 2000 micro-volume spectrophotometer (Thermo Scientific).

2.2 Microsatellites Analyses

Fourteen microsatellite loci previously derived from for *P. argus* were amplified in 171 individuals collected from Banco Chinchorro (N = 91) and Sian Ka’an (N = 80) MPAs ((Diniz et al. 2005; Diniz et al. 2004; Tringali et al. 2008). Amplification of three PCR multiplex reactions followed the methods described in Chapter 3 using fluorescently PCR products (6-FAM®, NED®, VIC® and PET®; Applied Biosystems). Reactions were performed in a final volume of 5 µl. Amplification products were genotyped using an ABI 3730xl automatic DNA sequencer (Applied Biosystems) at the University of Manchester DNA Sequencing Facility. Microsatellite profiles were examined using GeneMapper® v3.7
Figure 1: Map of study Sites and K-means clustering analysis. A) Regional map of the study area with the sampling sites located within the inset in panel B. B) Approximate locations of sampling sites in Sian Ka’an and Banco Chinchorro marine reserves in Mexico. The NASA/GSFC Scientific Visualization Studio provided flow data from the ECCO2 model for the visualization Caribbean ocean currents. C) Plot of Bayesian Information Criterion (BIC) values used for selecting the number of clusters for the discriminant analysis of principle components (DAPC) method. The lowest BIC values indicate the optimal numbers of clusters. D) Subdivision of clusters according to the DAPC method. Unique genetic clusters are indicated with different colours (red = cluster 1, green = cluster 2, and blue = cluster 3).

Genetic diversity estimated as observed heterozygosity ($H_O$), expected heterozygosity ($H_S$), the inbreeding coefficient, and deviation from Hardy-
Weinberg equilibrium (HWE) were computed in GENODIVE v2.0b23 (Meirmans and van Tienderen 2004). The HWE analysis used the least squares method and was tested with 50K permutations. Linkage disequilibrium (LD) between loci was tested using GENEPOP on the Web v4.2 (Raymond and Rousset 1995; Rousset 2008). (Markov chain parameters: dememorization number 10K, number of batches 1K, and number of iterations per batch 10K). Microchecker (van Oosterhout et al. 2004) was used to detected the possibility of null alleles and allele scoring error due to either large allele dropout or stutter.

Temporal levels of population age structuring in *P. argus* was examined by grouping individuals into 10 mm size classes based upon growth and size at maturity research (Ehrhardt 2008; Maxwell et al. 2013). Significance tests for interactions between size classes and sampling locations were calculated by a permutational multivariate analysis of variance (PERMANOVA) calculated in the R-package VEGAN using the function adonis (Dixon 2009; Oksanen et al. 2013). A PERMANOVA is an alternative to the analysis of molecular variance (AMOVA) that allows for significance testing among crossed and nested factors (Anderson 2001). The PERMANOVA was calculated using a distance matrix of squared Euclidian distances among all individuals and was run with 50K permutations. The R-package DEMETRICS was used to calculate Nei’s *G*<sub>ST</sub> (Gerlach et al. 2010) and corrections were made for loci that deviated from Hardy Weinberg Equilibrium by following the methodology of (Goudet et al. 1996). Levels of significance (*P*-values) for genetic differentiation were calculated using 10K bootstrap resamplings. The Benjamini and Hochberg correction (Benjamini and Hochberg 1995), which
controls for the false discovery rate (FDR), was used as a correction against type I
errors among the pairwise comparisons. Allelic Richness and hierarchical levels of
genetic differentiation ($F_{ST}$) were calculated in the R-package HIERFSTAT (Goudet
2005) using 50K permutations. The contribution of allelic richness within each size
class to the total allelic richness the populations was calculated with MOLKIN v2.0
(Gutierrez et al. 2005). Significance tests between size classes were conducted using
an AMOVA run in GENODIVE following the methods outlined by (Michalakis and
Excoffier 1996). The multivariate statistical method, the discriminant analysis of
principle components (DAPC) was used to identify clusters of genetically similar
individuals among size classes (Jombart et al. 2010). The DAPC analysis does not
rely on any particular population genetics model and is tolerant to deviations from
HWE, null alleles, and linkage disequilibrium (Jombart et al. 2010). Since we did
not know a priori how many populations were present within our size class data, we
first used the find.clusters function to run $K$-means clustering and selected the best
supported number of clusters using the Bayesian Information Criterion (BIC) for the
values of $K$. We then followed a recently described DAPC based method
(Therkildsen et al. 2013), 1) to identify the most likely number of clusters among all
samples and 2) calculate the mean membership probability of each size class to the
different clusters. We categorized size classes with a mean membership probability
> 0.6 to one of the clusters as ‘pure’ and the others as ‘mixed’ (Therkildsen et al.
2013).
3. Results

A total of 171 individuals were genotyped for 14 microsatellite loci. Across all loci and populations $H_O$ was consistently lower than $H_S$ suggesting the potential for null alleles (Table 1). Genotypes across all loci were tested using MICROCHECKER and found no evidence of scoring errors due to large allelic dropout or stuttering and suggested null alleles were present at locus PAR7 in all

Table 1. Summary of size classes information of *Panulirus argus* with number of samples ($N$), average observed heterozygosity ($H_O$), average expected heterozygosity ($H_S$), inbreeding coefficient ($G_IS$), loci suspected of containing null alleles (Null), allelic richness ($A_R$), contribution to total allelic richness (CTR%), and mean posterior membership probability to each cluster (Cluster 1- Cluster 3). Values in bold indicate a positive contribution to total allelic richness or mean posterior probabilities > 0.6 to one of the clusters.

<table>
<thead>
<tr>
<th>Size Class</th>
<th>$N$</th>
<th>$H_O$</th>
<th>$H_S$</th>
<th>$G_IS$</th>
<th>Null</th>
<th>$A_R$</th>
<th>CTR%</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 to 90</td>
<td>42</td>
<td>0.614</td>
<td>0.691</td>
<td>0.113</td>
<td>Par7</td>
<td>7.988</td>
<td>-1.168</td>
<td>0.499</td>
<td>0.407</td>
<td>0.094</td>
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<td>90 to 100</td>
<td>34</td>
<td>0.570</td>
<td>0.696</td>
<td>0.181</td>
<td>Par2, Par7, Par9</td>
<td>8.396</td>
<td><strong>1.127</strong></td>
<td>0.321</td>
<td><strong>0.620</strong></td>
<td>0.059</td>
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<tr>
<td>100 to 110</td>
<td>42</td>
<td>0.604</td>
<td>0.693</td>
<td>0.128</td>
<td>Par7</td>
<td>8.044</td>
<td>-0.039</td>
<td>0.361</td>
<td>0.401</td>
<td>0.239</td>
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<td>110 to 120</td>
<td>22</td>
<td>0.591</td>
<td>0.690</td>
<td>0.144</td>
<td>Par7</td>
<td>8.154</td>
<td><strong>1.074</strong></td>
<td><strong>0.707</strong></td>
<td>0.200</td>
<td>0.092</td>
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</table>

size classes (Table 1). Locus PAR7 was removed from all additional statistical analyses, leaving a final microsatellite dataset of 13 loci. Analysis with GENEPOP found no evidence of linkage disequilibrium.

The PERMANOVA analysis found no evidence of population structure in *P. argus* between Banco Chinchorro and Sian Ka’an ($P = 0.139$) nor evidence of an interaction among sizes classes between Banco Chinchorro and Sian Ka’an ($P = 0.42$). These data suggest that patterns of genetic variation are similar between MPA’s, therefore, individuals from both locations were pooled into four size classes (Table 1). Allelic richness ranged from 7.99 to 8.15 and the contribution of each size class to the total allelic richness varied from -1.17% to 1.13%. The $K$-means
analysis suggested that clustering solutions with either two or three clusters generated the lowest BIC scores (Figure 1C). Both clustering solutions revealed the presence of ‘pure’ size classes that evoke cohorts. We proceeded with the three-cluster solution since this allowed for a greater amount of mixing among size classes and since previous population genetics studies of *P. argus* suggested this species has high levels of geneflow (Silberman et al. 1994) and mixing among subpopulations (Naro-Maciel et al. 2011). The DAPC method revealed a clear genetic separation among the three clusters identified by *K*-means clustering (Figure 1D).

![Figure 2: Heatmap of pairwise estimates of genetic differentiation (GST) of *Panulirus argus* in the Caribbean Sea.](image)

Pairwise estimates of differentiation are color-coded (light green = low values, dark green = high values) sorted by size class. GST values are displayed below the diagonal and *P*-values are displayed above the diagonal. Significant pairwise comparisons are in displayed in bold and contain an asterisk symbol (*)..

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Table 2. AMOVA analysis weighted across thirteen microsatellite loci in *Panulirus argus* for size classes pooled between Sian Ka’an and Chinchorro marine reserves. Significant *p*-values are in bold.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Variance component</th>
<th>Percent of Variation</th>
<th>F-statistics fixation indices (P-value)</th>
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<tbody>
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<td><strong>Size Classes Pooled</strong></td>
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<tr>
<td>Among Individuals</td>
<td>0.6251</td>
<td>0.1388</td>
<td><em>F</em>$_{IS}$ = 0.1388 (<em>P</em> &lt; 0.0001)</td>
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<tr>
<td>Among Size Classes</td>
<td>0.0244</td>
<td>0.0054</td>
<td><em>F</em>$_{ST}$ = 0.0054 (<em>P</em> = 0.0052)</td>
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</table>

The AMOVA analysis (Table 2) suggested evidence of population structure among individuals (*F*$_{IS}$ = 0.1388; *P* < 0.0001) and between size classes (*F*$_{ST}$ = 0.0054; *P* = 0.0052). Pairwise comparisons of genetic differentiation (*G*$_{ST}$) among size classes (Figure 2) found significant levels of differentiation (FDR corrected *P*-values ranging from 0.04 to 0.006) among size class 90 to 100 mm and all other size classes. The DAPC analysis of the *K*-means clustering results provided membership probabilities of each individual belonging to one of the three genetically unique clusters (Figure 3). Analysis of the mean membership probability of all individuals within each size class to each unique cluster provided additional evidence of population structuring (Table 1). Individuals within size class 80 to 90 mm were well mixed predominately between Cluster 1 and Cluster 2, whilst individuals in size class 90 to 100 mm were classified as ‘pure’ to cluster 2 (mean membership probability = 0.62). Individuals in size class 100 to 110 mm were mixed among all three clusters, whilst individuals in size class 110 to 120 mm were classified as ‘pure’ to cluster 1 (mean membership probability = 0.71).
4. Discussion

This study identified significant levels of genetic variation among four carapace length size classes of *P. argus* inhabiting two marine protected areas in Mexico. Microsatellite analysis showed variation among size classes, consisting of changes in levels of genetic differentiation, probability of assignment to genetically unique clusters, and in total contribution to allelic richness. The two size classes that contained the highest levels of allelic richness and total contribution to allelic richness (size class 90 to 100 mm and size class 110 to 120 mm) were also classified as ‘pure’ to cluster 2 and cluster 1 respectively. Data from spiny lobster growth and size at maturity estimates suggest that the sizes classes of lobsters that we sampled from Banco Chinchorro and Sian Ka’an MPAs most likely recruited to these MPAs during different times of the year (Lozano-Alvarez et al. 1991). Temporal variation among the genotypes of new larval recruits may explain these results (Selkoe et al. ...
Biophysical modelling studies of *P. argus* larval dispersal are in agreement with our findings. Two independent biophysical modelling studies of larval recruitment dynamics both suggested that spiny lobster populations in Mexico are highly dependent on larval recruitment from distant source populations (Briones-Fourzán et al. 2008; Kough et al. 2013). Variation among the genotypes of individual spiny lobsters that recruit from various source populations may explain the high levels of variation we observed among the size classes of spiny lobsters in our study. An alternative explanation is that natural selection may be acting on the new recruits after they settle in nursery habitat in Mexico. *Panulirus argus* is dependant on several different habitat types through its life history and conducts ontogenetic migrations from shallow hard-bottom nursery habitats to coral reefs (Butler et al. 2006). Complex selective processes acting on new recruits, juveniles, or adults may also explain the variation we observed in the adult population (Planes and Lenfant 2002). Directly testing the genotypes of new larval recruits in Banco Chinchorro and Sian Ka’an MPAs will be required to confirm the hypothesis that temporal variation among larval recruits is indeed responsible for the genetic differences we observed among size classes.

Our analyses suggest that temporal variation in levels of genetic differentiation may positively contribute to the total genetic diversity of *P. argus* within Mexican marine reserves. We also observed that the total contribution to allelic richness varies among size classes and in some cases can be negative (e.g. size classes 80 to 90 mm and 100 to 110 mm). Negative contributions to diversity have been explained by the diversity of the immigrant population being lower than
the mean total diversity or because the population is well mixed and not divergent (Petit et al. 2008). The $K$-means clustering analysis suggests that the latter case is the most likely since size classes 80 to 90 mm and 100 to 110 mm had the highest levels of mixing among all clusters.

The findings of this study reveal the usefulness of collecting size data from each individual. This sampling methodology is straightforward and can easily be applied to MPA monitoring efforts. Monitoring temporal patterns of genetic variation can be used to improve the resolution of spatial patterns of connectivity among networks of marine reserves and may be useful for early warning to losses of genetic diversity caused by overfishing or natural causes (Hauser 2002; Palumbi 2003; Selkoe et al. 2008). However, the specific environmental and selective forces that are shaping the patterns of temporal variation observed in this study remain uncertain and will require additional research to resolve. Future studies of temporal genetic variation in species of spiny lobster would benefit from long-term sampling strategies that include a wide variety of age classes (e.g. post-larvae, early benthic juveniles, juveniles, and adults), oceanographic environments (e.g. advective and retentive regions) and habitat types.

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Chapter 8

Genetic population structure of the Caribbean spiny lobster, *Panulirus argus*, between advective and retentive oceanographic environments

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Abstract

Ocean currents play an important role in shaping spatial patterns of genetic variation among populations since the dispersal of most marine species occurs during a pelagic larval phase. In this study, we made use of the most comprehensive sampling of lobsters ever made in the Caribbean to perform a detailed study of population differentiation in *P. argus* as related to oceanographic conditions in the Caribbean sea. We used published findings on patterns of *P. argus* larval dispersal predicted by a biophysical model to forecast which oceanographic regions had the highest levels of larval self-recruitment within the Caribbean seascape. We then explored associations between levels of kinship, genetic population structure, and potential barriers to larval lobster dispersal in these locations. The kinship analysis suggested that the majority of locations we sampled had significantly higher levels of siblings than expected (*P* < 0.05). The overall *F*<sub>ST</sub> was 0.0016 (*P* < 0.01) and suggested weak yet significant levels of structuring among sites. Despite the potential for high-levels of geneflow on spatial scales > 2000 km, there was substantial variation in geneflow among sites. Our results suggest that a simple isolation by geographic distance model is not useful for explaining levels of genetic differentiation in *P. argus*. The findings of our study suggest that the long-lived larvae of *P. argus* disperse among sites throughout their range frequently enough to homogenize the genetic population structure of this species, except for a few sites where self recruitment is enhanced by persistent offshore gyres.
1. Introduction

Marine population genetics studies often try to identify the ecological and physical processes that are responsible for shaping spatial patterns of genetic variation among populations (Selkoe et al. 2008). Ocean currents play an important role in these processes because dispersal of most marine species occurs during a pelagic larval phase (White et al. 2010). Oceanographic features such as persistent offshore gyres and counter currents can prevent the mixing and diffusion of larvae and, when combined with larval behaviour, can significantly increase local levels of self-recruitment (Cowen et al. 2007). In contrast, strong advective currents disperse larvae sometimes hundreds to thousands of kilometres from their natal source, which may connect distant populations or result in larval wastage if larvae are swept away from settlement areas (Butler et al. 2011, Kough et al. 2013). The interaction among oceanographic and biological processes may produce patterns of genetic differentiation that are spatially and temporally unstable and difficult to interpret (Selkoe et al. 2010). For example, population genetics studies of species with extensive larval dispersal frequently report the counterintuitive result that neighboring sites only a few kilometres from one another are genetically more dissimilar than distant sites that may be thousands of kilometres apart (Banks et al. 2007). These intriguing findings are known as “chaotic genetic patchiness” (Johnson & Black 1982).

However, marine population genetic studies that have incorporated oceanographic and environmental data directly into spatial analyses of genetic variation have uncovered ecologically relevant patterns of population connectivity
within the ‘chaotic genetic patchiness’ (Selkoe et al. 2006). This approach, called ‘seascape genetics’, has begun to reveal novel insights into the mechanisms responsible for shaping spatial patterns of genetic variation and connectivity among marine populations and has helped to guide the spatial management of commercial fisheries (Selkoe et al. 2008). Many seascape genetics studies have identified genetic structure associated with large-scale oceanographic features such as fronts, semi-permanent gyres, and strong boundary currents (Galarza et al. 2009).

However, the life history characteristics and behaviours of many marine organisms can greatly influence their dispersal potential, prompting the use of coupled biological-physical models (i.e. biophysical models that incorporate ocean circulation data with larval behaviour) (Paris et al. 2007). It is also likely that environmental factors and life history traits may interact synergistically to shape spatial patterns of genetic variation (Riginos & Liggins 2013). Accordingly, integrating biophysical modelling with environmental data has proven particularly important for detecting ecologically informative patterns out of the ‘chaotic genetic patchiness’, especially over small spatial scales (Selkoe et al. 2010; Teacher et al. 2013).

The Caribbean Sea is an ideal location to explore how population connectivity via larval dispersal can produce chaotic biogeographic patterns. Many marine species in the Caribbean have a high potential for larval dispersal among localities via the prevailing Caribbean current, which is largely continuous and unidirectional (Kough et al. 2013). Most flow enters the Caribbean near the southern Windward Islands and flows west/northwest through South and Central
America into the Gulf of Mexico and Straits of Florida (Florida Current) then rejoins to form the Gulf Stream that emerges from the Caribbean and into the Western Atlantic between Florida and the Bahamas. Yet, large, persistent gyres located in the Gulf of Honduras, Panama-Colombia sub region, off the southwest coast of Cuba and the north of the Bahamas are important oceanographic mechanisms that promote the local retention of larvae (Cowen et al. 2006).

Considering the complex oceanographic environment of the Caribbean, it is not surprising that the interpretation of chaotic genetic patchiness is improved by incorporating biophysical modelling into genetic analyses (Selkoe et al. 2008). For example, several seascape genetics studies of coral reef species have identified a major biogeographic break in the eastern Caribbean at the Mona Passage, where strong currents flow between Puerto Rico and Hispaniola (Baums et al. 2006; Hellberg 2009). Coral reef species the occur within the Panama-Colombian gyre may also be genetically isolated from the rest of the Caribbean (Salas et al. 2009). Studies such as these have greatly improved the interpretation of marine population genetics data, yet the majority of studies on seascape genetics in the Caribbean have focused on species with relatively short PLDs (e.g. corals and coral reef fish) and low levels of geneflow. Seascape genetics research on species with longer PLDs and higher levels of geneflow may help to improve our understanding of how large-scale drivers of environmental and physical variation shape spatial patterns of genetic variation (Iacchei et al. 2013). The Caribbean spiny lobster (Panulirus argus), which is found throughout shallow seas and coral reefs in the tropical West Atlantic, is an ideal species for such studies.
Like most marine species, the Caribbean spiny lobster has a complex life cycle. Adults inhabit coral reefs where they spawn; spawning is seasonal in the Northern Caribbean and Florida but occurs throughout the year in the Southern Caribbean (Kough et al. 2013). Spiny lobsters produce pelagic larvae that undergo ontogenetic vertical migration throughout their larval duration of approximately 5-12 months (Butler MJ et al. 2011). The larvae of Panulirus argus have the potential to disperse among lobster populations throughout the Caribbean given their long pelagic larval duration (PLD) and the strong flow of the Caribbean current.

However, a growing number of empirical and modelling studies suggest that larval swimming behaviours (e.g., ontogenetic vertical migration; OVM) coupled with retentive ocean currents, retain marine larvae near their natal environment and are important drivers of self-recruitment (Cowen et al. 2006; 2007; Butler MJ et al. 2011; Kough et al. 2013). Even though P. argus has one of the longest PLDs of any marine species, biophysical modelling simulations suggest that OVM substantially increases the potential for self-recruitment, particularly in retentive oceanographic environments (Butler MJ et al. 2011).

Within the Caribbean Sea, Silberman et al. (1994) found no evidence of genetic differentiation among sites with contrasting ocean currents or evidence of isolation by distance using mtDNA markers (Silberman et al. 1994). These findings supported the widely accepted hypothesis that P. argus is a single panmictic population throughout the Caribbean sea. A later study found strong divergences in mitochondrial DNA (mtDNA) sequences between populations from the Caribbean Sea and Brazil that were attributed to a barrier to larval connectivity created by the
Amazon and Orinoco river plumes (Sarver et al. 1998). More recent phylogenetic analyses suggest that Caribbean and Brazilian spiny lobster populations are most likely divergent species that have been isolated for ~ 16 million years (Tourinho et al. 2012). However, within the Caribbean it has proved difficult to detect consistent or strong spatial patterns of genetic population structure in *P. argus* that are associated with meso-scale oceanographic features.

Previous Caribbean-wide genetic studies have lacked the genetic methodologies or statistical power for detecting ecologically meaningful patterns of connectivity when faced with high levels of gene flow. Even the exchange of a few migrants between sites located in regions with high self-recruitment, at levels considered insignificant from a demographic perspective, may still provide sufficient levels of geneflow to obscure the detection of genetically differentiated populations (Waples 1998). Indeed, recent studies of larval connectivity that have incorporated genetic methods for tracking marine larvae using parentage and kinship analyses have provided empirical support for the pan-Caribbean hypothesis (Selkoe et al. 2006; Christie et al. 2010). The use of numerous polymorphic loci may also increase the statistical power to detect subtle patterns of population structure that may go undetected using mitochondrial DNA methods (Eytan & Hellberg 2010). For instance, a recent study of *Panulirus argus* in Belize using five polymorphic microsatellite loci suggested that fine-scale levels of genetic differentiation may occur in this region (Chapter 5).

In this study, we made use of a recently developed microsatellite multiplex protocol (Chapter 3) and the most comprehensive sampling of lobsters ever made in
the Caribbean to perform a detailed study of population differentiation in *P. argus* as related to oceanographic conditions in the Caribbean sea. We used published findings on patterns of *P. argus* larval dispersal predicted by a biophysical model (Butler MJ *et al.* 2011; Kough *et al.* 2013) to forecast which oceanographic regions had the highest levels of larval self-recruitment within the Caribbean seascape. We then explored associations between levels of kinship, genetic population structure, and potential barriers to larval lobster dispersal in these locations. Our sampling strategy included sites within: 1) retentive oceanographic environments located in persistent offshore gyres; 2) advective oceanographic environments located in the Caribbean current; 3) the Mesoamerican Barrier Reef where previous studies have suggested the potential for fine-scale levels of population differentiation; 4) the biogeographic break near the Mona Passage in Puerto Rico; and 5) Bermuda, an isolated island archipelago far to the north of the primary Caribbean distribution of *P. argus*. We address the following questions: 1) Is there evidence for population differentiation in *P. argus* within the Caribbean Sea, 2) How well do spatial patterns of genetic variation correlate with geographic distance, and 3) Is there any evidence of site-specific correlations between genetic differentiation or genetic diversity and oceanographic conditions.

2. Methods

2.1 Biophysical Modeling and Sampling Strategy

From September 2010 through October 2011 Caribbean spiny lobsters were sampled (*n = 30 – 502*) from each of 43 locations throughout the Caribbean as part
Table 1. Summary statistics including the country, location, local oceanographic environment, predicted proportion of local (within ~10km) self-recruitment (obtained from the *Panulirus argus* biophysical modeling results); number of samples (N$_S$), number of alleles (N$_A$), observed heterozygosity (H$_O$), total expected heterozygosity (H$_T$), allelic richness (A$_R$), and inbreeding coefficient (G$_{IS}$).

<table>
<thead>
<tr>
<th>Country</th>
<th>Site Name</th>
<th>Oceanographic Environment</th>
<th>Self-Recruitment</th>
<th>N$_S$</th>
<th>N$_A$</th>
<th>H$_O$</th>
<th>H$_T$</th>
<th>A$_R$</th>
<th>G$_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panama</td>
<td>San Blas</td>
<td>Advective</td>
<td>0.00</td>
<td>41</td>
<td>144</td>
<td>0.70</td>
<td>0.75</td>
<td>12.42</td>
<td>0.07</td>
</tr>
<tr>
<td>Cayman Islands</td>
<td>Grand Cayman</td>
<td>Advective</td>
<td>0.00</td>
<td>87</td>
<td>191</td>
<td>0.71</td>
<td>0.76</td>
<td>13.48</td>
<td>0.06</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>Mayaguez</td>
<td>Advective</td>
<td>0.02</td>
<td>38</td>
<td>147</td>
<td>0.60</td>
<td>0.76</td>
<td>12.81</td>
<td>0.21</td>
</tr>
<tr>
<td>Belize</td>
<td>Glover's Reef</td>
<td>Advective</td>
<td>0.04</td>
<td>33</td>
<td>142</td>
<td>0.73</td>
<td>0.74</td>
<td>12.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Belize</td>
<td>Caye Caulker</td>
<td>Advective</td>
<td>0.26</td>
<td>56</td>
<td>170</td>
<td>0.75</td>
<td>0.76</td>
<td>13.21</td>
<td>0.01</td>
</tr>
<tr>
<td>Venezuela</td>
<td>Los Roques</td>
<td>Retentive</td>
<td>0.72</td>
<td>74</td>
<td>188</td>
<td>0.71</td>
<td>0.74</td>
<td>13.49</td>
<td>0.04</td>
</tr>
<tr>
<td>Bahamas</td>
<td>Andros Island</td>
<td>Retentive</td>
<td>0.82</td>
<td>36</td>
<td>156</td>
<td>0.73</td>
<td>0.77</td>
<td>13.76</td>
<td>0.04</td>
</tr>
<tr>
<td>Belize</td>
<td>Sapodilla Cayes</td>
<td>Retentive</td>
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<td>60</td>
<td>173</td>
<td>0.75</td>
<td>0.77</td>
<td>13.42</td>
<td>0.03</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>Corn Islands</td>
<td>Retentive</td>
<td>0.98</td>
<td>81</td>
<td>185</td>
<td>0.75</td>
<td>0.76</td>
<td>13.20</td>
<td>0.01</td>
</tr>
<tr>
<td>Bermuda</td>
<td>Bermuda</td>
<td>*</td>
<td>*</td>
<td>75</td>
<td>183</td>
<td>0.75</td>
<td>0.75</td>
<td>13.18</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Biophysical modeling data is not available for Bermuda
of a separate study on the distribution and prevalence of *Panulirus argus* virus 1 (Moss et al. 2013). The sampling methodology is thoroughly described in Moss et al. (2013). We then used results from a biophysical model to select a subset of the sampling sites from the Moss et al. 2013 study that were located either in persistent offshore gyres or in advective regions of the Caribbean current (Figure 1). The methods describing the biophysical model for *Panulirus argus* have been described previously (Butler MJ et al. 2011; Kough et al. 2013). The biophysical model provided estimates of larval self-recruitment at each advective and retentive location. We then selected the locations with the highest and lowest levels of self-recruitment for genetic analyses of population structure (Table 1).

### 2.2 Genotyping

A total of 581 individuals from 10 locations within either advective or retentive regions of the Caribbean were genotyped using 15 microsatellite loci. All loci were validated as polymorphic and reliable for scoring in Chapter 3. Genotyping was performed using an ABI 3730xl automatic DNA sequencer (Applied Biosystems) at the University of Manchester DNA Sequencing Facility. Microsatellite alleles were scored manually with GeneMapper® v3.7 software package (Applied Biosystems). Microsatellite alleles were binned with the R-package MsatAllele version 1.02 (Alberto 2009).
2.3 Data Quality Checks

All individuals were checked for duplicate genotypes using the R-package ALLELEMATCH (Galpern et al. 2012). The probability of sampling an identical twin is extremely unlikely. Therefore, the occurrence of duplicate genotypes is most likely due to accidentally sampling the same individual more than once. Duplicate genotypes were found at Venezuela (N = 2) and Panama (N = 44). All duplicate genotypes were removed from subsequent analyses. Additionally, all samples from Panama were genotyped again to make sure no duplicate genotypes were missed due to scoring error. Scoring error was not detected in any of the samples from

Figure 1. Map of the Caribbean Sea and Bermuda showing the locations of the *Panulirus argus* sampling sites (•). The three sites in Belize are abbreviated (CC = Caye Caulker, GR = Glover’s Reef, and SC = Sapodilla Cayes).
Panama that were re-genotyped. Each microsatellite loci was examined with Microchecker to check for the presence of null alleles and allele scoring error due to either large allele dropout or stutter (Van Oosterhout et al. 2004). Tests for linkage disequilibrium among all loci were run with GENEPOP (Raymond & Rousset 1995; Rousset 2008). Two loci (Par7 and Argus2) showed evidence of null alleles at all locations and were removed from further FST based analyses, since null alleles can inflate levels of population structuring in these types of statistical analyses. No pairwise comparisons of loci were significant for linkage disequilibrium. Therefore, no further loci were removed from FST based statistical analyses, leaving a final dataset of 13 loci. All loci were used for multivariate analyses since these statistical models are not biased by deviations from HWE or linkage disequilibrium (Jombart et al. 2009).

2.4 Genetic Diversity and Population Structure

Summary statistics including observed heterozygosity (H₀), expected heterozygosity (Hₛ), the inbreeding coefficient (Fₛ), and departures from Hardy-Weinberg equilibrium (HWE) were tested for each locus using the R-package POPGENREPORTS. The allelic richness (Aᵣ), corrected for sample size using rarefaction) at each sample site was calculated in the R-package HIERFSTAT using the function allelic.richness and 50K permutations (Goudet 2005). Overall FST was calculated for 1) each locus and 2) for all loci over all sites using an analysis of molecular variance (AMOVA) in GENODIVE (Meirmans & Van Tienderen 2004;
Meirmans 2012). Missing data at any locus was replaced with randomly drawn alleles based on the overall allele frequencies. An infinite allele model was used based on Weir and Cockerham’s (1984) calculations of $F_{ST}$ (Weir & Cockerham 1984). The level of significance was tested using 50K permutations. Nei’s pairwise $F_{ST}$ between all pairs of populations was calculated in the R-package ADEGENET using the function pairwise.fst (Nei 1973). The $P$-values for all pairwise comparisons of population differentiation were calculated in GENODIVE with the log-likelihood $G$-statistic using a 50K permutations. The false discovery rate (FDR) was used as a correction against type I errors among the multiple pairwise comparisons (Benjamini & Hochberg 1995). To visualize the variation among pairwise estimates of $F_{ST}$ for all the study locations multidimensional scaling (MDS) plots were created using the cmdscale function in R. The statistical technique of MDS is also known as principle coordinates analysis (PCoA).

2.5 Spatial Genetic Analyses

Geographic maps using color to represent levels of allele frequency variation among all sites were created in R using the functions in colorplot in the R-package ADEGENET and map in R-package MAP. The use of color to visualize the spatial patterns of allelic variation has been described previously (Menozzi et al. 1978). The coordinates of the first two axes of the MDS plots were recoded into a color signal on the red, green, and blue color scale. The unique colors for each site were then overlaid onto of a geographic map of all sites. Ocean currents in the Caribbean were visualized using the NASA ECCO2 model. The methods for developing the
NASA ECCO2 model have been described previously (Menemenlis et al. 2008). The sites with similar colors are less genetically differentiated and sites with different colors are more genetically differentiated, based upon levels of $F_{ST}$.

2.5.1 Isolation by Genetic Distance

Isolation by genetic distance was analyzed in R. The function dist.genepop was used in the R-package ADEGENET to calculate pairwise comparisons of Nei’s genetic distance among all sites. Pairwise geographic distances among all sites were calculated using the R function dist. Isolation by genetic distance was tested in R with a Mantel test on the matrix of genetic distances and geographic distances using the function mantel.randtest and 10K permutations in the R-package ADEGENET. The slope of the trend line for the isolation by distance plot was calculated in R using a straight-line linear regression model with an implicit y-intercept. The function lm was used in R with the model isolation=lm (genetic.distance~geographic.distance). The slope of the trend line for the isolation by distance plots was then created using the function abline.

2.4.5 Spatial Principle Components Analysis

A spatially explicit analysis of genetic variation was conducted using the spatial principal component analysis method (sPCA) in the R-package ADEGENET. We first used the function chooseCN in ADEGENET to build a connection network where all of our study sites were connected to each other. We then the function spca in ADEGENET to conduct the sPCA analysis. This analysis
is designed to distinguish global spatial structure from local spatial structure within a georeferenced genetics dataset. Global structure occurs when neighboring sites are genetically similar and exhibit positive spatial autocorrelation. Local structure occurs when neighboring sites are genetically different and exhibit negative spatial autocorrelation. The spca function first computes Moran’s $I$ value to compute levels of spatial autocorrelation in the genetics dataset. The spca function then incorporates the Moran’s $I$ value with the levels of genetic variance among all sampling sites. The eigenvalues that contained the highest levels of both spatial autocorrelation and genetics variance were selected for interpretation using the function screeplot. The first eigenvalue containing global structure and the last eigenvalue containing local structure both met the criteria. We then ran a Monte-Carlo test to test for significant levels of both global and local spatial genetic structures. The function global.rtest was used to test global structures and the function local.rtest was used to test local structures for significance using a Monte-Carlo test with 50K permutations in the R-package ADE4. The results from the Monte-Carlo test suggested significant levels of local spatial structure were present ($P = 0.008$), whereas no evidence of global structure was found ($P = 0.866$). Therefore, only the local structure, associated with eigenvalue containing the highest levels of spatial autocorrelation was selected for the interpretation. Finally, the function interp from the AKIMA R-package was used to create an interpolated map of the levels of local spatial genetic structuring among our sampling sites.

2.6 Kinship Analysis
The R-package DEMERELATE was used to calculate the relatedness of individuals within all of our sampling sites. We used the function Demerelate within the R-package DEMERELATE to calculate the observed levels of full siblings and half siblings within each study site using genotype sharing method \( M_{xy} \) (Blouin et al. 1996). This method was preferred since it requires no prior knowledge of population allele frequencies and achieves the highest level of accuracy when locus specific levels of heterozygosity are \( \geq 0.75 \) as was the case with our microsatellite data. The function Demerelate analyzes levels of kinship using a logistic regression model to calculated thresholds for individuals being full-siblings or half-siblings. Randomized reference populations are then created based on using the alleles present within the sampling site and the same number of individuals. Chi-squared statistics were used to calculate whether the sampling site contained more siblings than expected compared to the randomized reference population. This process was repeated for each sampling site.

3. Results

3.1 Microsatellite Locus Characteristics and Conformity to HWE

The observed heterozygosity \( H_O \) and number of alleles per locus have been described previously for all the microsatellite loci used in this study (Chapter 3). Six loci (Par1, Par2, Par7, Par9, fwc04, and argus 2) showed heterozygote deficiencies and statistically significant deviations from HWE after corrections for multiple comparisons. Analysis with MICROCHECKER suggested the presence of null alleles in all loci that deviated from HWE (Table S1). No loci showed evidence of
scoring errors due to stutter or the dropout of large alleles. The six loci that were suggested to have null alleles were removed from further \( F_{ST} \)-based analyses since they have the potential to bias estimates of genetic differentiation. Once these loci were removed Puerto Rico was the only location that consistently deviated from HWE \( (\text{fwc05, fwc07, fwc08, fwc17, fwc18, argus2}) \). Caye Caulker deviated from HWE at loci fwc08 and fwc17. The remaining locations conformed to HWE at all loci. No evidence of linkage disequilibrium was observed among any combinations of loci.

3.2 Levels of Genetic Population Structure

The overall \( F_{ST} \) was 0.0016 \( (P < 0.01) \) and suggested weak yet significant levels of structuring among sites (Table S2). The AMOVA suggested that 95.5% of the total variation was found within individuals, 4.4% was found among individuals, and the remaining 0.1% found among sites. The AMOVA analysis suggested differences in allele frequencies among sites were significant in four microsatellite loci (Table S2). Likewise, the overall Jost’s D measure of genetic differentiation \( (D_{EST}) \) was 0.011 \( (P < 0.05) \) suggesting weak but significant levels of structuring. Pairwise comparisons of \( F_{ST} \) and \( D_{EST} \) among sites provided additional evidence of significant levels of genetic structuring (Table S3). The levels pairwise \( F_{ST} \) and \( D_{EST} \) among sampling sites were highly correlated \( (P < 2.2 \text{ e}^{-16}, R^2 = 0.93) \), therefore we only refer to \( F_{ST} \) in future analyses. A total of 13 out of 45 pairwise comparisons of \( F_{ST} \) were significantly different after corrections for multiple comparisons. Panama had the most significant pairwise differences among sites \( (N = 8) \). Puerto Rico,
Panama, Andros Island (Bahamas), and Sapodilla Cayes (Belize) were all significantly different from Nicaragua.

The multidimensional scaling plot (MDS) of the pairwise differences among sites in levels of $F_{ST}$ suggested that Panama, Andros Island (Bahamas), Puerto Rico, and Glover’s Reef (Belize) were distinct from all other sites. Caye Caulker (Belize), Sapodilla Cayes (Belize), Grand Cayman, Bermuda, and Nicaragua all clustered near the origin, suggesting levels of genetic differentiation were low among these sites. The relation between spatial variation of $F_{ST}$ among sites and ocean currents was visualized by recoding the MDS coordinates as a color and plotting the colors for each site onto a high resolution map of Caribbean ocean currents generated from NASA satellite data (Figure 2B). Sites with pairwise levels of $F_{ST}$ were recoded as brown colors similar colors and sites with multiple pairwise differences in $F_{ST}$ were assigned red, yellow, and green colors. This analysis suggested that sites located near the mean surface flow of the Caribbean current were consistently assigned similar brown colors (Figure 2B). Two sites, Panama and Bahamas, located in large gyre systems were assigned red and green colors, respectively. The Puerto Rico site located in an advective region that is distant from the main flow of the Caribbean current was assigned a yellow color.

3.3 Isolation by Distance and Levels of Genetic Diversity among Sites

The spatial analysis of genetic isolation by geographic distance found no correlation between genetic differentiation and geographic distance ($P = 0.51$; $R^2 = 0.01$; Figure 3). The linear regression model suggested that levels of genetic
Figure 2. Multidimensional scaling (MDS) plots of pairwise levels of $F_{ST}$ among the *Panulirus argus* sampling sites (A). Site names are abbreviated (PR = Puerto Rico, PN = Panama, GR = Glover’s Reef, BA = Bahamas Andros Island, CA = Grand Cayman Island, NIC = Nicaragua, SC = Sapodilla Cayes, CC = Caye Caulker, VZ = Venezuela, and BM = Bermuda). The Bermuda site (BM) is obscured by the Sapodilla Cayes site (SC). The unit of scale for the grid of both x and y axes is 0.2 and located in the top right corner of the plot. Color plot of the MDS scores (B). Each dot is a sampling site. The colors of the dots are generated by recoding the x and y coordinates of the MDS as a signal of color on a red, green, and blue, color palette. Sites with similar colors have similar levels of pairwise $F_{ST}$ and sites with different colors have different levels of pairwise $F_{ST}$. Ocean currents were visualized using the NASA ECCO2 model and were provided by the NASA/GSFC Scientific Visualization Studio. The white arrow indicates the direction of flow for the Caribbean and Gulf Stream currents.

Figure 3. Scatterplot showing no relationship between pairwise levels of Nei’s genetic distance and the geographic distance between *Panulirus argus* sampling sites. Geographic distance between sampling sites is measured in units of latitude.
diversity and levels of self-recruitment were not robustly correlated. No significant correlations among sites were detected for heterozygosity ($P = 0.15; R^2 = 0.17$), expected heterozygosity ($P = 0.32; R^2 = 0.02$), levels of inbreeding ($P = 0.24; R^2 = 0.07$), or allelic richness ($P = 0.07; R^2 = 0.39$) when compared to site-specific levels of self-recruitment.

### 3.4 Kinship Analysis

The kinship analysis suggested that all sampling sites with the exception of Puerto Rico had significantly higher levels of half-siblings than expected ($P < 0.05$). Half of the sampling sites (Caye Caulker (Belize), Nicaragua, Panama, Sapodilla Cayes (Belize), and Venezuela) had significantly higher than expected levels of full-siblings ($P < 0.05$; Figure 4). We calculated a corrected percentage of total siblings at each site by subtracting the observed number of total siblings from the expected number of siblings. The relationship between the corrected percentages of total siblings at each site was compared to $F_{ST}$ and total expected heterozygosity ($H_T$) using a linear regression model. (Figure S1A and S1B). The results of linear regression suggested that relationship between $F_{ST}$ and $H_T$ were both negative and insignificant ($P = 0.082; R^2 = 0.33$ and $P = 0.098; R^2 = 0.31$ respectively). Likewise the linear regression model found no evidence of correlation between biophysical modeling estimates of site-specific levels of self-recruitment and the corrected percentage of siblings at each site ($P = 0.37; R^2 = 0.11$; Figure S1C).
Figure 4. The proportion of full-siblings (gray bar) and half-siblings (hatched bar) for *Panulirus argus* at each sampling site that are greater than levels expected by chance. The expected levels of kinship were calculated using 1000 pairs of randomized populations at each sampling site. Asterisks next to the grey and hatched portions of the histograms indicate significant differences ($P < 0.05$) between observed and expected percentages of siblings for full – and half-siblings, respectively.

3.5 Spatially Explicit Genetic Analyses

The interpolation of mean pairwise $F_{ST}$ values among all sampling sites suggested levels of genetic variation among them are patchy over large spatial scales (Figure 5A). The sites in blue regions of the interpolated map have the lowest levels of genetic differentiation and the sites in red have the highest levels of genetic differentiation, based upon mean pairwise levels of $F_{ST}$. The spatial principle components analysis found significant levels of local structure suggesting that several sites in our study are more genetically different from neighboring sites than from distant sites ($P = 0.008$). The interpolation of the spatial principle component
**Figure 5.** Interpolated map of mean levels of pairwise $F_{ST}$ among *Panulirus argus* sampling sites (A). Red colors indicate highest levels of pairwise $F_{ST}$, white indicated medium pairwise differences, and blue indicates the lowest pairwise differences. The color-scale bar on the right indicates the mean pairwise $F_{ST}$ values for red, white, and blue colors. An interpolated map of a spatial principle components analysis (B). Levels of spatial genetic structure are calculated using multivariate statistics to model levels of spatial autocorrelation and genetic variation at each sampling site. The analysis is designed to calculate levels of global (neighboring sites are more genetically similar) or local (neighboring sites more genetically different) spatial genetic structures. Significant levels of local spatial genetics structure were found ($P = 0.008$), while levels of global spatial genetics structure were not suggested to be significant ($P = 0.87$). Therefore, the coordinates of the principle component with the highest levels of negative spatial autocorrelation were chosen for interpolation. The scale bar entitled “PC score” corresponds to the coordinate values of the principle component. Sites with similar colors are more similar and sites with different colors are more different in terms of local spatial genetic structure.

eigenvalue with the greatest amount of local structure suggested that the Glover’s Reef site in Belize was the most differentiated from the other sites in terms of negative spatial autocorrelation and genetic variance (Figure 5B).

4. Discussion

4.1 Caribbean Spiny Lobster Population Structure

Our study compared levels of genetic differentiation and genetic diversity of *P. argus* among sites in the Caribbean with either high or low levels of self-recruitment, as determined from biophysical modeling. Levels of population structure were low, but significant among sites. Significant pairwise differences were found among several sites using $F_{ST}$ and Jost’s $D_{EST}$ based methods to measure levels of genetic differentiation. Sites in Panama, Bahamas, and Southern Belize were consistently the most genetically differentiated from other sites. A genetic
isolation by geographic distance model provided no additional explanatory power. For example, sites in Venezuela and Bermuda had low levels of pairwise $F_{ST}$ and Jost’s $D_{EST}$ between them even though they are separated by > 2000 km. These findings suggest that the long-lived larvae of $P. argus$ disperse among sites throughout their range frequently enough to homogenize the genetic population structure of this species, except for a few sites where self recruitment is enhanced by persistent offshore gyres. Despite the potential for high-levels of geneflow on spatial scales > 2000 km, there was substantial variation in geneflow among sites. A striking example of this variation was observed in the Mesoamerican Barrier Reef in Belize. Pairwise levels of $F_{ST}$ were significantly different between the Sapodilla Cayes and Caye Caulker, which are separated by < 200 km. These high levels of variability coupled with the lack of genetic isolation by distance suggests that spatial patterns of geneflow in Caribbean spiny lobsters are more likely influenced by environmental or physical factors than simply geographic distance.

**4.2 Spatial Patterns of Geneflow**

Our results, though perhaps counterintuitive, indicate that some adjacent sites exhibit higher levels of genetic differentiation than more distant sites, which is in agreement with a growing body of population genetics research on species with extensive dispersal potential. Johnson and Black (1982) originally identified this phenomenon as “chaotic genetic patchiness”. Previous genetics studies of $P. argus$ in the Caribbean using allozyme electrophoresis found similar evidence of chaotic genetic patchiness over large spatial scales. Menzies and colleagues (1981) found no
evidence of genetic differentiation between Trinidad and Florida, but found
differences between sites in the Virgin Islands and Jamaica (Menzies 1981). On a
much smaller scale, a recent study using five microsatellites found evidence for
differentiation in lobsters sampled from Glover’s Reef and Hol Chan marine
protected areas in Belize that are separated by < 200km (Chapter 5). In contrast to
studies that use nuclear genetic markers, several studies that used mitochondrial
DNA markers (mtDNA) found no evidence of population differentiation in *P. argus*
on both Caribbean-wide and local scales (Sarver et al. 1998; Naro-Maciel et al.
2011). Comparisons among previous *P. argus* genetics studies are difficult because
different genetic markers were used, the spatial scales of each study varied, and the
statistical methods used for genetic analysis were inconsistent. Some studies have
provided evidence for population differentiation of *P. argus* among a few sites in
the Caribbean, but the evidence is insufficient to reject the widely supported
hypothesis that *P. argus* is a single, genetically homogenous population in the
Caribbean. To reject this hypothesis sufficient evidence must be gathered to
conclude that the complex spatial patterns that have been observed thus far are not
simply due to chaotic or random events.

Seascape genetics studies that have integrated physical, environmental, and
 genetics data have improved our understanding of the drivers of chaotic genetic
patchiness and have revealed that chaotic spatial genetic patterns are not always a
the result of random processes (reviewed by (Selkoe et al. 2008) and (Hellberg
2009)). Our results suggest that a simple isolation by geographic distance model is
not useful for explaining levels of genetic differentiation in *P. argus*. Indeed, sites
in Panama, the Bahamas, and Southern Belize were consistently distinct from the rest of the sites in our study. Complex bathymetry and persistent offshore gyres are thought to restrict larval dispersal, however, biophysical modeling estimates of local levels of self-recruitment were quite different in Panama, the Bahamas, and Belize. For example, levels self-recruitment were predicted to be negligible at the Panama site and at Glover’s Reef in Belize, whilst at the Andros (Bahamas) and Sapodilla Cayes (Belize) sites levels of self-recruitment were estimated to be > 80%. Perhaps an isolation by oceanographic distance model may provide insight into how local and regional scale hydrodynamics may influence spatial patterns of geneflow among spiny lobsters within the locations of our study (White et al. 2010).

Our findings suggest that Belize may be a particularly important location for designing future studies to uncover how environmental and physical oceanographic factors shape spatial patterns of chaotic genetic patchiness. Our sampling sites in Belize were located within a convergence zone between a retentive offshore gyre in the south and a particularly strong advective portion of the Caribbean current that where the majority of the flow moves through the relatively narrow Yucatan Channel (Butler et al. 2011). Convergence zones where oceanographic variability is high are regions where chaotic genetic patchiness is likely to occur, as demonstrated in a seascape genetics analysis of the long-distance dispersing sea urchin Centrostephanus rodgersii (Banks et al. 2007). Banks et al. (2007) suggested that an oceanographic convergence zone was responsible for shaping the fine-scale levels of chaotic genetic patchiness associated with negative levels of spatial
autocorrelation among sea urchin populations in Australia and New Zealand (Banks et al. 2007).

4.3 Spatial Patterns of Kinship

Incorporating kinship analysis and biophysical modeling estimates of larval dispersal did not clarify the environmental and physical mechanisms that were responsible for shaping the chaotic genetic patchiness observed in our study. Kinship analysis suggested that all of our sampling sites with the exception of Puerto Rico had significantly more half-siblings than expected, and half of the sampling sites had significantly more full-siblings than expected. The excess of siblings at our sites can be explained either by self-recruitment, sweepstakes recruitment, or by unknown behavioral and physical mechanisms that prevent the mixing of siblings throughout the larval pool (Iacchei et al. 2013; Christie et al. 2010). Our results are similar to those recently reported in a kinship analysis of the spiny lobster Panulirus interruptus along the southwest coast of North America (Iacchei et al. 2013). That study also found higher than expected levels of siblings at the majority of their study sites ranging from Baja California in Mexico to Santa Barbara along the south central coast of California, USA (Iacchei et al. 2013). Levels of kinship noted in that study were hypothesized to be highest in locations where upwelling is persistent and thus a barrier to recruitment from outside the local system. As a consequence, those locations were also the most genetically differentiated from other sites in their study. In contrast, we found no correlation between oceanographic environment and levels of kinship in Panulirus argus. Even
though Panama, had the highest levels of full-siblings and was the consistently the most genetically differentiated, this trend was not consistent among sites located in off-shore gyres. For example, Venezuela had the highest levels of total kinship (full-siblings plus half-siblings) yet was not well differentiated genetically from the majority of sites in our study. A similar trend was also observed at the Nicaragua site. Even though biophysical modeling predicts that levels of larval self-recruitment should be relatively high in Venezuela and Nicaragua, the combined results of our F_{ST}-based and kinship analyses suggest that connectivity among many locations in the Caribbean is sufficient to maintain high levels of geneflow, despite the potential for self-recruitment.

This hypothesis, that is the potential for both localized and long-distance recruitment in *P. argus*, is consistent with results of a previous biophysical modeling study indicating that the dispersal kernel of *P. argus* larvae is highly bimodal (Butler *et al.* 2011). Most (~60%) of their modeled larvae successfully settled within 200 km of their release site, but a large fraction of the larvae (~20%) nonetheless settled > 1000 km away. Other studies of population connectivity of coral reef species in the Caribbean indicate that even though retentive oceanographic environments may substantially increase the likelihood of self-recruitment, they are by no means ‘closed’ systems with respect to larval dispersal (Cowen *et al.* 2006; Christie *et al.* 2010). The levels of geneflow for the larvae that ‘leak out’ of retentive oceanographic environments may be sufficient to mask the signal of self-recruitment using traditional F_{ST}-based statistics (Christie *et al.* 2010). These hypotheses may explain why sites in Venezuela and Nicaragua were
genetically similar to several other locations in our study, despite evidence for high levels of self-recruitment and kinship. Future genetic studies to evaluate levels of parentage and kinship among adults and larval recruits at the same locations and between locations will be required to test these hypotheses (Christie et al. 2010).

4.4 Source Sink Dynamics

Spatial patterns in genetic structure may also be reflected in source-sink dispersal dynamics. Some locations may act as sources of larvae to other regions in the Caribbean, whereas other locations are most likely to be sinks that act as catchments of larvae from multiple regions in the Caribbean. This hypothesis is supported by recent biophysical modeling of *P. argus* larvae which predicts that certain regions in the Caribbean are sources of larvae that supply a disproportionately large percentage of larvae to the larval pool (Kough et al. 2013). In contrast, other regions of the Caribbean appear to provide disproportionally few larvae to the greater larval pool and therefore act as larval sinks (Kough et al. 2013). By classifying the sampling sites in our study as either sources or sinks of larvae based on results from Kough et al (2013), we can perhaps explain some of the spatial pattern of genetic variability observed in our study. For instance, Venezuela, Nicaragua, and northern Belize are considered to be sources of *P. argus* larvae (Kough et al. 2013) and these locations are also the most genetically similar. In contrast, our sampling locations in Puerto Rico, Panama, Bahamas, and Southern Belize were all in locations thought to be larval sinks and these locations were also the most genetically differentiated. However, Grand Cayman Island, which is
predicted to be a sink for *P. argus* larvae, had one of the lowest mean $F_{ST}$ levels. These findings suggest that larval source sink dynamics could be important drivers of spatial genetic variation in the Caribbean spiny lobster and thus merit further investigation. Temporal replication will be required to test this hypothesis. However, the low number of sampling sites in our study and lack of temporal replication limits our statistical power to make robust conclusions regarding how source sink dynamics are shaping levels of genetic variation among spiny lobster populations in the Caribbean.

6. Acknowledgements

This research was supported by National Science Foundation grants to M. Butler (OCE-0928930) and D. Behringer (OCE-0723662). We thank James Azueta and Isaias Majil at the Bermuda Fisheries Department for helping to collect samples in the Belize. NKT is supported by postgraduate fellowships from the Sustainable Consumption Institute and the Faculty of Life Sciences at the University of Manchester.

7. Literature Cited


Truelove NK, Preziosi RF, Behringer DC, Butler IV MJ (*In review*) Characterization of two microsatellite PCR multiplexes for high throughput genotyping of the Caribbean spiny lobster, *Panulirus argus*. *Conservation Genetics Resources*.


Supplementary Information

Figure S1. Correlations of the corrected percentages of siblings at each site and (A) levels of genetic differentiation measured by Fst, (B) total expected heterozygosity (Ht), and (C) probability of larval self recruitment. P-values and R² values were calculated using a linear regression model.
Table S1: Departures from Hardy Weinberg Equilibrium (HWE). The table below shows the $P$-values for each combination of sampling location and locus. Significant departures from HWE, after the sequential goodness-of-fit correction for multiple tests are shown in bold ($P < 0.008$). The suggested presence of null alleles after analysis with MICROCHECKER is indicated by the symbol (*). Loci shown in grey were excluded from $F_{ST}$ and Jost’s D analyses of genetic differentiation due to the majority of sites deviating from HWE or potentially containing null alleles.

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Table S2: Analysis of Molecular Variance (AMOVA) table. The AMOVA was calculated in the statistical genetics program **GENODIVE**. An infinite allele model was used with 50K permutations and F-statistics correspond to those defined by Weir and Cockerham (1984). Standard deviations were calculated by jackknifing over loci and confidence intervals were calculated by bootstrapping over loci (10K bootstraps). % Var = percent variance, F-stat = the type of F-statistic, F-value = the value of each F-statistic, P-value = level of statistical significance, F'-value = a standardized measure of population differentiation that is suited for comparisons among different types of genetic markers or between organisms. Values in **bold** are statistically significant ($P < 0.05$). When the P-value = 0 the level of significance is equivalent to $P < 0.0001$.

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Table S3: Pairwise comparisons of genetic differentiation among sampling sites. Pairwise F_{ST} values are located below the diagonal and pairwise Jost’s D values are located above the diagonal. Values marked in bold were significant after using a sequential goodness-of-fit correction for multiple tests. The level of significance is indicated by: * P<0.05; ** P<0.01; *** P<0.001.

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Chapter 9

Genetic evidence from the spiny lobster fishery supports international cooperation among Central American marine protected areas

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Running Title: Spiny lobster fishery in Central American marine protected areas

Key Words: Connectivity, Sustainable Fisheries, Conservation, Population Genetics, Kinship Analysis, Spatial Management

Prepared for submission to Conservation Biology

Contributions: NKT, JA, IM, KLC, SB, SC, RFP, DB, and MB designed the study. NKT, KLC, SG, AM, AAP, and MB collected the samples. NKT and SG conducted the laboratory work. NKT, RM, and RFP analyzed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

Marine protected areas (MPAs) have become an important ecosystem-based management approach to help improve the sustainability of the spiny lobster fishery (*Panulirus argus*). Information concerning levels of connectivity of spiny lobster populations among MPAs is severely lacking. The main objective of this study is to use genetic techniques to uncover spatial patterns of connectivity among MPAs in the Central American region of the Caribbean Sea. We specifically test the hypothesis that levels of genetic differentiation and connectivity may differ between spiny lobster populations located in MPAs within advective and retentive oceanographic environments. We found that levels of connectivity are high among spiny lobster populations residing in MPAs in Central America. Despite the high levels of connectivity among spiny lobster populations residing in Central American MPAs, overall $F_{ST}$ was low ($F_{ST} = 0.00013$) but significant ($P = 0.037$). In the Mesoamerican Barrier Reef (MBRS) northern MPAs contained significantly more individuals that were genetically determined outliers or migrants than southern MPAs ($P = 0.008$, $R^2 = 0.61$). The increased number of outliers in northern MBRS MPAs may have contributed to the higher levels of genetic differentiation observed in northern MPAs. Direct genetic testing of larvae and to adults will be required to confirm this hypothesis. The high levels of connectivity among MPAs provide additional evidence of the importance of international cooperation among MPAs. However, the uncertainty regarding the ecological and physical drivers of genetic differentiation in Northern MPAs implies that managers should hedge against uncertainty.
1. Introduction

The fishery for Caribbean spiny lobster, *Panulirus argus*, is one of the most economically important in the Caribbean and most stocks are considered to be either fully exploited or in decline (Fanning et al. 2011). A variety of management strategies have been applied throughout the Caribbean to try to mitigate these declines (Lipcius et al. 2008; Kough et al. 2013). Marine protected areas (MPAs) have become an important ecosystem-based management approach to help improve the sustainability of the spiny lobster fishery (Acosta & Robertson 2003; Maxwell et al. 2013). Several MPAs have been established in Central American Caribbean nations since the 1990’s with the dual objectives of improving commercial fisheries and conserving the biodiversity, particularly in coral reef ecosystems (Kramer & Kramer 2002; Cho 2005). Although the boundaries of many MPAs in the Caribbean have been demarcated to protect sensitive coral reef habitat, information on levels of connectivity among coral reef species within and among MPAs is severely lacking (Botsford et al. 2008; 2009). Improving our understanding of spatial and temporal patterns of population connectivity for coral reef species remains one of the grand challenges for the sustainable management of current MPAs and for designing the MPAs of the future (Sale et al. 2005). The United Nations Convention on Biodiversity’s target to protect 20% of the world’s oceans by 2020 urgently requires information on the connectivity of marine species to achieve this objective (Gaines et al. 2010).

Genetic techniques offer a variety of methods to directly and indirectly measure spatial and temporal patterns of connectivity in marine species (Hedgecock
et al. 2007). While there has been a particular focus on using genetic methods to improve our understanding of connectivity in species with short to medium pelagic larval durations (PLD), such as species of coral and coral reef fish, little is known about connectivity in coral reef species with extremely long PLDs (Butler MJ et al. 2011). Furthermore, few genetic studies have specifically focused on understanding spatial patterns of connectivity among networks of MPAs (Jones et al. 2009).

The Caribbean spiny lobster, *Panulirus argus*, is an ideal species for examining patterns of connectivity among networks of MPAs. Caribbean spiny lobster supports one of the economically most valuable fisheries in the Caribbean and has an extensive history of scientific research and fisheries monitoring data (Fanning et al. 2011). The species has one of the longest PLDs of any known marine species (~ 6-12 months depending on environmental conditions) and has long been suggested to be panmictic throughout the Caribbean (Silberman et al. 1994; Butler MJ et al. 2011). The poor relationship between larval recruitment and adult population levels in many locations in the Caribbean suggests that levels of self-recruitment are low and therefore local populations are likely to be dependent on recruitment from upstream source populations (Briones-Fourzán et al. 2008). However, recent biophysical modeling studies have challenged this hypothesis. Larval behavior coupled with complex hydrodynamics of the Caribbean oceanographic environment may lead to self-recruitment and levels may be particularly high in regions under the influence of retentive oceanographic environments (Butler MJ et al. 2011; Kough et al. 2013). Larvae that originate from source populations located in strongly advective oceanographic environments under
the influence of the Caribbean current are suggested to be dispersed 1000s of km from their natal source and have much lower levels of self-recruitment (Butler 2011). Therefore, MPAs located in retentive oceanographic environments where self-recruitment is suggested to be high may require management strategies that differ from MPAs located in advective oceanographic environments where levels of self-recruitment are suggested to be much lower (Butler MJ et al. 2011).

The network of MPAs in the Central American region of the Caribbean are an ideal location to test the hypothesis that spatial patterns of connectivity in spiny lobster may differ between MPAs located in advective and retentive environments. MPAs in the southern portion of the Mesoamerican barrier reef system (MBRS) are located in a highly retentive oceanographic environment strongly influenced by semi-permanent offshore gyres. In contrast, MPAs in the northern portion of the MBRS are in a highly advective oceanographic environment that can experience particularly strong surface flow where the Caribbean current is impinged by the Yucatán channel. Biophysical modeling studies of *P. argus* have suggested that lobster populations in the southern MBRS have higher levels self-recruitment than northern MBRS lobster populations (Butler et al. 2011). Additionally, northern MBRS lobster populations may be more reliant on larval recruitment from distant lobster populations located upstream of the Caribbean current (Briones-Fourzán et al. 2008; Kough et al. 2013).

The main objective of this research is to use both direct (*e.g.* kinship analysis) and indirect (*F*$_{ST}$-based analyses of genetic differentiation) genetic techniques to uncover spatial patterns of connectivity among MPAs in the Central
American region of the Caribbean Sea. The spatial scale of our study ranges from
the Bocas del Toro MPA in Panama to the Alacranes reef MPA in the Gulf of
Mexico. We specifically test the hypothesis that levels of genetic differentiation and
connectivity may differ between spiny lobster populations located in MPAs within
advective and retentive oceanographic environments.

2. Methods

2.1 Genotyping

A total of 348 adult individuals from 12 locations in Central America were
sampled from either MPAs or spiny lobster conservation areas (Figure 1). All
samples were collected directly from fishers as part of commercial fisheries
monitoring efforts within Central American MPAs and conservation areas. The
dates of sample collection ranged from June-July 2010 at the beginning of lobster
season for individuals collected in Belize, Honduras, and Alacranes Reef in Mexico.
Samples from Banco Chinchorro and Sian Ka’an MPAs in Mexico were collected
the following year in August 2011. Muscle tissue was taken from a single leg and
preserved in 96% ethanol. The samples were transported to the University of
Manchester and stored at 4°C until DNA extraction and genotyping were
performed. Genotyping was performed using 9 previously described microsatellite
loci that have been previously validated as polymorphic and easy to score (Chapter
3). Microsatellite genotyping was performed at the University of Manchester DNA
Sequencing Facility with an ABI 3730xl automatic DNA sequencer (Applied
Biosystems). Microsatellite alleles were scored with the
Figure 1. Map of marine protected areas that spiny lobsters were collected from in Central America. The left panel shows the entire spatial scale of the study and the right panel shows the marine protected areas (MPAs) in the Mesoamerican Barrier Reef System (MBRS) that spiny lobsters were collected from. All MPAs are highlighted in green. The Belize Fisheries Department spiny lobster monitoring site Sector 5 is highlighted in blue.

GeneMapper® v3.7 software package (Applied Biosystems) and binning of alleles was performed using the R-package MsatAllele version 1.02 (Alberto 2009).

2.2 Data Quality Checks

The R-package ALLELEMATCH (Galpern et al. 2012) was used to check for duplicate genotypes that may have accidently resulted from sampling the same individual twice. No duplicate genotypes were found. Each microsatellite loci was analyzed with MICROCHECKER to identify null alleles and detect allele scoring error due to either the dropout of large alleles or stutter. All combinations of loci were tested for linkage disequilibrium (LD) with GENEPOP and no evidence of LD was
detected (Raymond & Rousset 1995; Rousset 2008). Deviations from Hardy-Weinberg Equilibrium (HWE) among all loci and populations were tested with the population genetics software package GENODIVE (Meirmans & van Tienderen 2004). No loci consistently showed evidence of null alleles or deviations from HWE. Therefore, all 9 loci were included in statistical analyses of kinship and population differentiation.

2.3 Kinship Analysis

We used several estimators to investigate the relatedness of individuals within all MPAs and conservation areas. There is no consensus on which estimator is the most accurate, however all calculate relatedness using the allele frequencies and assume HWE. Pairwise comparisons of kinship among all individuals were first calculated in GENODIVE using the relatedness estimator of Loiselle et al. (1995) (Meirmans & van Tienderen 2004). To visualize the results of this analysis we ran a principle coordinates analysis (PCoA) on the pairwise matrix of relatedness in R using the function cmdscale. Individuals that share similar alleles and are more related to each other will cluster in similar locations in multivariate space. Individuals that don’t have many alleles in common or have higher levels of rare alleles, and are less related to other individuals will cluster in distant locations in multivariate space. This analysis was repeated using the Queller and Goodnight (1989) relatedness estimator and the results were similar. We tested for an overabundance of full-siblings and half-siblings within each MPA and conservation zone using the R-package DEMERELATE (Kraemer & Gerlach 2013). The function
Demerelate within the R-package **DEMERELEATE** was used to calculate the observed levels of full siblings and half siblings within each study site using genotype sharing method ($M_{xy}$) (Blouin et al. 1996). This method requires no prior knowledge of population allele frequencies and achieves the highest level of accuracy when levels of heterozygosity are high, as was the case with our microsatellite loci. The function Demerelate tests for an overabundance of closely related individuals by using a logistic regression model to calculated thresholds for individuals being full-siblings or half-siblings. The function Demerelate then creates site-specific randomized reference populations using only the alleles present within the site and the same number of individuals. Chi-squared statistics were used to compare the randomized population to the empirical populations in order to evaluate whether a particular site contained an overabundance or related individuals.

### 2.4 Genetic Diversity and Population Structure

Summary statistics of genetic diversity including the average number of alleles per locus, effective number of alleles, observed heterozygosity ($H_O$), expected total heterozygosity ($H_T$), the inbreeding coefficient ($G_{IS}$), and departures from Hardy-Weinberg equilibrium (HWE) were tested for each locus using **GENODIVE** (Meirmans & van Tienderen 2004). Allelic richness ($A_R$) was calculated using rarefaction to correct for the variable sample sizes among locations with R-package **HIERFSTAT** (Goudet 2005). The function allelic.richness and 50K permutations were used. Overall $F_{ST}$ was calculated using **GENEPOP** with the default settings (Raymond & Rousset 1995; Rousset 2008). **GENEPOP** calculates overall $F_{ST}$ using on Weir and
Cockerham’s (1984) calculations of $F_{ST}$ (Weir & Cockerham 1984). Pairwise comparisons of population differentiation among all locations were calculated in \textsc{genodive} with the log-likelihood G-statistic and 50K permutations to calculate $P$-values (Meirmans & van Tienderen 2004). The statistical program \textsc{sgof} was used to calculate the false discovery rate (FDR) and to correct against type I errors for all statistical tests that contained multiple pairwise comparisons (Benjamini & Hochberg 1995; Carvajal-Rodríguez et al. 2009). PCoA was used to visualize the variation among pairwise estimates of $F_{ST}$ among all locations with the \texttt{cmds} function in R.

Discriminant analysis of principal components (DAPC) was used to visualize levels of genetic population structure among lobsters from specific MPAs and conservation areas (Jombart et al. 2010). DAPC is a multivariate method that identifies genetic differentiation between groups by combing principal component analysis (PCA) with discriminant analysis. DAPC does not rely on a particular population genetics model and therefore is not limited by deviations from Hardy-Weinberg equilibrium or linkage disequilibrium. We applied the \texttt{dapc} function in the R-package \texttt{adegenet} to describe the genetic relationship among specific MPAs and conservation areas. The \texttt{dapc} function creates a model that partitions genetic variation into between-group and a within-group components. Synthetic variables, called discriminant functions, are then constructed to maximize variation between-groups and minimizing variation within-groups. Coordinates of the discriminant functions are then calculated for each individual and plotted in two dimensions. To avoid over-fitting, which could bias our results, we inferred the optimal number of
principle components to retain for the DAPC analysis using the function xvalDAPC in \texttt{ADEGENET} (Jombart 2008). A total of 20 PCs provided the highest classification success suggesting that adding additional PCs to the DAPC may lead to overfitting. Therefore, we retained 20 PCs for our DAPC analyses, which accounted for 52.7% of the total genetic variance.

2.5 Spatial Genetic Analyses

A spatially explicit analysis of genetic variation was conducted using the spatial principal component analysis method (sPCA) in the R-package \texttt{ADEGENET} (Jombart 2008). This analysis is designed to distinguish global spatial structures, defined as positive spatial autocorrelation and genetic variance, from local spatial structures, defined as negative spatial autocorrelation and genetic variance. We used the function chooseCN in \texttt{ADEGENET} to build a connection network among our study sites, allowing connectivity among all locations (Jombart 2008). We used the function spca in \texttt{ADEGENET} to conduct the sPCA analysis (Jombart 2008). As recommended by the author, we conducted a Monte-Carlo test to identify global or local spatial genetic structures in our dataset. We used the function global.rtest to test global structures and the function local.rtest to test local structures. A total of 50K permutations were used to test for significance. The Monte-Carlo test found significant levels of global structure ($P = 0.047$) and no evidence of local structure ($P = 0.794$). Therefore, only global eigenvalues were interpreted. We then used the function screeplot to identify the global eigenvalue with highest levels of both spatial autocorrelation and genetics variance. The first global eigenvalue of the
sPCA met these criteria and therefore was used for the interpretation of spatial patterns of genetic variation. The function interp from the AKIMA R-package was used to create an interpolated map of spatial genetic connectivity among lobster populations within specific MPAs and conservation areas using the coordinates of the first global eigenvalue (Akima 1996). The function interp from the AKIMA R-package was also used to create an interpolated map of mean pairwise levels of \( F_{ST} \) among lobster populations within the MPAs and conservation areas of our study (Akima 1996).

### 2.6 Genetically Determined Outlier and Migrant Analysis

Genetically determined outliers and migrants were determined by PCoA analysis of individual levels of kinship described in section 2.3. The function s.kde2 was used in the R-package ADEGENT to plot a density kernel around individuals that were highly related to each other. Individuals located in multivariate space outside of the density kernel were classified as either outliers or migrants respectively (Figure S1). Outliers were classified as individuals located outside the density kernel but still within the first square (in either positive or negative directions of the x and y axes) of multivariate space surrounding the center of PCoA grid. Migrants were classified as individuals outside the density kernel at distance of at least two squares from the center of Euclidian grid (in either positive or negative directions of the x and y axes) corresponding to the PCoA plot. A linear regression model was then used in R to test for an increased number of genetically determined outliers and migrants with latitude. The linear model was tested using the function lm for the
model migrants=lm (latitude~migrants). The function abline was used in R to calculate the slope of the trend line for linear regression model.

3. Results

3.1 General Summary Statistics

The microsatellite loci in this study were previously tested and validated as polymorphic and neutral. The number of alleles per locus ranged from 4 to 35. Rarefied levels of allelic richness were similar among all sampling locations and ranged from 8.3 to 9.6. The levels of observed heterozygosity (H₀) were generally slightly lower than expected total levels of heterozygosity (H₇). H₀ ranged from 0.622 to 0.727 and H₇ ranged from 0.680 to 0.769 over all populations and loci (Table 1). Analysis with MICROCHECKER found no evidence of stutter or the drop out of large alleles (van Oosterhout et al. 2004). No locus consistently deviated from HWE or consistently contained null alleles. The deviations from HWE at five sites for locus FWC04, two sites at locus FWC17, and one site at locus FWC08 were suggested to be caused by null alleles after analysis with MICROCHECKER (van Oosterhout et al. 2004).

3.2 Levels of Genetic Connectivity among MPAs

The PCoA analysis of individual pair-wise levels of relatedness values suggested that levels of genetic connectivity were high among all the MPAs within our study (Figure 2). There was substantial overlap of adults within each MPA. The majority of adults from all MPAs clustered in the same multivariate space near the
Table 1. Summary statistics that include the name of the marine protected area (MPA), total number of alleles (N), average number of alleles (Number), effective number of alleles (Effective Number), allelic richness (AR), observed heterozygosity (H<sub>O</sub>), total expected heterozygosity (H<sub>T</sub>), and inbreeding coefficient (G<sub>IS</sub>).

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<th>Number</th>
<th>Effective Number</th>
<th>AR</th>
<th>Ho</th>
<th>Ht</th>
<th>Gis</th>
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Figure 2. Principle coordinates analysis (PCoA) of all pairwise levels of kinship for spiny lobsters individuals that were sampled from marine protected areas (MPAs) throughout Central America. A filled circle represents each individual with a unique color corresponding to the specific MPA the individual was collected from. The 95% inertia ellipses surround the specific individuals collected from each MPA and represented by the same color as the individuals they surround. All the inertia ellipses have extensive overlap suggesting high levels of connectivity among MPAs. Note that there are several outlier individuals located in multivariate space well outside the 95% inertia ellipses. The individuals are likely to be migrants. The specific MPA the migrants were collected from is noted next to the migrant.

Origin of the graph and all of the 95% inertia ellipses that correspond to the variation among individuals within each MPA overlapped. A total of sixteen individuals were not closely related to any of the other individuals within the MPAs of our study and are potentially migrants that have recruited from populations that we were unable to sample. The results of the DAPC were in agreement with PCoA analysis of relatedness. The was considerable overlap among the 95% inertia ellipses of
individuals sampled from all the MPAs in our study, suggesting high levels of genetic connectivity among Central American MPAs (Figure 3).

Figure 3. A scatterplot of discriminant analysis of principle components (DAPC) analysis of the microsatellite data from *Panulirus argus* individuals collected from marine protected areas (MPAs) throughout Central America. Individual genotypes are represented by dots with a unique color for each MPA. The 95% inertia ellipse surrounds individuals from each specific MPA. Note the extensive overlap of 95% inertia ellipses suggesting high levels of connectivity among MPAs. The PCA eigenvalues represent the number of principal components containing 61.9% of the total genetic variation that were retained for the DAPC analysis. The DA eigenvalues represents the amount of genetic information contained in the first two principle components of the DAPC analysis that were plotted on the x and y axes.
3.3 Sibling Analysis

The sibling analysis in DEMRELATE found more significantly more half-siblings than expected in the majority of sites ($P < 0.05$) (Kraemer & Gerlach 2013). Caye Caulker, Glover’s Reef, Utila, and La Moskitia were the only MPAs that did not have significantly more half-siblings than expected (Figure 4). The proportions of full-siblings were significantly higher than expected in Alacranes Reef, Bancho Chinchorro, Sian Ka’an, and South Water Caye ($P < 0.05$).

**Figure 4.** Differences between observed and expected number of full and half sibling comparisons from individuals collected from specific marine protected areas (MPAs) in Central America. The proportions of full-siblings are represented by grey bars and half-siblings by hatched bars. The expected levels of kinship were calculated using 1000 pairs of randomized populations at each MPA. Asterisks next to the full and half-siblings represent significantly greater levels than expected by chance ($P < 0.05$).
3.4 Levels of Genetic Differentiation among MPAs

Overall $F_{ST}$ was low ($F_{ST} = 0.00013$) but significant ($P = 0.037$). Levels of $F_{ST}$ among MPAs were significant for 10 out the 78 pairwise comparisons (Table S2). The PCoA analysis of pairwise comparisons of $F_{ST}$ indicated that Alacranes Reef, Hol Chan, Caye Caulker, and Sapodilla Cayes were all outliers, suggesting that they may be more differentiated from the other MPAs. Sian Ka’an, Banco Chinchorro, Sector 5, South Water Caye, Glover’s Reef, Utila, and Bocas del Toro all clustered together suggesting they were not differentiated from one another (Figure 5, Figure 6A). The spatial principle components analysis and interpolation of mean pairwise $F_{ST}$ at each MPA both suggested that MPAs in the northern MBRS were genetically differentiated from MPAs in the southern MBRS and from Bocas del Toro in Panama (Figure 6).

3.5 Genetically Determined Outlier Analysis

Northern MPAs contained significantly more individuals that were genetically determined outliers or migrants than MPAs in the southern MBRS ($P = 0.008, R^2 = 0.61$, Figure 7). The increased number of outliers in northern MBRS MPAs may have contributed to the higher levels of genetic differentiation observed in northern MPAs by contributing more rare alleles to populations in northern MBRS MPAs.
4. Discussion

We found that levels of connectivity are high among spiny lobster populations residing in MPAs in Central America. This is not surprising given the extremely long PLD of spiny lobster resulting in extensive dispersal potential (Butler MJ et al. 2011). Despite the high levels of connectivity among spiny lobster populations residing in Central American MPAs, we found low but significant levels of genetic differentiation ($F_{ST}$ and SPCA) among MPAs in the MBRS. Since the levels of connectivity were high among lobster populations residing within all the MPAs that we surveyed it’s unlikely that genetic isolation due to a lack of connectivity explains the higher levels of genetic differentiation that we observed in lobster populations.
Spatial Principle Component Analysis

Mean Pairwise $F_{ST}$

$F_{ST}$
**Figure 6.** Interpolated map of mean levels of genetic differentiation among spiny lobsters collected from marine protected areas in Central America based upon (A) (mean pairwise $F_{ST}$) and (B) spatial analysis of principle components (SPCA). For clarity, the sampling locations are represented by a circle only in the larger MPAs (i.e. Miskito Cayes, Banco Chinchorro, and Alacranes Reef). The boundaries of the MPAs are represented by the black lines on top of the interpolation. The scale bar located on the right of each panel indicates the levels of genetic differentiation among lobsters from specific MPAs. Reds indicating higher values and blues indicating lower values.

![Interpolated map of mean levels of genetic differentiation among spiny lobsters](image)

**Figure 7.** A linear regression model representing a significant increase in the number of genetically determined migrants with latitude. The grey portion represents the 95% confidence intervals of the linear regression trend line.

![Linear regression model](image)

Our analysis of genetically determined migrants and outliers indicates an increase in immigration to local populations in more northern portions of the MBRS. An increase in migrants would explain the increased genetic differentiation among more northerly MPAs that we did not find among southern MPAs in Belize and Honduras.
Our findings of increased levels of migrants and outliers within local populations in the northern MBRS are supported by biophysical and oceanographic modeling studies of spiny lobster larval dispersal in the MBRS (Briones-Fourzán et al. 2008; Butler MJ et al. 2011). While the methodologies of these biophysical and oceanographic modeling studies of spiny lobster larval dispersal have differed, they have both suggested that while the potential for self-recruitment may exist, northern regions of MBRS are highly dependent on larval recruitment from distant source populations located upstream of the Caribbean current. In contrast, spiny lobster recruitment dynamics in the southern MBRS are more likely to be influenced by the retentive ocean currents in this region. Biophysical modeling suggests that lobster populations, particularly near the Sapodilla Cayes MPA, may be more dependent on self-recruitment from locally derived stocks (Unpublished data). However, in this study we were unable to compare the genotypes of new lobster larvae that recruited to a specific MPA to the genotypes of the adults residing within the MPA. Therefore, we infer that the rare individuals that appear to be highly unrelated to all the other individuals we sampled in Central America (e.g. the migrants in Figure S1 and Figure 2) could not have been generated from the genotypes of the lobsters that we sampled in Central American MPAs. Consequently, we must again infer that these individuals are migrants that may have originated from other regions in the Caribbean that we were unable to sample. Direct genetic testing of larvae and to adults will be required to confirm this hypothesis.
The increased abundance of immigrants and outliers is only one of several potential ecological and physical drivers that may explain the higher levels of genetic differentiation that we observed in lobster populations residing within Northern MPAs. For example, a population genetics study of spiny lobster species \textit{Panulirus interruptus} in California found that kelp habitat was an informative predictor of genetic differentiation ($F_{ST}$) (Selkoe et al. 2010). Sites with high levels of kelp cover tended to be the most genetically differentiated. The Caribbean spiny lobster, \textit{Panulirus argus}, is dependent on several habitat types throughout it’s life history. Postlarvae require shallow coastal nursery habitat where they settle into vegetation, particularly red macroalgae that can be found in seagrass and mangrove habitats (Butler et al. 2006). Later, the juveniles emerge from vegetation, become social, and aggregate within crevices. As spiny lobsters near maturity (1.5 yrs post-settlement), they migrate tens of kilometers from the coastal nursery to join the adult population on the coral reef (Butler et al. 2006). Environmental variation among these habitats may also be responsible for the pairwise differences in genetic differentiation that we observed among MPAs and cannot be ruled out (Teacher et al. 2013). However, the small sample sizes of our study did allow for sufficient statistical power to test the relationship among specific habitat characteristics within MPAs and levels of genetic differentiation.

The sibling analysis suggested that there were significantly more half-siblings in the majority of MPAs and significantly more full-siblings in half of the MPAs. Higher than expected number of siblings have been also been reported in other species of spiny lobster and for Caribbean spiny lobster (Iacchei et al. 2013).
The higher than expected levels of full and half-siblings may be explained by self-recruitment, a sweepstakes recruitment event, or an unknown mechanism that prevents larvae from mixing throughout their PLD. Biophysical modeling studies of Caribbean spiny lobster larval connectivity suggests self-recruitment may be common due to larval behavior coupled with local oceanographic characteristics (Butler MJ et al. 2011). Several population genetics studies of coral reef fish species in the MBRS, which have much shorter PLDs than spiny lobsters, have provided evidence of both self-recruitment and limited connectivity in the MBRS (Hogan et al. 2011; Puebla et al. 2012; Chittaro & Hogan 2012). The presence of siblings and half-siblings would be expected in regions where self-recruitment occurs (Iacchei et al. 2013). Sweepstakes recruitment events may also explain higher than expected levels of siblings and half siblings among discrete location (Christie et al. 2010).

There is growing evidence to suggest that self-recruitment and sweepstakes recruitment may be predominant ecologically processes that shape patterns of larval dispersal in many marine species (Cowen et al. 2007; Christie et al. 2010; Hogan et al. 2011). Again, direct comparisons of larval genotypes to adult genotypes will be required to directly test hypotheses regarding sweepstakes recruitment and self-recruitment among spiny lobster populations residing in Central American MPAs.

**Implications for Management**

The high levels of connectivity among MPAs provide additional evidence of the importance of international cooperation among MPAs in Central America. The increased abundance of genetically determined lobster migrants and outliers in
MPAs in the Northern MBRS suggest that this region may be more dependent on recruitment from upstream source populations than MPAs in the southern MBRS. The higher than expected levels of full siblings and half siblings provide additional support that self-recruitment, sweepstakes recruitment or both may be occurring in the region. Our findings present only a single snapshot in the complex spatiotemporal web of spiny lobster connectivity patterns. Temporal replication and comparisons of larvae to adults will clearly be required to understand if the patterns we observed are stable or simply a shifting mosaic over time (Hellberg 2009). Due to the uncertainty regarding the ecological and physical drivers of genetic differentiation that we observed in Northern MBRS MPAs, managers should conservatively plan for uncertainty (Selkoe et al. 2006). For example, if northern MBRS MPAs are indeed more dependent on larval recruitment from distant source population, overfishing of adults from those source populations may reduce levels of larval recruitment (Butler MJ et al. 2011). If sweepstakes events are common fisheries managers should conservatively plan for potential periods of reproductive failure, despite having large population sizes (Selkoe et al. 2006). Finally, if self-recruitment is indeed common in spiny lobsters, then locally based conservation efforts are more likely to succeed and conversely overfishing is likely to have a larger impact on recruitment success (Fanning et al. 2011; Butler MJ et al. 2011).

5. Acknowledgements

We are grateful for the logistical support provided by the Belize Fisheries Department biologists and rangers and staff at Glover’s Reef Marine Reserve managed by the Wildlife Conservation Society. We would particularly like to thank
James Azueta and Isaias Majil at the Belize Fisheries Department. Without their help and hard work this research project would not have been possible. We would also like to thank the Comisión Nacional de Áreas Naturales Protegidas in Mexico and particularly Maria del Carmen García Rivas for her assistance at Banco Chinchorro. At Hol Chan we would like to thank Miguel Alamilla and Kira Forman. At Glover’s Reef Fisheries Department we would like to thank Alicia, Luis Novelo, Elias Cantun, Samuel Novelo, Martinez, and Merve. At the Caye Caulker Fisheries Department we would like to thank Shakera Arnold, Ali, Aldo, and Islop. At the Belize City Fisheries Department in Belize City we would like to thank Wilfredo Pott and Barbi Gentle. In Caye Caulker we would like to thank Friederike Clever for her assistance collecting samples. At the Wildlife Conservation Society Glover’s Reef Marine Field Station we would like to thank Alex Tilley, Danny Wesby, Janet Gibson, Sarah Pacyna, Uncle, Mango Juice, and Home Alone. At Northeast Caye at Glover’s Reef we would like to thank Ali McGahey, Brian, and Warren Cabral. A research permit was issued by the Belize Fisheries Department. We are grateful for the assistance of Dr. Edwin Harris at Manchester Metropolitan University for invaluable laboratory experience. This research was supported by funding for a PhD fellowship for NKT from the Sustainable Consumption Institute and Faculty of Life Sciences at the University of Manchester, and by a grant (OCE-0928930) from the US National Science Foundation to MJB and DCB.

6. Literature Cited


Truelove NK, Preziosi RF, Behringer DC, Butler IV MJ (In review). Characterization of two microsatellite PCR multiplexes for high throughput genotyping of the Caribbean spiny lobster, Panulirus argus. Conservation Genetics Resources.


South Water Caye

Glover's Reef
**Figure S1.** Principle coordinates analysis (PCoA) on all pairwise levels of kinship of spiny lobsters sampled from marine protected areas (MPAs) throughout Central America (Panels A-L). Each panel (A-L) represents the results of PCoA analysis for lobsters at a specific MPA (the name of the MPA is located on the bottom left of the panel). The blue diamond in the middle is a density plot used to highlight individuals that are related and share similar alleles. Individuals highlighted in green are outliers that are not well related to majority of other individuals. The individuals highlighted in red are extreme outliers, whose genotypes could not have been generated from the other individuals that we sampled and therefore are likely to be migrants.

**Table S1.** Departures of each microsatellite locus from Hardy Weinberg Equilibrium (HWE). The table includes the $P$-values for each combination of marine protected area lobster population and locus. Significant departures from HWE are shown in bold, after the sequential goodness-of-fit correction. Potential loci with null alleles determined by analysis with MICROCHECKER are indicated by the symbol (*).

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Table S2. Pairwise comparisons of genetic differentiation ($F_{ST}$) among sampling sites. Pairwise $F_{ST}$ values are located both below the diagonal and above the diagonal for ease of finding comparisons among sampling sites. Negative $F_{ST}$ values are replaced with 0 for ease of reading the table. Mean pairwise $F_{ST}$ values for each sampling site are located on the bottom row. Values marked in bold were significant using the sequential goodness-of-fit correction for multiple tests.

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| Mean $F_{ST}$ | 0.00106          | 0.00095     | 0.00318        | 0.00525      | 0.00259       | 0.00790 | 0.00640        | 0.00403        | 0.00208  | 0.00172    | 0.00153     | 0.0013|
Chapter 10

High levels of connectivity and kinship among juvenile and adult yellowtail snapper populations (*Ocyurus chrysurus*) in the southern region of the Mesoamerican barrier reef

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**Running Title:** Genetic connectivity of *Ocyurus chrysurus*

**Key Words:** Coral Reef Fish, Self-Recruitment, Marine Conservation, Population Genetics, Microsatellites, Spatial Management

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**Contributions:** NKT, RFP, SB, and SC designed the study. SB and SC collected the samples. NKT conducted the laboratory work. NKT an RFP analyzed the data. NKT drafted the manuscript, which was refined by the co-authors.
Abstract

Many large predatory species of coral reef fish such as groupers and snappers have been severely overfished in the Caribbean. Yellowtail snapper (Ocyurus chrysurus) is often the last omnivorous species that fisheries target before they shift to functional herbivores, such as species of parrotfish. Sustainable management plans are urgently needed to promote the long-term resilience of the yellowtail snapper fishery. The objective of this study is to help resolve the appropriate scale of management for yellowtail snapper in the southern Mesoamerican Barrier Reef System (MBRS). We used 12 microsatellite markers to examine patterns of connectivity among juvenile and adult yellowtail snapper populations in Honduras and Belize. The results of $F_{ST}$ and kinship analyses suggest that levels of connectivity are high among yellowtail snapper populations in Honduras and Belize. Pairwise relatedness analyses of juveniles and adults collected from the same locations in the North Coast of Honduras were highly suggestive of self-recruitment. Despite finding evidence of high levels of connectivity, we found low but significant pairwise levels of genetic differentiation between many juvenile and adult populations. The genetic differentiation that we observed among juvenile and adult populations may be caused by larval recruitment dynamics rather than genetic isolation due to lack of connectivity. The high levels of connectivity among yellowtail snapper populations in the southern MBRS provide further evidence of the importance of international cooperation for the sustainable management of coral reef fisheries.
1. Introduction

Many large predatory species of coral reef fish such as groupers and snappers have been severely overfished in the Caribbean (Coleman et al. 2000). Once these larger predatory species have been depleted many fisheries move down the food chain and begin to target omnivores and eventually herbivores (Mumby et al. 2012). For example, yellowtail snapper (*Ocyurus chrysurus*) has become one of the most commercially important species in the Western Caribbean since many grouper fisheries are in severe decline or have completely collapsed (Aguilar-Perera 2006; Heyman and Granados-Dieseldorff 2012). Yellowtail snapper is often the last omnivorous species that fisheries target before they shift to functional herbivores, such as species of parrotfish (Mumby et al. 2012). Parrotfish are well defined as ecologically important species that play an important functional role in maintaining the health and resilience of coral reefs (Mumby 2009; Mumby et al. 2006). Therefore, by improving the sustainable management of the yellowtail snapper, the fishery may ultimately protect parrotfish species from becoming overfished.

There is a growing international demand for yellowtail snapper and sustainable management plans are urgently needed to promote the long-term resilience of this fishery (Ault et al. 2005). Yellowtail snapper has several advantageous life-history characteristics that make it likely to respond positively to management strategies over short time scales. The species has a fast growth rate and reaches maturity relatively early at two years of age (Ault et al. 2005). Yellowtail snapper spawn throughout the year in the Western Caribbean and are not known to form site-specific spawning aggregations that can be easily over exploited by
fisheries (Huijbers et al. 2013). However, the species also exhibits high levels of spatial complexity in its life cycle that presents significant challenges for spatial management. For example, juveniles often recruit to shallow seagrass and mangrove habitats, however they are not obligates to these environments (Nagelkerken and van der Velde 2004). As juveniles mature their home range begins to increase and individuals eventually recruit into the adult population that inhabits coral reefs and off-shore banks (Nagelkerken et al. 2000). The use of multiple habitats throughout its life history makes this species particularly vulnerable to habitat loss, since the loss of either mangrove, seagrass, or coral reef habitats are likely to reduce recruitment rates from one life-history stage to the next (Mumby et al. 2004).

Despite the growing demand and economic importance of yellowtail snapper for sustaining industrial and small-scale fisheries in the Western Caribbean, very little information exists concerning levels of adult or juvenile population connectivity in this region. Recent genetics studies of yellowtail snapper among Puerto Rico, the US Virgin Islands, and the Florida Keys found evidence of genetically unique subpopulations and limited connectivity in this region of the Caribbean (Saillant et al. 2012). It is unclear whether or not genetically unique subpopulations of yellowtail snapper exist in the Western Caribbean since no genetics studies have been conducted in this region. Biophysical modeling studies have played an important role in guiding the spatial management of this species (Cowen et al. 2006). Recent biophysical modeling studies in the Miskito Cayes (a remote and poorly studied chain of coral islands off-shore of northeastern Honduras) suggest that the yellowtail population in this region may be an important
source of new recruits to the Mesoamerican Barrier Reef System (MBRS; Steve Box, Unpublished data). The southern MBRS is located in a highly retentive oceanographic region under the influence of semi-permanent offshore gyres and biophysical modeling studies suggest that levels of larval self-recruitment are particularly high for a several coral reef species in this region (Butler IV et al. 2011; Cowen et al. 2006; Kough et al. 2013).

The objective of this study is to help resolve the appropriate scale of management for yellowtail snapper in the southern MBRS. We will investigate three questions regarding ecologically relevant levels of connectivity among yellow snapper populations in the Miskito Cayes, the north shore of Honduras, and Belize: (1) Is there evidence of limited connectivity or genetically unique subpopulations, (2) is there evidence of self-recruitment in southern MBRS, and (3) how well connected is the yellowtail snapper population in the Miskito Cayes to populations in the southern MBRS? Our findings will be used to specifically test hypotheses derived from previously biophysical modeling studies suggesting that levels of self-recruitment and population connectivity are high among adult and juvenile populations in the southern MBRS and Miskito Cayes.

2. Methods

2.1 Genotyping

A total 269 adult and juvenile yellowtail snappers were collected from 13 discrete locations in Belize and Honduras from August 2011 through March 2012 (Figure 1). Adult and juvenile yellowtail snapper were caught with hook and line and also purchased directly from fishermen if the exact GPS coordinates of the
Figure 1. Map of yellowtail snapper juvenile and adult sampling locations. The circles represent the sampling sites. Black circles = only adults were sampled, white Circles = only juveniles were sampled, and grey circles = both juveniles and adults were sampled.

fishing location were available. A fin clip of approximately 1 cm² was collected from each individual and stored in 100% ethanol. The samples were shipped to the University of Manchester and stored at 4°C upon arrival until DNA extraction and genotyping could be performed. We used 12 microsatellite markers have been described previously for yellowtail snapper and validated as polymorphic and easy to score (Chapter 4). Microsatellite fragment analysis was preformed at the University of Manchester DNA Sequencing Facility with an ABI 3730xl automatic DNA sequencer (Applied Biosystems). The GeneMapper® v3.7 software package
(Applied Biosystems) was used for scoring microsatellite alleles. The binning of alleles was conducted with the R-package MsatAllele version 1.02 (Alberto 2009).

2.2 Data Quality Checks

The R-package ALLELEMATCH was used to check for duplicate genotypes (Galpern et al. 2012). Duplicate genotypes may occur by accidentally sampling the same individual twice. Analysis with ALLELEMATCH found no duplicate genotypes. All microsatellite loci were analyzed with MICROCHECKER to assess levels of null alleles and to detect potential scoring errors causing the dropout of large alleles or stutter (van Oosterhout et al. 2004). We tested all combinations of loci for linkage disequilibrium (LD) with GENEPOP and no evidence of LD was found (Raymond and Rousset 1995; Rousset 2008). The population genetics software package GENODIVE was used to test all combinations of populations and loci for deviations from Hardy-Weinberg Equilibrium (HWE) (Meirmans and van Tienderen 2004). Locus OCH14, OCH2, and OCH6 consistently deviated from HWE and were suggested to contain null alleles. Therefore, these loci were not included in statistical analyses of kinship and $F_{ST}$ since these tests assume HWE and deviations from HWE may bias these statistical analyses. None of the other remaining 9 loci consistently showed evidence of null alleles or deviations from HWE (Table S1).

2.3 Kinship Analysis

We used two relatedness estimators to investigate levels of kinship and to test for an overabundance of siblings within juvenile and adult yellowtail sampling
There is no consensus on which relatedness estimator is the most accurate, however, both calculate relatedness among individuals using allele frequencies and perform best when levels of heterozygosity are high and populations conform to HWE (Oliehoek et al. 2006). We calculated pairwise levels of relatedness among all individuals in Genodive, which uses the relatedness estimator of Loiselle et al. 1995. The results of the analysis were visualized in R by performing a principle coordinates analysis (PCoA) on the matrix of pairwise relatedness values using the function cmdscale. PCoA is often used to visualize patterns within genetics data and works well with distance matrices (Jombart et al. 2009). The PCoA analysis of individual levels of kinship identifies similarities and differences among individuals based upon the number of alleles they have in common. For example, individuals that share similar alleles will cluster in similar multivariate space, while individuals that don’t have many alleles in common or contain rare alleles will cluster in distant locations in multivariate space (Christie et al. 2010). We used the R-package DemRelate to test for an overabundance of full-siblings and half-siblings within juvenile and adult yellowtail sampling locations (Kraemer and Gerlach 2013). Observed levels of full siblings and half siblings within each study site were first calculated by using the function Demerelate. This function uses genotype sharing method ($M_{xy}$) of Blouin et al. (1996) to identify full-siblings and half-siblings. Randomized reference populations comprised of the same alleles and number of individuals as the empirical sites were then generated. To test for an overabundance of siblings the proportions of full-
siblings and half siblings between the randomized and empirical sites were tested using Chi-squared statistics.

2.4 Genetic Diversity and Population Structure

Microsatellite summary statistics, levels of genetic diversity, and levels of genetic population differentiation were calculated in *GENODIVE* (Meirmans and van Tienderen 2004). The summary statistics and genetic diversity statistics included the average number of alleles per locus, effective number of alleles per population, observed heterozygosity ($H_O$), expected total heterozygosity ($H_T$), the inbreeding coefficient ($G_{IS}$), and departures from HWE. The levels of allelic richness ($A_R$) for each discrete yellowtail sampling location were calculated with the R-package *HIERFSTAT* using the function allelic.richness and selecting 50K permutations (Goudet 2005). The R-package *HIERFSTAT* uses rarefaction to correct for differences in sample sizes among locations.

Several AMOVA analyses and were conducted in *GENODIVE* to test for genetic differentiation among 1) each discrete yellowtail sampling site, 2) only among adult sampling sites, and 3) between all adults pooled into a single population and all juveniles pooled into a single population (Meirmans 2012). AMOVA analyses used the infinite allele model with 50K permutations. The $F_{ST}$ values of the AMOVA are based upon Weir and Cockerham’s (1984) calculation’s of $F_{ST}$, which corrects for differences in sample sizes among populations (Weir and Cockerham 1984). Pairwise levels of $F_{ST}$ were calculated among all discrete yellowtail sampling
locations in GENODIVE. The level of significance among the pairwise comparisons of $F_{ST}$ was calculated using the log-likelihood G-statistic and 50K permutations. The false discover rate (FDR) was used to test for type I errors among the multiple comparisons using the statistical program SGOF (Benjamini and Hochberg 1995; Carvajal-Rodríguez et al. 2009). PCoA was used to visualize the variation among pairwise population level estimates of $F_{ST}$ using the cmdscale function in R. This PCoA analysis differs from the PCoA analysis of individual levels of kinship, since it identifies differences in allele frequencies among populations whilst the PCoA of kinship identifies differences in shared alleles among individuals.

The multivariate statistical method discriminant analysis of principal components (DAPC) was used to visualize levels of genetic differentiation among individual yellowtail snappers from each discrete sampling location (Jombart et al. 2010). This method combines principal component analysis with discriminant analysis. DAPC summarizes levels of genetic differentiation between groups while minimizing within-group. DAPC is not limited by deviations from Hardy-Weinberg equilibrium or linkage disequilibrium, since it does not rely on any specific population genetics model (Jombart et al. 2010). All adult and juvenile yellowtail snappers were grouped into discrete populations based upon the location they were collected from. The dcp function in the R-package ADEGENET was then applied to these specific groupings. Retaining too many principle components (PCs) can lead to over-fitting the discriminant functions, which can lead to type I errors among the groupings. Therefore, to avoid over-fitting we used cross-validation to suggest the optimal number of PCs to retain for the DAPC analysis using the function
xvalDAPC in ADEGENET. The results of the cross-validation suggested that retaining 20 PCs would provide sufficient amounts of genetic information for DAPC to discriminate among groups whilst minimizing the potential of over-fitting. Therefore, we retained 20 PCs for the DAPC analysis, which amounted to 52.7% of the total genetic variance.

2.5 Spatial Genetic Analyses

The R-package AKIMA and the function interp were used to create a geo-referenced interpolated map of mean pairwise levels of $F_{ST}$ among each discrete yellowtail snapper sampling location (Akima 1996). The R-packages maps and mapdata were used to overlay a map of Honduras and Belize on top of the interpolated map using the functions filled.contour and map.

3. Results

3.1 General Summary Statistics

The number of alleles for each microsatellite locus ranged from 8 to 32. Levels of AR, $H_T$, and $G_IS$ did not vary considerably among sites (Table 1). $P$-values for deviations from HWE indicated that loci $OCH14$, $OCH2$, and $OCH6$ had a departure from HWE at nearly every sampling site. Analysis with MICROCHECKER suggested that loci $OCH14$, $OCH2$, and $OCH6$ potentially contained null alleles. Therefore, $OCH14$, $OCH2$, and $OCH6$ were not included in $F_{ST}$-based analyses and analyses of relatedness since deviations from HWE and null alleles have the
Table 1. Summary statistics that include the name of each discrete yellowtail snapper sampling location, the total number of alleles (N), the average number of alleles (Number), levels of allelic richness (AR), levels of observed heterozygosity (H₀), levels of total expected heterozygosity (Hₜ), and the inbreeding coefficient (Gₛ). Bold indicates locations where both juvenile and adult yellowtail snappers were sampled.

<table>
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<th>Hₜ</th>
<th>Gₛ</th>
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potential to bias levels of $F_{ST}$ among populations and the presence of null alleles can decrease the accuracy of statistical tests of relatedness. Further analysis with MICROCHECKER found no evidence of scoring error due to stutter or large allelic dropout. None of the remaining loci had consistent departures from HWE or consistently contained null alleles among the sampling locations (Table 1).

3.2 Relatedness of Juveniles and Adults

The 95% inertia ellipses of the DAPC analysis showed considerable overlap suggesting that levels of genetic connectivity were high among all juvenile and adult sites (Figure 2). Likewise, the PCoA analysis of the relatedness among individuals suggested that levels of genetic connectivity were high among all adult and juvenile sampling locations (Figure 3 A-D). No separation of the 95% PCoA inertia ellipses for any combination of juvenile and adult was observed suggesting high levels of genetic connectivity among all the juvenile and adult sampling locations of our study. While the majority of individuals were clustered near the origin of the x and y-axis, the outlier individuals were found along all dimensions. The large distance between the outlier individuals and the main cluster of individuals suggests that these individuals are not well related to any of the other individuals in the study (Figure 3 A-E). Two outlier individuals were observed one on the positive and one on the negative side of the x-axis at the Porvenir Bank site (Figure 3E). No outlier individuals were found in either juveniles or adults collected from the Asañas site or in juveniles from Cayos Cochinos located 10.55 km away (Figure 3F).
Figure 2. A scatterplot of discriminant analysis of principle components (DAPC) analysis of the microsatellite data from yellowtail snapper juveniles and adults sampled from Belize and Honduras. Individual genotypes are represented by dots with a unique color for each discrete juvenile and adult sampling location. The 95% inertia ellipse surrounds individuals from discrete sampling location. Note the extensive overlap of 95% inertia ellipses suggesting high levels of connectivity among all yellowtail snapper populations. The PCA eigenvalues represent the number of principal components containing 52.7% of the total genetic variation used for DAPC analysis. The DA eigenvalues represents the amount of genetic information contained in the first two axes of the DAPC scatterplot.
**Figure 3.** Principle coordinates analysis (PCoA) of all pairwise levels of kinship among juvenile and adult yellowtail snappers sampled from Belize and Honduras. A filled circle represents an individual yellowtail snapper. Blue = adults, Red = juveniles, and yellow = juveniles from Cayos Cochinos. The 95% inertia ellipses surrounds the specific individuals collected from each discrete sampling site. A = all juveniles and all adults, B = Caye Caulker adults and all juveniles, C = La Moskitia adults and all juveniles, D = North Coast of Honduras adults and all juveniles, E = Porvenir adults and Porvenir juveniles, and F = Asañas adults, Asañas juveniles, and Cayos Cochinos juveniles (located ~10.5 km from Asañas). Note that there are several outlier individuals located in multivariate space well outside the 95% inertia ellipses in A-E that are likely to be migrants.

### 3.3 Self-Recruitment

The PCoA coordinates and 95% inertia ellipses of juveniles and adults collected from the same location overlapped considerably. These results are highly suggestive of self-recruitment (Figure 3 E-F). The significantly higher than expected levels of half-siblings among juvenile and adult locations provides further evidence of self-recruitment \((P < 0.05;\) Figure 4). Although full siblings were suggested to occur at all locations, the observed levels were not significantly different than those expected by chance \((P > 0.05)\).

### 3.4 Genetic Differentiation Between Juveniles and Adults

The AMOVA analysis found significant differences among adults and juveniles whilst no significant differences were found when only adult sites were compared. When all juvenile and adults sampling locations were included in the AMOVA analysis overall \(F_{ST}\) was low 0.0036 and significant \((P = 0.0176;\) Table 2). When only the adult locations were included in the AMOVA \(F_{ST}\) remained low at 0.0024 and was no longer significant \((P = 0.096)\). When juveniles and adults were
Table 2. Analysis of molecular variance (AMOVA) of yellowtail snappers from Belize and Honduras. All Sites = comparisons from each discrete juvenile and adult sampling location, adult sites = only sites where adult yellowtail snapper were collected, and adults and juveniles = all adult individuals pooled into a single population compared to all juvenile individuals pooled into a single population.

<table>
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<th>Source of Variation</th>
<th>Nested in</th>
<th>%VAR</th>
<th>F-stat</th>
<th>F-value</th>
<th>P-value</th>
<th>F'-value</th>
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<td>Within Individual</td>
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<td>--</td>
<td>--</td>
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<tr>
<td></td>
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<td>Among Population</td>
<td>--</td>
<td>0.0036</td>
<td>F&lt;sub&gt;ST&lt;/sub&gt;</td>
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<td></td>
<td>Among Population</td>
<td>--</td>
<td>0.0024</td>
<td>F&lt;sub&gt;ST&lt;/sub&gt;</td>
<td>0.0024</td>
<td>0.0959</td>
<td><strong>0.0121</strong></td>
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<tr>
<td>Juveniles and Adults</td>
<td>Within Individual</td>
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<td>0.9685</td>
<td>F&lt;sub&gt;IT&lt;/sub&gt;</td>
<td>0.0315</td>
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<tr>
<td></td>
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<td>Population</td>
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</tr>
<tr>
<td></td>
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<td>--</td>
<td>0.0021</td>
<td>F&lt;sub&gt;ST&lt;/sub&gt;</td>
<td>0.0021</td>
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Figure 4. Differences between observed and expected number of full and half sibling comparisons from yellowtail snappers in Belize and Honduras. The proportions of full-siblings are represented by grey bars and half-siblings are represented by hatched bars. The expected levels of kinship were calculated using 1000 pairs of randomized populations representing yellowtail snappers from the North Shore of Honduras (juveniles and adults), La Moskitia, and Belize. Asterisks represent significantly greater levels of half-siblings than expected by chance ($P < 0.05$).

Compared from the same locations the AMOVA $F_{ST}$ remained low at 0.0021 and was significant ($P = 0.015$). These results suggest that levels of genetic differentiation are higher in juveniles than in the adults within our study (Table 2). Pairwise comparisons of $F_{ST}$ among all sampling locations found that 15 of the 120 total comparisons were significantly different from one another after FDR correction (Table 3). The PCoA of the pairwise comparisons of $F_{ST}$ among juv
Table 3. Pairwise tests for population differentiation among each discrete juvenile and adult yellowtail snapper sampling location. Value represent $P$-values were calculated using log-likelihood G-statistic with 50K permutations. Values in bold are significant using the sequential goodness-of-fit correction for multiple tests.

<table>
<thead>
<tr>
<th></th>
<th>Caulker Adult</th>
<th>Glovers Adult</th>
<th>Moskitia Adult</th>
<th>Asanas Adult</th>
<th>Green Grass Adult</th>
<th>Asanas Adult</th>
<th>Green Grass Adult</th>
<th>Omoa Juvenile</th>
<th>Izopo Juvenile</th>
<th>Green Grass Juvenile</th>
<th>Cochinos Juvenile</th>
</tr>
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<tr>
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<td>--</td>
<td>0.390</td>
<td>0.845</td>
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<td>0.159</td>
<td>0.648</td>
<td>0.014</td>
<td>0.017</td>
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<td>0.714</td>
<td>0.134</td>
<td>0.797</td>
<td>0.300</td>
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<td>0.376</td>
<td>0.353</td>
<td>0.329</td>
<td>0.425</td>
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<td>0.359</td>
<td>0.904</td>
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<td>0.578</td>
<td>0.362</td>
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<td><strong>0.023</strong></td>
<td><strong>0.047</strong></td>
<td>0.368</td>
<td>0.719</td>
<td>0.063</td>
<td>0.229</td>
<td><strong>0.007</strong></td>
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<tr>
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<td><strong>0.023</strong></td>
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<td>0.118</td>
<td>0.098</td>
<td><strong>0.022</strong></td>
<td>0.083</td>
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<td><strong>0.011</strong></td>
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<td>0.300</td>
<td>0.359</td>
<td><strong>0.047</strong></td>
<td>0.118</td>
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<td><strong>0.047</strong></td>
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<td>0.808</td>
<td>0.240</td>
<td>0.718</td>
<td>0.561</td>
</tr>
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<td>0.063</td>
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<td>0.497</td>
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<td>0.578</td>
<td>0.229</td>
<td>0.053</td>
<td>0.501</td>
<td>0.718</td>
<td>0.950</td>
<td>0.982</td>
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<td><strong>0.492</strong></td>
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<td>Asanas Juvenile</td>
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<td>0.704</td>
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<td>0.119</td>
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<td>0.561</td>
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<td><strong>0.011</strong></td>
<td>0.176</td>
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<td>0.767</td>
<td>0.544</td>
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<tr>
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<td>0.071</td>
<td><strong>0.011</strong></td>
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<td>0.184</td>
<td>0.116</td>
<td>0.605</td>
<td>0.963</td>
<td>0.856</td>
</tr>
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</table>
and adult sites provided additional evidence that several of the juvenile sites were genetically differentiated from adult sites (Figure 5). Juveniles at Porvenir, Bells Caye, West Caye, Green Grass and Izopo were distinct from all other sites. The majority of adult sites clustered together near the origin of the x and y-axis suggesting that levels of genetic differentiation were lower among adult locations than for juvenile locations. The green grass adult site, however, was distinct from all other locations. All of the juvenile and adult sites that were distinct from the main cluster of sites contained low levels of rare alleles, which may be contributing to the higher levels of genetic differentiation observed at these locations (Figure S1). The interpolated map of mean pairwise $F_{ST}$ provided additional of low levels of genetic differentiation among the adult sites and high levels of patchy genetic differentiation among the juvenile sites (Figure 6).

4. Discussion

4.1 Levels of Connectivity and Self-Recruitment

Even though the sample sizes of this study are small our results suggest that levels of connectivity are high among adult and juvenile yellowtail snappers that were sampled from several locations in Honduras and Belize. Pairwise relatedness analyses of juveniles and adults collected from the same locations in the North Coast of Honduras were highly suggestive of self-recruitment. The vast majority of juveniles and adults clustered in the same multivariate space, which is the expected pattern for self-recruitment. The sibling analysis, which found significantly more half-siblings than expected in juvenile and adult locations in Belize and Honduras,
Figure 5. Principle coordinates analysis (PCoA) plots of pairwise levels of $F_{ST}$ among discrete juvenile and adult yellowtail snapper sampling locations.
Figure 6. An interpolated map of mean levels of genetic differentiation (mean pairwise $F_{ST}$) among discrete juvenile and adults yellowtail snapper sampling locations. Circles represent the sampling locations. The scale bar located on the right indicates the levels of genetic differentiation among sampling locations. Red colors = higher $F_{ST}$ values and blue colors = lower $F_{ST}$ values.

provides additional evidence of self-recruitment in the southern MBRS and Miskito Cayes. Even though our results are highly suggestive of self-recruitment we cannot confirm self-recruitment in the Miskito Cayes or in Belize since we had no juveniles from those regions to make direct comparisons.

The results of several biophysical modeling and genetics studies of corals, coral reef fish, and spiny lobster are in agreement with the results of our study, suggesting that levels of self-recruitment may be particularly high for coral reef species in the southern MBRS (Butler IV et al. 2011; Cowen et al. 2006; Kough et al. 2013). Our results suggesting that self-recruitment may occur among
populations that also exhibit extensive levels of geneflow are supported by recent genetics studies that have reported similar patterns in the coral reef fish species in the MBRS and Bahamas (Christie et al. 2010; Hogan et al. 2011). An extensive population genetics survey of five species of coral reef fish from 120 sites along a 250km transect of MBRS in Belize found parent-offspring dispersal distances ranged from only 7 to 42 km, despite high levels of geneflow (Puebla et al. 2012). Several genetics studies of bicolor damselfish, *Stegastes partitus*, suggest that self-recruitment and sweepstakes recruitment may be the predominant ecological drivers that shape patterns of larval dispersal this species. Long-term genetics studies that have used genetic techniques to evaluate spatial patterns of connectivity in *Stegastes partitus* over several years found that local levels of self-recruitment can vary significantly among years (Hogan et al. 2011). A three year long genetics analyses of *Stegastes partitus* connectivity patterns among 8 locations in the MBRS suggested that although self-recruitment was common among all the populations they examined, site specific levels of self-recruitment at the spatial scale of an individual reef varied considerably. Their estimates of self-recruitment ranged from 0 to 50% for individual reefs. These findings highlight the spatial and temporal variability of self-recruitment over small spatial scales. When the spatial scale was increased to cover the area of Turneffe Atoll (~50km long and 16km wide), 65% of larvae produced from sites at Turneffe Atoll were suggested to return to populations in Turneffe (Hogan et al. 2011). Despite such high-levels of local retention of larvae, these levels were insufficient to drive genetic differentiation among sites in the MBRS due to isolation.
4.2 Detection of Migrants

Even though the results of our kinship analysis suggested that the larval dispersal potential of yellowtail snapper may be limited in the southern MBRS, several genetically rare individuals were detected that could not have been generated from the genotypes of the existing populations that we sampled. These individuals may be migrants that have arrived from another population that has a substantially different genetic structure from the individuals that we sampled in our study. Our findings are in agreement with recent studies of coral reef fish and spiny lobsters that have identified a small proportion of individuals whose genotypes differ so substantially that they could not have originated from any of the sampled populations of their study (Elphie et al. 2012; Hogan et al. 2011). The results of our pairwise analysis of relatedness among yellowtail snappers suggested that several sites received migrants that were not well related to individuals from any of the locations that we sampled in the Southern MBRS and Miskito Cayes. While most of the juveniles and adults we sampled tended to cluster in the same region of multivariate space, the individuals we identified as likely migrants were scattered away from the main cluster of individuals in multivariate space along all positive and negative axes. These results suggest that unsampled populations of yellowtail snapper may exist that have a very different genetic structure than the populations we sampled in the southern MBRS and Miskito Cayes. However, it should be noted that our methodology would be unable to detect migrants arriving from distant populations that have very similar allele frequencies to the populations we sampled,
since it is likely that those individuals would cluster in the same multivariate space as the majority of individuals in our study and go undetected. A much larger scale population genetics study of yellowtail snapper in the Eastern Caribbean has identified barriers to connectivity among populations and suggested that genetically unique stocks may exist in Puerto Rico, the US Virgin Islands and Florida Keys (Saillant et al. 2012). Since our study is the first population genetics study of yellowtail snapper in the Western Caribbean more research will be required to identify locations with limited connectivity to the MBRS or genetically unique stocks. Rare dispersal events among sites that share limited demographic connectivity may explain the presence of the few unrelated individuals that we observed in our study (Hellberg 2009). Larger scale studies of yellowtail snapper that include genetic analyses of new larval recruits will be required to test this hypothesis.

4.3 Levels of Genetic Differentiation

The overall levels of genetic differentiation observed in our study were low and did not provide evidence of genetically unique stocks or barriers to connectivity in the southern MBRS and Miskito Cayes. The AMOVA analysis found no evidence of population differentiation among adult populations and low but significant levels of genetic differentiation between juvenile and adult populations. The lack of genetic differentiation among adult locations suggests that larval dispersal among sites in the southern MBRS and Miskito Cayes is sufficient is sufficient to have an homogenizing effect on population structure (Wright 1931).
The high levels of connectivity that we observed among juvenile and adult sites using tests of relatedness suggest that the levels of genetic differentiation that we observed among juvenile and adult populations may be caused by larval recruitment dynamics rather than genetic isolation due to lack of connectivity. These findings are supported by recent genetic investigations of sweepstakes recruitment in marine species. Sweepstakes recruitment is broadly defined as a recruitment event where only a small number of adults successfully contribute to the next generation. For example, Christie et al (2011) suggested that sweepstakes recruitment was responsible for the significant differences in $F_{ST}$ they observed among juvenile and adult bicolor damselfish despite finding high levels of relatedness between juveniles and adults. An alternative explanation is that the small sample sizes at the juvenile sites may lead to type II errors or accentuate the rare alleles of an occasional migrant. For example, the addition of a few unique individuals with rare alleles may disproportionally increase levels $F_{ST}$ when samples sizes are low and all individuals are pooled into a single population. Clearly more juvenile and adult samples as well as sampling of new larval recruits will be required to confirm weather or not the differences in $F_{ST}$ we observed were due to low sample sizes, sweepstakes recruitment, or self-recruitment.

4.4 Implications for Management

The high levels of connectivity between the Miskito Cayes and all other locations that we sampled in the MBRS suggest that the management of the Miskito Cayes yellowtail fishery should be integrated into management plans for yellowtail
snapper fishery throughout the MBRS management region. The high levels of connectivity provide further evidence of the importance of international cooperation for the sustainable management of coral reef fisheries (Kough et al. 2013). Our results also provided evidence that self-recruitment may occur in the MBRS, highlighting the importance of locally based management. Long-term genetics studies will be required to improve our understanding of the complex spatial and temporal patterns of connectivity among yellowtail snapper populations in the Caribbean. As the magnitude and scale of coral degradation increases studies of population connectivity among coral reef species are urgently needed for the sustainable management of reefs and to ensure fisheries resources for future generations (Mumby et al. 2010). The sustainable management of the yellowtail snapper fishery in the MBRS has the potential to play an important role in preventing “fishing down the food chain” and may ultimately provide an ecological buffer to alleviate fishing pressure on herbivorous species of parrotfish (Mumby et al. 2012).

Acknowledgements

We thank James Azueta and Isaias Majil at the Bermuda Fisheries Department for helping to collect samples in the Belize. NKT is supported by postgraduate fellowships from the Sustainable Consumption Institute and the Faculty of Life Sciences at the University of Manchester.
Literature Cited


Supplementary Information

Table S1. Departures of each yellowtail snapper microsatellite locus from Hardy Weinberg Equilibrium (HWE). The table includes the *P*-values for each combination of discrete juvenile and adult sampling location and microsatellite locus. Significant departures from HWE are indicated in **bold**, using the sequential goodness-of-fit correction. Loci that may contain null alleles suggested by analysis with MICROCHECKER are indicated by the symbol (*). Microsatellite loci highlighted in grey were removed from statistical analyses of genetic differentiation due to departures from HWE in the majority of sampling locations and an overabundance of potential null alleles.

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<th>LSY5</th>
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<td><strong>0.003</strong></td>
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<td>0.292</td>
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<td>0.888</td>
<td>0.432</td>
<td>0.510</td>
<td>0.217</td>
<td>0.528</td>
<td>0.646</td>
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<td>0.221</td>
<td><strong>0.015</strong></td>
<td>0.157</td>
<td><strong>0.002</strong></td>
<td><strong>0.000</strong>*</td>
<td>0.605</td>
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<td>0.888</td>
<td><strong>0.047</strong></td>
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<td>0.650</td>
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<td>0.907</td>
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<td>0.562</td>
<td>0.517</td>
<td><strong>0.028</strong></td>
<td>0.644</td>
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<td>0.181</td>
<td>0.668</td>
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<td><strong>0.000</strong>*</td>
<td>0.208</td>
<td>0.056</td>
<td>0.195</td>
<td><strong>0.000</strong>*</td>
<td><strong>0.002</strong></td>
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Chapter 11

Thesis Conclusion

In the series of papers presented in this thesis I combined population genetics data from microsatellite markers with data from biophysical modeling to explore associations among levels of connectivity, genetic population structure, and potential barriers to larval dispersal in two species of spiny lobster and yellowtail snapper in the Caribbean. Even though microsatellites are one of the most popular types of genetic markers for population genetics studies, there are limitations associated with microsatellites that need to be taken into consideration (Dakin & Avise 2004; Selkoe & Toonen 2006; Chapuis & Estoup 2006). For example, null alleles which commonly occur in microsatellites for a wide range of species can artificially inflate levels of genetic differentiation when using $F$-statistics (reviewed by Chapuis & Estoup 2006). Briefly, a null allele is an allele present in an individual, but is not amplified in the PCR due to a mutation in primer-binding site (Selkoe & Toonen 2006). Heterozygous individuals can be incorrectly genotyped as homozygotes when a mutation occurs at the primer-binding site for one allele but not in the other. In the more rare case of a mutation occurring at the primer-binding sites for both alleles, the PCR will fail to amplify any of the alleles. Thus, null alleles can artificially reduce the number of heterozygotes, lower levels of genetic diversity, and may lead to deviations in Hardy-Weinberg equilibrium (HWE) among populations (van Oosterhout et al. 2004). Since the effects of null alleles are similar to those caused by inbreeding, it is important to either 1) remove loci containing
null alleles prior to calculating $F$-statistics or 2) use statistical methods to correct for their potential bias (Chapuis & Estoup 2006). These measures will minimize the probability of significant levels of population differentiation occurring due to null alleles and not from true population differentiation. Therefore, all microsatellite loci in this thesis were tested for the presence of null alleles with the genetics software MICROCHECKER (van Oosterhout et al. 2004). Loci suggested to contain null alleles by MICROCHECKER were removed from $F_{ST}$ based statistical analyses, with the exception of the loci used for the Caribbean spotted lobster *Panulirus guttatus*. Since nearly all loci for *P. guttatus* contained null alleles, the statistical software FREENA was used to minimize the bias caused by null alleles (Chapuis & Estoup 2006). Multivariate statistical techniques were also used test for population differentiation in all of the species studied in this thesis. Multivariate population genetics models are not biased by null alleles, therefore, all microsatellite loci were included in these types analyses (reviewed by Jombart et al. 2009).

Despite the substantial differences among the life histories of each species (Nagelkerken & van der Velde 2004; Butler et al. 2006), we observed some similarities in connectivity patterns among all the species that were investigated in this thesis, even though the spatial scales covered in each chapter varied considerably. The results of the kinship and outlier analyses for both species of spiny lobster (Chapters 6, 8, and 9) and yellowtail snapper (Chapter 10) consistently found high levels of connectivity among distant populations separated by hundreds or in the case of spiny lobsters, thousands of kilometers. These results are not surprising given the long pelagic larval durations (PLDs) of all the species that were
investigated in this thesis (Cowen et al. 2006; Butler et al. 2011). Levels of genetic
differentiation ($F_{ST}$) for both species of spiny lobsters were low between
populations located in the Mesoamerican Barrier Reef (MBRS) and Bermuda
separated by > 2000 km, highlighting how the interaction between strong ocean
currents and long PLDs can facilitate high levels of connectivity over large spatial
scales (Chapters 6, 8, and 9). Whilst the spatial scale that was examined for
yellowtail snapper (Chapter 10) was much smaller and only included the southern
MBRS, we found low levels of genetic differentiation among spiny lobster and
yellowtail snapper populations in this region.

Despite the high levels of connectivity among distant populations of spiny
lobsters and yellowtail snappers, there was substantial variation in geneflow among
the populations of each species. Striking examples of this variation were observed in
the MBRS for both spiny lobsters and yellowtail snapper. In the spiny lobster,
Panulirus argus, pairwise levels of $F_{ST}$ were low yet significantly different between
the Sapodilla Cayes and Caye Caulker in Belize, which are separated by < 200 km.
Bayesian statistical analysis using the genetics software package STRUCTURE
(Pritchard et al. 2000) found clear evidence of population structure in P. argus
between Hol Chan and Glover’s Reef marine protected areas (MPAs) in Belize, also
separated by < 200 km (Chapter 5). However, this was the only instance where
population structure was observed using STRUCTURE. No evidence of population
structure was observed in spiny lobsters and yellowtail snappers when more
individuals, sampling locations and microsatellite loci where analyzed in STRUCTURE
(Chapters 6 – 10). These counter-intuitive findings may result from several factors
that may reduce the statistical power to detect population structure in studies using < 50 loci (reviewed by Ryman & Jorde 2001). These factors include the number of individuals among groups, the magnitude of genetic differentiation, allele frequency distributions among populations, and the number of loci used (Ryman et al. 2006; Kalinowski 2010). Thus, adding more loci and more samples may not always increase the statistical power to detect population structure and in some cases may even decrease statistical power (Toonen & Grosberg 2011).

After correction for null alleles, $F_{ST}$ based and multivariate statistical techniques provided additional evidence of population structure that was not observed using Bayesian statistical techniques. For instance, a principle coordinates analysis of pairwise levels of $F_{ST}$ among discrete juvenile and adult yellowtail snapper sampling locations found substantial variation among juveniles and adults collected from the same location (Porvenir) off the northern coast of Honduras. Since the levels of connectivity were high among the majority of spiny lobster and yellowtail snapper populations that we surveyed, it’s unlikely that genetic isolation due to a lack of connectivity explains the higher levels of genetic differentiation over small spatial scales (Hogan et al. 2011; Christie et al. 2013). These results, though perhaps counterintuitive, indicating that some adjacent sites (or in the case of yellowtail snapper individuals from the same site) exhibit higher levels of genetic differentiation than more distant sites, is in agreement with a growing body of population genetics research on species with extensive dispersal potential. Johnson and Black (1982) originally identified this phenomenon as “chaotic genetic patchiness”. The consensus among several studies of ‘chaotic genetic patchiness’ in
marine species suggests these types of spatial patterns are surprisingly common in species with widespread dispersal and most likely the result of temporal variation in the genetic composition of new recruits (Johnson & Black 1982; Planes & Lenfant 2002; Selkoe et al. 2006; Iacchei et al. 2013). The results from the large-scale population genetics study of the spiny lobster (Panulirus argus) among several advective and retentive oceanographic environments throughout the Caribbean suggest that the long-lived larvae of P. argus disperse among sites throughout their range frequently enough to homogenize the genetic population structure of this species, except for a few sites where self-recruitment is enhanced by persistent offshore gyres (Chapter 8). Recent population genetics studies that have combined analyses of kinship and $F_{ST}$ have uncovered potential drivers of chaotic genetic patchiness among populations of marine species that exhibit high levels of connectivity (Iacchei et al. 2013). A similar methodology was used in to help explain chaotic patterns of genetic differentiation among spiny lobster populations in the MBRS (Chapters 9). The kinship and multivariate spatial analyses of spiny lobster populations residing in marine protected areas (MPAs) in the MBRS found significantly more genetically determined migrants and outliers in northern MPAs compared to southern MPAs (Chapter 9). Our findings of increased levels of migrants and outliers within local populations in the northern MBRS are supported by biophysical modeling studies suggesting that northern regions of the MBRS are more dependent on larval recruitment from distant source populations located upstream of the Caribbean current than southern regions of the MBRS (Butler et al. 2011). Since we were only able to obtain samples of yellowtail snapper from the
southern MBRS (Chapter 10) additional genetic research will be required to confirm if this trend applies to other coral reef species.

The sibling analyses (Chapters 8, 9, and 10) found significantly more siblings than expected by chance in the majority of spiny lobster and yellowtail snapper populations than were examined in this thesis. Higher than expected number of siblings have also been reported in populations of other species of spiny lobster and reef fish (Selkoe et al. 2006; Christie et al. 2013; Iacchei et al. 2013). Higher than expected levels siblings may be explained by self-recruitment, a sweepstakes recruitment event, or an unknown mechanism that prevents larvae from mixing throughout their PLD (Selkoe et al. 2006). Biophysical modeling studies of spiny lobster larval and coral reef fish connectivity suggests self-recruitment may be common due to larval behavior coupled with local oceanographic characteristics (Cowen et al. 2006; Butler et al. 2011). Several population genetics studies of coral reef fish species in the MBRS, which have much shorter PLDs than spiny lobsters, have provided evidence of both self-recruitment and limited connectivity in the MBRS (Hogan et al. 2011; Puebla et al. 2012; Chittaro & Hogan 2012). Sweepstakes recruitment events may also explain higher than expected levels of siblings that were found in this thesis (Christie et al. 2010). There is growing evidence to suggest that self-recruitment and sweepstakes recruitment may be predominant ecologically processes that shape patterns of larval dispersal in many marine species (Cowen 2000; Christie et al. 2010; Hogan et al. 2011).

The findings of this thesis highlight the importance of international cooperation for the sustainable management of ecologically and commercially
important coral reef species in the Caribbean. In every paper of this thesis that contained sampling locations in > 1 Caribbean nation, connectivity analyses suggest that populations of spiny lobsters and yellowtail snappers easily spanned international borders. Despite the high dispersal potential for each species that was investigated in this thesis, substantial spatial and temporal variation in levels of geneflow was found among populations of spiny lobsters and yellowtail snapper in the Caribbean.Whilst the detection of genetically unique migrants and outliers helped to explain the variation of levels of genetic differentiation among spiny lobsters from MPAs in the MBRS, the effects of self-recruitment were not as clear. Whilst a few sites where self-recruitment is enhanced by persistent offshore gyres were indeed genetically differentiated among spiny lobsters from advective and retentive oceanographic regions, some sites with high levels of self-recruitment exhibited no evidence of genetic differentiation. These results suggest that connectivity among many spiny lobster populations in the Caribbean is sufficient to maintain high levels of geneflow, despite the potential for self-recruitment. Whilst we detected trends that were highly suggestive of self-recruitment in yellowtail snapper, these data didn’t help to explain the chaotic genetic patchiness that was observed in the northern coast of Honduras. However, the findings of this thesis only present a single snapshot in the complex spatiotemporal web of connectivity patterns of spiny lobsters and yellowtail snapper. Temporal replication and comparisons of larvae to adults will clearly be required to understand if the patterns we observed are stable or simply a shifting mosaic over time (Hellberg 2009). Long-term genetic studies will help improve our understanding of how population
structure can persist in marine species with extended PLDs despite the potential for homogeneity caused by long-distance migration. For instance, density-dependent processes that affect the survival of new recruits are important drivers of spatial patterns of genetic population structure in both marine and terrestrial species (reviewed by Waters et al. 2013). Whilst density-dependent factors such as predation on new recruits, habitat availability, and disease can significantly alter the demographics of spiny lobster and coral reef fish populations in the Caribbean (Behringer & Butler 2009; Hixon et al. 2012; Wormald et al. 2013), the role that density-dependent processes played in shaping the spatial patterns of genetic differentiation that were observed in this thesis remains uncertain.

A great deal of the uncertainty regarding the environmental and ecological mechanisms driving the low levels of population structure that were observed in the species studied in this thesis can be resolved by using genomic techniques (reviewed by Davey et al. 2011; Ellegren 2014). Whilst microsatellites are more cost effective for screening a relative small number of loci (i.e. 10 – 20), the additional statistical power provided by genomic techniques capable of screening 1000s of loci and eventually entire genomes are likely to provide the necessary resolution to answer many of the outstanding questions of this thesis (reviewed by Slate et al. 2010; Narum et al. 2013). Genomic studies of marine species have begun to reveal cryptic population subdivision and local adaptation that previously went undetected using microsatellite markers (reviewed by Allendorf et al. 2010). For example, genomics analyses of population structure in several commercially important fish species in Europe provided evidence of fine-scale population structure whilst previous
analyses with microsatellites suggested genetic homogeneity across populations (Nielsen et al. 2012; Milano et al. 2014). As the costs of genomics studies continues to decrease it is likely that molecular ecologists in the near future will shift away from microsatellites and use primarily genomic techniques (Narum et al. 2013). However, until the uncertainties regarding the ecological and physical drivers of genetic differentiation among coral reef species in the Caribbean can be resolved, the findings of this thesis suggest that MPA managers should plan for uncertainty, whilst providing the flexibility for refinement as genomics research provides additional clarity.

References


