Assessment of Genetic Connectivity between Sudan and Saudi Arabia for Commercially Important Fish Species

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ABSTRACT

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Patterns of genetic connectivity can help answer key questions about the evolutionary ecology of fishes. This knowledge is particularly useful when considering the management and conservation of species that are impacted by fisheries. Population connectivity in ocean habitats is heavily influenced by environmental and oceanographic factors. These factors can lead to strong genetic differences within populations, causing fragmentation into smaller subpopulations. The Red Sea exhibits pronounced oceanographic gradients in temperature, chlorophyll, and salinity, which have been assessed in various species' populations and which have been found to have potential impacts on gene flow. The Red Sea also features strong cyclonic and anticyclonic eddies that may facilitate, or possibly inhibit, the transport of larvae throughout the Red Sea, potentially influencing gene flow themselves. The ability of oceanographic factors like eddies to structure wild fisheries populations in this region has yet to be fully determined. To address this, the genetic composition of two of the most highly fished species, (*Plectropomus areolatus* and *Plectropomus pessuliferus marisrubri*), in the Red Sea were evaluated utilizing genetic markers (polymorphic microsatellite loci). Samples from three geographically separate regions along the Saudi Arabian Red Sea coastline, as well as from Sudan, were analyzed to address latitudinal and cross-sea connectivity. I was able

to determine that little genetic differentiation exists within *Plectropomus* species across all regions of the Red Sea, indicating high gene flow for these species throughout. These findings highlight the ability of currents and eddies to transport larvae along and across the Red Sea. The results from this study also indicate that a single population of *P. areolatus* and a single population of *P. pessuliferus marisrubri* occurs in the Red Sea. The high degree of genetic flow suggests that each species should be managed as individual units. This study presents a plausible avenue for buffering the effects of overfishing currently occurring in Saudi Arabia; Saudi Arabian fish subpopulations may be reseeded by the Sudanese subpopulations.

Keywords: Microsatellites, population genetics, *Plectropomus,* Panmixia

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LIST OF ABBREVIATIONS

BCL	Bioscience Core Labs
CRS	central Red Sea
CSA	central Saudi Arabia
DNA	deoxyribonucleic acid
F_{is}	inbreeding coefficient
F_{st}	fixation index
H_o	observed heterozygosity
H_e	expected heterozygosity
HWE	Hardy-Weinberg Equilibrium
IBD	isolation by distance
KAUST	King Abduallah University of Science and Technology
LD	linkage disequilibrium
PCR	polymerase chain reaction
PLD	pelagic larval duration
N_a	number of alleles
NSA	northern Saudi Arabia
SAR	Saudi Arabian Riyals
SSA	southern Saudi Arabia
SU	Sudan

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1. INTRODUCTION

1.1 Population genetics

A population is broadly defined as a group of conspecifics that are capable of reproducing and inhabiting the same geographic area (Hoelzel and Dover 1991). One of the major aims of population genetics is to describe the amount of population structure present within a species population (Holsinger and Weir 2009). Marine species populations are not always one single, open population across the species entire geographic range, as originally proposed (Caley et al. 1996; Hellenberg 2009). Often populations are structured or subdivided in some way (Hellenberg 2009).

The overall population, or "metapopulation," includes all individuals within the species under investigation over the entire study area. The metapopulation can be divided into smaller local or subpopulations, depending on the amount of genetic differentiation in the subpopulation relative to the total population (often measured using the metric F_{st}) (Futuyma 2006). The subdivision of the metapopulation into subpopulations occurs discontinuously across a geographic range, due to reduction of genetic flow between the subpopulations (Schunter et al. 2011). Continuous populations, with no obvious substructure creating subpopulations, can still have different gene frequencies at different areas if the whole metapopulation is not panmictic (Futuyma 2006). Population structure occurs when populations deviate from Hardy-Weinberg equilibrium (HWE), or panmixia with the occurrence of non-random mating.

1.2 Conserving genetic diversity

Understanding genetic connectivity is important for conservation management because of its ability to identify reductions in gene flow between subpopulations (Selkoe and Toonen 2006; Allendorf et al. 2008). Reductions in gene flow between subpopulations can lead to species extinction by decreasing overall genetic diversity in a metapopulation. A low genetic diversity can inhibit a population's ability to adapt to environmental change, the consequence of which can be detrimental to the population's longevity (Segelbacher et al. 2003; Frankham et al. 2010; Sgro et al. 2011). In order to maintain genetic diversity within a species population, it is ideal to have an effective population size of at least several thousand individuals, ensuring evolutionary potential is maintained and sufficiently resilient to changing conditions (Selkoe and Toonen 2006; Sgro et al. 2010). For example, Ayre and Hughes (2004) found that genetic isolation in the Great Barrier Reef caused decreased genetic diversity in certain corals. The authors speculated this would limit isolated coral colonies capacity to respond to environmental change. These corals would then be more vulnerable to warming and bleaching. Genetic connectivity requires relatively few successful migration events for interbreeding and the homogenization of the gene frequencies in the population to occur (Slatkin 1985). To maintain high genetic diversity and enhance resilience management, efforts should be focused on maintaining migration routes between geographically separated subpopulations, particularly when managing low population densities.

1.3 Seascape genetics

The majority of species in marine environments have a bipartite life history (Treml et al. 2008). Migration occurs mostly during their planktonic stage (Treml et al. 2008). This is when larvae, depending on the species can spend anywhere from days to months in the water column before settling, eventually remaining relatively sessile as adults (Treml et al. 2008). Therefore, in seascape genetics, the exchange of individuals between geographically separate subpopulations primarily occurs at the larval stage (Cowen et al. 2006; Cowen & Sponaugle 2009). The degree of larval dispersal is critical for understanding population dynamics but can be difficult to measure. Parentage analysis is a direct measure of larval connectivity, where by larval recruits are matched to their parents by their genetic signature (e.g., Nanninga et al. 2015). The distance between where parent DNA is sampled, compared to where the offspring's DNA is sampled, is equivalent to the dispersal distance for species with relatively sessile adult phases (Selkoe and Toonen 2006). Using parentage analysis to determine larval dispersal in a metapopulation requires exhaustive sampling, making it costly and time consuming (e.g., Almany et al. 2017). Recently, Pinsky et al. (2017) showed that using population genetics to examine changes in allele frequencies based on isolation by distance (IBD) patterns can provide an ecologically relevant estimate of dispersal equivalent to estimates derived from parentage analysis. This indicates population genetics is a more practical way to measure larval dispersal because it requires significantly fewer samples that can be collected over a longer period of time.

Isolation by distance (IBD) (Wright 1943) is a concept that states, the more geographically separated the populations are, the more genetically differentiated they

become. In the marine environment, IBD correlations are typically weak, as there are often other factors besides geographic distance influencing gene exchange (Giles et al. 2015). For example, there are a wide variety of oceanographic factors that influence the transport of larval, including currents, primary productivity, temperature, salinity, and turbidity, among others. These environmental influences can either facilitate or impede dispersal, settlement, and reproduction of organisms in marine environments, in some cases acting as an environmental barrier to gene flow (Vandermeer & Goldberg 2003; Frankham et al. 2010; Allendorf et al. 2013). Environmental and geographical barriers have the potential to create genetically isolated populations by hindering gene flow through restricted movement and breeding, ultimately changing the structure of the population (Vandermeer & Goldberg 2003; Frankham et al. 2010; Allendorf et al. 2013). Therefore, when assessing species' genetic connectivity, oceanographic factors and geographic distances between subpopulations must be considered.

1.4 Red Sea oceanographic factors

At its widest point, the Red Sea basin is only 355 km across. It has an average depth of 490 mm and a maximum depth of approximately 2900 m (Raitos et al. 2013; Zhan et al. 2014; Rasul et al. 2015). The Red Sea is one of the most saline and warmest environments to host coral reefs (Zhan 2014); reefs span almost the entire Red Sea coastal zone (>5,000 km) (Raitsos et al. 2017). This body of water is an ideal place to study population connectivity due to the interesting oceanographic features created by the seasonal influx of colder, high nutrient water from the Gulf of Aden into the southern

region of the Red Sea via the strait of Bab-el-Mandeb (Quadfasal and Braunder 1993, Raitos et al. 2013, Zarokanellos et al. 2017). This water influx creates a latitudinal gradient in temperature, nutrients, cholorphyll a, and salinity (Raitos et al. 2013). Each of these environmental variables has the potential to structure populations. The water in the southern region of the Red Sea, below 17˚N latitude, is greatly affected by this seasonal influx of Indian Ocean water (Raitos et al. 2013). This water from the Gulf of Aden mixes with older, Red Sea waters, creating a more turbid, less saline, and highly productive environment (Raitos et al. 2013). Vast and relatively sudden environmental differences such as these have the ability to create recruitment failures or pulses (Frisch et al. 2016) that can alter population structure within the Red Sea.

The inflow of water through Bab-el-Mandeb, driven by evaporation, also causes stratification in the Red Sea. This, along with the thermohaline flux, drives eddy formations by causing a seasonal, buoyant flux and wind forcing, which can alter the location, intensity, and structure of an eddy (Chen et al. 2014). Mesoscale eddies have a significant biochemical impact in the functioning of the oceanic ecosystem (Zarokanellos et al. 2017). McGillicuddy (2016) showed that mesoscale eddies affect plankton patchiness, as well as the plankton community, having an immediate impact on the higher trophic levels which consume plankton and planktivores. In the Red Sea, mesoscale eddies also play an important role in transporting salt, heat, chemical and constituents (Chen et al. 2014). Zarokanellos et al. (2017) pointed out that anticyclonic eddies in the central Red Sea can redirect, or even block, the northward flow of the eastern boundary current. Currents have been shown to act as barriers to connectivity in marine organisms by influencing the direction of fish and larvae movement, in some cases trapping them in

a certain region (James et al. 2002). A current may also connect geographically separated subpopulations by enhancing transport.

1.5 Review of Red Sea connectivity

There have been reports of many different species having a genetically distinct Red Sea population, compared to the greater Indo-Pacific (*Acanthaster planci*: Benzie 1992; *Scylla serrata*: Fratini & Vannini 2002; *Neoniphon sammara, Chaetodon auriga, Cephalopholis argus, Acanthurus nigrofuscus*, and *Pygoplites diacanthus:* DiBattista et al. 2013). The distinct species populations found in the Red Sea can be explained by low global sea levels during the Pleiostocene period (2.6-.01 MYA), restricting gene flow between the Red Sea and the Indian Ocean. This is due to the historic lack of water movement through the strait of Bab-el-Mandeb, the only natural opening to the Red Sea (DiBatista et al. 2013). Previous studies within the Red Sea have focused on either non-commercial species or species popular in the aquarium trade, showing a general trend of genetic homogeneity across coral reef taxa (fishes, corals, and sponges) when examined across potential environmental and geographical barriers (Nanninga et al. 2014; Giles et al. 2015; Monroe 2015; Robitzch et al. 2015). Studies on coral species (*Pocillopora verrucosa* and *Stylophora pistillata*) have found no genetic breaks throughout their sampling regions (Monroe 2015; Robitzch et al. 2015). Other studies across the Red Sea, using only mitochondrial markers, have found continuous populations absent of genetic breaks across eight different reef fish species (DiBattista et al. 2013).

Nanninga et al. (2015) is currently the only study conducted in the Red Sea that directly measures larval dispersal using parentage analysis. In this study. Nanninga et al. (2015) found almost no self-recruitment (1.4% in 2012 and 0% in 2013) in Red Sea anemonefish on an isolated reef. Similar studies, conducted in Kimbe Bay, Papua New Guinea, focusing on multiple reef fish taxa have found high self-recruitment (40-64%) and low dispersal ranges on the scale of meters to several kilometers (Almany et al. 2007; Planes et al. 2009; Berumen et al. 2013). Nanninga and Berumen (2014) speculated the unique low self-recruitment found in the Red Sea may be due to an unfavorable habitat, if the propensity to disperse is a trait passed from parent to offspring. There is currently no evidence to support this theory. I speculate that the differences in dispersal found in Nanninga et al. (2015), compared to other reef fish dispersal studies done primarily in Papua New Guinea, may be due to strong oceanographic currents in the Red Sea, sweeping the larvae off their natural reef and dispersing them great distances. The lack of genetic structure found in most Red Sea species populations studies, is consistent with my theory of increased dispersal in this region due to strong oceanographic currents.

Only a limited number of publications have reported strong genetic breaks occurring in the southern Red Sea, where there is a pronounced, somewhat abrupt, change in temperature, salinity, turbidity, and chlorophyll concentrations. Froukh and Kochzuis (2007) first found the northern and southern Red Sea to have genetically distinct subpopulations for endemic cleaner wrasses (*Larabicus quadrilineatus).* Low fixation index values reported in this study suggest long range dispersal, but the IBD model shows dispersal distances of only 0.44-5.1 km for this species. Nanninga et al. (2014) and Saenz- Agudelo et al. (2015) also reported a distinct subpopulation in the southern Red

Sea within the endemic Red Sea anemonefish (*Amphiprion bicinctus)* metapopulation. The presence of a genetic barrier at 19°N latitude was attributed to the strong oligotrophic-euphotic zone (16°N-19°N) in southern region of the Red Sea, which prevented gene flow in *A. bicinctus*. Giles et al. (2015) found similar strong genetic differences at 16°N latitude, creating a separate southern Red Sea population in the *Stylissa carteri* sponge metapopulation. The differences in location of these genetic breaks are likely due to differences in the biology of the two species (e.g. wider environmental tolerance active swimming capability). Giles et al. (2015) is one of the only authors to examine cross-sea connectivity, finding a moderate amount of genetic differentiation between the Sudanese and Saudi Arabian coast of the Red Sea. The authors attributed this separation to the presence of a quasi-permanent, anticyclonic eddy in the central Red Sea near Yanbu, which transported the larvae across the Red Sea in pulses associated with the short lifespan of eddies (Giles et al. 2015). Eddies in the CRS appear to contribute to larval transport across the Red Sea (Raitsos et al., 2017). However, more empirical evidence is needed to examine to what degree larvae are able to migrate across the Red Sea, especially for commercially important species.

1.6 Overfishing in the central Red Sea

In the Saudi Arabian waters of the Red Sea, unregulated fishing is believed to have occurred since the early 1990's (Jin et al. 2012; Kattan et al. 2017). Although Sudan and Saudi Arabia are separated by only approximately 300 kilometers, the biomass of top predators in Sudanese waters is recorded as being three times higher than in Saudi

Arabian waters (Kattan et al. 2017). The overall fish biomass is 20% higher in Sudan, with overall fish abundance 62% higher than in Saudi Arabia. Kattan et al. (2017) attributed these results to overfishing in Saudi Arabia, potentially threatening genetic diversity by greatly reducing effective population sizes (Mora 2015). Fishery management can utilize population genetics to characterize units of fishing stocks by their genetic structure, as multiple genetically distinct populations or one panmictic population (Laikre et al. 2005). It has been postulated that Sudanese and Saudi Arabian populations may be genetically distinct in some species, potentially due to the presence of a deep sea trench (~2000m) in the middle of the Red Sea (DiBattista et al. 2015; Giles et al. 2015). This deep sea trench is an unsuitable habitat for shallow benthic species, possibly inhibiting larval exchange in species with limited dispersal potential that are unable to cross it (DiBattista et al. 2015). Alternatively, if the Saudi Arabian and Sudanese regions have sufficient migration of larvae, Sudan could act to buffer to the effects of overfishing on Saudi Arabian reefs by constantly reseeding the population.

1.7 Study species

Past studies examining genetic connectivity in the Red Sea have rarely included commercially important species in their assessment. To assess larval migration between the Sudanese and Saudi Arabian regions, I decided to use only important commercial species in this study. *Plectropomus areolatus* and *Plectropomus pessuliferus marisrubri* are the only two species of *Plectropomus* groupers known to inhabit the Red Sea (Ma et al. 2016). These two *Plectropomus* species are among the most highly sought after fish

species in markets around the Red Sea (DesRoiser 2011). Despite their socio-economic importance, *Plectropomus areolatus* and *Plectropomus pessuliferus marisrubri* are greatly understudied in this region (DesRoiser 2011). Until recently *P. p. marisrubri* was considered a Red Sea endemic subspecies of *P. pessuliferus*, along with *P. pessuliferus*, the Indian Ocean subspecies (Randall and Hoese 1986). Morphologically these species show little differentiation, apart from the larger total length of *P.p. marisrubri* (120 cm) (DesRosiers 2011) compared to *P. p. pessuliferus* (63 cm) (Heupel et al. 2010). Genetically these taxa appear to come from two deeply diverged lineages, supporting reclassification of these subspecies into two separate distinct species (Ma 2016). In any case, very little is known about *P. p. marisrubri.*

The scientific knowledge currently available for these two *Plectropomus* species are mostly from studies done in other regions of the Indo-Pacific. In general, Epinephelinae are known inhabit shallow reefs (3-30m), but have been observed as deep as 150m (Ferreira et al. 2008; Theirry et al. 2008). *Plectropomus* groupers typically have limited home ranges (.004-.12 km^2 for *P. areolatus* in Micronesia) (Hutchinson and Rhodes 2010), meaning the exchange of individuals between geographically separated areas, which genetically structures the population, is primarily driven by the larval stage. The larval stage for *Plectropomus* groupers lasts anywhere from 25-35 days (Frisch et al. 2016). During this time, larvae are dispersed into the water column where they can be transported by oceanographic currents. Once *Plectropomus* larvae settle on a reef, the individuals typically remain within one kilometer of that spot their whole life (Green et al. 2015). The only time Epinephelinae species undergo adult migrations is when they participate in seasonal spawning aggregations. During this time, *P. areolatus* tagged in

Papua New Guinea were observed traveling up to 23 km to take part in large seasonal spawning aggregations (Hutchinson and Rhodes 2010). A close relative to *P. p. marisrubri, P. laevis*, is known to travel much shorter distances, and spawns in smaller aggregations than *P. areolatus* from the greater Indo-Pacific (Frisch et al. 2016). We expect that *P. p. marisrubri* may do the same as *P. laevis*. The temporal and spatial predictability of *Plectropomus* spawning aggregations, coupled with their high market value of approximately 55 Saudi Arabian riyals (SAR) per kilogram (compared to 35SAR/kg for snapper and 15SAR/kg for tuna) make these species highly vulnerable to overfishing (Rhodes and Tupper 2008; DeRosiers 2011; Jin et al. 2012). Overfishing of spawning aggregations can create population-level changes by the removal of larger, more fecund individuals, negatively impacting reproductive output and skewing sex ratios (Heupel et al. 2010). Ultimately, the impact overfishing has on reproductive output can shift the genetic structure within the population, decreasing genetic diversity (Heupel et al. 2010).

The two Red Sea *Plectropomus* species included in this study are among the most commonly consumed species in the region (Burger et al. 2014), and are also common in the aquarium trade (Ferreira et al. 2008; Theirry et al. 2008). *Plectropomus pessuliferus marisrubri* are much larger, reaching a total length of 120 cm, while the *P. areolatus* size is around 56 cm. *Plectropomus pessuliferus marisrubri* is a much longer-lived species, living upwards of over ten years longer than *P. areolatus* (DeRosiers 2011). Both species are also protogynous hermaphroditic, aggregate spawners that become sexually mature around 2-3 years of age (DeRosiers 2011).

Additionally, two other commercially important snappers were included in the study. *Lutjanus kasmira* and *L. ehrenbergii* are widespread throughout the Indo-Pacific and are both commonly found in large schools throughout the Red Sea region (McMahon et al. 2012). These species both spawn throughout the year, becoming sexually mature after two years of age, around 20 cm length (Allen 1985). Both *L. kasmira and L. ehrenbergii* can reach a maximum length of about 35 cm and have a life span of approximately 10 years (Allen 1985). *Lutjanus* snappers typically show long range dispersal, with pelagic larval durations (PLDs) of 20-44 days (Zapata and Herrón, 2002). *Lutjanus ehrenbergii* has been recorded to make ontogenetic migrations of up to 30 km in the Red Sea (McMahon et al. 2012). *Lutjanus kasmira* ontogeny has yet to be assessed in the Red Sea. However, in French Polynesia ontogenetic shifts as long as a few hundred meters have been recorded for this species (Vignon 2012). *Lutjanus kasmira* can occupy much deeper depths than *L. ehrenbergii*; *L. kasmira* typically occupies depths of 1-40m, but can be found as deep as 265m, while *L. ehrenbergii* is known to occupy depth range of 5-20m (Russell et al. 2016). Though these species are common in markets and aquarium trade, especially in the Arabian Gulf, they are categorized as "least concern" on the IUCN Red List, due to their relatively low market value (~35 SAR/Kg) and prevalence outside the Arabian Gulf (Russell et al. 2016).

1.7 Aim of study

This study aims to examine the population structure of fishery species: *Plectropomus areolatus, Plectropomus pessuliferus marisrubri, Lutjanus ehrenbergii,* and *Lutjanus*

kasmira latitudinally along the Saudi Red Sea coastline and across-basin to Sudan, utilizing microsatellites. Microsatellites, or tandem nucleotide repeats, are a useful tool for determining genetic population structure and relatedness of organisms with complex reproductive strategies (Selkoe and Toonen 2006). Microsatellites were chosen over other potential markers due to their low cost and high mutation rates, which allow the markers to depict more recent genetic events compared to mitochondrial markers (Selkoe and Toonen 2006). Furthermore, no published studies have examined the dispersal patterns of fishery species in this region with microsatellites; only one previous study examined transport of larva across the Red Sea using microsatellites (Giles et al. 2015). Ultimately, microsatellites were used in this study to assess population structure and to infer broad-scale patterns. Results were then compared to previous genetic connectivity research done in this region as well as the physical oceanographic environment known for the central Red Sea region.

For connectivity across the longitudinal gradients in the Red Sea, it was hypothesized that the *Plectropomus* species samples from northern (NSA) and central (CSA) Saudi Arabia would be genetically similar, with a distinct genetically separate southern (SSA) subpopulation. This hypothesis is based on previous studies of Red Sea connectivity, and the vulnerability of these species to overfishing. No population differentiation between sites were expected for the *Lutjanus* species, based on their abundance throughout the region, and their high dispersal potential inferred from their longer PLDs. It was hypothesized that cross-sea connectivity would be greater in the *Lutjanus* species than in the *Plectropomus* species, due to the longer PLDs present in *Lutjanus* species that allows more time to disperse farther distances. The results from this thesis will provide valuable

information for the conservation of these species, as well as yield valuable knowledge on the connectivity patterns that occur within the Red Sea.

2. METHODS

2.1 Sample collection and DNA extraction

A total of 463 samples were used in this study (118 *Plectropomous pessuliferus marisrubri,* 137 *Plectropomus areolatus,* 104 *Lutjanus ehrenbergii*, and 104 *Lutjanus kasmira*). Collected from seven sites, five along 1000km of the Saudi Arabian coastline, and two areas off Sudan's 500km coastline. These samples were pooled into four locations: Sudan (SU), northern (NSA), central (CSA), and southern Saudi Arabia (SSA) (Figure 1, Table 1), based on the existing regional management framework (Department of Marine Fisheries 2008). All of the samples from Sudan were collected using hook and line between October 2015 and December 2016. The samples from Saudi Arabia were collected by spearfishing between 2 meters and 30 meters, or through purchase of individuals from fishermen and fish markets between June 2010 to February 2014. Fin clips were preserved in 96% ethanol and stored at -20℃. DNA was extracted from fin tissue using Qiagen DNeasy kits in accordance with the manufacturer's protocol (Qiagen Inc.). Approximately $3mm^2$ pieces of fin tissue were cut from fish samples and lysed in a hot water bath at 56°C for 4 hours. Samples were eluted twice in the last step of the manufactures protocol with 100µl of Qiagen AE Buffer (Qiagen Inc.). DNA concentrations were measured using a NanoDrop 2000 (ThermoScientific).

Figure 1. Map of collection areas (modified from DesRosier 2011). Lines represent population regions for this study designed according to Saudi Arabian Department of Marine Fisheries regional management divisions.

	Local	Coordinates	N(P.a.)	N(P.p.m.)
NSA	Umm Lujj	25° 01'N, 37° 16'E	41	0
NSA	Yanbu	24° 04'N, 38° 19'E	4	33
CSA	Thuwal	22° 05'N, 39° 12' E	26	30
CSA	Al Qunfidhah	19° 08'N, 41° 08' E	8	2
SSA	Jizan	16° 52'N, 42° 57' E	31	31
SU	Kebir Island	18° 91'N, 30° 28' E	19	9
SU	Dungunab	21° 19'N, 37° 19'E	20	13

Table 1. List of number (N) of each species (P.a.= *Plectropomus areolatus*, P.p.m.=*Plectropomus pessuliferus marisrubri*) in each of the 4 pooled sampling regions (NSA=Northern Saudi Arabia, CSA= Central Saudi Arabia, SSA= Southern Saudi Arabia, SU= Sudan) as well as each specific local site, along with coordinates.

2.2 Microsatellite DNA analysis

2.2.1 *Lutjanus*

Lutjanus samples were tested for amplification with primers developed for a third *Lutjanus* species, *L. carponatus,* developed by Harrison et al. (2014). Primers were tested in simplex using two samples (DNA concentrations 60.3 mg/ml and 59.8 mg/ml), with an amplification reaction of 5 µl of Qiagen Master Mix, 3 µl of distilled water, 1 µl of primer, and 1 µl of DNA. The following polymerase chain reaction (PCR) conditions were run on Eppendorf thermocylers:15 min initial denaturation at 95 °C, 5 cycles of 30 s at 95 °C, 90 s at 62 °C, and 60 s at 72 °C, then 5 cycles of 30 s at 95 °C, 90 s at 60 °C, and 60 s at 72 °C, then 20 cycles of 30 s at 95 °C, 90 s at 58 °C, and 60 s at 72 °C, followed by 30 min at 60 °C. Products were screened using a Qiaxcel DNA Screening Kit 2400 (Qiagen Inc.). Of the 21 microsatellite primers tested, 9 markers amplified for the *L. ehrenbergii* samples, with 11 markers amplified for the *L. kasmira* samples. Multiplexes designed by Harrison et al. (2014), excluding the primers that showed no amplification, were run with 6 samples for each species, with DNA concentrations ranging between 50-65 mg/ml. Reaction components for the multiplexes otherwise remained the same, containing 5 µl of Qiagen Master Mix, 3 µl of distilled water, 1 µl of a mixture of multiple primers, and 1 µl of DNA.

The resulting product was submitted to the Bioscience Core Lab (BCL) at King Abdullah University of Science and Technology (KAUST) for fragment analysis using an ABI 3730XL genetic analyzer (Applied Biosystems). Results were examined by uploading ABI files to Geneious 8.1.7 software (Kearse et al. 2012). Primer concentrations were step-wise adjusted based on average peak height to ensure equal amplification for each

primer. 96 samples for each species were run under the same conditions with the new primer concentrations. Products were again submitted to the BCL to determine fragment length. These results were also input into Geneious 8.1.7 (Kearse et al. 2012). Less than 70% amplification occurred in the 192 samples submitted for each locus, even after resubmission. For this reason, all *Lutjanus* samples were removed from analysis.

2.2.2 *Plectropomus*

Plectropomus areolatus and *P. p. marisrubri* samples were amplified using 23 microsatellite primer pairs, developed for *P. areolatus* by Almany et al. (2013). Each forward primer was fluorescently labeled with either PET, VIC, NED, or 6-FAM dyes, and separated into 7 different multiplexes for PCR amplification (Table 2). Each amplification reaction of 10 µl consisted of 5 µl of Qiagen Master Mix, 3 µl of distilled water, 1 µl of primer premix (see Table 2 reaction concentrations), and 1 µl of DNA. The reactions were amplified using the following PCR reaction cycle: a denaturation step of 95 °C for 15 min, followed by 35 cycles at 94 °C for 30 s, annealing at a locus-specific temperature (57, 60, or 63 °C) for 90 s, and an extension at 72 °C for 60 s, with a final extension set at 60 °C for 30 min. Resulting products were diluted (1:15) using Milli-Q water (EMD Millipore Corporation) before being submitted to the BCL. Amplified PCR products were screened using an ABI 3730XL genetic analyzer to determine fragment length (Applied Biosystems).

2.3 Genetic analysis

ABI files received from the BCL were input into Geneious 8.1.7 software (Kearse et al. 2012), where alleles were manually sized and scored by comparison to the internal size standard ladder GeneScan-500LIZ (Applied Biosystems Inc.). Files containing the number of base pairs in each allele, for each individual, were exported into Microsoft Excel™. Five *P. areolatus* and four *P. p. marisrubri* samples were removed from the data set for having less than 66% amplification across all alleles. GenAlex v 6.5 Excel add in (Peakall and Smouse 2012) was used to determine number of alleles (N_a), observed heterozygosity (H_o), and expected heterozygosity (*He*) for each population (Sudan, and North, Central, and South Saudi). In Genepop (Raymond and Rousset 1995; Rousset 2008), the presence of linkage disequilibrium (LD) and deviations from Hardy-Weinberg (HWE) was determined and the inbreeding coefficient (F_{is}) was calculated.

2.4 Summary statistics

Analysis of molecular variance (AMOVA) was performed in GenAlEx (Peakhall and Smouse 2012) to determine the presence of genetic differentiation for the total metapopulation (F_{st}), individuals to the subpopulations (F_{is}), and individuals to the total population (F_{it}). AMOVA was performed for each species using 999 permutations to determine population-wide fixation indices. Additionally, the pairwise fixation index (F_{st}) was calculated in GenAlEx (Peakhall and Smouse 2012) to determine and explain the degree of genetic differentiation between the geographically separated sampling regions. F_{st} (Wright 1965) measures the reduction in heterozygosity due to population structure.

F_{st} is the most commonly used metric for describing population differentiation, enabling easy comparisons to other studies (Meirmans and Hedrick 2011). To correct for possible type I errors, false discovery rate, a method of estimating the proportion of wrongly rejected null hypotheses (Benjamini and Hochberg 1995), was employed to confirm significant F_{st} values.

PDG spider 2.0 (Lischer and Excoffier 2012) was then utilized to convert the GenAlex file into a STRUCTURE format. STRUCTURE 2.2.3 (Pritchard et. al 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) modeled the population structure of both study species using Bayesian multi-locus clustering to obtain a visual estimate of population differentiation, while also indicating the possible origin for each individual. Preliminary runs for each species were made with 5,000 burn-in and 10,000 MCMC steps for K=1-5 with 3 iterations. The number of potential subpopulations (K) was determined in STRUCTURE HARVESTOR (Earl and VonHoldt 2012). Final simulations were run with 50,000 burn ins, 100,000 MCMC, K=1-5, with 5 iterations for each species. The results were imported into CLUMPAK (Kopelman et al. 2015) to create plots of the genetic structure for each species. Finally, self-assignment tests for *P. p. marisrubri* and *P. areolatus* were performed using GENECLASS 2 (Piry et al. 2004). First generation migrants were determined according to Paetkau et al. (2004). The significance of assignment tests were ascertained through comparisons of genotypes of cross-assigned individuals run with 10 000 simulations with area-specific allele frequencies (Rannala and Mountain 1997). Individual migrants were identified as beyond the $\alpha_{0.05}$ thresholds in the distribution tails. Analyses were run with type I error rate <0.05 for each of the

four potential subpopulations (NSA, CSA, SSA, and SU). Any individuals greater than the number of chance migrants were considered to be true migrants.

Name	Dye	Motif	Mix	[Rx]	T_a	*Plectropomus areolatus*					*Plectropomus pessuliferus marisrubi*				
						Size Range	*Na*	*Ho*	*He*	*Exclusion*	*Size Range*	*Na*	*Ho*	*He*	*Exclusion*
2.12	FAM	CA	1	0.01	57	130-136	5	0.438	0.521		130-140	2	0.211	0.248	
2.22	NED	CA	1	0.01	57	212-222	6	0.529	0.712		212-260	12	0.786	0.858	
7.90TG	PET	TG	1	0.01	57	177-191	8	0.648	0.755		177-228	10	0.828	0.848	
PaA1	NED	GT	2	0.011	60	216-296	24	0.668	0.885		211-296	14	0.622	0.866	
PaD1	VIC	TCTA	2	0.011	60	244-312	11	0.584	0.935		244-337	12	-	-	Low amplification
5.45	FAM	TG/CG	3	0.01	60	147-173	10	0.467	0.526		145-173	3	0.272	0.306	
D076	FAM	TCTA	3	0.01	60	319-387	14	0.626	0.826		319-415	9	0.794	0.812	
PaD2	PET	TAGA	3	0.015	60	182-210	6	0.674	0.515		182-280	9	0.616	0.716	
PaA3	FAM	GT	4	0.01	63	212-246	16	0.562	0.818		210-246	18	-	-	Low amplification
Pa109	PET	CA	5	0.012	63	167-207	12	0.414	0.733	Linkage disequilibrium	167-255	13	0.391	0.884	
Pb102	NED	TTG	5	0.003	63	216-231	6	-	-	Linkage disequilibrium	-	-	-	-	No amplification
Pb114	VIC	TG/GA	5	0.003	63	169-183	8	0.612	0.618		155-193	11	0.816	0.798	
Pb111	FAM	TTG	5	0.007	63	227-275	11	0.414	0.733		227-300	9	0.509	0.812	
Pa117	PET	CA	6	0.007	63	206-214	4	0.656	0.801		193-235	6	0.336	0.545	
Pa08	FAM	CA	6	0.007	63	222-272	16	0.682	0.804		222-280	14	-	-	Low amplification
Pa110	VIC	TG/GT	6	0.01	63	212-244	16	0.257	0.861		215-252	16	-	-	Low amplification
Pa05	VIC	TG	6	0.003	63	155-157	2	0.309	0.718		155-212	7	0.512	0.790	
Pc111	FAM	TACA	7	0.013	57	162-222	10	-	-		162-222	11	-	-	
Pd11	NED	TATC	7	0.003	57	113-201	12	-	-		113-201	11	-	-	Low amplification
Pd113	PET	TC/CA	7	0.02	57	249-315	27	0.655	0.908		249-315	25	-	-	Low amplification
Pd112	VIC	GT	7	0.007	57	204-238	16	-	-		204-238	12	-	-	
Pb120	PET	CAA	7	0.005	57	167-194	6	0.672	0.714		167-191	7	-	-	Low amplification
Pa122	NED	GT	7		57	193-279	25	0.449	0.853		203-247	24	-	-	Low amplification

Table 2. Microsatellites utilized for this study including their dyes, repeat motifs, multiplex mix, reaction concentrations ([R_x]), annealing temperatures (T_a), size ranges of loci, number of alleles (N_a), observed (H_o), and expected heterozygosity (H_e), and reasons for exclusion from final analyses.

3. RESULTS

3.1 Genetic analysis

3.1.1 *Plectropomous pessuliferus marisrubri*

Of the 23 microsatellite primers developed for *P. areolatus* that I tested on *P. p. marisrubri,* only 14 were used in the final analyses (Table 2). On average only about 30% of microsatellite primers amplify in closely related species (Pemberton et al. 1995; Zane et al. 2002). In this study, amplification was found in over half (14/23) of the markers. Six of the fourteen markers significantly deviated from HWE, which could be caused by selection, Wahlund effects, or null alleles. Analyses carried out, both with and without these loci, were almost identical. Since the metrics used in later analyses perform better with a larger number of loci, (Schunter et al. 2011) these markers were kept in the study.

3.1.2 *Plectropomus areolatus*

Out of the 23 markers developed for *P. areolatus* by Almany et al. (2013), 19 were used in the final genetic structure and population differentiation analysis (Table 2). Due to less than 40% amplification across all samples, one marker was removed. No significant linkage was found in Almany et al. (2013), unlike in my study. Significant linkage disequilibrium (LD) was found between *Pb102* and *Pa109*, and these loci also significantly deviated from HW ($p > 0.001$), resulting in their removal from the study. LD was also found between *5.45, 7.90TG* and *D076,* as well as *7.90TG* and *PaA1*. Linkage disequilibrium was found to be significant only for the southern region, therefore markers *5.45, 7.90TG, D076* and *PaA1* were not removed from the data set. Two other markers significantly deviated from HWE, which was not found in the original study that

used and developed these primers for these same species (Almany et al. 2013). It was suspected that the deviations were caused by differences in sample size between the two studies. Almany et al. (2013) included 782 individuals in their study for parentage analysis, compared to this population genetics study which included only 137 samples to address the study question. The Almany et al. (2013) study was also conducted in Papua New Guinea, a large geographic distance away from the Red Sea. It is likely these two populations have vast genetic differences due to isolation by distance.

3.2 Summary statistics

3.2.1 *Plectropomus pessuliferus marisrubri*

For the AMOVA, F_{st} was not significant (p-value > 0.05), while the other indices had p-values < 0.010 (F_{is}=0.230 and F_{it}=0.233). Population differentiation was determined using Fisher's probability tests for all pairs of populations, on the online web version of Genepop (Raymond and Rousset 1995; Rousset 2008). Significant genetic differences were found between southern Saudi Arabia (SSA) and central Saudi Arabia (CSA), as well as SSA and northern Saudi Arabia (NSA).

Significant F_{st} values were found only between the central and southern Saudi regions (p-value < 0.05). The suggested interpretation for these indices are defined as followed; values ranging from 0-0.05 indicates little genetic differentiation, 0.05-0.15 moderate differentiation, 0.15-0.25 great differentiation qualifying a genetic break and separate populations, and any value over 0.25 indicating very great genetic differentiation between populations (Bird et al. 2011). F_{st} values in this study are extremely low (0.012-0.020)

(Table 3), indicating only slight genetic differentiation with no clear genetic break. Further investigation was made using Bayesian modeling in STRUCTURE (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) and the Evanno method in STRUCTURE HARVESTOR (Earl and VonHoldt 2012). After 5 runs using K=1-5 it was determined that K=3 for *P. p. marisrubri* along the Saudi Red Sea coast to Sudan (Figure 3 and 4). There appears to be three different genetic lineages in the sample set, but there seems to be mixing of these lineages between all the geographic regions sampled, with no genetic breaks. Finally, the assignment tests showed only 26% of P. *p. marisrubri* individuals were assigned to the population they were collected from. Indicating 74 % of individuals were more likely to have migrated from another subpopulation. Individuals seem to have migrated from all populations to each subpopulation, resulting in a high level of gene flow (Table 4). Interestingly, a much larger number of potential migrants came from Sudan compared to all the other subpopulations (Table 4).

F_{st}		NSA	CSA	SSA	SU
	NSA	-	0.890	**0.010**	0.570
	CSA	0.012	-	**0.020**	0.110
	SSA	0.012	0.015	-	0.320
	SU	0.017	0.020	0.018	-

Table 3. *Plectropomus pessuliferus marisrubi* pairwise fixation index (F_{st}) reported below the diagonal, p-values reported above the diagonal. Significant p-values are in bold.

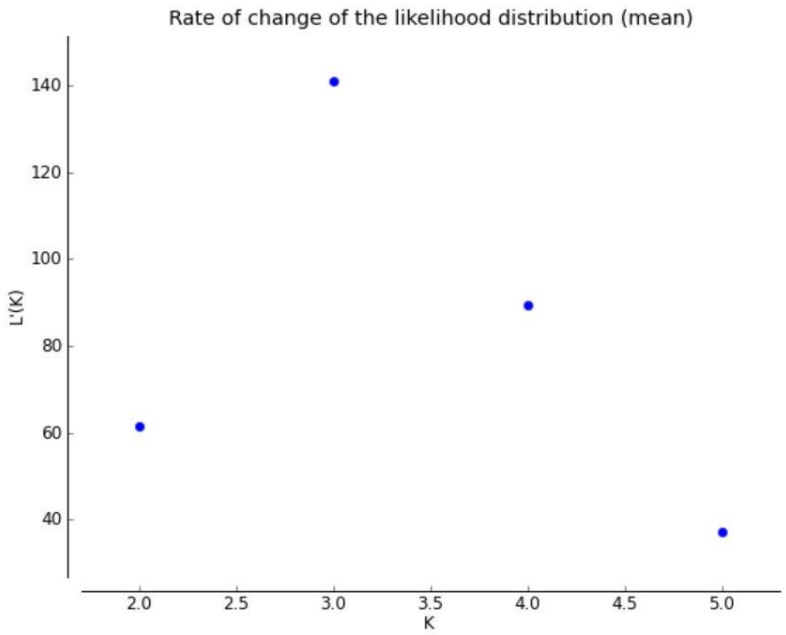

Figure 3. *Plectropomus pessuliferus marisrubri* K-plot. Highest rate of change (L'(K)) depicts the appropriate K-value (3) for subsequent structure plot.

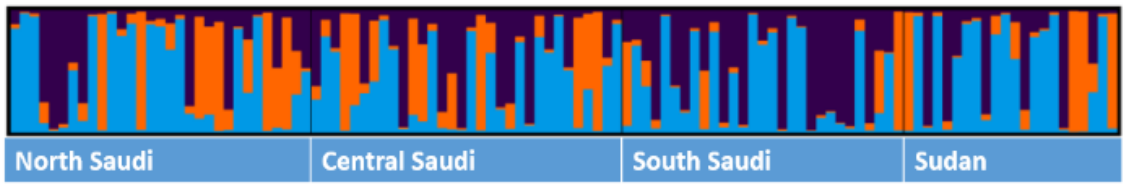

Figure 4. *Plectropomous pessuliferus marisrubri* STRUCTURE bar plot based on K=3. Plot indicates no clear genetic structuring patterns.

		Population Assigned to					% Assigned to Origin
		North Saudi	Central Saudi	South Saudi	Sudan	Unassigned	
Origin (Population Collected from)	North Saudi	4	6[1]	1[1]	20[1]	0	18
	Central Saudi	5	7	3[1]	17[1]	0	22
	South Saudi	4[1]	4	4	17[1]	0	19
	Sudan	6	3[1]	1	11	1	50
	Total	19[1]	20[2]	9[2]	65[3]	1	26

Table 4. Summary table of self-assignment test for *P. p. marisrubri*. The number of individuals assigned to each subpopulation (columns: origin, rows: assigned to) are shown. Superscript numbers represent how many individuals were first generation migrants. "Unassigned" represents the number of individuals that were not assigned to any population. Percent (%) assigned to origin is the percentages of individuals assigned to the region they were collected from.

3.2.2 *Plectropomus areolatus*

The same summary statistics were performed on both the data extracted from the *P. areolatus* samples, as the *P. p. marisrubri* samples. All of the fixation indices in the AMOVA were found to be significant with p-values <0.010, yet the highest resulting value was the F_{it} (0.345), followed by F_{is} (0.306) and F_{st} (0.056). Genetic differentiation assessed in Genepop (Raymond and Rousset 1995; Rousset 2008) was not found to be highly significant among any subpopulations using Fisher's method.

Pairwise genetic differentiation (F_{st}) index was found to be significant between Sudan and all the Saudi Arabian regions. Moderate genetic differentiation was found between CSA and Sudan (0.073), whereas low genetic differentiation was found between the SSA and SU (0.045), as well as NSA and SU (0.043) (Table 5). To confirm these findings,

Bayesian modeling of the total population was done in STRUCTURE (Pritchard et. al 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). Five iterations were made for K=1-5, and the most likely number of populations for *P. areolatus* for the entire study region was determined (K=2) in STRUCTURE HARVESTOR (Earl and VonHoldt 2012) (Figure 5 and 6). These results suggest a homogeneous population among the three Saudi regions, with moderate genetic separation occurring between Sudan and all other sites. Finally, the assignment tests showed only 38% of *P. areolatus* individuals were assigned to the population they were sampled from, indicating 62% of individuals were more likely to have migrated from another subpopulation. Individuals seem to have migrated from all populations to each subpopulation, again resulting in a high level of gene flow and potential mixing (Table 6).

F_{st}		NSA	CSA	SSA	SU
	NSA	-	0.051	0.340	**0.008**
	CSA	0.003	-	0.100	**0.009**
	SSA	0.025	0.026	-	**0.010**
	SU	0.043	0.073	0.045	-

Table 5. *Plectropomus areolatus* pairwise fixation index (F_{st}) reported below the diagonal, p-values reported above the diagonal. Significant p-values are in bold.

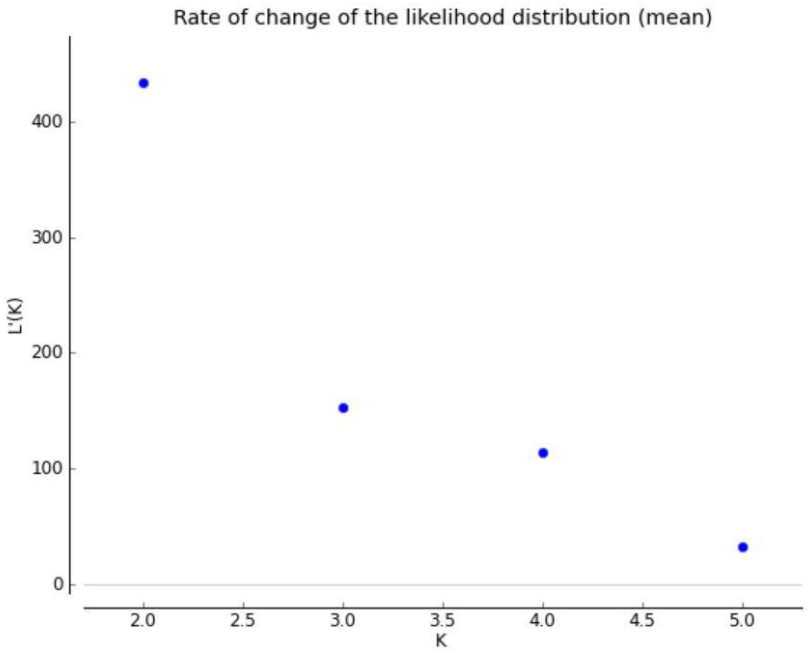

Figure 5. *Plectropomus areolatus* K-plot. Highest rate of change (L'(K)) depicts the appropriate K-value (2) for subsequent structure plot.

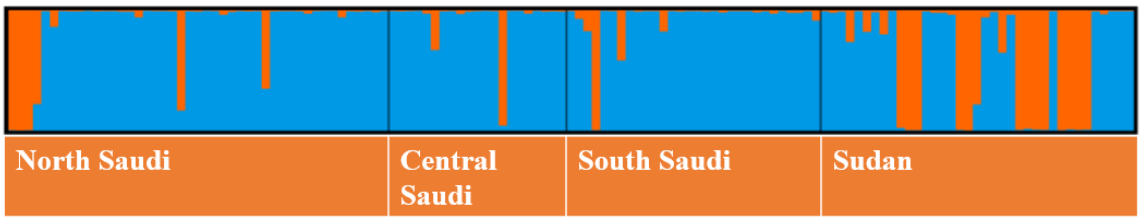

Figure 6. *Plectropomous areolatus* STRUCTURE bar plot based on K=2. Plot indicates possible moderate genetic difference between Sudan and Saudi regions.

Origin (Population Collected from)	Population Assigned to						% Assigned to Origin
		North Saudi	Central Saudi	South Saudi	Sudan	Unassigned	
	North Saudi	13	11^{2}	13^{1}	8^{3}	0	13
	Central Saudi	10^{2}	10	5^{1}	3^{2}	6	21
	South Saudi	8^{2}	5	10	8^{1}	0	19
	Sudan	8	3	6^{1}	22	0	28
	Total	39	29	34	41	6	38

Table 6. Summary table of self-assignment test for *P. areolatus*. The number of individuals assigned to each subpopulation (columns: origin, rows: assigned to) are shown. Superscript numbers represent how many individuals were first generation migrants. "Unassigned" represents the number of individuals that were not assigned to any population. Percent (%) assigned to origin is the percentages of individuals assigned to the region they were collected from.

4. DISCUSSION

In this study, I aimed to determine and compare the population structure within fishery species along longitudinal gradients along the Red Sea coastline, as well as across latitudes to Sudan. Slight genetic differentiation was found for *P. p. marisrubri* between southern Saudi Arabia (SSA) and central Saudi Arabia (CSA), as well as SSA and northern Saudi Arabia (NSA), but there was no support for differentiation between the Saudi regions and Sudan. In contrast, I found slight to moderate differentiation between Sudan and all the Saudi regions for *P. areolatus*, but no evidence of genetic differentiation among the Saudi regions. The lack of strong genetic differentiation found in this study coincides with most previous findings of little to no population isolation along most of the east coast of the Red Sea (>1500 km), with the exception of the southern region (Nanninga et al. 2014; Giles et al. 2015; Robitzch et al. 2015; Monroe 2015). My results indicate no strong isolation by distance or environmental factors structuring populations, and they suggest high gene flow throughout the Red Sea. These findings show promise that Sudan has the potential to reseed depleted grouper populations in Saudi Arabia. These results also suggest that larvae have the ability utilize eddies to transport themselves across the Red Sea.

4.1 *Plectropomus pessuliferus marisrubri*

Plectropomus pessuliferus marisrubri showed an overall genetically homogeneous population. P-values were significant (< 0.05) for the pairwise F_{st} index only between the SSA and the CSA and NSA regions (Table 2). However, these F_{st} values were low,

indicating only slight differentiation. In contrast to previous studies (Nanninga et al. 2014; Giles et. al 2015), there were no distinct genetic breaks found between these regions. Genetic mixing in the *P. p. marisrubri* metapopulation was apparent in the STRUCTURE plot (Figure 3) and evidenced by the random occurrence of all three potential subpopulations (K=3) within each geographic region. STRUCTURE HARVESTOR calculated K=3 for this species, indicating these three genetic populations seem to all be from the same genetic lineage. There is a possibility that these populations could be separate genotypes that evolved and mixed along the Red Sea. This could be due to strong longshore currents and eddies (Zarokanellos et al. 2017). The assignment test showed few (~25%) individuals occurring in their natal regions (Table 4). This may indicate a large amount of mixing occurring in the Red Sea, with little self-recruitment occurring in *P. p. marisrubri* populations, which has been observed in another Red Sea reef fish. Nanninga et al. (2015) found little to no self-recruitment of Red Sea anemonefish *(Amphiprion bicinctus).* Overall, the data suggests that within *P. p. marisrubri* there is significant gene flow throughout the Red Sea. This may be due to large-ranging larval dispersal and long pelagic larval dispersal times. .Larval life history traits and behavior could allow larvae to orient themselves and survive in the water column long enough to allow Red Sea currents to transport them great distances. The eastern boundary current is capable of carrying larvae up along the Saudi coastline, while mesoscale eddies can transport larvae across the Red Sea, contributing to the high mixing inferred by the lack of population structure observed (Chen et al. 2014; Raitsos et al. 2017).

4.2 *Plectropomus areolatus*

No strong genetic differences were found between regions for *P. areolatus*. Slight to moderate genetic structure was found between Sudan and Saudi Arabia (Table 5). The high number of probable migrants between each region calculated through the assignment test implies a high amount of gene flow for this species (Table 5). Interestingly, when compared to the results from the *P. p. marisrubri* assignment tests, there were significantly fewer probable migrants from Sudan for *P. areolatus*. This finding may support moderate differentiation between Sudan and Saudi Arabia, although the number of probable migrants coming out of each region for *P. areolatus* is comparable. Examining the STRUCTURE plot for *P. areolatus*, homogeneity is prevalent throughout the three Saudi regions (Figure 4). Furthermore, large genetic differentiation isn't apparent in the Sudanese region. Migratory events appear to occur across the Red Sea, between the Sudanese and Saudi regions. A first generation migrant between Sudan and southern Saudi was detected (Table 6). Assignment test also assigned many of the samples collected from Sudan to the Saudi regions, as well as some of the samples collected from the Saudi regions to Sudan (Table 6).

A similar amount of genetic differentiation between Sudan and Saudi regions (F_{st}=0.076) was found in a Red Sea sponge (*Stylissa carteri*). The authors attributed this to the presence of seasonal eddies in the central Red Sea (Giles et al. 2015). Moderate genetic structure could occur within a species with ample larval duration and dispersal abilities, depending on the timing of eddy formation in relation to spawning. The eddies in the central Red Sea have a lifetime of, on average, only 6 weeks, peaking in August and

February (Zhan et al. 2014). Across its species range, the season and time of month for *P. areolatus* spawning varies greatly (Rimmer et al. 2013) (see Table 7). Rimmer et al. (2013) studied spawning in *P. areolatus* within offshore sea cages in India, and found a variable six month spawning period ranging from June to December across the three year study. Spawning ceased during the hottest periods of the year when salinity was also at its peak (Rimmer et al. 2013). Many tropical fish taxa have shown inhibited reproduction above 30°C, and the author indicates *P. areolatus* has have a threshold temperature of 29-31°C. The threshold temperature of *P. areolatus* was found to be lower than other groupers, such as *Epinephelus fuscoguttatus* which had a threshold of 30-32°C (Rimmer et al. 2013). Red Sea *P. areolatus* are likely to have shifted, disrupted, or varying spawning timing across the Red Sea due to temperature, salinity extremes, and the environmental heterogeneity present. If *P. areolatus* spawning rarely occurs at the same time as the formation of eddies, responsible for transporting the larvae across the Red Sea, this could create moderate genetic differentiation found between Sudan and Saudi. Information on the precise timing of spawning in *P. areolatus*, as well as the formation of eddies in the central Red Sea, is needed to be certain. The greater proportion of migrants in the north, seen in the STRUCTURE plot, could be explained by the presence of a fairly regular cyclonic eddy near Al Wajh banks. This has the potential to move more larvae quickly across the Red Sea (Zhan et al. 2014).

Location	Spawning Season	Spawning time	References
Komodo National Park, Indonesia	September–February; April–July	New moon	Pet et al. (2005)
West Papua Province, Indonesia	September–January	Prior to new moon	Wilson et al. (2010)
Palau	January–September/December	New moon	Johannes et al. (1999)
New Ireland Province, Papua New Guinea	March–July; July–November	New moon	Hamilton et al. (2011)

Table 7. Spawning seasonality and timing of *P. areolatus* in different regions across its geographic species range.

4.3 Comparison of congeners

Two important factors are thought to cause a large genetically homogenous area: high larval dispersal abilities, and a continuous reef structure providing adequate habitat for settlement. First, in Papua New Guinea high dispersal capabilities have been seen in *P. areolatus*, although the vast majority of larvae (95%) were recorded to settle within 33 km from spawning sites (Almany et al. 2013). It is very plausible that dispersal distances

for *P. areolatus* in the Red Sea differ from dispersal distances in Papua New Guinea, possibly due to differences in oceanographic environment. The eddies present in the Red Sea can persist up to 9 weeks, and take approximately two weeks to move water masses across the Red Sea (Raitsos et al. 2017). Species with PLDs longer than two weeks are present in the water column before settling for enough time to use eddies to transport them across the Red Sea. However, pelagic larval durations (PLDs) may be shorter for many species in the Red Sea when compared to other regions where they also occur. Robitzch et al. (2016) found a decrease in PLD in three species of damselfishes along the environmental gradients present in the Red Sea. The authors found shorter PLDs for each species correlating to increased sea surface temperature and primary production, likely attributed to increased food availability and higher metabolic rates occurring in the southern region (Robitzch et al. 2016). This pattern needs to be assessed in more species to determine if PLDs decrease with increased temperatures for other families present in the Red Sea. Altered larval behavior has also been found with increased temperature, such as altered swimming behavior, decreased navigational skills, and reduced predator detection affecting offspring survival and increasing natal retention (Herbing 2002; Dixon et al. 2010). These suggest additional factors that could potentially cause population isolation found amongst some species in the southern Red Sea.

Alternatively, the Red Sea could be connected through the continuous reef structure found across nearly the entire coastal zone (>5000km), which is capable of supporting genetic homogenization through a stepping stone migration across a few short generations. If this were the case, we would expect to see great genetic differences

between Sudan and the central Saudi Arabian region, and little genetic differences between Sudan and the southern region of Saudi Arabia.

The genetic connectivity for *P. p. marisrubri* appears to supports Raitsos et al. (2017) long term connectivity estimates of simulated particles in the Red Sea. The study model, driven by satellite observations of the variable sea surface height in the Red Sea over 20 years, showed water masses were entrained from coral reefs by eddies, transporting particles across hundreds of kilometers across the Red Sea (Raitsos et al. 2017). This implies eddies and other types of surface currents may formulate physical pathways for gene flow in the Red Sea (Raitsos et al. 2017). The transport speed is important for larvae to reach a suitable habitat before energy resources are depleted, or metamorphosis happens. Raitsos et al. (2017) calculated that with water masses moving 25 cm/s (faster speeds of 30-100cm/s have been reported) particles could move greater than 250 km within two weeks' time. The majority of marine larvae have a pelagic larval duration greater than two weeks (Cowen and Sponaugle 2009). Therefore theoretically, water movement across the Red Sea can facilitate successful dispersal for most species.

The model may not be appropriate for all species. The. The moderate genetic differences we see in *P. areolatus*, and previously reported in *Stylissa carteri*, may be due to seasonal variability, which this model does not account for (Raitsos et al. 2017). Population structure could occur if there was a mismatch in the timing of spawning and the formation of seasonal eddies. If there were a small number of spawning aggregations that had aa great distance traveled between them, particles would be few and far between compared to Raitsos et al. 2017 particle dispersion model. This. This could potentially create genetic structuring of populations. Location and distance traveled to spawning

aggregations has yet to be determined for Red Sea *Plectropomus* species. *Plectropomus areolatus* has been recorded to travel up to 23 km to spawn in Micronesia, with the majority traveling less than 10 km to spawn (Hutchinson and Rhodes 2010). The Nassau grouper (*Epinephelus striatus*) has the current record of up to 240 km traveled to spawn in the Bahamas within aggregations of up to 100,000 individuals (Bolden 2000), indicating groupers are capable of very large and geographically distant aggregations. Spawning aggregation locations and distance traveled to them should be evaluated for Red Sea *Plectropomus* in future studies, in order to assess possible effects on population structure.

The dispersal capabilities of *P. p. marisrubri* have yet to be tested, although they are expected to produce smaller and more frequent spawning aggregations than *P. areolatus*, similar to their closer relative *P. laevis.* These smaller, more frequent spawning aggregations could cause less genetic structure by taking advantage of seasonal currents to further disperse larvae, with spawning occurring in more locations. This could explain the small degree of genetic differences determined between Sudan and Saudi Arabia for *P. p. marisrubri*, when compared to *P. areolatus*. However, smaller aggregations have a greater chance of becoming fished out, thus geographically separating aggregations, which could restrict gene flow. If *P. p. marisrubri* were historically overfished at certain locations, past geographically separating of the population could explain the larger K-value (K=3). Comparing the current life history knowledge available for *P. areolatus* and *P. p. marisrubri*, the two species seem very similar, although small differences in life history traits could have caused the slight contrast in genetic differentiation between populations within each species. These life history traits, especially those surrounding

spawning, such as identifying the time, location, and distance traveled to spawn, as well as the size of aggregations, should be addressed in future studies. This would enable us to compare the two species and infer which life history traits may affect population structure.

4.4 Conservation implications

The reduction of genetic diversity puts populations at higher risk for extinction, and reduces their capacity to adapt to environmental change (Frankham et al. 2010). Loss of biodiversity is occurring at an unprecedented rate, and marine environments are particularly vulnerable due to overfishing, habitat loss, and the effects of climate change (Mora and Sale 2011). As climate change continues, sea surface temperature is predicted to increase (Collins et al. 2010). Coral reefs of the Red Sea are already showing detrimental effects with increased frequency of bleaching events (Furby et al. 2013). Although the marine organisms inside the Red Sea are currently surviving, despite facing extremes of high temperatures and salinity, these organisms are living near the upper threshold of their tolerance (Furby et al. 2013). Unfortunately, being able to survive in an extreme set of environmental conditions does not necessarily indicate Red Sea organisms will be able to respond to increasing temperatures in the future, particularly at an increased speed of change (Furby et al. 2013). However, some researchers are optimistic that marine organisms being subjected to stress will provide an area where juveniles with higher resilience will be produced. These juveniles would have the ability to move to, and colonize, nearby areas facing temperature increases (Riegl et al. 2011).

The findings of this study show a pattern of predominantly low genetic structure. This work suggests that *Plectropomus* populations throughout the Red Sea are not at a high risk of genetic loss. The existing population connectivity between Sudan and Saudi Arabia shows that Sudan has the potential to reseed depleted grouper populations in Saudi Arabia. In terms of managing the population, the presumed connectivity suggests each species population could potentially be considered as a single unit. The population genetics of these commercially important species should continue to be monitored in the future, as overfishing is likely to increase. Sudan has already begun to see evidence of overfishing in the last few years (Klaus 2015). If the majority of new recruits are coming from Sudan into Saudi Arabia, both populations could crash if significant overfishing occurs in both countries.

4.5 Future research

Reduced availability of fish throughout the Red Sea is likely to have significant social and economic consequences for the desert Red Sea nations. This is especially true because the countries have minimal agricultural and livestock potential, currently featuring an average diet consisting of approximately three fish based meals a week (Burger et al. 2014). The coral reef community structure is also likely to be affected. A continual decrease in *Epinephelinae* could create a cascading effect, reducing ecosystem resilience, and should be investigated further. Microsatellites, like those used in this study, are only one measure of genetic differentiation. Different samples could be exhibiting extreme differentiation in other loci without detection through neutral loci

(Nosil et al. 2009). Next generation sequencing, using single nucleotide polymorphisms, has been found to show a much greater accuracy in predicting genetic differentiation (Bradbury et al. 2015). This method could also be applied in future studies.

In general the Red Sea is still considered an understudied region (Berumen et al. 2013). More research on currents and connectivity of species within the Red Sea is needed to decisively determine overall gene flow patterns. Future research should also focus on identifying timing and location of spawning aggregations, to better inform management decisions for *Plectropomus* groupers, as well as other important fishery species in the Red Sea. It is possible that the samples collected from the Saudi Arabian fish markets could have been from other regions outside our defined sampling regions. However, we are reasonably certain that fish purchased from small auctions, fish mongers, or directly from fisherman at a certain locality, were landed within that local region (DesRosier 2011). Most fishing in Saudi Arabia is done from small (10 m) fiberglass boats, with single 50 horsepower, 2-stroke outboard engines (Jin et al. 2012). Platforms to sell fishermen's catches are spaced tens to hundreds of kilometers apart (DesRoiser 2011). This suggests it is much more likely they sell closest to where fish are caught.

A previous study examined connectivity through parentage in Papua New Guinea and suggested a low dispersal range for *P. areolatus*, with 95% settling within 33 km of their parents (Almany et al. 2013). To date the longest dispersal distance of *Plectropomus* groupers is up to approximately 250 km for *Plectropomus maculatus* and *Plectropomus leopardus* along the Great Barrier Reef (Williamson et al. 2016). Given the results of my study, the wide species ranges across the Indo-Pacific, and the swimming abilities and

behavior of larvae, more distant dispersal is likely (Leis and Carson-Ewart 1999). An overall assessment of *P. p. marisrubri* life history traits is lacking. Larval dispersal duration, spawning mechanisms, and habitat preferences are potentially different between *P. p. marisrubri* and *P. areolatus*, and these difference can affect their population structure. Larval behavior has been evaluated in various *Epinephelus* species, and they have been found to have substantial regulation of their speed, depth, and direction at late stages of development (Leis and Carton-Ewart 1999). Most notably, studies examining *Plectropomus leopardus* larvae have found that the pelvic fin and dorsal fin spine emerge 5 days after hatching, (Masuma et al. 1993), so little control of movement is expected before this. Masuma et al. (1993) also found that *P. leopardus* did not begin to actively feed until about 15 days after hatching and started positioning themselves away from the surface of the water column only after 30 days at the time they begun to settle. Wright et al. (2008) determined that *P. leopardus* is capable of olfactory detection of amino acids and conspecifics, which is valuable to locate settlement habitat. It is clear that larvae of many coral reef fishes at the settlement stage have excellent swimming capabilities and sufficient sensory abilities giving them the potential to find settlement habitat. What is less clear is the point at which these abilities develop and if the larvae can actively alter their dispersal trajectories, and, if they can, what signals them to do so. The interaction of even subtle behaviors (such as controlling depth distribution) with ocean currents can greatly alter the outcome of dispersal patterns (Paris et al. 2007). These have and will be a continued focus of reef fish studies.

5. CONCLUSIONS

This study confirms that migration of larvae is occurring across the Red Sea, genetically connecting the two coastlines. Both *Plectropomus* species seemed to lacked strong genetic structure and to demonstrate wide dispersal ranges. This may be indicative of larvae's ability to transverse the Red Sea using currents to transport them long distances, as well as a lack of geographical barriers. These results give greater insight into eddies ability to transport larvae across large geographical distances (~350 km). Both *P. areolatus* and *P. p. marisrubri* are thought to have one population throughout the Red Sea, therefore they should be managed as one unit across nations. The existing population connectivity between Sudan and Saudi Arabia shows that Sudan has the potential to reseed depleted grouper populations in Saudi Arabia. Not only are the two coastlines genetically connected, they are genetically connected to a different degree in different species. To determine which attributes of each species is contributing to the differences seen in their population structure, further investigation into the life history of *P. areolatus* and *P. p. marisrubri* is needed. Studies related to *Plectropomus* grouper spawning in the Red Sea would give some much needed insight into the different genetic patterns presented in this study for each species. Overall this study provides a clearer understanding of genetic population structuring within the Red Sea, and the potential factors causing the patterns of high gene flow observed across taxa throughout the Red Sea.

REFRENCES

Allen, G.R. and Talbot, F.H., 1985. *Review of the snappers of the genus Lutjanus (Pisces: Lutjanidae) from the Indo-Pacific, with the description of a new species.* Honolulu, Hawaii: Bernice Pauahi Bishop Museum, pp.87&92.

Allendorf, F., Luikart, G. and Aitken, S. (2013). *Conservation and the genetics of populations*. Hoboken: John Wiley & Sons, pp.121-142.

Almany, G.R., Berumen, M.L., Thorrold, S.R., Planes, S. and Jones, G.P., 2007. Local replenishment of coral reef fish populations in a marine reserve. *Science, 316*, pp.742-744.

Almany, G.R., Hamilton, R.J., Bode, M., Matawai, M., Potuku, T., Saenz-Agudelo, P., Planes, S., Berumen, M.L., Rhodes, K.L., Thorrold, S.R. and Russ, G.R., 2013. Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. *Current Biology*, *23*, pp.626-630.

Almany, G.R., Planes, S., Thorrold, S.R., Berumen, M.L., Bode, M., Saenz-Agudelo, P., Bonin, M.C., Frisch, A.J., Harrison, H.B., Messmer, V. and Nanninga, G.B., 2017. Larval fish dispersal in a coral-reef seascape. *Nature Ecology & Evolution*, *1*, pp. 148. doi: 10.1038/s41559-017-0148

Ayre, D. J., & Hughes, T. P., 2004. Climate change, genotypic diversity and gene flow in reef-building corals. *Ecology Letters*, *7*, pp.273–278.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological), 57*, pp.289–300.

Berumen, M. L., Hoey, A. S., Bass, W. H., Bouwmeester, J., Catania, D., Cochran, J. E. M., Saenz-Agudelo, P., 2013. The status of coral reef ecology research in the Red Sea. *Coral Reefs, 32*, pp.737-748.

Benzie, J.A.H., 1992. Review of the genetics, dispersal and recruitment of crown-of-thorns starfish (Acanthaster planci). *Marine and Freshwater Research, 43,* pp.597-610.

Bird, C. E., Karl, S. A., Smouse, P. E., & Toonen, R. J., 2011. Detecting and measuring genetic differentiation. *Phylogeography and Population Genetics in Crustacea, 19*, pp.31-55.

Bolden, S.K., 2000. Long-distance movement of a Nassau grouper (*Epinephelus striatus*) to a spawning aggregation in the central Bahamas. *Fishery Bulletin-National Oceanic and Atmospheric Association, 98*, pp.642-645.

Bradbury, I. R., Hamilton, L. C., Rafferty, S., Meerburg, D., Poole, R., Dempson, J. B., Bernatchez, L., 2015. Genetic evidence of local exploitation of Atlantic salmon in a coastal

subsistence fishery in the Northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences, 72*, pp. 83-95.

Burger, J., Gochfeld, M., Batang, Z., Alikunhi, N., Al-Jahdali, R., Al-Jebreen, D., Aziz, M.A. and Al-Suwailem, A., 2014. Fish consumption behavior and rates in native and non-native people in Saudi Arabia. *Environmental Research, 133*, pp.141-148.

Caley, M.J., Carr, M.H., Hixon, M.A., Hughes, T.P., Jones, G.P. and Menge, B.A., 1996. Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics, 27*, pp.477-500.

Chen, C., Li, R., Pratt, L., Limeburner, R., Beardsley, R.C., Bower, A., Jiang, H., Abualnaja, Y., Xu, Q., Lin, H. and Liu, X., 2014. Process modeling studies of physical mechanisms of the formation of an anticyclonic eddy in the central Red Sea. *Journal of Geophysical Research: Oceans, 119*, pp.1445-1464.

Collins, M., An, S.I., Cai, W., Ganachaud, A., Guilyardi, E., Jin, F.F., Jochum, M., Lengaigne, M., Power, S., Timmermann, A. and Vecchi, G., 2010. The impact of global warming on the tropical Pacific Ocean and El Niño. *Nature Geoscience, 3*, pp.391-397.

Cowen, R.K. and Sponaugle, S., 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science, 1*, pp.443-466. https://doi.org/10.1146/annurev.marine.010908.163757

Cowen, R.K., Paris, C.B. and Srinivasan, A., 2006. Scaling of connectivity in marine populations. *Science, 311*, pp.522-527.

Department of Marine Fisheries, 2008. Fisheries statistics of Saudi Arabia: 2006. *Ministry of Agriculture, Department of Marine Fisheries*, Jeddah, Saudi Arabia.

DesRosiers, N.J.D., 2011. Growth and maturation of *Plectropomus* spp. in the Saudi Arabian Red Sea. MSc Thesis, King Abdullah University of Science and Technology, Saudi Arabia.

DiBattista, J.D., Berumen, M.L., Gaither, M.R., Rocha, L.A., Eble, J.A., Choat, J.H., Craig, M.T., Skillings, D.J. and Bowen, B.W., 2013. After continents divide: comparative phylogeography of reef fishes from the Red Sea and Indian Ocean. *Journal of Biogeography, 40*, pp.1170-1181.

DiBattista, J.D., Roberts, M.B., Bouwmeester, J., Bowen, B.W., Coker, D.J., Lozano- Cortés, D.F., Howard Choat, J., Gaither, M.R., Hobbs, J.P.A., Khalil, M.T. and Kochzius, M., 2016. A review of contemporary patterns of endemism for shallow water reef fauna in the Red Sea. *Journal of Biogeography, 43*, pp.423-439.

DiBattista, J.D., Rocha, L.A., Hobbs, J.P.A., He, S., Priest, M.A., Sinclair- Taylor, T.H., Bowen, B.W. and Berumen, M.L., 2015. When biogeographical provinces collide: hybridization

of reef fishes at the crossroads of marine biogeographical provinces in the Arabian Sea. *Journal of Biogeography*, *42*, pp.1601-1614.

Dixson, D.L., Munday, P.L. and Jones, G.P., 2010. Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology letters*, *13*, pp.68-75.

Earl, D.A., and Vonholdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources*, *4*, pp.359–361.

Falush, D., Stephens, M., and Pritchard, J.K., 2003. Inference of population structure using multi locus genotype data: linked loci and correlated allele frequencies. *Genetics*, *164*, pp.1567–1587.

Falush, D., Stephens, M., and Pritchard, J.K., 2007. Inference of population structure using multi locus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, 7, pp.574–578.

Ferreira, B.P., Gaspar, A.L.B., Samoilys, M., Choat, J.H. & Myers, R. 2008. *Plectropomus pessuliferus*. The IUCN Red List of Threatened Species 2008: http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T132775A3448422.en.

Downloaded on 26 June 2017.

Frankham, R., 2010. Challenges and opportunities of genetic approaches to biological conservation. *Biological conservation*, *143*, pp.1919-1927.

Fratini, S. and Vannini, M., 2002. Genetic differentiation in the mud crab Scylla serrata (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine Biology and Ecology*, *272*, pp.103-116.

Frisch, A.J., Cameron, D.S., Pratchett, M.S., Williamson, D.H., Williams, A.J., Reynolds, A.D., Hoey, A.S., Rizzari, J.R., Evans, L., Kerrigan, B. and Muldoon, G., 2016. Key aspects of the biology, fisheries and management of coral grouper. *Reviews in Fish Biology and Fisheries*, *26*, pp.303-325.

Froukh, T. and Kochzius, M., 2007. Genetic population structure of the endemic fourline wrasse (Larabicus quadrilineatus) suggests limited larval dispersal distances in the Red Sea. *Molecular Ecology*, *16*, pp.1359-1367.

Futuyma, D. (2006). *Evolutionary biology*. New York: W.H. Freeman, pp.224-243.

Furby, K.A., Bouwmeester, J. and Berumen, M.L., 2013. Susceptibility of central Red Sea corals during a major bleaching event. *Coral Reefs*, *32*, pp.505-513.

Gaube, P., McGillicuddy, D.J., Chelton, D.B., Behrenfeld, M.J. and Strutton, P.G., 2014. Regional variations in the influence of mesoscale eddies on near- surface chlorophyll. *Journal of Geophysical Research: Oceans*, *119*, pp.8195-8220.

Giles, E. C., Saenz-Agudelo, P., Hussey, N. E., Ravasi, T., & Berumen, M. L., 2015. Exploring seascape genetics and kinship in the reef sponge *Stylissa carteri* in the Red Sea. *Ecology and Evolution, 5*, 2487-2502.

Green, A.L., Maypa, A.P., Almany, G.R., Rhodes, K.L., Weeks, R., Abesamis, R.A., Gleason, M.G., Mumby, P.J. and White, A.T., 2015. Larval dispersal and movement patterns of coral reef fishes, and implications for marine reserve network design. *Biological Reviews*, *90*, pp.1215-1247.

Hamilton, R.J., Potuku, T. and Montambault, J.R., 2011. Community-based conservation results in the recovery of reef fish spawning aggregations in the Coral Triangle. *Biological Conservation*, *144*, pp.1850-1858.

Harrison, H.B., Feldheim, K.A., Jones, G.P., Mansour, H., Perumal, S., Williamson, D.H. and Berumen, M.L., 2014. Validation of microsatellite multiplexes for parentage analysis in a coral reef fish (*Lutjanus carponotatus*, Lutjanidae). *Conservation Genetics Resources*, *6*, pp.803-806.

Herbing, I., 2002. Effects of temperature on larval fish swimming performance: the importance of physics to physiology. *Journal of Fish Biology*, *61*, pp.865-876.

Hellberg, M.E., 2009. Gene flow and isolation among populations of marine animals. *Annual Review of Ecology, Evolution, and Systematics*, *40*, pp.291-310.

Heupel M.R., Williams A.J., Welch D.J., Davies C.R., Adams S., Carlos G., Mapstone B.D., 2010. Demography of a large exploited grouper, *Plectropomus laevis*: Implications for fisheries management. *Marine and Freshwater Research, 61*, pp.184-195.

Holsinger, K.E. and Weir, B.S., 2009. Genetics in geographically structured populations: defining, estimating and interpreting FST. *Nature Review Genetics*, *10*, pp.639.

Hoelzel, A. and Dover, G. (1991). *Molecular genetic ecology*. Oxford: IRL Press, pp.19-37, 167-207.

Hubisz, M.J., Falush, D., Stephens, M., and Pritchard, J.K., 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9, pp.1322–1332.

Hutchinson, N. and Rhodes, K.L., 2010. Home range estimates for squaretail coralgrouper, Plectropomus areolatus (Rüppell 1830). *Coral Reefs*, *29*, pp.511-519.

James, M.K., Armsworth, P.R., Mason, L.B. and Bode, L., 2002. The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. *Proceedings of the Royal Society of London B: Biological Sciences*, *269*, pp.2079-2086.

Jin, D., Kite-Powell, H., Hoagland, P. and Solow, A., 2012. A bioeconomic analysis of traditional fisheries in the Red Sea. *Marine Resource Economics*, *27*, pp.137-148.

Johannes, R.E., Squire, L., Graham, T., Sadovy, Y. and Renguul, H., 1999. Spawning aggregations of groupers (Serranidae) in Palau. *The Nature Conservancy Marine Research Series Publication, 1*, pp.1-144.

Kattan, A., Coker, D.J. and Berumen, M.L., 2017. Reef fish communities in the central Red Sea show evidence of asymmetrical fishing pressure. *Marine Biodiversity, 47,* pp.1-12.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28,* pp.1647-1649.

Klaus, R., 2015. Ecological patterns and status of the reefs of Sudan. MSc Thesis, Red Sea University. http://hdl.handle.net/123456789/232.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A. and Mayrose, I., 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources, 15,* pp.1179-1191.

Laikre, L., Palm, S., & Ryman, N., 2005. Genetic population structure of fishes: implications for coastal zone management. *Ambio, 34,* pp.111–119.

Leis, J.M. and Carson-Ewart, B.M., 1999. In situ swimming and settlement behaviour of larvae of an Indo-Pacific coral-reef fish, the coral trout *Plectropomus leopardus* (Pisces: Serranidae). *Marine Biology, 134,* pp.51-64.

Lischer, H.E.L., and Excoffier, L., 2012. PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics, 28,* pp.298–299.

Ma, K.Y., Craig, M. T., Choat, J. H., and van Herwerden, L., 2016. The historical biogeography of groupers: clade diversification patterns and processes. *Molecular Phylogenetics and Evolution, 100,* pp.21-30.

Masuma, S., Tezuka, N. and Teruya, K., 1993. Embryonic and morphological development of larval and juvenile coral trout, Plectropomus leopardus. *Japanese Journal of Ichthyology, 40,* pp.333-342.

McGillicuddy Jr, D.J., 2016. Mechanisms of physical-biological-biogeochemical interaction at the oceanic mesoscale. *Annual Review of Marine Science, 8,* pp.125-159.

McMahon, K.W., Berumen, M.L. and Thorrold, S.R., 2012. Linking habitat mosaics and connectivity in a coral reef seascape. *Proceedings of the National Academy of Sciences, 109,* pp.15372-15376.

Meirmans P.G., Hedrick P.W., 2011. Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources, 11,* pp.5–18.

Monroe, A., 2015. Genetic differentiation across multiple spatial scales of the Red Sea of the corals *Stylophora pistillata* and *Pocillopora verrucosa*. MSc thesis, King Abdullah University of Science and Technology.

Mora C. (ed), 2015. *Ecology of fishes on coral reefs.* Cambridge, England: Cambridge University Press, pp.16-27. .https://doi.org/10.1017/CBO9781316105412.004

Mora, C. and Sale, P.F., 2011. Ongoing global biodiversity loss and the need to move beyond protected areas: a review of the technical and practical shortcomings of protected areas on land and sea. *Marine Ecology Progress Series*, *434*, pp.251-266.

Nanninga, G.B. and Berumen, M.L., 2014. The role of individual variation in marine larval dispersal. *Frontiers in Marine Science*, *1*, pp.71. doi:10.3389/fmars.2014.00071

Nanninga, G. B., Saenz-Agudelo, P., Manica, A., & Berumen, M. L., 2014. Environmental gradients predict the genetic population structure of a coral reef fish in the Red Sea. *Molecular Ecology*, *23*, pp.591-602.

Nanninga, G.B., Saenz-Agudelo, P., Zhan, P., Hoteit, I. and Berumen, M.L., 2015. Not finding Nemo: limited reef-scale retention in a coral reef fish. *Coral Reefs*, *34*, pp.383-392.

Nosil, P., Funk, D. J., & Ortiz-Barrientos, D., 2009. Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, *18*, pp.375-402.

Paris, C. B., L. M. Chérubin, and R. K. Cowen. 2007. Surfing, spinning, or diving from reef to reef: Effects on population connectivity. *Marine Ecology Progress Series* 347, pp.285-300

Paetkau, D., Slade, R., Burden, M. and Estoup, A., 2004. Genetic assignment methods for the direct, real- time estimation of migration rate: a simulation- based exploration of accuracy and power. *Molecular Ecology*, *13*, pp.55-65.

Peakall, R., and Smouse, P.E., 2012. GenAlEx6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, *28*, pp.2537–2539.

Pemberton, J.M., Slate, J., Bancroft, D.R. and Barrett, J.A., 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, *4*, pp.249-252.

Pet, J.S., Mous, P.J., Muljadi, A.H., Sadovy, Y.J. and Squire, L., 2005. Aggregations of Plectropomus areolatus and Epinephelus fuscoguttatus (groupers, Serranidae) in the Komodo National Park, Indonesia: monitoring and implications for management. *Environmental Biology of Fishes*, *74*, pp.209-218.

Pinsky, M.L., Saenz-Agudelo, P., Salles, O.C., Almany, G.R., Bode, M., Berumen, M.L., Andréfouët, S., Thorrold, S.R., Jones, G.P. and Planes, S., 2017. Marine dispersal scales are congruent over evolutionary and ecological time. *Current Biology*, *27*, pp.149-154.

Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L. and Estoup, A., 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity, 95*, pp.536-539.

Planes, S., Jones, G.P. and Thorrold, S.R., 2009. Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences, 106*, pp.5693-5697.

Pritchard, J.K., Stephens, M., and Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics, 155*, pp.945–959.

Quadfasel, D. and Baudner, H., 1993. Gyre-scale circulation cells in the Red-Sea. *Oceanologica Acta, 16*, pp.221-229.

Raitsos, D.E., Brewin, R.J., Zhan, P., Dreano, D., Pradhan, Y., Nanninga, G.B. and Hoteit, I., 2017. Sensing coral reef connectivity pathways from space. *Scientific Reports*, *7*, pp.9338.

Raitsos, D.E., Pradhan, Y., Brewin, R.J., Stenchikov, G. and Hoteit, I., 2013. Remote sensing the phytoplankton seasonal succession of the Red Sea. *PloS one*, *8*, pp.e64909.

Randall, J.E. and Hoese, D.F., 1986. Revision of the groupers on the Indo-Pacific genus *plectropomus (*perciformes: serranidae*). Bernice Pauahi Bishop Museum*, pp.127-130.

Rannala, B. and Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences*, *94*(17), pp.9197-9201.

Rasul, N.M., Stewart, I.C. and Nawab, Z.A., 2015. *The Red Sea*. Boston, Massachusetts: Springer Berlin Heidelberg, pp. 1-28.

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

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

Rhodes, K.L. and Tupper, M.H., 2008. The vulnerability of reproductively active squaretail coral grouper (*Plectropomus areolatus*) to fishing. *Fishery Bulletin*, *106*, pp.194-204.

Riegl, B. M., Purkis, S. J., Al-Cibahy, A. S., Abdel-Moati, M. A., & Hoegh-Guldberg, O., 2011. Present limits to heat-adaptability in corals and population-level responses to climate extremes. *PLoS ONE*, *6*, pp.e24802.

Rimmer, M.A., Thampisamraj, Y.C., Jayagopal, P., Thineshsanthar, D., Damodar, P.N. and Toledo, J.D., 2013. Spawning of tiger grouper Epinephelus fuscoguttatus and squaretail coralgrouper Plectropomus areolatus in sea cages and onshore tanks in Andaman and Nicobar Islands, India. *Aquaculture*, *410*, pp.197-202.

Robitzch V., Banguera-Hinestroza, E., Sawall, Y., Al-Sofayani, A., and Voolstra C.R. 2015. Absence of genetic differentiation n the coral *Pocillopora verrucosa* long environmental gradients of the Saudi Arabian Red Sea. *Frontiers in Marine Science, 2*, pp.1-10.

Robitzch, V.S., Lozano-Cortés, D., Kandler, N.M., Salas, E. and Berumen, M.L., 2016. Productivity and sea surface temperature are correlated with the pelagic larval duration of damselfishes in the Red Sea. *Marine Pollution Bulletin, 105*, pp.566-574.

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

Russell, B., Smith-Vaniz, W.F., Lawrence, A., Carpenter, K.E., Myers, R. & Sparks, J.S. 2016. *Lutjanus ehrenbergii*. The IUCN Red List of Threatened Species 2016: http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T194407A2332631.en. Downloaded on 20 June 2017.

Saenz- Agudelo, P., Dibattista, J.D., Piatek, M.J., Gaither, M.R., Harrison, H.B., Nanninga, G.B. and Berumen, M.L., 2015. Seascape genetics along environmental gradients in the Arabian Peninsula: insights from ddRAD sequencing of anemonefishes. *Molecular Ecology, 24*, pp.6241-6255.

Schunter, C., Carreras- Carbonelle, J., Macpherson, E., Tintoré, J., Vidal- Vijande, E., Pascual, A., Guidetti, P. and Pascual, M., 2011. Matching genetics with oceanography: directional gene flow in a Mediterranean fish species. *Molecular Ecology, 20*, pp.5167-5181.

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

Selkoe, K. A., & Toonen, R. J., 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters, 9*, pp.615-629.

Slatkin, M., 1985. Genetic differentiation of transposable elements under mutation and unbiased gene conversion. *Genetics, 110*, pp.145-158.

Sgro, C.M., Lowe, A.J. and Hoffmann, A.A., 2011. Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications, 4*, pp.326-337.

Thierry, C., Sadovy, Y. & Yeeting, B. 2008. *Plectropomus areolatus*. The IUCN Red List of Threatened Species 2008: http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T64411A12779260.en.http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T64411A12779260.en. Downloaded on 26 June 2017.

Treml, E.A., Halpin, P.N., Urban, D.L. and Pratson, L.F., 2008. Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. *Landscape Ecology, 23*, pp.19-36.

Vandermeer, J. and Goldberg, D., 2003. Elementary population ecology: patterns of endemism for shallow water reef fauna in the Red Sea. *Journal of Biogeography, 43*, pp.423-439.

Vignon, M., 2012. Ontogenetic trajectories of otolith shape during shift in habitat use: Interaction between otolith growth and environment. *Journal of Experimental Marine Biology and Ecology, 420*, pp.26-32.

Williamson, D.H., Harrison, H.B., Almany, G.R., Berumen, M.L., Bode, M., Bonin, M.C., Choukroun, S., Doherty, P.J., Frisch, A.J., Saenz- Agudelo, P. and Jones, G.P., 2016. Large- scale, multidirectional larval connectivity among coral reef fish populations in the Great Barrier Reef Marine Park. *Molecular Ecology, 25*, pp.6039-6054.

Wilson, J., Rhodes, K.L. and Rotinsulu, C., 2010. Aggregation fishing and local management within a marine protected area in Indonesia. *SPC Live Reef Fish Information Bulletin, 19*, pp.7-13.

Wright, S., 1965. The interpretation of population structure by F- statistics with special regard to systems of mating. *Evolution, 19*, pp.395-420.

Wright, K.J., Higgs, D.M., Belanger, A.J. and Leis, J.M., 2008. Auditory and olfactory abilities of larvae of the Indo- Pacific coral trout Plectropomus leopardus (Lacepède) at settlement. *Journal of Fish Biology, 72*, pp.2543-2556.

Zane, L., Bargelloni, L. and Patarnello, T., 2002. Strategies for microsatellite isolation: a review. *Molecular Ecology, 11*, pp.1-16.

Zapata, F.A. and Herrón, P.A., 2002. Pelagic larval duration and geographic distribution of tropical eastern Pacific snappers (Pisces: Lutjanidae). *Marine Ecology Progress Series, 230*, pp.295-300.

Zarokanellos, N.D., Papadopoulos, V.P., Sofianos, S.S. and Jones, B.H., 2017. Physical and biological characteristics of the winter- summer transition in the Central Red Sea. *Journal of Geophysical Research: Oceans*. http://onlinelibrary.wiley.com/doi/10.1002/2017JC012882/full

Zhan, P., Subramanian, A.C., Yao, F. and Hoteit, I., 2014. Eddies in the Red Sea: A statistical and dynamical study. *Journal of Geophysical Research: Oceans, 119*, pp.3909-3925.