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Connectivity among populations of bicolor damselfish (Stegastes partitus) along the Mesoamerican barrier reef.

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CONNECTIVITY AMONG POPULATIONS OF BICOLOR DAMSELFISH (*Stegastes partitus*) ALONG THE MESOAMERICAN BARRIER REEF

By

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ABSTRACT

Connectivity is a critical measure affecting the evolution and ecology of species. The purpose of this thesis was to assess connectivity between populations of *Stegastes partitus* at seven sites along the Mesoamerican barrier reef. First, seven novel microsatellite markers were developed to allow the assessment of genetic population structure in *S. partitus* in conjunction with existing markers. Next, population structure and stability was evaluated at seven sampling locations encompassing both atoll and barrier reef regions along the Mesoamerican barrier reef. Results indicate significant but temporally unstable genetic population structure suggesting high but unpredictable patterns of gene flow between sites. Further study utilizing multi-locus genotype population assignment techniques revealed dispersal over relatively short distances and temporally variable dispersal direction. These results suggest population structure may be dependent on local processes and that local management may be effective for some reef fishes, however time-series data are necessary to fully understand population connectivity.

CO-AUTHORSHIP STATEMENT

This thesis includes materials reprinted from co-authored and submitted articles. In all cases the contribution of co-authors was primarily in an advisory capacity. The primary contributions, data collection and interpretation as well as the preparation of all manuscripts were performed by the author.

1- Thiessen RJ, Heath DD. (2006) Characterization of one trinucleotide and six dinucleotide microsatellite markers in bicolor damselfish, Stegastes partitus, a common coral reef fish. Conservation Genetics, DOI 10.1007/s10592-006-9207-9.

2- Thiessen RJ, Heath DD. (2007) Spatial and temporal instability in the genetic structure of adult and juvenile bicolor damselfish (*Stegastes partitus*) along the Mesoamerican barrier reef. (*Manuscript submitted to Molecular Ecology: August 2007*).

For my family and loving friends, whose enduring belief in me

continues to spur me onwards

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 General Introduction

1.1 Definitions

The terms dispersal, gene flow and connectivity will be used frequently in the following text, and since their meanings and the interaction between these processes are not necessarily intuitive, I will provide some definitions. Dispersal is simply the movement of individuals away from a starting location, typically their natal site (Mora and Sale 2002), and is integral to both gene flow and connectivity. Gene flow refers to the exchange of alleles between breeding populations, and therefore requires both the dispersal of individuals as well as their subsequent reproductive success. Dispersal and gene flow establish and maintain genetic and demographic links between neighbouring populations, and this is referred to as connectivity. Thus, each process is linked to the other (Figure 1.1) and by quantifying gene flow between populations one can infer the dispersal of individuals and estimate the connectivity between populations.

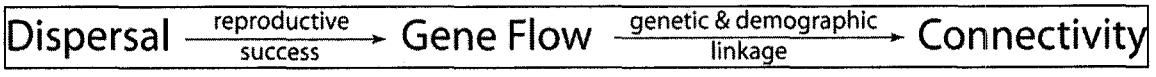

Figure 1.1 Relationships between dispersal, gene flow and population connectivity.

1.2 Gene Flow, Dispersal and Marine Population Connectivity

At neutral loci, the opposing forces of drift, which differentiates populations through the random selection of genotypes, and gene flow, which homogenizes allele frequencies between populations, produce genetic population structure (Wright 1931). As

gene flow between populations decreases, genetic differentiation will accumulate as the result of mutation, drift and natural selection, leading to locally adapted populations or possibly speciation events as reproductive barriers evolve (see Palumbi 1994 for review). Gene flow is difficult to quantify as it requires the assessment of both the movement of individuals and their subsequent reproductive success. These variables have the potential to be influenced by a host of life history traits including method of reproduction, vagility, territoriality, and most notably, dispersal potential.

In marine systems, many fish and invertebrates exhibit a bipartite life history including a dispersive pelagic stage and a relatively sedentary adult phase (Sale 1991). A number of evolutionary hypotheses have been proposed for why a pelagic life stage might exist, such as providing refuge of untended young from predation (Johannes 1978), as a means to overcome habitat instability over evolutionary timescales (Barlow 1981), or as a risk-spreading strategy (Doherty *et al.* 1985). Dispersal also facilitates the demographic linkage between neighbouring populations known as connectivity (Mora and Sale 2002). Ecologically, connectivity determines the extent to which populations are dependent on local or external processes for their patterns of growth and this in turn carries with it a number of conservation and management implications (Roberts 1997, Gaines *et al.* 2003).

Despite its evolutionary and ecological importance, connectivity in marine systems has been notoriously difficult to quantify. However, two general approaches exist: the quantification of gene flow between populations and measurements of dispersal between populations. The effects of gene flow on population structure have been assessed in a number of species with varying results. Some studies have shown little genetic structuring across vast geographic distances consistent with predictions of high levels of gene flow (e.g. Haney *et al.* 2007), while others have found significant genetic

structuring across geographically proximate sites and have attributed their findings to a variety of mechanisms (selection, population bottlenecks, sweepstakes chance matching, spatial autocorrelation, etc., see Larson and Julian 1999 for review). To circumvent the error associated with estimates of gene flow, some researchers have turned to direct measures of dispersal in order to quantify connectivity.

Direct estimates of dispersal are rare due to the relatively small size of larvae and the enormity of the environment through which they disperse, however significant progress has been made using natural (Thorrold 2001) and artificial (Almaney *et al.* 2007) chemical markers as well as genetic assignment techniques (Jones *et al.* 2005) to track larvae. Although these estimates provide a better idea of the actual numbers of dispersing individuals than those derived from indirect genetic estimates, they only address demographic connectivity and do not measure the reproductive success of dispersing individuals required for genetic connectivity between populations.

1.3 Genetic Tools

The genetic tools applied to the question of connectivity may explain some of the discrepancies between published estimates of population differentiation in reef fishes. Early genetic work often utilized protein allozymes and mitochondrial DNA markers which evolve relatively slowly. Their resolution of population differentiation is rather coarse and therefore may be more applicable to questions at an evolutionary rather than an ecological timescale. The question of connectivity requires the use of a rapidly evolving genetic marker that can track ecologically relevant genetic differentiation.

Microsatellites are tandem repeated regions within the non-coding DNA and mutate rapidly, providing a marker more suitable for determining ecologically relevant connectivity. These markers should be able to resolve the rapidly changing population structure likely present in reef fishes due to the constant influx of new individuals; and a variety of statistical procedures have been developed for this purpose. For example F_{ST} and exact tests allow us to quantify the differentiation between populations, while comparing pair-wise genetic distance measures such as Cavalli-Sforza and Edwards' (1967) chord distance (D_C) through time will allow us to examine the temporal stability of population structure. Furthermore, multi-locus assignment procedures can determine the natal population of an individual, allowing us to genetically "track" dispersal events. This thesis will utilize these procedures to examine connectivity among bicolor damselfish (*Stegastes partitus*) along the Mesoamerican barrier reef system.

1.4 Mesoamerican Barrier Reef System

The Mesoamerican Barrier Reef System (MBRS) is the second longest barrier reef in the world, extending from the Yucatan peninsula in Mexico to the Bay Islands of Honduras. The MBRS provides an excellent opportunity for the study of connectivity, as it is relatively one dimensional and thereby simplifies the interpretation of gene flow and dispersal information. Oceanographic processes critical to the understanding of dispersal and connectivity have also been characterized at fine spatial scales in this region (Sheng and Tang 2004, Ezer *et al.* 2005, Tang *et al.* 2006). Furthermore, recruitment, dispersal, genetic structure and patch dynamics of juvenile populations of reef fish in this area have been extensively studied through the "ecological connections among reefs" (ECONAR)

project, providing a background of scientific knowledge from which to proceed (Hepburn 2004, Chittaro 2005, Hogan 2007).

1.5 THE BICOLOR DAMSELFISH, *Stegastes partitus*

The bicolor damselfish (*Stegastes partitus*) is a small, sexually monomorphic pomacentrid that is widely distributed throughout the tropical western Atlantic at depths ranging from shallow back reefs to deep reef edges (0 – 100 meters) (DeLoach 1999). Adults establish small benthic territories and can maintain them for more than two years (Cole and Sadovy 1995). Spawning occurs daily throughout the year following a unimodal lunar cycle, with reproductive peaks from April to November (Robertson *et al.* 1988). Females can spawn every two days, choosing a nesting site established by a male and depositing between 500 and 5000 eggs in a monolayer over coral rubble or other hard surfaces approximately one hour before dawn (Robertson *et al.* 1988, DeLoach 1999). Males guard the nest by day (Knapp 1993), and after 3.5 days the eggs hatch and larvae move into the pelagic environment for 27-31 days before returning to the reef to settle (Robertson *et al.* 1988, Wellington and Victor 1989). Settlement pulses occur on a unimodal lunar cycle around the new moon (Robertson 1988, Robertson 1992).

1.6 THESIS OBJECTIVES

The purpose of this thesis is to characterize and assess spatial and temporal stability of population structure as well as describe the dispersal patterns of *S. partitus* along the MBRS over a two year period utilizing molecular genetic techniques.

1.7 Chapter 2 Objectives

Molecular markers used to study population demographics such as dispersal must be able to distinguish population-scale effects on a temporal scale relevant to such processes (Mora and Sale 2002). Most previous studies of reef fish population genetics have used allozymes or mtDNA markers to characterize population differentiation and infer migration and dispersal, however these markers are not sensitive to changes over short, ecologically relevant timescales (Shulman 1998). Microsatellite markers have a rapid rate of evolution, allowing the discrimination of events relating to demographically relevant processes and may provide valuable insights into the biology of coral reef fishes (Shulman 1998). Chapter 2 is a primer note reporting the development of novel microsatellite DNA markers for *S. partitus* which will be used in the genetic analysis for subsequent chapters. This chapter is published in *Conservation Genetics* as Thiessen and Heath (2006).

1.8 Chapter 3 Objectives

Genetic assessment of population structure and gene flow in coral reef fishes has produced a continuum of results ranging from significant structuring and restriction of gene flow (e.g. Carreras-Carbonell *et al.* 2007) to the complete absence of structure and very high gene flow (e.g. Haney 2007). The study species used in these evaluations often possess extremes in dispersal potential and results may not apply to reef fishes in general, however *S. partitus* has an intermediate dispersal potential and so should act as a

reasonable surrogate of an average reef fish. In Chapter 3, I use microsatellite allele frequency data to assess spatial and temporal population genetic structuring of *S. partitus*, a reef fish with intermediate dispersal potential, within and among seven sites along the MBRS. This chapter has been submitted for publication in *Molecular Ecology*.

1.9 Chapter 4 Objectives

Direct assessment of dispersal in reef fishes is difficult due to the size of dispersing larvae and the enormity of the pelagic environment, so estimates of dispersal have often relied on assumptions of passive drift and the effects of larval behaviour (Roberts 1997, Cowen *et al.* 2006). Recent advances in molecular genetic techniques provide a method of genetically assigning larvae to their natal populations and can provide important insights into actual dispersal patterns (Jones *et al.* 2005, Carreras-Carbonell *et al.* 2007). In Chapter 4, I use microsatellite allele frequency data alongside multi-locus genetic assignment techniques to infer the distance and directionality of dispersal in *S. partitus* among seven sampling sites. This chapter is in the preliminary stages of preparation for submission to *Science*.

1.10 References

Almany GR, Berumen ML, Thorrold SR, Planes S, Jones GP (2007) Local replenishment of coral reef fish populations in a marine reserve. Science, 316, 742-744.

Barlow GW (1981) Patterns of parental investment, dispersal and size among coral-reef fishes. Environmental Biology of Fishes, 6, 65-85.

Carreras-Carbonell J, Macpherson E, Pascual M (2007) High self-recruitment levels in a Mediterranean littoral fish population revealed by microsatellite markers. Marine Biology, 151, 719-727.

Chittaro PM (2005) An investigation of the use of otolith microchemistry to discriminate reef fish populations and assess the movement of individuals. PhD thesis, University of Windsor.

Cole KS, Sadovy Y (1995) Evaluating the use of spawning success to estimate reproductive success in a Caribbean reef fish. Journal of Fish Biology, 47, 181-191.

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science, 311, 522-527.

DeLoach N (1999) Reef fish behaviour: Florida, Caribbean, Bahamas, 1st edn. New World Publications Inc., Jacksonville, FL.

Doherty JP, Williams DM, Sale PF (1985) The adaptive significance of larval dispersal in coral reef fishes. Environmental Biology of Fishes, 12, 81-90.

Ezer T, Thattai DV, Kjerfve B, Heyman WD (2005) On the variability of the flow along the Meso-American barrier reef system: a numerical model study of the influence of the Caribbean current and eddies. Ocean Dynamics, 55, 458-475.

Gaines SD, Gaylord B, Largier JL (2003) Avoiding current oversights in marine reserve design. Ecological Applications, 13, S32-S46.

Haney RA, Silliman BR, Rand DM (2007) A multi-locus assessment of connectivity and historical demography in the bluehead wrasse (Thalassoma bifasciatum). Heredity, 98, 294-302.

Hepburn, RI (2004) Population genetic structure in coral reef fish: spatial and temporal genetic patterns of the bicolor damselfish (Stegastes partitus). MSc thesis, University of Windsor.

Hogan JD (2007) Behaviour, recruitment and dispersal of coral reef fish larvae: Insight into the larval life stage. PhD thesis, University of Windsor.

Johannes RE (1978) Reproductive strategies of coastal marine fishes in the tropics. Environmental Biology of Fishes, 3, 741-760.

Jones GP, Planes S, Thorrold SR (2005) Coral reef fish larvae settle close to home. Current Biology, 15, 1314-1318.

Knapp RA (1993) The influence of egg survivorship on the subsequent nest fidelity of female bicolor damselfish, *Stegastes partitus*. Animal Behaviour, 46, 111-121.

Larson RJ, Julian RM (1999) Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. California Cooperative Oceanic Fisheries Investigations Reports, 40, 94-99.

Mora C, Sale P (2002) Are populations of coral reef fish open or closed? Trends in Ecology and Evolution, 17, 422-428.

Palumbi SR (1994) Genetic divergence, reproductive isolation and marine speciation. Annual Review of Ecology and Systematics, 25, 547-572.

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science, 278, 1454-1457.

Robertson DR, Green DG, Victor BC (1988) Temporal coupling of production and recruitment of larvae of a Caribbean reef fish. Ecology, 69, 370-381.

Robertson DR (1992) Patterns of lunar settlement and early recruitment in Caribbean reef fishes at Panama. Marine Biology, 114, 527-537.

Sale PF (1991) Ecology of coral reef fishes. In: The ecology of fishes on coral reefs (ed. Sale PF), pp. 3-7. Academic Press, San Diego, CA.

Sheng J, Tang L (2004) A two-way nested-grid ocean-circulation model for the Meso-American barrier reef system. Ocean Dynamics, 54, 232-242.

Shulman MJ (1998) What can population genetics tell us about dispersal and biogeographic history of coral-reef fishes? Australian Journal of Ecology, 23, 216-225.

Tang L, Sheng J, Hatcher BG, Sale PF (2006) Numerical study of circulation, dispersion and hydrodynamic connectivity of surface waters on the Belize shelf. Journal of Geophysical Research, 111, DOI 10.1029/2005JC002930.

Thorrold SR, Latkoczy C, Swart PK, Jones CM (2001) Natal homing in a marine fish metapopulation. Science, 291, 297-299.

Wellington GM, Victor BC (1989) Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). Marine Biology, 101, 557-567.

Wright S (1931) Evolution in Mendelian populations. Genetics, 16, 97-158.

2.0 CHARACTERIZATION OF ONE TRINUCLEOTIDE AND SIX DINUCLEOTIDE MICROSATELLITE MARKERS IN BICOLOR DAMSELFISH, *Stegastes partitus*, A COMMON CORAL REEF FISH.

2.1 INTRODUCTION

Polymorphic and rapidly evolving genetic markers are useful tools in developing relevant conservation regimes for fish in that they provide information on critical population dynamic parameters, such as the level of genetic connectivity among populations. Many marine fish species, including most reef fishes, exhibit a pelagic larval phase in which their potential for long distance dispersal is high, leading to the expectation that populations will be highly connected (Roberts 1997). Recent evidence, however, has shown that there may be larval retention in certain reef systems, brought about by interactions of larval behaviour with oceanographic features such as eddies and gyres (Cowen *et al.* 2002; James *et al.* 2002). Resolving this issue and determining the level of population connectivity is critical for the effective management and conservation of marine populations and the development of marine reserves and marine protected areas. Microsatellite DNA markers are particularly useful tools in this respect because they provide the ability to detect subtle population differentiation over ecologically relevant spatial and temporal scales, allowing researchers to document population differentiation with high resolution. Although the bicolor damselfish (*Stegastes partitus*) is not a species of special concern in itself, it is a valuable surrogate for many reef fish

species as it shares many of their life history characteristics in terms of larval duration and potential for dispersal.

2.2 Materials and Methods

The microsatellite enrichment protocol of Fischer and Bachman (1998) was followed for this study. Genomic DNA was extracted from the pectoral fin tissue of seven *Stegastes partitus* using a Wizard DNA extraction kit (Promega, Madison, USA). Six micrograms of combined genomic DNA were digested with six units of RsaI restriction enzyme in a 50 μL volume for 2 h at 37° C. Digested product was ligated to the MluI adaptor complex (21-mer: 5'-CTCTTGCTTACGCGTGGACTA-3' and a phosphorylated 25-mer: 5'-pTAGTCCACGCGTAAGCAAGAGCACA-3') with 200,000 U T4-DNA ligase (New England Biolabs, Ipswitch, USA) in a total volume of 60 μL. The ligation reaction was incubated for two hours at room temperature. A short repeat biotinylated oligo probe (5'-biotin$(GACA)_4$-3') was added to the ligated DNA and the mixture denatured at 95° C for 5 minutes then chilled immediately on ice. This hybridization mixture was incubated at 45° C for 15 minutes. Further hybridization to streptavidin-coated magnetic beads (Roche, Indianapolis, USA) was carried out at room temperature on a slowly rotating wheel for 1 h. Beads were then washed once with 2x SSC and 0.1% SDS for 5 min at 25° C, once with 1x SSC for 5 min at 25° C and once with 1x SSC for 2 min at 40° C ($T_{M probe}$ - 5° C). Enriched DNA was then eluted and resuspended in NANOpure water. Post enrichment amplification was performed using 1x PCR buffer (Sigma-Aldrich, Oakville, Canada), 1.5 mM $MgCl_2$ (Sigma-Aldrich, Oakville, Canada), 200 μM each dNTP and 3 U Taq polymerase (Sigma-Aldrich,

Oakville, Canada), and 1 μM 21-mer adaptor primer (see above). Amplification was performed for 30 cycles of 94° C for 1 min, 56° C for 45 s, and 72° C for 45 s with a final extension at 72° C for 5 min. PCR product (100 ng) was ligated into pCR4-TOPO vector (Invitrogen, Burlington, Canada) following manufacturer's instructions and transformed into chemically competent *Escherichia coli* TOP10 cells. White colonies were selected, cultured, and plasmid DNA was prepared via lysis by boiling according to Sambrook *et al.* (1989). M13pUC primers (F: 5'-TTGTAAAACGACGGCCAGT-3' R: 5'-GGAAACAGCTATGACCATG-3') were used to amplify plasmid DNA and PCR products were sent to the Genome Quebec Innovation Centre (McGill University, Montreal, Canada) for sequencing.

2.3 Results and Discussion

Forty-seven clones contained microsatellites and 14 primer pairs were designed using Primer3 (Rozen and Skaletsky 2000) and NetPrimer (Premier Biosoft International) software. Allelic variability was initially evaluated using 32 individuals of *Stegastes partitus*, and PCR products visualized on 1.8% agarose gels. Of the 14 primer pairs, 7 produced polymorphic bands. Dye-labelled (IR-700, IR-800 MWG Biotech) forward primers were constructed and used to amplify the 7 polymorphic loci (Accession numbers DQ676492, DQ676493, DQ676494, DQ676495, DQ676496, DQ676497, DQ676498). PCR amplification (25 μL) was then performed on 38 individuals of *Stegastes partitus* under the following conditions: approximately 100 ng template DNA, 32 μM of FWD dye-label primer and 0.5 μM of REV primer, 200 μM of each dNTPs, 0.6 U Taq polymerase (Applied Biosystems, Foster City, USA), 1x PCR buffer and locus specific

concentrations of $MgCl_2$ (Table 2.1). PCR conditions were 94° C for 2 minutes, followed by 30 cycles of 94° C for 15 sec, annealing temperature (T_A; Table 2.1) for 15 sec, 72° C for 30 sec, and a final extension of 72° C for 90 sec. The size of PCR products was estimated using a LiCor 4300 DNA Analyzer with GeneImagIR 4.05 (Scanalytics, Inc). The number of alleles ranged from 5 to 31 and observed and expected heterozygosity ranged from 0.89 – 0.65 and 0.96 – 0.66 respectively (Table 2.1). Adherence to Hardy-Weinberg equilibrium was calculated with 10,000 permutations using Tools For Population Genetic Analysis (TFPGA) v1.3 (Miller 1997 Tools for population genetic analysis 3.1: A windows program for the analysis of allozymes and molecular population genetic data http://www.marksgeneticsoftware.net/tfpga.htm) and corrected for multiple tests using the sequential Bonferroni method (significance at $P_i \leq \alpha/(1 + k - i)$) (Rice 1989).

Six of seven loci conformed to Hardy-Weinberg equilibrium (Table 2.1), while one locus did not, showing significantly reduced heterozygosity. The presence of null alleles was detected using Microchecker v.2.2.3 (Van Oosterhout *et al.* 2004 http://www.microchecker.hull.ac.uk) for the non-equilibrium locus. Linkage disequilibrium was tested using Arlequin 3.0 (Excoffier *et al.* 2005) and no significant ($p > 0.05$) linkage disequilibrium was detected among any of the seven loci. Sequential Bonferroni corrections were applied to all multiple tests (Rice 1989). Four of seven primer pairs were also effective in amplifying loci in related taxa including other *Stegastes* species as well as *Microspathodon chrysurus* (Table 2.2).

Table 2.1 Characterization of seven novel microsatellite markers for *Stegastes partitus*. The number of individuals successfully amplified and GenBank accession numbers are given under the locus name. The indicated annealing temperature (T_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) are based on 38 genotypes.

Locus	Primer sequence	Repeat motif	Clone size (bp)	T_A (°C)	[$MgCl_2$] (mM)	Allelic range (bp)	No. of alleles	H_O	H_E
SpTG_{10}[†] (37/38) DQ676492	F: GTGACTCCCGTGACGTCTTC R: TTTAAACACAGTAATCAGAGAATGAGC	$(TG)_{10}$	350	54	1.5	168 - 280	31	**0.65**	0.96
SpTG_{16} (36/38) DQ676493	F: GTGAGACAGTGGGTCACCTG R: GTTTTCCCCCTCCTCACACT	$(TG)_{16}$	200	52	2.0	205 - 149	23	0.89	0.93
SpGGA_7 (35/38) DQ676494	F: CGATATGTTTAATGTGAGGAACG R: TTTCAGGAGGTAATAGTCCACCA	$(GGA)_7$	300	48	2.5	255 - 243	5	0.45	0.66
SpTG_8 (35/38) DQ676495	F: ACGCCGAAATGCATCTTAAT R: ACACATATTCCCTCGGTTTTT	$(TG)_8$	160	48	2.5	211 - 169	19	0.83	0.92
SpTG_{53} (35/38) DQ676496	F: GATGGCCTCTGGTGTAATGC R: CAACTGGGAAGGAGGTCAAG	$(TG)_{53}$	350	48	1.5	254 - 150	29	0.77	0.94
SpTG_{13} (37/38) DQ676497	F: CTTGTTCCTTGGCTTCTTGG R: TGATAGTGGCAAGCAATGGA	$(TG)_{13}$	338	49	2.5	236 - 228	5	0.51	0.70
SpGT_{10} (34/38) DQ676498	F: GGCCTGTTTAAAGGTCACCA R: CACCAACGAGCTACGGTGTA	$(GT)_{10}$	470	55	2.5	289 - 235	12	0.71	0.86

[†] Optimal PCR conditions for this locus were determined to be 35 cycles with denaturation, annealing and extension times of 30 sec, 30 sec and 45 sec respectively. Significant departure from Hardy Weinberg equilibrium ($P < 0.05$) indicated in bold and underlined.

Effective study of reef fish population dynamics often requires many microsatellite markers to allow resolution of relatively weak population structure. These seven novel microsatellites complement those already available (Williams *et al.* 2003) and will increase the pool of loci to a number that will allow powerful population analysis using genotype assignment tests. Successful assignment of juvenile fish to genetically characterized adult populations will provide a quantitive estimate of the rate and extent of larval dispersal. This information can then be applied to the effective design of conservation strategies including the delineation of the boundaries of marine protected areas.

Table 2.2 PCR amplification in damselfish genera *Stegastes* and *Microspathodon* using microsatellite primer sets developed for *S. partitus*. Dash indicates lack of amplification. Numbers indicate number of individuals amplified, and in parentheses the number of alleles detected.

Locus	*Stegastes leucostictus*	*Stegastes planifrons*	*Stegastes adustus*	*Microspathodon chrysurus*
SpTG10	-	-	-	-
SpTG16	-	-	-	-
SpGGA7	6 (2)	5 (2)	2 (2)	3 (1)
SpTG8	-	-	-	-
SpTG53	5 (5)	4 (7)	1 (1)	2 (2)
SpTG13	6 (2)	5 (2)	2 (1)	1 (1)
SpGT10	6 (4)	5 (4)	1 (1)	4 (1)

2.4 REFERENCES

Cowen RK, Paris CB, Olson DB, Fortuna JL (2002) The role of long distance dispersal versus local retention in replenishing marine populations. Gulf and Caribbean Research Supplement, p 1

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinfo Online 1:47-50

Fischer D, Bachman, K (1998) Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). Biotechniques 24:196-800

James MK, Armsworth PR, Mason LB, Bode L (2002) The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. Proc Royal Soc Lon. B 269:2079-2086

Rice WR (1989) Analyzing tables of statistical tests. Evol 43:223-225

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science, 278:1454-1457

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misnener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa New Jersey, p 365

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning. A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes, 4:535-538

Williams DA, Purcell J, Hughes CR, Cowen RK (2003) Polymorphic microsatellite loci for population studies of the bicolor damselfish, *Stegastes partitus* (Pomacentridae). Mol Ecol Notes, 3:547-549.

3.0 SPATIAL AND TEMPORAL INSTABILITY IN THE GENETIC STRUCTURE OF ADULT AND JUVENILE BICOLOR DAMSELFISH (*Stegastes partitus*) ALONG THE MESOAMERICAN BARRIER REEF

3.1 INTRODUCTION

The formation of genetic structure at neutral loci is primarily the result of the opposing forces of drift, which serves to differentiate populations through the random selection of genotypes, and gene flow, which homogenizes allele frequencies among populations (Wright 1931). If gene flow is restricted between two populations, genetic differentiation will result as the effects of drift, selection, and mutation accumulate. Such processes drive local adaptation (Plath *et al.* 2007), and eventually may lead to speciation as populations diverge and gene flow is further restricted by the evolution of reproductive barriers (Palumbi 1994). Clearly, gene flow has important evolutionary and ecological implications as it acts to erode genetic differentiation; however, it is often difficult to quantify as it involves not only the movement of individuals, but also their reproductive success. The reproductive success of migrants (i.e. gene flow) is influenced by a variety of life history traits, most notably those affecting the ability of an organism to disperse. As dispersal and gene flow increase, populations become demographically and genetically linked, leading to population connectivity. Understanding connectivity among populations is valuable in the management of marine populations as it quantifies their dependency on local processes (Roberts 1997, Gaines *et al.* 2003), and has important implications for ecology and evolution as it erodes the effects of local adaptation and the process of speciation (Palumbi 1994).

Many marine organisms have a pelagic larval phase, giving them the potential to disperse hundreds of kilometers in a single generation. The common perception is that this dispersal potential creates high levels of gene flow among populations, leading to genetic homogenization over large areas. Wright (1931) showed that for neutral genes, a single reproductively successful migrant between each pair of subpopulations every other generation should theoretically produce enough gene flow to homogenize these subpopulations over time. Given the high reproductive output of most marine species, it is thus logical to infer genetic homogenization, and this has been shown for many species (e.g., Shulman and Bermingham 1995, Haney *et al.* 2007). However, other studies have found contradictory results, showing fine scale genetic heterogeneity both through time and space (see Larson and Julian 1999 for review). Four main hypotheses have been put forth to explain the temporal and spatial heterogeneity in genetic composition of dispersing marine species: (1) localized post-settlement selection due to micro-geographic variation in environmental conditions (Johnson and Black 1982, Johannesson *et al.* 1995), or pre-settlement effects generating juvenile variability such as (2) spatial variability in cohort source (Roberts 1997, Purcell *et al.* 1996) (3) temporal variability in reproductive success of the source adult populations ("sweepstakes chance-matching"; Hedgecock 1994) and (4) temporal variability in cohort source as a result of stochastic processes (Cowen *et al.* 2006). These hypotheses are not mutually exclusive, and in fact may act jointly to produce the temporally unstable genetic structure sometimes reported in marine systems. Since these hypotheses generate specific predictions at both the juvenile and adult life stages, it is important to examine both life stages over various spatial and temporal scales to characterize population genetic structure in marine organisms with high dispersal capacity.

Stegastes partitus is a demersally spawning damselfish common throughout the Caribbean with a larval duration of approximately 30 days. It thus has a moderate to high dispersal capacity and is expected to have limited or no genetic structure. I chose *S. partitus* as a model organism to examine genetic connectivity among populations over time. This study was conducted in the Mesoamerican Barrier Reef System (MBRS), which extends from Mexico through Honduras and provides the opportunity to examine population genetic structure on various spatial scales. The one-dimensional nature of a near-continuous barrier reef makes it ideal for the study of connectivity and population structure as it simplifies the potential patterns of population connectivity. Also, the populations of reef fishes in this region have been extensively studied. Genetic population structure and stability of *S. partitus* was assessed using variable allozyme markers, and both fine-scale population structuring and temporal instability were demonstrated (Lacson *et al.* 1989, Lacson & Morizot 1991). Hepburn (2004) used microsatellite markers to show that juvenile *S. partitus* exhibited temporally unstable genetic structure at small geographic scales along the MBRS, attributing this to stochastic dispersal patterns and micro-geographic variation. Also, recruitment to these populations has been characterized over multiple years by the "Ecological connections among reefs" project, and otolith microchemical signatures have been used to infer dispersal movements and patch dynamics of *S. partitus* across the MBRS (Chittaro 2005, Hogan 2007). Furthermore, oceanographic processes relevant to population connectivity in this region have been characterized (Sheng and Tang 2004, Ezer *et al.* 2005, Tang *et al.* 2006).

Seven sites along the MBRS were sampled, encompassing both barrier reef and atoll regions, over two years and collected adult and recently settled juvenile *S. partitus* at

each site. Each fish was genotyped at 12 microsatellite loci in order to address three main questions: First, is there spatial genetic heterogeneity among populations of *S. partitus* in the MBRS? Second, are there genetic differences between adult and juvenile populations? Finally, is the genetic structure within and among these populations stable through time?

3.2 Materials and Methods

Study Species: The bicolor damselfish, *Stegastes partitus*, is a small, territorial, pomacentrid common on reefs throughout the tropical Western Atlantic (DeLoach 1999). Adults spawn demersally on a unimodal lunar cycle and the males provide paternal care, with seasonal reproductive peaks from April to November (Robertson *et al.* 1988). After hatch, (~3.5 days), pelagic larval duration ranges between 27-31 days (Robertson *et al.* 1988, Wellington and Victor 1989), and after settlement adults are sedentary (DeLoach 1999). This life history allows us to examine the population genetics of sedentary adults as well as recently dispersed juveniles to infer the nature of gene flow between populations.

Field Sampling: Adult and recently settled individuals were sampled by divers using hand nets from seven fore-reef sites each year (Figure 3.1) at approximately 10m depth. Adult individuals were selected by eye to be over 38mm fork length, as fish were found to be mature above 38mm based on gonadal development (R. Thiessen, unpublished data). Newly settled juveniles were identified as being less than 25mm fork length, corresponding to less than two weeks post-settlement (Hogan 2007).

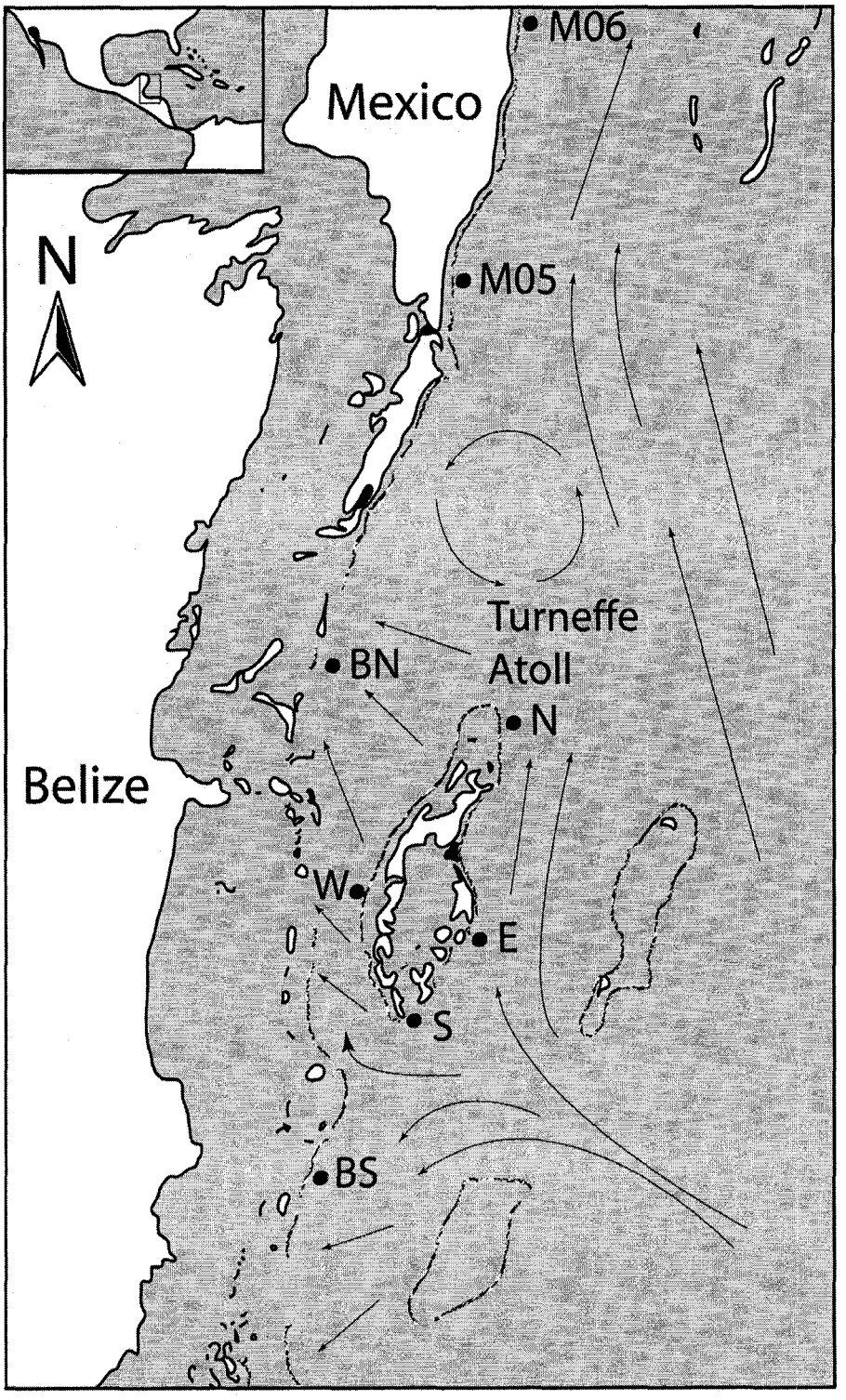

Figure 3.1 Sampling sites along the Mesoamerican barrier reef; BN = barrier reef North, BS = barrier reef South, N = Turneffe North, S = Turneffe South, E = Turneffe East, W = Turneffe West, M05 = Mexico (2005), M06 = Mexico (2006). Arrows indicate predominant hydrodynamic flow in the region as modelled by Ezer *et al.* (2005). Site M05 was only sampled in 2005, site M06 was only sampled in 2006.

Approximately 100 adults and 50 juveniles were collected at each site in both years. The size (fork length) of the adults ranged from 31-71mm, with 14 of 1168 individuals falling below the average size at maturity, and 10-30mm in juveniles, none of which were sexually mature. All samples were included in subsequent analyses. Due to logistical issues, the Mexico site sampled in 2005 (M05, Figure 3.1) could not be sampled in 2006, so a site was chosen further north for 2006 (M06, Figure 3.1). Sampling occurred from June 10 - July 5 in 2005 and April 19 - 29 in 2006. Although the time of sampling varies by 2 months between years, this should not bias our temporal results as both sampling periods occur within the peak spawning period for *S. partitus*. All fish were collected from a site in a single day except at east Turneffe in 2005, where adults were collected over a two week period and juveniles were collected in one day. Fish were held on ice until return to the field station where tissue samples were taken and preserved in 95% ethanol or a salt preservation solution (0.020 M EDTA, 0.025 M Sodium Citrate trisodium salt dehydrate, 5.3 M Ammonium sulphate) for 2005 and 2006 samples respectively.

Genotyping: Genomic DNA was extracted from pectoral fin tissue of 72-93 adult and 40-52 juvenile individuals per site using a Wizard DNA extraction kit (Promega, Madison, USA) or following the silica-based 96-well plate protocol of Elphinstone *et al.* (2003) for 2005 and 2006 samples respectively. Microsatellite loci were chosen from Williams *et al.* (2003) and Thiessen & Heath (2006), screened for suitability, and four were modified for size to facilitate running multiple loci on the DNA analyzer (Table 3.1). PCR amplification was then performed in 12μL reactions comprised of: approximately 100ng template DNA, 32 μM of forward dye-label primer and 0.5 μM of reverse primer, 200

μM of each dNTPs, 0.1 U Taq polymerase (Invitrogen, Burlington, Canada), 1x PCR buffer (provided by the manufacturer) and locus specific concentrations of $MgCl_2$ (Table

Table 3.1 Primer sequences, annealing temperatures, $MgCl_2$ concentration and base pair size ranges of amplified fragments for molecular markers used in microsatellite analysis of bicolor damselfish from MBRS. Primer sequences from Williams *et al.* (2003) and Thiessen & Heath (2006); primers marked with * were modified from the published primer sequence for size; optimal denaturation, annealing and extension times for primers marked with † were determined to be 30 s, 30 s, 45 s respectively; primers marked with ‡ use 35 PCR cycles.

Locus	Primer Sequence 5'–3'	T_A (°C)	[$MgCl_2$] (mM)	Allelic Range (bp)
*Sp*GATA$_{40}$	TTGCCTGCTGATAATTAACG ATGCCTCACAATGATGTATATTT	48	2.5	118-330
*Sp*AAT$_{40}$ *†	TGTTTCACCTGACATCCAAGA AGCCTCCCACTGAACACACT	48	2.5	121-211
*Sp*AAC$_{44}$ *	TGCTGTAAACCACCAGGAGA GCAAACAGAAGGAGCAGTGG	48	2.5	120-189
*Sp*AAC$_{33}$ *	TCACACCTGCTGAGTTCCTG CATGTACCTCCAATACAGGAAAAA	60	2.5	96-180
*Sp*AAC$_{41}$ *†	AGTCTGTGGTTTTGCCAACAT TGGTGCAGTTATTGCTTAGA	56	2.5	128-353
*Sp*TG$_{10}$ †‡	GTGACTCCCGTGACGTCTTC TTTAAACACAGTAATCAGAGAATGAGC	54	1.5	147-337
*Sp*TG$_{16}$	GTGAGACAGTGGGTCACCTG GTTTTCCCCCTCCTCACACT	52	2.0	131-225
*Sp*GGA$_{7}$	CGATATGTTTAATGTGAGGAACG TTTCAGGAGGTAATAGTCCACCA	48	2.5	229-262
*Sp*TG$_{8}$	ACGCCGAAATGCATCTTAAT ACACATATTCCCTCGGTTTTT	48	2.5	145-289
*Sp*TG$_{53}$	GATGGCCTCTGGTGTAATGC CAACTGGGAAGGAGGTCAAG	48	1.5	145-303
*Sp*TG$_{13}$	CTTGTTCCTTGGCTTCTTGG TGATAGTGGCAAGCAATGGA	49	2.5	216-242
*Sp*GT$_{10}$	GGCCTGTTTAAAGGTCACCA CACCAACGAGCTACGGTGTA	55	2.5	168-316

3.1). PCR conditions were 94°C for 2 min, followed by 30 cycles of 94°C for 15 s, annealing temperature (T_A; Table 3.1) for 15 s, 72°C for 30 s, and a final extension of 72°C for 90 s. The size of the PCR products was estimated using a LiCor 4300 DNA Analyzer with GeneImagIR 4.05 software (Scanalytics, Inc).

Genetic Analyses: Genotype data were separated into adult and juvenile populations for both 2005 and 2006 sampling seasons at each of the seven sampling sites. Exact tests for goodness of fit to Hardy-Weinberg equilibrium using the Markov Chain method (1000 permutation burn-in followed by 100,000 permutations) for each locus within each population were performed in ARLEQUIN v3.11 (Excoffier *et al.* 2005) and results were adjusted for multiple comparisons using the sequential Bonferroni correction (Rice 1989). Tests for linkage disequilibrium were performed on all 2005 and 2006 populations in ARLEQUIN v3.11 (Excoffier *et al.* 2005).

Genetic Structure and Temporal Stability Analyses: Pair-wise F_{ST} values were calculated to quantify the extent of differentiation between populations within each year using ARLEQUIN v3.11 (Excoffier *et al.* 2005) and pair-wise exact tests for differences in allele frequency distributions between populations were performed in TOOLS FOR POPULATION GENETIC ANALYSIS (TFPGA) v1.3 (MP Miller 1997 Tools for Population Genetic Analysis (TFPGA) 1.3: A windows program for the analysis of allozymes and molecular population genetic data. Computer software distributed by author). Exact test results were adjusted for multiple comparisons using the sequential Bonferroni correction (Rice 1989). Cavalli-Sforza and Edwards' (1967) chord distance (D_C) was calculated to estimate pair-wise genetic distance between all populations using the software POPULATIONS v1.2.28

(Langella 2002), and allelic richness was calculated using FSTAT v2.9.3.2 software (Goudet 1995). Average heterozygosity and allelic richness across loci were compared between adult and juvenile populations at the same site using paired two-tailed T-tests. Within each adult population, the largest and smallest 25% of individuals (N = 21-28 individuals for either size class) were tested for differences in allelic composition between groups using exact tests in TFPGA.

Measures of both genetic differentiation (F_{ST}) and genetic distance (D_C) were correlated with shortest water distance (km) to test for isolation by distance within years in both adult and juvenile populations using Mantel tests in GENALEX software (Peakall and Smouse 2006). Temporal stability of populations over the approximately 10 months separating the two sampling periods was assessed by two methods. First, exact tests were used to detect changes in allelic composition within each population over time, and were adjusted for multiple comparisons using the sequential Bonferroni correction (Rice 1989). Second, the stability of among-population genetic structure was assessed by testing for a correlation between adult pair-wise population divergence between years, measured with both F_{ST} and D_C values, using a Mantel test in GENALEX (Peakall and Smouse 2006). The temporal stability of juvenile populations between years as well as the correlation between juvenile population structure in 2005 and adult population structure in 2006 was assessed in the same manner. Patterns were consistent between measures of pair-wise population divergence, so only D_C values are reported due to their relative insensitivity to departures from Hardy-Weinberg equilibrium.

To test for sweepstakes effects I performed a linear regression of mean pair-wise D_C values against mean within-population relatedness for all juvenile populations in 2005 and 2006 individually as well as with both years combined. If population genetic

structure is driven by sweepstakes-chance matching, one would expect juveniles to be more related in genetically differentiated populations, as the observed differentiation would be the result of an influx of juveniles produced by a discreet subset of an adult population.

3.3 RESULTS

Genetic Analysis: For 2005 samples, 53 of 168 tests showed significant deviations from Hardy-Weinberg equilibrium after Bonferroni correction (Table 3.2). Locus *Sp*AAC$_{41}$ deviated from expected values in all but one population. Forty five percent (19/42) of the remaining deviations were attributed to two loci: *Sp*TG$_{10}$ and *Sp*TG$_{8}$. For 2006 samples, 45 of 168 tests showed significant deviations from Hardy-Weinberg equilibrium (Table 3.2). Locus *Sp*TG$_{10}$ deviated from expected values in 10 of the 14 populations. Forty percent (14/35) of the remaining deviations were attributed to two loci; *Sp*AAC$_{41}$ and *Sp*TG$_{8}$. Replicated analyses showed little qualitative change in results when *Sp*AAC$_{41}$, *Sp*TG$_{10}$ and *Sp*TG$_{8}$ were removed from the dataset, so all loci were included in subsequent tests. Approximately 4% (75/1848) of exact tests showed significant linkage disequilibrium after sequential Bonferroni correction, however no locus-pair showed consistent linkage at more than 16 of 28 populations over the two years sampled (Table 3.3).

Genetic structure: In 2005, adult F_{ST} values were low (global F_{ST} = 0.006 95% CI 0.002 – 0.013), but 12 of 21 pair-wise comparisons showed F_{ST} values significantly greater than zero (Table 3.4). Pair-wise comparisons involving adult Barrier South (BS) or adult

Table 3.2 Number of individuals (N), allelic richness (A), observed heterozygosity (H_O) and expected heterozygosity (H_E) for adult and juvenile populations collected from seven sites on the MBRS. Populations identified as in figure 3.1. Significant departures from Hardy-Weinberg equilibrium after sequential Bonferroni correction indicated in bold and underlined.

			Barrier Reef				Turneffe Atoll								Mexico	
Locus	Year		BN_{Adult}	BN_{Juv}	BS_{Adult}	BS_{Juv}	N_{Adult}	N_{Juv}	S_{Adult}	S_{Juv}	E_{Adult}	E_{Juv}	W_{Adult}	W_{Juv}	M_{Adult}	M_{Juv}
*Sp*GATA$_{40}$	2005	N	83	46	85	40	90	44	84	49	92	46	92	45	78	41
		A	21.6	21.9	22.6	22.7	24.2	20.7	21.2	20.0	23.8	20.5	23.9	25.4	19.3	24.8
		H_O	0.83	0.83	0.85	**0.78**	0.88	0.89	0.71	0.92	0.89	**0.85**	0.90	0.96	**0.73**	0.95
		H_E	0.96	0.96	0.96	0.96	0.96	0.95	0.95	0.95	0.96	0.95	0.96	0.97	0.94	0.97
	2006	N	63	44	69	42	66	35	87	46	79	40	71	48	87	45
		A	24.8	26.1	25.2	28.8	25.5	23.0	24.1	24.0	22.9	26.9	24.2	27.7	26.0	28.4
		H_O	0.78	0.82	0.81	0.86	**0.71**	**0.63**	**0.85**	**0.80**	**0.77**	0.90	**0.86**	0.90	**0.83**	0.84
		H_E	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.95	0.95	0.96	0.96	0.96	0.96	0.97
*Sp*AAT$_{40}$	2005	N	78	50	87	40	90	44	84	50	90	45	92	46	83	41
		A	12.4	11.9	12.7	14.1	13.2	14.1	13.8	14.0	13.0	13.4	13.2	13.1	14.2	12.8
		H_O	0.88	0.82	0.87	0.88	0.86	0.86	0.86	0.84	0.89	0.98	0.90	0.93	0.90	0.85
		H_E	0.90	0.90	0.89	0.93	0.87	0.89	0.90	0.90	0.89	0.91	0.91	0.90	0.90	0.90
	2006	N	70	44	71	46	73	48	87	46	79	42	76	48	91	42
		A	14.2	15.9	15.6	15.7	14.2	15.0	15.5	15.0	14.1	14.5	15.0	15.3	14.7	13.6
		H_O	0.89	0.89	0.89	0.91	0.85	0.77	0.84	0.80	0.86	0.88	0.87	0.81	0.84	0.81
		H_E	0.89	0.92	0.90	0.89	0.90	0.88	0.90	0.89	0.89	0.91	0.86	0.88	0.90	0.89

*Sp*AAC$_{44}$	2005	N	82	52	76	40	89	44	81	50	89	45	91	48	73	38
		A	8.0	6.8	7.3	7.9	6.9	4.9	6.2	7.8	6.3	7.1	7.0	6.5	7.7	8.0
		H_O	0.41	0.38	**0.28**	0.38	**0.28**	**0.20**	0.30	0.32	0.28	**0.33**	**0.18**	0.29	0.38	0.42
		H_E	0.46	0.43	0.64	0.45	0.53	0.59	0.29	0.37	0.31	0.54	0.35	0.34	0.43	0.51
	2006	N	73	36	85	47	69	48	90	44	88	41	80	48	85	48
		A	7.6	5.9	7.7	8.6	8.4	6.8	8.4	7.4	9.3	9.5	7.4	7.1	8.5	8.1
		H_O	0.33	0.17	0.29	0.32	0.36	0.42	0.34	0.30	0.35	0.37	0.35	0.27	0.35	0.35
		H_E	0.39	0.16	0.31	0.33	0.38	0.41	0.37	0.40	0.45	0.46	0.38	0.34	0.40	0.40
*Sp*AAC$_{33}$	2005	N	85	46	82	35	90	42	86	47	90	46	91	48	84	40
		A	12.6	11.8	19.0	12.7	12.8	13.2	11.9	11.2	13.9	11.3	12.2	13.1	11.3	12.8
		H_O	**0.65**	0.67	**0.82**	0.66	**0.73**	0.67	**0.67**	0.74	0.82	0.76	**0.69**	0.81	0.80	**0.75**
		H_E	0.90	0.90	0.94	0.86	0.90	0.89	0.87	0.89	0.90	0.87	0.90	0.90	0.87	0.89
	2006	N	68	38	87	47	73	47	90	42	89	41	81	39	93	48
		A	12.8	11.9	12.1	12.1	13.3	12.7	14.4	9.8	13.8	14.2	12.7	12.9	11.6	13.2
		H_O	0.78	0.84	0.79	0.83	0.74	0.91	0.80	0.74	0.87	0.73	0.70	0.72	0.77	0.71
		H_E	0.89	0.88	0.88	0.89	0.88	0.89	0.89	0.85	0.90	0.89	0.88	0.89	0.87	0.89
*Sp*AAC$_{41}$	2005	N	76	49	81	31	82	42	87	49	92	47	79	43	78	38
		A	21.6	19.1	22.2	17.3	17.3	17.5	22.9	19.2	22.8	22.0	17.5	20.9	20.8	17.8
		H_O	**0.55**	**0.61**	**0.62**	**0.42**	**0.48**	**0.43**	**0.82**	**0.47**	0.87	**0.81**	**0.51**	**0.53**	**0.63**	**0.50**
		H_E	0.95	0.95	0.95	0.95	0.94	0.93	0.95	0.93	0.96	0.96	0.94	0.96	0.95	0.92
	2006	N	68	44	81	46	69	45	88	48	72	41	75	48	85	37
		A	20.2	23.7	21.4	23.2	36.3	24.4	23.0	27.4	21.3	24.0	18.4	24.7	17.9	16.9
		H_O	**0.51**	0.93	**0.51**	0.80	0.71	0.96	**0.76**	**0.81**	**0.33**	0.85	**0.49**	0.81	0.34	0.57
		H_E	0.95	0.95	0.95	0.95	0.98	0.96	0.95	0.96	0.95	0.96	0.94	0.96	0.93	0.95

*Sp*TG$_{10}$	2005	N	82	40	86	36	79	43	82	39	73	43	67	45	73	39
		A	29.9	28.8	29.8	31.3	28.8	29.5	29.7	29.1	28.5	27.1	27.3	30.0	29.1	27.3
		H_O	0.66	0.60	**0.62**	0.78	**0.61**	**0.79**	**0.65**	**0.79**	0.53	0.58	**0.49**	**0.73**	0.74	0.64
		H_E	0.98	0.97	0.97	0.98	0.97	0.98	0.97	0.98	0.97	0.97	0.97	0.97	0.97	0.97
	2006	N	69	37	74	43	75	45	88	42	87	44	77	43	92	47
		A	29.3	31.3	34.1	28.8	37.7	30.0	34.0	33.0	35.7	31.8	34.2	30.0	34.8	32.2
		H_O	**0.41**	**0.68**	**0.61**	**0.63**	0.73	**0.58**	**0.69**	**0.64**	**0.75**	**0.64**	**0.70**	0.74	0.67	0.45
		H_E	0.97	0.98	0.97	0.97	0.98	0.97	0.97	0.97	0.98	0.97	0.98	0.97	0.97	0.98
*Sp*TG$_{16}$	2005	N	83	51	78	40	88	39	86	48	76	39	88	48	83	39
		A	20.0	21.2	23.1	22.2	20.1	20.5	20.9	21.4	19.3	19.0	21.8	22.6	20.1	22.7
		H_O	0.88	0.96	0.88	0.85	0.85	0.79	0.92	0.85	**0.80**	0.90	0.84	0.83	0.82	0.85
		H_E	0.94	0.95	0.96	0.956	0.95	0.95	0.95	0.96	0.94	0.94	0.95	0.96	0.95	0.96
	2006	N	64	44	81	47	73	48	89	46	81	38	79	47	79	48
		A	22.2	25.7	22.2	22.0	23.2	25.1	27.8	24.6	25.8	21.4	23.9	23.9	24.7	27.9
		H_O	**0.78**	0.82	0.88	0.85	**0.82**	0.92	**0.87**	**0.89**	0.89	0.79	0.85	0.81	**0.87**	0.92
		H_E	0.95	0.96	0.95	0.95	0.95	0.96	0.96	0.95	0.96	0.94	0.95	0.96	0.96	0.96
*Sp*GGA$_7$	2005	N	83	46	83	39	90	43	79	48	88	47	84	46	83	41
		A	4.4	4.5	4.5	3.6	4.6	4.0	4.5	4.5	4.0	4.8	5.7	4.1	7.3	4.8
		H_O	0.65	0.67	0.54	0.64	0.58	0.72	0.48	0.63	0.49	0.45	0.49	**0.20**	**0.59**	0.54
		H_E	0.58	0.68	0.60	0.55	0.59	0.62	0.61	0.60	0.56	0.64	0.58	0.48	0.71	0.61
	2006	N	66	41	80	46	60	47	90	37	74	43	72	37	89	48
		A	4.9	4.0	5.5	5.5	4.8	4.7	4.0	4.9	5.2	4.7	5.2	4.9	4.2	4.7
		H_O	0.62	0.56	0.63	0.46	0.55	0.57	0.57	0.49	0.43	0.60	0.58	0.65	0.49	0.46
		H_E	0.62	0.63	0.60	0.58	0.63	0.60	0.59	0.63	0.63	0.63	0.59	0.64	0.56	0.57

*Sp*TG$_8$	2005	N	78	51	86	38	87	37	78	42	81	32	79	47	85	41
		A	17.5	19.7	17.8	21.0	18.0	17.3	18.4	17.1	19.3	19.3	19.2	21.0	18.8	16.9
		H_O	**0.73**	**0.65**	0.77	**0.71**	**0.70**	**0.57**	**0.76**	0.60	**0.69**	**0.67**	**0.62**	**0.64**	**0.75**	**0.63**
		H_E	0.94	0.95	0.93	0.95	0.94	0.92	0.94	0.93	0.94	0.95	0.95	0.95	0.94	0.91
	2006	N	68	40	80	45	71	44	90	45	81	43	79	47	78	44
		A	21.5	20.8	22.3	20.4	21.4	17.1	23.5	19.0	21.6	19.8	24.9	20.0	20.1	19.9
		H_O	**0.69**	**0.70**	**0.68**	0.82	**0.59**	0.77	0.77	**0.58**	**0.69**	0.77	0.82	**0.72**	**0.64**	0.66
		H_E	0.94	0.93	0.95	0.93	0.94	0.93	0.95	0.94	0.95	0.93	0.95	0.94	0.95	0.93
*Sp*TG$_{53}$	2005	N	77	40	87	39	87	44	87	50	92	43	89	48	83	41
		A	22.4	20.6	20.6	20.7	20.5	24.0	22.5	23.1	21.5	17.1	21.1	23.5	21.2	19.6
		H_O	0.86	0.85	0.87	0.87	0.83	0.95	0.86	0.92	0.91	0.79	0.91	0.88	0.88	0.85
		H_E	0.94	0.93	0.93	0.93	0.93	0.94	0.94	0.93	0.94	0.91	0.94	0.94	0.94	0.93
	2006	N	68	35	87	47	71	47	87	46	90	44	81	41	93	42
		A	26.0	20.0	24.2	25.5	23.1	28.8	26.7	24.2	26.6	26.7	27.6	16.8	24.2	25.6
		H_O	**0.84**	**0.57**	0.87	0.89	**0.79**	0.96	0.91	0.89	0.90	0.82	**0.91**	**0.73**	0.85	**0.76**
		H_E	0.95	0.92	0.94	0.94	0.94	0.94	0.95	0.94	0.94	0.94	0.95	0.89	0.93	0.93
*Sp*TG$_{13}$	2005	N	82	50	85	25	83	28	85	45	91	46	90	45	82	36
		A	6.1	6.9	13.8	5.0	5.6	6.0	6.6	6.0	6.5	6.1	6.9	4.9	5.8	7.0
		H_O	0.70	0.80	0.74	0.68	0.67	0.64	0.64	0.60	0.62	**0.52**	**0.50**	0.58	0.53	0.67
		H_E	0.78	0.75	0.81	0.68	0.70	0.73	0.76	0.75	0.74	0.77	0.78	0.67	0.66	0.70
	2006	N	66	41	87	47	74	46	90	44	90	38	72	47	78	47
		A	6.6	6.8	7.2	6.7	7.1	6.7	6.8	6.8	6.9	8.6	5.0	6.5	7.2	5.9
		H_O	**0.61**	0.68	**0.77**	0.64	0.62	0.72	0.70	0.61	0.62	0.79	0.56	0.57	0.68	0.74
		H_E	0.79	0.76	0.72	0.78	0.74	0.72	0.74	0.78	0.73	0.74	0.70	0.73	0.79	0.71

*Sp*GT$_{10}$	2005	N	83	46	85	32	90	37	75	42	76	47	93	48	72	41
		A	13.3	13.5	12.8	13.6	12.3	11.2	12.8	12.3	13.3	13.8	12.8	13.0	11.1	12.5
		H_O	0.70	0.85	0.84	0.91	0.67	0.89	0.81	0.74	0.83	0.89	**0.78**	0.98	**0.74**	0.90
		H_E	0.76	0.89	0.89	0.88	0.70	0.89	0.89	0.90	0.89	0.89	0.90	0.89	0.88	0.90
	2006	N	67	36	84	36	61	42	81	44	82	38	79	39	93	36
		A	11.9	10.0	18.5	12.9	14.2	12.6	13.9	11.5	12.3	10.9	12.6	13.6	13.0	11.0
		H_O	0.82	0.69	0.77	0.72	0.87	0.83	0.83	**0.66**	0.85	0.92	0.86	0.82	0.83	0.72
		H_E	0.90	0.88	0.90	0.88	0.90	0.90	0.90	0.88	0.90	0.88	0.89	0.89	0.86	0.90

Table 3.3 Summary of linkage disequilibrium tests for 12 microsatellite markers at 7 adult and juvenile populations of *Stegastes partitus* over 2 years (N = 28 populations total). Values indicate the number of populations at which significant linkage disequilibrium was detected after sequential Bonferroni correction using exact tests.

	*Sp*GATA$_{40}$	*Sp*AAT$_{40}$	*Sp*AAC$_{44}$	*Sp*AAC$_{33}$	*Sp*AAC$_{41}$	*Sp*TG$_{10}$	*Sp*TG$_{16}$	*Sp*GGA$_{7}$	*Sp*TG$_{8}$	*Sp*TG$_{53}$	*Sp*TG$_{13}$
*Sp*AAT$_{40}$	0	-------									
*Sp*AAC$_{44}$	0	0	-------								
*Sp*AAC$_{33}$	0	2	0	-------							
*Sp*AAC$_{41}$	3	1	3	2	-------						
*Sp*TG$_{10}$	5	1	1	1	16	-------					
*Sp*TG$_{16}$	0	0	2	1	2	3	-------				
*Sp*GGA$_{7}$	0	0	0	0	0	0	0	-------			
*Sp*TG$_{8}$	1	1	0	1	6	8	2	0	-------		
*Sp*TG$_{53}$	0	0	0	0	0	3	1	0	0	-------	
*Sp*TG$_{13}$	0	0	1	0	1	1	0	0	0	0	-------
*Sp*GT$_{10}$	0	0	1	1	1	2	1	0	0	0	0

Table 3.4 F_{ST} values (below diagonal) and D_C values with significance of exact tests (above diagonal) for 2005 and 2006 adult populations of *Stegastes partitus* from seven collection sites along the MBRS. Populations identified as in Figure 3.1. Significant values after sequential Bonferroni correction indicated in bold and underlined. Negative F_{ST} values are not significantly different from zero.

		BN_{Adult}	BS_{Adult}	N_{Adult}	S_{Adult}	E_{Adult}	W_{Adult}	M_{Adult}
2005	BN_{Adult}	-------	**0.614**	**0.564**	**0.577**	**0.571**	**0.573**	**0.585**
	BS_{Adult}	**0.011**	-------	**0.598**	**0.613**	**0.609**	**0.606**	**0.626**
	N_{Adult}	0.002	**0.006**	-------	**0.600**	**0.592**	**0.563**	**0.587**
	S_{Adult}	0.001	**0.015**	**0.004**	-------	**0.569**	**0.580**	**0.582**
	E_{Adult}	-0.001	**0.012**	0.000	0.000	-------	**0.577**	**0.580**
	W_{Adult}	-0.003	**0.011**	**0.004**	-0.001	0.002	-------	**0.584**
	M_{Adult}	**0.003**	**0.014**	0.002	**0.005**	**0.003**	**0.001**	-------
2006	BN_{Adult}	-------	**0.246**	**0.281**	**0.251**	**0.247**	**0.245**	**0.236**
	BS_{Adult}	-0.001	-------	**0.252**	**0.232**	**0.245**	**0.246**	**0.231**
	N_{Adult}	0.002	-0.002	-------	**0.248**	**0.273**	**0.259**	**0.242**
	S_{Adult}	-0.001	-0.003	0.000	-------	**0.221**	**0.230**	**0.219**
	E_{Adult}	0.001	0.000	0.000	-0.003	-------	**0.237**	**0.228**
	W_{Adult}	0.001	0.000	-0.001	-0.001	0.000	-------	**0.233**
	M_{Adult}	0.001	-0.002	0.000	-0.002	-0.001	0.001	-------

Table 3.5 F_{ST} values (below diagonal) and D_C values with significance of exact tests (above diagonal) for 2005 and 2006 juvenile populations of *Stegastes partitus* from seven collection sites along the MBRS. Populations identified as in Figure 3.1. Significant values after sequential Bonferroni correction indicated in bold and underlined. Negative F_{ST} values are not significantly different from zero.

		BN_{Juv}	BS_{Juv}	N_{Juv}	S_{Juv}	E_{Juv}	W_{Juv}	M_{Juv}
2005	BN_{Juv}	-------	**0.663**	**0.662**	**0.634**	**0.650**	**0.676**	**0.648**
	BS_{Juv}	-0.005	-------	**0.648**	**0.640**	**0.683**	**0.673**	**0.665**
	N_{Juv}	0.003	**0.014**	-------	**0.639**	**0.651**	**0.691**	**0.681**
	S_{Juv}	-0.003	-0.003	**0.012**	-------	**0.640**	**0.651**	**0.658**
	E_{Juv}	-0.007	-0.010	0.000	-0.001	-------	**0.671**	**0.650**
	W_{Juv}	0.002	-0.001	**0.015**	0.001	0.003	-------	**0.671**
	M_{Juv}	-0.003	-0.002	**0.009**	0.001	-0.002	**0.011**	-------
2006	BN_{Juv}	-------	**0.302**	**0.294**	**0.301**	**0.295**	0.287	**0.298**
	BS_{Juv}	0.001	-------	0.280	**0.296**	**0.292**	**0.292**	0.277
	N_{Juv}	-0.003	-0.001	-------	**0.294**	**0.312**	0.279	**0.290**
	S_{Juv}	0.002	0.000	-0.001	-------	**0.321**	**0.301**	**0.311**
	E_{Juv}	0.002	0.000	0.001	0.004	-------	**0.304**	0.280
	W_{Juv}	0.000	0.002	-0.007	0.004	0.000	-------	0.271
	M_{Juv}	0.002	-0.003	-0.002	-0.003	-0.002	0.000	-------

Mexico (M) populations account for 75% of the significant F_{ST} values (Table 3.4). All 2005 pair-wise exact test comparisons showed significant differences in allelic composition between adult populations (Table 3.4). In 2006, adult F_{ST} values were even lower than in 2005 (global F_{ST} = 0.001 95% CI 0.000 – 0.002) with no pair-wise F_{ST} values significantly greater than zero (Table 3.4), though all pair-wise exact tests showed significant differences in allelic composition (Table 3.4). Among juvenile samples, 5 of 21 pair-wise F_{ST} values were significantly greater than zero in 2005, with 4 of those involving comparisons with the juvenile North (N) population (Table 3.5), and global F_{ST} = 0.003 95% CI 0.001 – 0.007. All exact tests showed significant differences in allelic composition between juvenile populations in 2005 (Table 3.5). No pair-wise F_{ST} values were significantly greater than zero for juvenile populations in 2006 and Global F_{ST} = 0.005 95% CI 0.001 – 0.011, though 15 of 21 pair-wise exact tests still showed significant differences in allelic composition (Table 3.5).

Only 2006 juvenile populations showed significant (P = 0.03) but negative isolation by distance; that is, genetic distance decreased with increasing geographic distance due to low genetic distances between Mexico (M) and all other sites. When these comparisons were removed, the relationship was no longer significant (P > 0.05).

Comparisons of genetic diversity between adult and juvenile populations at each site yielded no significant differences in either heterozygosity or allelic richness in 2005 (t-test, P > 0.05) while in 2006, the south Turneffe (S) juvenile population showed significantly lower average heterozygosity compared to the adult population (t-test, P = 0.01), however allelic richness showed no significant differences in any population comparison (t-test, P > 0.05). Regression of mean pair-wise D_C against mean within-population relatedness showed no relationship in 2005 (slope = -2.4, r^2 = 0.091, P = 0.51),

2006 (slope = -5.8, r^2 = 0.26, P = 0.24), or with both years combined (slope = -45.2, r^2 = 0.039, P = 0.50) (Figure 3.2). Exact tests showed differentiation between all juvenile and their corresponding adult populations in 2005, and in 6 of 7 comparisons in 2006 (Table 3.6). Only 2 of 14 pair-wise comparisons showed significant differences in allelic composition after partitioning adults by size. These differences were found at the barrier reef north (BN) and south Turneffe (S) sites (exact test, P ≤ 0.001), both in 2005.

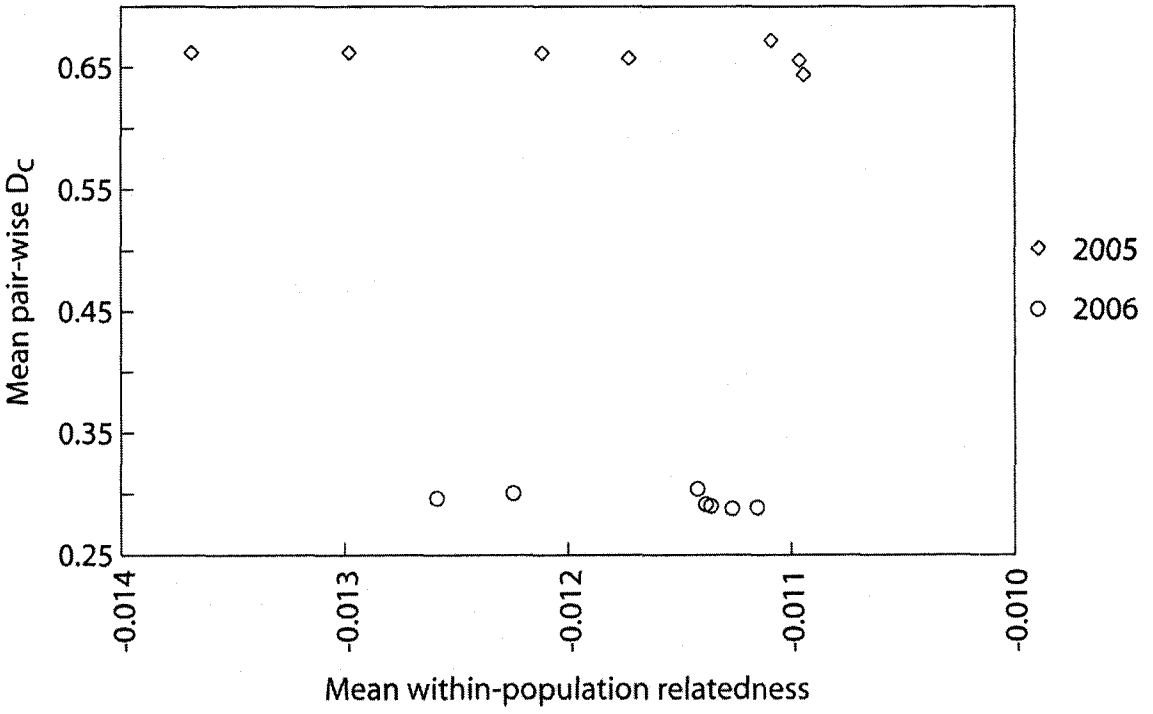

Figure 3.2 Scatterplot of average within-population relatedness versus mean pair-wise genetic distance (D_C) of juvenile populations of *S. partitus*. No relationship was found in 2005 (P = 0.51), 2006 (P = 0.24), or with both years combined (P = 0.50).

Temporal stability: Exact test results indicate all populations showed significant changes in allelic composition between sampling periods (P < 0.05). Mantel tests showed no significant relationship between 2005 and 2006 adult population structure (slope = -0.1372, r^2 = 0.0251, P = 0.330) (Figure 3.3a), or between juvenile population structure in

2005 to adult population structure in 2006 (slope = 0.1539, r^2 = 0.0215, P = 0.300) (Figure 3.3b). Comparisons of juvenile population structure across years also showed no significant relationship (slope = -0.3354, r^2 = 0.1828, P = 0.060) (Figure 3.3c).

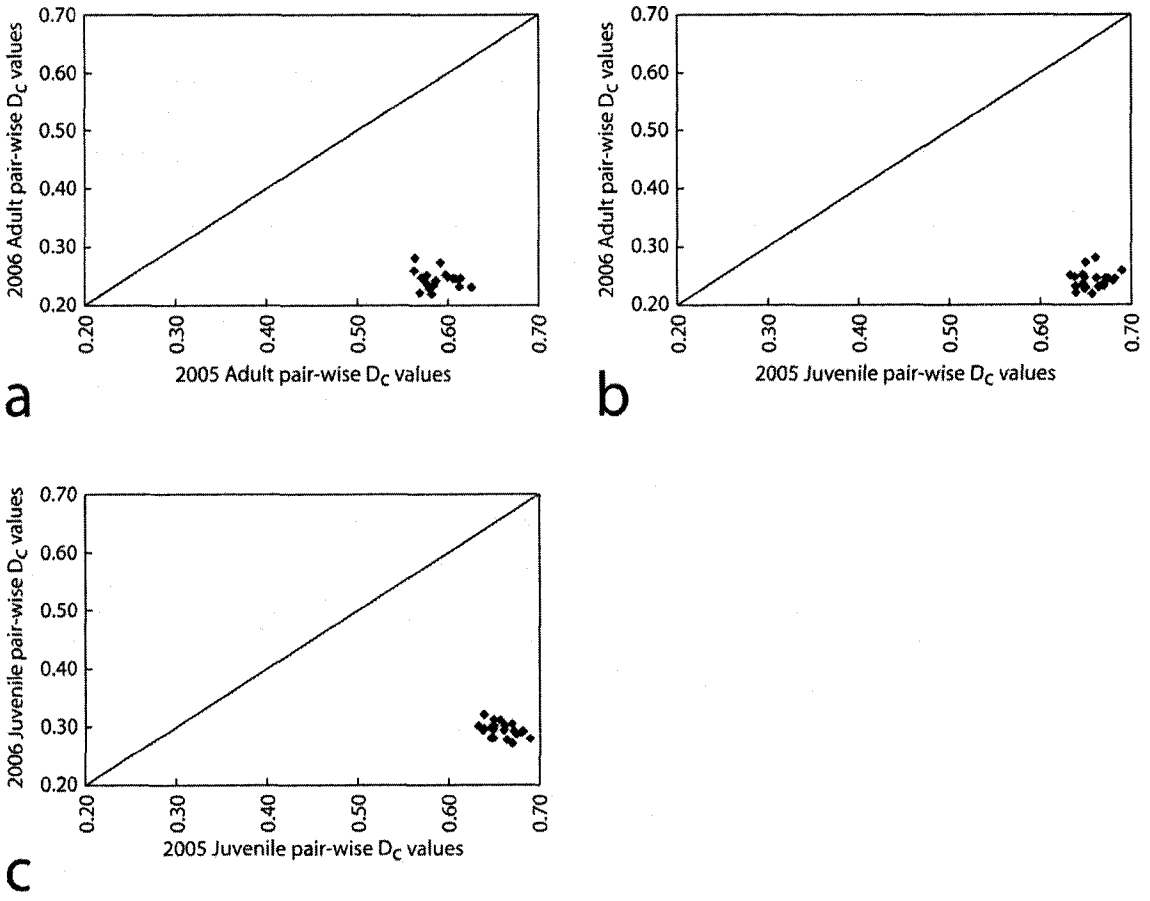

Figure 3.3 Comparison of pair-wise genetic distances between the two sampling years of *S. partitus*. Panel a: Scatterplot of 2005 and 2006 adult pair-wise D_C values (slope = -0.14, r^2 = 0.025, P = 0.33). Panel b: Scatterplot of 2005 juvenile and 2006 adult pair-wise D_C values (slope = 0.15, r^2 = 0.021, P = 0.30). Panel c: Scatterplot of 2005 and 2006 juvenile pair-wise D_C values (slope = -0.34, r^2 = 0.18, P = 0.06). Diagonal lines represent expected 1:1 among-population stability.

Table 3.6 Comparisons of juvenile and adult populations of *Stegastes partitus* collected from seven sites along the MBRS in 2005 and in 2006. Populations identified as in figure 3.1. Significant values after sequential Bonferroni correction indicated in bold and underlined. Negative F_{ST} values are not significantly different from zero.

	2005		2006	
	Fst	Exact test p-value	Fst	Exact test p-value
BN_{Adult} vs BN_{Juv}	-0.003	**0.001**	-0.002	**<0.001**
BS_{Adult} vs BS_{Juv}	0.003	**<0.001**	0.000	**0.001**
N_{Adult} vs N_{Juv}	0.001	**0.000**	0.000	**0.001**
S_{Adult} vs S_{Juv}	0.002	**0.002**	-0.001	0.007
E_{Adult} vs E_{Juv}	0.000	**<0.001**	0.003	**<0.001**
W_{Adult} vs W_{Juv}	0.000	**<0.001**	-0.003	**<0.001**
M_{Adult} vs M_{Juv}	**0.004**	**<0.001**	-0.003	**0.001**

3.4 Discussion

Given the high and continuous reproductive output of *S. partitus*, as well as their relatively long larval duration, it is somewhat surprising to find significant genetic structure among the populations sampled. Limited or a complete lack of genetic structure has been demonstrated for many species of reef fish in previous studies (Lacson and Morizot 1991, Bernardi *et al.* 2001, Haney *et al.* 2007). Allozyme analysis found no evidence of genetic population structure in *S. partitus* across the Caribbean (Lacson 1992), nor in the Pacific damselfish *Stegastes fasciolus* in the Hawaiian archipelago (Shaklee 1984). However, genetic heterogeneity was shown to exist among populations of *S. partitus* in the Florida Keys (Lacson *et al.* 1989), but was temporally unstable and previous differentiation was attributed to population bottlenecks (Lacson and Morizot 1991). This begs the question, why is there significant genetic structure among these

populations of *S. partitus*? Previous work used mitochondrial DNA or protein allozymes, both of which are functional markers which evolve slowly and only provide a very coarse picture of genetic structuring in these populations over evolutionary time. Microsatellite analysis, however, allowed me to explore the potential effects of pre- and post-settlement processes, cohort differentiation and oceanographic flow on population structure and assess the temporal stability of the observed genetic structure in an organism with high dispersal capacity over an ecologically relevant timeframe.

S. partitus populations along the MBRS exhibit genetic heterogeneity at small spatial scales. This was somewhat unexpected, but could be caused by geographic limits to dispersal causing genetic differentiation as geographic distance increases. However, patterns of differentiation between sites show no obvious spatial autocorrelation, as the magnitude of differences between geographically distant sites is similar to that of geographically proximal sites.

Genetic structure can be produced by both pre and post-settlement processes, such as selection or various forms of cohort stochasticity, and the analysis of juvenile genetic structure in comparison to that of the adult populations allow me to test predictions based on competing theories. If post-settlement processes, most notably selection effects, are the main driving factor in the differentiation of adult populations one would expect to see those effects intensify as the population ages, resulting in greater differentiation among older, adult populations than among juveniles. The results do not support this hypothesis, as juvenile and adult populations show similar magnitudes of genetic differentiation in both years (2005 adult global F_{ST} = 0.006 95% CI 0.002 – 0.013, juvenile global F_{ST} = 0.003 95% CI 0.001 – 0.007; 2006 adult global F_{ST} = 0.001 95% CI 0.000 – 0.002, juvenile global F_{ST} = 0.005 95% CI 0.001 – 0.011). Pre-settlement factors could include

either selection or cohort variation, or a combination of both. The data do not allow a direct assessment of pre-settlement selective processes; however, we can evaluate predictions based on cohort variation hypotheses. Cohort composition can vary either spatially or temporally, providing information on the likely mechanism of connectivity. Spatial variability would result from predictable larval dispersal from differentiated upstream sources to a settlement site and would likely produce relatively temporally stable juvenile and adult genetic structure. However, the data suggest that significant changes in genetic population structure occurred between years and life stages, indicating temporal variability in cohorts is a more likely influence on adult population structure than spatial variability in upstream sources.

I show considerable temporal change in population structure, both between sampling periods (2005 vs. 2006; Figure 3.3) and life stages (adults vs. juveniles; Table 3.6). Temporal genetic stability among populations is expected when either isolation or consistent, predictable patterns of gene flow (spatial variation in cohort source) are present (Purcell *et al.* 2006). Populations in the MBRS are unlikely to be isolated, as indicated by low F_{ST} values, and given the observed temporal changes in both adult and juvenile genetic structure, temporal variation in cohort structure is likely playing a role in generating the temporal instability in adult populations. This temporal variability in cohort composition may be the result of two mechanisms; the generation of different cohorts over time via sweepstakes-chance matching or changing sources of recruits as the result of stochastic processes.

Sweepstakes-chance matching is a mechanism capable of generating juvenile variability proposed by Hedgecock (1994). Under this model, high reproductive variance in adult populations caused by changing locally favourable conditions for spawning and

survival of larvae produces an instantaneous drift effect on larval populations, leading to spatial and temporal genetic patchiness as these larval cohorts recruit into adult populations. One prediction of this hypothesis is that we should observe reduced genetic variability in juvenile populations relative to the adults since larvae have been produced by only a subset of the total adult population. For example, Pacific oysters (*Crassostrea gigas*) show decreased genetic diversity in settling larvae attributed to variability in adult reproductive success (Li and Hedgecock 1998). The data however, do not indicate a reduction in genetic variability in juvenile populations relative to adults as measured by either observed heterozygosity or allelic richness at 12 microsatellite loci. A second prediction of this hypothesis is that cohorts should exhibit high relatedness. This was reported to occur in kelp bass (*Paralabrax clathratus*) populations in California, providing evidence for sweepstakes chance matching (Selkoe *et al.* 2006). If sweepstakes effects are driving the genetic structure observed in *S. partitus*, one would expect to see a positive relationship between average population genetic distance and within-population relatedness in recently settled juveniles, as differentiation would be driven by the influx of cohorts of highly related individuals. The results indicate no relationship between relatedness and genetic distance in either year, nor with years combined, hence it is unlikely that the patterns of genetic divergence and temporal instability I report are due to sweepstakes effects.

Finally, variation in recruit sources through time as a result of stochastic processes could also result in the pattern of genetic divergence observed in *S. partitus*. For this process, recruits arriving at a site over time would vary in their source population, due to stochastic processes affecting their survival or dispersal pathway (Cowen *et al.* 2000). If this were the case, one would expect to see temporally variable genetic structure in

juvenile and adult populations, and no relationship between adult and juvenile genetic structure. The results support this hypothesis, as I have shown temporally variable genetic structure in both adult and juvenile populations, and a lack of concordance between the two life stages; however, the nature of the process driving the variation in cohort composition is unknown.

Survival and growth of larval fish during the pelagic stage and their subsequent condition and persistence after settlement have been linked to larval feeding success (McCormick and Maloney 1992, Shima and Findlay 2002, McCormick and Hoey 2004, see McCormick 1998 for review). Furthermore, the strength of recruitment pulses has also been correlated to feeding and larval condition nearing the end of the larval duration (Bergenius *et al.* 2002), indicating that larval condition and survival, as influenced by the availability of food and the ability of larvae to exploit food resources in the pelagic environment, may generate cohort variability.

Temporal changes in oceanographic flow may also explain some of the patterns of differentiation seen in *S. partitus* populations along the MBRS, and have been shown to play a role in shaping population genetic structure in other species and systems. Although the effects of larval behaviour on dispersal trajectory can not be disregarded (e.g., Armsworth *et al.* 2001, Leis 2002, Irisson *et al.* 2004, Paris and Cowen 2004), current flow likely plays a large role in determining the direction and path of dispersal in reef fish (Hogan and Mora 2005). Abalone dispersal along the Great Barrier Reef is influenced by hydrodynamic features (McShane *et al.* 1988), and patterns of genetic variation in scallop populations were concordant with prominent currents along the east coast of Canada and the USA (Kenchington *et al.* 2006) suggesting that patterns of dispersal affecting population structure may be influenced by oceanographic flow, at least

in marine invertebrates. Extensive monitoring of oceanographic processes and recruitment patterns in the Florida Keys has also shown a significant link between fish and invertebrate recruitment and current regimes (Lee and Williams 1999). In this study, the majority of significant pair-wise genetic differences in 2005 included the barrier reef south (BS) and Mexico (M05) sites. The MBRS experiences considerable variability in flow, both in velocity and direction of currents seasonally, and also in conjunction with the formation of eddies and gyres associated with shifts in the Caribbean Current (Ezer *et al.* 2005, Tang *et al.* 2006). Predominant flow in the region is in a north-west direction (arrows, Figure 3.1), potentially pushing pelagic stage larvae northwards along the barrier reef, with a notable reversal in current direction occurring around Gladden Spit, located just south of the BS site (Ezer *et al.* 2005, Tang *et al.* 2006). Because of this divergence in current direction, flow in the vicinity of BS is generally more southward, away from the other sites, and flow around Turneffe is northwards (arrows, Figure 3.1). This could cause larvae produced by BS to be transported away from other sites, at least until competent swimming is achieved, and likewise the larvae produced by sites further north would be transported northward away from BS, explaining its observed genetic differentiation. Ezer *et al.* (2005) also note an eddy positioned between Mexico and the remainder of the sites (arrows, Figure 3.1), potentially entrapping dispersing larvae from more southerly sites and allowing the Mexico (M05) population to diverge. Much of the genetic structure observed in 2005 was absent in 2006 populations, possibly corresponding to changes in local flow due to the unusually active hurricane season in the Caribbean during the late summer and fall of 2005 (http://www.ncdc.noaa.gov/ oa/climate/ research/2005/hurricanes05.html). This may have altered patterns of connectivity in the region, facilitating gene flow between previously divergent

populations and resulting in the genetic homogeneity observed in 2006. Although my hypothesis remains untested, it is logical, given the genetic data and pertinent meteorological events, and consistent with modelled flow in the region (Ezer *et al.* 2005, Tang *et al.* 2006).

I have shown that both adult and juvenile genetic population structure varied significantly within and among populations through space and time, most likely due to pre-settlement stochastic processes. Cowen *et al.*'s (2006) model showed that patterns of dispersal were highly influenced by larval behaviour and oceanographic flow, and the study region experiences considerable variability in current patterns over time (Ezer *et al.* 2005, Tang *et al.* 2006), likely producing the low levels of differentiation and variability in genetic structure observed between these populations. Additionally, stochastic meteorological events such as the extreme hurricane events that occurred throughout the Caribbean during 2005 may disrupt local flow patterns and cause genetic homogenization, as was observed between sampling periods. This variability has important connotations for the management of coral reefs. As connectivity between populations is likely to vary through time, it is difficult to predict which populations should be protected and can be depended upon to produce propagules that would support exploited downstream areas or promote the recovery of populations in decline. It may be wise to employ a network of small reserves distributed throughout the region in order to increase the likelihood of protecting critical populations and account for the variability in connectivity through time.

3.5 References

Armsworth PR, James MK, Bode L (2001) When to press on or turn back: dispersal strategies for reef fish larvae. The American Naturalist, 157, 434-450.

Bergenius MA, Meekan MG, Robertson RD, McCormick MI (2002) Larval growth predicts the recruitment success of a coral reef fish. Oecologia, 131, 521-525.

Bernardi J, Holbrook SJ, Schmitt RJ (2001) Gene flow at three spatial scales in a coral reef fish, the three-spot dascyllus, *Dascyllus trimaculatus*. Marine Biology, 138, 457-465.

Cavalli-Sforza LL, Edwards AW (1967) Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics, 19, 233-257.

Chittaro PM (2005) An investigation of the use of otolith microchemistry to discriminate reef fish populations and assess the movement of individuals. PhD thesis, University of Windsor.

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

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science, 311, 522-527.

DeLoach N (1999) Reef fish behaviour: Florida, Caribbean, Bahamas, 1st edn. New World Publications Inc., Jacksonville, FL.

Elphinstone MS, Hinten GN, Anderson MJ, Nock CJ (2003) An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. Molecular Ecology Notes, 3, 317-320.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1, 47-50.

Ezer T, Thattai DV, Kjerfve B, Heyman WD (2005) On the variability of the flow along the Meso-American barrier reef system: a numerical model study of the influence of the Caribbean current and eddies. Ocean Dynamics, 55, 458-475.

Gaines SD, Gaylord B, Largier JL (2003) Avoiding current oversights in marine reserve design. Ecological Applications, 13, S32-S46.

Goudet J (1995) FSTAT (vers. 1.2): a computer program to calculate F-statistics. Heredity, 86, 485-486.

Haney RA, Silliman BR, Rand DM (2007) A multi-locus assessment of connectivity and historical demography in the bluehead wrasse (Thalassoma bifasciatum). Heredity, 98, 294-302.

Hedgecock D (1994) Temporal and spatial genetic structure of marine animal populations in the California current. California Cooperative Oceanic Fisheries Investigations Reports, 35, 73-81.

Hepburn, RI (2004) Population genetic structure in coral reef fish: spatial and temporal genetic patterns of the bicolor damselfish (Stegastes partitus). MSc thesis, University of Windsor.

Hogan, JD (2007) Behaviour, recruitment and dispersal of coral reef fish larvae: Insight into the larval life stage. PhD thesis, University of Windsor.

Irisson J-O, LeVan A, De Lara M, Planes S (2004) Strategies and trajectories of coral reef fish larvae optimizing self-recruitment. Journal of Theoretical Biology, 227, 205-218.

Johannesson K, Johannesson B, Lundgren U (1995) Strong natural selection causes microscale allozymes variation in a marine snail. Proceedings of the National Academy of Sciences of the United States of America, 92, 2602-2606.

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, Siphonaria sp. Marine Biology, 70, 157-164.

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

Kenchington EL, Patwary MU, Souros E, Bird CJ (2006) Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). Molecular Ecology, 15, 1781-1796.

Lacson JM (1992) Minimal genetic variation among samples of six species of coral reef fishes collected at La Parguera, Puerto Rico, and Discovery Bay, Jamaica. Marine Biology, 112, 327-331.

Lacson JM, Morizot DC (1991) Temporal genetic variation in subpopulations of bicolor damselfish (Stegastes partitus) inhabiting coral reefs in the Florida Keys. Marine Biology, 110, 353-357.

Lacson JM, Riccardi VM, Calhoun SW, Morizot DC (1989) Genetic differentiation of bicolor damselfish (Eupomacentrus partitus) populations in the Florida Keys. Marine Biology, 103, 445-451.

Langella O (2002) POPULATIONS 1.2.28 Logiciel de genetique des populations. Laboratoire Populations, genetique et evolution, CNRS UPR 9034, Gif-sur-Yvette, http://www.cnrs-gif.fr/pge.

Larson RJ, Julian RM (1999) Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. California Cooperative Oceanic Fisheries Investigations Reports, 40, 94-99.

Lee TN, Williams E (1999) Mean distribution and seasonal variability of coastal currents and temperature in the Florida Keys with implications for larval recruitment. Bulletin of Marine Science, 64, 35-56.

Leis JM (2002) Pacific coral-reef fishes: the implications of behaviour and ecology of larvae for biodiversity and conservation, and a reassessment of the open population paradigm. Environmental Biology of Fishes, 65, 199-208.

Li G, Hedgecock D (1998) Genetic heterogeneity, detected by PCR SSCP, among samples of larval Pacific oysters (Crassostrea gigas) supports the hypothesis of large variance in reproductive success. Canadian Journal of Aquatic Sciences, 55, 1025-1033.

McCormick MI (1998) Condition and growth of reef fish at settlement: Is it important? Austral Ecology, 23, 258-264.

McCormick MI, Hoey AS (2004) Larval growth history determines juvenile growth and survival in a tropical marine fish. Oikos, 106, 225-242.

McCormick MI, Maloney BW (1992) Effects of feeding history on the growth characteristics of a reef fish at settlement. Marine Biology, 114, 165-173.

McShane PE, Black KP, Smith MG (1988) Recruitment process in *Haliotis rubra* (Mollusca: Gastropoda) and regional hydrodynamics in southeastern Australia imply localized dispersal of larvae. Journal of Experimental Marine Biology and Ecology, 124, 175-203.

Palumbi SR (1994) Genetic divergence, reproductive isolation and marine speciation. Annual Review of Ecology and Systematics, 25, 547-572.

Paris CB, Cowen RK (2004) Direct evidence of a biophysical retention mechanism for coral reef fish larvae. Limnology and Oceanography, 49, 1964-1979.

Peakall R, Smouse, PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6, 288-295.

Plath M, Hauswaldt JS, Moll K, et al. (2007) Local adaptation and pronounced genetic differentiation in an extremophile fish, Poecilia mexicana, inhabiting a Mexican cave with toxic hydrogen sulphide. Molecular Ecology, 16, 967-976.

Purcell JFH, Cowen RK, Hughes CR, Williams DA (2006) Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. Proceedings of the Royal Society B, 273,1483-1490.

Purcell MK, Kornfield I, Fogarty M, Parker A (1996) Interdecadal heterogeneity in mitochondrial DNA of Atlantic haddock (Melanogrammus aeglefinus) from Georges Bank. Molecular Marine Biotechnology, 5, 185-192.

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

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science, 278, 1454-1457.

Robertson DR, Green DG, Victor BC (1988) Temporal coupling of production and recruitment of larvae of a Caribbean reef fish. Ecology, 69, 370-381.

Selkoe KA, Gaines SD, Caselle JE, Warner RR (2006) Current shifts and kin aggregation explain genetic patchiness in fish recruits. Ecology, 87, 3082-3094.

Shaklee JB (1984) Genetic variation and population structure in the damselfish, *Stegastes fasciolatus*, throughout the Hawaiian archipelago. Copeia, 3, 629-640.

Sheng J, Tang L (2004) A two-way nested-grid ocean-circulation model for the Meso-American barrier reef system. Ocean Dynamics, 54, 232-242.

Shima JS, Findlay AM (2002) Pelagic larval growth impacts benthic settlement and survival of a temperate reef fish. Marine Ecology Progress Series, 235, 303-309.

Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution, 49, 897-910.

Tang L, Sheng J, Hatcher BG, Sale PF (2006) Numerical study of circulation, dispersion and hydrodynamic connectivity of surface waters on the Belize shelf. Journal of Geophysical Research, 111, DOI 10.1029/2005JC002930.

Thiessen RJ, Heath DD (2006) Characterization of one trinucleotide and six dinucleotide microsatellite markers in bicolor damselfish, Stegastes partitus, a common coral reef fish. Conservation Genetics, DOI 10.1007/s10592-006-9207-9.

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

Wellington GM, Victor BC (1989) Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). Marine Biology, 101, 557-567.

Williams DA, Purcell J, Hughes CR, Cowen RK (2003) Polymorphic microsatellite loci for population studies of the bicolor damselfish, *Stegastes partitus* (Pomacentridae). Molecular Ecology Notes, 3, 547-549.

Wright S. (1931) Evolution in Mendelian populations. Genetics, 16, 97-158.

4.0 CONNECTIVITY AMONG BICOLOR DAMSELFISH, *STEGASTES PARTITUS*, ALONG THE MESOAMERICAN BARRIER REEF ASSESSED BY GENOTYPE ASSIGNMENT

4.1 INTRODUCTION

Population genetic structure as measured by neutral markers is maintained by a balance between the opposing forces of genetic drift and selection versus gene flow (Wright 1931). Thus, as gene flow among populations decreases, genetic differentiation will result as the effects of selection, mutation and drift accumulate. Ultimately, limited gene flow allows the possibility of local adaptation that may eventually lead to speciation as reproductive barriers between populations evolve. Clearly the estimation of gene flow and population divergence is key to understanding and predicting evolutionary and ecological processes (Thresher 1991).

Gene flow is generated by the successful reproduction of dispersing individuals, and as such, dispersal is the primary mechanism generating population connectivity in marine systems (Mora and Sale 2002). Many marine organisms have a bipartite life history, possessing both a dispersive pelagic larval stage and a relatively sedentary demersal adult stage (Leis 1991, Leis and Carson-Ewart 2000, Sale and Kritzer 2003). These pelagic larvae have the potential to disperse great distances each generation, theoretically providing high levels of connectivity between geographically distant populations (Roberts 1997). The spatial scale of dispersal and connectivity among populations also has important implications for the design of reserves and protected areas in terms of their size, placement and frequency in order to best conserve marine ecosystems and resources (Shanks *et al.* 2003). However, despite its importance,

dispersal is notoriously difficult to quantify in marine organisms due to the relatively small size of the larvae and the enormity of the environment through which they disperse (Mora and Sale 2002). Some progress in estimating dispersal has been made for reef fish by using artificial and natural chemical signatures to track larval movement (Jones *et al* 1999, Swearer *et al.* 1999, Thorrold 2001, Jones *et al.* 2005, Almaney *et al.* 2007), as well as biophysical modelling of larval dispersal strategies (Cowen *et al.* 2000, Armsworth *et al.* 2001, Irrisson *et al.* 2004, Cowen *et al.* 2006), however the extent of larval dispersal remains largely unknown. Recently, multi-locus genetic assignment methods have been used to identify migrant individuals and assign population membership in a variety of aquatic organisms (Jones *et al.* 2005, Carreras-Carbonell *et al.* 2007), and shows promise for characterizing dispersal in reef fish.

In this study I utilize multi-locus genotypes to assign newly settled *Stegastes partitus* to seven adult populations located along a 200 km stretch of the Mesoamerican barrier reef over two years. Adult *S. partitus* spawn demersal eggs that are cared for by the male until hatching in approximately 3.5 days, and larvae then spend approximately one month in the pelagic environment, providing ample opportunity for dispersal (Hogan 2007). I characterize patterns of dispersal between sampling sites and assess the average distance and net directionality of dispersal in each year with implications for population genetic structure and stability.

4.2 Materials and Methods

Approximately 100 adult and 50 recently settled juvenile bicolor damselfish were sampled by divers using hand nets from seven sites along the MBRS in June 2005, and

again in April 2006 (Figure 4.1). Fin tissue was removed from each fish, genomic DNA extracted and genotyping and population structure analysis were performed as reported in Chapter 3. Juveniles were assigned to adult populations of the same year based on their multi-locus genotype probabilities using a two step method. First I applied the Bayesian assignment method of Rannala and Mountain (1997), with probability estimates generated by 10,000 iterations of Monte Carlo resampling using the algorithm of Paetkau *et al.* (2004) in GENECLASS v2.0 (Piry *et al.* 2004). Next, the assignment procedure was repeated using the rank-based method where individuals are given a probability rank of belonging to one population relative to the other source populations, where the sum of these probabilities is equal to one. Individuals were considered assigned when they met two criteria: first, they must be assigned to at least one or more source populations with a probability greater than a pre-determined threshold (ranging between 75% - 95% in a sensitivity analysis) in the first Monte-Carlo based assignment procedure. Individuals with probabilities less than 5% were excluded from belonging to any of the source populations, and those between 5% and the threshold value were considered unassigned. The individuals successfully assigned to the sampled populations were then assessed using the rank-based method. Individuals were positively assigned to a specific source population if the likelihood rank of assignment to the highest ranked source population was greater than three times the likelihood rank of the second highest ranked population. This procedure further increased the stringency of assignment and eliminated individuals assigned to more than one population.

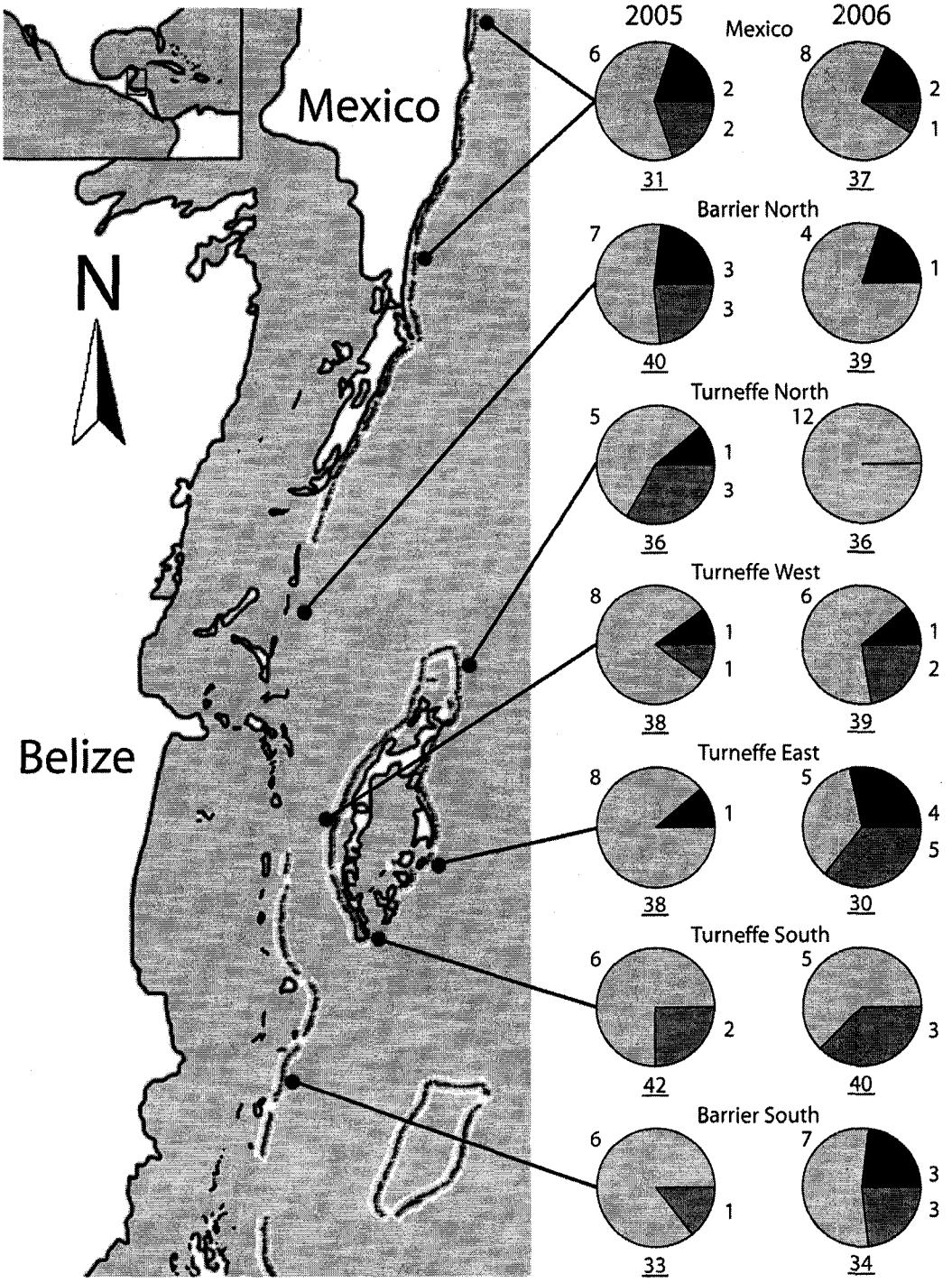

Figure 4.1 Genotype assignment of juvenile *Stegastes partitus* for 2005 and 2006 at seven sites along the Mesoamerican barrier reef. Pie charts represent the portions of individuals caught at each site that are self-recruiting (black), assigned to another site (light grey) or excluded from all populations (dark grey). Actual numbers of fish in each category are given by numbers associated with each segment. Number of individuals that failed to assign under the selected assignment criteria (85% threshold and 3:1 likelihood ratio) are underlined under each pie chart.

Between 6.2% and 27.8% of fish were successfully assigned depending on the selected threshold values. Increased stringency provided too few assigned individuals to allow proper assessment of dispersal patterns, while lower stringency, although increasing the number of fish assigned, yielded no novel paths of dispersal and increased the error associated with the assignment. Based on the sensitivity analysis results, all further analyses were performed on data obtained at the 85% threshold value (Table 4.1).

Table 4.1 Assignment test results at the 85% threshold level, documenting dispersal events of *Stegastes partitus* between seven sites along the Mesoamerican barrier reef in 2005 and 2006. Values indicate the number of fish dispersing from each origin population (site that juveniles were assigned to) to each endpoint population (site that juveniles were collected from). Populations identified as in Figure 3.1.

			Origin						
	Year	Site	BN	BS	N	S	E	W	M
Endpoint	2005	BN	3	0	1	1	3	1	1
		BS	1	0	0	1	1	3	0
		N	0	1	1	1	0	2	0
		S	1	0	2	0	3	0	0
		E	2	1	0	1	1	3	1
		W	2	0	0	1	4	1	1
		M	2	0	2	0	1	1	2
	2006	BN	1	2	0	0	1	1	0
		BS	0	3	0	0	4	2	1
		N	2	4	0	1	2	1	2
		S	1	2	0	0	1	0	1
		E	1	3	1	0	4	0	0
		W	1	4	0	1	0	1	0
		M	2	1	0	0	4	1	2

To assess the dispersal distance for both sampling years, dispersal distances were placed into eight categories ranging from zero to greater than 140 kilometres (Figure 4.2a and 4.3a). The distances between sampling sites were not evenly distributed, so to correct for biases in dispersal among distance categories, the expected frequencies of dispersal events if dispersal occurred equally at all distances, were calculated based on the proportion of dispersal pathways between sites possible in each distance category.

Corrected dispersal distance was measured as the difference between the observed and expected frequencies (Figure 4.2b and 4.3b). Absolute mean corrected dispersal distance was calculated and tested to see if it was significantly different from the null model of equal dispersal at all distances using a one-tailed one sample t-test.

Next, I characterized the directionality of dispersal by assigning each dispersal event to a direction bin, (in 22.5 degree increments from zero). These were tallied and the frequency of dispersal for each direction plotted (Figure 4.2c and 4.3c). Directional frequencies were corrected in the same manner as for distance (above) and net dispersal direction was then calculated as the sum of all corrected dispersal directions (open arrows; Figure 4.2c and 4.3c).

4.3 Results and Discussion

My data indicate that the distributions of dispersal distances in both 2005 and 2006 are significantly different from those expected by chance (t-test, $p < 0.05$), and that dispersal events occur over relatively short distances more often than expected (Figure 4.2 and 4.3). In 2005, 79% of dispersal occurred on scales less than 80 km and less than 4% of assigned individuals travelled greater than 140 km (Figure 4.2a). After correcting for the possible dispersal distance bias between sampling locations, I found the frequency of dispersal was higher than expected for distances less than 20 km, as well as distances between 41-80 km, while dispersal at distances greater than 140 km was rare (Figure 4.2b). In 2006, a similar pattern emerged: 69% of dispersal events were over distances less than 80 km while approximately 12% were longer than 140 km (Figure 4.3a). Fewer fish than expected travelled less than 20 km, however there was a strong bias towards

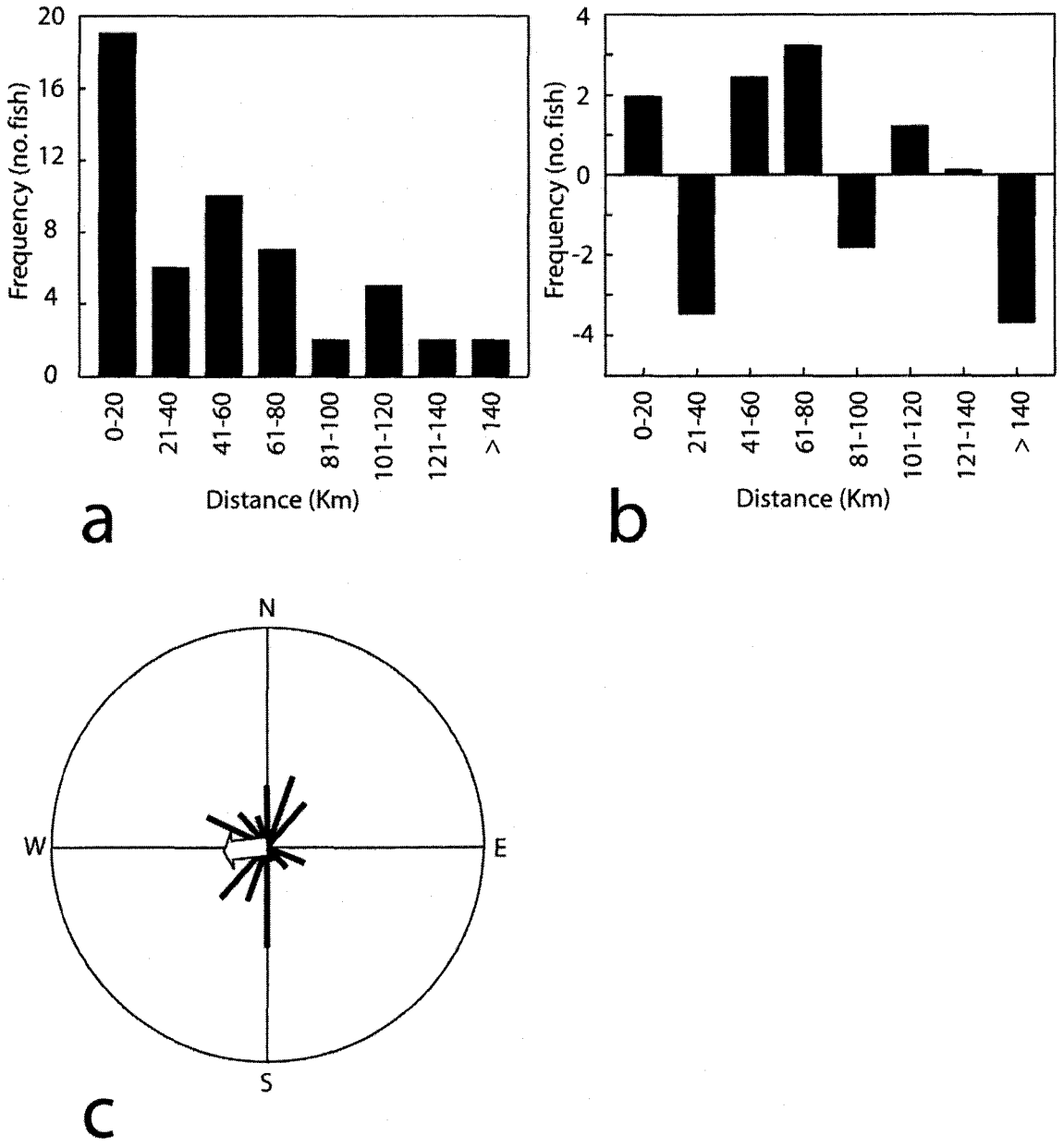

Figure 4.2 Dispersal distance distribution for juvenile *Stegastes partitus* in 2005 assessed by genotype assignment. **a.** Frequency of *S. partitus* dispersal at distances ranging from zero to greater than 140 km. **b.** Frequency of *S. partitus* dispersal at distances ranging from zero to greater than 140 km, corrected for distance bias between sites. **c.** Directionality of dispersal. Closed bars indicate direction of dispersal path, with the frequency of dispersal along each path represented by the length of the line. Open arrow represents net dispersal direction after correcting for directional bias between sites.

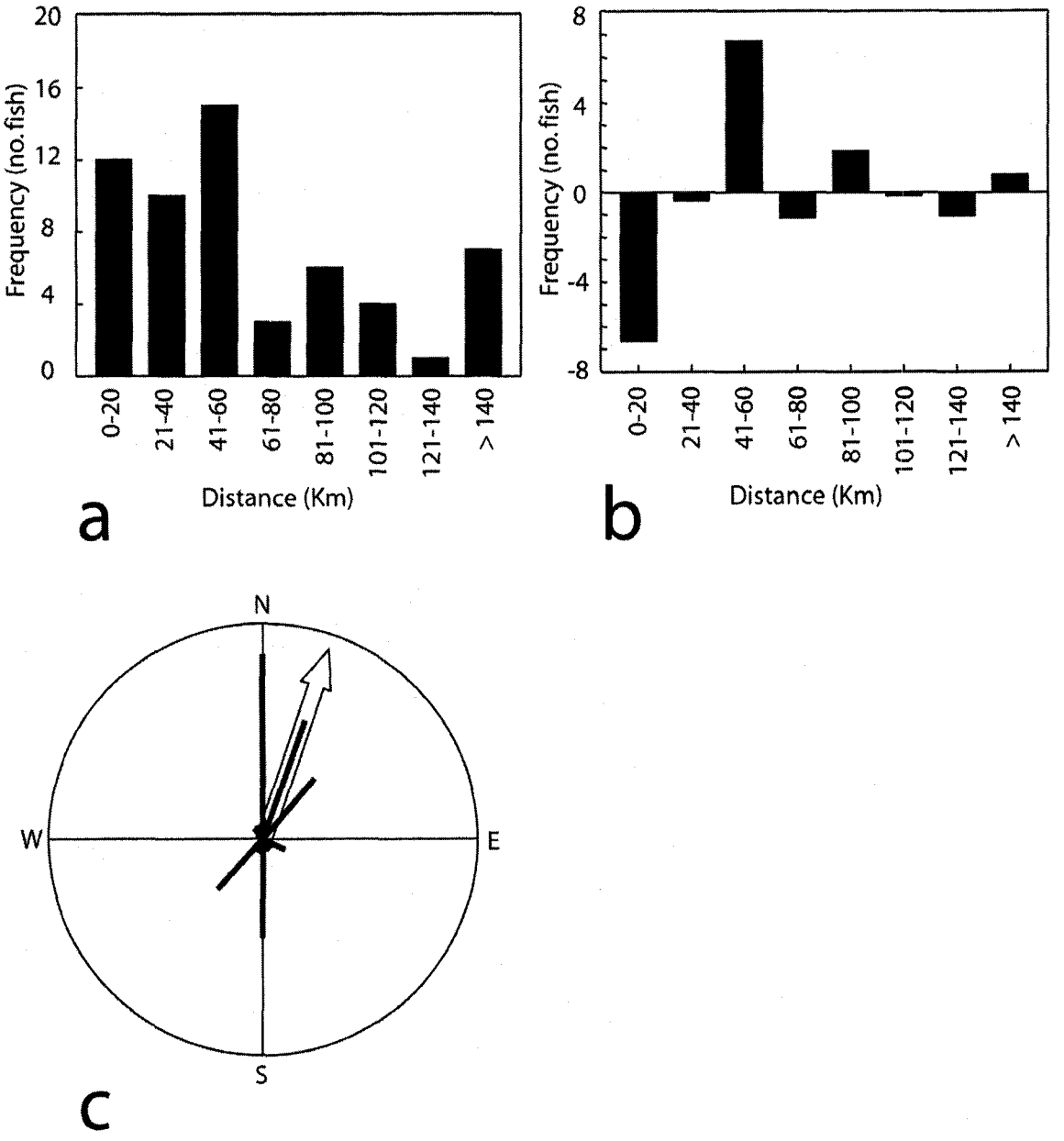

Figure 4.3 Dispersal distance distribution for juvenile *Stegastes partitus* in 2006 assessed by genotype assignment. **a.** Frequency of *S. partitus* dispersal at distances ranging from zero to greater than 140 km. **b.** Frequency of *S. partitus* dispersal at distances ranging from zero to greater than 140 km, corrected for distance bias between sites. **c.** Directionality of dispersal. Closed bars indicate direction of dispersal path, with the frequency of dispersal along each path represented by the length of the line. Open arrow represents net dispersal direction after correcting for directional bias between sites.

dispersal in the 41-60 km range (Figure 4.3b). Substantial local retention was also detected in both years, with approximately 15% of assigned individuals returning back to their natal populations in both years; however, the pattern of self-recruitment changed dramatically at some sites but was consistent at others (Figure 4.1). My results indicate that the distance of dispersal is also temporally variable, with an increase in the frequency of long distance dispersal events in 2006 relative to 2005. This may be the result of changes in the major on-shore flow, as oceanographic modelling has shown considerable variability of flow along the MBRS corresponding to the formation of eddies and gyres in the bay of Honduras (Ezer *et al.* 2005, Tang *et al.* 2006).

Overall, the majority of dispersal events documented by this study occurred at distances less than the average ecologically relevant interaction distance (145 km) based on passive drift proposed by Roberts (1997), and may be explained by the mortality and dilution effects experienced by larvae during the pelagic phase (Cowen *et al.* 2000). In fact, Cowen *et al.*'s (2000) biophysical model showed that at distances greater than 140 km, the number of larvae reaching downstream sources approaches zero when accounting for these effects. Cowen *et al.* (2006) suggested that effective dispersal distances in the Caribbean thus may be less than 100 km.

Long distance dispersal in marine organisms has always been assumed to be the rule rather than the exception; pelagic larvae acting as passive particles could potentially disperse hundreds of kilometres per generation, generating the expected high levels of population connectivity thought to exist (e.g. Roberts 1997). However, studies have shown that larvae are not so passive. They can utilize chemical, optical and auditory cues to locate and recruit to nearby reefs, and often have considerable swimming capabilities, potentially allowing them to control their trajectory (Leis and Carson-Ewart 2000,

Montgomery *et al.* 2001, Atema *et al.* 2002). Behavioural mechanisms have also been observed which may favour retention to local reefs, such as vertical migration and orientation within the benthic layers to avoid strong currents (Leis and Carson-Ewart 2000, Montgomery *et al.* 2001). Recent biophysical modelling efforts incorporating these new larval traits have shown that transport may not occur at the distances previously thought, and that these behaviours could act to decrease dispersal distances and maximize local retention (Cowen *et al.* 2000, Armsworth *et al.* 2001, Irisson *et al.* 2004, Cowen *et al.* 2006). Restricted dispersal has been reported in a number of empirical studies (Jones *et al* 1999, Swearer *et al.* 1999, Thorrold 2001, Gilg and Hilbish 2003, Jones *et al.* 2005, Almaney *et al.* 2007), and while rare long distance dispersal events may still influence genetic structure in marine systems, my results support the notion that the scale of connectivity even in highly dispersive species may be much smaller than previously thought. This paradigm shift has significant implications for marine reserve design. To effectively conserve and manage marine species, the goals of marine reserves are two-fold; they must protect the species inside their boundaries, as well as provide "subsidies" to exploited populations outside their borders via dispersal (Sale and Kritzer 2003). The spacing of protected areas is thus intrinsically linked to the effective dispersal distance of the organisms within them, and if dispersal is more local than distant as indicated by these results as well as those of others, this should be reflected in the design of reserve networks (Shanks *et al.* 2003). However, distance is not the only dispersal metric important to marine ecosystem management; the directionality of dispersal may influence population structure and stability, which are important considerations for the conservation and ecology of coral reefs.

Consistent biases in dispersal direction should lead to predictable gene flow, resulting in genetic structuring of populations along the axis of dispersal, while undirected or temporally variable dispersal should act to break down genetic structure. In 2005 dispersal was largely undirected among the seven sampling sites (Figure 4.2c). This may be the result of the disruption of local flow patterns by an unusually active hurricane season in the Caribbean during the summer of 2005 (http://www.ncdc.noaa.gov/oa/climate/research/2005/hurricanes05.html). This season of undirected dispersal also corresponds to the collapse of genetic structure in these same populations between 2005 and 2006 as described in Chapter 3. Regional hydrodynamics play an important role in reef fish dispersal (Roberts 1997, Cowen *et al.* 2000, Sanvincente-Anorve *et al.* 2000, James *et al.* 2002, Gaines *et al.* 2003, Cowen *et al.* 2006). Although larval behaviours will influence the distance and, to some extent, the trajectory of dispersal (Armsworth *et al.* 2001, Leis 2002, Irisson *et al.* 2004, Paris and Cowen 2004), choice experiments have shown that larvae may drift with currents if given the opportunity (Hogan and Mora 2005). Larval dispersal and recruitment events have been linked to local hydrodynamics in a number of systems, demonstrating the importance of currents in determining the directionality of dispersal in pelagic larvae (McShane *et al.* 1988, Lee and Williams 1999, Kenchington *et al.* 2006). Typical flow along the MBRS is in a northerly direction, however the hydrodynamics in the Caribbean region are temporally variable, with notable changes in current direction and speed associated with the formation of eddies and gyres in the Gulf of Honduras (Sheng and Tang 2004, Ezer *et al.* 2005, Tang *et al.* 2006). In 2006 there is a shift in the directionality of dispersal, with the major axis ending up in a north/south direction and significant net dispersal northwards consistent with typical flow (Figure 4.3c). The strong

directional bias in 2006 leads me to predict genetic population structure in a north/south direction in 2007. This pattern of alternating predictable and chaotic directionality of gene flow and recruitment would provide a resolution to two issues that arise in the study of dispersal in coral reef fishes: 1) the lack of consistency in patterns of genetic differentiation among published reports, and 2) the lack of adaptive advantage of dispersal under a complete stochastic model.

In summary, I have shown that the distance of dispersal in *S. partitus* is unexpectedly limited, and occurs most often at distances less than 80 km. Limited dispersal should lead to considerable genetic structuring among geographically separated populations, however this is not consistently the case. These same populations were shown to exhibit spatially and temporally variable genetic structure over a two year period (Chapter 3), and other studies have indicated that in many species dispersal potential is not necessarily a good predictor of genetic structure (e.g. Lacson *et al.* 1989, Lacson 1992, Hedgecock 1994, Shulman and Bermingham 1995, Moberg and Burton 2000, Bay *et al.* 2006). I propose that the absence of a clear relationship between dispersal distance and genetic structure in reef fish may be due in part to fluctuations in the direction of dispersal over time which periodically interrupt predictable patterns of gene flow and homogenize genetic structure between populations. My results therefore highlight the need for temporal replication, and indeed, extended time series data, in dispersal studies to evaluate the role of dispersal in connectivity among coral reef ecosystems.

4.4 REFERENCES

Almany GR, Berumen ML, Thorrold SR, Planes S, Jones GP (2007) Local replenishment of coral reef fish populations in a marine reserve. Science, 316, 742-744.

Armsworth PR, James MK, Bode L (2001) When to press on or turn back: dispersal strategies for reef fish larvae. The American Naturalist, 157, 434-450.

Atema J, Kingsford MJ, Gerlach G (2002) Larval reef fish could use odour for detection, retention and orientation to reefs. Marine Ecology Progress Series, 241, 151-160.

Bay LK, Crozier RH, Caley MJ (2006) The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Marine Biology, 149, 1247-1256.

Carreras-Carbonell J, Macpherson E, Pascual M (2007) High self-recruitment levels in a Mediterranean littoral fish population revealed by microsatellite markers. Marine Biology, 151, 719-727.

Cowen, RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science, 311, 522-527.

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

Ezer T, Thattai DV, Kjerfve B, Heyman WD (2005) On the variability of the flow along the Meso-American barrier reef system: a numerical model study of the influence of the Caribbean current and eddies. Ocean Dynamics, 55, 458-475.

Gaines SD, Gaylord B, Largier JL (2003) Avoiding current oversights in marine reserve design. Ecological Applications, 13, S32-S46.

Gilg MR, Hillbish TJ (2003) The geography of marine larval dispersal: coupling genetics with fine-scale physical oceanography. Ecology, 84, 2989-2998.

Hedgecock D (1994) Temporal and spatial genetic structure of marine animal populations in the California current. California Cooperative Oceanic Fisheries Investigations Reports, 35, 73-81.

Hogan JD (2007) Behaviour, recruitment and dispersal of coral reef fish larvae: Insight into the larval life stage. PhD thesis, University of Windsor.

Hogan D, Mora C (2005) Experimental assessment of the importance of swimming and drifting to the displacement of reef fish larvae. Marine Biology, 147, 1213-1220.

Irisson JO, LeVan A, De Lara M, Planes S (2004) Strategies and trajectories of coral reef fish larvae optimizing self-recruitment. Journal of Theoretical Biology, 227, 205-218.

James MK, Armsworth PR, Mason LB, Bode L (2002) The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. Proceedings of The Royal Society of London B, 269, 2079-2086.

Jones GP, Milicich MJ, Emslie MJ, Lunow C (1999) Self-recruitment in a coral reef fish population. Nature, 402, 802-804.

Jones GP, Planes S, Thorrold SR (2005) Coral reef fish larvae settle close to home. Current Biology, 15, 1314-1318.

Kenchington EL, Patwary MU, Souros E, Bird CJ (2006) Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). Molecular Ecology, 15, 1781-1796.

Lacson JM (1992) Minimal genetic variation among samples of six species of coral reef fishes collected at La Parguera, Puerto Rico, and Discovery Bay, Jamaica. Marine Biology, 112, 327-331.

Lacson JM, Riccardi VM, Calhoun SW, Morizot DC (1989) Genetic differentiation of bicolor damselfish (Eupomacentrus partitus) populations in the Florida Keys. Marine Biology, 103, 445-451.

Lee TN, Williams E (1999) Mean distribution and seasonal variability of coastal currents and temperature in the Florida Keys with implications for larval recruitment. Bulletin of Marine Science, 64, 35-56.

Leis JM (1991) The pelagic stage of reef fishes: the larval biology of reef fishes. In: The ecology of fishes on coral reefs (ed. Sale PF), pp. 183-230. Academic Press, California.

Leis JM, Carson-Ewart BM (2000) Behaviour of pelagic larvae of four coral-reef fish species in the ocean and an atoll lagoon. Coral Reefs, 19, 247-257.

Leis JM (2002) Pacific coral-reef fishes: the implications of behaviour and ecology of larvae for biodiversity and conservation, and a reassessment of the open population paradigm. Environmental Biology of Fishes, 65, 199-208.

McShane PE, Black KP, Smith MG (1988) Recruitment process in *Haliotis rubra* (Mollusca: Gastropoda) and regional hydrodynamics in southeastern Australia imply localized dispersal of larvae. Journal of Experimental Marine Biology and Ecology, 124, 175-203.

Moberg PE, Burton RS (2000) Genetic heterogeneity among adult and recruit red sea urchins, Strongylocentrotuts franciscanus. Marine Biology, 136, 773-784.

Montgomery JC, Tolimieri N, Haine OS (2001) Active habitat selection by pre-settlement reef fishes. Fish and Fisheries, 2, 261-277.

Mora C, Sale P (2002) Are populations of coral reef fish open or closed? Trends in Ecology and Evolution, 17, 422-428.

Paetkau D, Slade R, Burden M, 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, 55-65.

Paris CB, Cowen RK (2004) Direct evidence of a biophysical retention mechanism for coral reef fish larvae. Limnology and Oceanography, 49, 1964-1979.

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

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Science USA, 94, 9197-9201.

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science, 278, 1454-1457.

Sale PF, Kritzer JP. Determining the extent and spatial scale of population connectivity:decapods and coral reef fishes compared. Fisheries Research, 65, 153-172.

Sanvicente-Anorve L, Flores-Coto C, Chiappa-Carrara X (2000) Temporal and spatial scales of ichthyoplankton distribution in the southern Gulf of Mexico. Estuarine, Coastal and Shelf Science, 51, 463-475.

Shanks AL, Grantham BA, Carr MH. (2003a) Propagule distance and the size and spacing of marine reserves. Ecological Applications, 13, S159-169.

Sheng J, Tang L (2004) A two-way nested-grid ocean-circulation model for the Meso-American barrier reef system. Ocean Dynamics, 54, 232-242.

Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution, 49, 897-910.

Swearer SE, Caselle JE, Lea DW, Warner RR (1999) Larval retention and recruitment in an island population of a coral-reef fish. Nature, 402, 799-802.

Tang L, Sheng J, Hatcher BG, Sale PF (2006) Numerical study of circulation, dispersion and hydrodynamic connectivity of surface waters on the Belize shelf. Journal of Geophysical Research, 111, DOI 10.1029/2005JC002930.

Thorrold SR, Latkoczy C, Swart PK, Jones CM (2001) Natal homing in a marine fish metapopulation. Science, 291, 297-299.

Thresher, RE. 1991. Geographic variability in the ecology of coral reef fishes: evidence, evolution and possible implications. In: The ecology of fishes on coral reefs (ed. Sale PF), pp. 401-436. Academic Press, California.

Wright S. (1931) Evolution in Mendelian populations. Genetics, 16, 97-158.

5.0 General Discussion

5.1 Summary of Findings

Connectivity is an important measure for understanding the evolution, ecology and conservation of marine reef fishes. Genetic connectivity links populations through gene flow which affects the capacity of populations to adapt to local conditions and high gene flow may retard speciation. Demographic connectivity can affect population dynamics such as population growth rates, density, and overall persistence and therefore is important to conservation and management. Many reef fishes have the capacity to disperse great distances via a pelagic larval stage which initially led many to believe that populations should be "open" with little dependence on local processes, thus producing genetic homogeneity over large spatial scales. However, recent evidence indicates that these populations may be more demographically closed than would be predicted based on models of passive dispersal, and is supported by the results of this thesis.

In Chapter 3, I found that populations of *S. partitus* are spatially genetically distinct and show temporal variability, but do not conform to an isolation by distance model. The results of the multi-locus genotype assignment tests in Chapter 4 indicate that the observed genetic structure may have been the result of directional dispersal over moderate (<100 km) spatial scales. I have also shown that there are equivalent levels of genetic differentiation between adult and juvenile populations with no significant correlation of pair-wise genetic distance across years or developmental stage. This indicates that pre-settlement processes are likely acting to produce the observed temporal population genetic variation in adults, probably through changes in the genetic make-up

of settling cohorts. I believe that the variability among cohorts is produced by variable environmental conditions, notably the variable currents that have been modelled along the MBRS, as well as stochastic meteorological events, such as hurricanes, that disrupt local flow patterns.

Data in Chapter 4 support this hypothesis, as the net directionality of dispersal, which is likely influenced to a large extent by oceanographic flow, is variable through time. Furthermore, the sampling period during which I detected undirected dispersal corresponds to an unusually active hurricane season in the Caribbean, and is followed by a collapse in genetic structure in the subsequent sampling period.

Briefly, I believe that this thesis presents three major findings. First, *S. partitus* demonstrates significant and temporally unstable population genetic structure, potentially explaining the lack of agreement between estimates of genetic structure found in the literature. Second, dispersal is directed and predominantly over moderate spatial scales. Finally, there is considerable temporal variation in the dispersal direction, resulting in the observed temporally unstable population genetic structure. Overall these observations indicate that reef fish populations may be dependant on local processes such as local currents and meteorological events, which vary through time.

These results are consistent with previous studies of connectivity among reef fish populations along the MBRS. Variability and instability seem to be consistent attributes of these populations. Hepburn (2004) showed temporal instability in the genetic population structure of juvenile *S. partitus* along the MBRS at a bi-monthly scale; however the levels of population differentiation he observed were lower than those observed in the present study, likely as a result of using fewer microsatellite markers to resolve population structure. Hogan (2007) observed coherent larval recruitment at a

scale of 25 km, but recruitment was variable at larger scales and through time, indicating that while particular sites may consistently receive recruits from specific source populations, sources are temporally variable for most sites. This is further emphasized by examining putative source populations across studies, where Chittaro (2005) indicates that most recruits were produced by populations at the northern end of Turneffe Atoll, while data from Hogan (2007) and this dissertation have shown northern Turneffe populations to contribute very few new recruits to other sampled sites.

A consistent pattern of significant self-recruitment and short distance dispersal also emerges from this body of work. Chittaro (2005) estimates that 17% of larvae are self-recruiting to sites within Turneffe Atoll, corresponding closely with the 15% self-recruitment across study sites estimated in this study. Both Mora (2004) and Hogan (2007) show a bimodal distribution in terms of dispersal distances, with both short (30 – 60 km) and long (180 – 300 km) distance dispersal events favoured, with fewer individuals than expected dispersing over intermediate distances. The spatial scale of this study, as well as that of Chittaro (2005) did not extend over geographic distances large enough to measure these long distance dispersal events, however short distance dispersal seemed to be favoured in both cases. Together, this body of work has important implications for ecology and evolutionary biology as well as conservation and management.

5.2 Ecological and Evolutionary Implications

Temporal instability in population genetic structure and variation in dispersal direction imply that gene flow between populations is inconsistent through time. Genetic

instability makes the local adaptation of populations unlikely, and may instead lead to the "averaging" of traits to provide the general optimum across the species range (Warner 1997). This in turn suggests that studies of the evolutionary ecology of reef fishes should be at a distribution-wide scale in order to understand the processes which may influence the evolution of traits in these organisms (Warner 1997).

The insights into the effective dispersal distance in *S. partitus* provided in this thesis may also lend support to certain hypotheses regarding why reef fish have adopted a pelagic life stage. Johannes (1978) suggested that pelagic dispersal may provide larvae with a refuge from the intense predation experienced on the reef, while Doherty *et al.* (1985) postulate that larval dispersal is a risk-spreading strategy that insures that some members of a cohort will encounter suitable environments for survival. Both of these hypotheses are consistent with (although the latter is not obligate to) a shorter pelagic dispersal distance as they simply require that the larvae move into the pelagic environment, with dispersal potentially occurring as a bi-product. Dispersal distances should be considerably larger if the main purpose of the pelagic stage is to provide a mechanism for dispersal between patchily distributed habitat (Barlow 1981).

5.3 Conservation and Management

The use of marine reserves and protected areas as tools for the conservation and management of coral reef fishes has been widely suggested (e.g. Roberts 1997, Gell and Roberts 2003, Mora *et al.* 2006). However, successful application requires their size and spacing to be matched to the scales of movement of the organisms they are designed to protect (Gell and Roberts 2003, Mora *et al.* 2006). A significant knowledge gap exists

regarding the realized dispersal distance for most coral reef fishes, where most estimates of dispersal have been derived from biophysical modelling or inferred based on adult reproductive strategies or generalized larval behaviours (Armsworth *et al.* 2001, Leis 2002, Irisson *et al.* 2004, Cowen *et al.* 2006). The data from *S. partitus* provides a good generalized estimate of dispersal and connectivity for reef fishes, as it possesses the sedentary adult phase characteristic of many species and a pelagic larval phase of intermediate duration, which may be important to its dispersal capacity. Given its intermediate life history characteristics, *S. partitus* can act as a surrogate for reef fishes in general and can provide us with insights for their conservation and management. One caveat worthy of mention is that the population dynamics of *S. partitus* may not accurately reflect those of reef fishes with vagile adults, as this life history difference may alter connectivity patterns through adult movement, and alternative management strategies may be required for such species.

The results of Chapter 4 show that although long distance dispersal events likely do occur, the scale of dispersal in *S. partitus* is generally over tens of kilometres, not hundreds. This indicates that management at a local scale is warranted, and is likely to be effective for reef fish species with sedentary adults. The temporal variation in both population genetic composition and dispersal direction demonstrated in chapters 3 and 4 make it difficult for managers to pinpoint key source populations deserving special conservation status, and highlight the need for time-series data before management decisions are finalized. My data indicate that the length of time-series necessary to capture the variation within the MBRS will depend on the temporal scale of variability in physical processes occurring on the reef (e.g. monthly vs. seasonal vs. annual), however this may vary by system and may also be dependant on the biology of the species of

concern. Obtaining and analyzing all the pertinent data is a lengthy process, so I believe managers need to adopt an adaptive strategy that utilizes the most current information, but that can be refined as additional data become available.

Based on the data presented in this thesis, I believe a network of small reserves spaced on the order of tens of kilometres would be most effective along the MBRS. This should encourage sufficient population connectivity between protected areas to promote population sustainability within the reserve network itself, as well as providing propagules to areas outside reserves by accounting for temporal variation in dispersal direction and population structure through time.

5.4 Future Research Directions

The chapters of this thesis have addressed questions of the spatial and temporal variation in population structure as well as dispersal patterns of *S. partitus* along the MBRS. Based on my findings, I recommend the following future areas of research:

1. *Impacts of life history characteristics*

   I believe that a multi-species comparative study utilizing the techniques of multi-locus genotype assignment would yield insights into how life history variation factors into the realized dispersal of coral reef fishes. The examination of species with contrasting life history characters (spawning strategy, pelagic duration, larval physiology and behaviour) would allow the isolation of key biological factors relevant to connectivity.

2. *Scale of spatial and temporal variation*

Understanding of the spatial scale at which populations operate is a major knowledge gap in coral reef fish ecology (Sale 2002). Population characteristics are likely influenced by the life history traits of the species in question (Sale 2002) and the conclusions drawn from connectivity studies may depend on whether demographic or genetic connectivity is under consideration (Steneck *et al.* 2006). Therefore, it may be wise to incorporate multiple spatial scales in conjunction with the multi-species comparative studies proposed above to give us a better picture of the scale at which populations operate.

The results presented in this thesis indicate that temporal variation occurs over approximately 10 months; and Hepburn (2004) found temporal changes in juvenile genetic structure on a two month interval. Studies similar to these but employing shorter sampling intervals would give us an idea of the temporal scale of variation. Long term monitoring is also important if we are to understand any seasonal or annual cycles that may be occurring in these systems.

3. *Interdisciplinary studies*

Chapters 3 and 4 predict that some form of physical stochasticity is important to the observed population structure and dispersal patterns in *S. partitus*. Future work should strive to utilize interdisciplinary approaches to cross validate the results of genetic techniques with the predictions of oceanographic modelling, and examine if patterns of genetic connectivity can be predicted by physical processes.

5.5 REFERENCES

Armsworth PR, James MK, Bode L (2001) When to press on or turn back: dispersal strategies for reef fish larvae. The American Naturalist, 157, 434-450.

Barlow GW (1981) Patterns of parental investment, dispersal and size among coral-reef fishes. Environmental Biology of Fishes, 6, 65-85.

Chittaro, PM (2005) An investigation of the use of otolith microchemistry to discriminate reef fish populations and assess the movement of individuals. PhD thesis, University of Windsor.

Cowen, RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science, 311, 522-527.

Doherty JP, Williams DM, Sale PF (1985) The adaptive significance of larval dispersal in coral reef fishes. Environmental Biology of Fishes, 12, 81-90.

Gell FR, Roberts CM (2003) Benefits beyond boundaries: the fishery effects of marine reserves. Trends in Ecology and Evolution, 18, 448-455.

Hepburn, RI (2004) Population genetic structure in coral reef fish: spatial and temporal genetic patterns of the bicolor damselfish (Stegastes partitus). MSc thesis, University of Windsor.

Hogan JD (2007) Behaviour, recruitment and dispersal of coral reef fish larvae: insight into the larval life-stage. PhD thesis, University of Windsor.

Irisson JO, LeVan A, De Lara M, Planes S (2004) Strategies and trajectories of coral reef fish larvae optimizing self-recruitment. Journal of Theoretical Biology, 227, 205-218.

Johannes RE (1978) Reproductive strategies of coastal marine fishes in the tropics. Environmental Biology of Fishes, 3, 741-760.

Leis JM (2002) Pacific coral-reef fishes: the implications of behaviour and ecology of larvae for biodiversity and conservation, and a reassessment of the open population paradigm. Environmental Biology of Fishes, 65, 199-208.

Mora C (2004) Importance of dispersal in coral reef fishes. PhD thesis, University of Windsor.

Mora C, Andrefouet S, Costello MJ, *et al.* (2006) Coral reefs and the global network of marine protected areas. Science, 312, 1750-1751.

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science, 278, 1454-1457.

Sale PF (2002) The science we need to develop for more effective management. In: Coral reef fishes: dynamics and diversity in a complex ecosystem (ed. Sale PF), pp. 361-376. Academic Press, San Diego, USA.

Steneck RS, Cowen RK, Paris CB, Srinivasan A (2006) Response to: Connectivity in marine protected areas. Science, 313, 43-45.

Warner RR (1997) Evolutionary ecology: how to reconcile pelagic dispersal with local adaptation. Coral Reefs, 16, S115-S120.

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