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Population genetic structure in coral reef fish: Spatial and temporal genetic patterns of the bicolor damselfish (Stegastes partitus)

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Population genetic structure in coral reef fish: spatial and temporal genetic patterns of the bicolor damselfish (*Stegastes partitus*)

by

Russell Ian Hepburn

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Abstract

Dispersal in marine systems is of great importance within the context of ecology, evolution and conservation. Yet, in coral reef fish with pelagic larvae, little is known about their levels of connectivity, as it is very difficult to directly track these organisms through their deep-water dispersive stage. The dispersal of juvenile bicolor damselfish (*Stegastes partitus*), sampled from 16 sites on the Meso-American Barrier Reef System (MBRS), was inferred from allele frequency data based on six microsatellite DNA markers at three spatial scales: small (<20 km), medium (20 to 40 km), and large (100 to 300 km). Juvenile bicolor damselfish within the MBRS were found to possess genetic homogeneity at large geographic scales, with cryptic genetic structure detected at small and medium scales, most likely due to micro-geographic effects. The stability of the genetic structure in 12 of these sites was examined over annual and seasonal scales using the same suite of genetic markers. The genetic structure of these populations was found to be variable over both time-scales examined. From these results, it can be inferred that dispersal patterns of the bicolor damselfish are more stochastic than directed, and that temporally unstable genetic patchiness is present in newly recruited bicolor damselfish over the MBRS, possibly due to high reproductive variance. These findings are of importance for future research, as well as conservation and management strategies addressing connectivity in coral reef fish.

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GENERAL INTRODUCTION

Connectivity

Connectivity between coral reef fish populations is of fundamental importance in coral reef fish ecology, as the level of connectivity will determine if a population is dependent on local or non-local processes which, in turn, will impact management and conservation decisions at regional and international levels (Roberts, 1997). In marine systems, invertebrates commonly possess pelagic life histories (Pechenik, 1996), though this life history trait is seen in both temperate and tropical fishes (Moyle & Cech, 1996). It has been suggested that the evolutionary origin of the pelagic larval dispersal strategy was as a mechanism by which untended young can be sent to a safe refuge (Johannes, 1978), as a means to overcome habitats unstable over evolutionary time (Barlow, 1981), or as a risk-spreading strategy (Doherty *et al.*, 1985). Ecologically, the pelagic larval stage in coral reef fish, combined with the patchiness of their habitat, ensures that populations must, to varying extents, receive their recruits from other populations (Sale, 2002).

Connectivity is defined by Mora and Sale (2002) as "the demographic connection maintained between neighbouring populations of a species due to the migration of individuals (particularly dispersing larvae) between them." Connectivity is usually measured in terms of dispersal (e.g. tagging studies such as Swearer *et al.*, 1999), or gene flow (e.g. molecular-based approaches such as Doherty *et al.*, 1995). In order to use genetic tools to examine dispersal, and not gene flow, it is necessary to sample only juvenile fish to eliminate post-settlement selective processes.

Inherent to studies addressing levels of connectivity, is the issue of scale, both temporal and spatial. Sale (2002) states that "defining the spatial scales over which populations operate is the greatest lack in our understanding of coral reef fish ecology." He suggests that no single spatial scale will define all coral reef fish, as species vary in reproductive timing, mode of entry into the pelagic zone (eggs versus larvae), duration of larval stage and levels of larval behaviour. Therefore, investigations of multiple spatial scales when estimating dispersal in coral reef fish is of extreme importance. If connectivity is both variable and high between populations, including temporal scales is crucial, as any patterns detected in connectivity are less meaningful if the stability of these patterns is unknown.

Study species

The bicolor damselfish (*Stegastes partitus*: Pomacentridae) is found throughout the tropical western Atlantic (DeLoach, 1999; Robertson *et al.*, 1988). Individuals establish and defend a small territory within and around corals, sponges, rock outcroppings and conch shells, at depths ranging from deep reef shelf edges to shallow back reefs (DeLoach, 1999). This damselfish forms hierarchical colonies based on sex and size comprising all size classes and sexes, with the largest male being dominant (DeLoach, 1999). Male *S. partitus* grow to a maximum standard length (SL) of 60 mm, while females may reach 50 mm (Sadovy, 1986 in Cole & Sadovy, 1995). Both males and females reach sexual maturity at approximately one year of age and live for two to three years (DeLoach, 1999). Spawning occurs in unimodal lunar cycles over most of the year, with peaks from May to October (DeLoach, 1999; Robertson *et al.*, 1988). Females

spawn every two days and, after choosing a nest established by a male, they will lay 500 to 5,000 eggs, depending on body size (Knapp, 1993). Males guard the clutch diurnally, and the eggs hatch 3.5 days post-fertilization, after which the larvae begin a 27 to 31 day pelagic life stage (Wellington & Victor, 1989). Settlement pulses of new *S. partitus* recruits occur in a "lunar-cyclic" fashion on or around the new moon (Robertson, 1992).

To study connectivity it is critical to understand the life history traits that affect dispersal, and the bicolor damselfish has been well documented through the adult, juvenile and embryonic stages (e.g. Knapp, 1993; Robertson, 1992; Tolimieri *et al.*, 1998). The pelagic larval duration of *S. partitus* is in the upper-median range for damselfishes within the Caribbean (minimum 12 to 15 days *Abudefduf curacao*; maximum 25 to 39 days *Stegastes flavilatus*) (Wellington & Victor, 1989). By using published microsatellite DNA markers for the bicolor damselfish (Williams *et al.*, 2003), it is possible to resolve population genetic structure for this species to a greater degree than in previous work that used allozymes (Planes, 2002). For these reasons, the bicolor damselfish was chosen for the purposes of studying temporal and spatial genetic structure to infer connectivity.

Study area

The MBRS is the longest barrier reef in the Western hemisphere and is of great ecological importance as breeding and feeding grounds for marine mammals, reptiles, invertebrates and fishes (Mesoamerican Barrier Reef Systems Project (MBRS), 2003). Strong eastern ocean currents flow onto the MBRS from the Atlantic ocean, turning north along the reef system as they hit the Bay of Honduras. This redirection of flow results in

temporally transient gyres and eddies around the southern end of the MBRS (Sheng & Tang, 2004).

Objectives

The three chapters of this thesis explore the development and use of molecular techniques to investigate connectivity in coral reef fish populations. My thesis examines connectivity in the coral reef fish *S. partitus* by ascertaining the spatial and temporal stability of genetic structure within and among populations. It is comprised of three chapters, each written for separate publication. Chapter 1 infers dispersal of *S. partitus* from microsatellite allele frequency data at three spatial scales (<20 km, 20 to 40 km and 100 to 275 km) along the MBRS. This chapter is in final preparation for submission to *Molecular Ecology*. Using the same microsatellite markers, Chapter 2 explores the temporal genetic stability within and among populations of *S. partitus* at annual and seasonal scales over the MBRS. This chapter is in the preliminary stages of preparation for submission to *Molecular Ecology*. The third chapter is a primer note, submitted to *Conservation Genetics*, reporting the development of novel microsatellite DNA markers for the masked goby (*Coryphopterus personatus*) and the results of an initial analysis of population structure in this organism along the MBRS.

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CHAPTER 1

Population genetic analysis of juvenile bicolor damselfish (*Stegastes partitus*) at three spatial scales across the Meso-American Barrier Reef System

1.1 ABSTRACT

Dispersal in marine systems is of great importance within the context of ecology, evolution and conservation. To infer dispersal of the bicolor damselfish (*Stegastes partitus*) along the Meso-American Barrier Reef System (MBRS), the genetic structure of juveniles, sampled over 16 sites from three geographic scales, was examined using six microsatellite loci. Our results show genetic homogeneity of *S. partitus* populations at large geographic scales and the presence of cryptic genetic structure at small and medium geographic scales between neighbouring sites ($P<0.01$ and $P<0.006$) and between sites situated on windward/leeward sides of atolls ($P<0.005$; $P<0.01$). The overall homogeneous genetic structure of juveniles indicates that this species undergoes high larval dispersal. The structure seen at smaller scales may be driven by stochastic dispersal coupled with micro-geographic effects. Our findings provide a basis for the reconciliation of the contrasting results of genetic structuring in *Stegastes*.

1.2 INTRODUCTION

Like many marine organisms, most coral reef fish possess two distinct life history phases: a dispersive pelagic larval stage and a relatively sedentary demersal adult stage (Leis, 1991). There are varied hypotheses concerning the evolutionary and ecological significance of the pelagic larval dispersal strategy. Johannes (1978) proposed that larval dispersal was a mechanism to send untended young to a refuge, 'safe' from predators. Others have postulated that deep-water dispersal is an advantage in patchy environments, such as coral reefs, as a means to overcome habitats unstable over evolutionary time (Barlow, 1981) or as a risk-spreading strategy (Doherty *et al.*, 1985). Regardless of the reasons for the evolution of pelagic larval dispersal, it gives these organisms the potential for large-scale transport from their natal origins. Such dispersal is thought to be the exclusive means of linking populations across a patchy habitat (Ehrlich, 1975).

As directly measuring larval dispersal in the open ocean is very difficult (but see Leis *et al.*, 1996) most of our knowledge of coral reef fish dispersal is from indirect studies, such as plankton tows (Boehlert & Mundy, 1993; Leis, 1991; Leis, 1993), growth increments and micro-chemical signatures of the otoliths (Brothers & McFarland, 1981; Brothers & Thresher, 1985; Jones *et al.*, 1999; Shulman & Bermingham, 1995; Sponaugle & Cowen, 1994; Swearer *et al.*, 1999; Thresher *et al.*, 1989) and genetic analysis (reviewed by Planes, 2002). To date, plankton sampling has been the primary source of empirical data on coral reef fish dispersal. Key findings using this technique are that reef fish larvae have dispersal ranges of 10 to 100 km, (rarely >500 km) from natal reefs, and species with a pelagic spawning life history have larvae that are found farther from shore than the larvae of demersally spawning species (Boehlert & Mundy,

1993; Leis, 1991; Leis, 1993). Studies utilizing the daily growth increments in otoliths of tropical fish have found that, in general, there is a positive correlation between larval duration and species distribution range (Doherty *et al.* 1995). However, larval duration has been found to be of limited use as a predictor of dispersal (Brothers & McFarland, 1981; Brothers & Thresher, 1985; Shulman & Bermingham, 1995; Sponaugle & Cowen, 1994; Thresher *et al.*, 1989), primarily due to differences (from the family to the species level) in both the maximum age of larvae and plasticity in the larval duration (Brothers & Thresher, 1985; Sponaugle & Cowen, 1994; Thresher *et al.*, 1989). Determining the origin of larvae and inferring dispersal patterns via analysis of trace minerals found in the daily growth rings of otoliths has been successful in determining larval retention to natal or proximate reefs (Robertson, 1992). However, this technique provides only a minimal dispersal range and is also problematic due to high larval mortality (Planes, 2002; but see Swearer *et al.*, 1999). Although studies employing these techniques have made important contributions to our knowledge of coral reef fish dispersal, they have limitations in their ability to consistently predict larval dispersal patterns.

Population genetic analyses provide researchers an alternative method of inferring larval dispersal by using allele frequency data, under the assumption that allele frequencies reflect the conflicting forces of gene flow and genetic drift. Given restricted gene flow, the processes of selection, drift and mutation result in genetic differentiation, and this differentiation can be used to track patterns of dispersal.

A complicating factor that must be considered when genetic tools are used to infer dispersal is that dispersal may not equate to gene flow. If an individual successfully disperses, but does not contribute to the next generation due to post settlement mortality

or failure to reproduce, that individual will not contribute to gene flow between populations. This means that population genetic studies of coral reef fish based exclusively on adult specimens do not measure dispersal *per se*, but, instead, measure past gene flow. Population genetic studies that investigate gene flow are examining a different temporal scale than the one dispersal is operating at (generations versus larval duration). To properly examine dispersal using genetic methods, newly settled juveniles that have yet to undergo selective pressures of the demersal stage need to be studied.

The bicolor damselfish (*Stegastes partitus*: Pomacentridae) is a species abundant throughout most of the tropical western Atlantic (DeLoach, 1999). Spawning and settlement follow a unimodal lunar cycle from May to October (DeLoach, 1999; Robertson *et al.*, 1988). During a week-long reproductive cycle, female *S. partitus* demersally spawn every two days, laying up to 5,000 eggs (Knapp, 1993). *Stegastes partitus* has a planktonic duration of 27 to 31 days, and the age of settlers ranges between 31 and 35 days (approximately five weeks) (Robertson *et al.*, 1988). As a demersal spawner with a relatively lengthy pelagic larval duration, the bicolor damselfish would be expected to have moderate to high dispersal capabilities. As high dispersal rates are correlated with homogenization of allele frequencies and, therefore, the elimination of local genetic differences, it would follow that genetic differentiation should not be detected among populations of *S. partitus*. We investigated genetic differentiation in bicolor damselfish juveniles across the Meso-American Barrier Reef System (MBRS). Six polymorphic microsatellite markers were used to infer dispersal across three spatial scales: (1) small scale (neighbouring sites <20 km apart); (2) medium

scale (sites within atolls: i.e. (i) upstream versus downstream relative to ocean currents and (ii) windward versus leeward); and (3) large scale (among atolls).

1.3 MATERIALS AND METHODS

1.3.1 Sampling

The Meso-Amercian Barrier Reef System (MBRS) extends from Isla Contoy on the northern Yucatan Peninsula to the Bay Islands of Honduras (Fig 1.1). Bi-monthly collections were carried out targeting newly settled *S. partitus* (<2.5 cm) at 16 sites along the MBRS within 3 geographic regions: Roatan Island, Honduras (3 sites); Turneffe Atoll, Belize (7 sites); and Banco Chinchorro, Mexico (6 sites) (Fig 1.1). These three regions cover much of the MBRS and were chosen to maximize the geographic range of sampling over the reef system.

Sampling took place August13 to 24, 2003. Fish were collected by divers using hand nets, after anaesthetizing and immobilizing the individuals with clove oil (Munday & Wilson, 1997). Upon surfacing, the specimens were immediately placed in containers with 95% ethanol and labelled according to site and date.

1.3.2 DNA extraction and microsatellite analysis

DNA was extracted from caudal fin clips using the Wizard® Genomic Purification Kit (Promega Madison, WI) following manufacturer's protocol for DNA extraction from animal tissue. Microsatellite markers developed for *S. partitus* (Williams *et al.*, 2003) were screened for suitability (reliable amplification, suitable size ranges). Eight markers in total were chosen and six primer sequences were modified for size to facilitate running combinations of loci on the automated sequencer (Table 1.1). Polymerase chain reactions (PCR) were carried out in reactions comprised of: 2.5 μL 10X PCR Buffer (10 mM Tris-HCL (pH-8.4) 50mM KCL), 2.5 mM $MgCl_2$, 200 μM dNTP's, 0.05 μg of each primer,

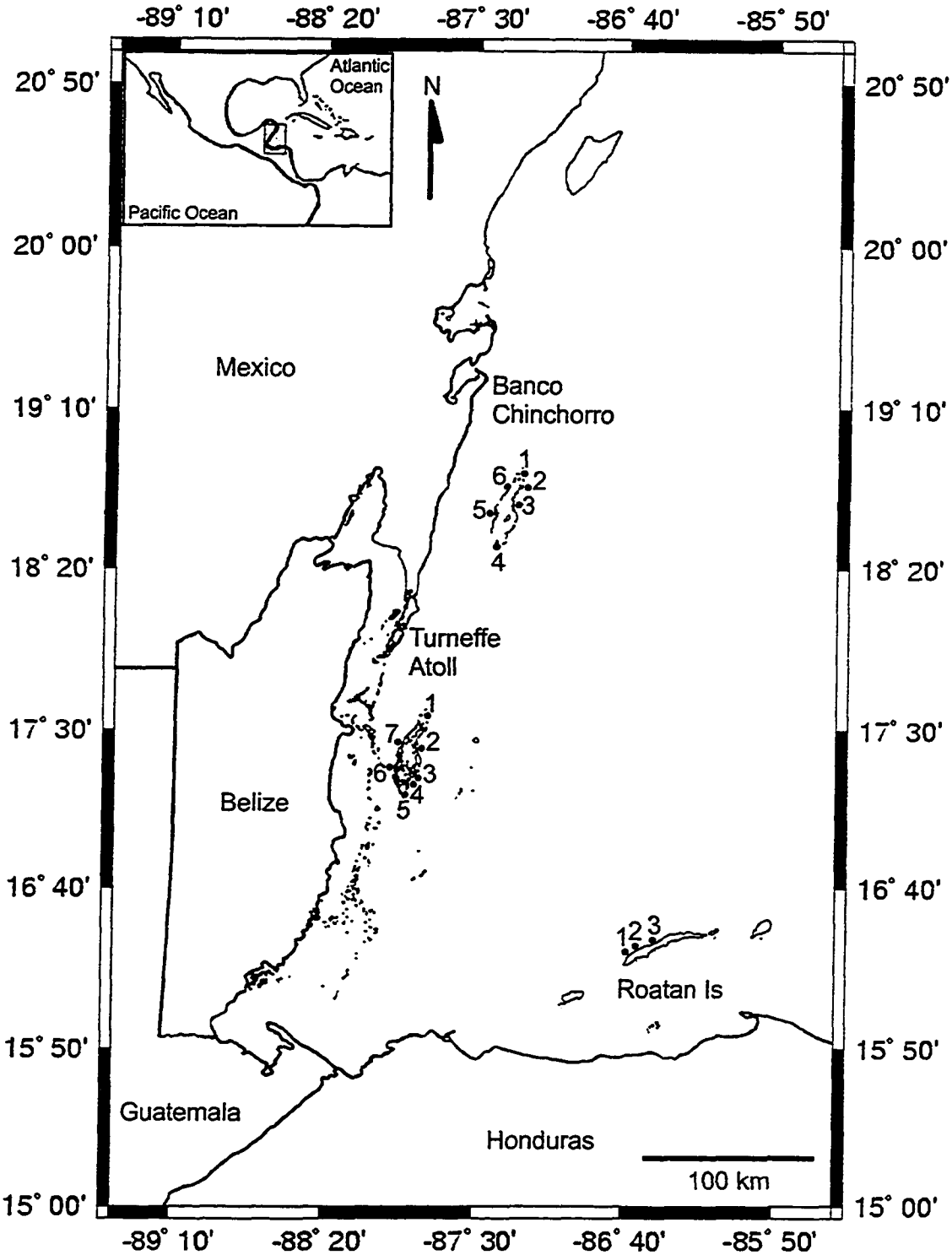

Fig 1.1. A map of the Meso-American Barrier Reef System (MBRS), depicting the sampling locations at the three atolls: Banco Chinchorro, Mexico; Turneffe Atoll, Belize; and Roatan Island, Honduras. Inset shows study area relative to Central America (shaded box).

Table 1.1. Primer sequences, annealing temperatures, and base pair size ranges of amplified fragments for molecular markers used in microsatellite analyses of juvenile bicolour damselfish from the MBRS. Primer sequences from Williams *et al.* (2003); primers marked with † were modified from the published primer sequence.

Locus Name	Primer sequence 5′-3′	T_a (°C)	Size range (bp)
SpAAT39	TGCCAAGTTAAACGTAGACAC CTCCCTTCAGTGTATTTCAGAA	59	140-220
SpGATA40	TTGCCTGCTGATAATTAACG ATGCCTCACAATGATGTATATTT	60	140-280
SpAAT9-2†	AGCCTCAAGGAACTTGTTGG GATCTTGTATGACTCTCAATGCTAAT	60	210-290
SpAAT40-1†	TGTTTCACCTGACATCCAAGA AGCCTCCCACTGAACACACT	57	250-310
SpAAC44-1†	TGCTGTAAACCACCAGGAGA GCAAACAGAAGGAGCAGTGG	60	100-165
SpAAC33-1†	TCACACCTGCTGAGTTCCTG CATGTACCTCCAATACAGGAAAAA	59	100-173
SpAAC42-1†	TGTTGAAGGGCAGGAAGC TCTCAACAAAATGTCCCATCAG	54	100-160
SpAAC41-1†	AGTCTGTGGTTTTGCCAACAT TGGTGCAGTTATTGCTTAGA	60	310-410

0.5 units DNA *Taq* polymerase, and 50-100 ng of genomic template DNA with ddH_2O added to bring the final reaction volume to 25 μL. PCR was performed on an MJ Research Tetrad DNA Engine model PTC-0225 (MJ Research Waltham, MA) with the following reaction profile: 2 minute initial denaturation (94°C); 35 cycles of 1 minute denaturation (94°C), 1 minute annealing (Table 1.1), 1 minute extension (72°C); 3 minute concluding extension cycle (72°C). Amplifications were analyzed for fragment size using a CEQ 8000 automated DNA sequencer with appropriate size standard (Beckman-Coulter, Fullerton, CA). Approximately 5% of all PCR reactions were replicated to verify repeatability.

1.3.3 Population structure

An exact test for goodness-of-fit to Hardy-Weinberg equilibrium was performed with the Monte Carlo method for each locus within each site (total 20,000 permutations) using TOOLS FOR POPULATION GENETIC ANALYSES (MP Miller (1997) Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author). To account for multiple, simultaneous tests, Hardy-Weinberg results were adjusted for significance using the sequential Bonferroni correction procedure (Rice, 1989).

All further analyses were performed using ARLEQUIN version 2.0 (Schneider *et al.* 2000), unless otherwise stated. Three spatial scales were tested for genetic differentiation: (1) large scale (among atolls); (2) medium scale (sites within atolls: i.e. (i) upstream versus downstream relative to ocean currents and (ii) windward versus leeward); and (3) small scale (neighbouring sites <20 km apart). Pairwise F_{ST} was

calculated to estimate the degree of population differentiation between and among sites and atolls (Weir & Cockerham, 1984). An hierarchical analysis of molecular variance (AMOVA) was carried out to partition observed variance into among-groups (atoll versus atoll), among-populations within groups (site versus site), and among-individuals components, as described in Excoffier *et al.* (1992).

Population structure was assessed using STRUCTURE software (version 2.0, Pritchard *et al.*, 2000). The admixture model with correlated allele frequencies was used, as the groups were expected to be genetically similar. A 'burn-in' of 100,000 runs, followed by 100,000 replicates were executed to ensure accurate estimation of model probability to estimate the number of populations (K) (Pritchard *et al.*, 2000). The smallest value of K that captured the major structure of the data was determined to be the most probable number of populations sampled (Pritchard *et al.*, 2000).

Pairwise exact tests for differences in allele frequency distributions between and among sites, for all three spatial scales, were completed (20,000 permutations; (Raymond & Rousset, 1995) using TFPGA 1.3. The sequential Bonferroni correction method (Rice, 1989) was again used to adjust significance levels.

An unrooted neighbour-joining cluster analysis (with Cavalli-Sforza and Edwards' (1967) chord distance (Dc)) was performed for the medium spatial scale (sites within atolls: i.e. (i) upstream versus downstream relative to ocean currents and (ii) windward versus leeward) using POPULATIONS (version 1.2.14 O. Langella, Centre National de la Recherche Scientifique, Laboratoire Populations, Génétique et Evolution, Gif sur Yvettev; www.cnrs-gif.fr/pge/bioinfo/populations) and visualized using TREEVIEW (Page, 1996). Cavalli-Sforza and Edwards' (1967) chord distance was chosen

as it has been demonstrated to produce accurate tree topology for closely related populations (Angers & Bernatchez, 1998). The resulting tree was bootstrapped among loci, with replacement, with 2,000 permutations.

1.4 RESULTS

1.4.1 Genetic variation

The eight microsatellite loci showed considerable allelic variation (3-26 alleles). Of the 128 tests performed, 26 showed significant deviation from Hardy-Weinberg equilibrium after sequential Bonferroni correction, all due to heterozygote deficiencies (Table1.2). Two loci, SpAAT9-2 and SpAAT39, were responsible for 24 of the 26 significant departures of Hardy-Weinberg equilibrium (Table 1.2). Analysis using MICRO-CHECKER (van Oosterhout *et al.*, 2004) indicated that null alleles were the most likely cause of Hardy-Weinberg disequilibrium and those two loci were removed from subsequent analysis.

No significant pairwise F_{ST} values were found after correcting for multiple simultaneous comparisons (Rice, 1989) at any of the three spatial scales. Isolation by distance over the MBRS was not apparent when geographic distance was plotted against pairwise F_{ST} values. Molecular variance between atolls, between sites within atolls, and among individuals was influenced exclusively by variation among individuals (100%), with negligible or no variance explained by between atolls (0.04%) or by sites within atolls (0.0%). The analysis of population structure was unable to conclusively designate the number of populations (K) within the MBRS sampling area. Our smallest estimated negative natural logarithm (-ln) probability value was for K=1, suggesting that we sampled one population over the MBRS. However, estimated -ln probability values for K=2, 3 and 4 were close to that of K=1 (Fig 1.2).

Table 1.2. Sample sizes (*N*), number of alleles (*A*), and observed and expected heterozygosity (H_O and H_E) at eight microsatellite loci for juvenile *S. partitus* collected from 16 sites along the MBRS within 3 geographic regions: Roatan Island, Honduras (3 sites); Turneffe Atoll, Belize (7 sites); and Banco Chinchorro, Mexico (6 sites). Significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni correction are underlined in boldface.

		Turneffe Atoll (TA)							Banco Chinchorro (BC)						Roatan (RO)		
Locus	Site	1	2	3	4	5	6	7	1	2	3	4	5	6	1	2	3
SpAAC42-1	*N*	9	30	25	25	25	20	20	15	14	9	8	23	25	35	24	26
	A	13	22	23	21	17	16	17	13	15	9	13	19	19	21	19	17
	H_O	1.00	0.87	0.96	0.92	0.96	0.80	0.95	1.00	0.79	1.00	1.00	0.91	**0.76**	0.83	0.92	0.92
	H_E	0.95	0.95	0.96	0.95	0.94	0.94	0.94	0.94	0.95	0.92	0.96	0.95	0.94	0.93	0.93	0.92
SpAAT40-1	*N*	9	30	25	25	24	20	19	14	13	9	8	22	24	33	23	25
	A	9	14	14	14	15	10	14	12	12	11	9	15	14	14	11	15
	H_O	0.89	0.83	0.84	0.92	0.88	0.80	0.79	0.86	1.00	0.89	0.75	0.77	0.88	0.73	0.87	0.84
	H_E	0.90	0.87	0.87	0.92	0.92	0.84	0.92	0.89	0.90	0.93	0.93	0.90	0.91	0.88	0.90	0.89
SpAAC44-1	*N*	9	30	25	26	25	19	20	15	14	9	10	22	25	36	24	26
	A	3	9	8	6	6	6	8	6	5	5	7	8	8	9	6	9
	H_O	0.44	0.33	0.40	0.15	0.28	0.26	0.35	0.53	0.36	0.67	0.70	0.41	0.44	0.36	0.29	0.35
	H_E	0.47	0.33	0.42	0.32	0.30	0.29	0.40	0.51	0.44	0.56	0.58	0.46	0.48	0.35	0.34	0.35

		Turneffe Atoll (TA)							Banco Chinchorro (BC)						Roatan (RO)		
Locus	Site	1	2	3	4	5	6	7	1	2	3	4	5	6	1	2	3
SpGATA40	*N*	9	29	24	26	25	20	20	15	14	9	10	22	25	36	23	25
	A	7	13	12	14	12	11	12	18	11	11	10	11	12	14	13	11
	H_O	0.89	0.86	0.92	0.88	0.92	0.95	0.85	0.80	0.57	0.67	0.60	0.82	0.84	0.94	0.74	0.84
	H_E	0.93	0.95	0.95	0.96	0.96	0.96	0.97	0.97	0.96	0.97	0.97	0.96	0.96	0.96	0.98	0.94
SpAAC33-1	*N*	9	30	25	26	25	19	21	15	14	9	10	22	25	36	24	26
	A	7	13	12	14	12	11	12	11	11	11	10	11	12	14	13	11
	H_O	0.78	0.70	0.80	0.88	0.76	0.79	0.95	0.73	0.93	0.78	0.80	0.91	0.84	0.81	0.83	0.81
	H_E	0.89	0.86	0.90	0.89	0.83	0.86	0.88	0.86	0.90	0.95	0.86	0.89	0.87	0.89	0.89	0.90
SpAAC41-1	*N*	9	27	24	24	22	19	18	13	13	6	10	22	25	34	22	25
	A	12	24	24	24	24	21	23	17	17	11	15	22	17	26	22	24
	H_O	0.89	0.89	0.96	0.88	0.90	0.84	0.94	0.79	1.00	1.00	0.90	0.77	0.80	0.82	1.00	0.96
	H_E	0.97	0.96	0.95	0.96	0.96	0.96	0.97	0.96	0.96	1.00	0.97	0.95	0.95	0.96	0.95	0.97
SpAAT9-2	*N*	9	30	25	26	24	19	21	15	14	9	10	22	24	36	24	27
	A	7	13	12	14	12	11	12	18	11	11	10	11	12	14	13	11
	H_O	**0.67**	0.63	**0.52**	**0.65**	0.71	**0.56**	**0.67**	0.73	**0.43**	**0.56**	**0.60**	**0.59**	**0.71**	0.72	**0.38**	**0.67**
	H_E	0.98	0.96	0.97	0.96	0.97	0.97	0.97	0.96	0.96	0.97	0.94	0.97	0.98	0.96	0.97	0.97

		Turneffe Atoll (TA)							Banco Chinchorro (BC)						Roatan (RO)		
Locus	Site	1	2	3	4	5	6	7	1	2	3	4	5	6	1	2	3
SpAAT39	*N*	9	29	25	26	25	20	19	15	14	8	8	23	25	35	22	25
	A	11	20	21	21	21	18	19	14	12	11	11	21	19	21	14	20
	H_O	0.56	**0.48**	**0.68**	**0.65**	**0.56**	0.75	**0.47**	0.73	**0.64**	**0.38**	0.63	**0.74**	**0.56**	**0.46**	**0.45**	**0.64**
	H_E	0.98	0.95	0.95	0.95	0.95	0.96	0.96	0.94	0.94	0.99	0.97	0.96	0.95	0.95	0.93	0.96

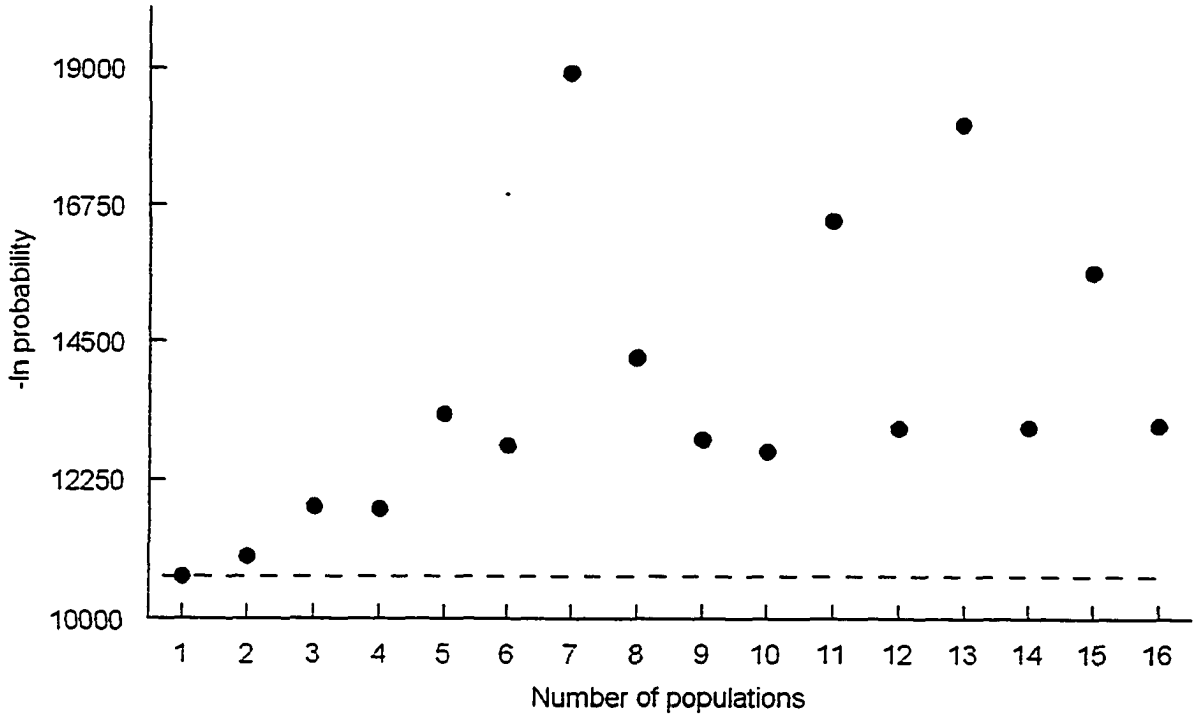

Fig. 1.2 Negative natural logarithm (-ln) probabilities, generated using STRUCTURE software, plotted against *K* values (number of populations specified *a priori*). Smallest value of *K* is considered 'correct' if several estimates are similar. Dashed line indicates the negative natural logarithm value for *K*=1 population for comparative purposes.

Exact tests for differences in allele frequency distribution between neighbouring sites within atolls showed significant genetic differentiation within the island of Roatan ($P<0.01$) and within Banco Chinchorro ($P<0.006$) (Table 1.3). Significant differences were also seen between windward versus leeward sites within Banco Chinchorro (sites two and five $P<0.005$), and for sites located up- or downstream of each other within Roatan (sites one and three $P<0.01$) relative to predicted ocean currents (Sheng & Tang, 2004) (Table 1.4).

The neighbor-joining trees support the divergence detected by the exact test of allele frequency distributions between windward and leeward sites of Banco Chinchorro, with strong bootstrap values, and also group the windward and leeward sites of Turneffe Atoll, with low bootstrap values (Fig 1.3 C and D). No divergence was seen between the upstream and downstream sites of Banco Chinchorro or Turneffe Atoll (Fig. 1.3 A and B).

Table 1.3. Exact test pairwise comparisons of neighbouring sites within each atoll. Values significant after Bonferroni correction are shown in underlined boldface.

Banco Chinchorro		Turneffe Atoll		Roatan Island	
Sites	Exact test probability	Sites	Exact test probability	Sites	Exact test probability
1 vs 2	0.101	1 vs 2	0.862	1 vs 2	0.269
2 vs 3	0.715	2 vs 3	0.146	2 vs 3	**0.014**
3 vs 4	0.977	3 vs 4	0.429		
4 vs 5	0.273	4 vs 5	0.872		
5 vs 6	**0.006**	5 vs 6	0.636		
6 vs 1	0.043	6 vs 7	0.518		
		7 vs 1	0.476		

Table 1.4. Individual and combined (parentheses indicate combined sites) pairwise exact tests probability results for medium scale geographic comparisons between windward/leeward sites and up/downstream sites. For Roatan, only up/downstream comparisons were possible.

	Upstream (U)/Downstream (D)					Windward (W)/Leeward (L)					
Turneffe Atoll		U(1)	U(2)	D(4)	D(5)			W(2)	W(3)	L(6)	L(7)	
	U(1)	-				U(1)	W(2)	-				W(2)
	U(2)	0.862	-			U(2)	W(3)	0.146	-		0.257	W(3)
	D(4)	0.545	0.486	-		D(4)	L(6)	0.782	0.123	-		L(6)
	D(5)	0.196	0.412	0.872	-	D(5)	L(7)	0.774	0.219	0.518	-	L(7)
Banco Chinchorro		U(1)	U(2)	D(3)	D(4)			W(2)	W(3)	L(5)	L(6)	
	U(1)	-				U(1)	W(2)	-				W(2)
	U(2)	0.101	-	0.355		U(2)	W(3)	0.715	-		0.114	W(3)
	D(3)	0.775	0.715	-		D(3)	L(5)	**0.005**	0.203	-		L(5)
	D(4)	0.548	0.213	0.977	-	D(4)	L(6)	0.786	0.545	**0.006**	-	L(6)
Roatan Island		U(1)	(2)	D(3)								
	U(1)	-										
	(2)	0.269	-									
	D(3)	**0.013**	**0.014**	-								

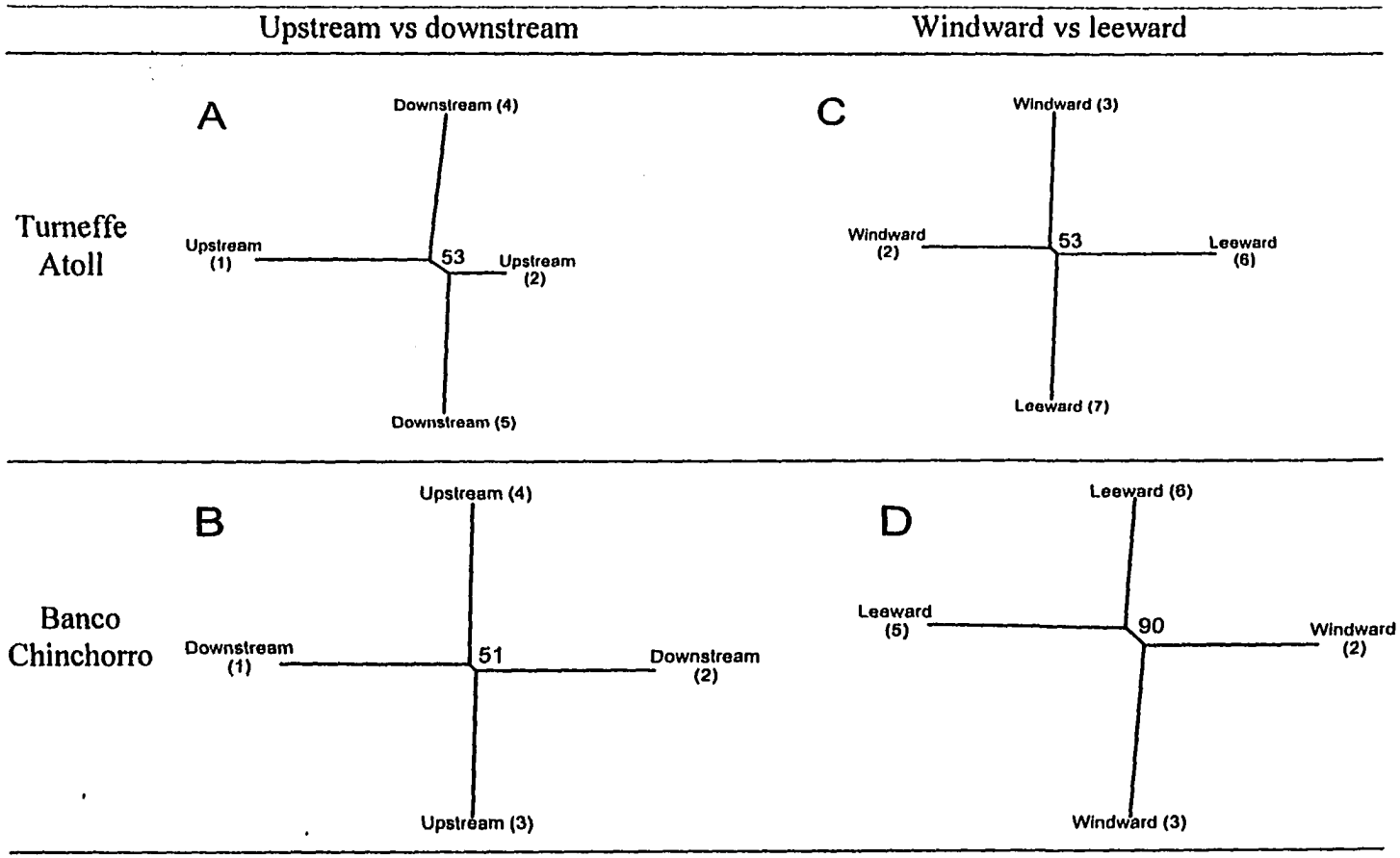

Fig 1.3. Unrooted neighbour-joining tree for juvenile *S. partitus* comparing upstream versus downstream and windward versus leeward sites (site number in brackets) from Turneffe Atoll and Banco Chinchorro. Trees based on Cavalli-Sforza and Edwards (1967) chord distance (Dc), calculated using 6 microsatellite loci. The data were bootstrapped 2000 times with replacement over loci.

1.5 DISCUSSION

Genetic structure in juvenile bicolor damselfish along the MBRS was not found at large geographic scales (between atolls) but was detected at medium (windward/leeward and up/downstream sites) and small geographic scales (between neighbouring sites). Similar genetic patterns at comparable geographic scales have been documented in previous studies of Pomacentridae (Bernardi *et al.*, 2001; Doherty *et al.*, 1995; Lacson, 1992; Lacson & Morizot, 1991; Planes *et al.*, 1993; Planes & Doherty, 1997; Shaklee, 1984), but, to our knowledge, this is only the second to examine microsatellite genetic data in coral reef fish (van Herwerden *et al.*, 2003), and the first to do so for a Caribbean species.

Our findings of low genetic divergence over the MBRS are in agreement with most past studies of *Stegastes* species in the Western Pacific and Atlantic oceans (Lacson, 1992; Lacson & Morizot, 1991; Shaklee, 1984). Shaklee's (1984) allozyme study found no evidence of genetic structuring in populations of *S. fasciolatus* within the Hawaiian archipelago. Within the Caribbean, populations of *S. partitus*, *S. planifrons*, *S. leucostictus* and *S. dorsopunicans* from Puerto Rico and Jamaica were shown to be genetically homogeneous, based on allozyme data (Lacson, 1992). Using allozymes, Lacson and Morizot (1991) established that *S. partitus* sampled in the upper Florida keys exhibited no population genetic structure, and concluded that the population was highly dispersed with geographically extensive gene pools. Shulman and Bermingham's (1995) study, however, detected 'very strong' structuring in *S. leucostictus* over the Caribbean using mitochondrial restriction fragment length polymorphisms (RFLP). As mitochondrial DNA is a functional marker (Planes, 2002), the intensity of the structure

detected may have been a measurement of selective, not dispersive, processes. Lacson's (1989) allozyme study of *S. partitus* detected large-scale structure in the Florida keys, which they concluded was most likely the result of a population bottleneck caused by a storm event earlier in the season. It may also have been due to two other factors: non-random sampling, as the individuals targeted for collection were from populations exhibiting variable prevalence rates of damselfish neurofibromatosis; and the use of a potentially functional marker. To date, there is little consensus on the genetic structure of coral reef fish populations within the Caribbean and Eastern Pacific oceans and this may be confounded by marker choice and/or possible sampling bias.

Overall, damselfish species in the Indo-Pacific appear to have higher genetic heterogeneity than Western Pacific and Atlantic damselfish species (Bell *et al.*, 1982; Doherty *et al.*, 1995; Planes & Doherty, 1997; Planes *et al.*, 1998, but see Bernardi *et al.*, 2001; Planes *et al.*, 1993). Two studies examining populations of *S. nigricans* along the Australian Great Barrier Reef (GBR) (Doherty *et al.*, 1995) and New Caledonian Island lagoon (Planes *et al.*, 1998) found significant genetic structure over large geographic scales. The genetic structure in the New Caledonian lagoon populations may be due to complex hydrodynamics separating the east and west coast reefs, combined with the partial segregation of the lagoon waters from the open ocean (Planes *et al.*, 1998). In contrast, the damselfish *Dascyllus aruanus* (Planes *et al.*, 1993) and the three-spot dascyllus (*Dascyllus trimaculatus*) (Bernardi *et al.*, 2001) of French Polynesia showed genetic homogeneity at all spatial scales until Indo-West Pacific populations (>1000 km) were included in the comparisons.

Genetic structuring within the MBRS was detected at medium and small scales, possibly due to micro-geographic effects. Our neighbour-joining trees, significant pairwise exact tests for allele frequency differences at small and medium scales, and lack of large-scale genetic differentiation indicate that we may be detecting the presence of cryptic genetic signatures underlying a primarily stochastic genetic structure over the MBRS. Research examining genetic structure in damselfish species is divided on the presence of localized genetic structure. Studies of two species of damselfish around the island of Moorea in French Polynesia showed no genetic structure at medium or small scales when analyzed with either allozymes (Planes *et al.* 1993) or mtDNA (Bernardi *et al.* 2001). Lacson (1989), however, found genetic differentiation between bicolor damselfish populations in the upper Florida keys on a very localized scale (<600m), but, as previously discussed, this structure may be due to population perturbation from a storm event, sampling bias and/or the genetic marker used. Small-scale heterogeneity was also detected in populations of *S. nigricans* along the GBR but, due to sampling design, the researchers were unable to discern if this was characteristic of the reefs sampled or of small spatial scales (Doherty *et al.* 1995). Small-scale processes may play a much more important role in the dispersal of larvae than currently thought, primarily due to high variability in local flow conditions brought about by deep-water and coastal current interactions (Cowen, 2002). Additionally, genetically differing cohorts of settling larvae may contribute to genetic differentiation within a small geographic area, as seen in the surgeonfish, *Naso unicornis*, off the island of Moorea in French Polynesia (Planes *et al.*, 2002). Genetic pulses were detected in settling larvae that, while comprised of individuals from multiple spawning events, were found to include up to 10% of related

individuals. These pulses were genetically different from the adults and juveniles already residing on the reef, which Planes *et al.* (2002) suggested was a result of post-settlement mortality and/or movement. Regardless of the forces creating these differences, the result is a temporally and spatially stochastic blend of recruits into the local gene pool. Our results suggest that these two processes may be occurring along the MBRS, creating localized genetic heterogeneity.

Within the context of coral reef fish, two fundamental processes determine genetic differentiation: selection and dispersal. Selection pressure on new recruits is high, with mortality estimated to be up to 88% and varying widely between cohorts (Jones *et al.*, 1991). Studies sampling adults would include individuals that had undergone this selective pressure. By sampling juvenile *S. partitus*, we minimized the potential effects of post-settlement selection. Dispersal in coral reef fish has been extensively studied (reviewed by both Cowen, 2002 and Leis, 1991), and two paradigms have developed regarding the dispersal mechanisms of these organisms. As the larvae of many coral reef fish species possess potentially dispersal-altering behaviours, including photo and auditory reception/stimulus and motor control (buoyancy control and strong swimming abilities) (reviewed by Armsworth *et al.*, 2001 and by Leis & McCormick, 2002; Losey, 2003; Siebeck & Marshall, 2001) the concept of active transport (behaviour-directed dispersal by larvae) has gained popularity in the last decade (Jones *et al.*, 1991; Swearer *et al.*, 1999, but see Mora & Sale, 2002). If *S. partitus* larvae disperse in a directed manner in the MBRS, a more consistent pattern of genetic differentiation would be expected. Traditionally, passive transport (larvae dispersed by abiotic factors, primarily hydrodynamic processes) was thought to be the fundamental mechanism of

coral reef fish dispersal (Sale, 1991). The lack of large-scale genetic structure found for *S. partitus* on the MBRS suggests that they are stochastically dispersed. Based on this, we can predict that the small-scale genetic structure found will be only as stable as the circumstances that created it and, therefore, any genetic structuring along the MBRS may be expected to be temporally transient.

The juvenile bicolor damselfish of the MBRS were found to have an homogeneous genetic structure, indicating that the larvae of this species are highly dispersed. Although potential dispersal-altering behaviours have been shown to exist in coral reef fish larvae (reviewed by Armsworth *et al.*, 2001 and by Leis & McCormick, 2002; Losey, 2003; Siebeck & Marshall, 2001), there is no genetic evidence that settling juvenile bicolor damselfish from the MBRS practice site selection, at least within the resolving power of the six microsatellite loci used in this study. Most probable is that the larval dispersal is more random than directed and, therefore, the genetic structure of these organisms is stochastic through space and time. Evidence of underlying genetic structure was found at a micro-geographic scale and is predicted to be temporally unstable, possibly due to changing current flow dynamics and/or genetic signatures of settlement pulses. At the larval stage, reproduction and life span of coral reef fish cover different time scales, ranging from weeks to years, temporal scales are of great importance when examining the dispersal of these animals. Species that possess protracted spawning seasons will likely display intra-annual and even intra-seasonal patterns of recruitment, and this increases the opportunity for small-to medium-scale processes to introduce extensive variability to recruitment (Cowen, 2002). Thus, there is a clear need for

investigations of dispersal in the bicolor damselfish over multiple spatial and temporal scales to more completely understand the dispersal in this species.

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CHAPTER 2

Temporal genetic variability in juvenile bicolor damselfish (*Stegastes partitus*) across the Meso-American Barrier Reef System

2.1 ABSTRACT

A fundamental goal in understanding connectivity of coral reef fish populations is determining the mode of dispersal of the organism. Genetic tools, such as microsatellite markers, can provide insight into coral reef fish connectivity by examining the genetic signals of settling larval cohorts over time. To investigate annual and seasonal population genetic variability in bicolor damselfish juveniles (*Stegastes partitus*), we collected individuals from three atolls across the Meso-American Barrier Reef System (MBRS) in 2002 (early summer) and 2003 (early and late summer). Six polymorphic microsatellite loci were used to determine the genetic structure of the sampled populations. We found genetic signatures of newly recruited bicolor damselfish to be variable over time, indicating that the dispersal for this species is stochastic. This variability may result from "sweepstakes-chance matching," where the survival of larval cohorts to settlement depends on their being at the right place at the right time, primarily by chance. Our results indicate that chaotic genetic patchiness is present in newly recruited bicolor damselfish over the MBRS, possibly due to high reproductive variance. These findings are of importance for future research as well as management strategies involving coral reef fish.

2.2 INTRODUCTION

Many benthic marine invertebrates and demersal fishes produce dispersive, pelagic larvae (Ball & Chapman, 2003; Caley *et al.*, 1996; Sale, 1991). It has been hypothesized that this strategy may give the unattended larvae a relatively 'safe' area in which to develop (Johannes, 1978) and/or impart advantages in patchy environments, functioning as a risk-spreading strategy (Doherty *et al.*, 1985) or as an adaptive mechanism in response to habitats unstable over evolutionary time (Barlow, 1981). Ultimately, this life history stage provides larvae the opportunity to mix and disperse prior to settling onto suitable habitats (i.e. recruitment). In coral reef fish, larval dispersal may be directed or stochastic in nature, depending on the physical and/or biological processes acting on the larvae.

Recruitment patterns in coral reef fish with dispersive, pelagic larvae have been historically determined primarily through ecological larval recruitment surveys (e.g. Lozano & Zapata, 2003; Miller *et al.*, 2001; Robertson, 1992; Robertson *et al.*, 1988; Tolimieri *et al.*, 1998). These surveys have found that many species of coral reef fish in the Pacific have high variability in recruitment (review by Doherty, 1991). While several Caribbean species display consistent spatial and temporal recruitment patterns, they vary annually in intensity (Miller *et al.*, 2001; Robertson, 1992; Robertson *et al.*, 1988; Tolimieri *et al.*, 1998). These surveys have increased our understanding of the population dynamics of these organisms, and while variation in abundance over time may be an indication of temporal variation in dispersal, other methods are needed to better infer the nature of the temporal component of connectivity among reefs.

Genetic techniques, such as the use of allozyme and microsatellite markers, can provide insight into coral reef fish connectivity by examining the genetic signals of settling larval cohorts over time. It has been found that larval cohorts have a clear genetic signal, readily discernable from existing genetic signatures (juvenile and adult) on the reef (Planes *et al.*, 2002). However, in terms of connectivity (i.e. gene flow), the interpretation of this genetic signal will depend on how it is created. Stochastic, or passive, dispersal will result in genetic patterns that change unpredictably over time, while stable (i.e. self-recruitment or directional) dispersal will result in more consistent temporal patterns. Thus, determining the nature of the genetic patterns of recruits over time can reveal the nature of dispersal employed by the organism, and, hence, the connectivity among populations.

The bicolor damselfish (*Stegastes partitus*: Pomacentridae) is a species abundant throughout the tropical western Atlantic (DeLoach, 1999). Both females and males reach sexual maturity at approximately one year of age and then spawn for up to three years (DeLoach, 1999). Spawning and settlement follow a unimodal lunar cycle from May to October, with female *S. partitus* demersally spawning up to 5,000 eggs every two days from a week-long reproductive cycle (Knapp, 1993). Peak recruitment usually occurs during early to late summer (but see Tupper & Hunte, 1994) and is spatially and temporally consistent, though annual abundance can vary (Miller *et al.*, 2001; Tolimieri *et al.*, 1998).

We investigated the temporal genetic variability in bicolor damselfish juveniles across the Meso-American Barrier Reef System (MBRS). Six polymorphic microsatellite markers were used to determine the stability of the genetic structure of juvenile bicolor

damselfish populations at two temporal scales: (i) annual (2002 versus 2003) and (ii) seasonal (early summer 2003 versus late summer 2003). This analysis will provide an understanding of the temporal stability in the genetic signals of newly recruited coral reef fish, and allow a critical evaluation of dispersal and connectivity among populations of *S. partitus* along the MBRS.

2.3 MATERIALS AND METHODS

2.3.1 Sampling

Collections were carried out targeting newly settled *S. partitus* (<2.5 cm) at 12 sites along the MBRS, which extends from Isla Contoy on the northern Yucatan Peninsula to the Bay Islands of Honduras (Fig 2.1). Sampling took place June 12 to 16, 2002 on Turneffe Atoll, Belize (three sites) and on Banco Chinchorro, Mexico (three sites). In 2003, sampling took place June 16 to 19 and August 13 to 24 on Turneffe Atoll, Belize (four sites), Banco Chinchorro, Mexico (four sites), and Roatan Island, Honduras (three sites) (Fig 2.1). Fish were collected by divers using hand nets, after anaesthetizing and immobilizing the individuals with clove oil (Munday, Wilson, 1997). Upon surfacing, the specimens were immediately placed in containers with 95% ethanol and labelled according to site and date.

2.3.2 DNA extraction and microsatellite analysis

DNA was extracted from caudal fin clips using the Wizard® Genomic Purification Kit (Promega, Madison, WI) following manufacturer's protocol for DNA extraction from animal tissue. Six microsatellite markers developed for *S. partitus* (Williams *et al.*, 2003) were chosen and modified to adjust fragment size, in order to facilitate running combinations of loci on the automated sequencer (see Chapter 1 for marker screening information). Polymerase chain reactions (PCR) were carried out in reactions comprised of: 2.5 µL 10X PCR Buffer (10 mM Tris-HCL (pH-8.4) 50mM KCL), 2.5 mM $MgCl_2$, 200 µM dNTP's, 0.05 µg of each primer, 0.5 units DNA *Taq* polymerase, and 50-100 ng of genomic template DNA, with ddH_2O added to bring the final reaction volume to 25 µL. PCR was performed on an MJ Research Tetrad DNA Engine, model PTC-0225 (MJ

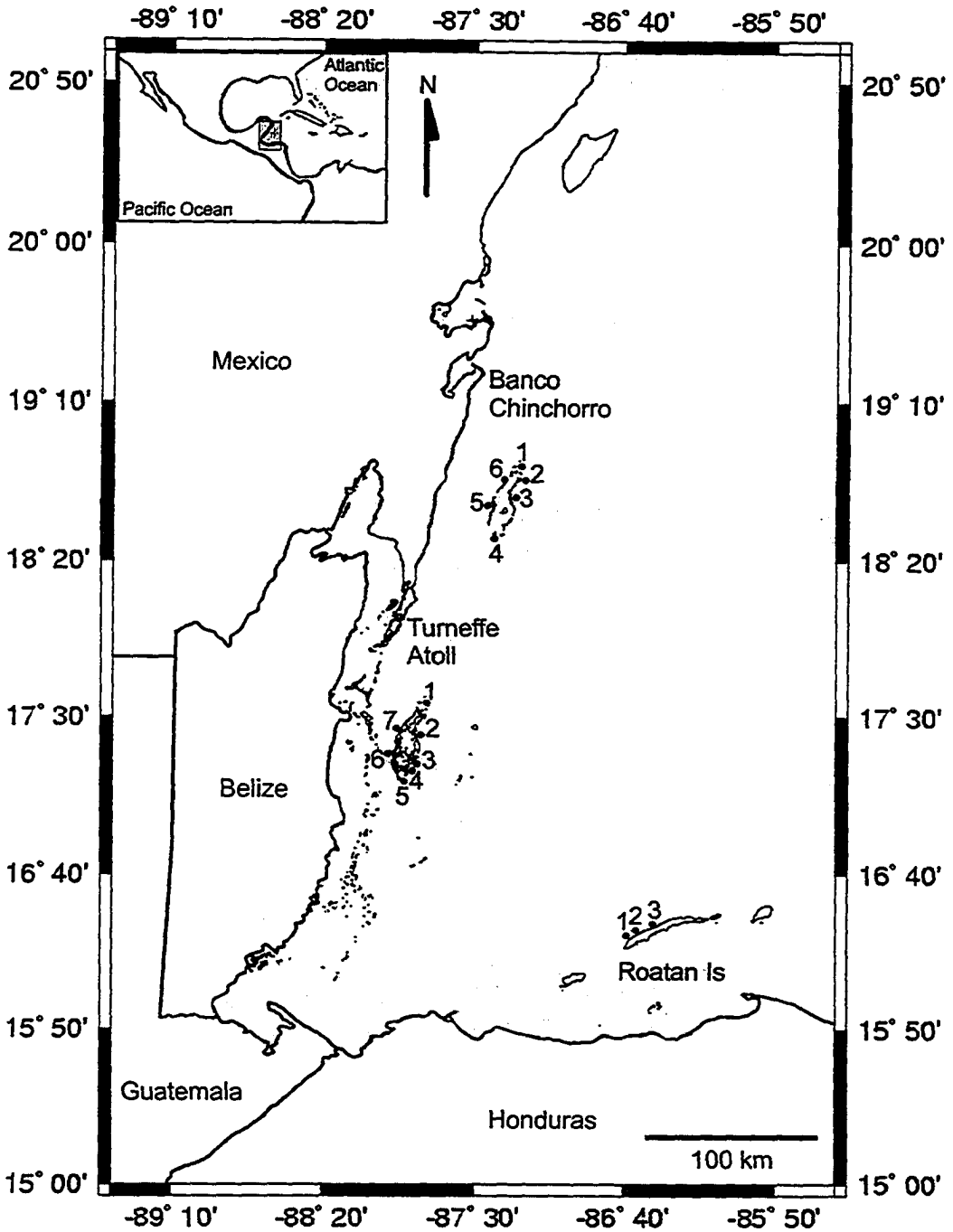

Fig 2.1. A map of the Meso-American Barrier Reef System (MBRS), depicting sampling locations within 3 geographic regions: Banco Chinchorro, Mexico; Turneffe Atoll, Belize; and Roatan Island, Honduras. See text for sampling site details. Inset shows study area relative to Central America (shaded box).

Research, Waltham, MA), with the following thermal cycle profile: 2 minute initial denaturation (94°C); 35 cycles of 1 minute denaturation (94°C), 1 minute annealing with variable temperatures (see Chapter 1), 1 minute extension (72°C); 3 minute concluding extension cycle (72°C). Amplifications were analyzed for fragment size using a CEQ 8000 automated DNA sequencer with appropriate size standard (Beckman-Coulter, Fullerton, CA). Approximately 5% of all PCR reactions were replicated to verify repeatability.

2.3.3 Population structure

For the annual temporal analysis, sites three and four in Banco Chinchorro, as well as sites one and two in Turneffe Atoll, were combined, after exact tests for allele frequency differences indicated that the sites were not significantly genetically differentiated (P=0.20-0.90). Similarly, for the seasonal temporal analysis, Banco Chinchorro sites three and four, and sites one and two in Turneffe Atoll (late only) were combined (see Table 2.1 for sample numbers of each site and temporal period).

An exact test for goodness-of-fit to Hardy-Weinberg equilibrium was performed with the Monte Carlo method for all loci within each site (total 20,000 permutations) using TOOLS FOR POPULATION GENETIC ANALYSES (MP Miller (1997), Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author). To account for multiple, simultaneous tests, Hardy-Weinberg results were adjusted for significance using the sequential Bonferroni correction procedure (Rice, 1989).

Table 2.1. Sample sizes (*N*), number of alleles (*A*), and observed and expected heterozygosity (H_O and H_E) at six microsatellite loci for juvenile *S. partitus* collected from 12 sites along the MBRS within 3 geographic regions: Roatan Island, Honduras (3 sites); Turneffe Atoll, Belize (5 sites); and Banco Chinchorro, Mexico (5 sites) during 2002 and 2003. Significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni correction are underlined in boldface.

				Banco Chinchorro (BC)				Roatan (RO)			Turneffe Atoll (TA)				
Locus		Year	Season	2	3†	5	6	1	2	3	1	2‡	3	4	6
SpAAC33-1	*N*	*2002*	*Early*		15	26	26				12		24	22	
	A				8	12	13				10		12	12	
	H_O				0.87	0.85	0.81				1.00		0.83	0.77	
	H_E				0.84	0.84	0.87				0.85		0.87	0.84	
	N	*2003*	*Early*	26	8	25		23	17	25		45	13	28	18
	A			10	7	13		13	8	9		14	8	14	10
	H_O			0.81	1.00	1.00		0.96	0.88	0.76		0.89	1.00	0.75	0.89
	H_E			0.85	0.82	0.87		0.85	0.85	0.84		0.88	0.82	0.89	0.85
	N		*Late*	14	19	22	25	35	24	26		40	25	26	19
	A			11	14	10	12	14	14	10		14	12	14	9
	H_O			0.93	0.79	0.91	0.84	0.83	0.88	0.81		**0.68**	0.80	0.88	0.79
	H_E			0.87	0.87	0.87	0.85	0.87	0.87	0.88		0.84	0.87	0.88	0.82

				Banco Chinchorro (BC)				Roatan (RO)			Turneffe Atoll (TA)				
Locus		Year	Season	2	3†	5	6	1	2	3	1	2‡	3	4	6
SpAAC41-1	*N*	*2002*	*Early*		15	26	26				12		24	23	
	A				16	23	21				11		25	21	
	H_O				1.00	0.85	0.88				0.75		0.88	1.00	
	H_E				0.90	0.92	0.93				0.85		0.95	0.94	
	N	*2003*	*Early*	25	8	24		23	18	26		45	12	25	17
	A			20	12	20		21	19	24		27	15	18	20
	H_O			**0.72**	1.00	**0.75**		0.91	0.94	0.88		0.84	0.83	0.88	0.88
	H_E			0.93	0.91	0.93		0.92	0.93	0.95		0.94	0.91	0.92	0.94
	N		*Late*	13	16	22	25	33	22	25		37	24	24	19
	A			16	16	21	17	25	22	23		25	23	23	21
	H_O			1.00	0.93	0.77	0.80	0.88	1.00	0.96		0.84	0.96	0.88	0.84
	H_E			0.92	0.92	0.92	0.92	0.94	0.93	0.95		0.94	0.93	0.94	0.94
SpAAC42-1	*N*	*2002*	*Early*		15	25	26				12		24	23	
	A				13	21	20				12		18	16	
	H_O				1.00	0.88	0.96				0.75		**1.00**	0.82	
	H_E				0.91	0.93	0.92				0.88		0.91	0.91	
	N	*2003*	*Early*	22	8	24		23	16	26		45	13	26	18

				Banco Chinchorro (BC)				Roatan (RO)			Turneffe Atoll (TA)				
Locus		Year	Season	2	3†	5	6	1	2	3	1	2‡	3	4	6
SpAAC42-1	*A*	*2003*	*Early*	18	10	17		14	15	19		20	15	22	15
continued...	H_O			0.86	0.88	0.88		0.87	0.94	0.77		0.87	0.85	0.85	0.89
	H_E			0.93	0.89	0.91		0.88	0.90	0.92		0.92	0.90	0.94	0.91
	N		*Late*	14	17	23	25	34	24	26		40	25	25	20
	A			14	12	19	19	21	18	16		23	23	20	16
	H_O			0.79	1.00	0.91	0.76	0.88	0.92	0.92		0.85	0.96	0.92	0.85
	H_E			0.91	0.90	0.93	0.92	0.92	0.91	0.89		0.93	0.94	0.93	0.92
SpAAC44-1	*N*	*2002*	*Early*		15	26	26				12		24	23	
	A				4	9	8				3		7	10	
	H_O				0.27	0.46	0.35				0.17		0.33	0.52	
	H_E				0.24	0.45	0.40				0.16		0.30	0.56	
	N	*2003*	*Early*	26	8	25		23	18	26		45	13	28	18
	A			9	1	7		5	8	9		8	3	9	9
	H_O			0.46	0.00	0.40		0.43	0.61	0.42		0.40	0.31	0.43	0.44
	H_E			0.43	0.00	0.35		0.40	0.54	0.37		0.35	0.27	0.43	0.47
	N		*Late*	14	19	22	25	35	24	26		40	25	26	19
	A			5	10	7	8	9	7	7		10	8	6	5
	H_O			0.36	0.68	0.41	0.44	0.40	0.29	0.31		0.35	0.40	0.15	0.26

				Banco Chinchorro (BC)				Roatan (RO)			Turneffe Atoll (TA)				
Locus		Year	Season	2	3†	5	6	1	2	3	1	2‡	3	4	6
SpAAC44-1	H_E	*2003*	*Late*	0.37	0.55	0.45	0.44	0.37	0.33	0.31		0.41	0.41	0.28	0.24
SpAAT40-1	*N*	*2002*	*Early*		14	24	23				12		23	22	
	A				11	13	12				11		13	14	
	H_O				0.86	0.96	0.88				0.92		0.83	0.81	
	H_E				0.89	0.86	0.87				0.87		0.87	0.91	
	N	*2003*	*Early*	22	7	24		21	18	24		38	12	25	18
	A			12	7	13		11	10	12		12	9	12	12
	H_O			0.86	0.71	0.83		0.70	0.94	0.71		0.79	0.91	0.85	0.83
	H_E			0.86	0.82	0.87		0.81	0.82	0.89		0.88	0.87	0.83	0.89
	N		*Late*	13	17	22	24	32	23	25		40	25	25	20
	A			11	12	14	13	13	11	14		14	14	13	10
	H_O			1.00	0.82	0.77	0.88	0.78	0.87	0.84		0.80	0.84	0.92	0.75
	H_E			0.86	0.88	0.87	0.89	0.86	0.88	0.86		0.87	0.83	0.90	0.83
SpGATA40	*N*	*2002*	*Early*		15	26	26				12		24	23	
	A				13	24	24				12		22	19	
	H_O				0.80	0.81	0.88				0.92		1.00	0.83	
	H_E				0.90	0.94	0.95				0.90		0.94	0.92	

				Banco Chinchorro (BC)				Roatan (RO)			Turneffe Atoll (TA)				
Locus		Year	Season	2	3†	5	6	1	2	3	1	2‡	3	4	6
SpGATA40	*N*	*2003*	*Early*	26	8	24		23	18	26		45	13	28	17
continued...	*A*			26	14	23		24	17	22		26	15	23	20
	H_O			**0.81**	1.00	0.88		0.83	0.94	0.81		0.89	1.00	0.93	0.94
	H_E			0.94	0.92	0.94		0.95	0.93	0.94		0.95	0.91	0.94	0.93
	N		*Late*	14	19	22	25	35	23	25		39	24	26	20
	A			15	17	19	20	25	24	18		24	21	23	20
	H_O			**0.57**	**0.63**	0.82	0.84	0.91	0.78	0.84		0.82	0.92	0.88	0.95
	H_E			0.92	0.91	0.93	0.94	0.95	0.95	0.92		0.93	0.93	0.94	0.94

All further analyses were performed using ARLEQUIN version 2.0 (Schneider *et al.*, 2000), unless otherwise stated. Two temporal scales: (i) annual (2002 versus 2003) and (ii) seasonal (early summer 2003 versus late summer 2003) were tested to resolve the genetic stability of both single populations and all populations over time.

To test a single population's genetic stability over time, an exact test for allele frequency differentiation was carried out at each temporal scale for each population (20,000 permutations; Raymond & Rousset, 1995) using TFPGA. Pairwise exact tests for differences in allele frequencies were also performed using TFPGA (20,000 permutations) to examine the genetic stability among populations within each temporal scale. The sequential Bonferroni correction method (Rice, 1989) was used to adjust significance levels of among-population pairwise comparisons. Cavalli-Sforza and Edwards' (1967) chord distances (Dc) were calculated for each between-population comparison within each time period. The resulting among-population chord distances were then compared in a pairwise manner, at each temporal scale. The pairwise Dc comparisons were plotted against one another to determine if they were predictive (i.e. if early summer Dc values between populations correlate to late summer Dc values between the same populations). An unrooted neighbour-joining cluster analysis was carried out with Cavalli-Sforza and Edwards' (1967) chord distance (Dc) using POPULATIONS (version 1.2.14, O. Langella, Centre National de la Recherche Scientifique, Laboratoire Populations, Génétique et Evolution, Gif sur Yvettev; www.cnrs-gif.fr/pge/bioinfo/populations) and visualized using TREEVIEW (Page, 1996). Cavalli-Sforza and Edwards' (1967) chord distance was chosen as it has been demonstrated to produce accurate tree topology for closely related populations (Angers & Bernatchez,

1998). The resulting tree was bootstrapped among loci, with replacement, with 2,000 permutations. To establish if same-site clustering within neighbour-joining trees (e.g. TA site 1, 2002 clustering with TA site 1, 2003) was due to random chance or was indicative of consistent genetic patterns (i.e. temporal genetic stability), we calculated the number of same-site pairs expected due to random chance alone (10,000 permutations) for both the annual and seasonal neighbour-joining trees.

2.4 RESULTS

The six microsatellite loci exhibited considerable variation among populations (1-27 alleles) (Table 2.1). Of the 161 tests performed, 5 showed significant deviation from Hardy-Weinberg equilibrium after sequential Bonferroni correction (Table 2.1).

Exact tests for differences in allele frequency distribution for single population comparisons detected only one significant genetic differentiation annually (Turneffe Atoll, site one, 2002-2003 ($P<0.004$)) and one seasonally (Turneffe Atoll, site four, early versus late summer ($P<0.05$)) (Table 2.2). No significant differences in allele frequency distributions were found in the annual or seasonal among-populations pairwise exact tests after Bonferroni correction. However, it is of interest to note that when results were compared prior to Bonferroni correction, none of the comparisons sustained their significance over either temporal period tested (i.e. annual, 2002 vs. 2003 or seasonal early vs. late) (Table 2.3).

If the Dc values from the early sampling period are predictive of the values from the late sampling period, the pairwise data points would be expected to be correlated with a positive slope. Regression analysis (SYSTAT, SPSS, Inc., Chicago, IL) found no significant relationship in either the annual ($P>0.11$) or seasonal ($P>0.08$) pairwise Dc comparisons (Fig 2.2).

The neighbour-joining trees for among-population annual and seasonal comparisons had extremely low bootstrap values (Fig 2.3). The number of same-site clusters (i.e. Banco Chinchorro site three and Turneffe Atoll site three) in the neighbour-joining tree for annual comparisons was not significantly different from that expected by

Table 2.2. Exact test probabilities of allele frequency differences for within-population comparisons for juvenile bicolor damselfish at two temporal scales. Significant results are underlined in boldface.

Temporal Scale	Population	Exact test probabilities
Annual	BC-3	0.504
2002/2003	BC-5	0.578
	BC-6	0.373
	TA-1	**0.004**
	TA-3	0.983
	TA-4	0.130
Seasonal	BC-2	0.331
Early/Late 2003	BC-3	0.830
	BC-5	0.277
	RO-1	0.538
	RO-2	0.454
	RO-3	0.763
	TA-2	0.084
	TA-3	0.196
	TA-4	**0.047**
	TA-6	0.591

Table 2.3. All significant (P<0.05) pairwise comparisons before Bonferroni correction for exact tests of allele frequency differences at annual (2002-2003) and seasonal (early-late) temporal scales. Significant results are underlined in boldface.

Annual	Sites Compared		2002	2003
	BC-3	TA-1	**0.003**	0.446
	BC-6	TA-4	**0.036**	0.286
	BC-5	BC-6	0.847	**0.030**
	BC-6	TA-3	0.587	**0.041**

Seasonal	Sites Compared		Early	Late
	RO-1	BC-5	**0.001**	0.771
	TA-2	RO-1	**0.004**	0.589
	TA-2	BC-2	**0.007**	0.864
	TA-4	RO-1	**0.014**	0.325
	TA-2	BC-5	**0.016**	0.205
	RO-1	BC-2	**0.026**	0.793
	RO-2	BC-2	**0.045**	0.786
	BC-5	BC-2	0.131	**0.025**
	RO-3	BC-5	0.417	**0.020**
	RO-3	RO-2	0.739	**0.024**
	TA-6	BC-3	0.784	**0.018**
	TA-2	RO-3	0.793	**0.026**
	TA-3	RO-3	0.867	**0.026**

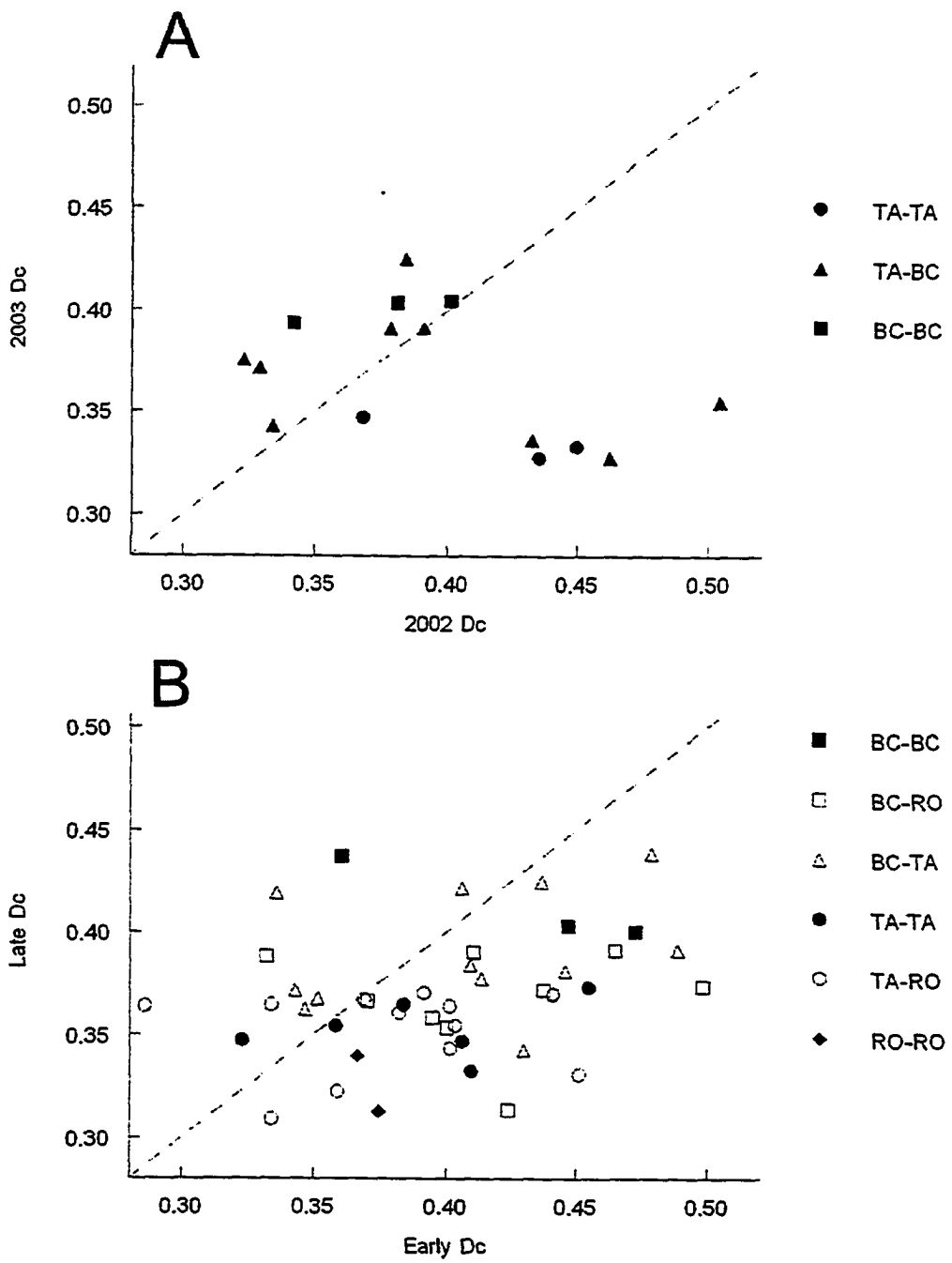

Fig 2.2. Scatter plots of genetic distance between pairs of sampling sites (calculated for two sampling dates). Annual comparisons in graph (A) and seasonal comparisons in graph (B). Dashed line represents one-to-one line expected if genetic distance values remain static over time. Distance values are Cavalli-Sforza and Edwards (1967) chord distance (Dc), calculated using six microsatellite loci.

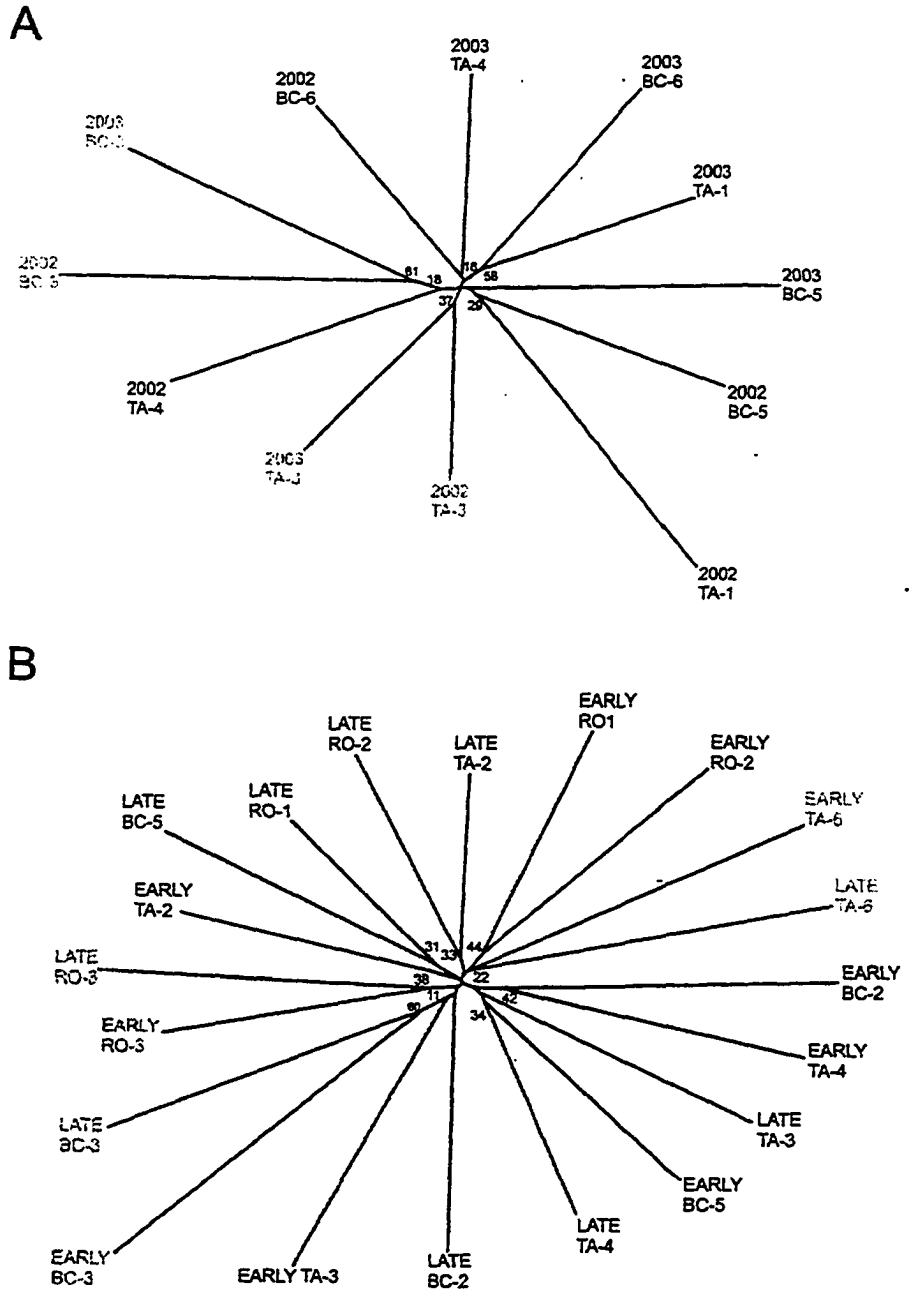

Fig 2.3. Unrooted neighbour-joining tree for juvenile *S. partitus* at two temporal scales, annual (A) and seasonal (B). Same-site clusters indicated by shaded areas. Trees are based on Cavalli-Sforza and Edwards (1967) chord distance (Dc), calculated using six microsatellite loci. The data were bootstrapped 2,000 times, with replacement, over loci.

chance ($P>0.15$). The three same-site clusters in the neighbour-joining tree for seasonal comparisons (Turneffe Atoll site six, Roatan Island site three and Banco Chinchorro site three), however, were significantly different from the number of same-site pairings expected by chance ($P<0.01$).

2.5 DISCUSSION

Our results indicate that chaotic genetic patchiness is present in newly recruited bicolor damselfish over the MBRS. Temporal genetic variability was found in within- and among-population comparisons at both annual and seasonal temporal scales, though there is evidence indicating that some seasonal genetic stability may exist in areas along the MBRS. The variability of the genetic signature over time indicates that dispersal patterns for larval *S. partitus* are primarily random in nature.

At present, there is a dearth of studies examining temporal genetic stability in tropical marine systems. Work to date has focused primarily on invertebrate species, with many of these organisms displaying temporal genetic variability (Arnaud-Haond *et al.*, 2004; Larson & Julian, 1999). Very few studies have examined genetic stability over time in tropical fish species. Planes and Lecaillon (1998) employed allozymes to examine founder effects on two species of snapper (*Lutjanus fulvus* and *L. kasmira*) and a grouper (*Cephalopholis argus*) introduced from French Polynesia to the Hawaiian archipelago in the 1950's. Their strategy used the ancestral and introduced populations as *de facto* temporal samples (t_0 and t_1, respectively) by assuming the source populations were stable and underwent no significant variation for 40 years. They found that the genetic signals between ancestral and introduced populations differed, possibly due to genetic drift caused by the small number of introduced fish or due to strong selection resulting from water temperature differences between regions, which is not a factor in the MBRS. Rhodes *et al.* (2003) employed three microsatellite markers to examine the population genetic structure of the camouflage grouper (*Epinephelus polyphekadion*) over a two-year period spanning a 5,000 km geographic scale. Three regional groups

were found, which is not unexpected at such a large spatial scale (see Bernardi *et al.*, 2001; Doherty *et al.*, 1995; Planes *et al.*, 1993; Planes & Fauvelot, 2002) and one of these groups displayed allelic differentiation over time. The authors speculated that the temporal structure detected may have been due to sampling multiple spawning aggregations (Wahlund effect), which, again, was not a factor in our study. Bernal-Ramirez *et al.* (2003) used six microsatellite markers and mtDNA analysis to determine the temporal stability of genetic structure in the New Zealand snapper (*Pagrus auratus*), based on comparisons with a previous allozyme study (Smith *et al.*, 1978). Both the microsatellite and allozyme results revealed strong spatial structuring between the North and South Islands of New Zealand due to oceanographic effects, and showed temporal stability over a 22 year period. The MBRS does not have any obvious oceanographic barriers to dispersal, which might have provided a more temporally stable genetic structure.

Only one published study has examined genetic temporal stability in populations of coral reef fish. Lacson and Morizot (1991) used allozymes to examine the temporal stability of population genetic structure that had been detected in *S. partitus* during a previous study (Lacson *et al.*, 1989) in the Upper Florida Keys. No temporal stability was detected, with previously differentiated populations undergoing almost complete genetic homogenization during the three years between studies. Such patterns of temporal change in genetic structure may be explained by Hedgecock's (1994) "sweepstakes-chance matching" hypothesis, where cohorts of larvae may or may not successfully settle depending on their being at the right place at the right time purely by

random chance. Under such a system, genetic structure over time would be expected to be unstable.

Our results are in agreement with the findings of Lacson and Morizot (1991), showing high levels of homogenization among the temporal scales. However, unlike Lacson and Morizot (1991), our study did find some evidence of genetic stability, though only at the seasonal temporal scale (Fig 2.3), which they did not test. Such chaotic genetic patchiness can be attributed to natural selection and/or chance acting before settlement, or natural selection occurring after settlement (Larson & Julian, 1999). By confining our sampling to only newly recruited bicolor damselfish, we were able to minimize the potential effect of post-settlement mortality. This approach means that the genetic patterns we observed were likely created before settlement, either by selective or chance occurrences during the pelagic life history stage.

Another potential contributing factor to genetic patchiness is high reproductive variance. Organisms with high fecundity and high levels of early mortality are likely to have elevated reproductive variance, due to random matches of larval cohorts with the optimal environmental conditions (Hedgecock, 1994). As the bicolor damselfish has relatively high fecundity (Knapp, 1993), and, since coral reef fish larvae have high early mortality (Jones *et al.*, 1991), we can expect high reproductive variance. The population genetic structure of white shrimp (*Litopenaeus setiferus*) was examined over four years using six microsatellite markers in populations along the Gulf of Mexico and the south-eastern seaboard of the United States. The authors found "differentiation underlying broad-scale genetic homogeneity" very similar to ours, and attributed their findings to high reproductive variance (Ball & Chapman, 2003). Similarly, an allozyme-based study

of white seabream (*Diplodus sargus*) populations in the Mediterranean Sea revealed populations undergoing rapid genetic change over time (Lenfant & Planes, 2002). This rapid change was partly attributed to the effects of high variability of reproductive success.

Most research on temporal genetic stability has been focused on temperate marine fish stocks of economic importance, including species such a cod (*Gadus morhua*) ((Ruzzante *et al.*, 1996; Ruzzante *et al.*, 2001), salmon (*Onchorhynchus spp.*) (Fillatre *et al.*, 2003; Waples & Teel, 1990), rockfish (*Sebastes jordani*) (Julian, 1996) and walleye pollock (*Theragra chalcogromma*) (Olsen *et al.*, 2002). Temporal genetic stability has been found in populations of cod and salmon, possibly because these species are philopatric, which may drive genetic isolation and hence differentiation. Rockfish and walleye pollock, however, have been shown to have unstable genetic structure over time, conceivably due to their non-migratory spawning, which may result in a stochastic genetic structure among sampling sites or 'populations.' *S. partitus*, while displaying nest fidelity (Knapp, 1993), does not practice philopatry and it is of interest to note that our findings are in agreement with studies of temperate rockfish and walleye pollock, which exhibit similar spawning behaviours.

We found that the genetic signatures of newly recruited bicolor damselfish were variable over time, indicating that dispersal patterns for this species are likely stochastic. Our findings of genetic temporal variability and patchiness, as well as evidence of random dispersal patterns for *S. partitus*, are important considerations for future management strategies and research on coral reef fish. Also, this study has implications for published studies of genetic stability in other potentially reproductively variable coral

reef fish species, since examinations of temporal genetic stability are rare in the coral reef fish literature.

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CHAPTER 3

Polymorphic microsatellite loci for the masked goby, *Coryphopterus personatus* (Gobiidae)

3.1 INTRODUCTION

The masked goby (*Coryphopterus personatus*) is a common benthic fish found on reefs in the Caribbean and eastern Pacific oceans. The masked goby has the potential for large-scale dispersal via pelagic spawning and a planktonic larval stage. In the past, larval dispersal in coral reef fish has been assumed to be primarily passive (Roberts, 1997; Sale, 1991), although recent studies show that the effects of both biotic (larval behaviour, such as swimming abilities and buoyancy control) and abiotic factors (eddies, gyres and storm events) need to be more completely understood in order to better comprehend dispersal processes in coral reef fish (Jones *et al.*, 1999; Swearer *et al.*, 1999; Taylor & Hellberg, 2003). Understanding dispersal processes is of critical importance to population management and/or creation of reserves, and polymorphic genetic markers are key to evaluating such processes.

Given restricted gene flow, the processes of selection, genetic drift and mutation result in genetic differentiation, and such differentiation can be used as a tool to track patterns of dispersal. To date, the vast majority of coral reef fish population genetic studies have used allozyme markers and mitochondrial DNA sequences to infer dispersal rates based on gene flow estimates (see review by Planes, 2002). Although the results of these studies have, for the most part, found wide-spread dispersal in a number of coral reef fish species, there is conflicting evidence of genetic structure in coral reef fish populations (Doherty *et al.*, 1995; Lacson *et al.*, 1989; Shulman & Bermingham, 1995). One possible explanation may be that functional markers, such as allozymes and mitochondrial DNA, may be under selection, and this selection could be misinterpreted as genetic drift effects, profoundly affecting interpretation of dispersal rates (Planes, 2002).

Two factors enable microsatellite markers to discriminate population genetic differences at higher resolution than either allozyme or mitochondrial DNA markers. First, they generally possess mutation rates orders of magnitude higher than either allozyme loci or mitochondrial DNA and, second, they are believed to be generally selectively neutral (Feral, 2002). As part of a genetic survey of coral reef fish over the Meso-American Barrier Reef (MBRS) we developed and characterized five polymorphic microsatellite loci for the masked goby and tested these markers on three other *Coryphopterus* species: *C. lipernes*, *C. glaucofrenum*, *C. eidolon*, as well as two goby species outside of the *Coryphopterus* genus: *Elacatinus evelynae*, and *Gnatholeps thompsoni*.

3.2 METHODS

3.2.1 Primer development

Genomic DNA for primer development was extracted from masked goby muscle and fin tissue using Wizard® Genomic Purification Kits (Promega, Madison, WI) following the manufacturer's protocol for DNA extraction from animal tissue. An enriched microsatellite library was constructed using a protocol modified from Hamilton *et al.* (1999) using paired combinations of the restriction enzymes *Hae*III, *Rsa*I, *Bst*UI, and *Hin*cII (New England Biolabs, Beverly, MA) and the biotinylated oligonucleotide probes $(GACA)_6$, $(CATC)_6$ and $(AGAT)_6$. The enriched fragments were cloned into a p-Gem vector and subsequently transformed into competent JM109 *E. coli* (Promega, Madison, WI). Positive clones (188 GACA, 66 CATC and 20 AGAT) were sequenced on a Beckman Coulter CEQ 8000 DNA sequencer and analyzed using the CEQ 8000 sequence analysis module (Beckman-Coulter, Fullerton, CA). Sixteen of the positive clones were suitable for primer design (using PRIMER3 (Rozen & Skaletsky, 2000) and NETPRIMER (Premier Biosoft International, http://www.premierbiosoft.com)). Amplified fragments were screened for variability, and the primers for potentially polymorphic markers were modified with the addition of the pUC M13 forward sequence (5'-GTAAAACGACGGCCAGT-3') on the 5' end of the forward primer. When combined with a dye-tagged pUC M13 primer during polymerase chain reaction (PCR), this procedure yields dye-labelled fragments suitable for scoring using an automated sequencer without requiring specific dye-labelled primers.

3.2.2 Sampling and DNA extraction

DNA for genotyping was extracted from muscle and fin tissue of 50 individuals collected from Turneffe Atoll, Belize (17 individuals), Banco Chinchorro, Mexico (17 individuals), and Roatan Island, Honduras (16 individuals), using Wizard® Genomic Purification Kits (Promega, Madison, WI), as above. PCR was carried out in reactions comprised of: 2.5 μL 10X PCR Buffer (10 mM Tris-HCL (pH-8.4) 50mM KCL), 2.5 mM $MgCl_2$, 200 μM dNTP's, 0.05 μg of each primer, 0.5 units *Taq* DNA polymerase, and 50-100 ng of genomic template DNA (plus ddH_2O to 25 μL total reaction volume). PCR was performed with the following reaction profile: 2 min initial denaturation (94°C); 35 cycles of: 1 min denaturation (94°C), 1 min annealing, 1 min extension (72°C); 3 min concluding extension cycle (72°C). Genotypes were scored for fragment size (minus the 17bp pUC M13 modification) using the Beckman Coulter CEQ 8000 DNA sequencer, with appropriate size standards, and the CEQ 8000 fragment analysing software.

3.2.3 Analysis

An exact test for goodness-of-fit to Hardy-Weinberg equilibrium was performed with the Monte Carlo method for each locus within each site (total 20,000 permutations) using TOOLS FOR POPULATION GENETIC ANALYSES (MP Miller (1997) Tools for population genetic analyses (TFPGA) 1.3: A Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author). To account for multiple, simultaneous tests, Hardy-Weinberg results were adjusted for significance using the sequential Bonferroni correction procedure (Rice, 1989). We used the software STRUCTURE (Pritchard *et al.*, 2000) to infer population structure of the masked goby sampled across the MBRS.

3.3 RESULTS

The five microsatellite markers developed for *C. personatus* yielded from 5 to 38 alleles in the individuals collected from the three atolls within the Meso-American Barrier Reef System (MBRS) (Table 3.1). We used the software STRUCTURE (Pritchard *et al.*, 2000) to infer population structure of the masked goby sampled across the MBRS and, based on this preliminary dataset, were able to detect evidence of population structure (Fig 3.1).

Table 3.1. Microsatellite loci developed for the masked goby (*Coryphopterus personatus*). T_a = annealing temperature, N_A = number of alleles, N_S = number of samples. Species screened for intra- and inter-genus amplification (five samples except C_e, two samples): C_l = *C. lipernes*, C_g = *C. glaucofrenum*, C_e = *C. eidolon*, G_t = *Gnatholeps thompsoni*, E_e = *Elacatinus evelynae*. MB designates multiple banding and dash indicates no amplification.

Locus Name Accession No.	Repeat motif	Primer sequences 5′-3′	T_a (°C)	Size range (bp)	N_A (N_S)	C_l	C_g	C_e	G_t	E_e
COPE05 AY738124	$(GATA)_n(GACA)_n$	†GGATGCCCTTATGTCACTGAA TGACACTTCCAACAAGTTTCTGA	58	104-317	38 (48)	2	-	MB	MB	-
COPE09 AY738125	$(GACA)_n$	†CCCCTCTTCCTATTGGCTCT CGCCATCAGACTGAAGAATG	61	157-183	7 (27)	3	3	1	-	-
COPE10 AY738126	$(TAGA)_n(TGGA)_n(TAGA)_n$	†GTGTCAAAGGCCAAACTGCT TGGGGTCAGTAGCAGAGTCC	57	143-273	18 (32)	3	1	MB	MB	MB
COPE12 AY738127	$(GT)_nGG(GT)_n$	†ATCACTGACGCAGCTTCACC GCTAACCAGATGCAACATGC	61	78-97	5 (39)	2	1	2	MB	MB
COPE14 AY738128	$(CATC)_n$	†GATGGATTGAGAGATGGATAGGT TGATCTGTAGCGAATGTCATTGT	59	146-206	11 (34)	MB	-	-	MB	MB

† = primer which received pUC M13 modification.

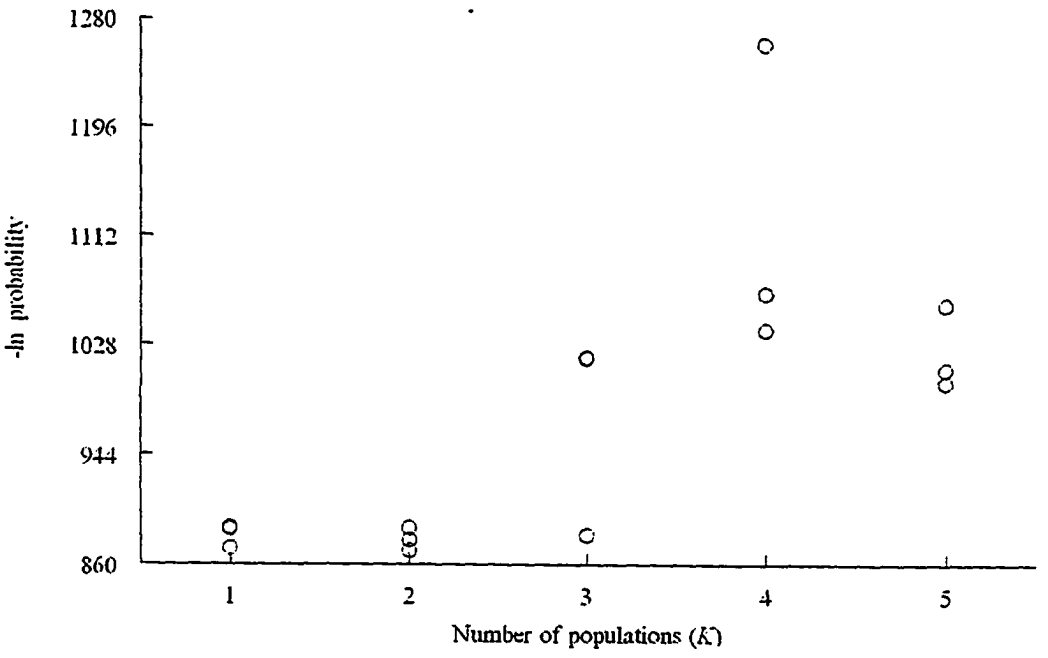

Fig 3.1. Three replicates for each *K* value (number of populations specified *a priori*) plotted against negative natural logarithm (-ln) probabilities generated using STRUCTURE software. Lowest -ln values for *K* correspond to the most probable number of populations in sample.

3.4 DISCUSSION

Under the assumption of panmictic dispersal, the number of populations (K) should be equal to one. Because our results do not distinguish between one or two populations, we conclude that despite a limited sample size, our markers detected evidence of population structure among the masked gobies in the MBRS. Our findings indicate that these markers will be very useful for examining the population genetic structure in this species.

The five loci varied in amplification success when used on the five other species (Table 3.1). The two inter-genus species either did not amplify, or the PCR resulted in multiple banding, suggesting that, with optimization, some primers may be of use in those species. Within the *Coryphopterus* genus, three loci (COPE05, COPE09, COPE10) exhibited polymorphism, though variability decreased as species divergence increased (see Thacker and Cole 2002 for *Coryphopterus* phylogeny).

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CONCLUDING REMARKS AND RECOMMENDATIONS

Determining the level of connectivity among populations is fundamental to effective conservation and management policy. This is particularly important in coral reef fish communities, where patchy habitats predominate and larval exchange is the primary mechanism for dispersal. Past studies in coral reef fish have done much to increase our understanding of the mechanisms of connectivity, such as larval durations, larval behaviour and larval movement (through tagging studies), but fundamental questions remain unanswered. To help address these questions, this thesis examined the spatial extent and temporal stability of genetic structure in juvenile bicolor damselfish populations in the MBRS.

My population genetic analysis of juvenile bicolor damselfish over the Meso-American Barrier Reef (MBRS) had two primary goals: (1) to infer dispersal at three spatial scales (between sampling sites (<20 km), within atolls (20 to 40 km) and among atolls (100 to 300 km)) and (2) to determine temporal genetic stability within and among populations over the MBRS. Juvenile bicolor damselfish from the MBRS were found to possess genetic homogeneity at large geographic scales (100 to 300 km), with cryptic genetic structure detected at small (<20 km) and medium scales (20 to 40 km) (Chapter 1). I concluded that this pattern of genetic structure was most likely the result of highly localized geographic and/or hydrographic effects and, therefore, should be temporally unstable. This prediction was validated (Chapter 2), indicating a primarily stochastic larval dispersal and emphasizing the importance of including temporal scales in studies

examining the genetic structure of coral reef fish. Additionally, I developed novel microsatellite DNA markers for the masked goby (*Coryphopterus personatus*) for use in future population genetic studies (Chapter 3).

Based on my results, I recommend the following future research directions, in order to gain further insight into the connectivity of coral reef fishes using molecular genetic methods:

1) Studies similar to mine, but employing multiple species with a diverse range of life history traits, should be carried out to determine how life history affects dispersal, and, therefore, patterns in the genetic structure of coral reef fish populations. Such studies should include species with contrasting life histories that potentially impact connectivity. For example, coral reef fish with less dispersive life history characteristics, such as short pelagic larval duration, demersal spawning behaviour and limited reproductive timing, would be expected to have stronger genetic structure than those with highly dispersive life history traits.

2) The possibility of temporal cycles or trends is of critical interest for biologists examining connectivity in reef fish. In this study, I found increasing genetic variability within sites over the time scales examined (Chapter 2), indicating that studies of spatial and temporal genetic structure spanning longer timeframes could provide insight into potential cyclic trends, as well as an empirical estimate of the magnitude of such variation. A more quantitative analysis of temporal variation

in population genetic structure should be done in conjunction with the first recommendation, as biotic and abiotic processes would be expected to influence and interact with species differently, depending on their dispersive life histories.

3) Determining the origins of new recruits is of particular importance in investigating larval dispersal and, hence, reef connectivity. Parental assignment of juvenile coral reef fish would enable researchers to determine the origin of larvae, and, thus, provide highly accurate information about the extent and patterns of their dispersal paths. Based on the weak genetic structure detected in this study, (Chapters 1 and 2) the characterization of parental populations would require the use of more genetic markers than those used here, in order to provide the resolution required to assign juvenile fish to their natal site with high precision.

4) My results suggest that local hydrodynamics and micro-geographic effects may have a greater influence on population genetic structure in coral reef fish than was previously thought (Chapter 1). This indicates a pressing need for a more interdisciplinary approach, combining in- and offshore hydrodynamic modeling with molecular-based studies of connectivity. By integrating both types of investigations, we could gain a greater understanding of the processes affecting and/or creating the patterns of genetic structure, and, therefore, connectivity throughout a reef system.

This thesis shows the value of population genetic analyses as a tool for the better understanding coral reef fish population dynamics. The decline of coral habitat and reef fishes worldwide underscores the importance of obtaining insight on connectivity between populations, and it is critical that researchers use every available tool to allow informed, meaningful management decisions.

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