UNIVERSIDAD DE COSTA RICA

SISTEMA DE ESTUDIOS DE POSGRADO

CONECTIVIDAD GENÉTICA DEL PEZ DAMISELA BICOLOR *Stegastes partitus* (OSTEICHTHYES: POMACENTRIDAE), EN ARRECIFES CORALINOS EN EL SISTEMA ARRECIFAL MESOAMERICANO, COSTA RICA Y PANAMÁ

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar al grado de Magister Scientiae en Biología

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DEDICATORIA

En memoria de Luis Angel Salas y Priscilla Zamora

Coral Reefs, "*Vast kaleidoscoscope-ic shelves of the underwater continent*"

-Kamau Brathwaite

"*No, yo me niego al mar desconocido,*
muerto, rodeado de ciudades tristes,
mar cuyas olas no saben matar,
ni cargarse de sal y de sonido:
Yo quiero el mío mar, la artillería
del océano golpeando las orillas,
aquel derrumbe insigne de turquesas,
la espuma donde muere el poderío"

-El Gran Océano, Pablo Neruda

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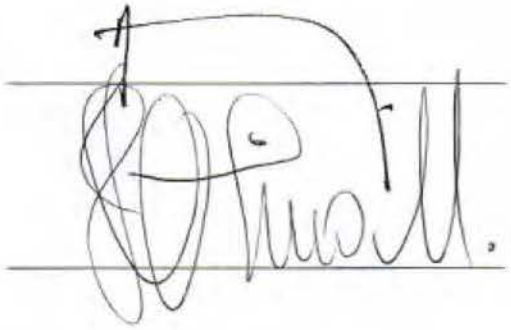

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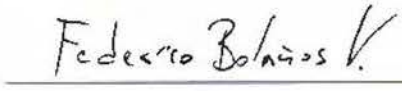

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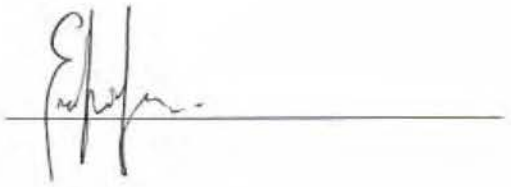

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PREFACIO

Conectividad en poblaciones de peces de arrecife

Los arrecifes de coral constituyen uno de los ecosistemas más diversos del planeta (Connell 1978) y proveen refugio y alimento a invertebrados y peces. Ecológicamente, los peces son importantes para mantener el equilibrio de los arrecifes (Hixon 1997), y económicamente sustentan las industrias del turismo, la pesca artesanal y la industria ornamental (Sale 2002). Los diferentes intereses que existen sobre los peces de arrecife, así como la necesidad de proteger este recurso, hacen urgentes las medidas de manejo, tales como delimitar zonas de pesca, de turismo y reservas marinas. Para lograr un manejo efectivo, es fundamental entender los procesos involucrados en la estructuración de las poblaciones de peces.

Capacidad de dispersión de los peces de arrecife

La mayoría de los peces de arrecife poseen un ciclo de vida con dos fases: una larva pelágica con alta capacidad de dispersión y un adulto relativamente sedentario y asociado al bentos. Estos organismos son altamente fecundos, producen de 10 000 a más de 1 000 000 huevos por hembra (Sale 1980), pero la mortalidad es muy cercana al 100%, y la mayoría ocurre durante el estadio pelágico, lo cual posiblemente afecta la demografía de las poblaciones adultas. La larva es planctónica y generalmente posee una morfología diferente del adulto (Victor 1991). Al cabo de un tiempo, se realiza una transición del plancton al bentos, denominada **asentamiento** (Leis 1991). En este momento, suele ocurrir una metamorfosis en que los peces desarrollan color, adquieren escamas y se dan cambios en el comportamiento (Victor 1991). Muchas larvas no se establecen de inmediato en los arrecifes coralinos, sino que cambian de hábitat y profundidad con el crecimiento. Los juveniles más pequeños observables en el hábitat arrecifal se denominan **reclutas** (Leis 1991).

De aproximadamente 100 familias de peces de arrecife conocidas, únicamente cuatro y una especie de una quinta familia carecen de larvas pelágicas (Leis 1991). Con base en estudios de otolitos, se ha podido determinar que la duración larval va desde 9 hasta más de 100 días, dependiendo de la especie (Leis 1991). El movimiento de los organismos

desde una localidad en la cual se originaron, a través del desove de los adultos, hacia otras localidades, se conoce como **dispersión**. Ésta es una de las fases más importantes del ciclo de vida de las especies, afectando su evolución y persistencia. Las larvas de peces (ictioplancton) de arrecife tienen altas capacidades de dispersión. Pueden ser encontradas desde las aguas cercanas a un arrecife coralino, hasta cientos de kilómetros mar afuera (Leis 1991). Por ejemplo, se han encontrado larvas de lábridos en medio del Océano Pacífico (Leis 1983). La dispersión determina la conectividad entre poblaciones, lo cual es un concepto importante para el manejo de áreas protegidas marinas. La **conectividad**, es el lazo demográfico que se mantiene entre poblaciones de una especie, debido a la migración de individuos o larvas entre ellas.

Los factores que podrían afectar la dispersión de larvas de peces de arrecife son: a) las temporadas y los lugares de desove, b) las corrientes marinas y la batimetría de la costa, c) la duración larval, d) el comportamiento, el crecimiento y la tasa de mortalidad en el océano (Leis 1991). Su distribución en el mar puede ser explicada por una combinación de todos estos factores. Las corrientes realizan un papel importante en dispersar las larvas marinas, pero también pueden retenerlas a través de remolinos o giros (Cowen 2002). Además, las larvas de peces de arrecife poseen una fuerte capacidad de nado (Stobutzki & Bellwood 1997) y algunas especies la tienen desde los primeros estados de desarrollo (Fisher *et al.* 2000). Aún no se conoce con certeza la influencia de estas capacidades y del régimen de condiciones locales para modificar su dispersión.

Debido a las grandes capacidades de dispersión, por mucho tiempo se consideró que las poblaciones de peces de arrecife eran de tipo abierto, en que las larvas provienen de poblaciones distantes (ej. Scheltema 1986). Sin embargo, se ha demostrado que pueden retenerse en su arrecife natal. Este concepto se denomina **autorreclutamiento**, es decir, la nueva cohorte de peces proviene en su mayoría o totalmente de individuos originados en la misma población (Mora & Sale 2002). Jones *et al.* (1999) marcaron masas de huevos de *Pomacentrus amboinensis* con tetraciclina, y estimaron que entre el 15 y 60% de las larvas volvieron al arrecife donde había ocurrido el desove, en Lizard Island, Australia. Swearer y colaboradores (1999), utilizando diferencias en la tasa de crecimiento y concentraciones de elementos traza en otolitos, estimaron que un 44.5% de los peces señorita cabeza azul,

Thalassoma bifasciatum de la isla caribeña de St. Croix, se habían desarrollado dentro de las aguas costeras y por lo tanto eran resultado de autorreclutamiento.

Se ha propuesto también, que las poblaciones de peces de arrecife forman metapoblaciones, donde se da una mezcla entre autorreclutamiento y a la vez dispersión de algunas larvas hacia diversos lugares. Según el concepto original de metapoblación de Levins (1969, 1970), deben existir parches de hábitat discretos, el movimiento de individuos entre los parches debe darse pero no debe ser excesivo, y debe existir una probabilidad de extinciones locales (extinción de poblaciones en los parches). En peces de arrecife, según Sale *et al.* (2006), se cumplen los dos primeros criterios. Los autores proponen que la dinámica de poblaciones locales está determinada en gran parte por cierto grado de autorreclutamiento, así como el reabastecimiento de larvas que provienen de poblaciones externas. Este concepto es importante de conocer para entender cómo encajan las diferentes escalas espaciales en que operan las poblaciones de peces de arrecife. Se requiere más investigación respecto a las proporciones de autorreclutamiento, y la magnitud de conectividad entre poblaciones.

Entre las estrategias que se han utilizado para responder estas preguntas, se encuentra el desarrollo de modelos que predicen conectividad de poblaciones en distintas localidades. El modelo de Roberts (1997) que fue pionero en este campo, propuso que las poblaciones de peces en el Caribe estaban ampliamente conectadas y podían recibir larvas de las localidades corriente abajo. Este estudio ha sido criticado por Mora & Sale (2002) porque se basó completamente en un modelo muy básico de corrientes en el Caribe, y no tomó en cuenta la biología de los organismos, tal como migración vertical de las larvas o sus capacidades de nado, ya que las larvas no se comportan como partículas inertes. Cowen y colaboradores (2000) dieron un paso adelante, pues al evaluar el intercambio larval de una damisela alrededor de la isla caribeña de Barbados, su modelo incluyó el efecto de la difusión y la mortalidad larval. El descubrimiento más importante a través de este modelo fue que algunos arrecifes, tal como el de Barbados, no necesariamente constituyen una fuente suficiente de larvas para los arrecifes que se encuentran corriente abajo, debido a que la mayoría de larvas mueren en el trayecto. Estos autores sugirieron que bajo estas condiciones, las poblaciones de los peces de arrecife maximizan el grado de retención

larval para evitar la pérdida de sus hijos. Cowen y colaboradores (2006), recientemente desarrollaron un modelo matemático más avanzado que incluye aspectos de la biología de las especies, tales como el comportamiento larval y las tasas de mortalidad. Además mapearon la disponibilidad de hábitat en todo el Caribe e identificaron cuatro distintas regiones de conectividad, entre ellos el Caribe oeste, donde se localiza el Sistema Arrecifal Mesoamericano (SAM), y el Giro de Panamá-Colombia (GPC), donde se encuentran los arrecifes de Costa Rica y Panamá (CR-PAN). Ellos predicen que el GPC tendría mayores niveles de retención que la otra región. Los modelos matemáticos son útiles para predecir dispersión, pero es necesario desarrollar técnicas para validar los resultados por medio de estudios que midan la dispersión in situ (Sale & Kritzer 2003). Para cumplir este objetivo, los biólogos han utilizado marcadores artificiales (e.g., teñir embriones en tetraciclina, cf. Jones *et al.* 1999) y naturales (química de los otolitos, marcadores genéticos, Swearer *et al.* 1999, Purcell *et al.* 2006), los cuales proveen una idea de las escalas de dispersión y origen de las larvas. Ciertos tipos de marcadores genéticos son muy efectivos para determinar las rutas dispersoras de las larvas.

Marcadores genéticos como herramienta para estudiar la conectividad entre poblaciones

El estudio de la estructura genética es un método útil para inferir la conectividad entre poblaciones, analizando las frecuencias alélicas. Existen cuatro procesos que afectan la frecuencias alélicas en las poblaciones: mutación, migración, selección natural y deriva genética (Hartl & Jones 2005). Para los estudios poblacionales, se asume que las frecuencias alélicas reflejan el flujo genético entre poblaciones. El flujo genético es el intercambio de genes entre poblaciones, producto de la dispersión o migración de los individuos (Hartl & Jones 2005). Si hay poco flujo genético, los procesos de selección, deriva y mutaciones actuarán por aparte en cada población, lo que da como resultado distintas frecuencias alélicas.

Para estudiar estructura genética poblacional, se han utilizado numerosos marcadores moleculares. La variación en los marcadores moleculares, tales como isoenzimas y ADN mitocondrial entre subpoblaciones, ha sido utilizada para identificar

niveles de migración en peces de arrecife (Mora & Sale 2002, Planes 2002). Varios estudios han mostrado un alto nivel de flujo genético entre poblaciones separadas por miles de kilómetros (Lacson 1992, Planes 1993 y 1998, Doherty et. al. 1995, Shulman & Bermingham 1995). Es importante notar que los estudios demográficos requieren marcadores moleculares altamente polimórficos, con altas tasas de mutación, que permitan inferir eventos genéticos recientes en las poblaciones, tales como la dispersión larval. Las poblaciones reciben larvas y se suplen en una escala de meses o años, no en escalas de cientos o miles de años. Las isoenzimas y ciertas secuencias del ADN mitocondrial no son los mejores marcadores para discernir eventos recientes, particularmente en poblaciones grandes (Mora & Sale 2002).

Los microsatélites son marcadores genéticos que se han utilizado escasamente en peces arrecifales. Son secuencias de ADN no mayores a 6 pares de bases repetidas en tándem, en uno o más sitios del genoma. Pueden ser altamente polimórficos (Hancock 1999), y probablemente son neutrales o poco afectados por la selección (Mora & Sale 2002). Dentro de los métodos genéticos disponibles, constituyen uno de los mejores para estudiar variaciones a nivel ecológico, ya que las tasas de mutación son rápidas, por lo que es posible detectar variaciones a corto plazo. Los microsatélites hipervariables co-dominantes expresan muchos alelos para cada locus (más de 50), y por eso estos sistemas permiten una precisa discriminación genética entre poblaciones, e inclusive individuos.

Recientemente, se ha introducido métodos estadísticos más avanzados, que permiten analizar toda la información de múltiples loci de marcadores moleculares que contienen una gran cantidad de alelos (tal como los microsatélites). Inclusive algunos de estos métodos permiten cuantificar los individuos que migran de una población a otra. Con las pruebas de asignación, es posible asignar reclutas de origen desconocido a poblaciones de adultos de origen conocido, con base en los genotipos de sus microsatélites. Este método fue introducido para el grupo de peces de arrecife por Mora (2004) y permite estimar la proporción de peces "locales" que se están reclutando en la nueva cohorte, y además es útil para determinar hacia dónde van las larvas. Se basa en el principio de que los genotipos larvales deben ser similares a los de su población originaria. Este método ha sido exitoso para determinar el origen de poblaciones de salmones (Castric & Bernatchez 2004), y en

otros vertebrados como lagartijas (Berry et al. 2004). Mora (2004) lo aplicó en poblaciones de la damisela bicolor *Stegastes partitus*, sin embargo su número de muestras totales y de marcadores de microsatélites fue muy bajo, por lo que recomendó utilizar un mayor tamaño de muestra y un mayor número de marcadores, para aumentar el éxito de las pruebas de asignación.

Aún se desconocen los grados de dispersión larval local (e.g., escalas < 50 km) y regional (en Centroamérica, escala > 1000 km) para la damisela bicolor, *Stegastes partitus*. Los microsatélites constituyen un marcador genético muy útil para el estudio de la dispersión, especialmente con los nuevos métodos estadísticos que se han desarrollado que aprovechan la gran cantidad de información genética producida por estos marcadores.

En esta contribución, procuramos estudiar los patrones de dispersión de la damisela bicolor, *Stegastes partitus*, a diferentes escalas espaciales. Este trabajo se divide en tres capítulos. El primer capítulo investiga si los juveniles de *Stegastes partitus* regresan a su arrecife natal, es decir si se da "autorreclutamiento", en arrecifes de Belice. Se explora la utilidad de las pruebas de asignación, y se estima qué proporción de larvas podría estar regresando a su arrecife original. El segundo capítulo investiga los patrones de dispersión a una escala espacial mayor, entre poblaciones del Sistema Arrecifal Mesoamericano (SAM) y poblaciones de Costa Rica y Panamá (CR-PAN), mediante el estudio de la estructura genética poblacional. En este capítulo exploramos si la predicción del modelo de dispersión de larvas de Cowen *et al.* (2006) para el Caribe, se cumple para poblaciones de *Stegastes partitus*. Los autores sugieren que la región CR-PAN se encuentra separada del SAM. Ambos capítulos investigan estas preguntas a través del estudio con marcadores de microsatélite, y análisis de genética poblacional. El tercer capítulo reseña las conclusiones, así como las recomendaciones para la conservación mediante el diseño de áreas marinas protegidas (AMP). Las AMP prometen ser una herramienta muy efectiva, tanto para el manejo de las pesquerías tropicales como la conservación de la biodiversidad marina. En ellas se da la oportunidad de combinar ambos intereses, pues protegen organismos marinos a la vez que pueden proporcionar una fuente de reclutas para las zonas de pesca (Sale 2002). El conocimiento de la conexión que hay entre los arrecifes, dado por el flujo de larvas, es una pieza importante en el diseño de las AMP.

En el primer capítulo, se utilizaron pruebas de asignación genética para investigar si los juveniles se asientan su arrecife natal. Estas pruebas utilizan información genética para determinar las poblaciones de origen de individuos. Se determinó que el método parcialmente bayesiano de Rannala y Mountain (1997), aplicado en combinación con el algoritmo de re-muestreo de Monte Carlo de Paetkau (2004), era el más exacto, porque determinó correctamente el origen de una muestra de embriones de origen conocido. Posteriormente y utilizando este método, se determinó el origen de juveniles recolectados en dos arrecifes en el sector este y oeste de Turneffe, Belice, para estimar en qué proporción existe autorreclutamiento, y comparar si las proporciones varían entre arrecifes. Se recolectaron juveniles recientemente asentados en una hectárea de cada arrecife (este y oeste). Para caracterizar las potenciales poblaciones de origen, se utilizaron muestras de adultos de siete arrecifes distribuídos a lo largo del Sistema Arrecifal Mesoamericano (SAM) incluyendo el este y oeste de Turneffe. Se analizaron los genotipos con 12 loci de microsatélites. El nivel de diferenciación genética de estas poblaciones fue mínimo, con valores de F_{ST} global de 0.003, lo que dificultó la labor de discernir el límite entre las poblaciones muestreadas, y consecuentemente, limitó la capacidad de determinar el origen de los juveniles. Fue posible estimar un intervalo de autorreclutamiento, aproximadamente de 14 a 24%. La magnitud del autorreclutamiento fue muy similar para el este y el oeste de Turneffe. Este resultado indica que ca. 76-86% de las larvas se dispersan a otras localidades. Estos resultados, en combinación con el bajo nivel de diferenciación genética, indican que el autorreclutamiento a esta escala espacial es en muy bajas cantidades, y que existe un alto intercambio de larvas entre las poblaciones muestradas. La poca diferenciación genética de las poblaciones en el SAM, fue la motivación para ampliar el estudio a una escala espacial mayor, a nivel regional.

El segundo capítulo analizó la estructura genética de las poblaciones adultas de *Stegastes partitus* a una escala espacial de 1000 km, que incluye la región del Sistema Arrecifal Mesoamericano (SAM) y la región de Costa Rica y Panamá (CR-PAN). Para caracterizar la estructura genética, se realizaron pruebas exactas, pruebas de F_{ST}, Análisis de Varianza Molecular (AMOVA), distancia genética y aislamiento por distancia. El estudio encontró evidencia de una débil, pero significativa diferenciación genética entre estas dos

regiones, tal como lo predice un modelo bio-oceanográfico de dispersión larval. Las poblaciones dentro de CR-PAN exhibieron mayor diferenciación genética que las del SAM, y estos patrones de diferenciación no pudieron ser explicados por barreras geográficas ni por aislamiento por distancia. La mayoría de diferencias significativas se dieron entre los adultos de Manzanillo (Costa Rica) y otras poblaciones adultas, tanto de la región del SAM como de CR-PAN. Los datos sugieren que la fragmentación del hábitat puede jugar un rol en la conectividad entre poblaciones, restringiendo la dispersión larval. Sin embargo se encontró inestabilidad en la estructura genética al comparar entre cohortes, por lo cual se deduce que factores adicionales, tales como estocasticidad en las fuentes de donde provienen las larvas, pueden estar contribuyendo en los patrones presentes. Los resultados indican que los arrecifes coralinos de la región de Costa Rica y Panamá deben ser de interés y esfuerzo para la conservación, debido a su relativo aislamiento, niveles de degradación y disponibilidad de hábitat.

Esta investigación brindó dos conclusiones principales. Primero, las damiselas *Stegastes partitus* del Caribe tienen capacidad de dispersarse hasta 1000 km, similar a otros peces de la misma familia, sin embargo, existe un pequeño, pero significativo nivel de restricción en el flujo genético entre las regiones del SAM y CR-PAN, el cual concuerda con las predicciones de un modelo bio-oceanográfico de dispersión. Segundo, menos del 25% de los peces juveniles regresan a su arrecife natal, y se da un alto intercambio de larvas entre poblaciones, lo que disminuye el nivel de diferenciación genética. Debido a que el nivel de conectividad genética entre las poblaciones de *Stegastes partitus* es bastante alto, con un gran nivel de mezcla entre poblaciones, pero también con restricción en el flujo genético, se puede concluír que sus poblaciones se comportan como metapoblaciones marinas. Esto es, cuando las poblaciones locales habitan parches de hábitat, y la dispersión entre parches no es tan baja como para aislarlas, ni tan alta como para que sean considerados una sola población. En el caso del SAM, realmente se comportan más como metapoblaciones funcionales, esto es, cuando el hábitat es continuo, sin embargo se pueden delimitar subpoblaciones a lo largo de este tracto de arrecife continuo. En términos de conservación, para proteger especies con ámbitos de distribución y ciclo de vida similar a *Stegastes partitus*, se recomienda diseñar redes de áreas marinas protegidas, que mantengan

las redes de poblaciones naturales interconectadas. El tamaño y espaciamiento entre éstas dependerá de la capacidad de dispersión de la especie de interés, así como el nivel de fragmentación del hábitat. Debido a que la fragmentación de hábitat puede restringir los niveles de conectividad, se recomienda también impulsar el manejo sostenible fuera de las áreas protegidas marinas, en coordinación con las comunidades costeras, para mantener su continuidad y que existan porciones de arrecife saludable donde pueda ocurrir el asentamiento de los organismos marinos.

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CAPITULO I

Patrones de retención larval en la damisela bicolor, *Stegastes partitus*, por medio de pruebas de asignación[1]

[1] "Assessing larval retention patterns in the bicolor damselfish, *Stegastes partitus*, using assignment tests" Este articulo respeta el formato de la revista "Molecular Ecology" donde será enviado para su publicación.

ASSESSING LARVAL RETENTION PATTERNS IN THE BICOLOR DAMSELFISH (STEGASTES PARTITUS) USING ASSIGNMENT TESTS

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ABSTRACT

The ability of marine larvae to recruit back to their natal populations is a key evolutionary factor in shaping marine populations, providing an opportunity for local adaptation. The magnitude and scale of larval retention is of critical importance for defining units of conservation for reef fishes. By using genotype assignment techniques, we estimated the proportion of self-recruitment in *Stegastes partitus*, the Caribbean bicolor damselfish with a long (30-day) pelagic larval phase. Adults and recently settled juveniles were collected from seven reefs located on Turneffe atoll and the Mesoamerican barrier reef, and were genotyped at 12 microsatellite loci. Assignment tests and F_{ST} values suggest that sampled populations are highly admixed. We assessed different assignment techniques and determined that the partial bayesian approach of Rannala and Mountain (1997), combined with Paetkau (2004) Montecarlo resampling algorithm, performed better than others, even though the populations are not in equilibrium. Using this approach we determined that the range of self-recruitment was between 14-24% occurring on two reefs located at the East and West of Turneffe atoll; however the determination of the most likely source population was limited by the low degree of genetic differentiation among the putative source populations. The level of self-recruitment did not differ significantly between the eastern and western sites, despite an expectation for differences in oceanographic retention regimes. The high levels of dispersal (>75%) are probably a product of the life-history of this species: long pelagic duration, high abundance and year-round reproduction. Our results contribute to the understanding of the evolutionary persistence of dispersal, since a combination of dispersal and retention provides the opportunity for both local adaptation and dispersal-based risk-spreading. This study also analyzes the accuracy of different assignment tests for natural populations with low genetic differentiation.

KEYWORDS
Population assignment, self-recruitment, *Stegastes partitus*, Pomacentridae, coral reef fish, Mesoamerican reef

1.1 Introduction

Dispersal and retention patterns have important consequences for the dynamics and viability of populations. They influence the distribution and range of species (Bowler & Benton 2005) and determine the rate at which species evolve (Kinlan & Gaines 2003). Dispersal can prevent habitat saturation and reduce intraspecfic competition, reduce inbreeding likelihood, facilitate range expansion and the colonization of new habitats and enhance species persistence by reducing the risk of local extinction (Pechenik 1999). However, it is also a risky strategy since it can lead to short-term dispersal-related mortality with no guarantee of finding suitable habitat (Gadgil 1971), and in the long term, may prevent adaptation to local conditions (Pechenik 1999). Thus it is unclear whether selection would favor recruitment near the parents, where a suitable environment is likely, or dispersal as a risk-avoidance strategy.

Given that dispersal may be risky, the extended pelagic larval stage found in many marine organisms can be of little advantage. For coral reef fishes, 96% of the approximately 100 families have a pelagic larval stage (Leis 1991). Some of those larvae have been collected thousands of kilometers away from parental habitats (Victor 1991). The likelihood that most larvae traveling away from a reef will find a suitable site during the narrow timeframe of settlement competency may be low. Additionally, how reef fishes have diverged in more than 3000 species, if extended pelagic stage may preclude local adaptation? Possibly, larvae have mechanisms that enhance retention to natal reefs. Coral reef fish larvae have strong swimming abilities (Stobutzki & Bellwood 1997) even in the early developmental stages (Fisher *et al.* 2000). Swimming behavior may contribute to self-recruitment, by vertical migrations within stratified currents (Paris & Cowen 2004). This kind of dispersal-avoidance behaviors raise the question of why extended pelagic larval stages still persist in so many marine taxa. Perhaps the pelagic ontogenetic stage is maintained for other reasons, such as the protection of larvae from predation (Strahmann *et al.* 2002), or it is a persistent a plesiomorphic trait (Bonhomme & Planes 2000). Large-scale dispersal clearly occurs in some coral reef fish, but whether or not self-recruiting individuals are the

majority of the settling juveniles is not resolved. Perhaps selection against obligate larval dispersal may favour a mixed retention-dispersal strategy.

Evidence demonstrating that coral reef fish larvae are retained in substantial numbers is growing. Direct studies of retention using otolith tagging with tetracycline, have shown 15-60% self-recruitment in *Pomacentrus amboinensis* at Lizard Island, Australia (Jones *et al.* 1999); while 1/3 of the *Amphiprion polymnus* juveniles from Kimbe Bay, Papua New Guinea were retained within a two hectare natal area (Jones *et al.* 2005). Almany *et al.* (2007) tagged offspring using maternal transmission of stable isotopes and reported approximately 60% self-recruitment for two coral reef fish species at Kimbe Bay. Given the growing evidence for natal retention of coral reef fishes, self-recruitment may in fact form a significant proportion of many settling cohorts. Tagging studies are costly and logistically difficult when fishes are very abundant and widely distributed in continuous habitat, so studies using indirect, but more logistically feasible techniques, may be more applicable.

Indirect evidence for larval retention through otolith microchemistry and genetics is becoming relatively common. Swearer *et al.* (1999), using otolith trace element data, showed that 44.5 % of bluehead wrasse (*Thalassoma lucasanum*) originated within the waters of the island St. Croix. However, that species lacks genetic differentiation across the Caribbean basin based on microsatellite data (Purcell *et al.* 2006, Haney *et al.* 2007). Studies involving allozymes, mitocondrial sequences and microsatellite markers have shown some examples where coral reef fish with relatively long pelagic larval durations (20-60 days), can have limited gene flow at scales of hundreds of kilometers or less (e.g., Planes *et al.* 1998, Rocha *et al.* 2005, Gerlach *et al.* 2007). These authors suggest larval retention, habitat selection and settlement preferences as possible explanations for these results. While some studies attribute the degree of dispersal to the duration of the pelagic larvae (Doherty *et al.* 1995, Shulman & Bermingham 1995), others indicate no clear relationship unless species lacking a pelagic stage are included (Planes *et al.* 1998, Bay *et al.* 2006). Recent studies rely on genotype assignment techniques and parentage analysis that are, in contrast with traditional genetic methods, able to quantify migrant fish. Using

this method, Carreras-Carbonell *et al.* 2007, found that a mean of 66% of *Tripterygion delaisi* recruits settled in their natal population, and Gerlach *et al.* 2007 could determine that 58% of the recruits of *Apogon doederleni* recruited back to their natal reef. Parentage analysis also helped confirm the self-recruitment estimate of panda clownfish *Amphiprion polymnus* using tetracycline marking (Jones *et al.* 2005). Assignment methods are now standard tools in molecular ecology and are used widely to study dispersal patterns (Berry *et al.* 2004; Manel *et al.* 2005). Genotype assignment methods help to assign or exclude reference populations as possible origins of individuals, based on their multilocus genotypes (Piry *et al.* 2004). With this technique, is possible to quantify amounts of moving larvae, therefore it can be determined if the majority of larvae self-recruit to natal reefs, and understand the importance of each strategy.

Here we describe a genotype assignment analysis using large samples sizes and 12 microsatellite marker loci to estimate the proportion of self-recruited versus dispersed larvae in cohorts of the bicolor damselfish (*Stegastes partitus*) at two reefs located in Turneffe atoll in the Mesoamerican Barrier Reef System (MBRS). Since there are many assignment methods available (Manel *et al.* 2005), we first evaluate them, and choose the most robust to estimate larval retention at a local scale (within a hectare). Our analyses provide new estimates of self-recruitment, and provide valuable direction for the use of this kind of tests to study coral reef fish connectivity.

1.2 MATERIALS AND METHODS

1.2.1. Study sites

Our sampling sites are located on reefs around Turneffe Atoll (TA) and the Belize barrier reef (Fig. 1.1), in the Mesoamerican Barrier Reef System (MBRS). TA is a chain of islands, 48 km long and more than 16 km wide, with three semi-enclosed shallow inner lagoons. The atoll is separated from the barrier reef by a 10-16 km wide and 275-300 m deep

channel, with atolls to the East and South (Fig. 1.1). The reef surrounding TA is almost continuous, segmented by approximately 20 narrow channels.

The sea surface water circulation surrounding the atoll and the barrier reef has a predominant northwestward flow, as a direct influence of the Caribbean Current (Sheng & Tang 2004, Tang *et al.* 2006) and the Gulf of Honduras cyclonic circulation (Ezer *et al.* 2005). MBRS can show variability in the flow patterns affected by propagation of Caribbean eddies, creating strong southward or westward flow depending on the eddy direction (Ezer *et al.* 2005).

1.2.2. Study species

The bicolor damselfish, *Stegastes partitus* (Pomacentridae), is a very abundant coral reef fish species found throughout the Caribbean. It inhabits shallow coral reefs and isolated patch reefs in deeper water. Although *S. partitus* lacks of a specific economic or conservation interest, it is abundant and shares life history traits with many other coastal marine fish. Male and female bicolor damselfish defend small territories (Robertson *et al.* 1988) and are relatively sedentary. The bicolor damselfish produces pelagic larvae with a mean duration of one month (Wellington & Robertson 2001). The first 5 days larvae are in a preflexion stage, after that time they develop the caudal fin (flexion and postflexion stages), achieving a better ability to swim and control vertical migration (Paris & Cowen 2004). Spawning occurs all year long, with peak activity during summer, usually within 3-5 days after the full moon (Robertson *et al.* 1988). The females deposit demersal eggs in male-guarded nests (Knapp & Warner 1991), and the larvae hatch and disperse after a 3-5 day incubation period (Robertson *et al.* 1988).

1.2.3. Sampling

We studied recently settled juveniles of bicolor damselfish, *Stegastes partitus* from two reefs, located in east and west TA. A total 297 juveniles were collected at the east, and 238 at the west. Oceanographic modeling has shown the leeward side (west TA) has a higher retention index of surface waters (Tang *et al.* 2006), potentially increasing self-recruitment rates. Fish were sampled between June and August 2005, corresponding to the peak recruitment months in the Caribbean. The sampling at each reef was in an approximate area of about 1 ha along the fore reef, at about 10-15 meters depth. Fish were measured for fork-length and fin clipped. The tissue was stored in 99% ethanol for later DNA extraction.

The bicolor damselfish juveniles from east and west TA were compared to adult reference populations collected in the same period, from seven localities along the Mesoamerican Barrier Reef System (MBRS), including east and west TA (Fig. 1). Sample sizes varied from approximately 70 to 270 fish per reef (Table 1). We targeted our adult sampling effort at the TA sites (mostly the east and west sites) to correspond with our juvenile sampling, since this study was primarily designed to quantify self-recruitment. Since juveniles recruit within 30 days of spawning, we collected juveniles always before or within 30 days after adult collection, to ensure they could originate from the sampled adults. Fish larger than 4.0 cm in fork-length were classified as adults, as they generally have developed gonads.

Additionally, we collected embryos from nests under terracotta tiles placed in male territories at both the east and west TA sites. These collections were included to provide criteria for evaluating the appropriate genotype assignment analysis and to estimate assignment error. We collected 15 parents and 86 embryos from the east, and 8 parents and 18 embryos from the west. The advantage of embryo collections is that we can identify their parental population.

1.2.4. DNA extraction and microsatellite analysis

Genomic DNA was extracted from fin clips using the Wizard® DNA extraction kit (Promega, Madison, WI, USA) following the manufacturer's protocol for DNA extraction from animal tissue. Fish fin tissue was digested in 500 µL of Nuclei Lysis Solution and 10 µl of Proteinase K (20 mg/mL). Individual eggs were digested whole in 100 µL of Nuclei

Lysis Solution and 2 µl of Proteinase K (20 mg/mL), and the Wizard kit protocol was scaled down by a factor of five.

Each fish was genotyped at a total of twelve microsatellite loci (Table 1). PCR amplification was performed in 12 µL reactions consisting of: 100 ng template DNA, 1x PCR buffer (500 mM potassium chloride and 100 mM Tris-HCl, pH 8.3 at room temperature), locus-specific concentrations of $MgCl_2$ (Table 1), 200 µM of each dNTP, 32 µM of dye-labeled forward primer, 0.5 µM of reverse primer and 0.1 U Taq polymerase (Applied Biosystems, Foster City, USA). PCR conditions were: initial denature at 94°C for 2 min, followed by 30-35 cycles of denaturing at 94°C for 15 s, locus-specific annealing temperature (Table 1) for 15 s, extension at 72 °C for 30 s, and a final extension of 72°C for 90 s. Microsatellite allele sizes were determined by using a LiCor 4300 DNA analyzer, and scored using GeneImagIR 4.05 (Scanalytics, Inc).

1.2.5. Locus characteristics

Allelic richness was calculated with FSTAT v2.9.3.2 (Goudet 1995). We performed exact tests in ARLEQUIN v3.11 (Excoffier *et al.* 2005) to assess departures from Hardy-Weinberg equilibrium (Guo & Thompson 1992). The presence of null alleles and large allele drop-out was tested using Microchecker v.2.2.3 (Van Oosterhout *et al.* 2004). FSTAT was employed to test for linkage disequilibrium (Goudet 1995).

1.2.6. Adult and juvenile genetic structure

We estimated basic population parameters for the adult and juvenile samples, since genotype assignment power is dependant on the degree of genetic differentiation (Manel *et al.* 2005). To detect differences in allelic frequencies, we performed global exact tests of allele frequency differentiation (Raymond & Rousset 1995) among source populations with TOOLS FOR POPULATION GENETIC ANALYSIS (TFPGA) v1.3 (Miller 1997). We also estimated global and pairwise F_{ST} for the juvenile versus adult (source) populations, employing the

software MSA (Dieringer and Schlötterer 2003). We also calculated pairwise F_{ST} between the East and West parental stock, and the East and West embryo populations. Sequential Bonferroni corrections were applied to all multiple tests (Rice 1989).

1.2.7. Genotype assignment analyses

We ran genotype assignment analyses to classify the origin of each juvenile fish collected from both east & west TA, using the adult populations as potential populations of origin. We first evaluated different assignment methods available in GeneClass version 2.0 (Piry *et al.* 2004) and STRUCTURE v 2.2.3 (Pritchard *et al.* 2000), in order to choose the one that provided more accurate results.

1.2.7.1.Choosing assignment method

In order to find out which method is more accurate, we first ran all tests, using a sample of pre-dispersal embryos. The populations where embryos originated are known, since we collected their parents. We had two different populations of origin, the east and west TA. Since embryos must be assigned back to their original parental population, the results were used to find which is the most accurate test. This would be the one that less falsely conclude the origin of embryos. The available methods have different assumptions, so we also checked on them before choosing. Additionaly, some authors have been tested them under data simulations (Paetkau *et al.* 2004, Cornuet *et al.* 1999), so we also considered the conclusions made by them.

The diferent methods tested were 1) Fully Bayesian assignment, available in STRUCTURE program. In order to do assignment, one must first determine the real number of genetically differentiated populations. In this case we used adult populations, because we needed first to pool all adult populations to define the population clusters where embryos can be assigned. We applied one million Markov Chain Monte Carlo (MCMC) repetitions (burning=100,000), under the assumption of "correlated allele frequencies" (Pritchard *et al.* 2000).

Other methods used, available in GeneClass2, were 2) Partial Bayesian assignment (Rannala and Mountain 1997), 3) Frequency-based assignment (Paetkau et al 1995), and two distance-based methods: 5) Cavalli-Sforza & Edwards (1967), and 6) Goldstein *et al.* (1995); (Fig 1.2). Each method was sub-tested under three different scenarios a) Classification of most likely population by ranks, and classification of most likely population by probabilities of exclusion, which reject populations that are below a certain threshold. We used two exclusion methods, b) Cornuet et al (1999), and c) Paetkau et al (2004; Fig 1.3). These tests differ in the Monte-Carlo resampling algorithms used to calculate the probabilites. In both resampling algorithms, we used 10 000 simulated individuals These four methods were tested to classify each embryo, using the male parents from east and west TA, as potential populations of origin.

1.2.7.2. Running assignment test under different different thresholds

The best method was identified as the one that produced the lowest number of false assignment (i.e. wrongly conclude the embryo population of origin), which also agreed on predictions made by data simulations. After choosing the best method, we used a two-step assignment approach: a) To detect individuals that didn't come from sampled populations, an exclusion method was used. Individuals were excluded when the probabilities of being assigned to any population were lower than a threshold. Since choosing the threshold of exclusion is an arbitrary decision, we tried with different thresholds: excluding when the probability of belonging to a population is less than 0.001, 0.005, 0.01, 0.05 and 0.1. We didn't try excluding with probabilities above 0.1, because we considered that is not sufficiently low.

b) Next, we assigned all remaining genotypes to one of the seven potential populations, using a rank-based assignment. We can simply choose the highest rank, but with the exclusion method applied previously, we observed that many of our samples had high probabilities of assignment to more than one population. Therefore, fish were assigned to the most likely population if it was at least twice as likely as any of the other reference populations. However, we again find that choosing how many times more likely is an

arbitrary decision, so this was repeated using ratios of 3, 4, 5, 10, 20, 50 and 100 times more likely than any of the other reference populations. When fish are unassigned, they were judged as "failed assignments", that is their genotypes were ambiguous and could not be either assigned to, nor excluded from, the reference populations (e.g. a fish that does belong to the sampled populations, but the rank of the most likely population, is not sufficiently larger than the other ranks).

1.2.7.3. Estimation of self-recruitment

Based on those analyses, we classified individuals as 1) **excluded** from all sampled populations, 2) **assigned** to one of the sampled populations, and 3) **unassigned** if genotype was neither excluded nor assigned. Fishes were considered self-recruited when they were assigned to the adult population from the same place (i.e. a juvenile from the east, if it has the adult east population as the more likely). The proportion of self-recruitment to a reef, was calculated as:

$$= \frac{\text{No. juveniles self-recruiting to population X}}{\text{Total no. of assignable juveniles from population X}} * 100$$

1.3. RESULTS

1.3.1. Locus characteristics

Average allelic richness across all loci was 21.87 ± 11.34, allelic richness per locus and population is shown in Table 1. Average heterozygosity across all loci was 0.85 ± 0.16, loci SpAAC44, SpGGA7 showed the lowest average heterozigocity values per loci, 0.46 and 0.63 respectively (Table 1.1). Deviations from Hardy-Weinberg equilibrium (HWE) were found at 5 out of 12 loci (Table 1.1). Tests for HWE across microsatellite loci and populations showed significant deviations for 45 out of 108 comparisons, after sequential Bonferroni correction. Three loci accounted for 49% of the deviations: SpAAC41 and

SpTG10, (which deviated in all but two populations), and SpTG8 that deviated in all but one population (Table 1.1). Microchecker results suggested the presence of null alleles in all loci but SpAAT40 and SpTG53. Only loci SpAAC41, SpTG10 and SpTG8 showed null alleles in all populations. The three loci that consistently deviated from HWE were removed from the dataset for all subsequent tests, but assignment tests were conducted with and without these loci. No significant linkage disequilibrium was detected for any locus pair after sequential Bonferroni corrections ($P > 0.05$).

1.3.2. Adult and juvenile genetic structure

The adult source populations had a small but significative degree of genetic differentiation, revealed by exact tests and F_{ST} values. Exact tests revealed highly significant differences ($P<0.01$) in allelic frequencies for all pairwise comparisons of adult populations. The adult source populations had a global F_{ST} of 0.003 ($P<0.001$, n=1127 adults). We also calculated population structure parameters between source (adults) and assigned (juvenile) populations. Exact tests resulted in significant differences ($P<0.01$) for all pairwise comparisons between adult and juvenile samples. The F_{ST} values between the juvenile and adult populations are shown in Table 1.2.

1.3.3. Assignment tests

1.3.3.1. Choice of assignment method

These are the results per assignment method: STRUCTURE analyses indicated the presence of only 1 cluster (k=1); thus, any further results with the fully-bayesian approach were not analyzed.

For the diverse array of GeneClass2 assignment methods, we have a) The embryos correctly excluded from the wrong population (Fig 1.4), using the two kinds of probability calculations. b) The number of embryos correctly classified (Fig. 1.5), using the rank classification. Based on these results, we choose Paetkau's (2004) as the best exclusion

method, with Rannala and Mountain (1997) partial-bayesian simulation, because embryos were less likely to be wrongly (falsely) excluded from their source (parental) population (Fig 1.4). Additionally, this is the most efficient method based on simulations conducted (Piry *et al.* 2004, Paetkau 2004). Although this method assumes Hardy-Weinberg equilibrium, others that don't, such as Cornuet (1999), are prone to falsely exclude many embyros (Fig 1.4). Goldstein and Cavalli-Sforza genetic distance methods did not perform better in any case (Fig 1.4).

Additionally, we chose Rannala and Mountain's (1997) simulation, as the best rank classification method, because it performed well relative to other methods (Fig 1.5), with correct assignment rates greater than 75%.

1.3.3.2. Running the assignment test under different thresholds

Using Paetkau (2004) exclusion method with Rannala & Mountain (1997) algorithm, less than 3.73% of all juveniles from east and west, belonged to populations other than the seven ones sampled (Table 1. 3). Thus, the choice of threshold probability was not critical; very few individuals were excluded at all values (Table 1.3). We chose 0.05 because is a standard often used in biology.

Using Rannala & Mountain (1997) rank classification method, we found far more sensitivity to the choice of a rank (Figure 1.6). At ratios above 10, the proportion of assignable individuals drops below ~10% of the total sampled individuals. At ratios above 10, the estimated self-recruitment rate becomes somewhat unstable, likely due to the very low sample size of successfully assigned juvenile fish. Ratios of 5 or less, shown more stable results due to a larger sample size.

1.3.3.3. Estimation of self-recruitment

We estimate 20 to 24% of juveniles self-recruited to the TA East, and between 14 and 19% self-recruited to West TA, using ratios of 5:1 or less, that lies within a reasonable sample size (Fig 1.6). This estimate is based on the number of self-recruited individuals divided by

the total number of assignable fish. In Tables 1.4 and 1.5, we give more detailed results of the self-recruitment analysis, under the chosen threshold (0.05) using various ranks.

Since three loci (SpAAC41, SpTG10 and SpTG8; Table 1.1) showed major deviations from Hardy-Weinberg equilibrium, we ran our analysess with those loci removed (described above). However, when we included all 12 loci in our analyses, the patterns of self-recruitment did not change: 24% or less for the TA East site, and 18% or less for the TA West site.

1.4. DISCUSSION

The results of this genotype assignment analyses show better performance of Paetkau's (2004) Monte Carlo resampling algorithm and Rannala and Mountain's (1997) partial bayesian criteria. The accuracy of these assignment tests has been previously demonstrated with simulated datasets (Cornuet *et al.* 1999, Paetkau *et al.* 2004), or using well-differentiated populations with few migrants (Eldridge *et al.* 2001; Manel *et al.* 2002, Berry *et al.* 2004). Nevertheless, to our knowledge, this is the first time this method is used for real population assignment systems with very low levels of genetic differentiation and non-equilibrium populations. For example, the lowest F_{ST} value used in Berry *et al.* (2004) was 0.04, which still represents substantial isolation. In contrast, the global F_{ST} of the present study is 0.003. Our assignment success may be due to the very high levels of polymorphism at our marker loci, which may compensate for the low genetic differentiation. The importance of markers with high levels of variation has been highlighted in various studies (Estoup *et al.* 1998; Bjørnstad & Røed 2002, Berry *et al* 2004). Although the genotype analysis using the embryo data helped choose the most appropriate method, they cannot be directly compared to the juvenile analyses, since the self-recruitment estimation used a larger sample size and more reference populations, which probably makes the estimation more accurate.

The analyses showed that self-recruitment in *S. partitus* populations at the east TA and west TA sites in 2005 was relatively low (probably between 14-24%) at the local scale (within

one hectare). These values are similar to what has been reported for another pomacentrid, *Amphiprion polymnus*, where approximately 32% of the larvae settled within a two-hectare area (Jones *et al.* 2005). The self- recruitment similarities in those two species are surprising since *A. polymnus* has a shorter pelagic larval duration (9-12 days) than *S. partitus*. However, pelagic larval durations are not always good predictors of larval dispersal (Bay *et al.* 2006). For example, Almany *et al.* (2007), found similar rates of self-recruitment for two reef fishes with long (38 day) and short (11 day) pelagic larval durations, using a tagging technique that provide very unequivocal estimates. It is likely then, that the larval behaviors may be playing a more important role than pelagic larval durations (PLDs), in such a way that self-recruitment rates are not very different between fishes with different PLDs.

It is interesting that the east and west TA site self-recruitment rates are similar, despite the different oceanographic regimes. Oceanographic models for the summer months at TA show that the retention indexes of near surface particles are 1.3 to 2 times higher at the West site than the East site (Tang *et al.* 2006). If *S. partitus* larvae behaved as near-surface particles, then higher self-recruitment at the West site would be expected. However, few coral reef fish larvae, even in the early pre-flexion stage, are found in the near-surface layer, and most of them are distributed between 15 and 60 meters (Cowen 2002), which indicates their abilities to do vertical migrations in the water column. Clearly we need more resolution in bio-oceanographic models that take into account the layers of the water column as well as larval behavior and mortality. The highest resolution biophysical model available at present has a minimum resolution of 50 km (Cowen *et al.* 2006).

Although self-recruitment is occurring at TA's reefs, it did not constitute a major component of summer recruitment in 2005. The data indicates that approximately 76 to 86% of the cohorts are arriving from elsewhere. Two factors likely affect retention rates at these sites: 1) the bathymetry of TA, 2) the biology of *S. partitus*. TA's fore-reefs are abundant, highly connected and occur throughout the whole atoll (Gibson & Carter 2003). Thus, it is very likely that *S. partitus* larvae will find suitable reef habitat for settlement anywhere on the atoll's fore-reefs, especially since it is a widely distributed species with

broad habitat preference (De Loach 1999). Also, only in the summer months of 2005, at least 5-6 reproduction events were observed (pers. obs.). These life history traits enhance the chances of multiple larvae dispersing. In recent years there has been a paradigm shift in the understanding of reef fish dispersal, with a new emphasis on self-recruitment and larval retention (e.g. Jones *et al.* 1999, Cowen *et al.* 2006). But what is the importance and magnitude of self-recruitment for most reef fish species? High amounts of dispersal have been also found in many reef fish species, including wrasses like *T. bifasciatum* (Purcell *et al.* 2006, Haney *et al.* 2007), squirrelfishes like *Myripristis jacobus* (Bowen *et al.* 2006 a), *M. berndti* (Craig *et al.* 2007), pygmy angelfishes of genus *Centropyge* (Bowen *et al.* 2006 b, Schultz *et al.* 2007), trumpetfish *Aulostomus* (Bowen *et al.* 2001), parrotfish (Geertjes *et al.* 2004), and *Stegastes partitus* (present study).

In conclusion, this study has shown that approximate levels of 14-24% self-recruitment ocurr within Turneffe atoll reefs, combined with a large proportion of larvae dispersing elsewhere, which in turn produces a high degree of population mixture. The high mixture precludes well-differentiated populations, a condition that limits the number of individuals that can be assigned. Due to the amount of unassignable individuals, these self-recruitment numbers are only an approximation, and needs to be interpreted with caution. The most important pattern that emerges with this study is the high degree of mixture, which is higher than the amounts of self-recruitment. We recommend to keep exploring more genetic methods for the challenging task of estimating dispersal trajectories in l e populations of high-dispersing species, combining different molecular markers.

In terms of conservation and management, the damselfish populations in Turneffe probably act as a functional metapopulation, that is, a metapopulation with no visible spatial boundaries (Kritzer & Sale 2006). *Stegastes partitus* is widely distributed (DeLoach 1999), and coral-reef habitat is more or less contiguous within Turneffe (Stoddart 1962). Our data support that these are not separate local populations, and the levels self-recruitment, combined with a little, but existing degree of genetic differentiation, suggests it is neither a panmictic population. The high proportion of migrant juvenile fish indicates that there is high connectivity at least at the scale of this study, and hence, conservation efforts must

include broad spatial areas to maintain the genetic pool of the species. Since very large marine protected areas are difficult to establish and hard to manage, a network of marine protected areas, to preserve species with similar life histories, is likely the best choice.

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1.7. FIGURES AND TABLES

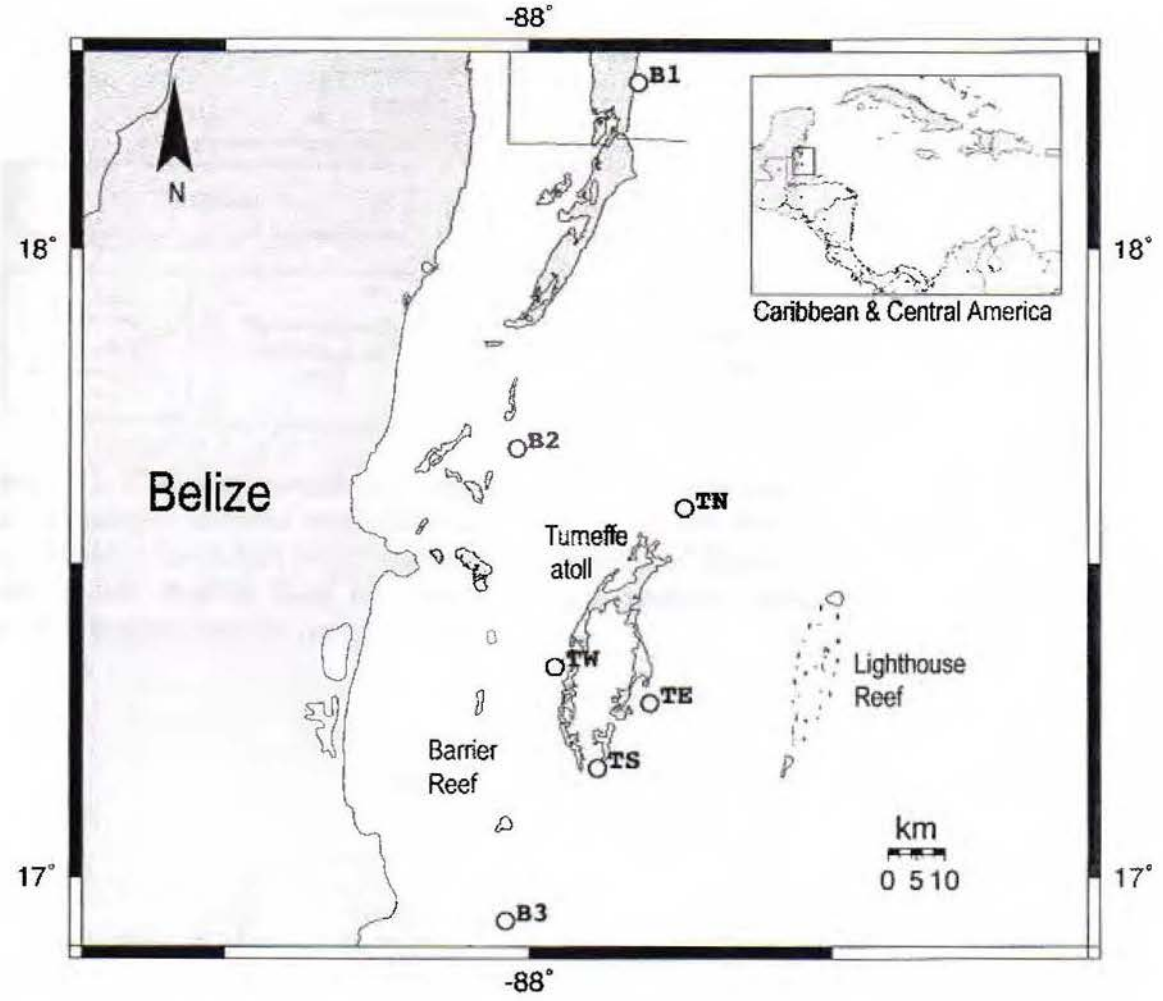

Figure 1. 1. Study sites at Turneffe atoll and the Mesoamerican Barrier Reef. B1: Barrier reef, site 1; B2: Barrier reef, site 2; B3: Barrier reef, site 3; TN: North Turneffe, TE: East Turneffe, TW: West Turneffe, TS: South Turneffe. Map made using online map creation http://www.aquarius.geomar.de/

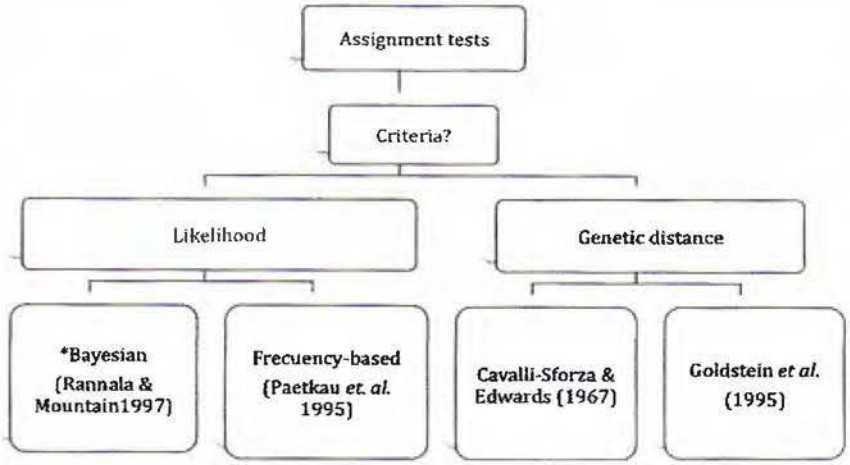

Figure 1.2. Choosing assignment method, step one; using partial Bayesian, frequency based and two genetic distance methods (Fully bayesian is not shown here, see results). Embryos were employed to detect which method assigns most of them to their real origin. Methods are based in two distinct tipes of criteria for data analysis, likelihood and genetic distance. Bayesian method was the most accurate.

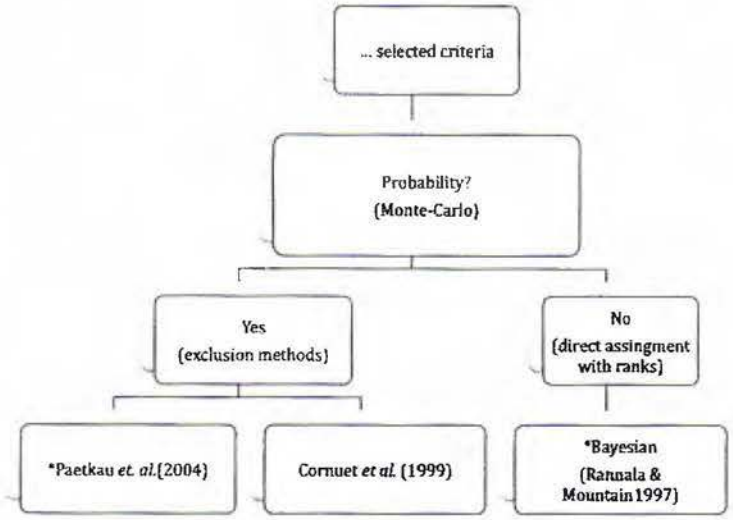

Figure 1.3. Choosing assingment method, step two. Embryos were employed to detect which method assigns most of them to their real origin. The second step was to calculate or not an associated probability to each assignent method. If a probability is calculated, it is possible estimate the confidence of the most likely populations chosen by the program. It also provides an opportunity to exclude populations of origin. Paetkau *et al.* (2004) method was the best exclusion method. Direct assignment, without associated probabilities is the step one from Fig 1.2.

Table 1.1 Number of genotypes (n), allelic richness (a), observed (Ho) and expected heterozygosity In parentheses, number of fish genotyped. Bolded values indicate significant deviations from HWE after sequential Bonferroni correction (P<0 05). First five primers developed by Williams *et al.* (2003) and the others by Thiessen & Heath (2007). Locus names same as in genebank, with the initials Sp before the names in the table. [MgCl2] is 1.1 mM for all loci except SpTG10, SpTG16, SpTG53, with 0.66 mM. Anealing temperature is indicated as T_A.

		$GATA_{40}$	AAT_{40}	AAC_{44}	AAC_{33}	AAC_{41}	TG_{10}	TG_{16}	GG_{47}	TG_{8}	TG_{53}	TG_{13}	GT_{10}
	T_A	*56*	*49*	*48*	*60*	*56*	*55*	*52*	*48*	*48*	*48*	*49*	*56*
E_a	n	273	271	271	271	269	266	266	272	273	269	271	269
(273)	Ho	0.91	0.89	0.35	0.80	0.76	0.62	0.87	0.58	0.75	0.91	0.80	0.83
	He	0.96	0.89	**0.49**	0.89	**0.95**	0.98	0.95	0.57	**0.94**	0.94	0.78	0.89
	A	30.35	15.45	11.16	16.41	28.03	44.74	27.11	4.89	24.21	31.85	8.28	16 29
N_a	n	164	163	163	163	163	154	164	164	164	162	163	163
(164)	Ho	0.88	0.84	0.36	0.77	0.65	0.61	0.90	0.55	0.75	0.86	0.69	0.87
	He	0.96	0.89	**0.39**	0.88	**0.95**	**0.97**	0.95	0.63	**0.94**	0.93	0.74	0.89
	A	31.50	15.91	11.14	14 32	25.44	42.68	25.78	5.69	25.22	29.10	6.67	15.53
S_a	n	181	180	180	181	180	179	177	181	181	181	180	179
(181)	Ho	0.83	0.87	0.33	0.75	0.84	0.63	0.92	0.59	0.78	0.91	0.65	0.78
	He	**0.96**	0.90	0.33	**0.88**	**0.97**	**0.97**	0.95	0.64	**0.94**	0.95	0.74	**0.88**
	A	29.08	15.85	10.46	14 99	32.86	40.90	28.84	5.45	25.08	33.52	7.41	15.20
W_a	n	268	268	267	268	268	262	267	265	268	268	267	261
(268)	Ho	0.88	0.88	0.30	0.75	0.67	0.60	0.88	0.66	0.69	0.88	0.72	0.81
	He	**0.96**	0.90	**0.48**	**0.89**	0.95	**0.98**	**0.95**	0.64	**0.95**	0.94	0.79	0.90
	A	31.74	15.20	11.56	15.32	25.80	44.77	28.93	6.62	25.30	29.13	7.74	15.96
$B1_a$	n	78	80	72	81	76	72	80	80	82	80	79	70
(84)	Ho	0.73	0.93	0.36	0.79	0.62	0.71	0.83	0.59	0.76	0.88	0.61	0.76
	He	0.94	0.91	0.41	0.87	**0.95**	0.97	**0.95**	**0.72**	**0.94**	0.94	0.66	**0.89**
	A	24.10	17 11	9.63	14 24	25.62	43.35	25.37	9.41	23.53	29.09	6.74	13.50
$B2_a$	n	82	77	81	84	75	81	82	83	77	77	81	82
(75)	Ho	0.83	0.88	0.41	0.64	0.53	0.64	0.88	0.64	0.74	0.86	0.69	0.79
	He	**0.96**	0.90	**0.67**	**0.90**	**0.95**	**0.98**	0.94	0.57	**0.94**	0.94	0.76	0.90
	A	27.24	14.29	13.06	14.43	27.39	40 98	26.66	4.71	22.25	32.43	7.38	17.48

Table 1.1 Continued

		$GATA_{40}$	AAT_{40}	AAC_{44}	AAC_{33}	AAC_{41}	TG_{10}	TG_{16}	GG_{47}	TG_{8}	TG_{53}	TG_{13}	GT_{10}
$B3_a$	n	73	75	65	70	59	75	66	72	74	75	71	74
(82)	Ho	0.82	0.88	0.32	0.83	0.76	0.63	0.88	0.54	0.76	0.88	0.70	0.82
	He	**0.96**	0.90	0.39	**0.93**	0.95	**0.98**	0.96	0.60	0.93	0.94	0.73	0.88
	A	28.08	14.52	11.44	22.31	25.00	46.19	31.88	5.76	22.80	28.38	9.30	15.15
E_j	n	295	289	297	293	295	285	293	296	294	297	296	297
(297)	Ho	0.91	0.88	0.35	0.76	0.86	0.58	0.91	0.64	0.74	0.89	0.65	0.84
	He	0.96	0.90	**0.43**	**0.88**	**0.96**	**0.97**	0.95	0.62	**0.94**	0.94	0.73	0.90
	A	30.265	15.795	10.435	14.7	27.588	44.08	28.345	5.158	23.911	31.667	7.138	17.355
W_j	n	236	231	238	237	235	237	234	238	238	237	238	237
(238)	Ho	0.92	0.87	0.37	0.81	0.80	0.59	0.90	0.74	0.74	0.89	0.66	0.81
	He	0.96	0.90	**0.49**	**0.88**	**0.95**	**0.97**	0.95	**0.65**	**0.95**	0.94	0.71	**0.89**
	A	31.05	15.92	11.76	15.68	25.95	45.35	27.93	5.33	24.49	32.54	7.15	15.54

Table 1.2. Pairwise F_{ST} and between adult and juvenile populations. F_{ST} values significant after sequential Bonferroni correction ($P<0.05$) are bolded.

Populations		Juveniles	
		East TA	West TA
	East TA	0.0006	**0.0019**
	North TA	0.0002	0.0007
	South TA	0.0009	0.0018
Adults	West TA	0.0012	**0.0021**
	Barrier reef 1	**0.0053**	**0.0035**
	Barrier reef 2	**0.0076**	**0.0076**
	Barrier reef 3	**0.0093**	**0.0090**

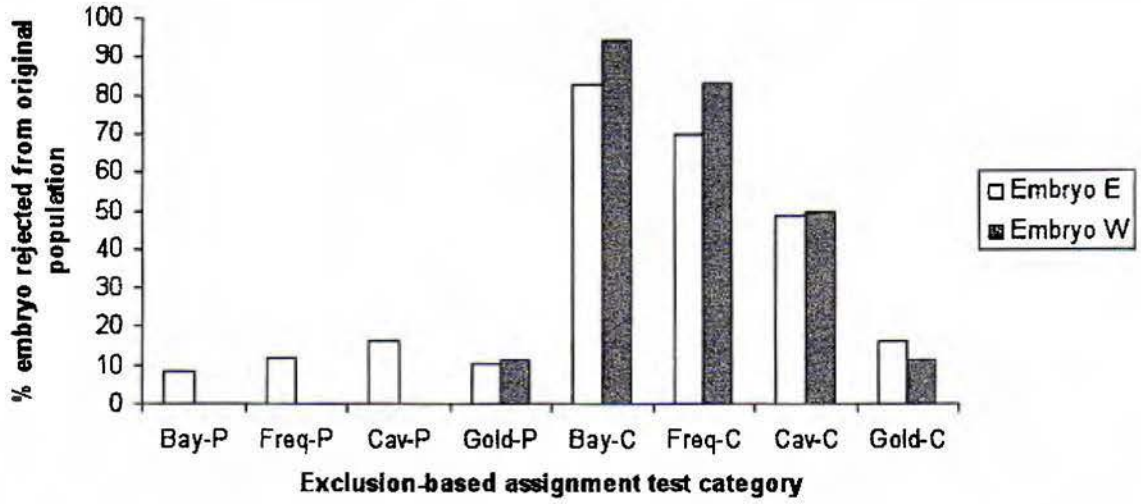

Figure 1.4. Proportion of embryos rejected from the true male parents, for the East and West sites, under different assignment techniques.

Genetic distance method: Bay: Rannala and Mountain (1997) Bayesian method, Freq: Paetkau (1995) Frequency-based method, Cav: Cavalli-Sforza & Edwards (1967) distance based method, Gold: Goldstein (1967) distance based method. Probability computation for exclusion: P: Paetkau et al (2004) Monte Carlo resampling algorithm, C: Cornuet et al. (1999) Monte Carlo resampling algorithm.

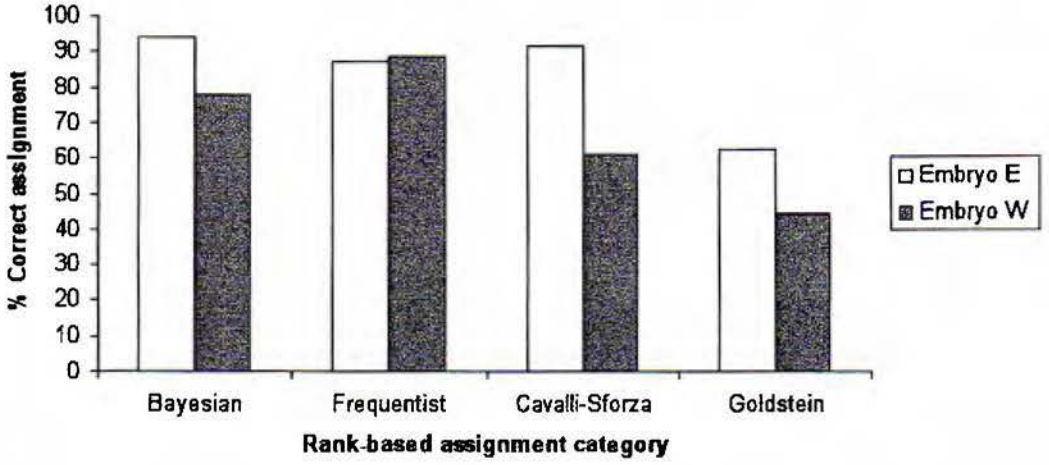

Figure 1.5. Proportion of embryos correctly assigned to their true male parents, for the East and West sites, under different assignment tests. Genetic distance method: Bayesian: Rannala and Mountain (1997) method, Frequentist: Paetkau (1995) Frequency-based method, Cavalli-Sforza & Edwards (1967) distance based method, Goldstein (1967) distance based method.

Table 1.3. Proportion of recently settled fishes that do not self-recruit to the sites East and West of Turneffe, and overall percentage of fishes (JE and JW together) that do not correspond to any of the sampled populations, under various exclusion thresholds

Threshold	East	West	Not from sampled populations
<0.001	0.00	0.00	0.00
<0.005	0.67	0.00	0.00
<0.01	0.67	2.10	0.00
<0.05	3.70	7.98	0.93
<0.1	10.44	13.03	3.73
Total no. juveniles sampled	**297**	**238**	**535**

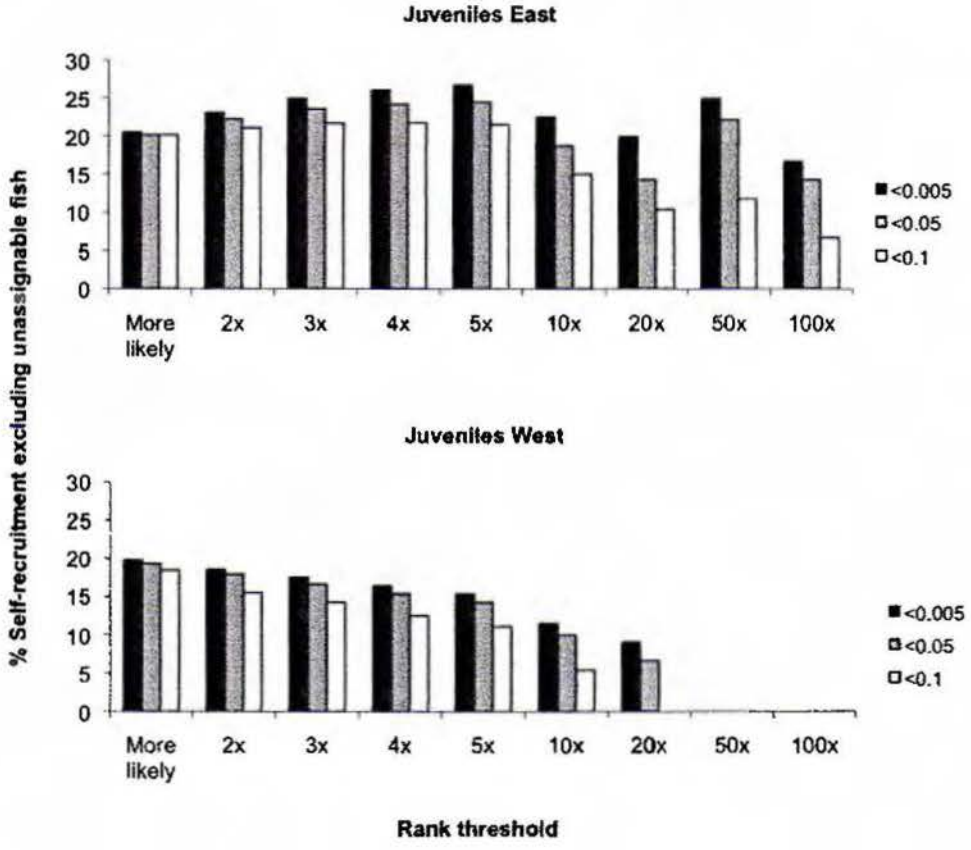

Figure 1.6. Self-recruitment rates at the East and West are compared using different thresholds for Paetkau's exclusion method (<0.005, <0.05, <0.1), and for the rank assignment method (Just more likely population, if population is two times more likely 2x, if it is three times more likely 3x, four times more likely 4x, 5x... 100x).

Table 1.4. Self-recruitment results in detail for the recently settled juveniles of the East site, under a threshold case of 0.05. The total of sampled juveniles from East is 297 and only one was excluded from all sampled populations under this threshold. That fish is accounted in the number of fish that belong to other sources, and is also accounted as an assignable fish.

Rank	Self-recruited East	Other sources	Assignable fish	% Self recruitment from assignable fish
More likely	60	237	297	20.20
2x	33	115	148	22.30
3x	22	71	93	23.66
4x	17	53	70	24.29
5x	14	43	57	24.56
10x	6	26	32	18.75
20x	3	18	21	14.29
50x	2	7	9	22.22
100x	1	6	7	14.29

Table 1.5. Self-recruitment results in detail for the recently settled juveniles of the West site, under a threshold case of 0.05. The total of sampled juveniles is 238 and only four were excluded from all sampled populations under this threshold. Those are accounted in the number of fish that belong to other sources and are also accounted as an assignable fish.

Rank	Self-recruited West	Other sources	Assignable fish	% Self recruitment from assignable fish
More likely	46	192	238	19.33
2x	23	105	128	17.97
3x	14	70	84	16.67
4x	10	55	65	15.38
5x	8	48	56	14.29
10x	3	27	30	10.00
20x	1	14	15	6.67
50x	0	9	9	0.00
100x	0	7	7	0.00

CAPÍTULO II

Conectividad genética local y regional en poblaciones de un pez de arrecife del Caribe [2]

[2] "Local and regional genetic connectivity among populations of a Caribbean coral reef fish" Este artículo respeta el formato de la revista "Marine Biology" donde será enviado para su publicación.

LOCAL AND REGIONAL GENETIC CONNECTIVITY AMONG POPULATIONS OF A CARIBBEAN CORAL REEF FISH

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ABSTRACT

Coral reef fish dispersal along the coral reefs in Costa Rica, Panama (CR-PAN) and the Mesoamerican Barrier Reef System (MBRS) regions, was assessed by determining the genetic structure of bicolor damselfish (*Stegastes partitus*) populations within and among those regions. Published models of larval movement in the Caribbean predict that the CR-PAN region would be demographically isolated from the MBRS region. To test this, adults were sampled from five reefs in Costa Rica and Panama, and from four reefs along MBRS. Between region F_{ST} (F_{ST} = 0.0030, $p < 0.005$) and exact test ($x^2 = 74.34$, d.f. = 18, $p < 0.0001$) results indicated that there is weak, but significant genetic differentiation between regions, suggesting some restriction in connectivity along the Central American coastline, as predicted by bio-oceanographic models. Additionally there is among-site genetic structure in the CR-PAN region, relative to the MBRS and between regions, suggesting higher self-recruitment within CR-PAN. This pattern may be explained by local differences in habitat quality and availability that reduce connectivity levels.

KEYWORDS
Stegastes partitus, microsatellites, connectivity, dispersal.

2.1. Introduction

Most coral reef fishes have a pelagic larval stage with potential to disperse long distances (Leis 1991). Because adult reef fishes are relatively sedentary (Sale 1980), the pelagic stage is an important link for population connectivity between patchily distributed habitats. This has implications for reef fish populations at evolutionary and ecological scales (Leis and McCormick 2002). Connectivity plays key roles in local adaptation and speciation, population replenishment, and the likelihood of local extinction. Connectivity is also important for coral reef management; for example it provides a mechanism for no-take reserves to enhance fish production outside their borders (Botsford et al. 2001; Hilborn et al. 2004; Kritzer and Sale 2004). Caribbean reef ecosystems are experiencing serious decline (Gardner et. al 2003) and need urgent management action. The reefs fringing the eastern coast of Central America, especially the ones within the Mesoamerican Barrier Reef System (MBRS), constitute critically important regions of biodiversity concentration. A quantitative understanding of patterns of connectivity in that region is of particular relevance for management and conservation.

Based on coupled bio-physical modelling of oceanographic data, habitat availability and larval behavior of coral reef fish in the Caribbean, four distinct regions of population isolation have been identified: the eastern Caribbean, the western Caribbean, the Bahamas-Turks and Caicos Islands, and the region at the periphery of the Panama-Colombia gyre (Cowen et al. 2006). Based on this, the Panama-Colombia reefs are expected to be isolated from the remainder of the Caribbean, due to limited larval exchange (Cowen et al. 2006). This region is predicted to have higher self-recruitment than the others, due to low importation from upstream locations (Cowen et al. 2006). Additionally, an oceanographic-genetic model designed for low-dispersal species, also predicted a cluster of genetically similar populations within the Panama coastal region (Galindo et al. 2006). Therefore, the Panama-Colombia region is a good candidate for elevated self-recruitment (Cowen et al. 2006; Galindo et al. 2006) and thus genetic differentiation. However limited genetic data exists to test this possibility for species with higher dispersal potential.

Genetic markers can be used to indirectly estimate levels of self-recruitment, since genetic differentiation is highly sensitive to migration (Hellberg et al. 2002). For example, many reef-associated species in the Indo-Pacific show significant levels of genetic differentiation at small and large scales (e.g. Planes et al. 1998; Bernardi et al. 2001; Planes and Fauvelot 2002; Magalon et al. 2005; Gerlach et al. 2007), as do some species from the Caribbean, including gobiids (Shulman and Bermingham 1995; Taylor and Hellberg 2003), wrasse and damselfish (Shulman and Bermingham 1995). Although there is a tendency for species with short pelagic larval duration (PLD) to show higher levels of population differentiation (e.g. Doherty et al. 1995, Riginos and Victor 2001), there is not always a clear relationship between PLD and dispersal (Shulman and Bermingham 1995). Indeed in some cases the relationship only seems significant when comparing species with and without a larval dispersal stage (Bay et al. 2006); and some species with high dispersal abilities can still show limited dispersal (Barber et al. 2000).

Research testing the Panama-Colombia gyre as a genetically isolated region for high-dispersive species is scarce. Large-scale studies accross the Caribbean suggested an overall lack of genetic differentiation for the bluehead wrasse, *Thalassoma bifasciatum* (Purcell et al. 2006, Haney et al. 2007). Similarly, the stoplight parrotfish, *Sparisoma viride*, shows high overall gene flow, and weak genetic differentiation along the SE Caribbean (Geertjes et al. 2004). However, neither of those studies sampled the Panama-Colombia region, perhaps missing an important area of isolation. A microsatellite marker study in the hamlets *Hypoplectrus nigricans* and *H. puella* sampled from Panama, Bahamas and the MBRS, demonstrated that both species manifest highly significant genetic differences among sites (Puebla et al. 2008). In those cases, the pattern of divergence seemed to reflect an ecological speciation similar to that of the wrasse *Halichoeres bivittatus* (Rocha et al. 2005). A study using microsatellite markers in *Stegastes partitus* sampled within the Panama-Colombia gyre found high genetic connectivity at sites separated by up to 800 km (Ospina-Guerrero et al. 2008), while Hepburn et al. (2009) found a similar pattern of limited genetic divergence in *S. partitus* among sites across the MBRS. However, no study has directly tested the possibilty that the Panama-Colombia

coastal region may constitute a genetically isolated region of elevated self-recruitment.

Cowen et al. (2006) model predicts a meridional biogeographical break at the northern edge of the Nicaraguan rise. Therefore, we can hypothesize that any larva originating in the Panama-Colombia gyre that tends to move away, would probably go towards Nicaragua, San Andres Island and/or Jamaica, but would not go towards the Mesoamerican reef, due to that break. An additional study targeting the isolation of the Panama-Colombia gyre with other regions is necessary.

Here we use microsatellite marker data for the bicolor damselfish (*S. partitus*) to assess genetic divergence within and between two regions: a) the Mesoamerican barrier reef system (MBRS) and b) reefs in Costa Rica-Panama (CR-PAN), located within the area of the Panama-Colombia gyre. This study has two main objectives: 1) To test for large-scale genetic isolation of the CR-PAN region from the MBRS, as predicted by bio-physical oceanographic models (e.g. Cowen et al. 2006), 2) to test for small spatial scale genetic structure within the CR-PAN and MBRS regions and relate such structure to potential local barriers to gene flow.

2.2. MATERIALS AND METHODS

2.2.1. Study sites

Two Caribbean regions, the CR-PAN, extending ~120 km along the southeastern Central American coast, and a 220 km stretch of the MBRS, comprise the study area (Fig. 2.1). The CR-PAN region falls within the oceanographic regime of the Panama-Colombian gyre, and we use the CR-PAN region as a proxy representative of the gyre region defined by Cowen et al. (2006). The two regions are separated by ~1100 km, with intervening reefs in Honduras, Guatemala, Nicaragua and Colombian islands (San Andres, Providencia, etc). There is a ~120 km reef gap, made up of of high-energy sandy beaches in northern Costa Rica (Cortés and Jiménez 2003). Populations from each region are affected by two separate

oceanographic gyres along the Central American coast that may contribute to genetic isolation.

CR-PAN: The Costa Rica fringing reefs are small and patchy (Fig. 2.1), and continue their development in Panama (Cortés and Jiménez 2003). The main near-shore current flows from northwest to southeast (Roberts 1997), creating small eddies in the opposite direction (Cortés 1998). Local rivers, such as La Estrella, Sixaola and Changuinola, develop freshwater plumes and sediment input that may create unsuitable conditions for migrants. Fish diversity of this region is low relative to other Caribbean areas, possibly due to the poor reef development and low structural complexity (Perry and Perry 1974, Phillips and Pérez-Cruet 1984, Fonseca and Gamboa 2003, Fonseca et al. 2006). The effect of these habitat conditions on reef fish connectivity is unknown, but it may reduce opportunities for emigration out of the environment, increasing self-recruitment and also may select against incoming migrants. The two Panama sites (BO, KE, see Fig. 2.1 and Table 2.1) are fringing and patch reefs within the Archipelago of Bocas del Toro, and are located within a semi-enclosed lagoon. Waves and currents have a strong effect outside of the archipelago, but the major islands act as barriers, decreasing wave action and moderating tides (Collins et. al. 1996). Therefore, population isolation inside the lagoon may be expected. The three sample sites located in Costa Rica are located near the coast and in reef patches (Table 2.1).

MBRS: The barrier reef of the MBRS is located approximately 20 to 40 km from the mainland, separated by a shallow lagoon. The barrier reef extends approximately 220 km with well-developed reefs (Fig 2.1). Since barrier and atoll reefs are separated from the mainland, they may be less directly exposed to the effect of rivers and runoff than in CR-PAN. The MBRS region is strongly affected by severe weather events and it is located in the Caribbean hurricane belt (Wells 1988). Water circulation has a predominant northwestward flow, influenced by the Caribbean Current (Sheng and Tang 2004, Tang et al. 2006). However, MBRS displays highly variable flow patterns driven by propagation of Caribbean eddies, creating strong southward or westward flow depending on eddy direction

(Ezer et al. 2005). Three of the four MBRS sampling sites are within the barrier reef and the remaining is on the esat coast of Turneffe atoll (Fig. 2.1 and Table 2.1).

2.2.2. Study species

We chose the bicolor damselfish, *Stegastes partitus*, as our study species because they do not move large distance as adults but have an extended pelagic larval phase. They are a very abundant species in the Caribbean basin, so it is easy to collect representative samples from many locations. Adults are territorial, demersal spawners and reproduce year-round on a lunar cycle, with seasonal reproductive peaks from April to November (Robertson et al. 1988). The male defends nests (Knapp and Warner 1991) and eggs hatch after 2-5 days (Robertson et al. 1988) to produce pelagic larvae with a pelagic larval duration of about a month (Wellington and Robertson 2001). Although *S. partitus* is not of particular economic or conservation interest, it is abundant and shares some life history traits with other coastal marine organisms, including coral reef fishes important for the aquarium trade that belong to the same family (e.g. *Chromis cyanea*, *Microspathodon dorsalis*; Sadovy 1992).

2.2.3. Sampling design

We sampled adult damselfishes from five CR-PAN sites and four MBRS sites (Fig. 2.1). Between May and November 2006, a total of 595 adult *Stegastes partitus* were captured from the coral reefs in Central America, 285 samples from CR-PAN, and 310 from MBRS. Sample sizes ranged from 42 to 96 fishes per site (Table 2.2). Fish were fin clipped and the tissue was stored in a salt preservation solution (0.020M EDTA, 0.025M Sodium citrate trisodium salt dehydrate, 5.3 M Ammonium sulphate: "RNAlater"), for DNA analysis. Individuals over 40 mm fork-length were considered adults, since they showed developed gonads above 38mm (Thiessen 2007). To test for genetic stability across cohorts at one site in CR-PAN region (Manzanillo (MA)), we sampled 121 recently-settled juvenile bicolor damselfish (Fig. 2.1), collected at the same time as the adults in MA.

2.2.4. DNA extraction and microsatellite analysis

Genomic DNA was extracted from fin clips following the silica-based 96-well plate extraction protocol (Elphinstone et al. 2003). DNA quality was verified using electrophoresis with 1.8 % agarose gels. Each fish was genotyped at a total of twelve microsatellite loci, (developed by by Williams et al. (2003) and Thiessen and Heath (2007); Table 2.2). PCR amplification was performed in 12 μL reactions consisting of: approximately 100 ng template DNA, 1x PCR buffer (500 mM potassium chloride and 100 mM Tris-HCl, pH 8.3 at room temperature), locus-specific concentrations of $MgCl_2$ (Table 2.2), 200 μM of each dNTP, 32 μM of dye-labeled forward primer, 0.5 μM of reverse primer and 0.1 U Taq polymerase (Applied Biosystems, Foster City, USA). PCR conditions were: initial denature at 94°C for 2 min, followed by 30-35 cycles of denaturing at 94°C for 15 s, locus-specific annealing temperature (Table 2.2) for 15 s, extension at 72 °C for 30 s, and a final extension of 72°C for 90 s. The microsatellite allele sizes were determined using a LiCor 4300 DNA analyzer, and scored using GeneImagIR 4.05 (Scanalytics, Inc).

2.2.5. Genetic analysis

Allelic richness was calculated using FSTAT v2.9.3.2 software (Goudet 1995). Exact tests for Hardy-Weinberg equilibrium using the Markov Chain method (1000 permutation burn-in followed by 100,000 permutations; Raimond and Rousset 1995) for each locus within each population were performed in ARLEQUIN v3.11 (Excoffier et al. 2005). Linkage disequilibrium between all pairs of loci was examined with FSTAT, and tests for pairs of linked loci per population were performed with ARLEQUIN. Microchecker v.2.2.3 was used to detect the presence of null alleles and large allele drop-out (Van Oosterhout et al. 2004).

2.2.6. Genetic structure

Global and pair-wise exact tests for differences in allele frequency distributions among populations were performed with TOOLS FOR POPULATION GENETIC ANALYSIS (TFPGA) v1.3 (Miller 1997), with 100 batches and 20000 permutations per batch. Global

and pair-wise F_{ST} values were estimated to quantify the extent of differentiation between populations, using MSA v4.05 (Dieringer and Schlötterer 2003). Within- and between-region (MBRS and CR-PAN) global F_{ST} values were also calculated. An analysis of molecular variance (AMOVA) between regions and among-populations within regions was implemented in ARLEQUIN v3.11 (Excoffier et al. 2005).

Cavalli-Sforza and Edward's (1967) chord distance (D_C) was computed to estimate pair-wise genetic distance between all populations in POPULATIONS v1.2.28 software (Langella 2002). Measures of genetic distance (D_C) were assessed for correlation with shortest water distance (km) to detect patterns of isolation by distance in adult populations, applying Mantel tests in GENALEX software (Peakall and Smouse 2006). The isolation by distance patterns were also examined with F_{ST} and $F_{ST}/(1-F_{ST})$, across all data and also within each region. Sequential Bonferroni corrections were applied to all multiple tests (Rice 1989).

Genetic clustering was assessed using STRUCTURE v 2.2.3 (Pritchard et al. 2000), with one million Markov Chain Monte Carlo (MCMC) repetitions and 500,000 burn-in runs. Since *S. partitus* larvae can remain in the plankton for extended periods of time, the admixture ancestry model was used with correlated allele frequencies to improve clustering in populations with high gene flow.

To evaluate the stability of the genetic structure across cohorts, we used the sample of recently settled juveniles from MA in comparisons with the adult population samples. Specifically we tested for genetic differentiation using exact tests and pairwise F_{ST} comparisons. The results for the juvenile MA sample were compared to those for the adult MA samples

2.3. RESULTS

2.3.1. Genetic analysis

Deviations from Hardy-Weinberg Equilibrium (HWE) were found at 5 of the 12 loci following sequential Bonferroni corrections. Locus *Sp*TG$_{10}$ displayed deviations in

88% of the populations, while loci *Sp*AAC$_{41}$ and *Sp*TG$_8$ both presented deviations in 66% of the populations. *Sp*GATA$_{40}$ and *Sp*TG$_{16}$ also showed deviations from HWE, but only in 33% of the populations. In all cases, departures from HWE were due to homozygote excess, but they were not attributable to genotyping errors (based on Microchecker results). However, Microchecker identified null alleles in all populations for loci *Sp*TG$_{10}$ and *Sp*TG$_8$. Null alleles were present in only a few populations for all other loci, but they could not be distinguished from Wahlund effects. Linkage disequilibrium analyses concluded no significant global linkage between pairs of loci, after sequential Bonferroni correction. Approximately 2.3% (14/594) of exact tests showed significant linkage disequilibrium within each population, after sequential Bonferroni correction. Due to HWE deviations, loci *Sp*TG$_{10}$, *Sp*AAC$_{41}$ and *Sp*TG$_8$, were removed from all genetic structure analysis.

2.3.2. Genetic structure

Large scale (between regions) analyses: There were highly significant differences in the allelic frequencies, as shown by global exact test ($x^2 = 85.98$, d.f. = 18, $p < 0.0001$). Six out of 20 (33%) between-region pairwise comparisons were significant (Table 2 3). The exact test between MBRS and CR-PAN regions was highly significant ($x^2 = 74.34$, d.f. = 18, $p < 0.0001$). Global F_{ST} across all populations was low, yet significant ($F_{ST} = 0.006$; $p = 0.0001$). The F_{ST} between the MBRS and CR-PAN regions was 0.0030, and significant ($p = 0.0001$). However, AMOVA detected no significant between-region component ($F = 0.0004$, df = 1, $p = 0.39$, 0.05% variance). There was a significant among-site within-region effect ($F = 0.0046$, df = 7, $p < 0.0001$, 0. 46% variance), as well as among-individuals within-sites ($F = 0.0464$, df = 586, $p < 0.0001$, 4.62% variance) and among individuals ($F = 0.0513$, df=595, $p<0.0001$, 94.87% variance). The populations sampled did not follow a pattern of isolation by distance ($r^2 = 0.008$, $p = 0.220$). The relationship did not improve when the genetic distance metric was F_{ST} or $F_{ST}/(1-F_{ST})$. Pairwise exact tests and F_{ST} values for population comparisons between regions showed little genetic differentiation, with the exception of MA - MBRS sites pair (Table 2.3). And if MA was removed, the

pairwise F_{ST} between the regions MBRS and CR-PAN becomes non-significant (F_{ST} = 0.0002; p=0.2922). The chord genetic distance, D_C, showed a weak separation among all sites (Table 2.3). Analysis using STRUCTURE indicated the presence of only 1 cluster (K=1); however STRUCTURE is not a powerful tool for detecting weak genetic structure.

Within MBRS analysis: The global F_{ST} within the MBRS was 0.0004 and non-significant (p = 0.2575). However, the global exact test for allele frequency distribution variation among populations within the MBRS was significant (p<0.0001). Pairwise tests of allele frequency differences resulted in 2 out of 6 (33.3%) being significant differences within the MBRS after sequential Bonferroni corrections (Table 2.3). All pairwise F_{ST} values between MBRS, were low and non-significant F_{ST} values (Table 2.3). The populations within MBRS did not follow an isolation-by-distance pattern (Fig. 2.2), and the relationship did not improve when the genetic distance metric was F_{ST} or $F_{ST}/(1-F_{ST})$

Within CR-PAN analysis: Within CR-PAN, the global F_{ST} was 0.0101 and statistically significant (p < 0.0001), and the global exact test of allele frequency distribution variation was significant (p < 0.0001). Five out of ten (50%) of the pairwise exact test pairwise comparisons were significantly different within the CR-PAN (Table 2.3). Four out of ten Pairwise F_{ST} values among CR-PAN were significant after sequential Bonferroni corrections (Table 2.3). The populations within CR-PAN did not follow an isolation by distance pattern (Fig. 2.2), and the relationship did not improve when the genetic distance metric was F_{ST} or $F_{ST}/(1-F_{ST})$

MA juvenile and adult comparison: We found that pairwise F_{ST} and exact test comparisons between MA juveniles and most adult populations were low and not significant (p>0.05). Also, we found that the MA juvenile population was significantly different by F_{ST} and exact tests from the MA adult population (Table 2.4).

2.4. DISCUSSION

The current study found evidence of weak but significant genetic differentiation

between MBRS and CR-PAN regions, and apparently MA adult population is responsible of a high degree of divergence, and needs to be further studied. Our field data agrees with bio-physical modeling predictions that CR-PAN populations would be relatively isolated from MBRS (Cowen et al. 2006). Low F_{ST} values among regions are common in marine fishes (O'Reilly et al. 2004), and may reflect a large effective population size (DeWoody and Avise 2000). It is likely that *S. partitus* does have very large effective population sizes in both the MBRS and CR-PAN regions, and hence our low F_{ST} values may reflect limited genetic drift, rather than substantial gene flow between these regions. It is unlikely that the low level of regional genetic structure in our study was due to lack of statistical power, since our analyses detected local genetic structure. Our results are in concordance with other genetic studies of bicolor damselfish that report limited or no genetic structure in regions such as Puerto Rico and Jamaica (Lacson 1992), in Colombia (Ospina-Guerrero et al. 2008) and across the MBRS (Hepburn et al. 2008). The genetic structure observed at the whole sampling area did not follow an isolation-by–distance model of divergence. In other studies with *H. flavolineatum* and *S. partitus*, it was noted that the eastern and the western Caribbean were different in terms of isolation by distance: the former shows isolation by distance patterns at distances < 1000 km, whereas the latter doesn't show any pattern (Purcell et al. 2006; Purcell et. al. 2009). This was attributed by the authors to differences in oceanographic regimes in the two regions (Cowen et al., 2000, 2003, 2006; Purcell et al 2009). Our study shows that the absence of an isolation-by-distance pattern found in the Western Caribbean, also includes the area of CR-PAN, located far south. The degree of isolation of populations located between West Caribbean "connectivity regions" (sensu Cowen et al. 2006) may be weaker (but present), than the isolation between East Caribbean ones.

We also found higher among-site genetic structure in the CR-PAN region (relative to the MBRS and between regions), suggesting higher self-recruitment within CR-PAN. The pattern of genetic structure within the CR-PAN region was not explained by geographic factors, nor did it conform to an isolation-by-distance model of divergence. It is interesting to note that MA adult population was consistenty different with the other sites.

Other work has suggested MA site has different geological origin (Fonseca-Escalante, comm. pers.), and also it holds a different community composition of actinomycetes in coastal sediments, compared to Cahuita (Rojas-Jimenez comm. pers). It will be important to do additional work in this site. Other notable geographic barriers within CR-PAN do not seem to explain the patterns of genetic structure. For example, we would have expected fish populations from Bocas del Toro inner lagoon in Panama to be isolated from the other sites in the study area, due to the effects of restricted water circulation and the Sixaola River (on the Costa Rica-Panama border) runoff. However, those populations did not show substantial genetic isolation from the Costa Rica sites. Although rivers such as the Amazon and Orinoco have been shown to act as freshwater barriers to marine fish distribution (Floeter et al. 2008), they create very large scale freshwater plumes and substantial coastline modification (2300 km; Rocha 2005), and yet there is still some degree of connectivity across those rivers (Collette and Rützler 1977). In contrast, the Sixaola River has only a ~35 km plume, thus it is perhaps not surprising that the river does not contribute to the genetic structure observed in the CR-PAN region.

The patterns we observed in genetic structure at the local scale may be explained by differences in habitat quality and availability between the MBRS and CR-PAN sites: CR-PAN has less developed reefs and are patchily distributed. Habitat variability directly affects the reproductive biology and recruitment success of the bicolor damselfish. Baums et al. (2006) showed that larval competency affects recruitment success and thus affects connectivity more in fragmented reefs than in continuous ones, because larvae have a broader settlement window if suitable habitat is abundant. This is supported by oceanographic modeling showing that for continuous reefs in the Mesoamerican region, connectivity patterns were not affected by variation in the duration of the pre-competent period (Paris et al. 2007). Since the CR-PAN reefs are patchily distributed and the reef system is less developed than that in MBRS, connectivity may be locally limited. However, the substantial genetic divergence between the adult and recently-settled juveniles sampled at Manzanillo (MA), Costa Rica, indicates that the processes driving the genetic structure in CR-PAN are not likely only related to temporally stable and predictable

habitat conditions. It is more likely that the observed genetic structure among the CR-PAN sites (as well as the limited structure identified at the MBRS sites) is due to stochastic processes (e.g., Hepburn et al. 2008). Temporal genetic instability of bicolor damselfish populations has been reported in previous studies (Lacson and Morizot 1991; Thiessen 2007; Hepburn et al. 2008), and may be a fundamental property of such systems (Hepburn et al. 2008).

In conclusion, the current study showed some restriction in connectivity between our two sampled regions (e.g., MBRS vs. CR-PAN), with substantially higher genetic divergence within one of our regions (CR-PAN). MA adult population in Costa Rica shows higher genetic divergence than other sites, and future studies with temporal sampling will help reveal its dynamics. These results support published bio-physical model predictions, and highlight the Panama-Colombia gyre as a possible isolating mechanism within the western Caribbean. Our data further suggest that habitat fragmentation may play a role in the widely reported local-scale genetic structure in coral reef fishes, although a lack of across-cohort stability indicates that additional factors must be contributing to the structure. Our results indicate that the coral reefs of the CR-PAN region may be of particular conservation concern due to its relative isolation, high levels of degradation, and its limited extent.

The bicolor damselfish populations living in the MBRS probably constitute "functional metapopulations", (sensu Kritzer and Sale 2006), and probably constitute typical metapopulations in CR-PAN, the difference between both being the contiguous habitat available. Management actions must take in account how local processes and habitat distribution affect connectivity. The protection of the species (or other ones with similar life cycles) would be best achieved with networks of medium sized marine reserves. A very small reserve would not be capable to cover the distribution range of the species, and a very large one would be difficult to manage. The design would depend on the habitat distribution. To design networks of marine reserves where habitat is continuous, optimal reserve size and spacing will increase with the dispersal kernel of the species (Jones et al. 2007). Where habitat is fragmented, optimal reserve size and spacing will be constrained by

the size and spacing of habitat fragments, because dispersal potential cannot be achieved fully (Jones et al. 2007). It is also important to note that networks of marine reserves placed in areas with extensive habitat, must be combined with proper management outside marine protected areas, to maintain healthy reef habitat and preclude the fragmentation that can reduce connectivity.

2.5. ACKNOWLEDGEMENTS

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2.7. FIGURES AND TABLES

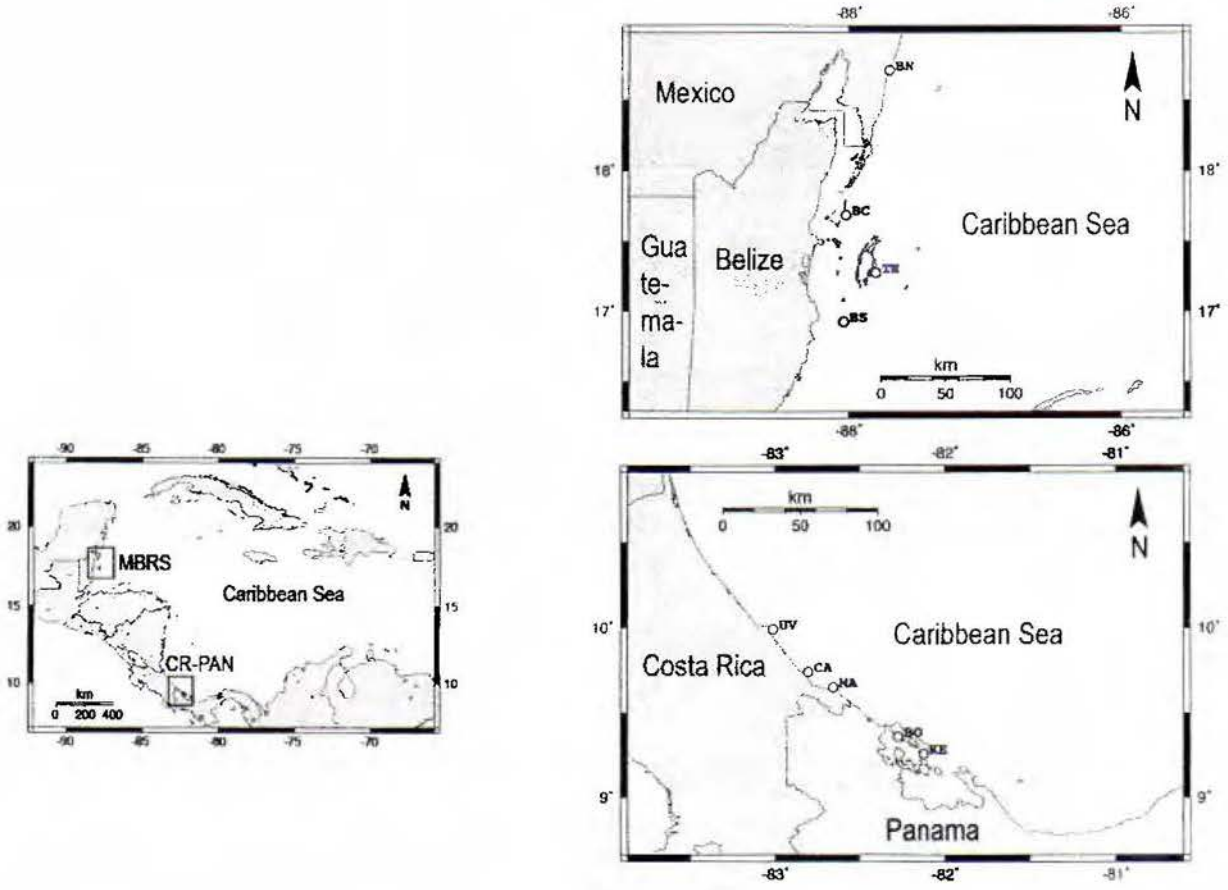

Figure 2.1. Sampling locations. Left: Mesoamerican Barrier Reef System (MBRS) and Costa Rica-Panama (CR-PAN) regions. Lower right: MBRS sites (BN=Barrier North, BC=Central Barrier, BS=Barrier South). Upper right: CR-PAN sites (UV=Uvita Island, CA=Cahuita, MA=Manzanillo, BO=Bocas, KE=Coral key). BO and KE are within the Bocas del Toro province in Panama. Maps made using online map creation http://www.aquarius.geomar.de/

Table 2.1. Description of sampling sites at each region.

Region	Site	Description
MBRS Offshore reefs except coastal BN	Barrier North (BN)	Fringing forereef with spur and grooves, dominated by Montastrea and gorgonians, 10 m.
	Barrier Central (BC)	Forereef platform, dominated by Montastrea and gorgonians, 10 m.
	Barrier South (BS)	Forereef platform dominated by Montastrea and gorgonians, 10 m.
	Turneffe East (TE)	Forereef platform, windward side of the atoll, dominated by Montastrea and gorgonians, 10 m
CR-PAN Fringing and patch coastal reefs	Uvita Island (UV)	Fringing forereef, dominated by sponges and massive Siderastrea and Montastrea corals, 8 m
	Cahuita (CA)*	Shallow patch reef in the lagoon, constructed of live and dead *Acropora palmata* colonies, < 5m
	Manzanillo (MA)	Fringing forereef, located in a carbonate platform, with low relief, 10 m
	Bocas (BO)	Shallow leeward fringing reef, dominated by *Acropora cervicornis*, 3-7 m
	Coral Key (KE)	Patch reef with a slope, constructed of diverse array of corals, 8 m

* Forereef platforms in Cahuita were explored, however *S. partitus* was very scarce at those locations

Table 2.2. Number of individuals (N), allelic richness (A), observed heterozygosity (H_O) and expected heterozygosity (H_E) for adult populations of *Stegastes partitus* collected from Costa Rica, Panama and the Mesoamerican Barrier Reef System. Significant departures from Hardy-Weinberg equilibrium after Bonferroni corrections are bolded and underlined. Locus names same as in genebank, with the initials Sp before the names in the table. [MgCl2] is 1.1 mM for all loci except SpTG10, SpTG16, SpTG53, with 0 66 mM. Anealing temperature is indicated as T_A. Initial names are indicated in Fig 2.1

Site		*Sp* $GATA_{40}$	*Sp* AAT_{40}	*Sp* AAC_{44}	*Sp* AAC_{33}	*Sp* AAC_{41}	*Sp* TG_{10}	*Sp* TG_{16}	*Sp* GG_{47}	*Sp* TG_{9}	*Sp* TG_{53}	*Sp* TG_{13}	*Sp* GT_{10}
T_A		56	49	48	60	56	55	52	48	48	48	49	56
BN	n	83	85	81	88	83	87	75	84	77	88	77	87
(n=88)	Ho	**0.82**	0.82	0.37	0.78	**0.35**	**0.70**	**0.88**	0.50	**0.64**	0.85	0.68	0.84
	He	0.96	0.90	0.41	0.87	0.93	0.97	0.96	0.56	0.94	0.93	0.78	0.86
	A	26.29	14.17	8.86	12.11	18.42	37.03	25.52	4.28	20.14	25.12	7.33	13.25
BC	n	58	64	65	61	63	62	59	61	62	61	61	61
(n=65)	Ho	**0.78**	0.91	0.32	0.77	**0.52**	**0.37**	0.80	0.64	**0.71**	0.84	0.62	0.80
	He	0.96	0.89	0.36	0.89	0.94	0.96	0.95	0.60	0.94	0.95	0.77	0.90
	A	25.78	15.00	8.06	13.20	20.80	30.33	23.47	4.84	21.29	25.91	6.87	12.16
BS	n	67	67	78	78	73	69	73	73	74	78	78	69
(n=78)	Ho	0.82	0.90	0.28	0.81	**0.55**	0.64	0.89	0.63	**0.65**	0.87	0.77	0.81
	He	0.96	0.91	0.29	0.89	0.94	0.97	0.95	0.60	0.95	0.94	0.72	0.89
	A	25.42	16.49	7.63	12.53	22.14	37.49	23.22	5.80	21.80	25.25	7.55	14.20
TE	n	73	71	79	79	65	77	75	67	74	79	79	74
(n=79)	Ho	0.78	0.87	0.35	0.86	**0.34**	**0.73**	0.89	0.45	0.69	0.92	0.62	0.86
	He	0.95	0.90	0.47	0.89	0.94	0.97	0.96	0.61	0.94	0.94	0.71	0.90
	A	23.38	13.88	9.84	13.64	21.71	37.15	26.67	5.38	22.45	27.53	6.43	12 61
UV	n	47	40	47	47	47	46	46	47	47	47	47	46
(n=47)	Ho	**0.89**	0.88	0.40	0.77	0.89	**0.61**	0.89	0.68	0.66	0.81	0.60	0.78
	He	0.96	0.91	0.46	0.89	0.96	0.97	0.93	0.67	0.93	0.93	0.74	0.90
	A	27.89	13.90	10.16	14.35	27.75	33.91	19.55	4.97	20.43	26.59	6.77	15.10
CA	n	48	38	48	47	48	47	48	45	48	48	48	48
(n=48)	Ho	0.88	0.87	0.23	0.77	0.88	0.60	0.88	0.49	0.69	0.83	0.60	0.77
	He	0.95	0.88	0.29	0.87	0.96	0.97	0.95	0.56	0.92	0.90	0.77	0.90
	A	21.67	13 00	9.13	12.39	26.45	35.95	24.51	5.53	18.31	24.26	7.73	14.32

Table 2.2. Continued.

Site		*Sp* $GATA_{40}$	*Sp* AAT_{40}	*Sp* AAC_{44}	*Sp* AAC_{33}	*Sp* AAC_{41}	*Sp* TG_{10}	*Sp* TG_{16}	*Sp* GG_{A7}	*Sp* TG_{8}	*Sp* TG_{53}	*Sp* TG_{13}	*Sp* GT_{10}
MA	n	96	92	96	96	93	93	96	95	93	93	96	86
(n=96)	Ho	0.95	0.84	0.59	0.77	0.95	0.68	0.97	0.57	**0.73**	0.95	0.59	0.87
	He	0.96	0.91	0.60	0.87	0.96	0.98	0.96	0.65	0.94	0.94	0.65	0.90
	A	27.37	15.01	8.99	12.22	26.11	37.83	26.67	5.07	20.91	26.15	6.03	15.04
BO	n	50	52	52	51	52	51	52	51	51	52	52	52
(n=52)	Ho	0.90	0.88	0.42	0.82	**0.79**	**0.57**	**0.77**	0.47	**0.75**	0.96	0.56	0.85
	He	0.96	0.90	0.41	0.86	0.96	0.97	0.95	0.56	0.95	0.94	0.67	0.89
	A	26.16	14.53	10.12	13.47	24.64	31.97	24.05	4.73	25.35	26.68	6.59	12.10
KE	n	42	42	42	42	41	42	42	42	41	42	42	41
(n=42)	Ho	0.88	0.86	0.24	0.81	0.88	**0.55**	0.83	0.45	0.78	0.95	0.71	0.78
	He	0.96	0.91	0.27	0.88	0.95	0.97	0.96	0.68	0.95	0.94	0.71	0.92
	A	25.21	14.71	8.43	12.61	19.90	32.78	27.01	5.90	21.76	26.71	8.71	14.77

Table 2.3. F_{ST} values (below diagonal) and D_C values with significance of exact tests (above diagonal) for adult populations of *Stegastes partitus* in the MBRS and CR-PAN, within-region comparisons are shaded. Significant values after sequential Bonferroni correction indicated in bold and underlined.

	MBRS				CR-PAN				
	BN	BC	BS	TE	UV	CA	MA	BO	KE
BN	*	0.212	0.189	0.200	0.217	0.222	**0.214**	0.214	0.223
BC	0.001	*	0.222	**0.224**	0.239	0.232	**0.247**	0.241	**0.257**
BS	0.000	0.000	*	**0.230**	0.246	0.237	**0.238**	0.236	0.252
TE	0.000	0.001	0.001	*	0.240	0.246	**0.243**	**0.252**	0.252
UV	0.002	0.005	0.005	0.003	*	0.256	**0.238**	**0.258**	0.262
CA	0.000	0.000	0.001	0.003	0.005	*	**0.253**	0.255	0.253
MA	**0.013**	**0.016**	**0.019**	**0.018**	0.005	**0.014**	*	**0.266**	**0.247**
BO	0.002	0.001	0.000	0.002	**0.010**	0.004	**0.026**	*	0.263
KE	0.002	0.003	0.003	0.003	-0.001	0.003	**0.009**	0.006	*

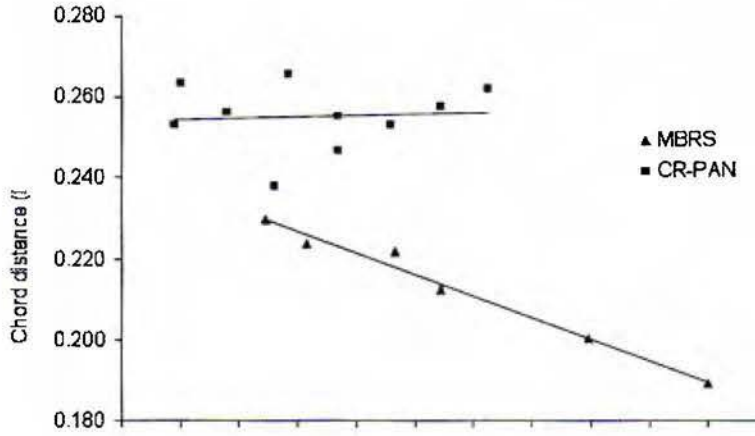

Figure 2.2. Geographic distance vs. Chord distance for adult populations in CR-PAN, p=0.410, r^2=0.008 and the MBRS, p=0.030, r^2=0.981. Same pattern was found when the distance used was Dc, Fst or Fst/1-Fst, but when using Fst the significance for MBRS ibd is lost (p=0.09)

Table 2.4. Pairwise D_C values with significance of exact tests, and pairwise F_{ST} values, between Manzanillo juveniles and each adult population of *Stegastes partitus* in the MBRS (shaded) and CR-PAN. Significant values after sequential Bonferroni correction indicated in bold and underlined.

Population	D_C	F_{ST}
BN	0.178	0.001
BC	0.206	0.001
BS	0.193	0.002
TE	**0.203**	0.002
UV	0.215	0.001
CA	0.211	0.000
MA	**0.205**	**0.010**
BO	**0.238**	**0.007**
KE	0.217	0.001

CAPÍTULO III

Conclusiones generales

3.1. Conclusiones generales

El conocimiento de las escalas de la dispersión larval y de la conectividad entre poblaciones marinas son esenciales para resolver los mecanismos de especiación y para proporcionar una adecuada protección de las poblaciones (Sale 2002). El concepto de conectividad genética se refiere al movimiento de genes entre poblaciones. Los genomas de las poblaciones conectadas genéticamente difieren muy poco. En especies con un tamaño efectivo de población grande, como las de los peces marinos, son necesarios unos pocos migrantes por generación para mantener la conectividad genética, y prevenir la diferenciación que se forma a través de la deriva (Palumbi 2003). Sin embargo, unos pocos peces no son suficientes, por ejemplo, para mantener una pesquería fuera de los bordes de un AMP. La conectividad demográfica (en términos de manejo y pesquerías, no genética), en contraste, es el movimiento de individuos entre poblaciones, en cantidades suficientemente grandes para ser importantes en la pesca, el manejo, y procesos ecológicos. (Cowen 2002). Es importante reconocer esta distinción a la hora de interpretar los estudios de conectividad (Steneck *et al.* 2006). Aunque la conectividad genética no siempre implica conectividad demográfica, se pueden realizar inferencias sobre ésta. Por ejemplo, cuando existen grandes diferencias genéticas entre poblaciones, se puede concluir que estas se encuentran demográficamente aisladas, porque no intercambian ni unos pocos migrantes por generación. Además los marcadores altamente polimórficos en conjunto con métodos innovadores tal como los análisis de asignación, permiten cuantificar el número de individuos que se dispersan a diferentes localidades.

Los resultados de este trabajo indican que las poblaciones de *S. partitus* no son unidades discretas cerradas debido al alto flujo genético que hay entre varias poblaciones. Además, si bien una porción de las poblaciones de la damisela bicolor puede ser retenida a una escala espacial de una hectárea, al menos tres cuartas partes de sus larvas se dispersan mayores distancias. Sin embargo, sus poblaciones tampoco se pueden considerar suficientemente homogéneas o abiertas, porque también existe cierta restricción en el flujo

genético, por ejemplo entre ciertos grupos de poblaciones, y entre las regiones del SAM y de CR-PAN.

El Capítulo 1 demostró niveles de autorreclutamiento de hasta 24%, a pesar de la poca diferenciación genética. Aunque las pruebas de asignación permiten cuantificar las cantidades de intercambio larval, existen muchas limitaciones metodológicas cuando hay un alto nivel de flujo genético, pues se dificulta la caracterización de las poblaciones de referencia. Dado que la mayoría de los peces de arrecifes caribeños exhibe bajos niveles de diferenciación, las pruebas de asignación deben ser utilizadas con cautela. Es importante buscar nuevas alternativas para determinar con mayor exactitud el origen de juveniles, tales como los análisis de parentesco. Desafortunadamente, estos análisis requieren muestrear la mayoría de la población parental, lo cual no es siempre posible cuando el rango de distribución de una especie es muy amplio, o es muy abundante. Se requiere estudiar especies modelo que cumplan con ciertas características. Otra alternativa, es combinar métodos genéticos con métodos de otras disciplinas, como la microquímica de otolitos o experimentos de pequeña escala con marcaje de larvas (i.e, directamente, con isótopos o tintes). Los resultados de este capítulo reflejan en mayor medida la alta capacidad de dispersión de *S. partitus* y el alto grado de mezcla de las poblaciones muestreadas. Un factor que probablemente promueve la mezcla de las poblaciones en el SAM, es la influencia de huracanes, capaces de cambiar los sistemas de corrientes. El año 2005 fue un período de alta incidencia de huracanes en esta región (NOAA 2008).

En el capítulo 2, se demostró que las larvas de la damisela bicolor tienen capacidad de dispersarse por lo menos 1000 km, en cantidades suficientes para establecer conectividad genética, aunque existe cierta restricción en el flujo genético, que posiblemente restringe los niveles de conectividad demográfica entre las regiones del SAM y CR-PAN, y en poblaciones particulares como Manzanillo, Costa Rica. Estas dos regiones pueden considerarse como unidades de conservación independientes, para el manejo de pesquerías, pero no así para el mantenimiento de la diversidad genética. Además, se demostró que otros factores tales como la fragmentación del hábitat podrían reducir los niveles de conectividad dentro de una región.

Los resultados de ambos capítulos, en conjunto, reflejan los dos extremos de la distribución de propágulos, e ilustran la diferencia entre conectividad genética y demográfica. Si bien los bajos niveles (cercanos a cero) de diferenciación genética encontrados en todo el estudio podrían ser resultado de la homoplasia[3] de los marcadores, esta situación es poco probable porque se utilizaron suficientes microsatélites (9-12 loci), y es altamente improbable que todos ellos exhiban la misma homoplasia. Además, como se mencionó anteriormente, valores bajos F_{ST} son comunes en poblaciones de peces marinos por sus grandes tamaños efectivos de población (O'Reilly *et al.* 2004).

Los adultos de la damisela bicolor en Belice alcanzan al menos 5 años de edad (Caldow & Wellington 2003), aunque pueden vivir más de 7 años (Caldow & Wellington 2003), y se reproducen durante todo el año. Los individuos pueden llegar a tallas de 3.5-4 cm (donde se observan gónadas desarrolladas, obs. pers.) en pocos meses (McGeehe 1995). Por lo tanto, la población adulta está compuesta de múltiples cohortes que podrían provenir de distintos arrecifes en cada evento de reclutamiento. Este patrón se ha demostrado en diversos estudios demográficos y genéticos realizados en las poblaciones de la damisela bicolor en el arrecife Mesoamericano y en los Cayos de Florida. Por ejemplo Hogan (2007), encontró que los patrones de reclutamiento de *S. partitus* eran coherentes hasta una escala de 25 km, pero presentaban variabilidad temporal. El reclutamiento es variable en el espacio y el tiempo (e.g. Tolimeri *et al.* 1998), y esto se refleja también en cambios en la estructura genética, tal como lo demuestra el II capítulo, así como estudios de Hepburn (2004) en poblaciones de damiselas bicolor del arrecife Mesoamericano y Lacson y Morizot (1991) en los Cayos de la Florida.

En cuanto a la conservación y el manejo, el comprender el movimiento de las larvas en el mar abierto es una pieza importante para completar nuestro conocimiento en modelos de las pesquerías y el manejo costero (Palumbi 1999). En el ámbito de manejo, el uso de las reservas marinas como herramienta para la conservación de la biodiversidad marina ha sido ampliamente sugerido (e.g. Roberts 1997, Gell y Roberts 2003, Mora *et al.* 2006). Es

[3] Homoplasia: Similaridad debido a evolución convergente, pero con orígenes independientes (Brooks & McLennan 1991). En el caso de microsatélites, que varias muestras exhiban los mismos tamaños de alelos, y que hayan llegado a esta similaridad de manera independiente, por homoplasia.

fundamental que el tamaño de las reservas marinas se adecúe a la distancia de dispersión de los organismos para los cuales está diseñada a proteger (Mora *et al.* 2006). Con la información recopilada hasta la fecha para *Stegastes partitus,* sabemos que menos del 24% de los peces se retienen en arrecifes de Turneffe a una escala de 1 ha, y que existe un alto nivel de mezcla entre poblaciones (presente estudio, cap I). Conocemos que, aunque algunas larvas pueden dispersarse a distancias de hasta 1000 km, manteniendo poblaciones genéticamente casi homogéneas, también existen sutiles niveles de restricción el en flujo genético, que indican que posiblemente hay limitada conectividad demográfica a esta escala (cap II). Más aún, otros autores (Cowen 2006, Chittaro 2005) sugieren que es posible que la distancia de dispersión de la mayoría de los individuos de esta especie, a nivel demográfico, sea <50 km. Las poblaciones no son cerradas gracias al intercambio de unos cuantos migrantes por generación a grandes distancias, pero tampoco son completamente abiertas, como se ha demostrado en ambos capítulos y en otros trabajos. Los bordes de las poblaciones son difíciles de definir debido a la inestabilidad temporal en la estructura genética poblacional de esta especie (Lacson & Morizot 1991, Hepburn 2004, Cap II). Es claro que no podemos definir con claridad arrecifes fuente o receptoras de larvas, por la compleja variación espacio-temporal en la estructura genética poblacional existente. Sin embargo, es posible deducir que los individuos de *Stegastes partitus* forman metapoblaciones, ya que cumplen con los parámetros mencionados en el prefacio: deben existir parches de hábitat discretos y el movimiento de individuos entre los parches debe darse pero no debe ser excesivo (Sale et al 2006). Se ha acuñado también la definición de "metapoblación funcional" (Kritzer & Sale 2006), que es cuando existe continuidad de habitat, pero las poblaciones adultas forman "parches" discretos conectados por dispersión larval. Dependiendo del escenario espacial, *Stegastes partitus* entonces conforma metapoblaciones funcionales (en secciones extensas y continuas, tal como las encontradas en el SAM), o metapoblaciones simples (posiblemente como en los arrecifes de CR-PAN, donde el desarrollo arrecifal suelen ser más discontinuo, separado por grandes extensiones arenosas o bocas de ríos).

Para conservar especies con ciclos de vida y ámbitos de distribución similares a esta especie, lo más recomendable sería diseñar redes de áreas marinas protegidas que tomen en cuenta la distancia de dispersión de la especie. Aún existe gran debate en este tema, pero como recomendación general, para que estas puedan proveer suficientes larvas a otras reservas se recomienda que, entre menos sea la capacidad de dispersión de una especie, menos sea la distancia (Jones *et al.* 2007). En el caso de *Stegastes partitus*, sería aproximadamente una distanca de 50 km. Sin embago, varios autores recomiendan variar las distancias (Kaplan & Botsford 2005), por ejemplo entre 10-200 km (Palumbi 2004), para cubrir el ámbito de variación de distintas especies marinas. En el caso de que el hábitat se encuentre fragmentado, como en Costa Rica y Panamá, se debe tomar en cuenta no solamente la distancia de dispersión, sino también el espaciamiento entre parches (Jones *et al.* 2007), de manera que se garantice que un AMP supla a otra AMP con suficiente cantidad de larvas, manteniendo las poblaciones saludables. Las anteriores recomendaciones poseen grandes dificultades para ser aplicadas en la realidad, debido a que para diseñar redes de reservas marinas se debe tomar en cuenta factores sociales, económicos y políticos. La recomendación final es tomar en cuenta esto en conjunto con los demás factores, en un modelo que permita diseñar la red de AMPs más balanceada, que sea validado y consultado en talleres con las comunidades, usuarios y demás interesados. Se debe tomar en cuenta que reservas pequeñas podrían mantener una población de *Stegastes partitus* por el grado de autorreclutamiento, sin embargo no sería suficiente, porque no se darían los niveles de diversidad genética actual, que se mantienen por el alto intercambio con múltiples poblaciones. Lo más importante será crear (y manejar efectivamente) más AMPs, para "conectar los puntos", y tomar medidas de manejo fuera de las AMPs, para no reducir los niveles de conectividad por la fragmentación del hábitat.

A partir de este trabajo algunas recomendaciones para futuras investigaciones son:

a) Realizar un muestreo genético uniformemente a través de la región de estudio, como se ha recomendado recientemente en estudios de genética del paisaje (Schwartz & McKelvey 2008), en vez de muestrear poblaciones predefinidas por los científicos. La

razón es que muchas poblaciones se encuentran distribuidas de manera continua y no forman estas poblaciones artificiales. Posteriormente, se puede utilizar un programa (e.g, STRUCTURE, BAPS, etc. para definir los bordes reales de las poblaciones y buscar posibles barreras geográficas (Schwartz & McKelvey 2008). Para poder aplicar este método, se recomienda estudiar primero la escala de los patrones espaciales de autocorrelación para definir el mejor diseño, ya que éste puede afectar los resultados (Schwartz & McKelvey 2008). Este tipo de muestreo ofrece la ventaja de un mayor poder de detección de aislamiento por distancia y de inferencia de distancias de dispersión. Palumbi (2003) recomienda muestrear 2-5 veces la distancia de dispersión larval para detectar patrones de aislamiento por distancia. El presente estudio abarcó un área 20 veces la distancia de dispersión de la mayoría de *S. partitus* (~ 50 km), pero no se muestrearon poblaciones intermedias en Nicaragua, Honduras, San Andrés, o Jamaica, donde el modelo de Cowen (2006) supone que se podrían desviar las larvas de Costa Rica. Es recomendable que el muestreo sea además de manera jerárquica en distintas escalas espaciales, para discernir cuál es la escala de movimiento larval más importante. En síntesis, se recomienda para mayor resolución de la escala de dispersión, el muestreo continuo y jerárquico. Se puede tomar como base este estudio y otros con *Stegastes partitus*, que sugieren que es importante extender el estudio para cubrir todo el Caribe y definir bordes de regiones, y además concentrarse en alguna región de interés para estudiar los patrones más finos, tales como la variación de las fuentes larvales.

b) Mantener un programa de monitoreo temporal, pues si la estructura genética débil se preserva en el tiempo, podría indicar la existencia de un grado de autorreclutamiento persistente (Hedgecock *et al.* 2007). Si alternativamente se presentara variabilidad temporal, la mejor manera de explorarlo es muestreando finamente en el tiempo (en días), para determinar la estructura genética de cada cohorte por separado y las razones que la causan. Existe una tendencia en poblaciones marinas de tener estructura genética significativa en larvas, reclutas o poblaciones a escalas finas, carente de patrones espaciales y que cambia a través del tiempo. Esto sugiere que las larvas no están bien mezcladas en el plancton. Este muestreo permitiría identificar las causas de heterogeneidad

espacio-temporal, sean cambios en patrones de migración de larvas, selección natural que ocurre en los parches de plancton, o incluso la estocasticidad producto del éxito reproductivo de algunos adultos que resultan en una estructura familiar detectable (Selkoe *et al.* 2006). Recomendamos continuar estudiando la población de Manzanillo, tanto adultos como juveniles para investigar si la estructura poblacional adulta se mantiene, y si se ven más cambios en la estructura de juveniles.

c) Combinar distintos tipos de marcadores genéticos para complementar la información, o utilizar un mayor número de loci. Pueden usarse marcadores, tales como los los Polimorfismos de la Longitud Amplificada del Fragmento (AFLPs, que permiten un mayor número de loci), condición ideal para las pruebas de asignación que cuantifiquen movimiento larval y su dispersión (Campbell *et al.* 2003). Sin embargo, se deben considerar algunas limitaciones logísticas, pues estos marcadores son útiles cuando se utilizan en grandes cantidades y se presentan bandas diagnósticas por población.

d) Comparar diferentes organismos con distintos ciclos de vida durante el mismo período de muestreo es útil para detectar barreras de dispersión, importantes para manejo y conservación.

e) Utlizar modelos matemáticos que simulen la dispersión de los peces y hacer investigación multidisciplinaria en colaboración con oceanógrafos, matemáticos y especialistas en comportamiento larval. Existen modelos oceanográficos detallados para algunas partes del mundo, y en colaboración con otros expertos, se puede planear de antemano el muestreo para probar diversas hipótesis sobre el movimiento larval, con el fin de calibrar estos modelos. Recomendamos continuar haciendo pruebas con el modelo de Cowen *et al.* 2006, o los modelos oceanográfico-genéticos, como el de Galindo (2006)

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