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

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

Connectivité Génétique dans la Langouste Blanche Del Caribe (*Panulirus argus*) au Belize

NATHAN K. TRUELOVE[1,2]*, EMILY BURDFIELD-STEEL[1], SARAH GRIFFITHS[1], KIM LEY-COOPER[3], RICHARD PREZIOSI[1], MARK J. BUTLER IV[4], DONALD C. BEHRINGER[5,6], STEPHEN BOX[7], and STEVEN CANTY[7]

*[1]University of Manchester, Michael Smith Building, Faculty of Life Sciences, Oxford Road, Manchester M13 9PT, United Kingdom. *Nathan.Truelove@postgrad.manchester.ac.uk. [2]Sustainable Consumption Institute, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom. [3]Curtin University, Department of Environment and Agriculture, Perth, Australia, WA 6845. [4]Old Dominion University, Department of Biological Sciences, Norfolk, Virginia 23529 USA. [5]University of Florida, School of Forest Resources and Conservation, Gainesville, Florida 32653 USA. [6]University of Florida, Emerging Pathogens Institute, Gainesville, Florida 32610, USA. [7]Centro de Ecología Marina de Utila, Oficina 401-403, Edificio Florencio, Blvd Suyapa, Tegucigalpa, Honduras.*

ABSTRACT

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

KEY WORDS: Spiny lobster, *Panulirus argus*, genetics, microsatellites, marine reserves, connectivity, Belize

INTRODUCTION

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

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

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

Silberman et al. (1994) conducted the first Pan-Caribbean study of *P. argus* using mtDNA markers, sampling 259 individuals from nine sites: Los Roques, Venezuela; Martinique; Antigua; Turks and Caicos; Jamaica; San Blas, Panama; Dry Tortugas, Florida; Miami, Florida; and Bermuda.

They analyzed levels of genetic differentiation by separating sites based upon:

i) Isolation by distance,
ii) Contrasting ocean currents, and
iii) Continental vs. insular.

None of their three models provided evidence of genetic differentiation, lending credence to the widely accepted hypothesis that *P. argus* is a single genetically homogenous population throughout the western tropical Atlantic.

Biophysical Modelling

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

The regional differences in larval dispersal caused by the interaction among OVM and advective and retentive oceanographic currents could potentially be a driver of spatial genetic patterns in *P. argus*. However, the previously mentioned genetic studies using allozyme and mtDNA markers failed to detect significant differences in *P. argus* genetic patterns between advective and retentive oceanographic environments. Why were these previous studies unable to detect any spatial genetic patterns? Is it possible that the high levels of mixing and gene flow were sufficient to mitigate the effect of the oceanographic environments? An alternative explanation is that the previous studies had limited resolution to detect subtle genetic signals due to:

i) Small sample sizes (~30 per site),
ii) Sampling only one site within each oceanographic environment, and
iii) The use of genetic markers with too few polymorphic loci.

Seascape Genetics

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

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

Study Questions

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

MATERIALS AND METHODS

Sampling Locations

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

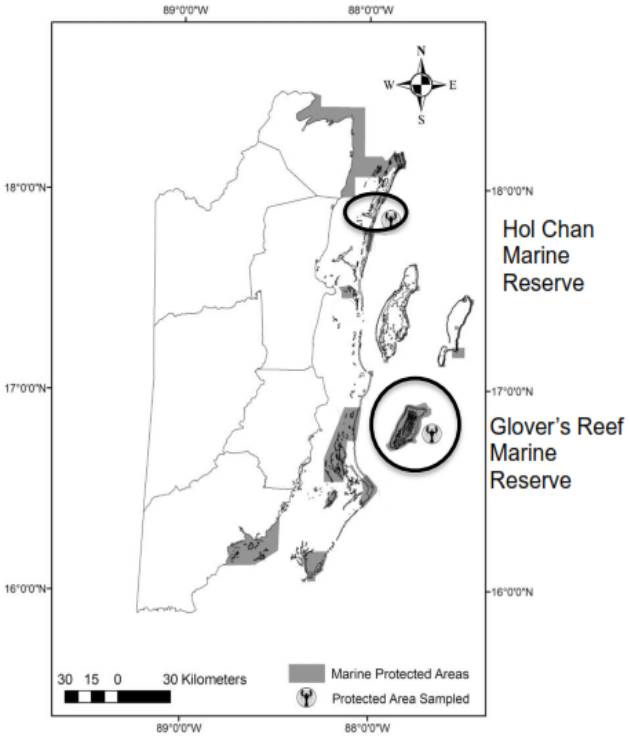

Figure 1. Map of marine protected areas in Belize. Samples were collected at Glover's Reef and Hol Chan reserves (located inside the black circles).

Sample collection

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

DNA Extraction and Microsatellite amplification

Genomic DNA was isolated from muscle tissue using the ISOLATE Genomic DNA Mini Kit (BIOLINE). DNA quality and quantity was assessed by a NanoDrop 2000 micro-volume spectrophotometer (THERMO SCIENTIFIC). Primers for 5 microsatellite loci (Table 1) were simultaneously amplified by multiplex PCR with a Qiagen Type-it Microsatellite PCR kit. PCR reactions took place in a 25 mL reaction volume containing 20 - 100 ng DNA, 1μM forward and reverse primers (5' end labeled with fluorescent dye, Cyc5/Cyc5.5) in 1x QIAGEN Multiplex PCR Master Mix containing HotStar *Taq* DNA Polymerase, and 3 mM $MgCl_2$. Primers were optimized under following conditions: DNA polymerase was activated in an initial activation step (95°C for 5 min), followed by 28 thermocycles of denaturation (95°C for 30 s), annealing (60°C for 90 s), and extension (72°C for 30 s), and a final extension (30 min at 60°C). Florescent- labeled PCR products were size-separated and analyzed in a CEQ 8000 Genetic Analysis System (Beckman Coulter). Allele peak profiles were identified at each locus with alleles designated by their size in base pairs. Binning of allele size was carried out using the CEQ 8000 Genetic Analysis System software. All fragment sizes were pre-analyzed by the software and checked by eye.

Statistical Analysis

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

RESULTS AND DISCUSSION

Microsatellite Loci

A total of 16 individual lobsters from Hol Chan marine reserve and 41 lobsters from Glover's Reef were scored for five microsatellite loci to explore levels of gene flow between the marine reserves. Results from the CEQ 8000 Genetic Analysis System software indicated that the multiplex PCR worked well and fragment sizes were similar to those previously described by Diniz et al. (2006; see Table 2). To investigate the potential for null alleles,

Table 1. Microsatellite primers and allele sizes.

Loci	Sequence (5' - 3')	Number of Alleles	Size Range (Base Pairs)	GenBank Accession Number
Par3	F: TTACCGGGTTGACAGGAGAC R: GTCCGTGTGGTCCGATATTC	9	98-138	AY526337
Par6	F: GAAGTTTCCCTAATGTTCGTCCT R: GCAAACAGTGGACCGAGAGA	4	83-95	AY526340
Par7	F: TGGGTAACGGTAAGACTATTGA R: CAGACAGATGGACGGAGAGA	11	117-157	AY526341
Par9	F: CCCTGACTTTCTTGTTAAACTCG R: TCAGTCTATCCATCTATCTAACCATC	4	155-183	AY526343
Par10	F: CAAGCAAAGCACAGAAGCAT R: AACCAGCGTTCCAGTCAGTT	15	242-386	AY526344

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

Locus	Hol Chan					Glover's Reef					F_{IS}	F_{ST}
	Samples	Alleles	H_O	H_E	P_{HWE}	Samples	Alleles	H_O	H_E	P_{HWE}		
Par3	16	26	1.000	0.824	0.843	41	54	0.925	0.785	0.112	-0.196	0.021
Par6	16	26	0.692	0.559	0.786	41	44	0.545	0.622	0.494	-0.002	0.019
Par7	16	12	0.00	0.783	0.001	41	28	0.214	0.814	<0.001	0.822	-0.021
Par9	16	20	0.10	0.100	< 0.001	41	8	0.000	0.425	0.143	0.639	0.787
Par10	16	28	0.50	0.857	0.003	41	22	0.454	0.809	0.003	0.439	0.018

calculations of observed heterozygosity, expected heterozygosity, and Hardy-Weinberg equilibrium were conducted (Table 2). The number of alleles ranged from 12 - 28 for individuals from Hol Chan marine reserve and 8 - 54 for Glover's Reef marine reserve. The increased number of alleles present at Glover's reef is most likely an artifact of increased sample size rather than actual population structure. The low observed heterozygosities and deviation from Hardy-Weinberg equilibrium (i.e., assuming random mating, no mutation, no drift, no migration; $p < 0.001$) suggests the presence of null alleles (those that fail to amplify during PCR) at Par7 and Par9. The small number of alleles present and 100% non-overlapping allele frequencies at Par9 provided further evidence of null alleles at this locus. As a conservative measure to minimize the effect of fragment scoring error due to null alleles, Par7 and Par9 were excluded from statistical power analyses and Bayesian models of population structure.

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

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

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

Biological Implications

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

It is an oversimplification to suggest that local hydrodymanics and larval behavior are the only factors responsible for the observed patters we found. The availability of suitable nursery habitat is crucial for the survival of *P. argus* larvae and may ultimately limit their

Figure 2. STRUCTURE assignment test for *Panulirus argus* individuals from Hol Chan (black) and Glover's Reef (grey) populations. The probability of correct assignment of individuals from Hol Chan was ~90% and > 95% for Glover's Reef.

successful recruitment. Spatial analyses of nursery habitat availability should also be incorporated into future genetic analyses of *P. argus* connectivity. Similarly, marine reserves have been designed throughout the Caribbean to conserve critical nursery and spawning habitats for *P. argus* and the effects of these conservation strategies should be taken into account. Additionally, one must account for the effect that protection from fishing has on *P. argus* genetic structure. Acosta et al. (2003) found a remarkable 20x increase in spiny lobster abundance in un-fished patch reefs after only five years of protection in Glover's Reef marine reserve. Information concerning the increases in lobster abundance in the no-take area of Hol Chan has yet to be published and could potentially provide additional support for these genetic findings.

Implications for Marine Reserves

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

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