

Ecological and genetic data indicate recovery of the endangered coral *Acropora palmata* in Los Roques, Southern Caribbean

A. L. Zubillaga · L. M. Márquez · A. Cróquer ·
C. Bastidas

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Abstract The rapid decline of *Acropora cervicornis* and *Acropora palmata* has often been linked with coral reef deterioration in the Caribbean; yet, it remains controversial whether these species are currently recovering or still declining. In this study, the status of ten populations of *A. palmata* in Los Roques National Park (LRNP), Venezuela is presented. Six of these populations showed signs of recovery. Ten 80 m² belt-transects were surveyed at each of the ten reef sites. Within belt-transects, each colony was measured (maximum diameter and height) and its status (healthy, diseased or injured) was recorded. Populations in recovery were defined by a dominance of small to medium-sized colonies in densities >1 colony per 10 m², together with 75% undamaged colonies, a low prevalence of diseases (<10%), and a low density of predators (0.25 snails per colony). Based on allozyme analysis of seven polymorphic loci in four populations ($N = 30$), a moderate to high-genetic connectivity among these populations ($F_{ST} = 0.048$) was found with a predominance of sexual over asexual reproduction ($N^* : N = 1$; $N_{go} : N = 0.93-1$). Both ecological and molecular data support a good prognosis for the recovery of this species in Los Roques.

Keywords *Acropora palmata* · Population density · Size classes · Diseases · Sexual reproduction · Gene flow

Introduction

During the past three decades, the rapid decline of Elkhorn and Staghorn corals, *Acropora palmata* and *Acropora cervicornis* has been a major concern among coral reef scientists because of their importance as reef builders in the Caribbean (Aronson and Precht 1997, 2001; Jackson et al. 2001; Bruckner 2002). The massive mortality of Caribbean acroporids was attributed to a combination of factors including hurricanes, eutrophication, sedimentation, bleaching, and more importantly, a white band disease (WBD) epizootic event that occurred during the 1980s (Gladfelter 1982; Aronson and Precht 1997, 2001). Total and partial mortality of a large number of colonies of *A. palmata* had significant impacts at the individual (reduction in reproductive output and survivorship), population (decrease in colony densities), and ecosystem levels (increase of erosion and bioerosion rates, replacement of species, loss of spatial heterogeneity, and therefore biodiversity) (Aronson and Precht 1997; Bruckner 2002). In view of the sudden decline of *A. palmata*, its former role as a keystone and structural species in Caribbean coral reefs (Bruckner 2002; Patterson et al. 2002) and its current critical status, this species was recently listed under the United States Endangered Species Act as ‘threatened’ (Anonymous 2006).

Three decades after the mortality event, it remains unclear whether *A. cervicornis* and *A. palmata* are recovering or continue declining. While several studies have shown evidence of moderate recovery in different sites (Grober-Dunsmore et al. 2006; Mayor et al. 2006), others still claim that this species has failed to recover or that it is

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A. L. Zubillaga (&) · A. Cróquer · C. Bastidas
Depto. Biología de Organismos, Universidad Simón Bolívar,
Apartado 1080-A, Caracas, Venezuela
e-mail: alzubillaga@gmail.com

L. M. Márquez
Centro de Biotecnología, Instituto de Estudios Avanzados,
Apartado 17606, Caracas, Venezuela

far from recovering its former role as major reef builder of shallow habitats in the Caribbean (Knowlton et al. 1990; Porter et al. 2001; Bruckner 2002; *Acropora* Biological Review Team 2005). This controversy is mainly due to the lack of studies incorporating not only ecological but also genetic data (Bruckner 2002; *Acropora* Biological Review Team 2005). Ecological data (such as population density, size structure, health condition of colonies, etc.) are obviously necessary to evaluate the current status of a population and to some extent its potential for further recovery. Genetic data can provide an indication of how likely it is that a population goes extinct. The level of connectivity, or gene flow, provides a measurement of the chance of seeding and recolonization of a population by individuals coming from other populations (Roberts 1997; Cowen et al. 2000; Hellberg et al. 2002). Also, estimates of genetic and genotypic diversity serve as proxies for the ability of the population to respond to environmental changes and epidemic diseases (Altizer et al. 2003; Reed and Frankham 2003; Reusch et al. 2005).

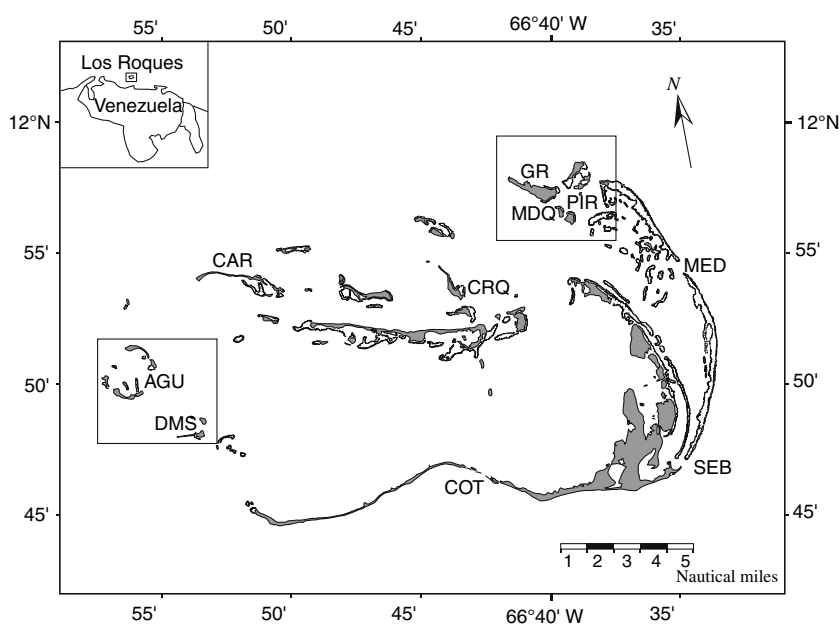
In this study, both ecological and genetic data were used to evaluate the status of *A. palmata* at Los Roques National Park (LRNP), Venezuela. The high cover of dead *A. palmata* stands in the reef crests of Los Roques (>70%) provide evidence of the former dominance of this species at the study sites. Thus, the presence of new and healthy colonies at these sites where populations declined dramatically can be used as an indication of recent colonization and potential for recovery. Density of colonies and the size structure of ten populations were estimated, and in four of them genetic diversity, genetic connectivity, and the relative contribution of sexual reproduction were evaluated.

Materials and methods

Field surveys and ecological estimations

Between August 2003 and August 2004 a series of surveys were conducted in the surf zone of ten coral reef sites at LRNP ($11^{\circ}44'45''$ – $11^{\circ}58'3''$ N, $66^{\circ}32'42''$ – $66^{\circ}52'57''$ W): (1) Gran Roque (GR: $11^{\circ}56'50.4''$ N, $66^{\circ}39'49.1''$ W), (2) Madrisquí (MDQ: $11^{\circ}56'23.8''$ N, $66^{\circ}39'37.5''$ W), (3) Cayo Pirata (PIR: $11^{\circ}55'77.1''$ N, $66^{\circ}39'15.5''$ W), (4) Crasquí (CRQ: $11^{\circ}52'49''$ N, $66^{\circ}43'16.2''$ W), (5) Carenero (CAR: $11^{\circ}53'21.3''$ N, $66^{\circ}50'35.8''$ W), (6) Dos Mosquises Sur (DMS: $11^{\circ}47'48.7''$ N, $66^{\circ}53'02.3''$ W), (7) Cayo de Agua (AGU: $11^{\circ}49'07''$ N, $66^{\circ}55'51.5''$ W), (8) Boca de Cote (COT: $11^{\circ}45'9.22''$ N, $66^{\circ}42'2.63''$ W), (9) Sebastopol (SEB: $11^{\circ}46'79.3''$ N, $66^{\circ}34'85.6''$ W), and (10) Boca del Medio (MED: $11^{\circ}53'77.1''$ N, $66^{\circ}39'15.5''$ W) (Fig. 1). At each site, within the natural habitat of *A. palmata*, ten 20-m long by 4-m wide belt transects (replicates) were set to determine: (1) the number of colonies; (2) the maximum diameter and height of each colony (live tissue + dead skeleton); (3) the status of each colony (diseased, bleached, marks of predation or injured); and (4) the number of *Coralliophila abbreviata* snails (a coral predator) per colony. Individual size of adults and juveniles were estimated by multiplying their height and maximum diameter. Although this criterion might have overestimated the actual size of certain individuals by including both living and dead portions of the colony, the amount of living tissue was always larger than the dead portion. Nevertheless, dead portions were taken into account when establishing the health status of each colony, as mentioned above.

Fig. 1 Map of Los Roques National Park showing the ten sites of *Acropora palmata* populations for ecological studies and the four for genetic studies (squares). DMS Dos Mosquises Sur, AGU Cayo de Agua, CAR Carenero, CRQ Crasquí, GR Gran Roque, MDQ Madrisquí, PIR Pirata, COT Boca de Cote, MED Boca del Medio, and SEB Sebastopol. Image: Institute of Marine Remote Sensing



For putative recruits, only the maximum diameter was used as a size estimator. Although minimum reproductive size is quite variable among coral species (Harrison and Wallace 1990), estimated size at puberty (colony size at maturation) for *A. palmata* is 1,600 cm² of living tissue (Soong and Lang 1992). Colonies between 60 and 1,600 cm² have low-reproductive potential, while colonies below 15–60 cm² are not reproductively active (Soong and Lang 1992). Thus, adult colonies were considered to be those above minimum reproductive size (>1,600 cm²), juveniles those that just reached minimum reproductive size (>60–1,600 cm²), and recruits those below minimum reproductive size (<60 cm²). Rounded edges with paled polyps distinguished putative sexual recruits from patches of remaining tissue produced by partial mortality. The density of adults, juveniles and recruits was compared across sites by a Kruskal–Wallis test given that the data did not have a normal distribution and the variance was heterogeneous. Paired comparisons for each size class across sites were performed with a Mann–Whitney test corrected by Bonferroni's method.

The number of size classes for the frequency histograms and their range in size were chosen to maximize the number of colonies within each class considering the data of all *A. palmata* populations together. Distribution of size classes, community structure (cover and composition), colony density, and the overall condition of colonies were used to assess the status of *A. palmata* at each reef site.

Sample collection and allozyme analysis

For allozyme analyses, samples were taken at DMS and AGU (south-western sector of LRNP, 5.1 km apart), and at PIR and GR (northeast sector of LRNP, 1.8 km apart). Northeast and southwest sectors are separated from one another by ~30 km. Samples for allozyme analysis were collected only at these sites because they had the largest numbers of *A. palmata* colonies, allowing a large sample size, and because they were located in opposite areas of the Archipelago (Fig. 1), and were therefore expected to be the most likely of all ten populations to show restrictions to gene flow. At each population, i.e., reef site, 30 fragments no larger than 20 cm² were collected from *A. palmata* colonies that were at least 5 m apart. Since the study involved estimating the contribution of asexual reproduction for the population as a whole, the collection of samples 5 m apart avoided sampling pieces of fragmented colonies that did not disperse and thus did not contribute significantly to the coral cover and population density. Samples were collected from colonies located both within and outside the belt transects to cover more extensive areas (~800 m²) at each site. Samples were collected in properly labeled (date, site of collection, and number) plastic bags. To avoid protein deg-

radation, all samples were stored in liquid nitrogen in the field immediately after collection. Once in the laboratory, the samples were kept in a –80°C ultra freezer until standard procedures for extraction and electrophoretic analyses were used (Richardson et al. 1986). Based on the resolution, polymorphism and reliability of scoring, 7 of 14 enzymes that showed activity were used for routine screening: GPI (glucose-6-phosphate isomerase E.C. 5.3.1.9), HK (hexokinase E.C. 2.7.1.1), MDH (malate dehydrogenase E.C. 1.1.1.37), VL (peptidase substrate valil-leucine E.C. 3.4.11/13), LGG (peptidase substrate leucilglycine E.C. 3.4.11/13), GLUDH (glutamate dehydrogenase E.C. 1.4.1.3), and GDH (glucose dehydrogenase E.C. 1.1.1.47). Electrophoreses were carried out on cellulose acetate gels (Cellogel). LGG was run in Citrate-phosphate buffer pH 6.4; GDH and HK were scored from Tris-maleate buffer pH 7.8; and GLUDH, GPI, MDH, VL from TG 8.1 buffer (Tris-glycine). Departures of genotypic frequencies from those expected under conditions of Hardy–Weinberg equilibrium (HW) were evaluated for each locus with an approximation to the Fisher's exact test using TFGA software (Miller 1997) and a level of significance of $\alpha = 0.05$ corrected with Bonferroni's method. The magnitude of genetic differentiation was estimated using hierarchical F_{ST} analysis with sectors and populations (Weir and Cockerham 1984), based on allele frequencies for individual populations. Genetic diversity was expressed as allelic richness (average number of alleles per locus), average number of effective alleles, percentage of polymorphic loci, and expected heterozygosity (H_e).

The number of genetically differentiated *A. palmata* populations (k) was estimated by employing a Bayesian approach, implemented in the program *Structure* v. 2 (Pritchard et al. 2000; Falush et al. 2003). The program uses a clustering method to assign individuals with similar multilocus genotypes to probable common populations. The number of populations were inferred from multilocus genotypes by running *Structure* with one million Markov Chain Monte Carlo (MCMC) repetitions (burn in = 100,000). Since *A. palmata* larvae can stay for long periods in the plankton, the 'admixture ancestry model,' was run under the assumption of 'correlated allele frequencies' rather than 'independent allele frequencies' to improve clustering of closely related populations (Falush et al. 2003; Baums et al. 2005a).

The contribution of sexual versus asexual strategies within each population was evaluated for multilocus genotypes using (1) the ratio of N^*/N (maximum expected number of individuals produced sexually/number of individuals sampled) calculated from the allelic frequency for each reef and (2) the number of genotypes observed (N_{go}) over N (total number of individuals), following reported procedures (e.g., Johnson and Threlfall 1987; Uthicke et al.

1998). Although 30 individuals may be a relatively small sampling size to assess the contribution of sexual and asexual reproductive strategies, this number represented up to 55% (PIR) of the total number of colonies at these sites.

The probability of identity (P_{ID}); i.e., the probability that two individuals drawn at random from a population have the same genotype at multiple loci (Waits et al. 2001) was calculated using the software GIMLET v. 1.3.3 (Valiere 2002). An overall P_{ID} was obtained after sequentially multiplying P_{ID} values over all loci. In this case, we considered only biased estimation, because all populations had the same number of individuals ($N = 30$) and it was not necessary to correct for differences in sample sizes (Waits et al. 2001).

Results

A high abundance of *A. palmata* colonies in healthy condition, a predominance (42–74%) of small (0.1–50 cm²) to medium size classes (50–4,550 cm²), and a low density of predators characterized six (AGU, DMS, PIR, GR, CAR, and CRQ) of the ten study sites evaluated at LRNP (Table 1, Fig. 2). In the remaining four sites, old stands of dead *A. palmata* were mostly covered by different algal guilds (48 ± 19%, mean ± SE) and there were no signs of re-colonization. Concomitantly, colony density was significantly different across sites (Kruskal–Wallis test, $H = 52.61$, $df = 9$, and $P < 0.05$) with density varying from 0 to 3.4 individuals per 10 m² ($N = 10$). For adults, juveniles, and recruits separately, there were also differences across sites ($df = 9$, $P < 0.05$, Kruskal–Wallis test $H = 43.82$, 41.99, and 39.56, respectively). Colony density was highest at AGU (Mann–Whitney, $P < 0.05$, corrected by Bonferroni); also the largest colonies (4,550.1–16,500 cm² and >16,500 cm²) were found at this site (Fig. 2).

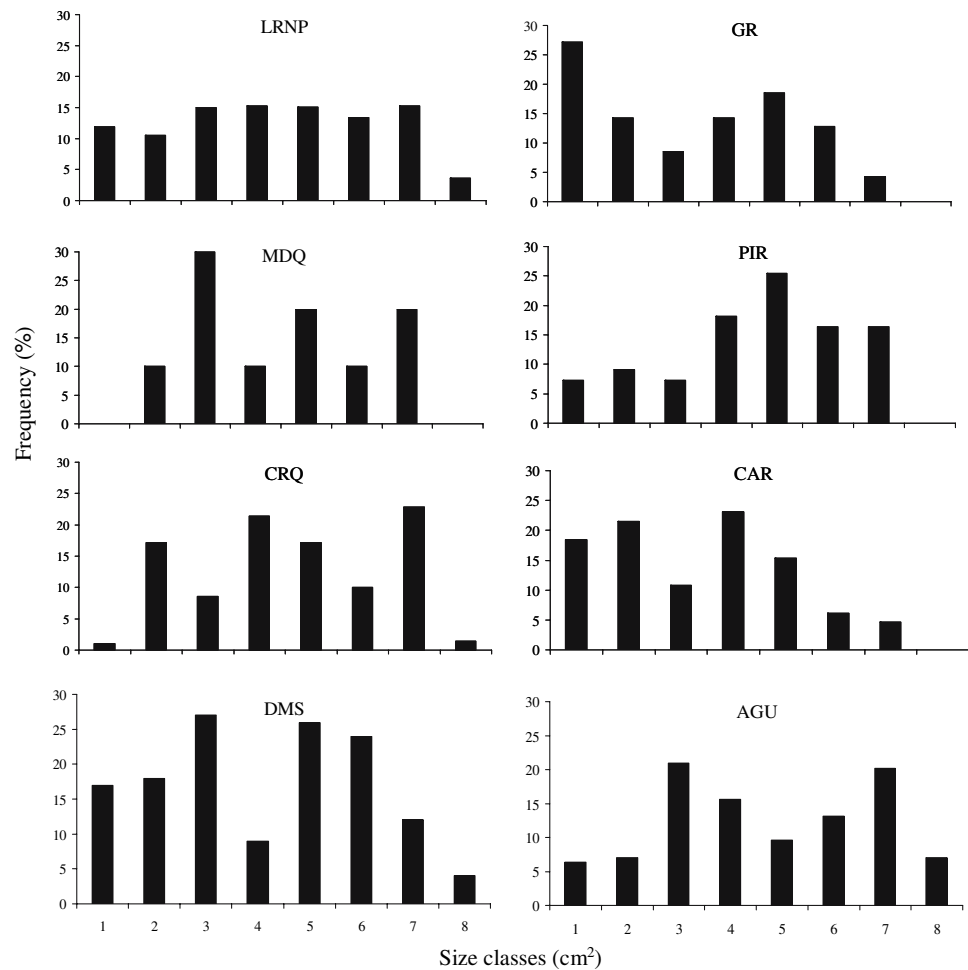
Most colonies within each population (70–89%) were undamaged, with no signs of disease (e.g., white band, white pox or patchy necrosis), predation (damselfish, worms or gastropods) or partial mortality (sedimentation, algae overgrowth) (Table 1). When *A. palmata* colonies were present, the prevalence of WBD ranged from 0.39% (AGU, $N = 257$) to 10% (MDQ, $N = 10$). Only at DMS and PIR and in low percentages (3%, $N = 137$ and 5.5%, $N = 55$, respectively) there were colonies affected by the ciliate *Halofolliculina* sp. that causes skeletal eroding band disease and has recently been reported for the Caribbean (Cróquer et al. 2006). Predation was the most common cause of partial mortality (4–20%); however, it had a low impact on colonies, as only 2–10% of injured colonies had qualitatively more than 75% of their surface damaged (data not shown). The frequency of colonies with *C. abbreviata*,

Table 1 *Acropora palmata* absolute density of colonies (indv 10 m⁻²), prevalence of diseases (%), percentage of injured colonies, and density and frequency of the gastropod *Coralliophila abbreviata* for ten *A. palmata* population at Los Roques National Park. Site abbreviations as in Fig. 1

Reef sites	Total colonies	Density of colonies (indv/10 m ²)				Injured colonies (%)				Prevalence of diseases (%)		Injured + diseased (%)		<i>Coralliophila abbreviata</i>	
		Adult		Juvenile	Recruit	Total	Pomacentrids	Predation	Algae	Sediment	WBD	Ciliates	Density (snail/colony)	Frequency (%)	
		Adult	Juvenile	Recruit	Total	Pomacentrids	Predation	Algae	Sediment	WBD	Ciliates	Density (snail/colony)	Frequency (%)		
MED	3	0.04	0.01	0.03	0.04							0.25	25.00		
COT	4	0.04	0.01		0.05										
SEB	6	0.04	0.01	0.03	0.08										
MDQ	10	0.04	0.06	0.03	0.13	20.00			10.00			30.00			
PIR	55	0.24	0.36	0.09	0.69	7.27	1.82			1.82	5.45	23.63	1.81		
CRQ	64	0.26	0.41	0.16	0.80	4.68				4.69		18.74	7.81		
CAR	66	0.11	0.38	0.34	0.83	9.37				3.03		10.60	10.60		
GRQ	73	0.16	0.44	0.31	0.91	7.57	4.24		1.50	1.41		21.11	2.73		
DMS	137	0.53	0.8	0.38	1.71	10.17			0.73	1.40	3.00	16.76	0.72		
AGU	257	1.39	1.49	0.34	3.22	1.17	15.93			0.39		17.49	8.17		

WBD white band disease, Ciliates *Halofolliculina* sp. Empty spaces are zeros

Fig. 2 *Acropora palmata*. Size structure of each population ($N \geq 10$ colonies) at LRNP ($N = 675$). (a) DMS ($N = 137$), (b) AGU ($N = 257$), (c) CAR ($N = 66$), (d) CRQ ($N = 64$), (e) GR ($N = 73$), (f) MDQ ($N = 10$), and (g) PIR ($N = 55$). Size classes (1) 0–3 cm², (2) 3.1–50 cm², (3) 50.1–115 cm², (4) 115.1–225 cm², (5) 225.2–1,250 cm², (6) 1,250.1–4,550 cm², (7) 4,550.1–16,500 cm², and (8) >16,500 cm². Site abbreviations as in Fig. 1



a gastropod particularly prone to feeding on corals, which damages living tissues (Miller 2001; Miller et al. 2002; Baums et al. 2003), ranged from 0.72 to 10.6% among the six populations with a representative number of *A. palmata* (Table 1). In these populations, the density of this gastropod varied between 0.1 and 0.25 snails per colony (Table 1).

Electrophoretic analysis of seven polymorphic allozymes showed high levels of genetic diversity in all four populations of *A. palmata* analyzed at LRNP. The number of alleles per locus in all populations ranged from two to a maximum of four (Table 2), with a mean number of alleles per locus of 2.6–2.9, 2.1–2.5 effective alleles, and 0.5–0.6 for observed H_e (Table 2). In most cases (20 of 28), genotypic frequencies for each population and locus conformed to those expected from HW predictions, which is a good indication of panmixia within all populations. The only exception was the population at DMS, where five of the seven loci evaluated were not in HW equilibrium (Table 3). These deviations were mainly due to heterozygote deficits and may reflect ‘chaotic genetic patchiness,’ caused by poor mixing of larval pools (Selkoe et al. 2006).

Analysis of multilocus genotypes also indicated a high-genetic diversity with a total of 118 unique genotypes of 120 individuals, and a concomitantly $N_{go} : N$ ratio equal or close to 1 in all populations. The P_{ID} indicated a low probability (8.51×10^{-5}) of misidentifying colonies as clone mates. According to this result, sexual reproduction has been more important than asexual reproduction in all populations for a spatial scale of 5 m between samples. Again, only at DMS, two multilocus genotypes repeated twice each. This indicates a low level of asexual reproduction at DMS. The maximum expected number of individuals produced sexually belonging to the observed genotypes (N^*) was always equal to the number of individuals sampled (N), which also supported the predominance of sexual over asexual reproduction in the four *A. palmata* populations of LRNP analyzed with allozymes.

The hierarchical F_{ST} analysis of allele frequencies revealed a moderate to high level of connectivity between the south-western and north-eastern populations of the archipelago (nesting sites within sectors, $F_{ST} = -0.030$). Pairwise comparisons among populations also indicated high connectivity but with some restriction in gene flow,

Table 2 *Acropora palmata* allele frequencies for seven loci and genetic diversity estimators (mean) of four populations (sites) in Los Roques National Park

Locus	Allele	Northeast		Southwest	
		GR	PIR	DMS	AGU
<i>N</i>		30	30	30	30
GPI	1	0.25	0.05	0.27	0.17
	2	0.32	0.47	0.48	0.27
	3	0.43	0.48	0.25	0.56
MDH	1	0.05	0.15	0.20	0.23
	2	0.56	0.50	0.42	0.50
	3	0.37	0.35	0.33	0.27
	4	0.02		0.05	
GUDH	1	0.55	0.48	0.32	0.43
	2	0.45	0.47	0.52	0.52
	3		0.05	0.16	0.05
GDH	1	0.55	0.25	0.43	0.20
	2	0.37	0.63	0.37	0.62
	3	0.08	0.12	0.20	0.18
HK	1	0.35	0.22	0.48	0.44
	2	0.57	0.78	0.52	0.56
	3	0.08			
LGG	1	0.35	0.10	0.03	0.43
	2	0.57	0.53	0.73	0.57
	3	0.08	0.37	0.24	
VL	1	0.70	0.23	0.30	0.60
	2	0.30	0.65	0.55	0.30
	3		0.12	0.15	0.10
Heterozygosity		0.53	0.53	0.58	0.55
<i>N</i> alleles/locus		2.62	2.75	2.87	2.62
<i>N</i> effective alleles		2.16	2.13	2.45	2.21

N number of individuals

Table 3 *Acropora palmata* *D* values [(*H_o*–*H_e*)/*H_e*] indicating heterozygote deficit (negative number) or excess (positive number) for each locus and population

Populations	Loci						
	GPI	MDH	GLUDH	GDH	HK	LGG	VL
Gran Roque	0.36	0.15	–0.40	–0.17	0.01	–0.05	–0.22
Pirata	0.38	–0.46	–0.16	–0.37	0.06	0.15	–0.16
Dos Mosquises Sur	–0.35	–0.42	–0.40	–0.49	0.12	–0.52	0.29
Cayo de Agua	0.36	0.15	–0.22	–0.22	0.07	0.07	–0.39

Bold numbers indicate allelic frequencies deviating significantly from Hardy–Weinberg expectations based on Fisher's exact test after Bonferroni's correction

considering an F_{ST} of 0.048, which was significantly different from zero. Also, three of the four pairwise comparisons between distant populations showed significant genetic differences, GR–AGU being the exception. On the other

hand, the Bayesian approach did not identify distinct genetic populations among reef sites. According to Pritchard et al. (2007) this result is not surprising, as testing for frequency differences between predefined groups can be more powerful than applying *Structure*.

Discussion

In six of ten study sites (AGU, DMS, PIR, GR, CAR, and CRQ), small (0.1–50 cm²) to medium size colonies (50–4,550 cm²) predominated (42–74% of colonies) over large-sized colonies (4,550–16,500 cm²), population densities were above 1 colony per 10 m²; 75% of colonies were undamaged and both disease prevalence (<10%) and density of predators (0.25 snails colony^{–1}) were low. Overall, these results point toward a recovery of *A. palmata* in LRNP.

Although the presence of small colonies could be interpreted as a good sign of recovery, it is impossible to ensure that these populations will thrive unless they continue growing. Growing populations are expected to have large numbers of small colonies, but populations with high-mortality rates can also have large numbers of small colonies and low or even negative growth rates. Comparisons between the former size structure of *A. palmata* in Los Roques before the massive mortality event with current size structures would be required to actually provide irrefutable evidence for recovery; unfortunately, such data do not exist. Future efforts should focus on estimating the survivorship and growth rates of small colonies to determine the fate of these populations.

Throughout the wider Caribbean, the density of *A. palmata* has remained below 1 colony per 10 m² since their decline (Bruckner 2002; *Acropora* Biological Review Team 2005) and only a few sites have densities above that [(e.g., Colombia: 6 colonies per 10 m², Mexico: 7.6 colonies per 10 m² (Jordán-Dahlgren 1992), and Florida: 8–10 colonies per 10 m² (Bruckner 2002)]. In the present study, *A. palmata* was found at densities varying between 1.7 and 3.4 colonies per 10 m² in six of ten sites. Both the average live coral cover of *A. palmata* in LRNP (3.19%), and that at particular sites, such as AGU (10.5%) (Zubillaga et al. 2005), DMS (4.37%), PIR (2%), CAR (1.8%), and GR (0.3%) also support the proposition that this species has begun to recover. However, even at these sites, the cover of this species seems to be far from its former dominance on the reef crests of LRNP. Limited data of *A. palmata* densities at the study sites before its decline indicate that this species covered up to 100% of the reef crests at LRNP (Grajal 1981), as it did in many other Caribbean sites (Aronson and Precht 2001). Indeed, old-dead, bioeroded, and overgrown *A. palmata* frameworks constitute

the main component (>70%) of hard substrata at the study sites.

Whilst signs of recovery in *A. palmata* live cover have been observed in some Caribbean reef sites, in other areas such as Discovery Bay, Jamaica (Quinn and Kojis 2005), and the Florida Keys Marine National Sanctuary (FKMNS) population decline has continued to levels equal to or below 1% of cover since 1994 (Patterson and Ritchie 2004). The apparent success of recovery by *A. palmata* at LRNP might be the result of the lack of severe anthropogenic impacts (sedimentation, coastal development, sewage, etc.), hurricanes, storms, and emerging coral diseases (i.e., white pox and patchy necrosis), which have been recognized as major threats to the remaining populations in the Florida Keys and the US Virgin Islands (Patterson et al. 2002; Bythell et al. 2004; Patterson and Ritchie 2004; Rogers et al. 2005). Also noteworthy is the low density (0.25 snails per colony) of *C. abbreviata* that were found actively preying upon *A. palmata* in LRNP. Overall, *C. abbreviata* were present on 5.6% of the sampled colonies ($N = 675$), which is lower than other studies across the Caribbean (e.g., 18% for Puerto Rico, Bruckner et al. 1997; 10–20% for Florida, Baums et al. 2003; 6.1% for US Virgin Islands, Grober-Dunsmore et al. 2006). This gastropod has been considered a potential threat to *A. palmata* when there are more than 23 snails per colony (Miller et al. 2002), as it removes up to 6.5 cm² of coral tissue snail⁻¹ day⁻¹ (Bruckner et al. 1997) and it has a special preference for acroporids, agaricids, and the *Montastraea* species complex (Knowlton et al. 1990; Miller 2001). *C. abbreviata* is not only an important predator, but also a potential vector for diseases such as white pox and patchy necrosis in acroporids (Williams and Miller 2005). Thus, the high proportion (70–89%) of undamaged colonies in the study sites might be a consequence of low-anthropogenic impacts and/or low density of *C. abbreviata*.

Despite the good prognosis for recovery in six of the sites, four did not show signs of recovery. The recovery of impacted populations of corals is a complex process, as it depends on a variety of factors and how they interact in space and time. Adult stock size and fecundities, hydrodynamic features that influence the transport of larvae and patterns of early mortality may influence the abundance of recruits and therefore the process of recovery (Hughes et al. 2002). It is possible that sites with no recovery in Los Roques have a limited input of larvae either because of low fecundity that results from a small population of large-reproductive corals, or because of particular hydrodynamic processes that prevent accumulation of larvae or settlement at these sites. It is also likely that substratum differences among sites promote differential recruitment rates and/or that post-settlement mortality is different across sites. Several studies have shown high temporal and spatial variability in recruitment that result from the interaction of different

biotic and abiotic processes, which often produces patchy distribution of new settlers and patchy recovery of depleted populations (Vermeij 2006, Adjeroud et al. 2007).

The allozyme analysis showed that *A. palmata* in LRNP: (1) use sexual reproduction as the main strategy of colonization at the spatial scales analyzed; (2) have a relatively high-genetic diversity and (3) reef sites are highly interconnected. These three factors have important consequences and may play a central role in determining the successful recovery of this species in LRNP. Relatively high levels of connectivity (gene flow) and genetic diversity were considered a good prognosis for population recovery because: (1) the exchange of larvae creates and maintains high levels of genetic diversity, which are crucial in terms of resilience after disturbance; (2) migrants may carry new alleles that will be integrated into the population through reproduction, creating new gene combinations on which selection can potentially act; (3) the spread of selectively advantageous alleles may confer resistance to bleaching or other physiological stress; (4) high-gene flow increases local effective population sizes thereby enhancing the ability to resist rapid random changes in allele frequencies from one generation to the next through drift; and (5) high-gene flow indicates that reseeding of locally extinct or damaged populations from surrounding populations is possible (van Oppen and Gates 2006).

It has been stressed that *A. palmata* is recovering mainly by asexual reproduction (Highsmith 1982); due to the reduced genetic diversity resulting from this reproductive process, populations of this species might be highly susceptible to emergent epizootics that could prevent their final recovery (Bruckner 2002). Contrary to expectations, the allozyme analyses showed a predominance of sexual rather than asexual reproduction in the population of *A. palmata* at LRNP. These results support the argument of Szmant and Miller (2006) that the importance of sexual reproduction has been underestimated for *A. palmata*. While asexual reproduction might play an important role in the colonization of *A. palmata* at local or small spatial scales (Highsmith 1982), sexual reproduction could be more important in determining a regional-scale recovery (Bruckner 2002). Ecological studies have linked occasional hurricanes to high levels of asexual reproduction *A. palmata* (Highsmith et al. 1980; Fong and Lirman 1995). However, using a multiple linear regression, Baums et al. (2006) found that shelf area, rather than hurricane incidence, predicted better the genotypic richness of *A. palmata* populations and thus concluded that habitat effects are more important defining clonal structure in this species. Either way, Los Roques is located below the hurricane belt and its reefs and islands are the peaks of underwater mountains, with a very narrow shelf (Méndez 2002).

The results from Los Roques were consistent with the high genetic and genotypic diversities observed in the east-

ern Caribbean by Baums et al. (2005b, 2006). Baums et al. (2006) found that within provinces (west and east Caribbean) genotypic diversity was negatively correlated with colony density and inferred from this that denser populations may be the result of asexual recruitment. However, all sampling sites at Los Roques presented high-genotypic diversity, even the site with the highest density estimate, AGU with 0.32 colonies per m². This density estimate was equivalent to that of Horseshoe in Florida (0.25 colonies per m²) that had the lowest genotypic diversity ($G_o = 1.00$) in Baums et al. (2006). Thus, dense populations are not always the result of asexual recruitment in *A. palmata*. Also, the genotypic diversity examined within the relatively small area of Los Roques was homogeneously high compared to the markedly different patterns of genotypic diversity observed by Baums et al. (2006) over small spatial scales. It is unlikely that these differences are due to differences in sampling design, but rather to a truly high level of sexual recruitment in Los Roques. In both studies the size of the sampled areas was very similar (800 m² this study vs. ~707 m² in Baums et al. 2006). Although colonies were sampled at least 5 m apart in this study, a couple of repeated genotypes were detected; and Baums et al. (2006) found putative clones more than 30 m apart. When coral populations rely heavily on asexual reproduction, sampling colonies a few meters apart still detects a high number of repeated multilocus genotypes (e.g., Bastidas et al. 2002).

The moderate to high level of connectivity found among sub-populations (sampling sites) of *A. palmata* in Los Roques can be attributed to the mode of reproduction of this species (gamete broadcasting), the relatively small area of this archipelago (2,210 km²), and the oceanographic conditions that may allow the interconnectedness of its superficial waters and eastern and southern barriers that may help to retain larvae. Connectivity between Los Roques and other localities in the Caribbean would be expected to be high, given the results of Baums et al. (2005a) indicating high connectivity among *A. palmata* populations in the eastern Caribbean. Moreover, based on the surface current patterns in the Caribbean, Los Roques may constitute an 'upstream' reef area that supplies coral larvae to reefs 'downstream' (Roberts 1997). However, it is necessary to actually evaluate this level of connectivity, because patterns of connectivity do not necessarily follow predictions based on mode of reproduction and surface current patterns. For instance, Severance and Karl (2006) found that populations of the Caribbean broadcast spawner *Montastraea annularis* were genetically isolated whereas those of its sister species *Montastraea faveolata* were highly interconnected, in spite of nearly identical larval dispersal capability. Acroporids in the Indo-Pacific also show the whole spectrum of connectivity, from very little (*Acropora tenuis*) (Márquez et al. 2002), moderate to low (Benzie et al. 1995; Whitaker

2004), moderate to high (Ayre and Hughes 2004; Smith-Keune and van Oppen 2006), and very high connectivity (*Acropora hyacinthus* and *Acropora cytherea*) (Márquez et al. 2002).

In summary, ecological and molecular results from this study highlight the dominance of small colony sizes at six of ten sites and the predominantly sexual origin of the colonies of *A. palmata* in Los Roques. Although size structure alone cannot establish whether or not these populations are growing; and connectivity with other sites in the Caribbean remains to be tested, the central argument is that clonal propagation is playing a relatively small role in the dynamics of these populations. This supports the contention that *A. palmata* is recovering at LRNP, and suggests a good prognosis for regaining its former status as a dominant species in the reef crests in at least six reef sites at the scale surveyed (i.e., 800 m² per site). However, management efforts must be sustained because emergent pathogens, human impacts, epizootics of pathogens and/or natural predators may still delay or prevent the final recovery of this species in Los Roques and the Caribbean.-

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