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Scleractinian coral recruitment to reefs physically damaged by ship groundings

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Abstract. The southeast Florida reef system faces a number of stress factors, among which ship groundings are one of the most physically damaging. Portions of the Florida reef tract located near Port Everglades, Broward County, Florida, USA have been damaged by ship groundings. In 2004, physical damage of more than 30,000 m^2 was caused by the groundings of two large cargo ships, the MV *Eastwind* and MV *Federal Pescadores.* The present study was designed to measure differences of scleractinian coral recruitment patterns (recruit diversity and richness) and rates to these injured sites in comparison to undamaged reef sites. Coral recruitment rates were measured on unglazed ceramic tiles deployed for a period of one year from February 2007 to February 2008 at five different locations: three control sites (including a high coral cover site), and the two ship grounding sites. Morphology and genetic markers including CO1 and cytb were used to identify the coral recruits. A whole genome amplification kit (REPLI-g, Qiagen) was used to obtain sufficient amounts of DNA. Results revealed low recruitment rates (0.5-2.7 recruits m^{-2} yr^{-1}) to the studied sites, suggesting a low potential for recovery of the damaged areas.

Key words: reef groundings, coral recruitment, genetic markers

Introduction

Coral reefs around the world have been affected by anthropogenic changes in the environment, resulting in a general pattern of decline worldwide. A substantial number of these stressors also affect the Florida coral reef system, and ship groundings have been found to be particularly destructive (Jaap 2000; Collier et al. 2008). Reef areas located near Port Everglades, Broward County, FL have been severely impacted due to the location of a portion of the port anchorage area between two major reef tracts: the middle and outer reefs. Navigational error and inclement weather have resulted in a number of ship groundings and anchor damage events. From 1993 to 2006, there were 11 groundings and 6 anchor drag cases, resulting in more than 40,000 m^2 of coral reef injury in the vicinity of Port Everglades (Collier et al 2007, Banks et al. 2008). In 2004 the most severe impacts were groundings of two cargo ships: the MV *Eastwind* in March and the MV *Federal Pescadores* in October, which destroyed more than 30,000 m^2 of reef (Melendez 2004; Flesher 2004).

Coral recruitment is a vital step in the natural recovery process of injured reefs. Thus, understanding the process of coral recruitment to these areas is essential in evaluating the ability of reefs to recover (Smith 1992; Tougas and Porter 2002; Glassom et al. 2004). At 26°N latitude, Broward County is near the northern range limit for many scleractinian coral species, which may lead to an assumption of low recruitment rates and thus slow recovery. The present study was designed to estimate scleractinian coral recruitment rates and patterns (species diversity and richness) to damaged reef sites three years after the initial impacts of the two cargo ships MV *Eastwind* and MV *Federal Pescadores*. It also aimed to determine differences in coral recruitment patterns and/or rates between undamaged parts of the reef and sites which were destroyed by ship groundings. This study has provided the first estimation of scleractinian coral recruitment rates on reef sites off the coast of Broward County, FL.

One of the challenges of coral recruitment studies is properly identifying coral recruits. Previous researchers have typically been able to morphologically categorize recruits only to the family level because of the small size (< 1 to several mm) of many recruits (Baggett and Bright 1985; Harriott 1992; Tougas and Porter 2002). However, genetic markers have been used to overcome the difficulty in morphological identification of early life stages (gametes, larvae, juveniles) of a number of marine

organisms (Neigel et al. 2007). The cytochrome *c* oxidase subunit 1 (CO1) mitochondrial gene has been used to identify coral recruits settled on ceramic tiles (Shearer and Coffroth 2006) and revealed a great advantage in the ability to identify recruits as small as 1 to 2 mm in diameter. In this study, genetic markers including CO1 and cytochrome b (cytb) genes were used to supplement the morphological identification of coral recruits to a lower taxonomic level.

Material and Methods

Coral recruitment rates were measured on unglazed ceramic tiles deployed off the coast of Broward County for a period of one year from February 2007 to February 2008. A total of 480 ceramic plates (8 per array, Fig. 1) were deployed, resulting in 22.3 m^2 of settling area under study.

Figure 1. Settlement plate array design. Twelve arrays deployed to each of 5 sites for a period of one year.

The settlement plate arrays were deployed at five sites (12 arrays per site): three control sites (including one high coral cover site) and two ship grounding sites (*Eastwind* and *Federal Pescadores*) (Table 1). After 12 months on the reef, the plates were collected, transported in coolers and frozen (-20° C) until microscopic and genetic examination. The tiles were examined for coral recruits under a dissecting microscope at 12X magnification, and the recruits found were identified by morphological characteristics to family or genus level according to the identification keys in Budd et al. (2006) and Smith (1948).

Each scleractinian spat was then scraped with a razor blade and preserved in a saturated sodium chloride 2.5M EDTA, 20% dimethyl sulfoxide (DMSO) buffer for genetic analysis. DNA extraction was completed with a DNeasy isolation kit (Qiagen). For samples with low concentration of extracted DNA, a whole genomic DNA was amplified with REPLI-g kit (Qiagen). The CO1 was amplified by the polymerase chain reaction (PCR) using coral–specific primers and cycling conditions suggested by Fukami et al. (2004).

The cytb and ITS genes were amplified using newly designed primers; CYTBF: 5'-GGGTGTTT TTTGTCBATGCATTAT-3', CYTBR: 5'CCCAATT TATTTGGTATCGAACGCA-3', ITSF:5'GGGGAC AGAGMGTCGGAT-3', ITSR:5'-TCCGGGKAGAA AGTGCTTCT-3'.

Table 1. Location of five reef sites in Broward County, FL; CS1 = control site one, CS2 = control site two, HC1 = high coral cover site, EW = *Eastwind* and FP = *Federal Pescadores*

SITE	LATITUDE	LONGITUDE	DEPTH (m)	REEF TYPE
CS1	26°09.625'N	80°05.306'W	6.5-7.5	INNER
CS2	26°10.073'N	80°05.265'W	6.5-7.5	INNER
HC1	26°08.857'N	80°05.763'W	6.5-7.5	RIDGE COMPLEX
EW	26°07.042'N	80°05.549'W	9.0-10.0	INNER
FP	26°06.747'N	80°05.504'W	11.5-12.5	INNER

The PCR protocol for the above primers was 35 cycles at 94°C for 45s, 50°C for 45s, and 72°C for 90s. Following the work of Shearer and Coffroth (2004), the restriction fragment length polymorphism (RFLP) patterns of the CO1 gene were used for partial identification of the recruits. First, virtual RFLP patterns for major scleractinian coral species were created with a Web-based nucleic acid analysis tool (http://workbench.sdsc.edu/) using CO1 sequences available in GenBank. The CO1 RFLP patterns of recruits were created by Taq1 restriction digestion of the amplified gene and then compared to the virtual patterns of known species. Because of the low interspecific variation in CO1 gene sequences among scleractinian corals (especially in the family Faviidae), the CO1 RFLP was useful to differentiate only two of the coral species from the genus *Porites*. CO1 and cytb genes acquired from coral recruits that were not distinguished by RFLP patterns were sequenced and blasted to find the best possible match in GenBank. In case of low concentration or low purity of the PCR results, products were cloned with TOPO TA Cloning kit (Invitrogen). The final identification was made based on the combined morphological analysis RFLP patterns and BLASTN queries (www.ncbi.nlm.nih.gov/BLASTN).

Results

A total of 33 coral spat were found on 478 tiles (two tiles were lost). The overall recruitment rate was estimated to be 1.5 recruits m^{-2} yr^{-1}, ranging from 0.5 recruits m^{-2} yr^{-1} at the *Federal Pescadores* ship grounding site to 2.7 recruits m^{-2} yr^{-1} at both the control site 1 and high coral cover site (Fig. 2). A one-way analysis of variance showed no significant difference among recruitment rates from the five studied sites, likely due to a small sample size. The lowest number of recruits settled on tiles located at the *Federal Pescadores* ship grounding site (2 recruits) and the highest number of recruits were found on tiles from control site 1 (11 recruits) and the high coral cover site (10 recruits) (Table 2).

The identification process of scleractinian coral recruits using genetic markers was successful for all but one sample. The small size of the recruits, which in several cases were less than 1 mm in diameter, resulted in extraction of very small amounts of DNA. Another challenge was related to recruit DNA contamination with zooxanthellae DNA. The easiest markers to be obtained from this poor quality DNA were CO1 and cytb mitochondrial genes.

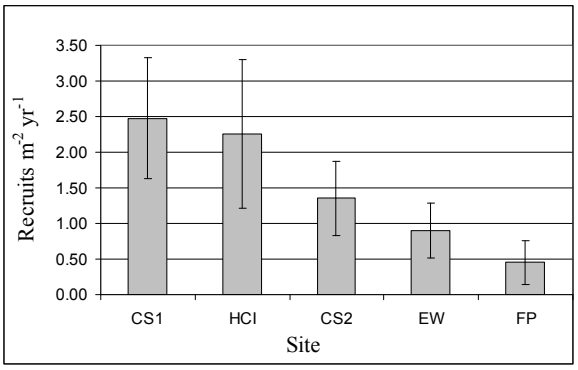

Figure 2. Scleractinian coral recruitment rates found on settlement tiles located on five reef sites in Broward County, FL. Mean values are calculated per array (n=12) with error bars indicating standard errors. CS1 = control site one, CS2 = control site two, HC1 = high coral cover site, EW = *Eastwind* and FP = *Federal Pescadores*.

Amplification of nuclear genes such as ITS turned out to be more challenging and was only successful for a few of the largest recruits. One method of improving results was to amplify the whole genome using a REPLI-g kit from Qiagen which allowed an increase in the concentration of DNA template (Table 3). This technique showed potential to be a useful tool when very small samples such as gametes, larvae and other early life stages are involved, since target genes could still be amplified.

Table 2. Comparison of scleractinian coral recruitment rates and patterns among five reef sites. CS1 = control site 1, CS2 = control site 2, HC1 = high coral cover site, EW = Eastwind grounding site, and FP = Federal Pescadores grounding site.

SITE	NUMBER OF RECRUITS	NUMBER OF GENERA	NUMBER OF Porites spp.	SHANNON WEINER DIVERSITY INDEX	TOTAL RECRUITS m^{-2} yr^{-1}
CS1	11	3	10	0.92	2.96
HCI	10	3	7	1.10	2.69
CS2	6	4	4	1.36	1.61
EW	4	1	4	0.00	1.08
FP	2	1	2	0.00	0.54

The restriction digestion performed on the CO1 gene (total 742 bp) with Taq1 restriction enzyme resulted in 4 different RFLP patterns (Fig, 3). Patterns designated with letters B (2 fragments: 446, 296 bp) and C (3 fragments: 330, 298, 116 bp) were species-specific for *Porites astreoides* and *Porites porites*, respectively. Patterns A (2 fragments: 613, 129 bp) and D (3 fragments: 374, 296, 72 bp) were characteristic of multiple species, so recruits with these two patterns were chosen for better resolution via CO1 and cytb sequencing analysis.

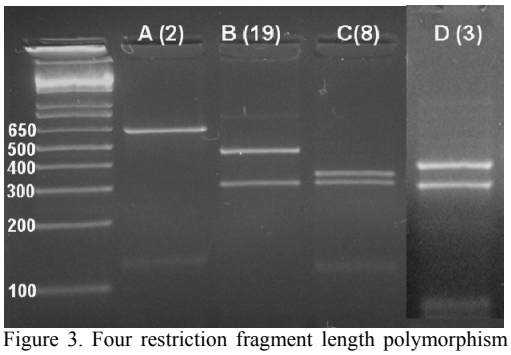

Figure 3. Four restriction fragment length polymorphism patterns named A, B, C, and D created from the CO1 gene amplified from scleractinian coral recruit DNA samples and digested with Taq1 restriction enzyme. The number in parenthesis indicates the number of times the pattern was found.

Table 3. DNA concentrations extracted from coral recruits, and DNA concentrations of the same samples after whole genome amplification determined by Nanodrop ™

SAMPLE	DNA (ng*µL^{-1})	DNA AFTER REPLI-g (ng*µL^{-1})
CS154	0.42	341.85
HCI52	1.36	321.90
CS144	1.69	218.00
CS212	3.59	250.94
CS146	3.86	333.86
CS261	6.50	246.38
HC126	10.17	270.72

There were a total of four coral recruit genera found on the tiles. The most dominant coral species recruiting to all five sites were *Porites astreoides* and *Porites porites* comprising 82% of all the recruits. Twelve percent of the coral recruits consisted of species from the genus *Agaricia,* and the remaining 6% (two recruits) were *Montastraea cavernosa* and *Colpophyllia natans*. The last two mentioned were the only two recruits of reef framework-building corals. These recruits were found on tiles from the high coral cover site which had the highest diversity (all four genera represented) of all the studied sites (Table 2).

Discussion

Overall coral recruitment rates and patterns

The present study on reef sites off Broward County, FL demonstrated extremely low recruitment rates, between 0.5 to 2.7 recruits m^{-2}. The low recruitment rates to these reefs were not surprising due to their location on the northern extent of the Florida reef tract which is characterized by relatively low coral cover.

In Florida and the Caribbean coral recruitment rates have been declining most likely as a consequence of the overall decline of coral population densities (Porter et al. 1988; Jaap et al. 2000; Santavy et al. 2005, Hughes and Tanner, 2000). Through meta-analysis, Gardner et al. (2003) have estimated an 80% reduction in coral cover in three decades between 1977 and the present for the entire Caribbean basin. Extensive coral cover losses certainly have led to a decrease in coral breeding stocks, thus resulting in reduced larval supply and low recruitment rates.

The most dominant coral species recruiting to the studied reef sites were *Porites astreoides* and *Porites porites* which constituted 82% of all the recruits. The next dominant species were from the genus *Agaricia* (12%). The trend of dominant recruitment of agariciid and poritiid corals has been documented in several other studies of coral recruitment on Florida and Caribbean reefs (Bak and Engel 1979; Rogers et al. 1984; Smith 1997; Hughes and Tanner 2000; Tougas and Porter 2002; Shearer and Coffroth 2006). The recruitment patterns found in previous studies have been explained by either: a) the existence of two different life histories exhibited by scleractinian corals (broadcasting vs. brooding) (Bak and Engel 1979) or b) the Allee Effect of low population densities (Knowlton 2001).

The first theory states that coral species which are massive, long-lived, reef frame-builders and usually broadcast spawn their gametes into the water have low recruitment rates and are represented by a low number of juvenile colonies. The low recruitment rate, however, is then compensated by lower post-settlement mortality and good competing and surviving abilities (Bak and Engel 1979; Smith 1992 1997; Hughes and Tanner 2000). In contrast, small-sized, short-lived species that brood their larvae exhibit higher recruitment rates but also suffer from higher post-settlement mortality rates. The adult community structure with the dominance of massive broadcast spawning species like *Montastraea* spp. and *Diploria* spp. over small, brooding species such as *Porites* spp, *Agaricia* spp. and *Favia* spp. is thus the result of the two opposite life strategies.

The Allee Effect mostly affects broadcasting species which generally do not self-fertilize, so high gamete densities are necessary for successful fertilization and reproduction (Knowlton 2001). Low population densities of these species lead to asynchronous spawning events (limited hormonal regulation), low gamete densities and consequently to the increase in reproductive failure. Brooding species can self-fertilize, and their larvae can settle within hours to days of release. Therefore, they are more likely to persist through periods of low densities. The Allee Effect creates a major shift in the structure of coral adult communities from those dominated by framework builders toward those dominated by non-framework opportunists such as *Porites*. Both the differing life history strategies and the Allee Effect could be the cause of *Porites* dominated recruitment patterns in Broward County, FL.

Recovery process in terms of recruitment rates on ship grounding sites

Ship groundings on coral reefs cause not only serious damage to the reef builders, but also result in a loss of habitat for other animals, consequently leading to barren areas. The problem, however, may be further intensified by other stress factors (climate change, disease, pollution, eutrophication and sedimentation) which decrease coral population size, impact the size of coral breeding stocks, larval supply and recruitment rates, and increase post-settlement mortality rates, consequently resulting in unlikely recovery of the damaged sites.

The present coral recruitment data showed that a small number of coral recruits settle on tiles located on the studied ship grounding sites suggesting a very slow recovery process and unlikely recovery success. Additional studies of coral recruitment involving artificial and natural substrates are required to confirm the low recovery potential of these and other physically damaged sites in Broward County, FL.

Genetic identification

This study demonstrated that genetic markers help overcome the difficulty in morphological identification of early life stages such as small juvenile coral recruits. We confirmed the usefulness of the CO1 gene in partial species or family level identification of scleractinian coral recruits as it was developed by Shearer and Coffroth (2006). RFLP analyses of the CO1 gene allowed differentiation between at least two species in the coral genus *Porites*. The low nucleotide polymorphism in CO1 was, however, insufficient to distinguish some closely related coral species (Shearer and Coffroth 2007; Neigel 2007), but a better genetic marker has not yet been developed for this purpose.

This study tested other genetic markers including cytochrome b and internal transcribed spacer region (ITS) for species identification of corals in the early developmental stage. Coral-specific primers were designed and tested in order to avoid amplification of zooxanthellae genes. Nuclear markers were difficult to amplify from the small amounts of DNA. Similar to the CO1 gene, the cytb gene showed limited genetic variation, which limits the number of species specific RFLP patterns. However, the combination of obtaining both gene sequences allowed better species

identification, which could be improved with more markers.

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