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Monitoring of Coral Larval Recruitment on Artificial Settlement Plates at Three Different Depths Using Genetic Identification of Recruits (Guadeloupe Island)

Suivi du Recrutement Larvaire des Coraux sur des Substrats Artificiels Disposés à Trois Différentes Profondeurs avec Identification Génétique des Recrues (Guadeloupe)

Seguimiento del Reclutamiento de Larvas de Coral en Sustrato Artificial Instalado a Tres Diferentes Profundidades, Mediante la Identificación Genética de las Larvas (Isla de Guadeloupe)

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ABSTRACT

Coral mortality has been increasing over the last 30 years in the Caribbean, making coral recruitment critical for sustaining coral reef ecosystems and contributing to their resilience. Dynamics of coral communities is partly controlled by larval recruitment. However, the accurate identification of coral recruits remains difficult (size < 2 mm). Genetic markers permit to distinguish coral species commonly observed in Caribbean studies. The objective of the study was to examine the temporal variability in the recruitment of corals larvae from 2010 to 2012 in Guadeloupe Island. For that, twenty terracotta settlement plates were fixed on a grid and immersed every month at 5 m, 10 m and 20 m depth. COI and ITS region as a genetic marker were used for the identification of coral recruits. Larvae settlement was observed all year round and presented an important peak between March and June. Temporal variability in the density of coral recruits occurred at all depths. Among the 276 recruits collected, genetic identification revealed seven different species. The highest settlement was shown by *Agaricia* sp. and *Porites astreoides* at all depths followed by *Favia fragum*, *Madracis* sp., and other *Porites* sp. Settlement appeared to be inversely related to depth. The taxonomic composition of the recruits reflects the structure of juvenile but not adult coral communities. To conclude, the initial phase of the life cycle plays an important role in the establishment and survival of Scleractinian corals. The understanding of the mechanisms of post-settlement mortality of recruits could improve the knowledge necessary to maintain coral populations.

KEY WORDS: Coral larvae recruitment, Caribbean reefs, terracotta plates

INTRODUCTION

Coral reefs are among the most diverse and complex ecosystems in the world in terms of biodiversity and productivity but also the most fragile. The vulnerability of coral reef ecosystems to environmental changes have accelerated their decline for several decades (Gardner et al. 2003, Hoegh-Guldberg et al. 2007, Pandolfi et al. 2003). In the Caribbean, coral mortality has been increasing over the last 30 years making coral recruitment more significant than ever for sustaining coral reef ecosystems and contributing to their resilience. Recruitment pattern is an important factor for the future of coral communities. Recruitment begins with the first attachment of coral *planula* larvae (larval settlement) to reef substrate and is successful when the environment is favorable for its survival.

Caribbean studies on coral recruitment are restricted to observations of young coral colonies at a visible size of 2 - 5 cm and the recruitment of juveniles (Harriott 1985, Vermeij and Sandin 2008), which are generally at least one year post-settlement. Many previous studies have concerned larval recruitment (Babcock and Mundy 1996, Banks and Harriott 1996, Gleason 1996, Harriott 1985, Hughes et al. 2000, Kenyon 2008, Kojis and Quinn 2001, Quinn and Kojis 2005, Rubin et al. 2008, Wallace and Bull 1982). An important question concerns the relative importance of larval recruitment versus post-recruitment events. Episodes of mortality may occur between the first stage of settlement (invisible to the naked eye) and the recruitment of juveniles (visible). Thus, the first stage of development represents an important issue to study.

Two important processes influence the abundance and distribution of corals — the patterns of larval settlement and the subsequent post-settlement mortality. Also, artificial settlement substrate has been used to study larval settlement on the reefs. To avoid confusion between larval settlement on plates and natural substrata recruits, the term "larvae" will be used to discuss only settlement onto plates (size < 1 cm).

Artificial settlement plates have been widely used (Babcock and Mundy 1996, Banks and Harriott 1996, Gleason 1996, Harriott 1985 ; Hughes et al. 2000, Kenyon 2008, Okamoto et al. 2008, Quinn and Kojis 2005, Rubin et al. 2008, Wallace and Bull 1981). Most studies encompassed a subset of variables among the material of the settlement support, the size, the shape, the texture, the orientation in the field, the time of preconditioning, the type of fixation, the duration and the depth of the study, and the type of site. There is little information on the variation of recruitment taking into account all these parameters and especially in the Caribbean (Kojis and Quinn 2001, Rogers et al. 1984, Smith 1992, Tomascik 1991, Vermeij 2006).

The accurate identification of the early stages of recruits following settlement remains difficult (size < 2 mm). At small size, coral recruits can be at least identified to the family level or the genus level using morphological criteria of the skeletons after bleaching (Babcock et al. 2003, Baird and Babcock 2000, Harriott 1999, Kojis and Quinn 2001). The destruction of tissues does not permit genetic analyses of these samples. Genetic analyses can provide pertinent information on parentage of new recruits, larval sources and genetic connections among reefs, ultimately achieving a fundamental understanding of recruitment process (Shearer and Coffroth 2006). Then, the preservation of the tissue of coral recruits for genetic analyses appears judicious.

Molecular biology techniques have been used to identify larvae of numerous organisms (Andre et al. 1999, Evans et al. 1998). In the Caribbean, only Shearer and Coffroth (2006) analyzed recruits of scleractinian corals using a PCR-restriction fragment length polymorphism (RFLP) method for species identification. To identify species of coral recruits, we used a combination of the mitochondrial marker Cytochrome c Oxidase subunit I (COI) and the nuclear ribosomal Internal Transcribed Spacer 1 and 2 fragments sequenced and a comparison with Genbank sequences database.

To understand the dynamics of coral communities on reefs in Guadeloupe Island, we studied three main stages of development of corals: larvae, juveniles and adults. The present study is focused on the results of larval recruitment. The first objective was to select a suitable support to study larval recruitment. The second was to examine the temporal variability in the recruitment of coral larvae from 2010 to 2012. Then, we investigated distribution of larval recruitment along a depth gradient. A genetic method was also used to identify newly settled *planulae*, which allowed us to compare the temporal variation in species composition and their distribution according to depth.

MATERIAL AND METHODS

Study sites

Guadeloupe Island is located Lesser Antilles by 16° 30'N and 61°30'W. Two islands (Grande-Terre and Basse-Terre) separated by a narrow channel called "Rivière Salée" compose Guadeloupe. In the north, this channel is connected to the Bay of the Grand Cul-de-Sac Marin, occupied by one of the most important barrier reefs of the Lesser Antilles. This barrier is 30 km long and encloses a lagoon of about 11,000 ha. (Figure 1). The leeward coast of Basse-Terre presents the largest marine biodiversity of the island whereas coral communities only form veneer reefs. Pigeon Islets (61°47'290W, 16°10'000N) represent a hotspot of biodiversity and are located in a marine protected area. Coral communities have been studied for 23 years on this site (Bouchon et al. 2008). This site has been retained to test natural and artificial substrata for larval settlement during two months.

Subsequently, a monthly monitoring of larval settlement was conducted in the channel of the "Passe-à-Colas" (61°34'238W, 16°21'315N) in the Bay of the Grand Cul-de-Sac Marin. Recruitment plates were set up on the west side of the pass at 5 m, 10 m, and 20 m deep. Studies on coral communities were already available for that area (Bouchon and Laborel 1990, Bouchon et al. 2008).

Coral Settlement on Different Material

A set of 288 plates (200 mm x 200 mm) composed by unglazed terracotta, dead coral skeletons of *Acropora palmata* (Lamarck, 1816), glass, and Plexiglas were fixed to a support grid horizontally set up at 15 m deep and raised 15 cm above the bottom. Settlement plates were collected after two months, observed under dissecting microscope, and settled coral larvae were counted. Data concerning the number of larvae on plates were compared with a Kruskal-Wallis test.

Figure 1. Location of Guadeloupe island in the Caribbean area and the study reefs site: Pigeon Islets and the Bay of the bay of the Grand Cul-de-Sac Marin.

Temporal Larval Settlement Patterns

Following the results of the previous experiments, unglazed terracotta was chosen as the most suitable material for coral larval settlement. The plates used in this experiment were 200 mm x 200 mm x 150 mm. Numerous pits and grooves cover one side (up to 1 mm deep and 1 mm wide) providing a rough texture, the other side being smooth These rough and smooth side were alternatively oriented up and down. Twenty plates were fixed on a support grid (1.5 m x 1 m) isolated from the bottom by 4 cm thick PVC spacers, in order to allow larvae to move freely underneath the plate. Grids were immersed every month on the reef channel side, (from July 2010 to March 2012 and set up horizontally 15 cm above the bottom (Figure 2). One grid was placed at three different depths: 5m, 10 m and 20 m. Each grid remained two months in place. When collected, all settlement plates were kept in seawater and transported to the laboratory. The plates were examined with a UV Torch (FL-1-NightSea™) to detect coral recruits by their fluorescence (light excites fluorescence of green fluorescent protein in the tissue of recruits) (Schmidt-Roach et al. 2008). The recruits were then examined with a dissecting microscope. Live settled larvae were also photographed. Data were standardized to represent the number of larvae per square meter of colonized substrate during two months.

Figure 2. A coral settlement grid settled on the reef at Passe-à-Colas.

Genetic Identification Analysis

Every larval recruit were removed from the settlement plates using a hypodermic needle and preserved separately in 95-100% ethanol. To proceed to the DNA extraction, samples were left to dry at ambient temperature during 24 hours for the complete evaporation of alcohol. DNA of the entire body of the recruits (276 specimens in total) was then extracted according to the protocol of Gentra Puregene®. PCR amplification were performed in 25 µl volume solutions with 1 µl ADN, 1 U *Taq* polymerase and a final concentration of 0,2 mM of each dNTP, 10 mM Tris-HC, 50 m KCl, 2,5 MgCl2 and 0,2 µM of each primer. For the COI region, the modified primers used LCOI1490-HCOI2198 and the thermal cycling protocol, were described by Shearer and Coffroth (2008). For the ITS region, the primers used were ITS1-ITS4 (white et al. 1990), ITSZ1-ITSZ2 (Forsman et al. 2008) and ITS1S-ITS2S (Chen et al. 2004). PCR thermal cycling was 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 50°C for 90 s and 72°C for 90 s, ending with a final phase of 72°C for 10 min. The PCR products were sequenced with both PCR primers.

RESULTS

The number of coral larvae recruiting onto the plates differed significantly between the types of substrate (Kruskal, $p < 0{,}05$). Settlement was higher on unglazed terracotta followed by glass, coral skeleton, and plexiglass (Figure 3). Following this result, unglazed terracotta plates were used for the study of temporal variability of larval settlement.

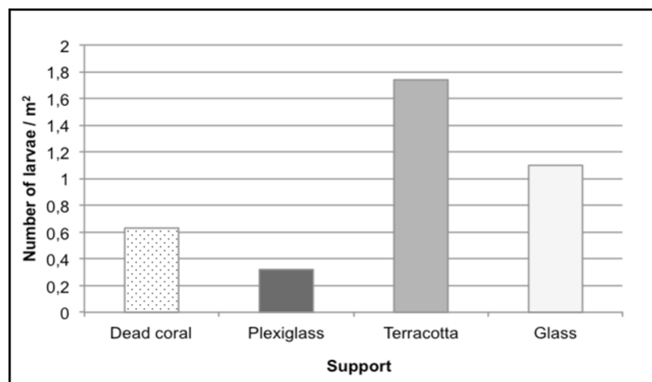

Figure 3. Number of larvae per square meter for plates of several materials: dead coral, plexiglass, unglazed terracotta, and glass.

The number of coral larvae recruiting onto the plates differed significantly between the types of substrate (Kruskal, $p < 0{,}05$). Settlement was higher on unglazed terracotta followed by glass, coral skeleton and plexiglass (Figure 3). Following this result, unglazed terracotta plates were used for the study of temporal variability of larval settlement.

Over a 20 months period, 276 recruits of coral larvae have settled. Coral settlement was observed all the year round and presented peaks of recruitment in March, April, June and September (Figure 4). Main fluctuations are of seasonal order. The annual recruitment varied from a minimum of 11 larvae/m^2 to a maximum of 21 larvae/m^2. Larvae recruitment decreased with depth between 5 m and 20 m (Figure 5). There were five times more larvae settled at 5 m (155 larvae) than at 20 m (24 larvae). On the channel side, depth can be considered as a proxy for the decrease of light, and hydrodynamic factors and the augmentation of sedimentation rate. These ecological factors are likely to play an important role on coral recruitment and distribution.

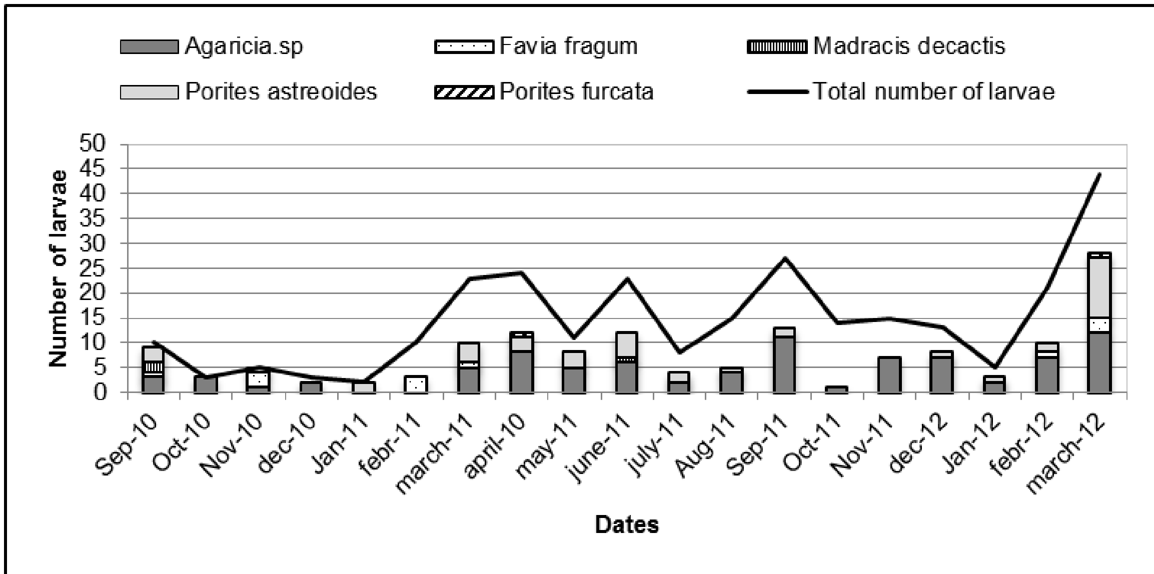

Figure 4. Temporal distribution of the total number of coral larvae and the proportion of identified larvae on plate over the 20-months study period.

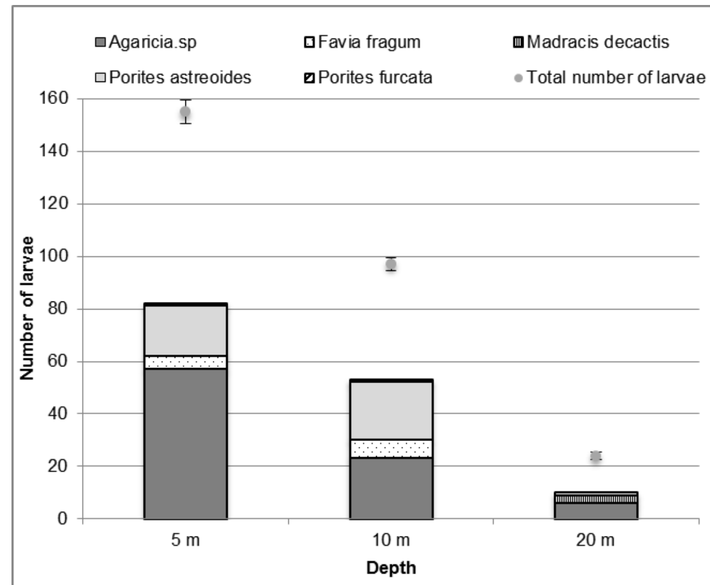

Figure 5. Distribution of the total number of larvae and identified larvae on settlement plates according to depths.

Table 1. Numbers and relative abundance of observed coral larvae.

	Species	Number of larvae	Percentage of larvae
Identified	*Agaricia spp.*	86	31
	Favia fragum	12	4
	Madracis decactis	3	2
	Porites astreoides	42	15
	Porites furcata	2	1
Non-identified		131	47
Total		276	100

The genetic identification of coral larvae helped to identify unambiguously 145 recruits (53%) but unfortunately 130 recruits (47%) could not be identified. Among the larvae identified, 31% belonged to the genus *Agaricia*, 15% to the species *Porites astreoides* Lamarck 1816, 4% to the species *Favia fragum* (Esper, 1795), 2% to the species *Madracis decactis* (Lyman, 1859) and 1% to the species *Porites furcata* Lamarck 1816 (Table 1). The number of *Agaricia* spp. and *Porites astreoides* the most abundant larvae settled, decreased with depth (Figure 5). *Madracis decactis* known to be lucifugous recruited only at 20 m. *Porites furcata* and *Favia fragum* recruited between 5 and

10 m. From September 2010 to March 2012, the number of recruits per month vary according to species (Figure 4). Only *Agaricia* spp. and *Porites astreoides* recruit all the year round.

DISCUSSION

In Guadeloupe Island, the larval recruitment varied according to the type of settlement plate used. The type of substrate used must be taken into account when designing coral recruitment experiments and when comparing the results of studies using different types of settlement plates. Each surface of settlement plates present advantages and disadvantages. Plexiglass plates had the lowest recruitment rate. As noticed by Wallace and Bull (1981), it appeared that the plexiglass plates did not provide a good surface on which larvae could cement themselves. Terracotta plates attracted the largest number of larvae. These plates require little preparation and provide a surface partly protected from the effect of predation with their rough texture. Moreover, the color of these plates makes the search of larvae easier. Plates of glass attracted more recruits than plates of plexiglass and plates of dead coral. As plexiglass, the smooth face of glass plates prevents the larvae from cementing.

In the present study, few recruits where found on dead coral plates contrarily to other studies (Birrell et al. 2005, Carleton and Sammarco 1987, Harriott 1985). Surprisingly, this type of settlement plate could better reflect the field situation and the habitat usually available to new recruits. One of the disadvantages of the coral plates was that they are laborious to produce for the fabrication of standardized units in term of size and thickness. Another disadvantage is that the color and the presence of old corallites on dead coral plates do not permit the accurate observation of larvae. Finally, the best surface for recruitment studies in Guadeloupe was found to be unglazed terracotta plates because of their convenience, the facility of larvae observation, and their success in attracting larvae.

The annual recruitment rate is lower in Guadeloupe Island (11 to 21 recruits/m^2/year) compared to those observed in other Caribbean reefs. Rogers et al. (1984) and Kojis and Quinn (2001) in the U.S Virgin Islands recorded respectively an average of 33 and 89 to 180 recruits/m^2/year[1]. In Barbados, Tomascik (1991) observed an average of 265 recruits/m^2/year. Anyway, these data are relatively old, and it can be envisaged that coral recruitment may have declined since that time.

As in the present study, these authors found the same dominant species among the recruits, that is *Agaricia* spp., *Porites* spp., *Madracis decactis* and *Favia fragum*. Rylaarsdalm (1983) also observed the dominance of these species of larval recruits in Jamaican reefs. Thus, *Agaricia* spp. and *Porites* spp. appear to be important species contributing to the recruitment rate in Caribbean coral reefs (L.U., Unpublished data, Roger et al. 1984).

Recruitment rates are known to vary greatly within reef systems (Harriott and Fisck 1987), within a same site (Baggett and Brigth 1985), and among reefs at different latitudes (Hughes et al. 1999). In the present study, the settlement was observed all the year round and presented two seasonal peaks of recruitment in March, April, June, and September. These months could correspond to intense spawning periods for these species. All the recruits observed on the plates belonged to species known as brooders that spawn almost all the year round (Chornesky and Peters 1987, Goodbody-Gringley and Putron 2009, Szmant-Froelich 1985, Van Moorsel 1983, Vermeij et al. 2003). The broadcast spawners, which contain the main framework builders on Caribbean reefs, were found to be absent from the plates.

While some studies demonstrated a significant tendency for successful recruitment (Birkeland et al. (1981) and also survival of recruits according to increasing depth (Birkeland 1977), our results revealed that settlement was inversely related to depth. This can be explained by a high sedimentation rate and a high concentration of total suspended particulate matter (SPM) at the bottom of the channel (20 m) of Passe-à-colas. Suspended particulate matter in the water column and the associated reduction in light intensity are environmental factors that may play a significant role in the settlement behavior of planktonic coral larvae as well as on the growth and the survival of larval recruits. Sediments deposited on the reef substratum have been shown to inhibit the settlement of coral larvae and to damage newly settled corals (Babcock and Davies 1991, Babcock and Mundy 1996, Birrell et al. 2005), as was the case in the present study.

The genetic analysis used to identify coral larvae did not provide all the expected results. First, DNA extraction failure could be due to insufficient quantity of DNA in our sample or to the inhibition of PCR reaction by mucus, photosynthesis pigment or other DNA precipitates. Second, the accurate identification could not be 100% reliable for some species and so the results were not presented. One of the most important problems encountered during the study was the presence of symbiotic dinoflagellates that live in abundance in the endodermis of the coral cells. DNA from these symbionts could contaminate coral DNA preparation (van Oppen et al. 2000b). This problem occurred when comparing sequences of recruits with genbank sequences: the obtained sequences matched with multiple clades of zooxanthellae. An alternative method would consist of purifying the coral tissues by centrifugation (Hunter et al. 1997). However, this method does not guarantee a complete absence of symbiont DNA, which is a problem for sensitive techniques such as PCR. Coral-specific primers have been employed successfully to amplify mitochondrial (Romano and Palumbi 1996, van Oppen et al. 2001a) and nuclear DNA (van Oppen et al. 2000a). But the identification of recruits would have necessitate to test all the coral specific primers, which was not possible in our case considering the few quantity of DNA collected (< 3 ng/µl).

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