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Final Project Report
Project Title

Recruitment of two species of the reef building coral *Montastraea*: Factors that affect post-settlement survivorship

Alina M. Szmant
Center for Marine Science
University of North Carolina at Wilmington

for the
U.S. Environmental Protection Agency
Region IV

Correspondence Address:
5600 Marvin K. Moss Lane
Wilmington, North Carolina 28409
Phone: (910) 962-2362
Fax: (910) 962-2410
Email: szmanta@uncwil.edu

1.0 Introduction

1.1 Problem Definition/Background

Coral species belonging to the genus *Montastraea* are primary reef-builders in the Florida Keys and Caribbean where they generally dominate the adult coral community within every reef habitat and zone. Three species, *M. annularis, M. franksi*, and *M. faveolata* form a sibling species complex formerly thought to be the single species named *M. annularis.* A fourth species, *M. cavernosa*, is more distantly related. *M. faveolata* grows into enormous colonies several meters high that form major buttresses. Many of these colonies are hundreds of years old, and have large cavities that are important shelter and substrate for reef fishes and invertebrates. The remaining species have a more moderate-sized colony size approximately 1 m in diameter. Small recruits of *M. cavernosa* are relatively common on Florida coral reefs, but small (< 10 cm), young colonies of the other species are rare, suggesting that these corals have been unable to successfully recruit to Florida reefs in recent decades (Chiappone and Sullivan 1996; Smith 1997; Miller et al 2000).

The sibling species complex has suffered a major decline in live cover over the past two decades due to bleaching stress and diseases (Porter and Meier 1992; Ginsburg et al. 2002; EPA Coral and Hard-bottom Monitoring studies). If recruitment rates continue to be as low as those observed over the past decades, the populations of these corals on Florida reefs will decline to a point of local ecological extinction (Hughes and Tanner 2000). Given the structural and ecological importance of these corals to Florida and Caribbean reefs, it is important to understand the factors that affect their ability to achieve successful sexual recruitment.

There are still serious limitations in our understanding of the processes that determine coral recruitment success. This requires detailed studies of coral settlement behavior and post-settlement survivorship (Miller et al. 2000), which are hampered by the small size and low visual contrast of newly settled coral recruits and the difficulty of visually detecting small juvenile corals in more cryptic and architecturally complex habitats. Recruitment success is influenced by many biological and physical factors, including hydrographic conditions, reproductive strategies of individual species (Harrison and Wallace, 1990), larval substrate preference (Carleton and Sammarco, 1987), and post-settlement mortality (Sammarco, 1980). Numerous studies suggest that the interactions with competing encrusting invertebrates and algae are important to post-settlement survivorship (Harrison and Wallace 1990; Babcock and Mundy 1996; Dunstan and Johnson 1998; Carlon 2001) yet few studies have been able to approach this problem in any detail because of the difficulty in observing newly settled corals.

Our recent work (Szmant and Miller 2006) has circumvented the obstacle of detecting very young natural recruits by using the same approach used successfully by Babcock and Mundy (1996). Because newly settled corals are so small, they cannot be seen in

the field with the naked eye (or even with a magnifying glass) for many months to a year, a critical time-frame in the life history of a coral. Obviously this is the most vulnerable time of life for a settled coral, and yet we have little understanding of the conditions or factors that might promote increased survivorship. We have been collecting *Montastraea* spawn in the field, fertilizing the gametes and culturing the larvae in the laboratory for observation and eventual settlement. Various pre-conditioned substrates were used for larval settlement (limestone plates, clay tiles, and natural reef rubble). After settlement, spat settlement was mapped under a microscope, patterns characterized and survivorship monitored. With this approach we were able to determine survivorship rates of only 1-2 % over the first three months after settlement (Szmant and Miller 2006). We also determined that the larvae settled in an aggregated pattern, suggesting that micro-habitats have cues that attract the larvae to settle onto them.

1.2 Present Project Overview

The objective of this project was to build on and continue experimental studies with cultured *Montastraea faveolata* larvae to investigate factors that contribute to, or reduce, post-settlement mortality. Our proposed approach was to map the settlement patterns of larvae onto limestone plates preconditioned for several months at each of three reef sites (Algae Reef, Sand Island and Molasses Reef). We would also characterize the composition of the encrusting community on the settlement plates to specifically investigate biological interactions thought to promote larval settlement (e.g. cover by certain types of crustose coralline algae (CCA)) and/or reduce post-settlement survivorship (e.g. cover by macroalgae, encrusting invertebrates such as bryozoans and ascidians that overgrow the coral spat; CCA that overgrow the coral spat). The mapped settlement plates were to be attached to the substrate at each of three reefs that differ in their environmental characteristics (described below). A comparison of the survivorship patterns of spat on such plates would provide an indication of environmental factors important to spat survivorship.

The initially proposed plan was modified, as explained below, when Hurricane Frances prevented us from collecting spawn from *M. faveolata*. We were able, however, to raise a small number of larvae of *M. cavernosa* and *M. annularis*, which allowed us to revise the experimental plan to compare settlement and survivorship of these two species at the three reef sites.

2.0 Materials and Methods

2.1 Collection of spawn for the culture of *Montastraea* larvae

The three *M. annularis* sibling species are hermaphroditic and broadcast their gametes into the water column where development takes place (Szmant 1986, 1991). During a

few, highly predictable nights a year they release gamete bundles (eggs plus sperm) that form large slicks on the ocean surface. After fertilization, the embryos take a minimum of 3 to 5 days to develop into competent planula larvae (Szmant et al. 1997; Szmant and Miller 2006) but most larvae attempt to settle within the first two weeks if they are in the appropriate environment (Szmant and Meadows 2006). *M. cavernosa* is gonochoric (colonies are of a single sex), and spawns clouds of eggs and sperm into the water column during the same time frame as the other species. Based on the lunar cycle (Szmant 1991) we predicted that these species would spawn between September 3 to 5, 2004, and planned to capture with collecting nets the spawn of the bundle-releasing species as it was released, while spawning colonies of the gonochoric species inside of large coolers.

The work funded by this grant was originally supposed to focus on *M. faveolata*, but Hurricane Frances was affecting the Florida Keys exactly during the spawn period and thus we were not able to collect spawn in the field even though we attempted to do it under terrible weather and diving conditions the last night of the spawn season.
In anticipation of bad weather during the spawning period, a number of large pieces of *M. annularis*, *M. faveolata* and *M. cavernosa* were collected just before the storm closed down our field operations. The corals were kept in separate coolers per species at the field laboratory. Normally, the spawn from multiple colonies are combined to achieve high rates of cross-fertilization since these species cannot self-fertilize. Thus it was hoped that multiple colonies per cooler would spawn and yield larvae.
The night of September 3rd only one male colony of *M. cavernosa* spawned. The next night, one male and one female colony spawned, and the fertilized eggs were pipetted out of the cooler for culture. The night of September 5th, two colonies of *M. annularis* spawned, and fertilized eggs were similarly pipetted out for culture. None of the *M. faveolata* colonies spawned.

2.2 Revised experimental objectives

Although the funded project was supposed to examine settlement and post-settlement survivorship of *M. faveolata*, we had no choice but to change the plan to work with the two coral species for which we had successfully collected some spawn. The experimental plan was accordingly revised to be a comparison of survivorship of the two *Montastraea* species, co-settled onto the same experimental plates. *M. cavernosa* are commonly found as small recruits on Florida reefs, while *M. annularis* are not. The eggs and larvae differ in mean size (*M. cavernosa* ca. 100 um larger in diameter), and thus size at settlement was hypothesized to be a factor affecting survivorship.

2.3 Larval culture

The fertilized eggs were transferred to 4 L polycarbonate chambers filled with filtered reef water, and maintained on orbital shaker tables. Water in the chambers was changed once or more per day if they became cloudy or accumulated debris. After four

days, the larvae were determined to be able to settle by using a settlement assay in 6-well plates with small pieces of CCA known to induce settlement.

2.4 Station selection and experimental design

The main objective of this study was to experimentally determine the conditions that foster the best post-settlement survivorship. The two factors to be investigated were:

(a) reef exposure: offshore shallow fore-reef habitat vs. more protected patch reef habitat
(b) 'no-take' vs 'take/fished' management status.

The rational for this is that the main physical variables that are known to coral community structure, and hypothesized to also affect coral survivorship, are wave exposure and water depth (reviewed in Done 1983). More exposed areas have more water circulation, and less sediment build-up, and at shallower exposed depths, there is also more light, all conditions that favor coral growth. However, previous work (Miller et al. 2000), recent personal observations and several studies underway by others suggest that coral recruitment in general is higher on Florida Keys patch reefs, even though the environmental conditions seem to be less optimal for corals (higher algal cover, more turbid, less circulation). With regard to management status, fish grazing is more intense on the offshore reef zones, and known to be important to coral recruitment and dominance (Hixon 1997). While no major difference in herbivorous fish density or grazing pressure have been demonstrated between SPA and non-SPA sites in the FKNMS, the increased abundance of other fish guilds within SPAs may play an important role in the community structure of the encrusting community where juvenile corals start their life (e.g. Birkeland 1977; Day 1983; Fairfull and Harriott 1999).

The three sites selected for this experiment were: Molasses Reef: forereef within the Wellwood restoration site (SPA), Sand Island: forereef (non-SPA) less than 1 km from the Molasses reef site; Algae Reef (large patch reef area) (Figure 1). Settlement plates were deployed in the 5 to 6 m depth range.

We initially proposed more reefs and two depths per site, but with a lower replication (18 plates) per station. Reviewers expressed concern about high variability in survivorship making it difficult to detect differences among treatments, and thus the number of sites/habitats was reduced to increase replication to 36 plates per site (one depth). Increasing replication at all of the originally proposed sites/depths was not an option because the available resources would not allow for the analysis of more settlement plates than originally proposed. A short-coming of this design, however, is that there is no replication of reef types. Given that this is the first attempt of a study of this nature in the Florida Keys, we recognized that we would document major patterns of survivorship, without being able to statistically attribute differences in survivorship between reefs to their reef type or management status. If large differences were found, that would provide justification for a larger more replicated study in future years.

Justification for site selection is as follows: (1) The Wellwood site at Molasses has been used the past two years for pilot studies of plate deployment and spat survivorship. It is a site that is being intensely studied by the FKNMS as part of their restoration program. There is interest in trying to (and we are attempting to) seed restoration modules with coral larvae to accelerate coral recovery at the site. So any knowledge gained about juvenile coral survivorship at this site will have direct management application. Further, Molasses reef is a SPA where no fishing is allowed, which makes it a good selection for testing hypotheses regarding the value of no-take reserves to community recovery. (2) The Sand Island site is just north of the Molasses site, and a similar reef structure and depth, but it is open to fishing. Other groups funded by NOAA have been conducting fish studies at all of these sites, and thus a comparison of coral spat survivorship can be conducted taking into account the possible contribution of fish communities to coral recruitment. (3) The Algae Reef patch reef site is ca. 10 km north of the other two sites, but there is no suitable patch reef closer to Molasses and Sand Island. White Banks, which is closer, is too heavily visited by snorkelers to be used as an experimental site. Other nearby patch reefs are too shallow and have too little vertical relief, and we consider depth and relief important factors to try to standardize among sites. Algae Reef, while more distant, has similar relief and depth to the first two reefs, but being further inshore, is more protected and has less wave action and current. It is also within a more lagoonal water mass than the two above reefs (more turbid, higher nutrients; Szmant and Forrester 1996). In the EPA-funded NCORE study, the algal community structure was compared at all three of these sites.

2.5 Plate conditioning and experimental design

Settlement plates were made from quarried Key Largo limestone cut into 8 x 8 x 2 cm pieces. Each plate had a hole drilled into the center to serve for attachment, and a numbered metal tag cable tied to it for identification. Each field attachment site was tagged with the same number so that each plate was always returned to the exact same location and orientation. Settlement plates were 'conditioned' before they were used for settlement. Conditioning consists of deploying them at their field sites for a sufficient time for a bio-film and fouling community to become established on their surfaces. Chemical cues produced by the fouling community are necessary to provide settlement cues for the larvae. The nature of the fouling community on the substrate is known to affect settlement behavior and is hypothesized to be a factor affecting post-settlement survivorship. Substrate characteristics were another factor we proposed to investigate. We have limited ability to control what organisms settle and grow on each settlement plate, but we know from experience that exposure time is an important factor affecting fouling community structure. We deployed half the plates per site 9-11 months before settlement (September to December, 2003), and the other half only three months before use (May 2004), thus providing two fouling community composition treatments at each station.

At each site, 36 plates (108 plates total) were attached to the substrate using the method of Mundy (2000). Mundy found that angle of plate attachment did not affect natural recruitment to his experimental plates, but others have found substrate relief to be important. We deployed our plates to substrates with relief of 20+ cm above the reef plane to reduce sediment scouring.

2.6 Plate settlement and maintenance

Because of the hurricane conditions described above, we were not able to collect the amount of *M. faveolata* spawn needed to culture sufficient larvae for the experimental plan approved for this project. Rather, we were able to raise ca. 40,000 larvae of *M. cavernosa*, and another 10,000 *M. annularis* larvae. Furthermore, because of the weather, we were not able to do the settlement at Carysfort Lighthouse where there is an ample of clean reef water to maintain the plates in, but instead had to do the settlement at our field lab on shore where we had a limited clean seawater supply. It was therefore decided that we had sufficient larvae to settle only 13 plates per site, and we selected the qualitatively best plates per site for the experiment. Criteria for selecting the plates were that they had a good cover of healthy CCA and also low cover of fleshy filter feeding invertebrates (sponges, bryozoans, ascidians) that would make it difficult to keep the plates healthy in coolers under laboratory conditions. The selected plates were suspended directly into 60 L plastic coolers filled with filtered seawater and 1/3 of the larvae of each species were added to each cooler. The cooler seawater was changed twice daily with fresh oceanic water for three days, avoiding loss of unsettled larvae from the coolers by siphoning the water from the cooler through a 120 uM mesh sieve. Once the larvae were given sufficient time to become firmly attached to the substrate cooler water was changed daily for another 4 days, during which time the spat lay down a basal skeleton. We wanted the spat to be well attached to the plates before any further manipulation. It is estimated than ca. one-tenth of the larvae added to the coolers settled on the plates. The rest were still swimming at the surface of the cooler water at the end of the 3 day settlement period.

The plates with spat were removed from the coolers and placed into a re-circulating seawater system consisting of a 200 L sump-reservoir outfitted with a submersible pump that distributed the seawater into 12 L plastic containers serving as aquaria. Outflow from the containers gravity flowed back to the sump. The sump had ca. 30 cm of clean reef rubble that served to filter and denitrify the seawater before it was pumped back to the containers. Each container held 8 settlement plates. The seawater in the system was checked daily for salinity and temperature. De-ionized water was added to the sump to make up for evaporation. Half of the volume of the sump was replaced with fresh offshore seawater twice per week to maintain good water quality.

2.7 Plate spat mapping

Spat location was mapped under a Leica MZFLIII fluorescent dissecting microscope at

12 x or higher magnification. Especially in the earliest stages, the newly settled corals are very small (ca. 0.5 mm in diameter), very pale in color, and often cryptic in their position on the plate. We used a fluorescent microscope to help locate the spat because they fluoresce green under the microscope; this helps reveal their position even when tucked into a crack or partially obscured by another organism. We also used white light (to confirm identity), and generally switched back and forth between the two light sources. An acrylic grid-guide with a 1 cm x 1 cm grid [100 x 1-cm^2 cells] scribed into the plastic was used for orientation. Settlement plates, which had a hole drilled in their center, were placed into a plastic container with seawater, supported on a rack that had a stainless screw bolt sticking upwards out of the center. The bolt centered the plate over the rack, and the acrylic grid was placed over the plate, also centered with the bolt. In this way the grid was always aligned the same way over the plate. The location of each spat was marked onto data sheets with a similar grid printed on it, and assigned to a given species (*M. cavernosa* or *M. annularis*). That way the fate of individual spat could be followed at each re-survey. Both sides of each plate were mapped. This was a time consuming and labor intensive task, and limits the number of replicate plates that can be studied. It took ca. 1.5-3 hours to do the initial mapping of each plate. Re-surveys generally took less time. With one fluorescent microscope, it took three researchers doing two to three hours shifts over one week to do the initial survey. In retrospect, it would have been impossible to map the originally proposed 108 settlement plates as completely as this set of 39 was mapped. The plates would have had to be sub-sampled for mapping.

2.8 Mapping of community structure

Two methods were used to characterize the community structure of the plate surfaces, one which provided an overall assessment of substrate characteristics for each plate surface, and a second one that provided a more detailed description of the microhabitat where coral larvae settled.

Photographs were taken of each settlement plate once per month for four months on the days they were collected for spat mapping. Both sides of the plates were photographed using a Sony Cyber-shot DSC-P10 digital camera. Plates were submerged in seawater in a plastic dish placed on a copy stand to ensure the same distance was kept from camera to plate throughout the photographs. The photos included rulers along both side edges of the plate, along with a label with plate number and plate surface (upper/lower). Two halogen lamps were used to provide even and reproducible illumination. Digital files were imported into Microsoft Office 2003 PowerPoint, and an 11 x 11 grid was superimposed over each plate. The substrate type/organism under each of the 100 points where gridlines intersected was identified using the categories: microfilm (single cell algae), turf algae (filamentous algae), macroalgae (foliose and corticated algae), encrusting red algae (e.g. *Peyssonnelia*), CCA (e.g. *Lithothammnion*), sponges, bryozoans, tunicates, worm tubes, foraminiferans and 'other'.

The second method consisted of characterizing, under a microscope, the microhabitat close to and away from where coral spat settled. As for the mapping, each plate was covered with a transparent plastic grid divided into 100 x 1 cm^2 cells. After mapping the spat, we selected the ten cells with the greatest number of spat and 10 cells with no spat, on each side (top and bottom) of the plates. No-spat ('control') cells were randomly selected among the spat-free cells using the following method. We first used the random number generator in SPSS Inc. to select 10 numbers within the range of the total number of spat-free cells. Spat-free cells were then numbered, beginning with the upper left corner of the grid from left to right, and those with the given random numbers were selected. The same procedure was used for choosing among spat cells with the same number of spat when only a subset had to be selected. Settlement was too low on the tops of 31 plates and on the bottom sides of 7 plates to obtain the full ten spat cells. One plate had no settlement on the top side and was discarded from the analysis.

Estimations of substrate characteristics were made for each of the selected cells. Substrate types used were the same as for the photographic analysis, but with further discrimination of the CCA. CCA were further characterised into 4 subgroups based on morphological characteristics of surface cells. Group 'A' included all species with large epithelial cells (> 10 µm in diameter) showing no particular spatial arrangement and large trichocytes (> 20 µm) usually clumped together. Group 'B' included all species with large epithelial cells (> 10 µm) forming fan-like arrangements and no (or very small) trichocyte. Group 'C' were all species with small epithelial cells (<5 µm) forming no particular spatial pattern and no (or very small) trichocyte. CCA species not fitting into these categories were classified as 'other CCAs'. The cover of each substrate type (or CCA group) was visually assigned into one of seven categories (0, 1-4, 5-19, 20-39, 40-59, 60-79, 80-100 %). Average percent cover was then estimated from the midpoint of the cover category for each square.

2.9 Plate re-surveys

Eleven of the mapped plates (the ones with the most settlement) per station were returned to their tagged field positions after the initial mapping. They were carefully retrieved at ca. monthly intervals, in October, November and December 2004, and in May 2005. The plates were supported for moving by sliding them onto a stainless steel threaded rod through their center holes, and inserting a 1 cm wide PCV spacer-ring in between plates to keep them from scraping each other. They were returned to the laboratory submerged in coolers of clean seawater, re-mapped and photographed as described above, and then re-deployed usually within 24 hours. The plates were maintained in the re-circulating seawater system while in the shore laboratory.

2.10 Quality Assurance, Quality Control procedures

A QA/QC concern of this project was that the spat mapping and community structure

data collected by individual observers and at different time intervals are consistent with each other. To satisfy this data quality concern, we established a QA program committed to reducing and minimizing random and systematic errors and to producing accurate, high quality, reproducible data. One team of three observers was assigned to map larval settlement and a second team, also of three observers, collected all of the microhabitat data. Before the initial plate mapping, a week was spent in training during which time a single plate was mapped or characterized by each of the observers on each team. Discrepancies among observers were identified and discussed until there was agreement. We also had two people read each of several plates during the initial mapping effort, to make sure we were being consistent. Again, any discrepancies were re-examined and discussed to make sure whomever had erred could improve their work quality. We did not have time to read each plate twice.

A second concern was consistency in the relocation over time of individual spat on settlement plates, and on assigning each spat to a given species. When observers were tired, the spat could be mapped into the wrong cell on the data sheet, or some weakly fluorescent spat could be missed. Sometimes spat located in cryptic locations would be seen during one survey if they were expanded, but missed during a later one if they contracted into a hiding spot. In addition, an unexpected problem arose because we had expected to be able to reliably distinguish between spat of *M. cavernosa* and *M. annularis* based on both polyp size and fluorescence pattern. Larvae of *M. cavernosa* are on average > 100 um larger in diameter and more fluorescent than those of *M. annularis*, and in fact, some *M. annularis* were difficult to find because they had so little fluorescence. For most of the spat, this assignment could be made with confidence, but there was a troubling number of them that we were not sure of, and in several cases this resulted in reversal of species assignment between surveys. Luckily, the spat of the two coral species became more distinct with age, and likely, we also got better at recognizing small differences among them. While we were very concerned about this problem, it turns out that it only involved ca. 5 % of the total spat, and thus was not a major factor affecting the outcome of the experiment.

Our solution to there problems was to correct the raw data as follows: (a) The species identify of a given spat, if there was a change in designation from one survey to the next, was fixed based on the last survey in which that spat was observed since we felt more confident in making the assignment during the later mapping efforts. The earlier settlement maps and data were corrected to reflect this revised designation. (b) It was assumed that it was unlikely that there would be natural recruitment of *Montastraea* larvae to the experimental plates after they were first returned to the field, because it would have been too long after spawning. Therefore, if *Montastraea* spat were found on the plates during later surveys that were not observed during the initial mapping, we assumed that they were present all along but missed. This assumption is supported by several lines of evidence: There were also spat that were present during the initial mapping, missing during the second mapping, and re-located during the third and later mappings. Thus, we feel that in spite of our best efforts and great care in mapping, it

was possible for a polyp to contract and hide from us, or to be hidden by some other growth on the plate. Secondly, the newly found spat were of the same size and developmental stage (number of tentacles, skeletal deposition, acquisition of zooxanthellae) as those mapped initially, and it was unlikely that a newly settled spat could have caught up to ones settled a month earlier. Therefore, the initial settlement data were corrected to include settlers that were initially missed but found during later surveys.

The second type of data collected were the spatial analysis of the photographs. All of these data were generated by a single observer.

2.11 Data analysis

Spat settlement analysis: Statistical analyses comparing initial settlement among plate surfaces and stations were performed using SigmaStat v2.03. Two-factor ANOVAs using reef site and plate side (top and bottom) as factors were tested for each coral species. Although the design was balanced, the data for both species were neither normal nor had equal variance due to the large differences in raw settlement numbers among plates. For *M. cavernosa* settlement, a one-factor ANOVA among reef sites was performed for only the bottom sides of the plates because these had the higher numbers of settlers. The data passed both normality and equal variance tests.

Spat survivorship analysis: SigmaStat v2.03 was also used for statistical analyses of survivorship data. Two-factor ANOVA was performed for each coral species for each monthly sampling period using reef site and plate side as factors. None of the eight ANOVA data sets passed the test for normality, but all but three sets passed equality of variance.

Community structure: Differences in specific substrate type of settlement plates between sites were tested using the Kruskal-Wallis test. To determine associations between the number of spat and plate substrate characteristics, we conducted principal component analyses for each side of the plates (sites pooled). Relationships between the number of spat and the PC scores were then examined using Spearman Rank Order correlation. Differences in specific substrate type between spat and control microhabitats (i.e. cells with spat versus without spat) were analysed for each site and plate side using Wilcoxon's signed-ranks test. Analysis of microhabitat characteristics was also conducted using multivariate discrimination techniques developed by Clarke & Warwick (2001). Data were square root transformed prior to the calculation of the similarity between plates using the Bray-Curtis similarity index. The resulting similarity matrix was used to perform non-metric multidimensional scaling (MDS). An *a priori* analysis of similarities test (ANOSIM) was used to compare spat and control microhabitat characteristics for each side of the settlement plates at each site, and between the top and bottom sides of settlement plates (microhabitats and sites pooled). The SIMPER procedure was performed to obtain the contribution of substrate types to

the Bray-Curtis similarity measure for statistically significant clusters of plates (Clarke 1993).

3.0 Results

The results presented here are a preliminary analysis of the data. A manuscript in preparation for submission to Ecological Monographs will contain the final analysis of the data. Only the initial community structure analysis is presented here. We are still refining and completing the statistical analyses to detect relationships between substrate community structure, larval settlement and spat survivorship.

3.1 Settlement Patterns

There were larvae of *M. cavernosa* than of *M. annularis* to begin with, and so it is not surprising that there were more settlers of *M. cavernosa* on the plates than of *M. annularis* (Table 1). At all three sites, more larvae attached themselves to the bottom side (as positioned in the field during the pre-conditioning period) than to the upper wards-facing surfaces, and this difference in settlement preference was statistically significant ($p < 0.001$). It is important to point out that the plates were in a vertical position during the settlement period, and thus the preference for the plate undersides was due to some substrate characteristic of the community growing on the plate undersides rather than due to light or plate orientation per se.

In addition, more larvae settled onto the plates from Sand Island than the other two sites, and more settled on Molasses Reef plates than on Algae Reef plates. Only the difference between Sand Island and Algae Reef was statistically significant, due to large variance at each site, and relatively low 'n' (13 per site). Given that the plates from each site were settled separately in three separate coolers (as opposed to intermingled within the three coolers), we cannot distinguish whether this inter-reef difference in settlement density was due to the Sand Island plates being more attractive to the coral larvae, or some artefact of the initial health and quantity of the batches of larvae added to each of the three coolers, or environmental conditions during settlement.

In all, a total of ca. 4300 settlers of *M. cavernosa* and 1150 of *M. annularis* were accounted for and followed in this study, distributed over 39 settlement plates at three reef sites. The number of settlers per plate varied greatly within each site (Figure 2), with a coefficient of variation of ca. > 50 %.

3.2 Substrate characteristics of settlement plates: photographic method

Average substrate composition for the top and bottom sides of the settlement plates at each site are presented in Figures 3a & 3b. The community structure of plate tops was made up mostly of microfilm and CCA, which together made up almost 70 % of the cover. Encrusting red algae and turf made up the next two most abundant groups. On

the plate bottoms, the community structure was also dominated by the same four groups, but several encrusting colonial invertebrates were more abundant (bryozoans and tube-building polychaetes). Overall community structure between plate tops and bottoms was significantly different ($p < 0.001$); the organisms responsible for this top-bottom difference will be addressed in the next section. On the top sides, where coral settlement was significantly higher at Sand Island, only turf cover was significantly higher at Sand Island (Figure 3a). On the bottom sides, there was no significant difference in settlement among sites, and only minor differences in community structure with this method of analysis. A Principal Component Analysis (PCA) of the community structure data (of the three sites pooled) did not yield any correlation between overall plate community structure and the number of coral larvae that settled on the plates (Figure

Plate tops had more turf cover and CCA type A, while plate bottoms had more bryozoan and tunicate cover, and also, a higher cover of *Peysonnelia*-like fleshy encrusting red algae.

3.3 Substrate characteristics of settlement plates: microhabitat characteristics

Two types of comparison were made with this data set: (1) a comparison of community structure between 1 cm^2 'cells' (microhabitats) where corals settled and the randomly selected cells in which they did not, and (2) a more detailed comparison between the community structure of upper and lower plate surfaces.

A comparison of substrate composition within spat and no-spat microhabitats is presented in Figures 5a to 5c. On the top sides of plates, microfilm microhabitat was more abundant within the spat cells compared to the no-spat ones, but Wilcoxon's signed-ranks tests indicated significant differences at Algae Reef only (Figure 5a). Turf algae were significantly more abundant within the no-spat cells at Sand Island. Encrusting red algae were also significantly more abundant in the no spat cells at Molasses Reef. On the bottom sides, where most of the coral larvae settled, CCA were more abundant within the spat cells at all sites, but this difference was not significant at any of the three sites. The only significant difference was for turf algae at Algae Reef.

When the CCA are subdivided by taxa/group, on plate bottoms CCA type B was consistently more abundant within spat cells, but again, this difference was only significant at Algae Reef. Overall, plates tops had a higher percent cover by CCA type A, while plate bottoms had higher percent cover of CCA type B (Figure 6a to 6c).

Using MDS ordination plots (Figure 7a tops and 7b bottoms), there was no difference in substrate characteristics between spat and no-spat cell microhabitats at any of the three sites, with the exception for the top sides on Molasses Reef (ANOSIM $r = 0.094$, $P = 0.048$). The average dissimilarity of all pair-wise coefficients between spat and no-spat

cells at Molasses was 29.65; 40% of this dissimilarity was due to differences in microfilm, with this substrate type being more abundant in the spat cell microhabitat.

In contrast, when pooling spat and no-spat microhabitats among sites, the MDS ordination plot separated the data points into two distinct clusters which corresponded with the top and bottom sides of the plates (Figure 8). With these more detailed data, there was a highly significant difference in microhabitat substrate composition between the top and bottom sides of plates (ANOSIM $r = 0.551$, $P < 0.001$). The top sides were dominated by microfilm and CCA type A, whereas the bottom sides were dominated by encrusting red algae and CCA type B (Table 2). A SIMPER analysis showed that these four substrate types contributed >70 % to the dissimilarity between top and bottom sides.

3.4 Post-settlement survivorship

Only 11 of the 13 plates per site settled with coral larvae were returned to the field after the initial mapping to follow survivorship. The mean number of spat of the two coral species per plate when deployed in September 2004, and the number of spat relocated during each resurvey are summarized in Table 3. The high variability in initial settlement density among plates at each site and the relatively low n of 11 precluded our being able to statistically detect differences in survivorship among sites. Given this variability, it was decided to follow the population of coral spat as a whole rather than on a plate by plate basis. Figure 9 presents the time course of coral spat at each site for each species, and also plotted by plate surface. Figure 10 presents the data plotted as a percentage of initial settlement at each site, the two plate surfaces combined. Finally, Figure 11 plots the percent survivorship over each interval of measurement: in this plot the number of spat alive at the beginning of the interval was used to calculate the percent survivorship during that interval. Two trends are apparent in these figures: Survivorship was lowest for both coral species over the first 30 days (< 50 %), and survivorship was lowest at Algae Reef (< 20 % by 30 days; only a few percent surviving after 60 days). Also, percent survivorship was lower for *M. annularis* at both Sand Island and Molasses Reef. Because of the low settlement by both species on the plate tops at all sites, there were few spat to follow after the November resurvey. Percent survivorship increased greatly for those spat that survived the first 30 days (Figure 11), at least for the next 60 days. But few spat survived the winter: only 2 to 4 % were still alive when the plates were resurveyed for the last time the following May 2005.

4.0 Discussion and Conclusions

Bad weather due to several hurricanes precluded us from executing the original research plan, but fortunately, we were able to raise sufficient larvae of two *Montastraea* species to be able to settle a small number of settlement plates for each of the three research sites. The revised research plan, in addition to addressing the original topic of

post-settlement survivorship, devoted more attention to the substrate composition of the settlement plates, and of the relationship between settlement and substrate composition. This direction was decided upon because of previous and other complementary work that had shown a strong preference of larvae of *M. faveolata* to settle on the undersides of field-conditioned settlement plates, as well as bioassays using small pieces of a number of CCA types, that showed that some CCA had the property of inducing settlement of *Montastraea* larvae, and that *Montastraea* larvae would not settle unless presented with some sort of substrate such as CCA (Szmant and Miller 2006).

The results of the present settlement experiments confirmed earlier results that larvae of two additional species of *Montastraea* preferentially settle on the undersides of field-conditioned settlement plates (e.g. Figure 2), and that this preference is not due to orientation of the substrates because the plates were in a vertical position when presented to the larvae. Thus, there is some property of the undersides of the plates which is attractive to the larvae and/or inductive of larval metamorphosis.

Much of the effort in characterizing the community structure of the organisms growing on the plate undersides vs tops had the intent of determining what component(s) of community structure was responsible for this attraction/induction phenomenon. The methods used, both analysis of high resolution photographs, and detailed microscopic examination of the microhabitats within which coral larvae settled, were successful in discriminating between the community structure of the tops and undersides of the settlement plates (e.g. Figure 8), yet it was not clear which component of the bottom community was primarily responsible for the higher settlement on those surfaces. Microscopic examination of the substrate immediately under each newly settled polyp showed that ca. 80 % of the larvae settled directly on microfilm. Crustose coralline algae are thought to be important inducers of coral settlement (e.g. Morse et al 1996; Heyward and Negri 2001; Harrington et al 2004). However, while additional statistical analysis of the microhabitat data (in progress; not shown here) shows that there is more CCA type B in cells with spat settled in them than in no-spat cells, the differences in percent cover are small and don't seem to be biologically significant.

The percent of laboratory-cultured larvae that settled onto the plates was low (ca. 20 %) and similar to the percent reported by other investigators using this approach (Babcock and Mundy 1996). It is not clear what happened to the remaining larvae: some were still swimming when the settlement phase was terminated after 48 hours. The rest might have died or have been eaten by filter feeders are small predators living on the settlement plates. These plates were made of quarried Key Largo limestone, and had extensive pits and crevices inhabited by a wide variety of small invertebrates, including polychaetes and crustaceans which could prey on the larvae. A serious limitation of the settlement system was the lack of adequate circulation. Only small airstones and a water change once per day were used to provide some circulation during this period. The original plan called for culturing and settling the larvae using the Carysfort

Lighthouse, where we would have had plenty of fresh seawater to flow through the coolers, but again, the hurricanes pre-empted this plan.

An interesting facet of the revised research plan was a comparison of the settlement and survivorship of the two *Montastraea* species, which have very different reproductive and recruitment patterns. *M. cavernosa* has separate sexes, and it is thought that their eggs are fertilized before they are released. Their eggs are larger (ca. 450 um diameter) than those of *M. annularis* (ca. 300 um). The latter are hermaphrodites which release sperm-egg bundles that break up in the water column where fertilization takes place. *M. cavernosa* larvae develop competency within three days which those of *M. annularis* take an extra day or more. *M. cavernosa* are commonly observed as recruits on Florida coral reefs, while *M. annularis* are not.

We had ca. five times more larvae of *M. cavernosa* to use in the experiments, so the absolute differences between species cannot be used for comparison of patterns. No difference In settlement pattern was observed between the two species, and in fact, many incidences of co-mingled settlement within a single 1 cm^2 cell were observed. The newly settled polyps of *M cavernosa* were slightly bigger than those of *M. annularis*. *M. cavernosa* polyps were observed to develop larger tentacles and grow faster than *M. annularis* polyps. The larger size of the *M. cavernosa* polyps might have contributed to a higher success in capturing food, and possibly to their higher percent survivorship. Several observations were made under the microscope of *M. cavernosa* spat capturing small prey animals that were attracted to the polyps by the microscope light. No such observation was made for *M. annularis* polyps. Growth rates of both species were very slow compared to those we have observed for newly settled polyps of brooding species such as *Favia fragum* and *Agaricia humilis*. After 3 months, Montastraea polyps had not budded and were barely 0.5 mm in diameter, and not much bigger after 9 months. By comparison, settlers of *Agaricia* or *Favia* can have budded to form small colonies with numerous polyps and be 2-5 mm in diameter after a few months.

The main objective of this study was to determine whether we could learn more about the factors affecting coral post-settlement survivorship. It was hypothesized that offshore reefs that were protected from fishing would have higher coral survivorship than reefs exposed to fishing or more inshore reefs with poorer water quality and/or circulation. While survivorship was low at all three sites, the highest survivorship was observed on settlement plates attached to Sand Island, an offshore reef but not one protected from fishing. The natural substrate on this reef is highly grazed and has a high cover of CCA and turf, and lower macroalgal cover, compared to the nearby Molasses Reef, which is a protected area (Szmant in prep). The lowest post-settlement survivorship occurred at Algae Reef, the more inshore site, where the water is frequently quite turbid, and the reef has high algal cover. The settlement plates at this site had a higher sediment cover than those at the two offshore reefs, and this alone could have been responsible for the lower spat survivorship. It is interesting that the

settlement plates pre-conditioned at this site also induced the lowest level of larval settlement in our laboratory system.

The low percent survivorship by *Montastraea* settlers after 9 months (< 3 percent) means that there would need to be very high initial settlement by coral larvae for there to be survivors left to recruit after a few years. The density of settlement on the experimental plates used in this study was in the range of 5,000 to 10,000 per m^2, which is probably much higher than one could expect for natural settlement. If survivorship continued to be in the range of ca. 2 percent per year, for there to be one coral recruit of these species per m^2 five years after settlement, initial larval settlement would need to have been in the range of 625,000 per m^2. The one year we succeeded in seeding and deploying settlement plates with larvae of *Acropora palmata* showed that this species grows faster, buds much sooner, and has much higher survivorship than do the *Montastraea* spp. spat (Szmant and Miller 2006). The obvious conclusion is that the small size of the eggs and resulting larvae, and low growth potential of the newly settled *Montastraea* spat lead to these small corals being overgrown or preyed on by other benthic organisms. Their small size and cryptic settlement behaviour might limit their access to food (plankton capture or photosynthate from their symbiotic algae). The polyps were observed to acquire zooxanthellae within a couple of weeks of settlement, but if the polyps are living on the dark undersides of the plates (and similar locations on natural substrates), then the zooxanthellae may not be able to make much of a nutritional contribution to their hosts.

Much remains to be learned about the early life history of newly settled coral polyps, and the factors that affect their survivorship. It is clear that some environments will be more conducive to survivorship, and our experience is that survivorship can vary greatly from year to year. A similar settlement-post-settlement study conducted in 2002 yielded much lower survivorship levels. Another study conducted in Puerto Rico the summer of 2005 also yielded much lower survivorship where we expected it to be higher. However, 2005 was a severe bleaching year, and so it is not surprising that newly settled corals died just as nearby adults did.

5.0 Acknowledgements

This project required the hard work and dedication of a large number of people. Dr. Maggy Nugues helped with all aspects of the execution and especially important, with the statistical planning and analysis of the data. Darcy Lutes, Ben Mason and Melissa Meadows were the dedicated field team responsible for monthly sampling and microscope analysis, and data analysis. Chris Rader, Adam Jones and Erin Looney helped during the first two months of the project when we were working almost around the clock. Dr. Margaret Miller and her team of students and post-docs were instrumental in collection of spawn and many aspects of the project. Drs. Mary Alice Coffroth, Monica Medina, and Rob Whitehead also helped out when needed during the early aspects of the work. To all of the above I owe a great debt of gratitude for a job well done.

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Table 1. Summary of the total number of coral larvae settled per coral species, per site and settlement plate surface. There were 13 settlement plates pre-conditioned at each site. AR = Algae Reef; SI = Sand Island; ML = Molasses Reef. Significantly more larvae settled on the plate under surfaces (as oriented while pre-conditioning in the field) than on the upward facing surfaces for both species at all three sites. However plates were oriented vertically during the laboratory settlement period, indicating that larvae were selecting plate surface based on substrate composition and not orientation.

Site	*M. cavernosa*		*M. annularis*	
	Top	Bottom	Top	Bottom
AR	291	902	45	190
SI	328	1605	32	482
ML	67	1146	18	392
All Sites	686	3653	95	1064

Table 2. SIMPER analysis of microhabitat assemblages on the settlement plates showing the abundance of the substrate type that contributed most to the dissimilarity between the top and bottom sides of the plates (microhabitats and sites pooled). The average dissimilarity calculated using the Bray-Curtis index of similarity was 40.58 %.

	Top side Mean	Bottom side Mean	Cumulative (%)
Microfilm	55.31	50.42	31.2
CCA group A	11.52	0.78	47.4
Encrusting red algae	3.75	10.88	61.4
CCA group B	1.89	7.47	70.9
Turf algae	5.93	2.64	77.1
Worm tube	0.84	4.33	82.9
Foraminiferans	2.95	0.07	87.2
Bryozoans	0.51	2.21	91.2

Table 3. Summary of mean larval settlement and survivorship. Values are the mean numbers (± s.d.) of larvae settling per 100 cm^2 of settlement plate surface on the 11 settlement plates mapped in September 2004 that were re-deployed after mapping (only 10 for Sand Island because one plate was lost after the October re-survey) and re-surveyed at monthly intervals through December 2004, and again in May 2005. Top and Bottom refer to the plate surfaces.

Station	Date	*M. cavernosa*		*M. annularis*	
		Top	Bottom	Top	Bottom
Algae Reef	Sept	23.5 ± 34.6	77.5 ± 29.3	4.0 ± 5.8	15.8 ± 12.4
	Oct	2.3 ± 2.7	3.5 ± 2.2	0.5 ± 1.2	0.5 ± 0.9
	Nov	0.7 ± 0.8	1.1 ± 1.0	0.2 ± 0.4	0.5 ± 0.7
	Dec	0.5 ± 0.7	0.5 ± 0.5	0.1 ± 0.3	0.1 ± 0.3
	May	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sand Island	Sept	28.3 ± 21.7	104.2 ± 51.1	2.8 ± 4.0	33.6 ± 24.3
	Oct	11.0 ± 8.3	52.0 ± 37.1	0.5 ± 1.0	10.4 ± 9.7
	Nov	5.7 ± 4.8	37.9 ± 32.2	0.1 ± 0.3	6.3 ± 7.3
	Dec	2.6 ± 2.5	23.1 ± 23.5	0.1 ± 0.3	3.3 ± 4.8
	May	0.9 ± 1.7	3.7 ± 6.8	0.0 ± 0.0	0.2 ± 0.6
Molasses	Sept	5.5 ± 3.2	97.3 ± 67.6	1.6 ± 1.3	31.6 ± 21.5
	Oct	2.0 ± 2.3	33.3 ± 30.3	0.3 ± 0.6	8.2 ± 10.3
	Nov	1.3 ± 1.3	23.2 ± 25.9	0.0 ± 0.0	4.6 ± 7.2
	Dec	0.6 ± 1.0	16.7 ± 22.0	0.0 ± 0.0	3.2 ± 5.6
	May	0.1 ± 0.3	3.4 ± 5.0	0.0 ± 0.0	0.4 ± 0.7

Figure 1. Map of the Key Largo area of the Florida Keys showing the locations of the three study sites.

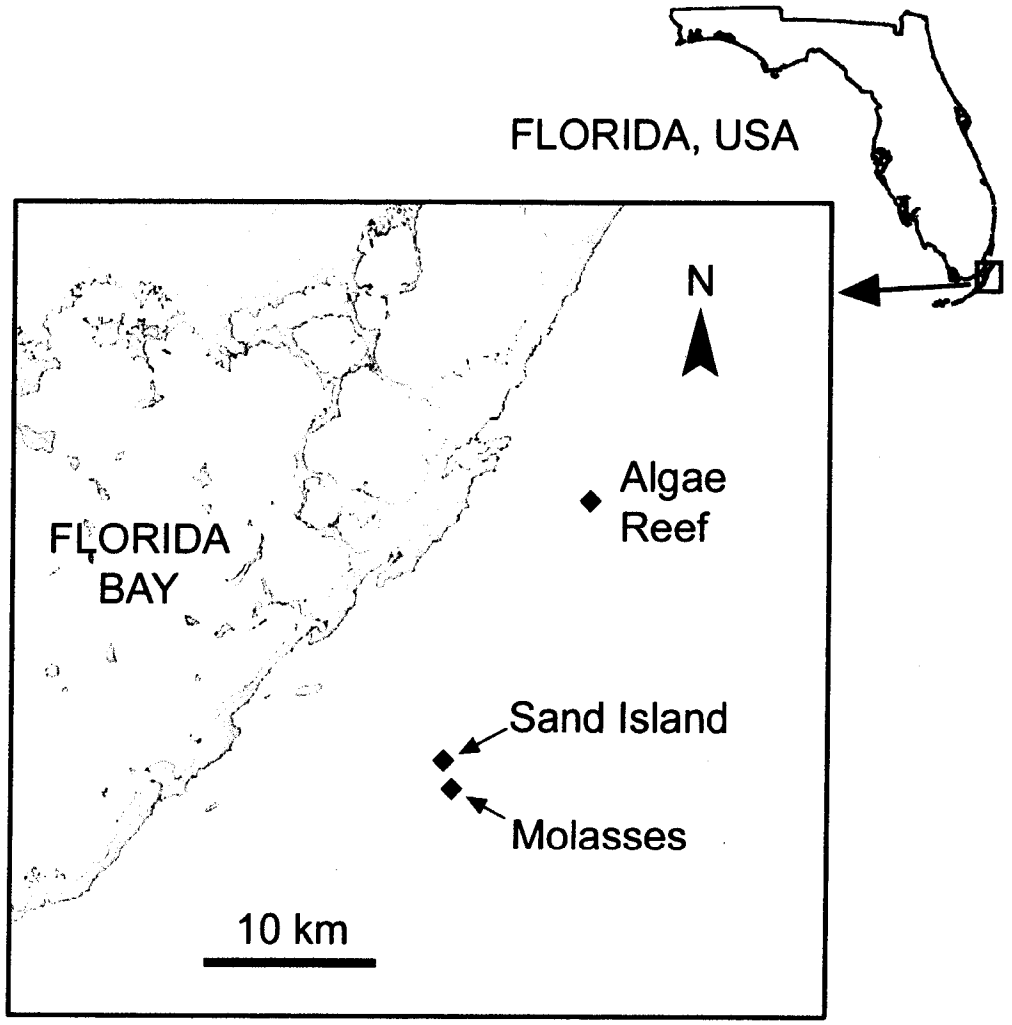


Figure 2. Mean (± s.d.) number of coral larvae settled per plate surface on settlement plates aged *in situ* at each study site (n = 13 plates per site). Settlement plates were 10 cm x cm in surface area, and 2 cm thick. Larvae that settled on plate sides were not censused. Upper panel: *Montastraea cavernosa*; Lower panel: *M. annularis*.

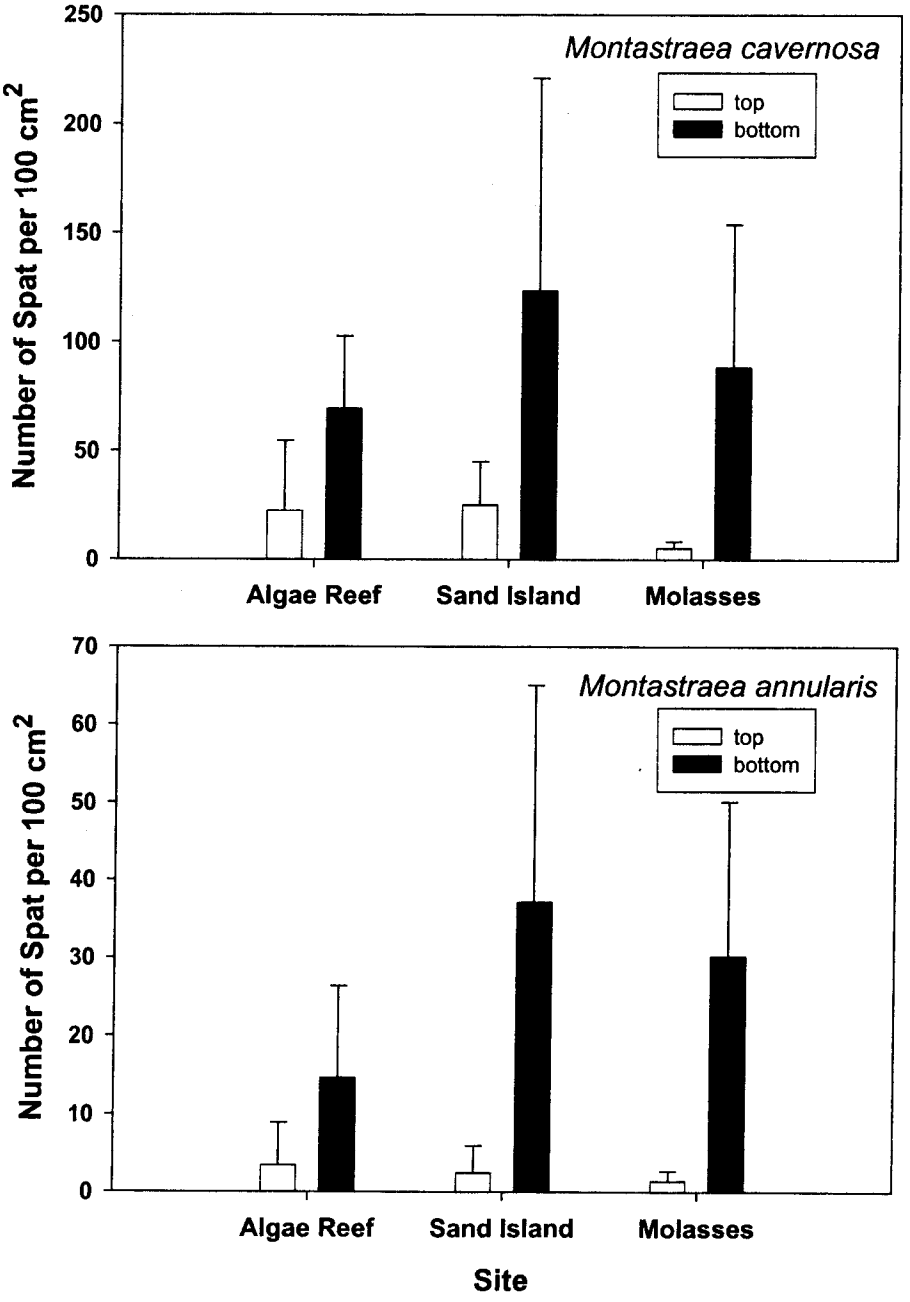
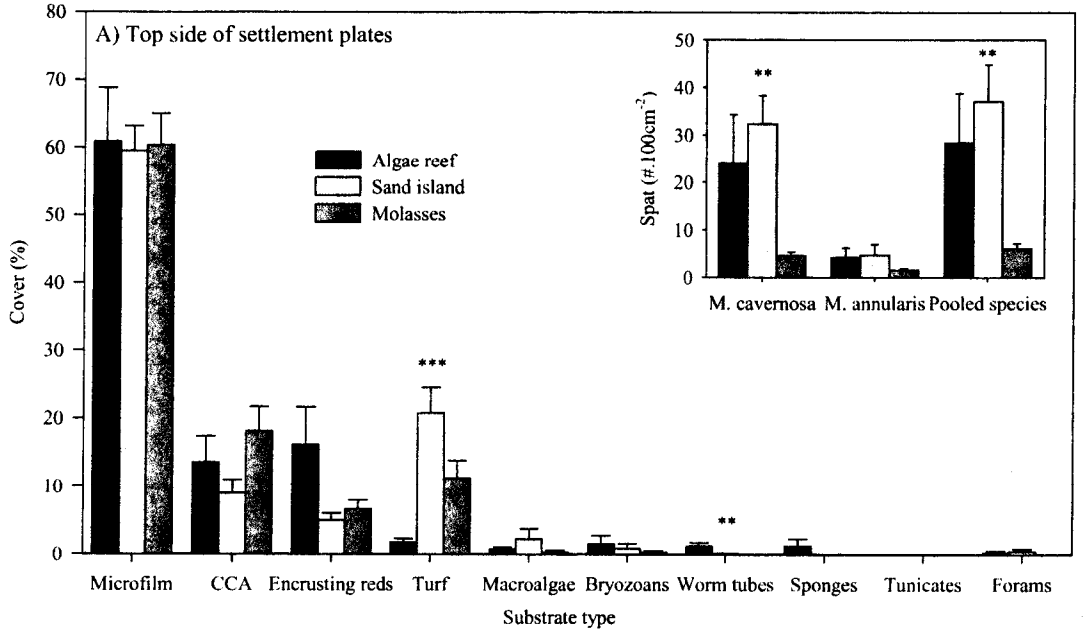


Figure 3. Percent cover of each substrate type for the (A) top and (B) bottom sides of settlement plates at the three study reefs. The inset graph shows spat settlement. Data are means (% ± S.E.M.) of 11 plates. For each graph, sites were ordered from left to right by increasing abundance of coral spats to facilitate visual comparisons between spat abundance and substrate characteristics. Significant differences between sites were tested using Kruskal Wallis test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



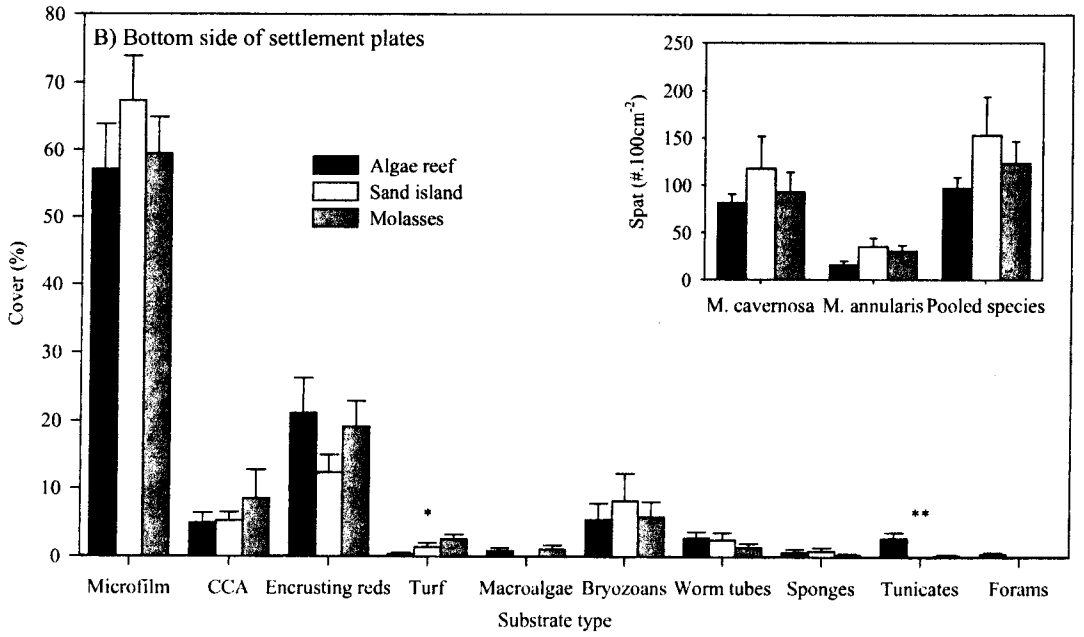
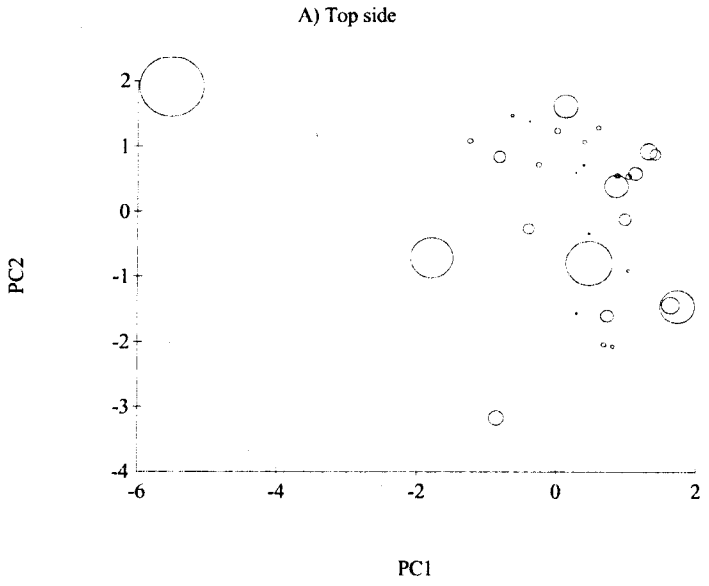


Figure 4. Plots of principal components analysis for the (A) top and (B) bottom sides of settlement plates (pooled sites) based on substrate characteristics. Each point represents data for one plate (n = 33 plates for each graph). The number of coral spats (top side only for (A) or bottom side only for (B)) was superimposed on each data point. The size of each circle is proportional to the number of spats present on the plate. In (A), PC1 explained 27.3 % of the variation; PC2 18.5 %. In (B), PC1 explained 24.2 % of the variation; PC2 19.5 %. No obvious pattern can be seen in the distribution of plates with high spat densities, suggesting no relationship between plate substrate characteristics and spat settlement success.



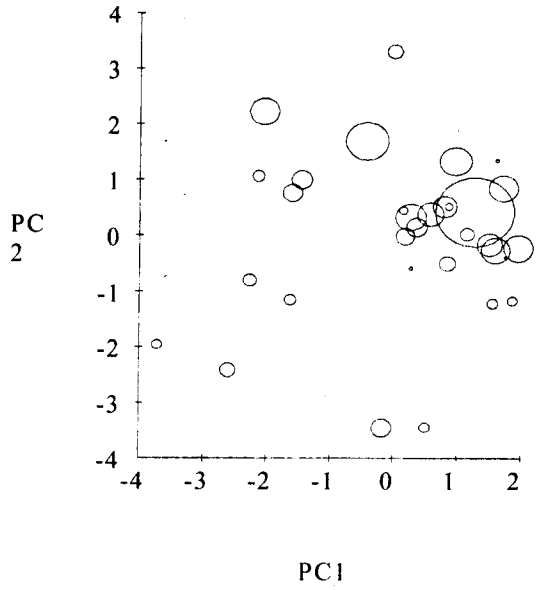


Figure 5. Characteristics of spat and control microhabitats for the top and bottom sides of settlement plates at the three study sites. Data are means (% cover ± S.E.M.) of 12 to 13 plates (n = number of plates). Significant differences between microhabitats were tested using Wilcoxon's signed-ranks tests. * $P < 0.05$, ** $P < 0.01$. CCA = crustose coralline algae (pooled groups). See Figure 6 for data on each CCA group.

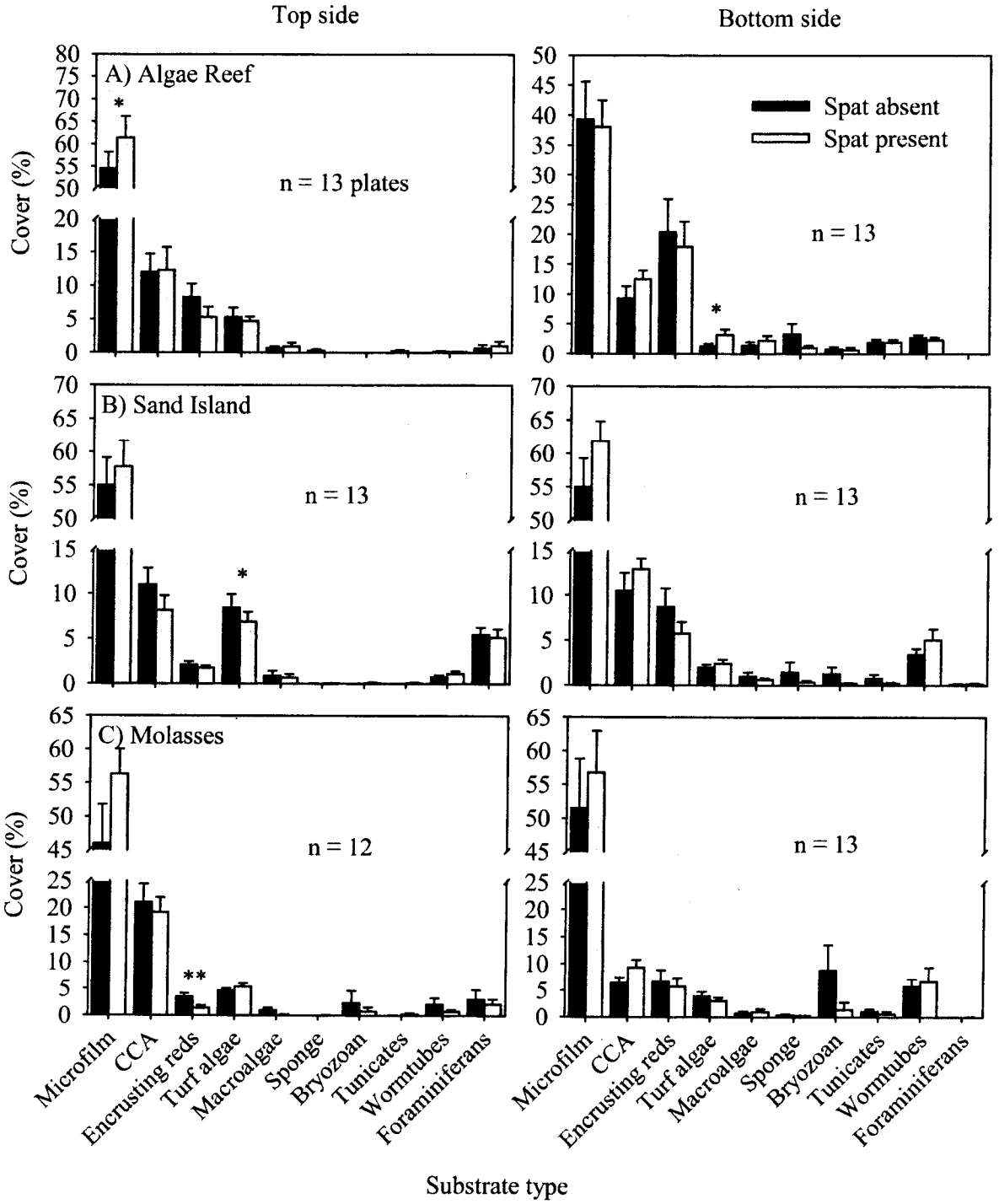


Figure 6. Percent cover of coralline algal groups in spat and control microhabitats for the top and bottom sides of settlement plates at the three study sites. Data are means (% ± S.E.M.) of 12 to 13 plates (n = number of plates). Statistical test and significance levels as in Figure 5.

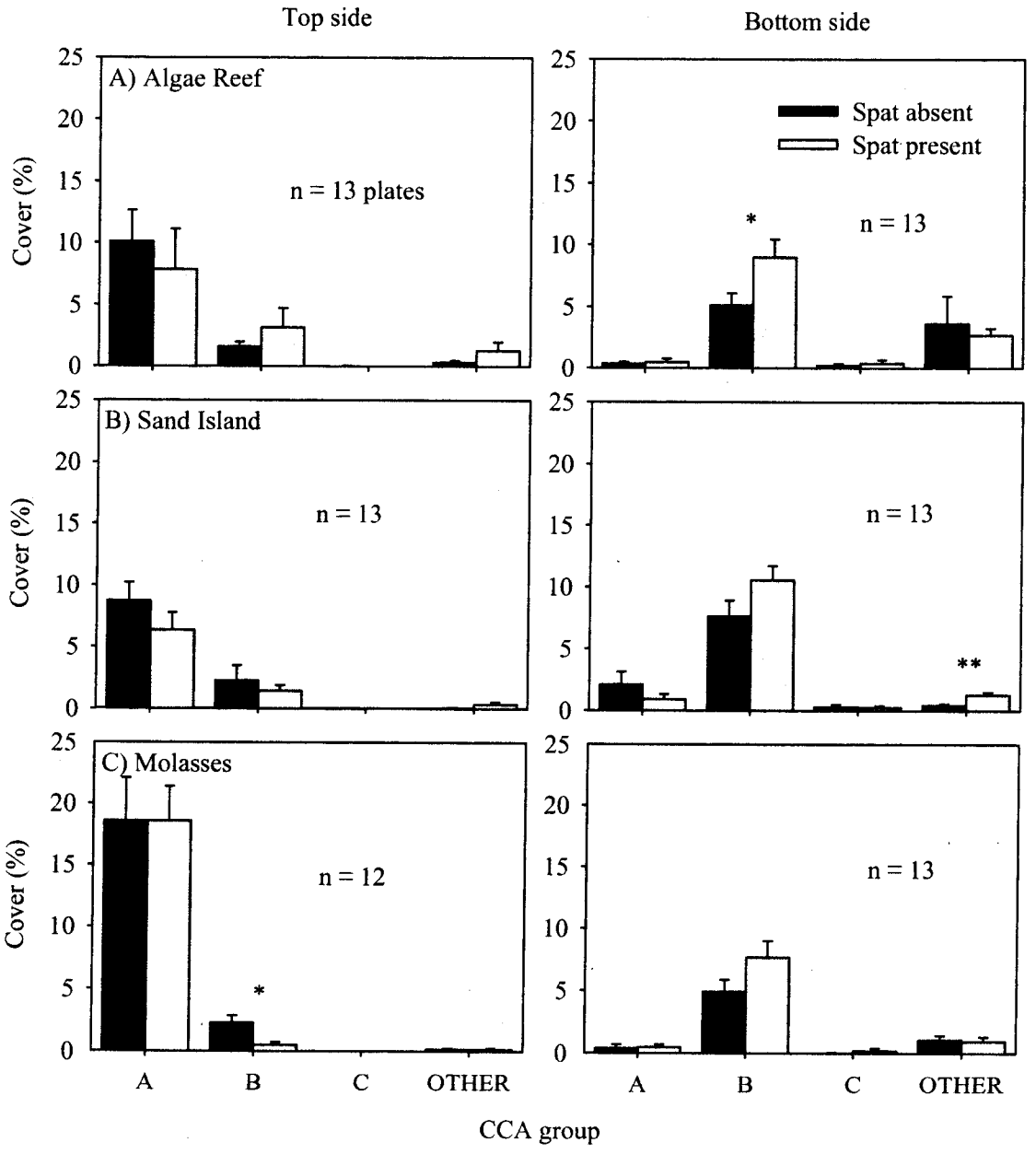
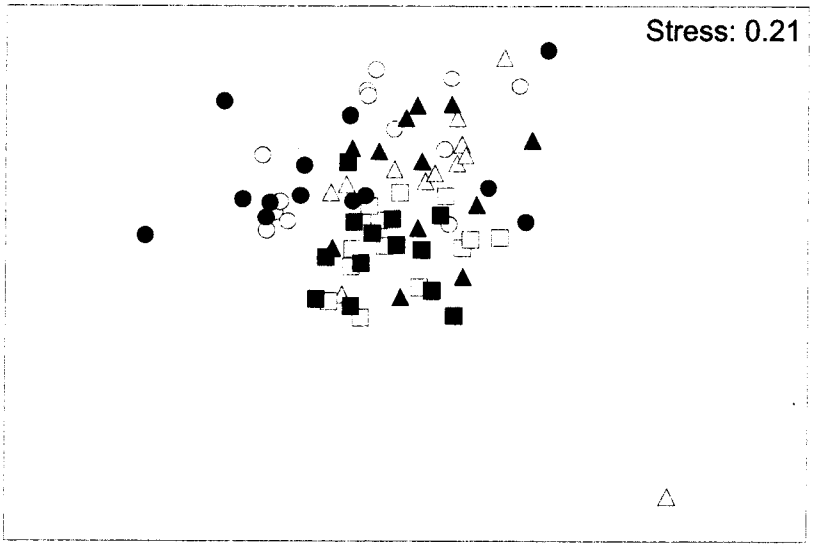


Figure 7. First two axes of the MDS ordination plot for spat (filled symbols) and no-spat (open symbols) microhabitats at Algae Reef (circles), Sand Island (squares) and Molasses (triangles) for (A) top and (B) bottom sides of the settlement plates. Each data point represents the averaged data for 1 to 11 1 cm^2 squares within a plate.

A) Top side (n = 76 plates)



B) Bottom side (n = 78 plates)

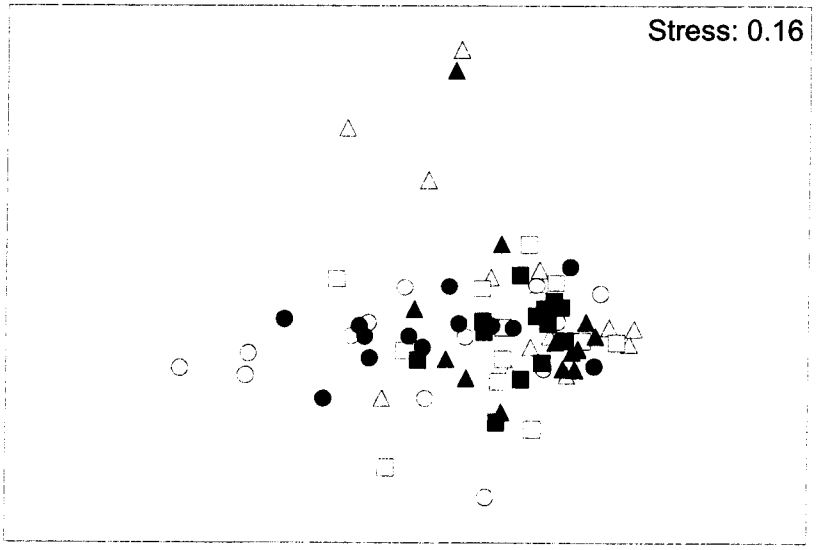


Figure 8. First two axes of the MDS ordination plot for the microhabitat characteristics in top (filled symbols) and bottom (open symbols) sides of the settlement plates at Algae Reef (circles), Sand Island (squares) and Molasses (triangles). Spat and control microhabitats were pooled. Each point represents the pooled data from 1 to 11 1 cm^2 squares within a plate (n = 154).

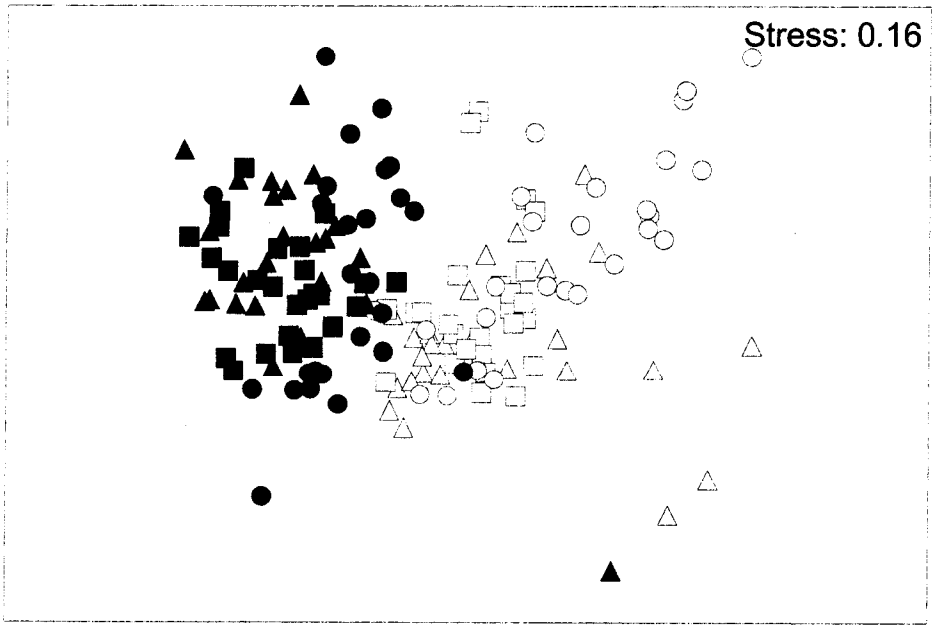


Figure 9. Total number of coral settlers initially mapped on 11 (10 for Sand Island) pre-conditioned settlement plates and total number of spat surviving until each of the subsequent re-surveys. After the initial mapping, plates were returned to their original reef site and then brought back into the laboratory for re-mapping at approximately one month intervals for three months, and then after nine months. Data are plotted for each plate surface for each reef site. Upper panel: *Montastraea cavernosa*; Lower panel: *M. annularis*.

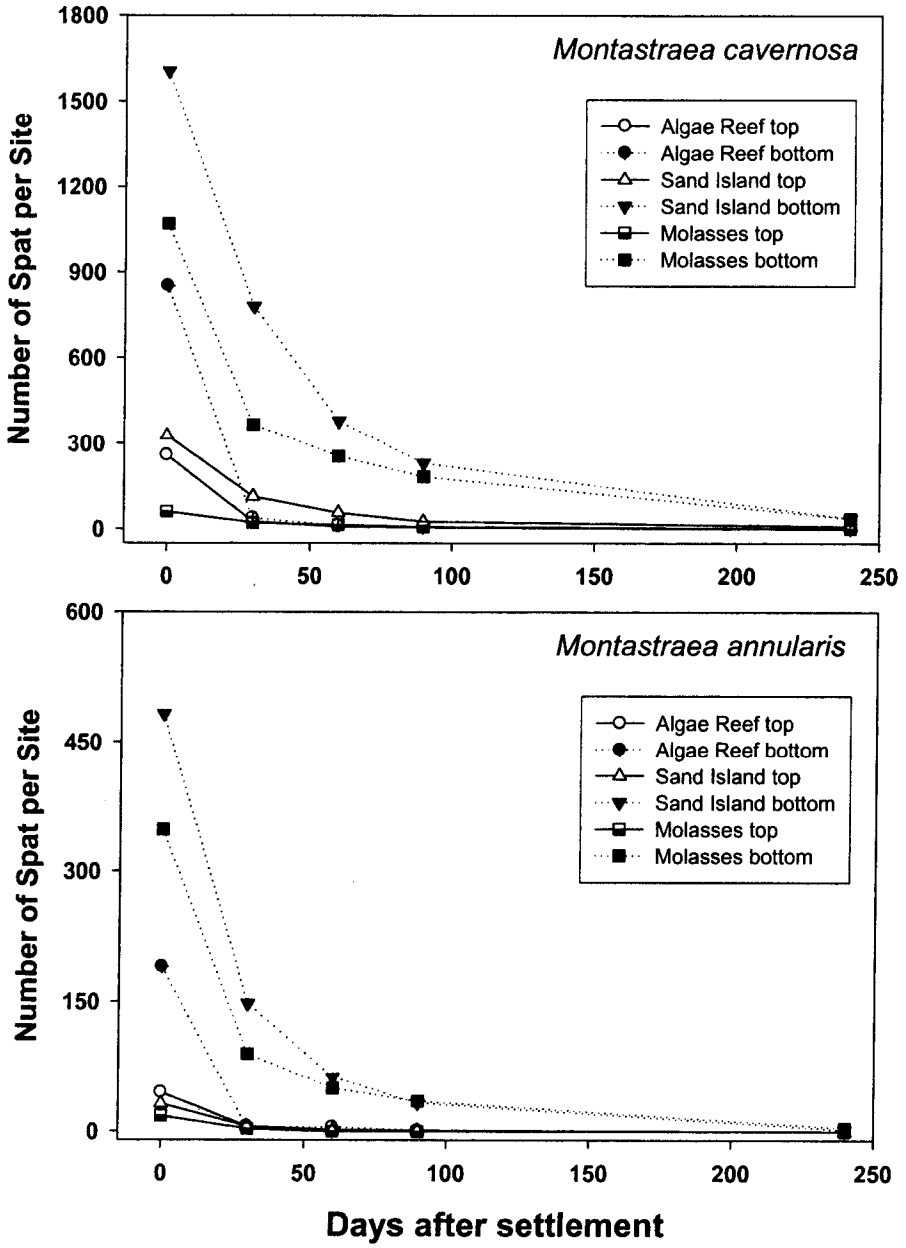


Figure 10. Time-course of percent survivorship of coral spat seeded onto pre-conditioned settlement plates, at each of three reef study sites, and for the two coral species studied. Survivorship for each time interval is based on the initial number of settlers. Data are for the top and bottom plate surfaces combined. Overall percent survivorship was higher for *M. cavernosa* but there were fewer settlers to begin with of *M. annularis*..

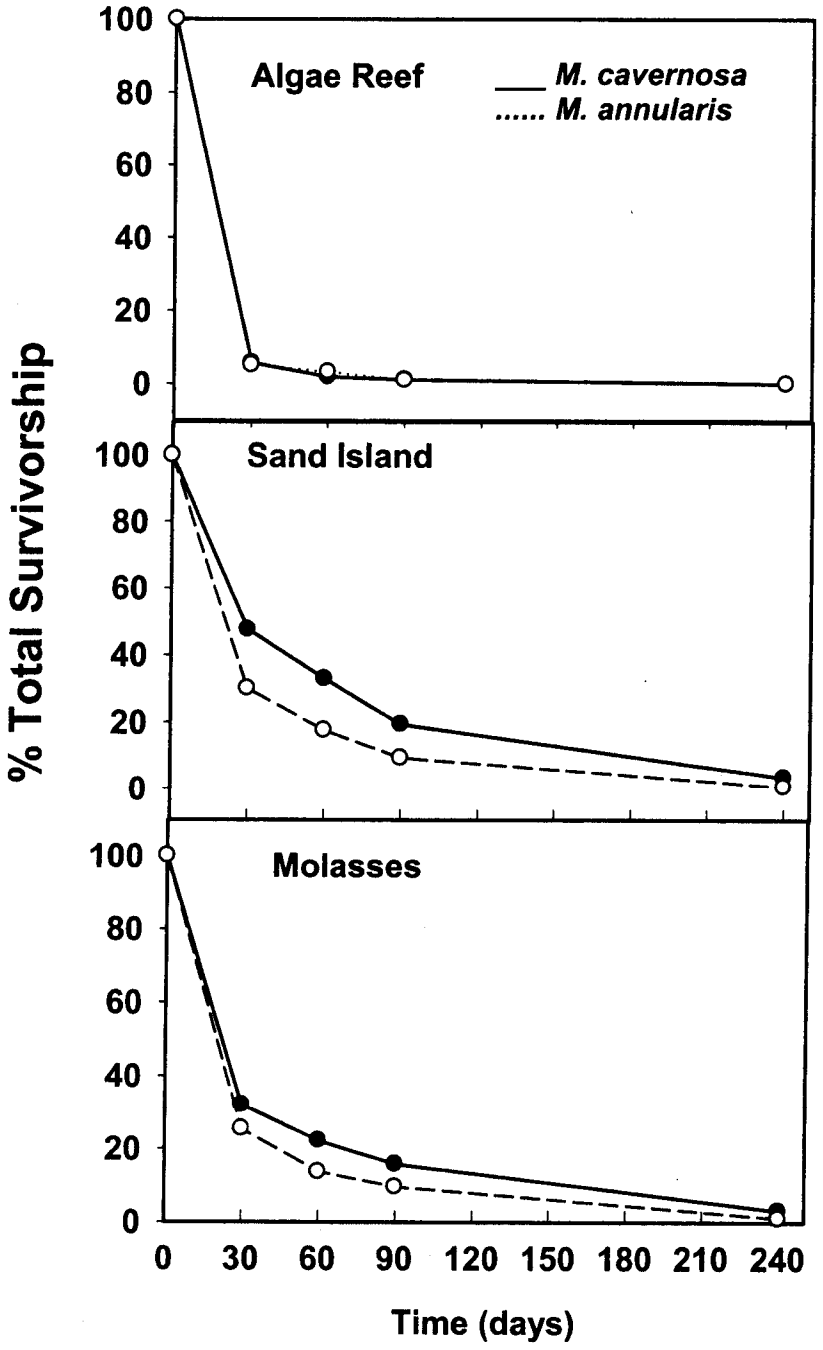


Figure 11. Time-course of progressive percent survivorship of coral larvae seeded onto pre-conditioned settlement plates, at each of three reef study sites, and for the two coral species studied. Survivorship after the initial mapping was calculated based the number present during the previous survey, not on the initial settlement. Data are for the top and bottom plate surfaces combined. Survivorship was lowest during the first 30 days after settlement but increased for those settlers that survived the first 30 days.