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Memphis Project Annual Report July 2000 - July 2001

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Executive Summary

The United States nuclear submarine MEMPHIS grounded in approximately 30 feet of water on a southeastern Florida coral reef off Broward County in February 1993. This grounding caused extensive physical and biological damage to the reef substrate and to the coral community. As part of a mitigation plan, in July 2000 a three-year experimental restoration project was initiated. This is a report of the first 12 months of that project.

In order to gain insight into optimal methodology for restoring corals to a damage site, the project compares settlement, growth, and survival rate of corals amongst artificial reefs treated with potential attractants (iron, quarry rock, transplants, and no-attractant controls). Further, in order to examine appropriate structural design for restoration, the reefs are divided into four treatments of structural complexity. This allows the determination of the interactive effects of four different fish communities on coral settlement and growth. In addition, the work will investigate the potential role of microbial biofilms as settlement precursors. The transplant treatments are identical replicates (same numbers of each species). This will allow the determination of species specific differential survival and growth rates of coral transplants. Finally, the four complexity treatments will yield insight into fish community restoration methodology (the hypothesis here is that multiple refuge size is required for a diverse coral reef community).

The experimental design consists of 160 small (1.13 m) Reef Balls which are organized into 40, 4-module reef units (quads) each in a square configuration with 3 m sides. Each quad will have one Reef Ball with one of the four attractant treatments (iron, quarry rock, coral transplants, or only concrete). Each Reef Ball will have two standardized settlement plates (with the attractant treatment of the Reef Ball) affixed so that observations can be reproducible. The 40 quad are divided into four different levels of structural complexity. One set of 10 quads has the void spaces of all the Reef Balls empty. One set has the void spaces of all filled with structure offering small refuge (plastic caging). Another set of 10 has the void spaces of all filled with large refuge (concrete block). The final set of 10 quads is mixed and has one Reef Ball empty, one with large refuge, and the last two with small refuge. In addition, biofilm discs will be attached to 20 quads, 5 from each treatment. These discs will be removed at 1, 3, 7, 14, 21 and 60 day intervals and examined microscopically for biofouling.

An estimated 146 dives have been made for the project during the first year of the study. One hundred and sixty Reef Balls, 433 settlement plates, and 1500 biofilm discs were constructed July-August 2000. The settlement plates and biofilm discs were constructed at the same time, from the same cement trucks, as the Reef Balls to ensure the concrete mixture was the same for all three structures. The Reef Balls were deployed in November 2000. However, many Reef Balls were not deployed on designated sites and extensive delay to the research was incurred due to the time needed to adjust the positions.

Coral species were selected for transplantation (*Montastrea cavernosa* and *Meandrina meandrites*) and donor colonies located. The first 4 transplants were drilled out of donor colonies using a hydraulic drill in March 2001. The holes in the donor corals were filled with concrete plugs and before-and-after photographs were taken of each site. The cores were then taken to the Reef Ball site and epoxyed into the appropriate Reef Balls. The last of the 80 transplants were completed in July 2001.

All the settlement plates and biofilm discs were coated with concrete and attractants (iron filings, calcium carbonate sand) or concrete without attractants (controls) in July 2001 and will be deployed late July early August. Some preliminary study on methodology for the biofilm study has been completed; however, research data have not been collected to this point; all the work completed this year involved setting up the study. Quarterly data collection will be initiated in September 2001.

Reef Ball Construction and Deployment

Construction of Reef Balls™ began on July 31, 2000 and was completed August 18, 2000. The construction involved a total of 494 labor hours and concluded with the completion of 168 Reef Balls (suitable for deployment), 433 settlement plates and 1500 biofilm discs. The settlement plates and biofilm discs were constructed at this time to ensure the concrete mixture was the same for Reef Balls and attractant structures. Approximately 40 additional Reef Balls were constructed that were rejected due to flaws. The Reef Balls and settlement plates were stored at Nova Southeastern University's Oceanographic Center (NSUOC) until deployment.

On September 12, 2000 NSUOC personnel surveyed the deployment site to map the reef edge and search for hard bottom between the reefs. Deployment of the Reef Balls took place on November 17, 2000. The first quad (group of four Reef Balls) was deployed at approximately 0700 and the 40th quad was deployed at approximately 1420.

NSUOC personnel attempted to map the grid of quads from November 27, 2000 to January 4, 2001 using SCUBA divers with slates. After six dives, the Reef Balls did not appear to be in a recognizable pattern and eight quads could not be found. To obtain a more detailed map and find the missing quads, NSUOC personnel chose a day (January 6, 2001) with very good surface-to-bottom visibility and live-boated over the grid area. DGPS coordinates were recorded each time the boat passed over a quad. The coordinates were then entered into a program written specifically for the purpose of charting the grid. Divers then swam the grid with a laminated hard-copy of the chart and signaled the boat each time they were at a quad. The signal was to submerge the dive flag and then hold the flag directly over the quad. The boat came up to the flag, paused and the DGPS coordinate was recorded. This was accomplished on January 11-12, 2001 and the missing eight quads were found. The coordinates were reentered into the charting program to acquire an accurate map of the grid area. It was determined that approximately 16 quads would need to be repositioned to obtain the requisite 30-meter separation. In addition, a number of individual Reef Balls within quads were not spaced correctly. NSUOC personnel started repositioning the Reef Balls on February 21, 2001. This involved 2-3 divers and 4-5 100 lb. lift bags for each Reef Ball. The bags were attached to a Reef Ball, inflated and the divers maneuvered the Reef Ball to obtain the correct spacing. This work was completed on February 21, 2001; however an additional two Reef Balls were later found that require correct spacing (Figure 1). Broward County DPEP and NSUOC personnel started moving quads to obtain the requisite 30 meter separation distance on March 3, 2001 and completed the task on June 5, 2001. Approximately 29 dives were made to move 80 Reef Balls. There appear to be two sets of quads that still do not have the correct 30-meter separation distance, but exact measurements have not been made at this time. Each quad is labeled with a 3x5 inch plastic laminated tag containing the quads specific number. The DGPS coordinates and label numbers are listed in Table 1.

Figure 1. Example of Reef Balls that need to be repositioned (Quad 19). Transplant coral cores are visible in front right Reef Ball.

Coral Transplantation

From January 26 to February 8, 2001, 89 concrete plugs were made to fill the holes in donor corals that would be formed by removing the drilled transplant coral cores. On April 5, 2001 the plugs were shortened to better fit the donor corals. The coral transplantation work began on September 12, 2000 with a dive on the second reef, near the Memphis grounding site, to assess the area for suitable species and numbers of donor corals. *Montastrea cavernosa* and *Meandrina meandrites* were selected as transplant species due to availability and colony size. The first four colonies were drilled using a Stanley Hydraulic drill on March 14, 2001. The holes in the donor corals were filled with concrete plugs and before-and-after photographs were taken. The cores were then taken to the Reef Ball site and epoxyed into the appropriate Reef Balls (Figure 2).

Figure 2. *Meandrina meandrites* core transplanted into a Reef Ball.

Table 1. Final DGPS coordinates of the Reef Ball Quads

Latitude	Longitude	Quad Label
26 03.369	80 05.798	1
26 03.342	80 05.797	2
26 03.324	80 05.798	3
26 03.305	80 05.804	4
26 03.285	80 05.804	5
26 03.261	80 05.800	6
26 03.241	80 05.804	7
26 03.226	80 05.799	8
26 03.205	80 05.798	9
26 03.190	80 05.799	10
26 03.375	80 05.770	11
26 03.360	80 05.779	M1 (will be re-labeled 12)
26 03.288	80 05.744	13
26 03.273	80 05.782	14
26 03.170	80 05.801	15
26 03.177	80 05.784	16
26 03.161	80 05.777	17
26 03.142	80 05.759	18
26 03.143	80 05.778	19
26 03.161	80 05.756	20
26 03.183	80 05.760	21
26 03.202	80 05.764	22
26 03.219	80 05.748	23
26 03.226	80 05.756	24
26 03.250	80 05.754	25
26 03.267	80 05.762	26
26 03.272	80 05.744	27
26 03.286	80 05.758	28
26 03.312	80 05.758	29
26 03.326	80 05.760	30
26 03.344	80 05.767	31
26 03.357	80 05.750	32
26 03.190	80 05.777	33
26 03.207	80 05.779	34
26 03.224	80 05.780	35
26 03.242	80 05.777	36
26 03.257	80 05.812	37
26 03.291	80 05.782	M4 (will be re-labeled 38)
26 03.320	80 05.780	M2 (will be re-labeled 40)
26 03.307	80 05.778	M3 (will be re-labeled 39)
26 03.373	80 05.749	Extra RBs (3 Reef Ball 'quad' w/ a broken RB)
26 03.382	80 05.746	Extra RBs

Corals for donors and controls (non-drilled colonies) were mapped (Figure 3), tagged and photographed from April 2, 2001 through June 11, 2001. Requirements for controls were specimens of the appropriate size for both donors and transplants. The trigger mechanism on the drill malfunctioned in April and the drill was taken in for service. Technical problems continued with the drill finally resulting in a new drill being sent to NSUOC on June 5, 2001. Drilling of donor corals resumed on June 15, 2001 and was completed on July 6, 2001. Coral cores were placed in the appropriate Reef Balls the day each core was drilled. The cores were photographed and secured into the Reef Balls with epoxy from June 19 to June 24, 2001. Donor corals were photographed and plugged from June 24 to July 10, 2001. Approximately 61 dives were made to setup the coral transplantation and monitoring aspects of the project.

Complexity Fill

Plastic cage material and cinder block were use for the small fill and large fill respectively. Approximately 50 m of plastic cage material (2 cm grid) were cut into triangular shapes, rolled into cones and the cones were tie-wrapped into the Reef Balls by divers. The cinder blocks were dropped overboard at the location of each quad assigned to have large fill. Divers then collected the block and placed them inside the Reef Balls. This work began on May 7, 2001 and was completed on July 6, 2001.

Coral Attractants

Approximately 330 settlement plates have been coated with the appropriate attractants (iron granules, quarry rock, or plain concrete) and stored at NSUOC. The plates will be cemented onto the Reef Balls in July-August 2001.

Biofilm Research

Approximately 1500 biofilm discs were made during the Reef Ball's construction of the same concrete mix. The discs have been coated with the appropriate attractants (iron granules, quarry rock, or plain concrete) and are stored at NSUOC. The discs will be attached in an array (Figure 4) on the Reef Balls in July-August 2001.

A preliminary technique study of biofilm attachment to the discs was accomplished in November 2001 and the results were presented at American Society Limnology and Oceanography Conference 2001 in Albuquerque, NM (Figure 5).

Research Data

Research data have not been collected to this point as all the work completed this year was required for setting up the study

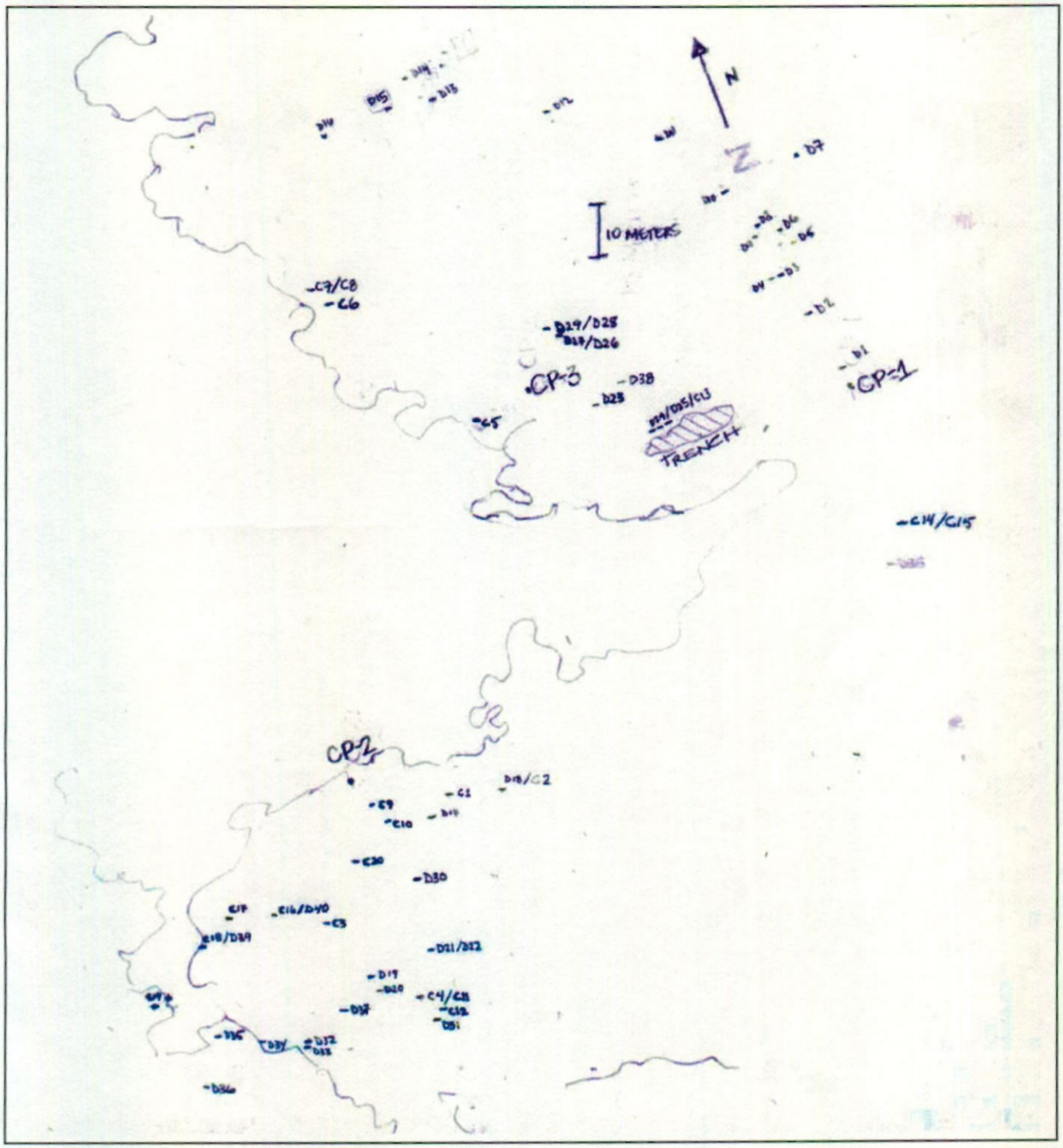
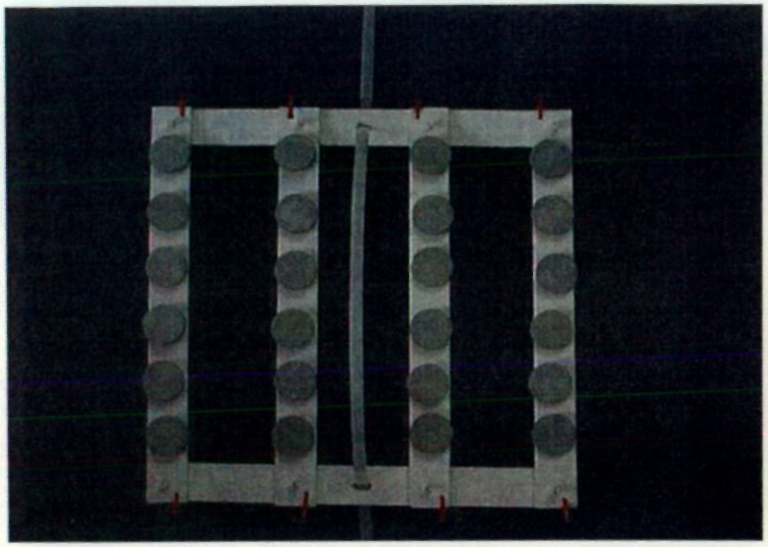
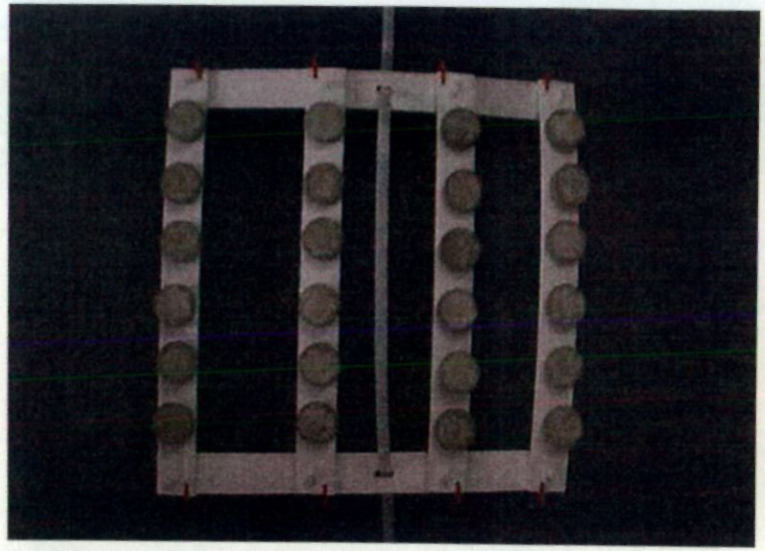


Figure 3: Map of second reef depicting site of donor (D) and control (C) corals (note area of USS Memphis grounding trench and DPEP damage control pins: CP1, 2, & 3).



A.



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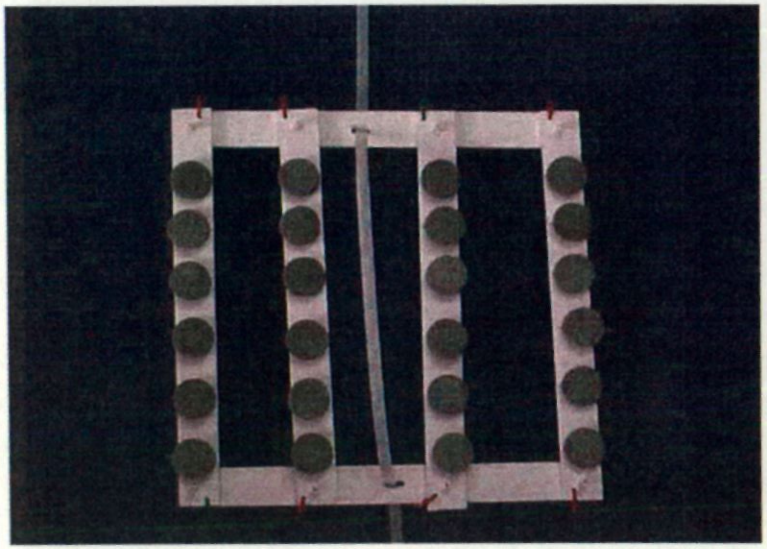

C.

D.

Figure 4. Photos of biofouling disc arrays. A. concrete discs only, B. concrete discs with quarry rock, C. concrete discs with iron powder, and D. glass slides.

Preliminary Analysis of Initial Microfouling of a Nearshore Artificial Reef in Broward County, Florida

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Introduction

The majority of artificial reef studies have focused on the interaction between fish density and artificial physiography. Although colonization of artificial reef structures has been studied to varying degrees (Gascon and Miller 1981; Bonsack and Sutherland 1985; Haughton and Aiken 1989), relatively few studies have quantitatively described the development of "attached" microbial or algal assemblages on artificial reef structures (i.e. Reef-balls, Fig.1).

Development of microbial and algal films is important because they directly occupy the substratum and provides secondary biotic space in the form of "lower story" habitats (Dayton 1971). Additionally, chemosensory recognition of microbial and algal biofilms is thought to play a role in inducing settlement and metamorphosis of a wide variety of marine invertebrate larvae, at least in the early stages of substratum colonization or succession (Morse *et al.* 1994). Given the biological significance of these communities to overall reef productivity, we examined the first steps of fouling by microbial and algal biota during the initial days of immersion of Reef-ball substrates.

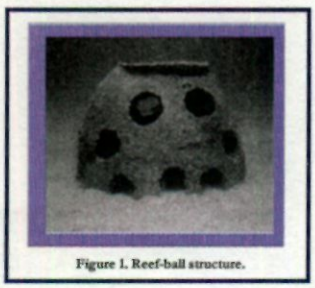

Figure 1. Reef-ball structure.

Study locale

The nuclear submarine USS MEMPHIS grounded in 30 ft of water on a coral reef off the coast of southeast Florida in February 1993. This grounding caused extensive physical and biological damage to the reef substrate and to the coral community. Broward County authorities have partnered with the National Coral Reef Institute at Nova Southeastern University to perform hypothesis-based research to document pre-restoration site conditions and outcomes. This includes the installation of restoration structures (Reef-balls) and monitoring of changes in the biological and ecological characteristics over time. In October 2000, Reef-balls were deployed .05 miles offshore of Hollywood Beach on a sandy bottom site between the second and third reef lines (Fig. 2). This area is situated adjacent to the grounding site of the USS MEMPHIS.

An additional site (Fig. 2), a hardbottom reef area, was also chosen for biofilm plate deployment.

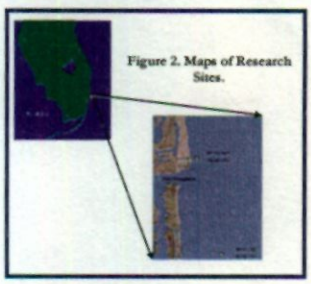

Figure 2. Maps of Research Sites.

Methods

Reef-ball Samples

- Samples were collected from Reef-ball structures after immersed for 4 weeks.
- Samples were mixed with 2µm filtered seawater. The mircobiota were fixed in 4% formaldehyde and stained with DNA specific fluorochrome DAPI.
- Bacterial and algae were enumerated by epifluorescence microscopy.

Biofilm Plate Samples

- Biofilm plates (constructed of Reef-ball concrete) were submerged for 18 days.
- After collecting, biofilm plates were examined under a dissecting microscope for settlement of small invertebrates.
- For examination of the biofilm, 3.5 cm^2 of plate surface was scraped off and mixed with 2µm filtered seawater. The mircobiota were fixed in 4% formaldehyde and stained with DNA specific fluorochrome DAPI.
- Bacterial and algae were enumerated by epifluorescence microscopy.
- Plates were prepared for SEM by dehydrating through an acetone series and drying with HMDS. After palladium coating, samples were examined with a ISI-DS-130 dual state SEM.

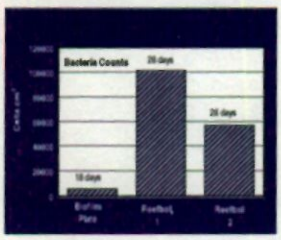

Figure 5a. Number of attached bacteria cm^{-2} determined by epifluorescence microscopy.

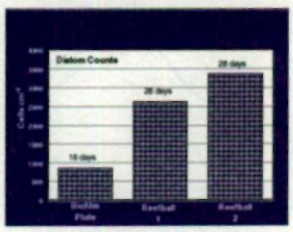

Figure 5b. Number of diatoms cm^{-2} determined by epifluorescence microscopy.

SEM Images

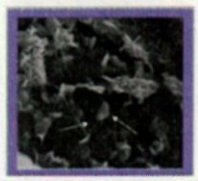

Figure 3a. Unidentified biota with attachment structures. Possibly bacteria distorted by the fixation procedures or the chemical nature of the concrete substrate.

Figure 3b. Scanning electron mircograph of fungi colonizing the biofilm plate.

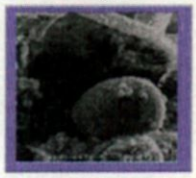

Figure 3c. Scanning electron mircograph of an invertebrate egg found on biofilm plate.

Results

Colonization of Substrate

- Initial microfouling on Reef-balls and biofilm plates consisted of fungi (Fig 3a), diatoms, filamentous algae, and bacteria (Fig. 4 a,b and c).
- The number of bacteria on the biofilm plate reached 5535 cells cm^{-2} after 18 days of submergence, whereas, bacterial numbers on the Reef-balls were >50,000 cells cm^{-2} but <120, 000 cells cm^{-2} after 28 days of submergence (Fig. 5a).
- Diatoms settled on the biofilm plate (848 cells cm^{-2}/18 days) as well as the Reef-ball structures (>2500 cells cm^{-2}/ 28 days, Fig. 5b).
- No obvious bacterial cells were observed by SEM. This was probably due to (a) the low number of bacteria in the film and (b) the excessive precipitation of concrete components during SEM preparation. Visualization of bacteria was possible with epiflourescence microscopy (Fig. 4 a,b, and c).
- Early secondary colonizers on the biofilm plate were mainly composed of copepods, small molluscs, and polychaetes.

Epifluorescence Images

Figure 4 a,b, and c. Micrographs (1000X) of microbiota stained with DNA specific flourochrome DAPI.

Discussion

Our preliminary results demonstrate that bacteria and algae colonized Reef-ball concrete surfaces and the number per unit area appeared to increase with time (Fig. 5a,b) However, the number of bacterial colonizers was low compared to film formation on other substrates i.e. glass slides, 10^{6} cells cm^{-2} day^{-1} (???), or polystyrene petri dishes, 10^{6}-10^{7} cells cm^{-2} day^{-1} (Maki *et al.* 1989). Initial substrate conditions may not have been attractive to microbial colonizers, in part due to the potential interaction with the harsh environment of the concrete surface (pH >11, per. com. Reefball Int.), which may have affected biofilm formation and attachment preferences.

Microbial and algal films are well known as probable settlement inducers of benthic marine invertebrates, in addition, to providing secondary biotic space and shelter. Therefore, the period of submersion and subsequent biofilm formation may be of paramount importance when assessing the colonization of secondary fauna on these artificial reef structures.

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Figure 5.