1-1-2002

Memphis Project Annual Report: July 2001 - July 2002

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Submitted to:
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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 second year of that project. However, the annual reports are cumulative in text, data and analyses and therefore this report also contains information from the first year's work.

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 investigates 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 has Reef Balls with the four-attractant treatments, one Reef Ball per attractant (iron, quarry rock, coral transplants, or only concrete). Each Reef Ball has two standardized settlement plates incorporating the attractant treatment of the Reef Ball. The 40 quads 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, for two months, biofilm discs were attached to 20 quads, 5 from each treatment.

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. The final arrangement was achieved in June 2001. Settlement plates were attached in August 2001. Quarterly data collection was initiated in October 2001. In excess of 520 individual SCUBA dives have been made to date.

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 were deployed in August 2001. These discs were removed at 1, 3, 7, 14, 21 and 60 day intervals and examined microscopically for biofouling. Data analysis was completed in July 2002. The control discs and those incorporating the calcium carbonate did not differ in the settlement rate of bacteria or diatoms. However, the discs with iron filings had a significantly slower settling rate than both the calcium carbonate and control discs.

A study was also initiated to examine the potential for a red coralline alga (*Hydrolithon boergesenii*) to act as an attractant for coral settlement on restoration concrete structure. Settlement plates were formed into tent shaped modules by the addition of a concrete base and deployed at four sites, eight modules per site. One site was on a field of coral rubble with abundant *H. boergesenii.* A second nearby site on a sand field was selected as control, and cleaned of incidental algae coated rubble for a 10m radius. The third site was adjacent to hard bottom on a rubble field with abundant *H. boergesenii*. Site four, the control for #3 was located on hardbottom with abundant hard corals, but which lacked *H. boergesenii* or abundant rubble. Our hypothesis is: if *H. boergesenii* provides a coral attractant, more hard coral should recruit to plates surrounded by the algae then in control areas without the presence of the algae.

Caging and concrete fill was added to the Reef Balls in May-July 2001 to acquire differential complexity. As hypothesized, at 12 months, there are different fish assemblages associated with the differing fill. For total abundance of fish, the empty reef balls did not differ from those with small fill however both these treatments were significantly less than either mixed or large fill which did not differ from each other. With species richness, the empty reef balls had fewer species than those with small fill which, in turn, had fewer than either mixed or large fill treatments which did not differ from each other. An understanding of the potential interaction of these differing assemblages with coral recruitment and mortality awaits photographic analysis of the settlement plates.

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. After nine months of sampling, 100% of the *M. cavernosa* and 71% of the *M. meandrites* transplants maintained their original tissue surface area or showed evidence of an increase in surface area. The remaining 29% of the *M. meandrites* transplants have shown varying degrees of partial tissue mortality. The donor colonies have experienced 100% colony survival. The core hole sites have not regenerated tissue over the concrete plugs. However, there has been little tissue die back from the plug sites and so regeneration remains possible. Although it is too early in the study to draw firm conclusions, the species specific differences in transplant growth and mortality may be an important consideration in future coral reef restoration efforts.

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INTRODUCTION

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 second year of that project. However, the annual reports are cumulative in text, data and analyses and therefore this report also contains information from the first year's work. A breakdown of the annual task accomplishment is provided in the project timeline and the quarterly reports (see Appendix).

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. 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). In addition, two sub-studies investigate the potential role of microbial biofilms as settlement precursors and the potential of a coralline red algae (*Hydrolithon boergesenii*) to act as an attractant to restoration-concrete structure.

METHODS AND MATERIALS

Experimental Design

The experimental design consists of 160 small (1.13 m) Reef Balls that are organized into 40, 4-module reef units (quads) each in a square configuration with 3-m sides. Each quad has Reef Balls with the four-attractant treatments, one Reef Ball per attractant (iron, quarry rock, coral transplants, or only concrete). Each Reef Ball has two standardized settlement plates incorporating the attractant treatment of the Reef Ball. The 40 quads 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 were attached to 20 quads, 5 from each treatment. These discs were removed at 1, 3, 7, 14, 21 and 60-day intervals and examined microscopically for biofouling.

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 approach the desired 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 required correct spacing (Figure 1). Broward County DPEP and NSUOC personnel started moving quads for the desired 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 were two sets of quads that still did not have the full 30-meter separation distance but it was decided to take this into account, if necessary, in statistical evaluations and proceed with the study. Each quad is labeled with a 3x5 inch plastic laminated tag containing the quad's specific number. The DGPS coordinates and label numbers are listed in the appendix.

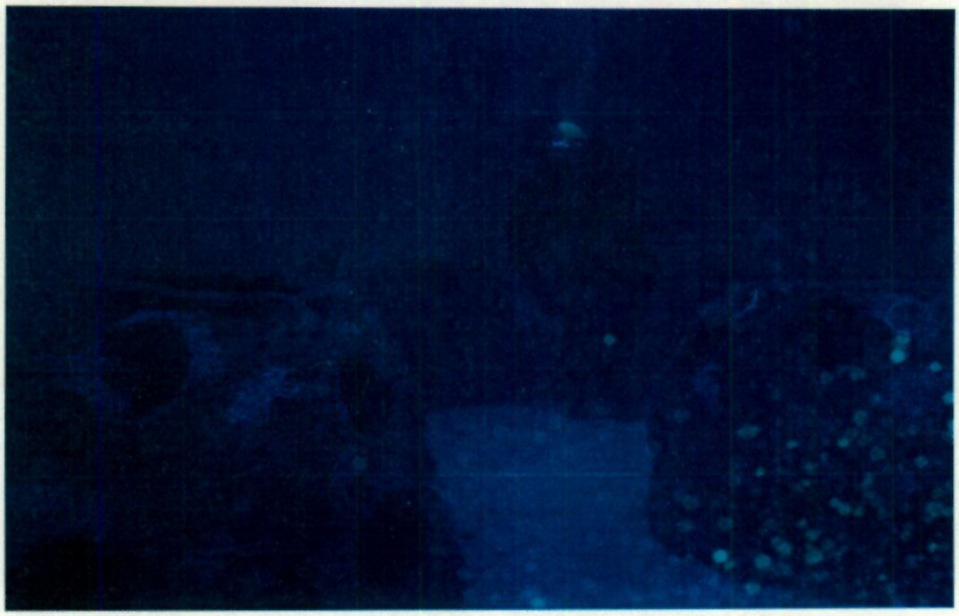


Figure 1. Example of Reef Balls that needed to be repositioned (Quad 19). Transplant coral cores are visible in front right Reef Ball.

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).

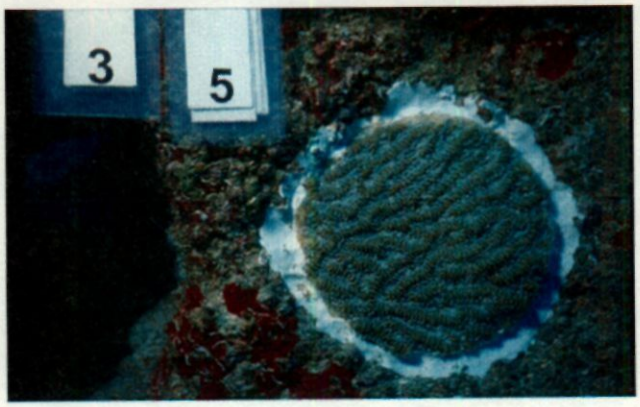


Figure 2. *Meandrina meandrites* core transplanted into a Reef Ball.

Corals for donors and controls (non-drilled colonies) were mapped (see Appendix), 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.

Photographs, taken at quarterly intervals, are being used to determine coral growth over the course of the study. The first complete monitoring session for 2001 was June-July. Monitoring consisted of a photographic (slide) image of each study coral. Photographic images of the transplants, the core hole sites (in the donor corals), and the small controls are recorded using a Nikonos V camera with a 28 mm lens and close up kit. Photographic images of the entire donor colonies and the large control colonies are recorded using a Nikonos V camera and a 20 mm lens with a 0.75 m^2 PVC framer marked in 10 cm increments. The resulting slide images are scanned using a Hewlett Packard Photosmart S20 slide scanner. SigmaScan Pro4 image analysis software (Jandel Scientific Corporation) is being used for the analysis of the slide data. Individual slides have been calibrated using a ruler, included in the image, then digitized and measured in order to determine tissue growth or retreat over time. This photographic technique has allowed growth to be measured continually using an accurate and non-invasive methodology. This technique is one of the few monitoring methods in which the coral colony is not sacrificed and in which changes in planar growth can be accurately assessed.

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 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 320 settlement plates were coated with the appropriate attractants (iron granules, quarry rock, or plain concrete) and cemented onto the Reef Balls in August 2001.

Biofilm Research

Approximately 1500 biofilm discs were made during the Reef Ball's construction of the same concrete mix. The discs were coated with the appropriate attractants (iron granules, quarry rock, or plain concrete) and stored at NSUOC. The discs were attached in an

array on extra Reef Balls deployed outside, but adjacent to, the main study area in 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 (Appendix). The biofilm discs were collected over a two-month period and analyzed. Data analysis was completed July 2002. A full discussion of the biofilm methods is included in an attached report (Appendix).

Red Algae as Coral Attractant Study

A study was initiated to examine the potential for using a red coralline alga (*Hydrolithon boergesenii*) to enhance recruitment to restoration structure. Settlement plates, made in July-August 2000 with the other settlement plates used on the Reef Balls, were formed into tent shaped modules by the addition of a concrete base (Fig. 3) and deployed July 18 and 19, 2002. The modules were placed in Broward County at four sites, eight modules per site. One site was on a field of coral rubble with abundant *H. boergesenii.* A second nearby site on a sand field was selected as control, and cleaned of incidental algae coated rubble for a 10m radius. The third site was on a rubble field adjacent to hard bottom. It also had abundant *H. boergesenii,* although on this site the algae appeared to be predominately restricted to the underside of rubble pieces. Site four, the control for #3 was located on the hardbottom with abundant hard corals but which lacked *H. boergesenii* or abundant rubble. Our hypothesis is: if *H. boergesenii* provides a coral attractant, more hard coral should recruit to plates surrounded by the algae then in control areas without the presence of the algae. The settlement plates will be examined quarterly for recruitment.

Figure 3. Example of settlement module.

RESULTS

Coral Transplants

After nine months of sampling, (image analysis is currently in progress on the last data set) there was a highly significant difference ($p<0.01$, G-test) between the two species of transplants in growth/mortality; 100% of the *M. cavernosa* (Fig. 4, 5) and 71% of the *M. meandrites* transplants maintained their original tissue surface area or showed evidence of an increase in surface area. The remaining 29% of the *M. meandrites* transplants have shown varying degrees of partial tissue mortality.

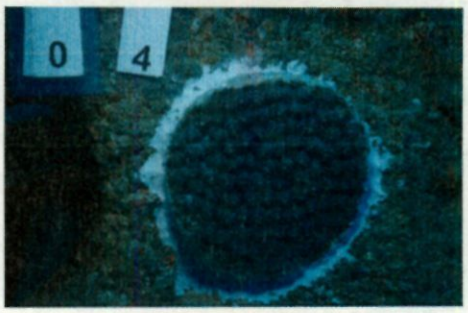


Figure 4: Transplant #4 on March 16, 2001

Figure 5: Transplant #4 on March 13, 2002. Note tissue growth over surface of Reef Ball on lower right.

The donor colonies have experienced 100% colony survival. The core hole sites have not regenerated tissue over the concrete plugs. However, there has been little tissue die back from the plug sites and so regeneration remains possible. These data will be presented at the International Society for Reef Studies in September (see Appendix).

Biofilm

The control discs and those incorporating the calcium carbonate did not differ in the settlement rate of bacteria or diatoms. However, the discs with iron filings had a significantly slower settling rate than both the calcium carbonate and control discs (see Appendix for full report and complete results).

Fish Assemblages

As hypothesized there are different fish assemblages associated with the differing fill. For total abundance of fish the empty reef balls did not differ from those with small fill however both these treatments were significantly less than either mixed or large fill ($p<0.05$, ANOVA, SNK) which did not differ from each other ($p>0.05$, SNK). With species richness, the empty reef balls had fewer species than those with small fill which, in turn, had fewer than either mixed or large fill treatments ($p<0.05$, ANOVA, SNK) which did not differ from each other ($p>0.05$, SNK) (see Appendix).

Settlement Plates

Several corals have recruited to and grown on the Reef-Ball-attached settlement plates sufficient to allow ready recognition as scleractinian corals. However, additional growth is required for more specific identification and additional recruitment is required to provide adequate numbers for rigorous statistical evaluation among settlement treatments. In the coming year, we intend to photograph and analyze growth with the same methodology used with the transplanted corals.

DISCUSSION

The similarity of the microbial fouling of concrete and calcium carbonate coated settlement plates is interesting. Concrete leaching affecting the results can still not be entirely discounted, as the thin layer of calcium carbonate sand may not have been sufficient to reduce any leachate from the settlement plate. The significant decrease in the rate of fouling of the iron-coated plates is unexpected. Possibly the filings were oxidizing and sloughing off too rapidly for the biofilm to be maintained. Until additional results provide a clear indication of coral settlement preferences, or lack thereof, the results of the biofilm study are difficult to evaluate relative to their potential role in coral recruitment.

Although it is too early in the study to draw firm conclusions, the species specific differences in transplant growth and mortality may be an important consideration in future coral reef restoration efforts.

The difference in fish assemblages associated with the differing Reef Ball fill treatments was anticipated. An understanding of the potential interaction of these differing assemblages with coral recruitment and mortality awaits the photographic analysis of the settlement plates. Likewise, the study on the potential of *H. boergesenii* to attract coral to restoration structure has just been initiated and results of this study also await future photographic analysis of settlement plates.

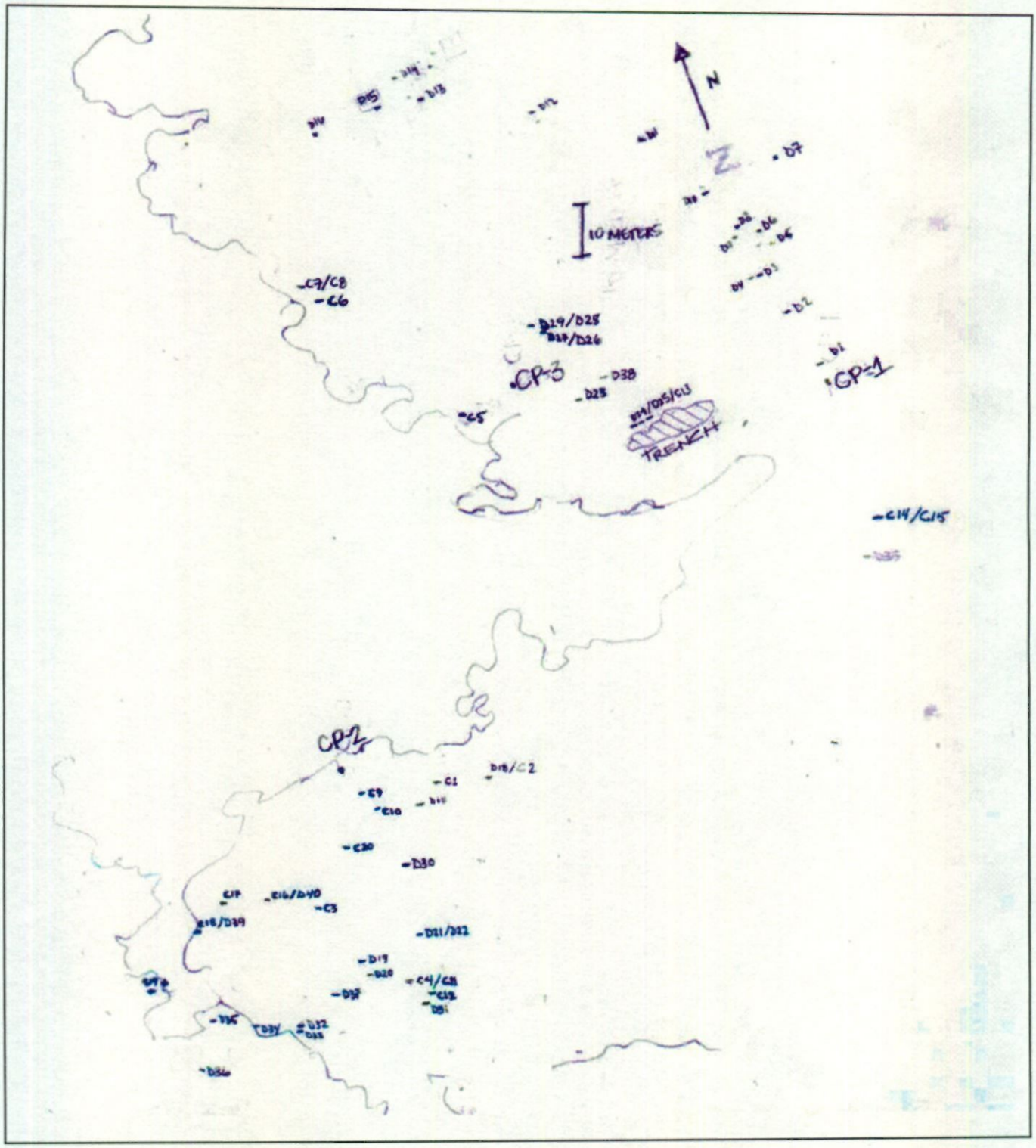
APPENDIX

Memphis Project Timeline

Task	Scheduled Completion	Actual Completion
Reef Ball Construction	N/A	Aug. 2000
1st Quarterly Report	Oct. 2000	Oct. 2000
Reef Ball Deployment	N/A	Nov. 2000
2nd Quarterly Report	Jan. 2001	Jan. 2001
3rd Quarterly Report	Apr. 2001	Apr. 2001
Final Reef Ball Positioning	N/A	June 2001
Coral Attachment and Assessment	N/A	June 2001
Reef Ball Complexity Fill	N/A	July 2001
4th Quarterly Report	July 2001	July. 2001
1st Yearly Report	July 2001	July 2001
Biofilm Disc Attachment	N/A	Aug. 2001
Biofilm Disc Retrieval	N/A	Sept. 2001
Coral Assessment	Sept. 2001	Sept. 2001
Fish Census	Oct. 2001	Oct. 2001
Final Plate Attachment	N/A	Oct. 2001
5th Quarterly Report	Oct. 2001	Oct. 2001
Coral Assessment	Dec. 2002	Jan. 2002
Fish Census	Jan. 2002	Jan. 2002
6th Quarterly Report	Jan. 2002	Jan. 2002
Coral Assessment	Mar. 2002	Mar. 2002
Fish Census	Apr. 2002	Apr. 2002
Biofilm Labwork Complete	Apr. 2002	April 2002
7th Quarterly Report	Apr. 2002	April 2002
Coral Assessment	June 2002	June 2002
Fish Census	July 2002	July 2002
Algal Attractant Plates Complete	July 2002	July 2002
8th Quarterly Report	July 2002	July 2002
2nd Yearly Report	July 2002	July 2002
Algal Plate Deployment	Aug. 2002	July 2002
Coral Assessment	Sept. 2002	
Fish Census	Oct. 2002	
9th Quarterly Report	Oct. 2002	
Coral Assessment	Dec. 2002	
Fish Census	Jan. 2003	
10th Quarterly Report	Jan. 2003	
Coral Assessment	Mar. 2003	
Fish Census	Apr. 2003	
Algal Plate Retrieval	Apr. 2003	
11th Quarterly Report	Apr. 2003	
Coral Assessment	June 2003	
Fish Census	July 2003	
Final Report	July 2003	

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	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 05.782	37
26 03.291	80 05.782	38
26 03.320	80 05.780	40
26 03.307	80 05.778	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



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).

Memphis Restoration, 1st Quarterly Report
(19 August – 18 October 2000)

The first quarter was a downtime for the project awaiting deployment.

Memphis Restoration, 2nd Quarterly Report
(19 October – 19 January 2000)

11/14-16/00
The contractors for Reefball deployment arrived and loaded the balls on a barged moored at the Navy's dock. Pat Quinn acted as a liaison between the contractors and NSU.

11/15/00
A meeting was held at NSUOC at 0900 with OC and Broward County personnel to discuss the logistics of the deployment scheduled for 11/17/00. In attendance were Ken Banks, Pamela Fletcher, Joe Ligas, and Lou Fisher from Broward County and Richard Spieler, Dave Gilliam, Pat Quinn, Elizabeth Glynn, Dan Fahy, Brian Walker, Paul Arena, Lance Jordan, and Lance Robinson from OC.

11/17/00
The reef balls were deployed. The Broward County boat Monitor was used to set buoys for the barge to use as marks for setting quads (four reef balls). Above-mentioned OC personnel were on board the Panacea. NCRI's boat Researcher was used to ferry news media to the site and back. The first quad was deployed at approximately 0720 and the last at approximately 1410.

11/27/00
Pat Quinn and Elizabeth Glynn dove on the site from R/V Researcher and started searching for the ends of the quad lines, labeling the quads and mapping the grid. Ten quads were labeled. Lance Robinson and Dan Fahy crewed the boat.

11/27/00 – 12/13/00
Multiple dive cancellations were required due to weather.

12/14/00
Pat Quinn and Elizabeth Glynn dove the site from the R/V Researcher to continue searching for the ends, mapping and labeling. Twelve more quads were labeled. Lance Robinson and Judy Robinson crewed the boat.

12/15/00
Richard Spieler and Ken Banks decided, due to forecast weather and holiday scheduling, to postpone any redeployment until the first of the year and that OCN personnel would accomplish a full site map by early January.

01/04/01
Pat Quinn and Elizabeth Glynn dove the deployment site trying to map the southern part of the "middle row". One dive was made and the rest were cancelled due to high winds and building seas. Brian Walker and Dan Fahy crewed Panacea.

01/06/01
Pat Quinn, Judy Robinson, and Brian Walker on Panacea surveyed the deployment site visually finding Reefballs from the surface and recording GPS coordinates.

01/07/01
Richard Spieler and Pat Quinn met to discuss the apparent disarray of the grid.

1/08/01
Richard Spieler and Pat Quinn discussed the arrangement of the grid with other members of the lab and scheduled additional diving to confirm the grid and obtain more detailed information on placement and distances.

01/11/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Judy Robinson made four dives on the deployment site measuring distances, retagging quads, and marking the quads for GPS coordinates. Thirty-five quads were mapped. Brian Walker piloted Panacea.

01/12/01
Pat Quinn and Elizabeth Glynn dove the deployment site to continue the work from 01/11/01 and finished mapping the last 5 quads. Paul Arena and Dan Fahy crewed Panacea.

01/12/01
Richard Spieler and Pat Quinn met to discuss the revised map of the grid.

01/15/01
Richard Spieler, Pat Quinn and the rest of the members of the lab met to discuss the arrangement of the quads and potential work schedule for moving the quads.

01/18/01
Meeting at DPEP.
Richard Spieler, Pat Quinn, and Elizabeth Glynn met with Ken Banks, Pamela Fletcher, Joe Ligas, Lou Fisher, and Dave Stout to discuss the number and logistics of moving quads.

Memphis Restoration, 3rd Quarterly Report
(20 January 2001 – 27 April 2001)

01/24/01
Pat Quinn and Joe Ligas discussed proposed cable lengths and techniques for moving the reef balls using balls located on the NSUOC property.

01/25/01
Richard Spieler, Richard Dodge, Andrew Rogerson, Pat Quinn, Elizabeth Glynn and Judy Robinson met with Dr. Aileen Morse to discuss the CCA extract and how it could be incorporated into the experimental design.

01/26/01
Pat Quinn and Elizabeth Glynn dove on the second reef near the Memphis grounding site and found abundant colonies of *Montastrea cavernosa* that should be suitable for coring and control monitoring. Dan Fahy piloted Panacea and Rob Baron crewed.
Pat Quinn and Elizabeth Glynn started making concrete plugs for the corals that will be cored.

01/29/01
Richard Spieler met with his lab personnel to discuss the upcoming deployment.

01/30/01
Broward County personnel met at NSUOC for testing of underwater communication masks to be used during the deployment. Joe Ligas gave Pat Quinn a brief lesson.

01/31/01
Email communication between Richard Spieler, Ken Banks, Pat Quinn and Elizabeth Glynn to set tentative dates to start moving the reef balls. Cables should arrive 02/05/01 and could start moving reef balls 02/06/01.

02/01/01
Joe Ligas, Pat Quinn and Elizabeth Glynn discussed updated procedures for moving the reef balls. Pat Quinn supplied coordinates and labeling scheme to Ken Banks via email.

02/05/01
Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.
Pat Quinn and Elizabeth Glynn finished making plugs for the donor corals. They also made a 'separator bar' for moving of the reef balls.

02/06/01
Ken Banks communicated with Elizabeth Glynn via email that he will schedule Thursday the 8th to start moving reef balls.
Richard Spieler and Elizabeth Glynn agreed to start coring the donor corals and this information was passed along to Pat Quinn via phone.

02/12/01
Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.

02/14/01
Richard Spieler, Pat Quinn, Elizabeth Glynn and Ken Banks discussed, via phone, starting to move the reef balls Sunday Feb. 18 weather permitting. Also discussed was movement of the quads to ensure all of the quads, direction and distance were considered.

02/19/01
Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.

02/21/01
Pat Quinn, Elizabeth Glynn, Dan Fahy, and Rob Baron worked on positioning Reef Balls within quads using four 100 lb. lift bags. Five dives were made using Panacea and 7 quads were positioned.

2/22/01
Pat Quinn, Elizabeth Glynn, Dan Fahy, and Rob Baron dove the 2nd reef north of Port Everglades looking for colonies of *Diploria clivosa* for coring. Two dives were made using Panacea.

02/28/01
Pat Quinn, Elizabeth Glynn, Paul Arena, Rob Baron, Brian Walker, Brian Buskirk, Dan Fahy and Fleur Harttung worked on positioning Reef Balls within quads using five 100 lb. lift bags. Three dives were made using Panacea and 8 quads were positioned.

03/03/01
Elizabeth Glynn and Lance Jordan assisted Ken Banks, Joe Ligas and Lou Fisher in trying out the methods discussed in the meeting of 01/18/01. Two Reef Balls were moved and the trip was terminated due to building seas.

03/09/01
Pat Quinn, and Elizabeth Glynn assisted Ken Banks, Joe Ligas, Pam Fletcher, and Lou Fisher in moving 2.5 quads (10 Reef Balls) to the correct locations. Two Reef Balls were moved at a time using two lift bags, two cum-a-longs, one 2x4 spreader bar and various cables. Quinn, Glynn, Banks and Ligas were divers while Fletcher and Fisher crewed the Monitor.

03/12/01
Richard Spieler, Richard Dodge, Pat Quinn, Elizabeth Glynn and Dave Gilliam met at NSUOC to discuss the use of the boats, photography equipment, methods, and personal schedules in relation to the Memphis project.

03/14/01
Pat Quinn, Elizabeth Glynn, Dr. Dave Gilliam, Susan Thornton and Brian Ettinger started drilling coral transplants from the 2nd reef (west of the Reef Ball deployment site). Glynn and Gilliam drilled, Thornton and Ettinger photographed and plugged corals and Quinn crewed Researcher with Capt. Lance Robinson. Eight cores were taken and placed in the Reef Balls to be attached later. Eight dives were made.

03/16/01
Pat Quinn, Elizabeth Glynn, Paul Arena, Brian Ettinger and Brian Walker epoxied the 8 coral transplants onto the Reef Balls. Two dives were made using Panacea.

03/19/01
Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab. Spieler and Quinn also tried various techniques for creating small fill in the Reef Balls

03/23/01
Pat Quinn and Elizabeth Glynn assisted Ken Banks, Joe Ligas and Lou Fisher in moving three quads to the correct locations. Two Reef Balls were moved at a time using two lift bags, two come-a-longs, one 2x4 spreader bars and various cables. Quinn, Banks and Ligas were divers while Glynn and Fisher crewed the Monitor. Five dives were made.

04/01/01
Pat Quinn, Elizabeth Glynn, and Dan Fahy assisted Ken Banks and Joe Ligas in moving three quads to the correct locations. An entire quad was moved at a time using three lift bags, four come-a-longs, three 2x4 spreader bars and various cables. Quinn, Fahy and Banks were divers while Glynn and Ligas crewed the Monitor. Three dives were made.

04/02/01
Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.
Elizabeth Glynn, Dan Fahy, Brian Buskirk, Fleur Harttung and Ryan Moyer dove the 2nd reef mapping donor corals for coring. Three dives were made using Panacea.

04/04/01
Elizabeth Glynn, Dan Fahy and Rob Baron surveyed the reef near the Memphis grounding looking for donor corals to core. One dive was made by Glynn and Baron using OSC's vessel Lucy Forman. Fahy crewed the Lucy.

04/09/01
Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.

04/10/01
Pat Quinn, Elizabeth Glynn and Dave Gilliam assisted Ken Banks and Joe Ligas in moving one quad to the correct location. Site location problems prevented more quads

from being moved. Quinn, Gilliam and Ligas were divers while Banks and Glynn crewed the Monitor. Multiple dives were made.

04/16/01
Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.

04/17/01
Pat Quinn, and Elizabeth Glynn assisted Ken Banks, Joe Ligas and Dave Stout in moving four quads to the correct locations. Quinn, Glynn, Banks, and Stout were divers while Ligas crewed the Monitor. Four dives were made.

04/23/01
Richard Spieler, Pat Quinn and Elizabeth Glynn discussed the Memphis project and projected work for the week with the other members of the lab. Spieler, Quinn and Glynn met again to discuss various techniques for attaching settlement plates and attractants to the reef balls. Method trials will be made for plate attachment using other Reef Balls from previous projects.

04/25/01
Pat Quinn, Elizabeth Glynn, Dave Gilliam, Dan Fahy, Jamie Vernacchio, Brian Ettinger and Rob Baron dove the 2nd reef near the Memphis grounding site mapping and tagging corals for coring or controls. Capt. Lance Robinson and Susan Thornton crewed the Researcher. Four dives were made.

04/27/01
Richard Spieler and Pat Quinn made a cone using ¾" plastic cage material to function as small fill in a Reef Ball. Spieler authorized the purchase of cage material for 60 cones.

Memphis Restoration, 4th Quarterly Report
(01 May 2001 – 19 July 2001)

05/01/01
Dr. Richard Spieler authorized purchase of 220 blocks for large fill and 65 ft. of ¾" plastic cage material for small fill in the RBs.

Dr. Richard Spieler and Pat Quinn discussed various types of iron product to be added to the settlement plates as an attractant. Dr. Spieler ordered two different types of iron products for testing.

05/04/01
Dr. Richard Spieler and Pat Quinn received the block and cage material.

05/07/01
Dr. Richard Spieler met with his lab personnel to coordinate the making of small fill using the cage material cut into cones. The cones were completed that week.
Dr. Richard Spieler, Pat Quinn and Elizabeth Glynn met to discuss the logistics of adding fill, drilling coral cores, and attaching settlement plates.

05/09/01
Dr. Richard Spieler, Pat Quinn and personnel from Industrial Divers discussed various mixtures of concrete that could be used to attach the settlement plates to the RBs while underwater.

05/11//01
Dr. Richard Spieler and Pat Quinn coated a settlement plate with cement and embedded calcium carbonate and iron. The plate was submerged in the marine environment as a methods test.

05/23/01
Pat Quinn and Rob Baron dove the Memphis RBs to practice attaching settlement plates and small fill. Sixteen block for large fill was dropped near a quad. One dive was made and the remainder of the work was cancelled due to thunderstorms. Paul Arena captained Panacea and Elizabeth Glynn crewed.

05/25/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Brian Buskirk dropped 32 blocks for large fill in 2 quads. Five dives were then made at the grounding site to map corals for donors to be drilled and controls from Panacea.

05/27/01
Elizabeth Glynn and Dan Fahy dove the grounding site to map donor corals from drilling. Two dives were made from the Lucy Forman.

05/30/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Brian Buskirk dropped 64 blocks for large fill in 4 quads. Quinn and Buskirk filled two quads with 16 blocks each. Elizabeth Glynn and Dan Fahy then dove the grounding site to map and tag donor corals. Four dives were made from Panacea.

05/31/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Brian Buskirk dropped blocks near the quads for large fill. Quinn and Buskirk filled four quads with 16 blocks each and measured the distance between specific quads.
Elizabeth Glynn and Dan Fahy then dove the grounding site to map and tag donor corals. Five dives were made from Panacea.

06/01/01
Pat Quinn, Elizabeth Glynn, Ken Banks, Joe Ligas and Lance Jordan moved RBs using Monitor. Quinn, Banks and Jordan made three dives moving quads 5 and 27. Ligas captained Monitor and Glynn crewed.

06/03/01
Elizabeth Glynn, Dave Gilliam, and Susan Thornton dove the grounding site to photograph donor and control corals. Two dives were made from Researcher. Lance Robinson captained.

06/04/01
Dr. Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.
Pat Quinn, Elizabeth Glynn, Dave Gilliam and Brian Ettinger dove the grounding site on the Lucy Forman to drill the donor corals. The drill did was not working properly and the day was aborted to troubleshoot the problem.

06/05/01
Pat Quinn, Ken Banks, Joe Ligas and Amy Hall moved RBs using Monitor. Quad 1 was moved and quads 5, 27 and 36 had individual balls repositioned. Four dives were made.
Elizabeth Glynn, Susan Thornton, Dave Gilliam and Brian Ettinger dove the grounding site to drill donor corals. Three dives were made from the Lucy Forman. The drill was not working properly and the day was aborted.
A replacement drill was obtained the next day.

06/06/01
Pat Quinn, Paul Arena, and Rob Baron made two trips on Panacea to drop 72 blocks for large fill on 12 quads.

06/08/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Brian Ettinger dove the grounding site to drill corals and place the cores in RBs. Five dives were made from the Lucy Forman.

06/11/01
Dr. Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.
Elizabeth Glynn, Dave Gilliam, Susan Thornton and Brian Ettinger dove the grounding site to finish photographing donor corals prior to drilling. Three dives were made from Researcher. Lance Robinson captained.

06/12/01
Pat Quinn, Elizabeth Glynn, Brian Ettinger and Kevin Helmle dove the grounding site to drill corals and place the cores in RBs. Four dives were made from the Lucy Forman.

06/13/01
Pat Quinn, Elizabeth Glynn and Dan Fahy dove the grounding site to drill donor corals and place the cores in RBs. Quinn and Glynn made three dives were made from the Lucy Forman and Fahy captained.

06/15/01
Pat Quinn, Elizabeth Glynn, Dave Gilliam, Brian Ettinger and Kevin Helmle dove the grounding site to drill donor corals and place the cores in the RBs. Five dives were made from the Lucy Forman. Due to lack of air and time, 6 cores were left inside a RB of quad 23 to be moved later. This completed drilling of corals to be used as attactants.

06/16/01
Elizabeth Glynn, Dave Gilliam and Brian Ettinger dove quad 23 to recover the cores left from the previous day. These cores were placed in their correct quads. This completed the distribution of corals to the RBs to be used as attractants. The corals will still need to be permanently attached and photographed. Glynn and Gilliam made one dive from the Lucy Forman and Ettinger captained.

06/18/01
Dr. Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.

06/19/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Mike Hoke dove the RBs to epoxy cores into the RBs and photograph. Four dives were made from the Lucy Forman.

06/20/01
Pat Quinn, Elizabeth Glynn, Judy Robinson, and Ryan Moyer dove the RBs to epoxy cores into the RBs, photograph the cores and place small fill in quads 5 and 14. Four dives were made from the Lucy Forman.

06/21/01
Pat Quinn, Elizabeth Glynn, Amy Hall and Ryan Moyer dove the RBs. Quinn and Hall made two dives to add fill to quads 6, 8 and 36. Glynn and Moyer made two dives to epoxy cores into the RBs and take photographs. Four dives were made from Panacea

06/22/01
Elizabeth Glynn, Dan Fahy, Brian Ettinger and Jamie Vernacchio dove the RBs to epoxy and photograph the cores in quads 9, 10, 15, 17, 23 and 24. Two dives were made from Panacea and additional work was aborted due to thunderstorms.

06/24/01
Elizabeth Glynn, Dan Fahy, Ryan Moyer and Heather Halter dove the RBs to epoxy and photograph the remaining cores. This completed the addition of corals to the RBs as attractants.
The personnel then moved to the grounding site to epoxy plugs into the donor corals and take photographs.
A total of four dives were made from the Lucy Forman.

06/25/01
Dr. Richard Spieler and Pat Quinn discussed the Memphis project and projected work for the week with the other members of the lab.
Pat Quinn, Elizabeth Glynn, Dan Fahy, Rob Baron, Fleur Hartung, and Amy Hall dove the Memphis RBs adding small and large fill to quads. Four dives were made from Panacea and additional work was aborted due to thunderstorms.

06/26/01
Dr. Richard Spieler, Pat Quinn and personnel from the Rinker Materials Corp. discussed the cement mix used to make the RBs, as this same mix will be used to attach the coral attractants to the settlement plates.
Elizabeth Glynn, Dan Fahy, and Amy Hall dove the 2nd reef near the Memphis grounding site to epoxy plugs into the donor corals and photograph donors and controls. Two dives were made at this location.
Pat Quinn and Rob Baron dove the Memphis RBs adding small and large fill to the quads. One dive was made at this location.
Panacea was used the entire day.

07/02/01
Dr. Richard Spieler and Pat Quinn coated a settlement plate with a cement mixture covered with calcium carbonate. When dry, the plate was placed in the marine environment as a methods test.

07/06/01
Pat Quinn, Elizabeth Glynn, Paul Arena and Fleur Hartung dove the RBs to add small and large fill to the quads. This completed filling the quads for the complexity aspect.
The personnel then moved to the grounding site to epoxy plugs into the donor corals and take photographs. Five dives were made from Panacea.

07/09/01
Pat Quinn and Judy Robinson discussed the substrate coatings to be used on the biofouling plates. It was determined to follow the same coating methods as used on the larger settlement plates. Dr. Andrew Rogerson agreed with this procedure.

07/19/01
Judy Robinson and NSUOC personnel constructed the biofouling plate arrays with just concrete, concrete and quarry rock, concrete and iron, and glass slides. Seventy-two biofouling plates with the appropriate substrate types and 24 glass slides make up the four arrays.

Memphis Restoration, 5th Quarterly Report
(20 July 2001 – 11 October 2001)

07/29/01
Richard Spieler and Pat Quinn discussed personnel assignments and logistics of deploying the 320 settlement plates on the quads. It was decided to put all 320 plates in the water before attaching the plates to the Reef Balls.

07/30/01
Pat Quinn, Brian Walker, Judy Robinson and Lance Robinson placed 96 settlement plates on Quads 1-10, 15 and 33. The plates were stood upright on the substrate and against the Reef Balls to which they will be later cemented. J. Robinson lowered the plates to Quinn and Walker who made 2 dives. L. Robinson captained Researcher.
After the plates were deployed, Richard Spieler and Pat Quinn met at NSUOC to discuss personnel and logistics for the following day.

07/31/01
Pat Quinn, Brian Walker, Judy Robinson, Lance Robinson and Amy Hall placed 104 settlement plates on Quads 16-27 and Q 13. Walker and J. Robinson lowered the plates to Quinn and Hall during one dive. Plates for the 13 quads were deployed on one dive. Twenty-four plates remained on board for three more quads so it was decided to return to NSUOC and load the remaining 96 plates. At the dock, Paul Arena replaced Amy Hall. Arena and J. Robinson lowered the plates to Quinn and Walker on two dives that completed the plate deployment. L. Robinson captained Researcher.
Andrew Rogerson and Judy Robinson met to discuss the deployment site of biofilm discs and a collection schedule.

08/06/01
Pat Quinn, Elizabeth Glynn, and Amy Hall started cementing settlement plates onto the Reef Balls. Two dives were made from the Lucy Forman. The first dive was on Quad 1 and the second dive on Quad 12.

08/08/01
Pat Quinn, Elizabeth Glynn and Brian Walker cemented settlement plates onto Reef Balls. Two dives were made from the Lucy Forman. The first dive was on Quad 31 and the second dive on Quad 11.

08/09/01
Pat Quinn, Elizabeth Glynn and Amy Hall cemented settlement plates onto Reef Balls. Two dives were made from the Lucy Forman. The first dive was on Quad 32 and the second dive on Quad 30.

08/10/01
Richard Spieler and Pat Quinn discussed the upcoming coral spawning and decided to have divers orient the plates in the correct position for settlement per the experimental design then, starting with the top plates, cement them onto the Reef Balls.

08/11/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Brian Buskirk positioned the remaining settlement plates on the Reef Balls in preparation of the upcoming spawning. Plates were laid flat on top of the Reef Balls and placed upright against the sides. In addition, top plates near the coral transplants were cemented on Quads 7-10, 14, 15, 28, 29 and 37. Three dives were made from Panacea.

08/12/01
Pat Quinn, Dave Gilliam, Brian Walker and Rob Baron cemented top plates onto Reef Balls. Four dives were made from Panacea attaching plates to Quads 2-10, 28, 29 and 39.

08/13/01
Richard Spieler and Pat Quinn met with other lab personnel to discuss actual procedures of mixing cement underwater and application of the settlement plates onto the Reef Balls. Andrew Rogerson and Judy Robinson met to discuss methodology for preparation of biofilm discs to be used in counting. It was agreed to use previous designed methods from the preliminary study.

08/14/01
Pat Quinn, Robin Sherman, Dan Fahy and Amy Hall cemented top plates onto Reef Balls. Four dives were made from Panacea attaching plates to Quads 13, 15, 17, 19 and 25-27. Paul Arena captained Panacea and Rob Baron lowered bags of cement to divers as needed.
Judy Robinson and Lance Robinson attached four arrays of 24 discs coated with different attractants to the Reefballs. One dive was made from the Researcher with Brian Ettinger as captain.

08/15/01
Richard Spieler, Pat Quinn, Dan Fahy, Amy Hall and Brian Buskirk cemented plates onto Reef Balls. Four dives were made during which the remaining top plates were attached and work began on cementing side plates. Paul Arena captained Panacea and Rob Baron lowered bags of cement to divers as needed.
Judy Robinson and Lance Robinson collected 12 discs from the Reef Balls. One dive was made from the Researcher with Brian Ettinger as captain.

08/17/01
Judy Robinson and Lance Robinson collected 12 discs from the Reef Balls. One dive was made from the Researcher with Brian Ettinger as captain.

08/19/01
Pat Quinn, Dave Gilliam, Dan Fahy and Brian Buskirk cemented plates onto Reef Balls. Four dives were made attaching plates to Quads 2-7, 14, 34, 35 and 37-40. Richard Spieler captained Panacea and Rob Baron lowered bags of cement to divers as needed.

08/20/01
Pat Quinn, Elizabeth Glynn, Dan Fahy, Amy Hall and Brian Buskirk cemented plates onto Reef Balls. Five dives were made attaching plates to Quads 8-10, 13, 15, 16, 18, 21-28, 31 and 33. Richard Spieler captained Panacea and Paul Arena lowered bags of cement to divers as needed.

08/24/01
Judy Robinson and Lance Robinson collected 12 discs from the Reef Balls. One dive was made from the Researcher with Brian Ettinger as captain.

08/28/01
Pat Quinn, Dan Fahy and Fleur Harttung surveyed the quads to ensure all plates were still attached and had been correctly placed. Of the 320 settlement plates attached to the Reef Balls, only 2 had come loose, one each on Quads 6 and 32. Both were side plates. Two dives were made from Panacea and Elizabeth Glynn crewed.

08/30/01
Judy Robinson and Lance Robinson collected 12 discs from the Reef Balls. One dive was made from the Researcher with Brian Ettinger as captain.

09/04/01
Dave Gilliam and Lance Robinson collected 12 discs from the Reef Balls. One dive was made from the Researcher with Brian Ettinger as captain.

09/12/01
Richard Spieler and Pat Quinn discussed preliminary methodology for conducting the fish community surveys.

09/20/01
Elizabeth Glynn, Dan Fahy and Susan Thornton photographed donor and control corals at the Memphis grounding site. One dive was made from Researcher with Brian Ettinger as captain and Dave Gilliam as crew.

09/25/01
Elizabeth Glynn, Dan Fahy and Fleur Harttung photographed donor and control corals at the Memphis grounding site. Two dives were made from Panacea with Pat Quinn as captain.

09/28/01
Elizabeth Glynn and Dan Fahy photographed donor and control corals at the Memphis grounding site. Two dives were made from the Lucy Forman with Pat Quinn as captain.

10/02/01
Elizabeth Glynn, Pat Quinn, Fleur Harttung and Brian Buskirk photographed transplant corals on the Reef Balls. Three dives were made from Panacea.

10/03/01
Elizabeth Glynn and Amy Hall photographed transplant corals on the Reef Balls. Two dives were made from the Lucy Forman with Pat Quinn as captain.

10/05/01
Richard Spieler and Pat Quinn met with lab personnel to discuss methodology and dive schedule of the fish monitoring.

10/06/01
Richard Spieler and Pat Quinn discussed final details of methodology and personnel for the fish monitoring.

10/07/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Robin Sherman conducted the first fish community monitoring on the quads. Four dives were made from Panacea and data were collected from Quads11-13, 16-34.

Memphis Restoration, 6th Quarterly Report
(15 October 2001 – 11 January 2002)

10/12/01
Richard Spieler and Pat Quinn met with lab personnel to review methodology and dive scheduling for the fish monitoring.

10/15/01
Pat Quinn, Elizabeth Glynn, Dan Fahy and Brian Buskirk finished the first fish community monitoring on the quads. Five dives were made from Panacea and data were collected from Quads1-10, 14, 15, and 35-40. Additionally, photographs were taken of the coral transplants on Quads 5-8.

10/16/01
Pat Quinn and Rob Baron finished cementing the settlement plates onto the Reef Balls of Quads 6 and 32. One dive was made from Panacea with D. Fahy as capt. and E. Glynn as crew.

12/05/01
Richard Spieler, Elizabeth Glynn and Pat Quinn met with lab personnel to review methodology and dive scheduling for the corol monitoring.

12/12/01
Elizabeth Glynn and Heather Halter photographed donor and control corals at the Memphis grounding site (CP2). Two dives were made from Panacea with P. Quinn as capt. and D. Fahy as crew.

12/14/01
Elizabeth Glynn, Brian Buskirk and Fleur Harttung photographed coral transplants on Quads 2-10, 14-17, 19, and 33-40. Two dives were made from Panacea with P. Quinn as capt.
Additionally, a thermograph was changed on Quad 36.

12/17/01
Elizabeth Glynn and Dan Fahy finished photographing the coral transplants on Quads 1, 11-13, 18, and 20-32. Two dives were made from Panacea with P. Quinn as capt. and A. Hall as crew.

01/07/02
Richard Spieler and Pat Quinn met with lab personnel to review methodology and dive scheduling for the second fish monitoring.

01/09/02
Richard Spieler, Pat Quinn and Lance Robinson finalized the schedule for conducting the fish monitoring on the following day.

01/10/02
Richard Spieler, Pat Quinn, Elizabeth Glynn, Dan Fahy, Brian Buskirk, Amy Hall, Fleur Harttung and Rob Baron conducted the second fish community monitoring on the quads. Seven dives were made from Researcher and data were collected from Quads 1-20 and 22-40. Lance Robinson captained Researcher.

01/11/02
Elizabeth Glynn and Dan Fahy finished photographing the donor and control corals at the Memphis grounding site. Pat Quinn and Fleur Harttung completed the second fish community monitoring and data were collected from Quad 21. Four dives were made from Panacea.

Memphis Restoration, 7th Quarterly Report
(11 January – April 11, 2002)

01/18/02
Richard Spieler and Pat Quinn discussed conducting a maintenance dive on the Reef Balls to reattach plates and quad labels.

01/22/02
Richard Spieler, Pat Quinn, Dan Fahy and Rob Baron reattached settlement plates on Quads 3, 7, 16, 30, 32, 33 and 38. Three dives were made from Panacea.

01/25/02
Elizabeth Glynn and Dan Fahy dove the Memphis grounding site to complete control coral photographs. One dive was made from Panacea with F. Harttung as Captain.

02/26/02
Pat Quinn and Elizabeth Glynn changed a thermograph on Quad 36 and examined Quad 32 for loose settlement plates. One dive was made from Panacea with Fleur Harttung as Captain and Dan Fahy as crew.

03/11/02
Richard Spieler, Elizabeth Glynn and Pat Quinn met with lab personnel to discuss scheduling for photographing the transplant, donor and control corals.

03/13/02
Elizabeth Glynn, Dan Fahy, Amy Hall and Fleur Harttung photographed transplant corals on the Reef Balls. Four dives were made from Panacea.

03/15/02
Elizabeth Glynn, Ryan Moyer and Brian Buskirk photographed donor and control corals at the Memphis grounding site (CP2). Two dives were made from Panacea with P. Quinn as Captain. Additional work was aborted to due injury, weather conditions and equipment problems.

03/16/02
Elizabeth Glynn and Dan Fahy photographed donor and control corals at the Memphis grounding site (trench). Two dives were made from the R/V Lucy Forman.

03/26/02
Elizabeth Glynn and Dan Fahy dove the Memphis grounding site (CP2) to photograph corals and record GPS coordinates for large donor and control corals. One dive was made from Panacea with Paul Arena as Captain and Rob Baron as crew.

03/28-03/29/02
Judy Robinson scraped, filtered and made slides of the accumulated substance on the remaining collected biofilm plates (4).

03/29/02
Elizabeth Glynn and Heather Halter dove the Memphis grounding site (CP1) to finish photographing corals and record GPS coordinates. One dive was made from Panacea with F. Harttung as Captain.

04/01/02
Richard Spieler and Pat Quinn met with lab personnel to review methodology and dive scheduling for the fish monitoring. Scheduled date to conduct the monitoring is 04/08/02.

04/02/02
Andrew Rogerson and Judy Robinson discussed the methods in which the slides will be counted. Four slides were examined and the bacterial cells counted.

04/07/02
Richard Spieler and Pat Quinn canceled the scheduled 04/08/02 monitoring due to weather. Pat Quinn contacted lab personnel and rescheduled for 04/10/02.

04/09/02
Richard Spieler and Pat Quinn canceled the scheduled 04/11/02 monitoring due to weather. Next scheduled date is TBD, weather contingent.

Memphis Restoration, 8th Quarterly Report
(12 April 2002 - 22 July 2002)

04/19/02
Richard Spieler and Pat Quinn met with lab personnel to discuss scheduling and procedures for the third fish community monitoring.

04/22/02
Pat Quinn, Elizabeth Glynn, Dan Fahy, Brian Buskirk, Rob Baron and Paul Arena conducted the third fish community monitoring on the quads. Six dives were made from the Panacea and data were collected from all 40 quads. Tags with the appropriate quad number were replaced on each quad.

05/02/02
Pat Quinn and Ryan Moyer dove on Quad 15 looking for coral recruits. Another dive was made on Quad 32 to reattach a settlement plate. The plate was found, and put in place, but was not attached due to time constraints returning to the port before Fleet Week closure. Two dives total were made from the Researcher with B. Ettinger as Captain and D. Gilliam as crew.

06/04/02
Pat Quinn and Dan Fahy reattached settlement plates on Quads 32 and 39. One dive was made from the Panacea with F. Harttung as Captain and R. Baron as crew.

06/13/02
Richard Spieler and Pat Quinn discussed potential designs for settlement plates to be used in the Crustose Coralline Algae (CCA) study.

06/17/02
Andrew Rogerson and Judy Robinson discussed the problem with cover slip detachment on some of the iron and $CaCo^2$ slides. Judy Robinson continued bacterial counts.

06/18-26/02
Pat Quinn worked on several potential design structures for settlement plates to be used in the CCA study.

06/19/02
Andrew Rogerson and Judy Robinson developed methodology to prevent cover slip detachment of the iron and $CaCo^2$ slides. Judy Robinson continued bacterial counts.

06/20/02
Judy Robinson completed bacterial counts on samples with no treatment. Remaining bacterial counts continue.

06/24/02
Judy Robinson completed bacterial counts on samples on glass slides. Remaining bacterial counts continue.

06/27/02
Judy Robinson completed bacterial counts on samples with $CaCo^2$ treatment. Remaining bacterial counts continue.

07/1/02
Andrew Rogerson and Judy Robinson discussed the bacterial density calculations. Judy Robinson completed bacterial counts with iron treatment.

07/2/02
Andrew Rogerson and Judy Robinson discussed the analysis and write-up for final report. Richard Spieler and Pat Quinn discussed finalized structure for settlement plates to be used in CCA study.

07/03/02
Elizabeth Glynn, Pat Quinn, Dan Fahy and Ryan Moyer photographed transplant corals on Quads 1-19 and 25-40. Three dives were made from the Panacea. The remaining dives were cancelled due to weather.

07/05/02
Elizabeth Glynn, Pat Quinn, photographed the tranplant corals on Quads 20-24. One dive was made from the Panacea with D. Fahy as Captain and R. Moyer as crew. The remaining dives were cancelled due to equipment failure.

07/05-15/02
Pat Quinn made 32 settlement plate modules to be used in the CCA study.

07/07/02
Elizabeth Glynn, Pat Quinn and Dan Fahy photographed donor and control corals at the Memphis grounding site. Three dives were made from the Thompson. An additional dive was made by Pat Quinn and Dan Fahy to photograph a coral recuit located on Quad 15.

07/10/02
Elizabeth Glynn, Pat Quinn and Dan Fahy finished photographing donor and control corals at the Memphis grounding site. Three dives were made from the Thompson.

07/17/02
Richard Spieler and Pat Quinn discussed potential deployment sites for the CCA settlement plate modules and logistics for the actual deployment.

07/18/02
Richard Spieler and Pat Quinn surveyed two potential study areas for deployment of the CCA modules. Eight modules were placed in the Site 1 Control area and 8 were placed in the Site 1 Study area. Two dives were made from Panacea with F. Harttung as Captain and P. Arena and D. Fahy as crew.

07/19/02
Richard Spieler, Pat Quinn and Robin Sherman dove Site 2 Control and Study areas to place sixteen CCA modules. Spieler, Quinn, Sherman and Dan Fahy then dove Site 1 Control to remove any rubble around the CCA control modules. Two dives were made from Panacea with F. Harttung as Captain and P. Arena as crew.

07/21/02
Richard Spieler and Pat Quinn discussed the logistics for conducting the fourth fish community monitoring on the quads.

07/22/02
Richard Spieler, Pat Quinn, Robin Sherman, Fleur Harttung, Dan Fahy, Paul Arena, Brian Buskirk and Kristy Foster conducted the fourth fish community monitoring on the quads. Six dives were made from the Panacea and data were collected from all 40 quads.

Descriptive Statistics: Fish Abundance Per Treatment

Empty

Mean	45.5
Standard Error	3.973308381
Median	38
Mode	38
Standard Deviation	25.12940866
Sample Variance	631.4871795
Kurtosis	6.043953305
Skewness	2.475899342
Range	109
Minimum	24
Maximum	133
Sum	1820
Count	40

Small

Mean	52.725
Standard Error	3.47241649
Median	49.5
Mode	44
Standard Deviation	21.96149019
Sample Variance	482.3070513
Kurtosis	0.375990243
Skewness	0.891065563
Range	97
Minimum	16
Maximum	113
Sum	2109
Count	40

Mixed

Mean	64.75
Standard Error	5.890752205
Median	51.5
Mode	48
Standard Deviation	37.2563882
Sample Variance	1388.038462
Kurtosis	9.162627706
Skewness	2.827267035
Range	188
Minimum	33
Maximum	221
Sum	2590
Count	40

Large

Mean	70.5
Standard Error	6.154527206
Median	56.5
Mode	52
Standard Deviation	38.92464778
Sample Variance	1515.128205
Kurtosis	3.281524666
Skewness	1.755520462
Range	178
Minimum	29
Maximum	207
Sum	2820
Count	40

Newman-Keuls test; variable log total abundance. Probabilities for Post Hoc Tests Error: Between MS = .02871, df = 153.00

	Treatment	{1}	{2}	{3}	{4}
1	Empty		0.067778	*0.000286*	*0.000022*
2	Small	0.067778		*0.037098*	*0.011203*
3	Mixed	*0.000286*	*0.037098*		0.428249
4	Large	*0.000022*	*0.011203*	0.428249	

Descriptive Statistics: Species Richness Per Treatment

Empty		*Small*	
Mean	12.65	Mean	14.325
Standard Error	0.547195648	Standard Error	0.529135119
Median	12	Median	14.5
Mode	10	Mode	14
Standard Deviation	3.460769145	Standard Deviation	3.346544333
Sample Variance	11.97692308	Sample Variance	11.19935897
Kurtosis	-0.573107357	Kurtosis	-0.245642041
Skewness	0.342625735	Skewness	-0.004485625
Range	14	Range	15
Minimum	6	Minimum	7
Maximum	20	Maximum	22
Sum	506	Sum	573
Count	40	Count	40

Mixed		*Large*	
Mean	16.275	Mean	17.35
Standard Error	0.631428478	Standard Error	0.581278432
Median	16.5	Median	17
Mode	17	Mode	17
Standard Deviation	3.993504341	Standard Deviation	3.676327599
Sample Variance	15.94807692	Sample Variance	13.51538462
Kurtosis	-0.161835169	Kurtosis	-0.35056848
Skewness	0.14029046	Skewness	0.273143176
Range	16	Range	15
Minimum	8	Minimum	10
Maximum	24	Maximum	25
Sum	651	Sum	694
Count	40	Count	40

Newman-Keuls test; variable Species Richness. Probabilities for Post Hoc Tests Error: Between MS = 10.808, df = 153.00

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

Judy Robinson and Andrew Rogerson

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™.

Development of microbial and algal films is important because they directly occupy the substratum and provide secondary biotic space in the form of "lower story" habitats (Dayton 1971). The physical nature of the substratum has been shown to be an influence on substrate selection by marine organisms in the laboratory (Crisp and Ryland 1960; Leitz and Wagner 1993) and in the field (Harriot and Fisk 1987; Todd and Keough 1994; Stoner 1994). The micro-scale features of substrate surfaces can differ in surface tension, hydrophobicity, and surface texture (Mihm and Banta 1981). Moreover, the nature to the microbial films could make an unattractive substratum attractive. These films may act as a physical presence between settled larvae and the immersed surface possibly enhancing adhesion. Crisp *et al.* (1985) reported that cypris larvae might not permanently attach to a surface in which their adhesive does not bind strongly, suggesting that extracellular material from bacterial films may mediate greater adhesion.

Additionally, chemosensory recognition of microbial films (Kirchman *et al.* 1982) and of some algal species (Morse *et al.* 1994), are 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. Zobell and Allen (1935) first documented the importance of microbial biofilms in the development of marine invertebrate communities. Since their early work, a variety of marine organisms have been reported to display varying responses to filmed and unfilmed surfaces. Brancato and Wollacott (1982) observed that three species of bryozoans selected "filmed" in preference to "non-filmed" substrata when given a choice. Moreover, when not offered a choice, *Bugula simplex* and *Bugula turrita* preferred not to settle on a non-filmed surface, whereas *Buglua stolonifera* settled on filmed as well as non-filmed surfaces. However, it is worth noting that available data concerning the necessity of primary organic and micro-floral films for triggering larval settlement are highly variable. Crisp and Ryland (1960) showed that biofilms were not an essential prerequisite for larval settlement, while Kirchman *et al.* (1982) demonstrated that a spirobin polychaete settled in response to multi-species microbial films. Maki *et al.* (1988, 1992) noted age-related effects both for larva and bacterial-organic films. However, these experimental designs did not permit the assessment of the respective contribution of initial substratum surface chemistry and the influence of previously settled conspecifics on subsequent settlement.

Considerable emphasis has been placed on the importance of supply and settlement of marine larvae in relation to patterns of distribution and abundance of juveniles and adults (Underwood and Fairweather 1989; Gleason 1996; Smith 1997). The events, factors, and processes affecting larval settlement are evidently pivotal to the

further development of a given assemblage. An understanding of settlement is, therefore, an essential pre-requisite to an appreciation of the overall population and assemblage dynamics. The nature of the mechanisms inducing or inhibiting settlement is of much interest, yet there are insufficient field data for a wide range of taxa. Given the biological significance of these micro-scale communities to overall reef productivity, we examined the first steps of fouling by microbial and algal biota during the initial immersion of Reef Ball substrates.

Methods

Study Area:

The study site is located in Broward County 0.5km offshore of the Hollywood Beach area. This location is situated at the grounding site of the USS MEMPHIS nuclear submarine that ran aground in 30 ft of water on a coral reef off southeast Florida. Biofilm plate arrays were deployed on four Reef Balls (Quad) in the north-east corner of the Memphis project site (figure1).

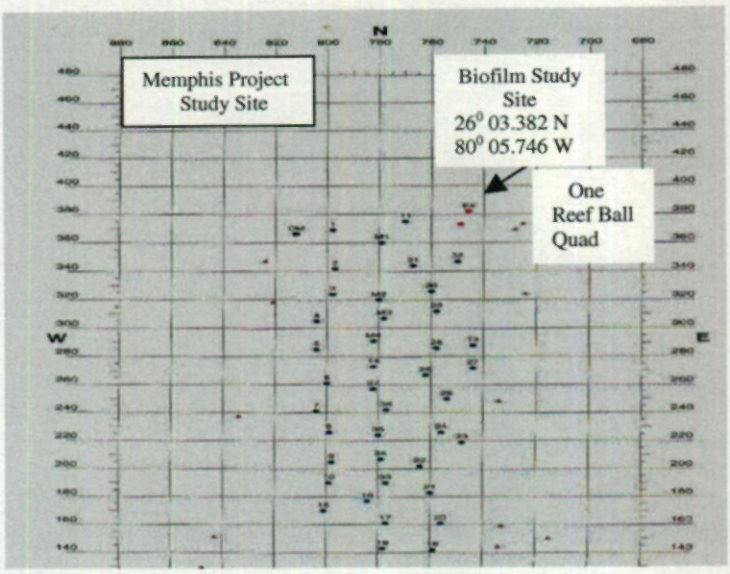


Figure 1. Map of study site.

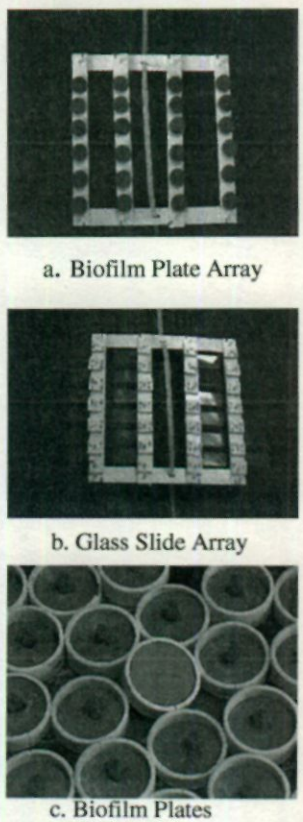
Experimental Design, Collection and Preparation of Samples

The design of the experiment involved four biofilm plate treatments (concrete only, concrete with $CaCo_3$ surface coating, iron powder surface coating, and glass slides) placed in three arrays, each consisting of 24 biofilm plates (figure 2a, one treatment per

array) and one array of 24 glass slides (figure 2b). One treatment array was attached to a Reef Ball within the Reef Ball quad. Three biofilm plates were collected from each treatment array at a given time interval (24h, 3 d, 7 d, 14 d, 21 d and 60 d).

Biofilm plates were 15 mm in diameter and constructed of the same concrete as the Reef Balls (figure 2c). Plates were soaked in distilled water for 2 weeks prior to deployment.

For examination of the biofilm, 3.53 cm^2 of the plate surface and 9.37 cm^2 of the glass slide surface were scraped to remove and mixed with 2 µm filtered seawater. The microbiota were fixed in 2% glutaraldehyde and then stained with the DNA specific flourochrome DAPI. Bacteria, algae, and other flora and fauna were enumerated by epifluorescence microscopy using UV illumination. In all cases, 50 random fields of view were counted per samples.



a. Biofilm Plate Array

b. Glass Slide Array

c. Biofilm Plates

Figure 2. Biofilm plate design.

Statistical Analysis

Analysis of bacteria and diatom treatments was performed by one-way ANOVA with the four treatments (3 replicates) selected from the full design. For growth rates based on cell count data, treatment effect was judged present if the F-statistic showed significance at $\alpha \leq 0.05$. If significant, then Tukey's HSD determined which treatments differed ($\alpha < 0.05$). Scatter plot and regression were fitted to pooled bacterial data summed across replicates within three substrate treatments. Nonparametric procedures (χ^2 approximation of Kruskal-Wallis $P \leq 0.05$) were employed

in lieu of parametric ANOVA when population variances were heterogeneous. Data analysis and graphing was completed using SPSS 11.0 ® software.

Results

Bacteria and diatoms were usually the most abundant organisms found on the biofilm plates. Significantly greater coverage of bacteria, diatoms and other associated flora and fauna was seen on plates that did not contain the iron surface treatment.

No significant differences in bacterial densities were found between treatments in most sampling intervals except for iron (figures 3-8, Tukey's HSD $\alpha = <0.05$). Highest rates of settlement were on concrete and $CaCo_3$ substrates at 14 and 21 days (figures 5 and 6, Tukey's HSD $\alpha < 0.05$). Bacterial densities increased through time in three treatments, concrete, $CaCo_3$, and glass slides, with pooled mean densities reaching > 800,000 cells cm^{-2} and remaining somewhat stationary after 14 days (figures 9 and 10). However, bacterial densities in iron treatments did not follow the same pattern as the other treatments and bacterial colonizers remained low compared to the other substrates (means $\leq$ 16,600 cells cm^{-2}, figures 1-8, and 11). Settlement was poorest on iron coated concrete in all trials.

Diatom populations fluctuated (figures 12-17) throughout the study with peak density at >80,000 cells cm^{-2} found on the concrete substrate at 21 days (figure 16). Yet, no significant difference existed between substrate types at 60 days except for iron. At day 60, mean densities had dropped to < 40,000 cells cm^{-2} (figure 17) in three treatments; no diatoms were reported in any iron treatments. Other autotrophic organisms also varied in numbers (figures 18-23) but day 60 showed no difference between substrate types (figure 23, χ^2 α =.102).

Early secondary micro-colonizers were mainly composed of known (i.e. nematodes and flagellates) and unidentified heterotrophs. The density of these consumers differed significantly between treatments over time (χ^2, α = .000, .050, .011, and .012) with treatments of concrete, $CaCo_3$, and glass slides exhibiting the greatest densities (figures 24-29), ranging from < 4000 cells cm^{-2} at 24 hours to >14,000 cm^{-2} at 60 days. The number of heterotrophic fauna remained very low on the iron substrate (figures 24-29) and after three days none were found (figures 26-29). Copepods, small molluscs, and small polychaetes comprised the remaining group of fauna associated with the majority of the biofilm plates.

Discussion

Microbial and algal films have been indicated as probable settlement inducers of marine benthic invertebrates, in addition, to providing secondary biotic space and shelter. Marine larvae are capable of responding to a variety of physico-chemical cues, some which may originate from organic films, during substratum exploration (Johnson *et al.* 1991; Anderson 1996; Johnson and Sutton 1994; Raimondi and Morse 2000). Larvae may respond to biofilms because they may reflect the physical regime that has acted on that surface over time (Wiecsorek and Todd 1998), relaying information about surface suitability for larval settlement and post-settlement survival. Therefore, the period of immersion and subsequent biofilm formation may be of paramount importance when assessing the colonization of secondary fauna on these artificial reef structures.

Results for three of the four treatments were not dramatically influenced by substrate type and bacterial numbers were similar. The single discrepancy in results was

at day 60 where one replicate had an exceedingly high count of 2,410,847 cells cm^{-2}. This suggests that even when organic films are developed over the same period under the same conditions, film formation can be patchy and cause important differences in colonization. Previous preliminary results (Robinson and Rogerson 2001) also demonstrated that bacteria and diatoms colonized surfaces of Reef Ball concrete and the count per unit appeared to increase with time (densities ranging between > 50,000 cells cm^{-2} to < 120,000 cm^{-2} and > 2500 cm^{-2} at 28 days, respectively). The number of bacterial colonizers were comparable to film formation on three of the four substrates in this study.

Colonization was uniformly low in all trails with iron surface coating. We hypothesize that initial substrate conditions may not have been attractive to microbial or micro- colonizers, in part, due to the potential interaction with the harsh environment, which may have effected biofilm formation and attachment preferences. Because iron concentrations on the substrate surface were extremely high and choice of this substrate for settlement was low, detailed interpretation of these results should be regarded with caution.

Microbial and algal films, which are ubiquitous in the marine environment, are probably not substratum specific, although some selectivity in extreme environments is likely (i.e. heavy metals, Basalobre 1993). Substrate type may be much less important in controlling the distribution and abundance of microbial colonizers than factors or events occurring after settlement, such as predation, disease, or migration. Our results revealed that, with the exception of iron coated concrete substrate, the development of the microbial film on the concrete substrates (concrete alone, concrete with $CaCo_3$) proceeded at a rate comparable to the control material (glass). The precise role of

biofilms in larval settlement remains unknown, but it is encouraging that the material used in the Reef Ball structures does not noticeably affect film formation.

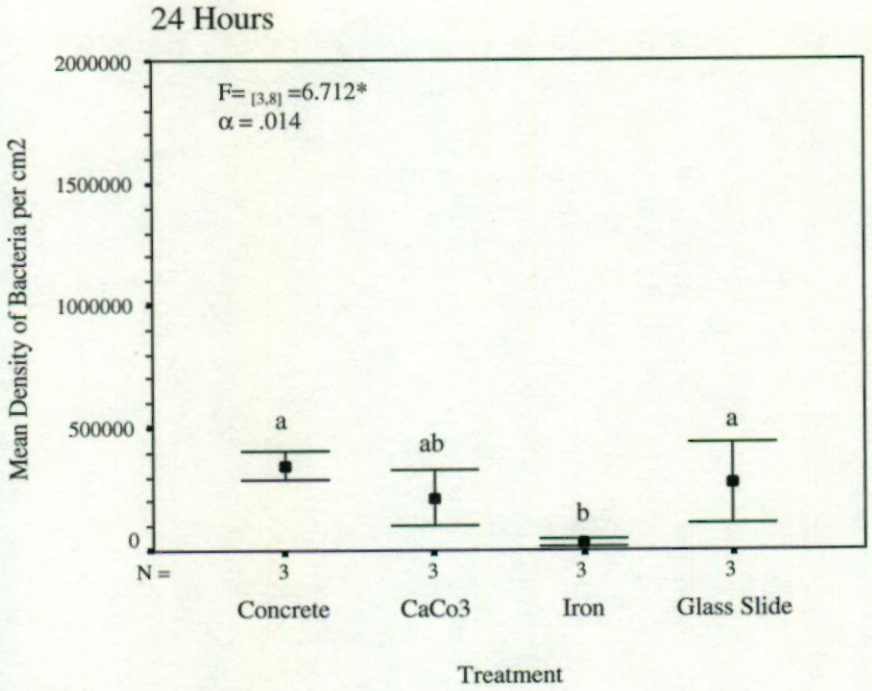


Figure 3.

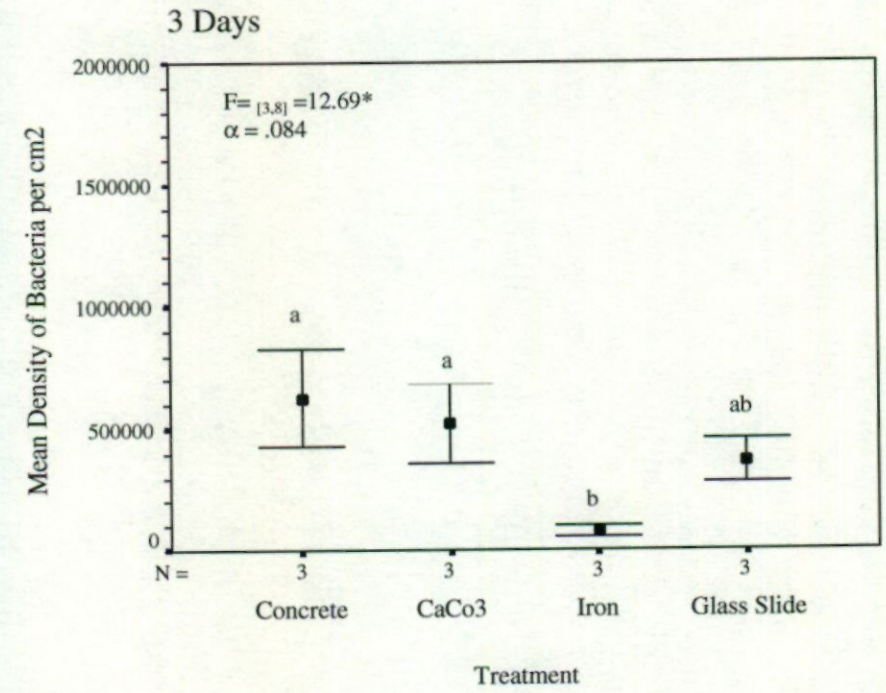


Figure 4.

Figures 3 and 4. Settlement responses of bacteria on the four substrates.
*Significant at P≤ 0.05, ANOVA. Lower case letters identify treatments not significantly different (Tukey's HSD P<0.05). Error bars show mean +/- 2.0 SE.

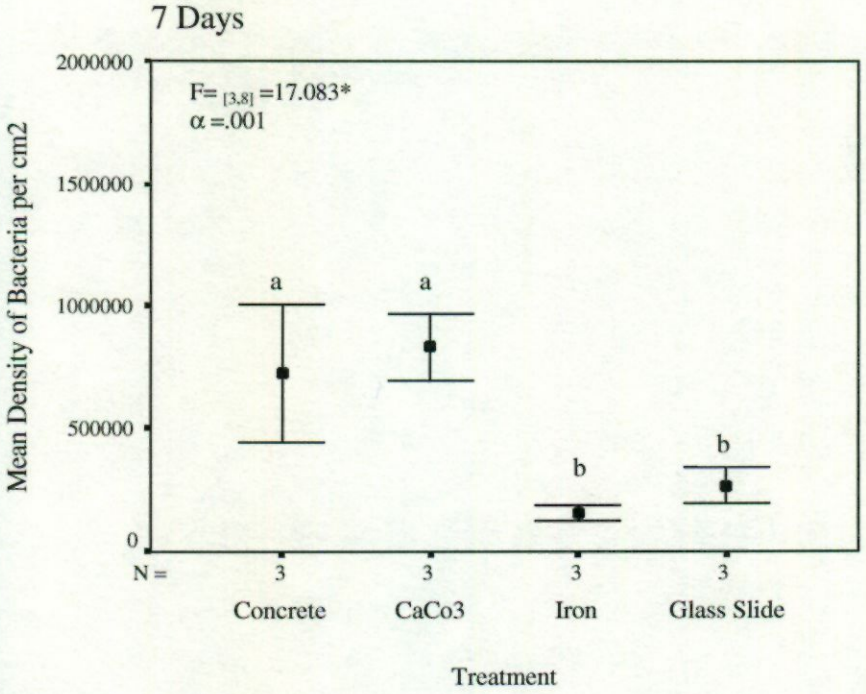


Figure 5.

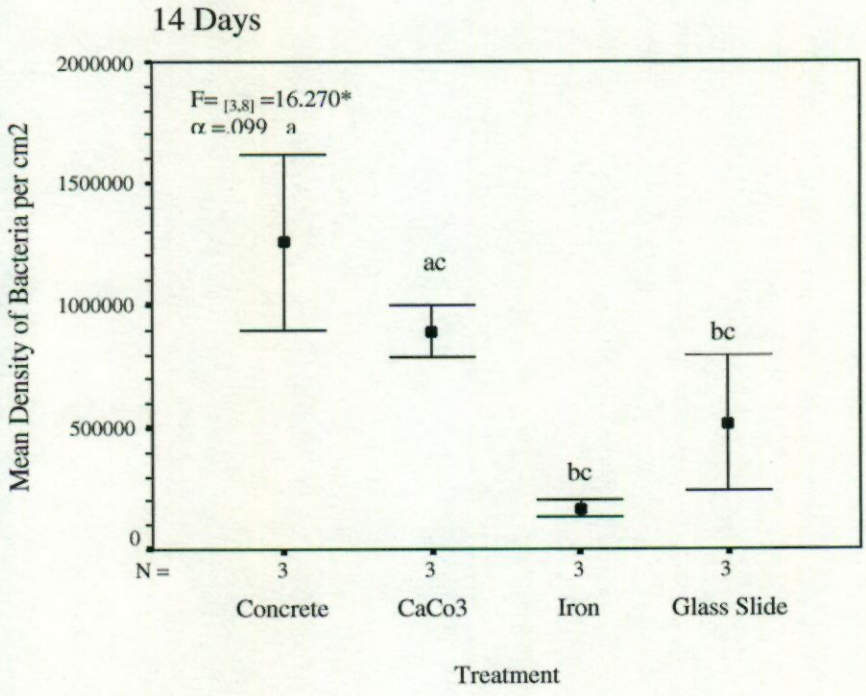


Figure 6.

Figures 5 and 6. Settlement responses of bacteria on the four substrates.

*Significant at P≤ 0.05, ANOVA. Lower case letters identify treatments not significantly different (Tukey's HSD P<0.05). Error bars show mean +/- 2.0 SE.

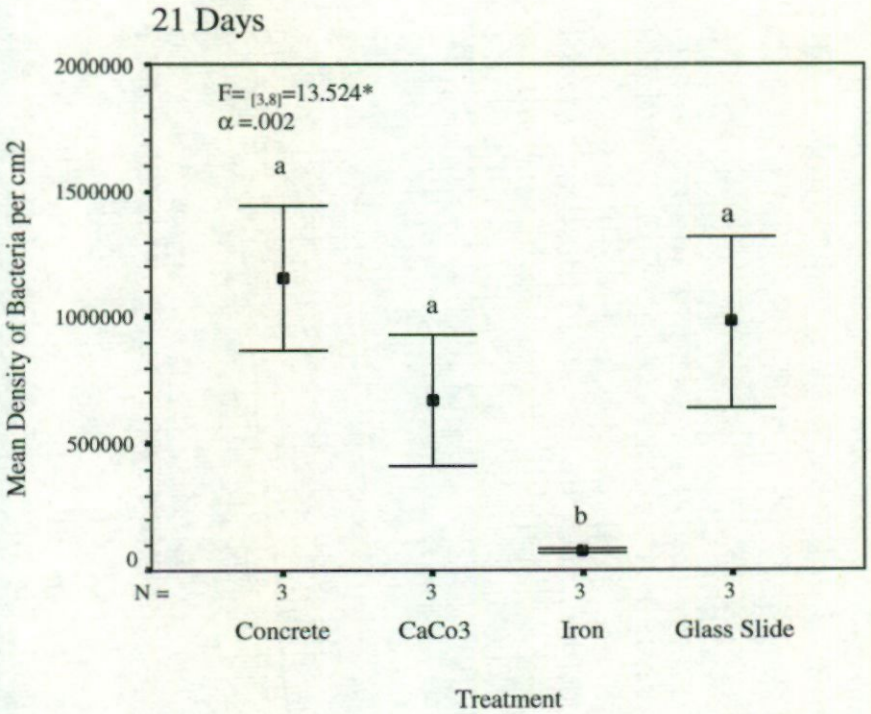


Figure 7.

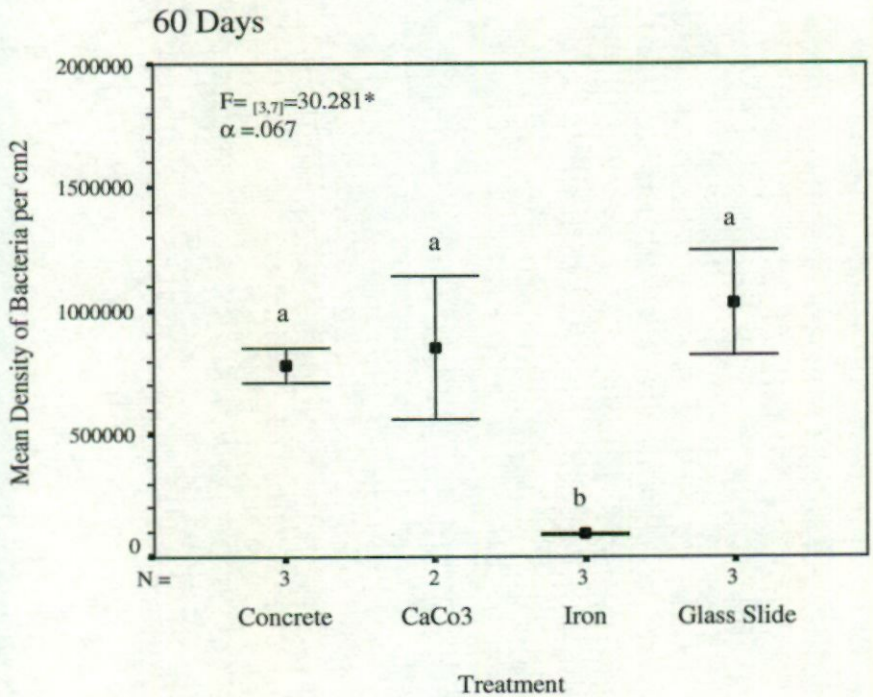


Figure 8. -- Figures 7 and 8. Settlement responses of bacteria on the four substrates.
*Significant at P≤ 0.05, ANOVA. Lower case letters identify treatments not significantly different (Tukey's HSD P<0.05). Error bars show mean +/- 2.0 SE. Replicate #3 at day 60 was excluded from the analysis.

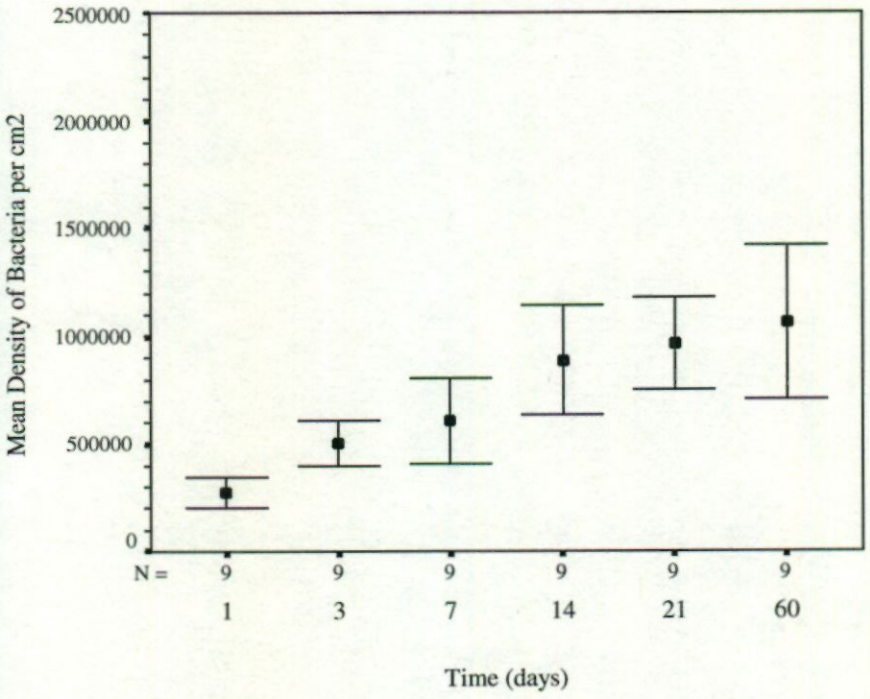


Figure 9. Mean densities of pooled bacterial counts over time: treatments concrete, $CaCo_3$, and glass slide. Error bars show mean +/- 2.0 SE.

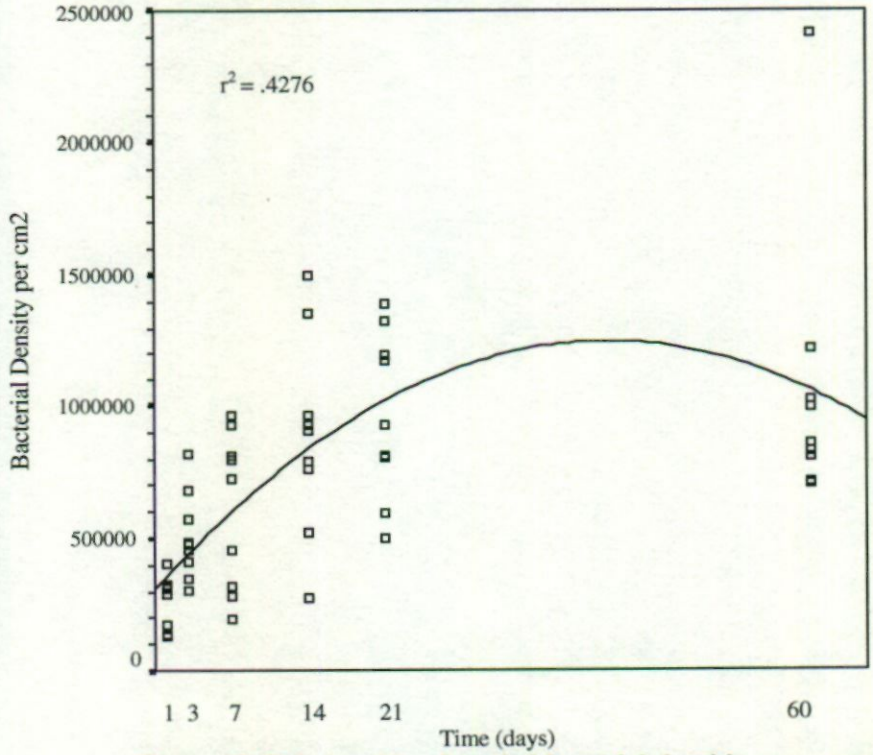


Figure 10. Relationship of pooled bacterial densities on concrete, $CaCo_3$, and glass slides treatments from initial deployment to day 60 (n=36).

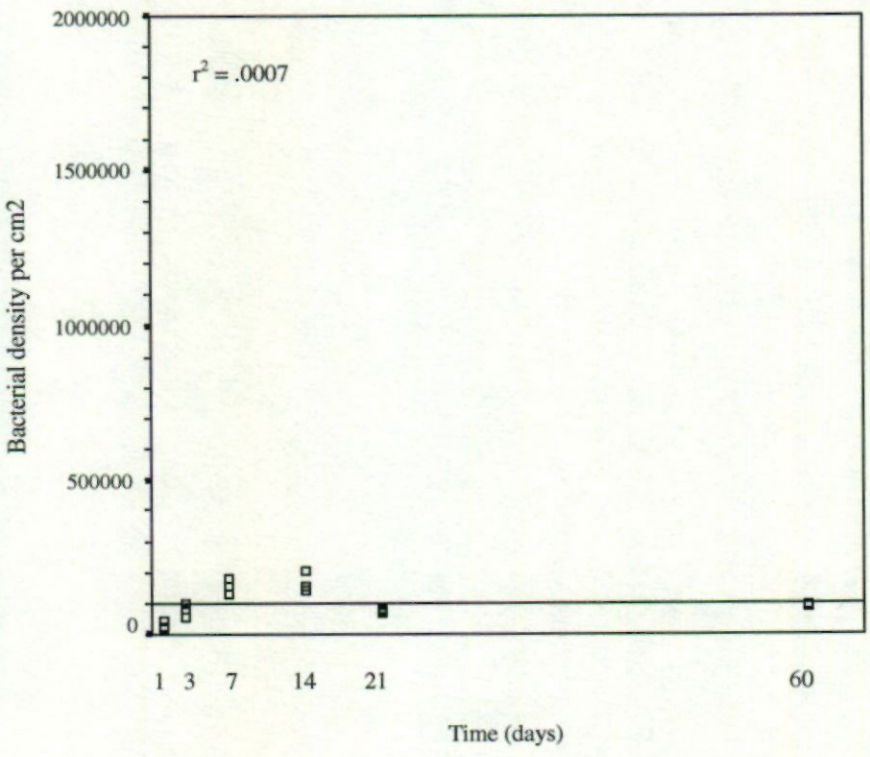


Figure 11. Relationship of bacterial densities on iron treatment from initial deployment to day 60 (n=18).

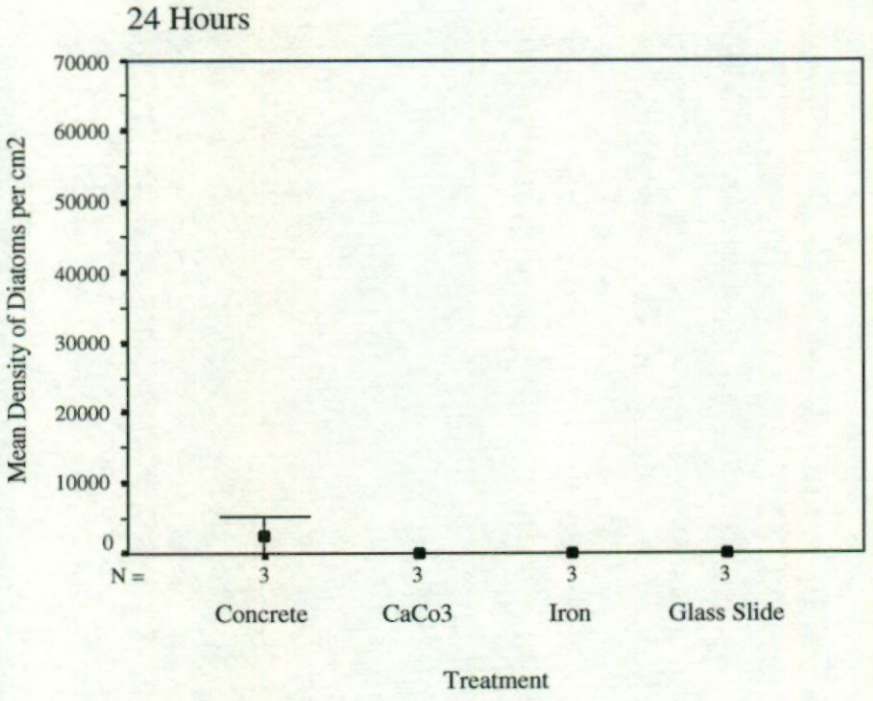


Figure 12.

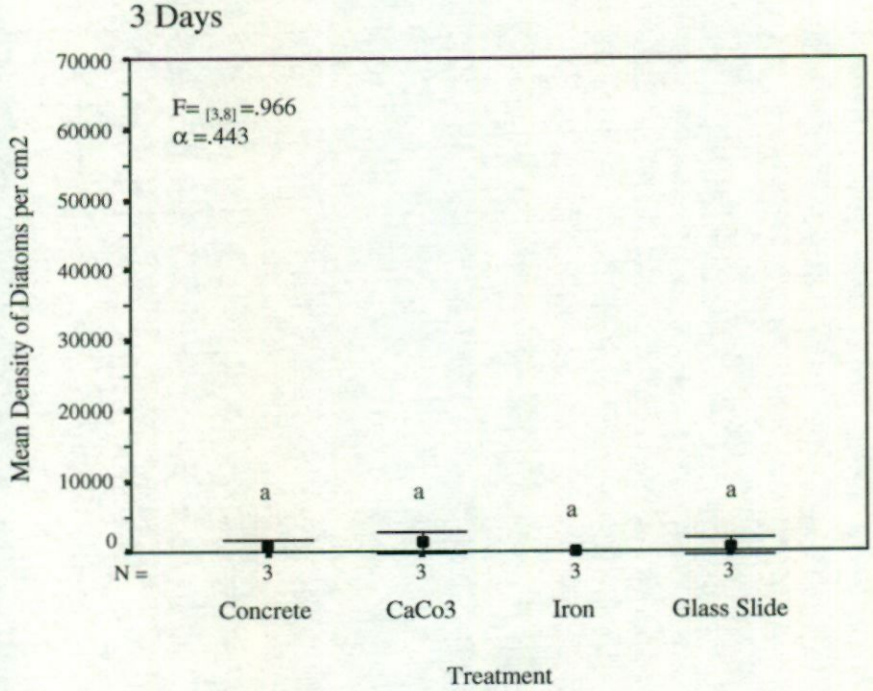


Figure 13.

Figures 12 and 13. Settlement responses of diatoms on the four substrates.

*Significant at P≤ 0.05, ANOVA. Lower case letters identify treatments not significantly different (Tukey's HSD P<0.05). Error bars show mean +/- 2.0 SE. No analysis was performed on 24 hour treatments.

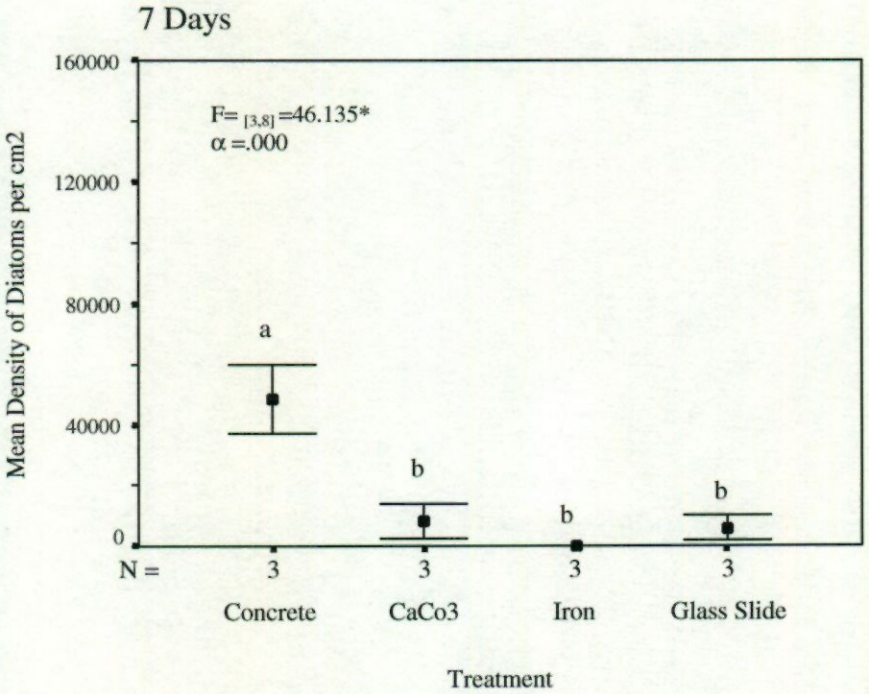


Figure 14.

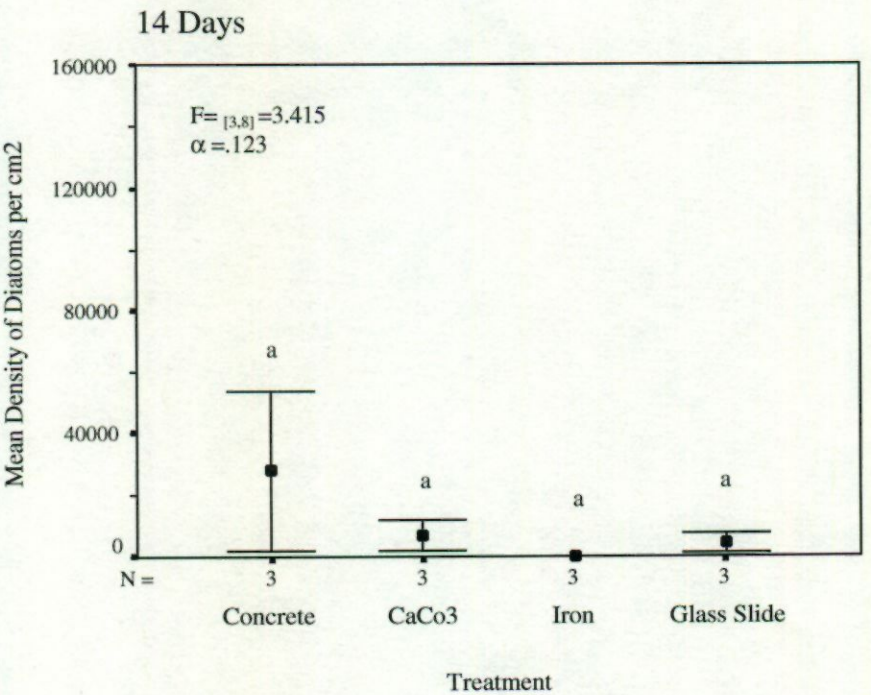


Figure 15.

Figures 14 and 15. Settlement responses of diatoms on the four substrates.
*Significant at P≤ 0.05, ANOVA. Lower case letters identify treatments not significantly different (Tukey's HSD P<0.05). Error bars show mean +/- 2.0 SE.

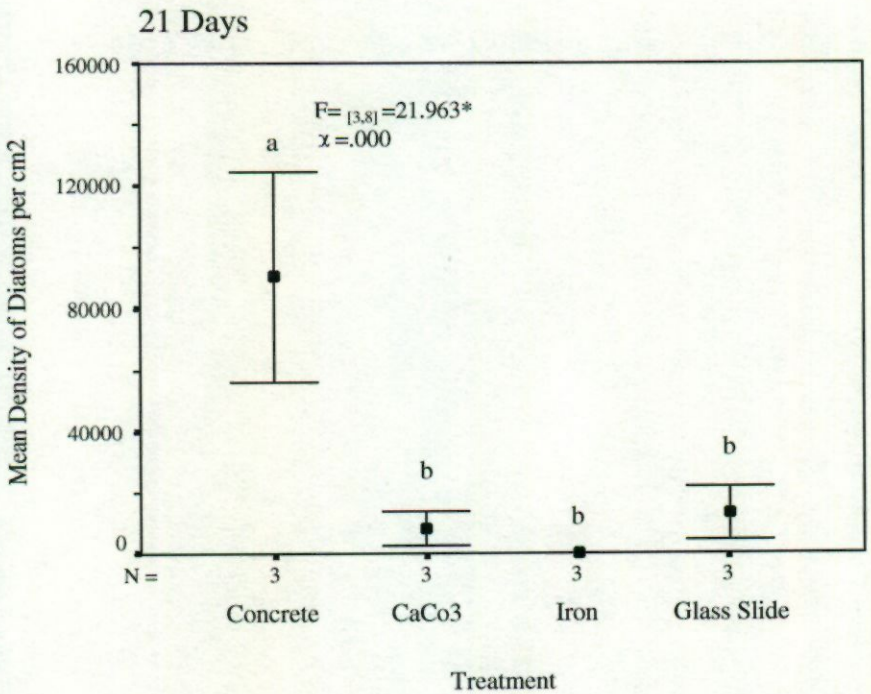


Figure 16.

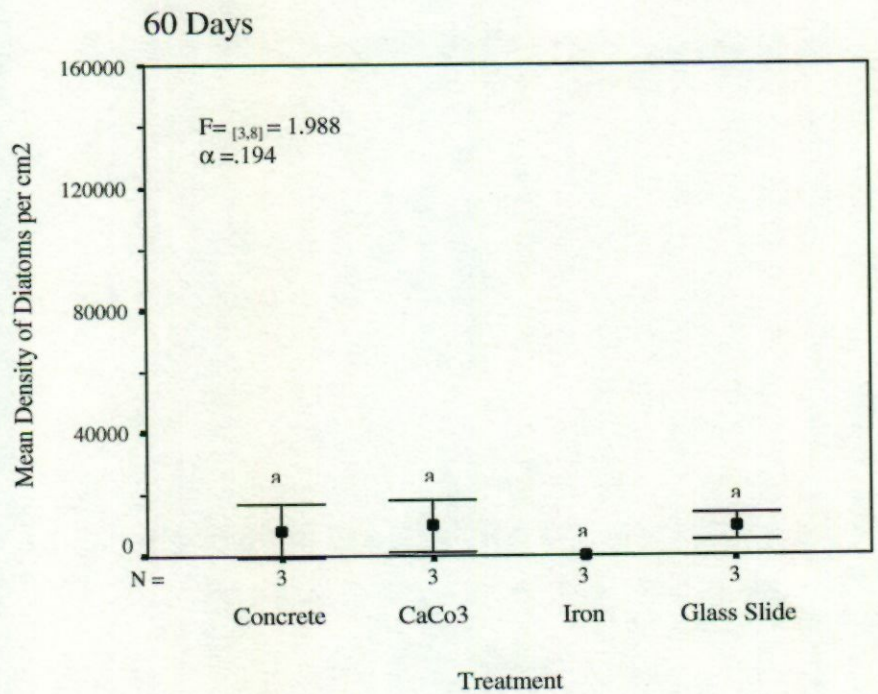


Figure 17.

Figures 16 and 17. Settlement responses of diatoms on the four substrates.
*Significant at P≤ 0.05, ANOVA. Lower case letters identify treatments not significantly different (Tukey's HSD P<0.05). Error bars show mean +/- 2.0 SE

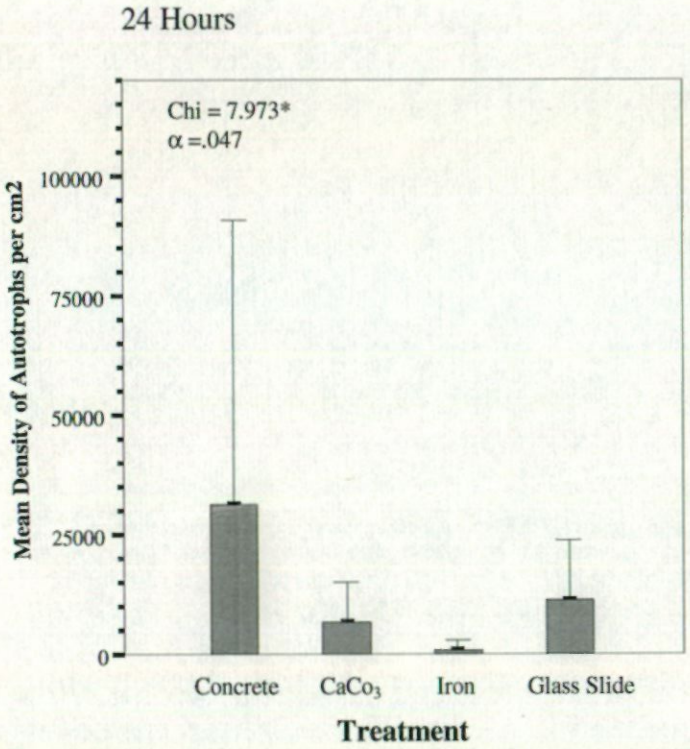


Figure 18.

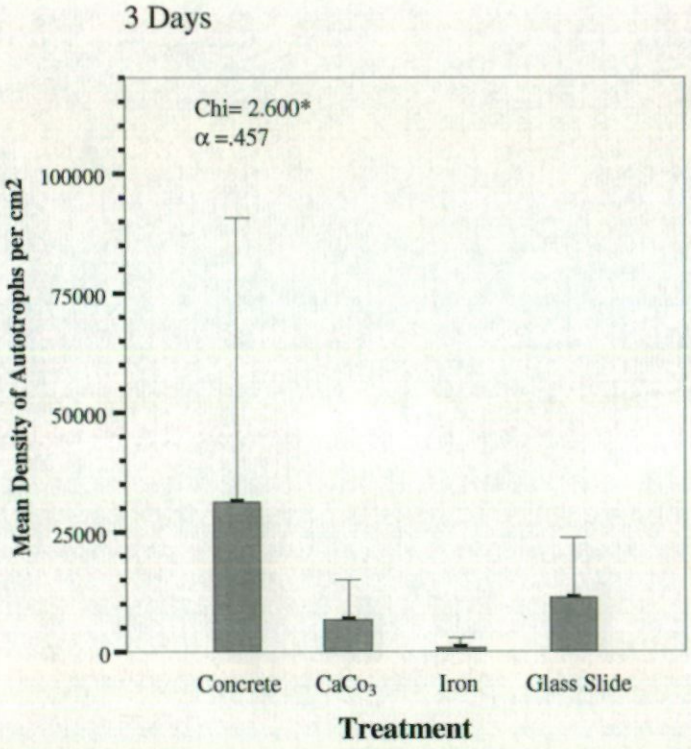


Figure 19.

Figures 18 and 19. Settlement responses of autotrophic organisms on the four substrates. Each bar shows the mean (+/- 2.0 SE) number of settlers per given treatment. *Significant at P= 0.05.

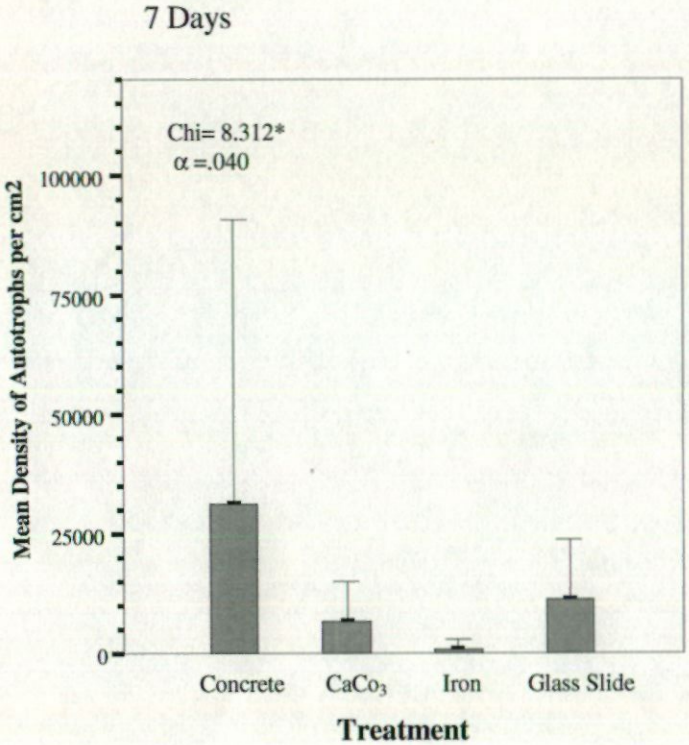


Figure 20.

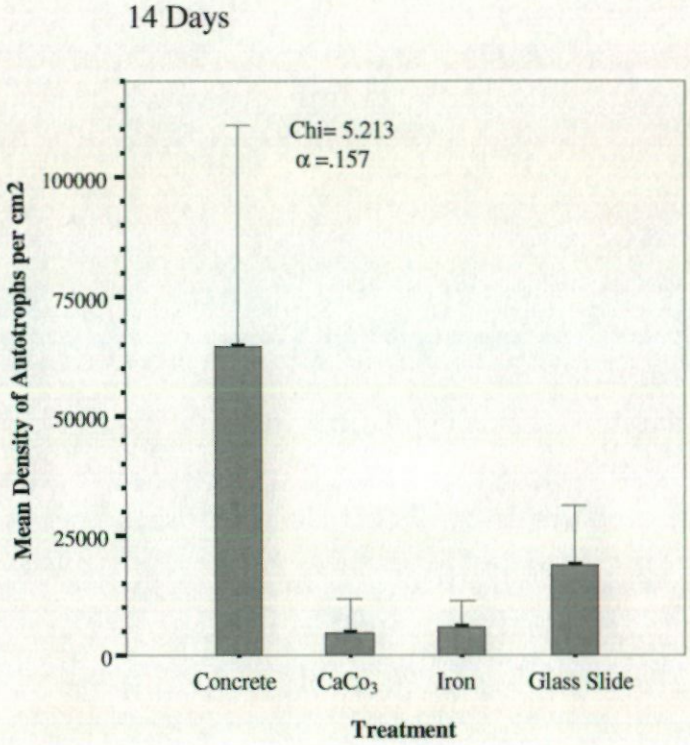


Figure 21.

Figures 20 and 21. Settlement responses of autotrophic organisms on the four substrates. Each bar shows the mean (+/- 2.0 SE) number of settlers per given treatment. *Significant at P= 0.05.

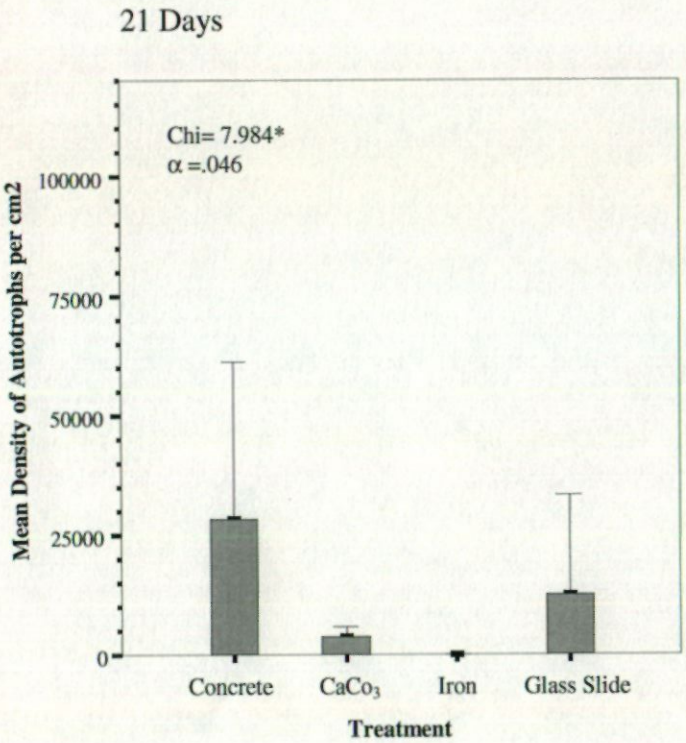


Figure 22.

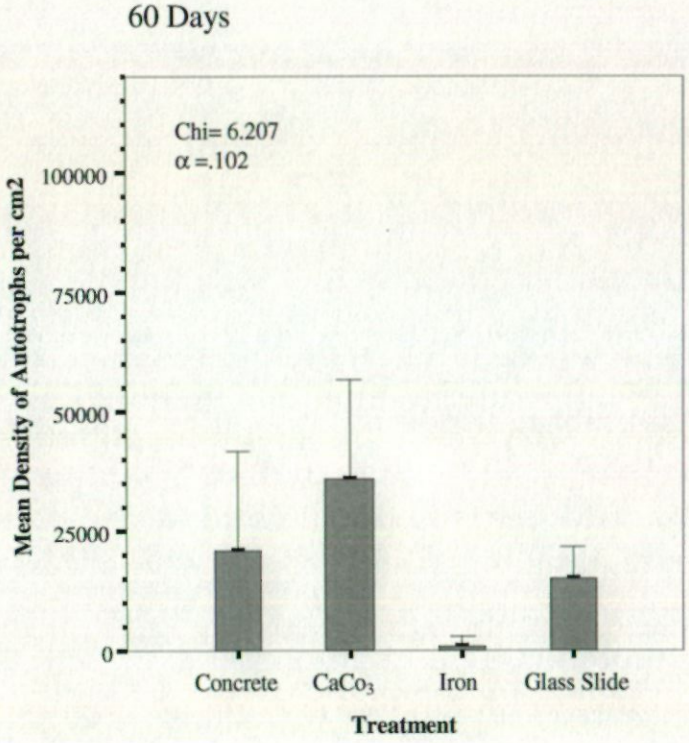


Figure 23.

Figures 22 and 23. Settlement responses of autotrophic organisms on the four substrates. Each bar shows the mean (+/- 2.0 SE) number of settlers per given treatment. *Significant at P= 0.05.

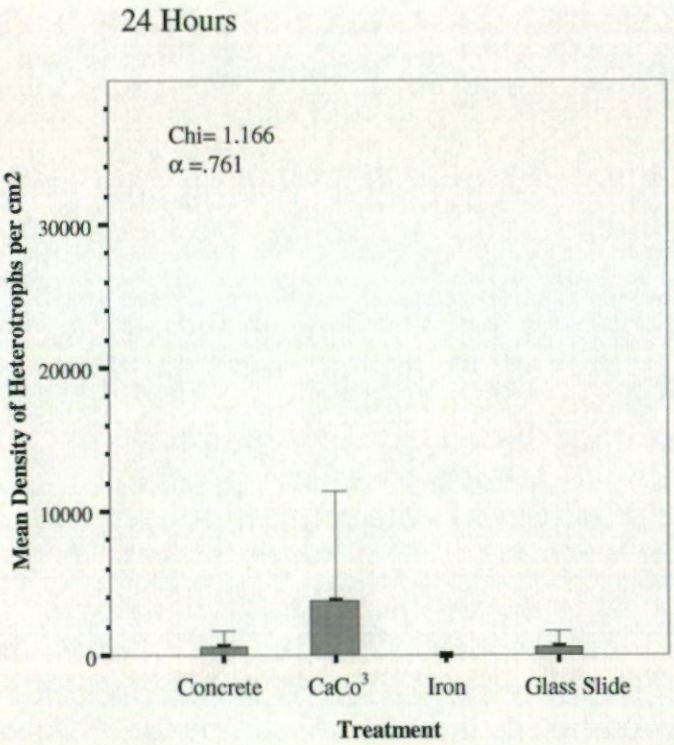


Figure 24.

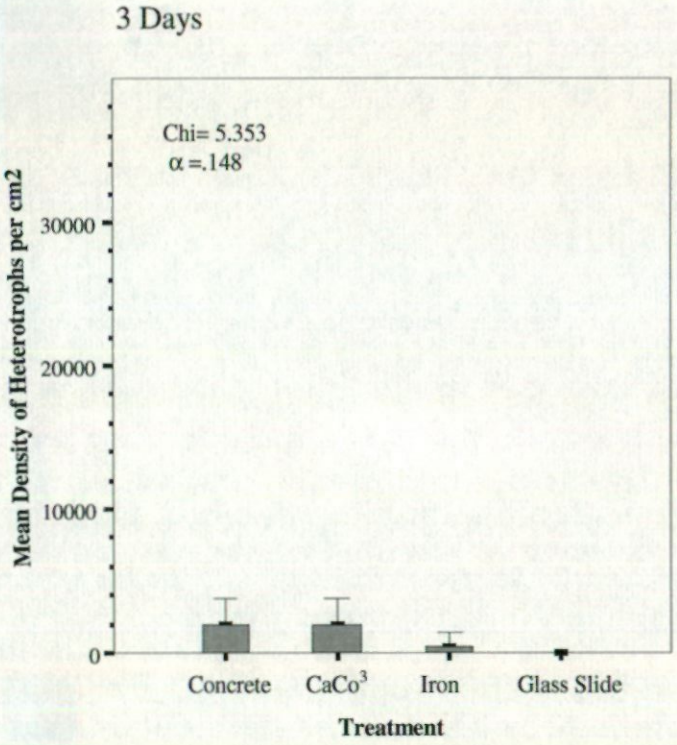


Figure 25.

Figures 24 and 25. Settlement responses of combined heterotrophic fauna. Each bar shows the mean (+/- 2.0 SE) numbers of settlers per given treatment. *Significant at P= 0.05.

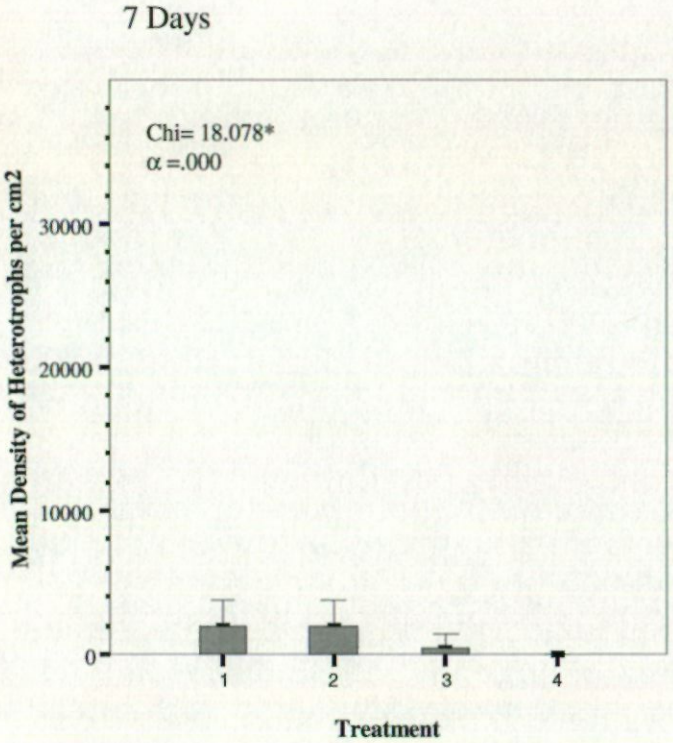


Figure 26.

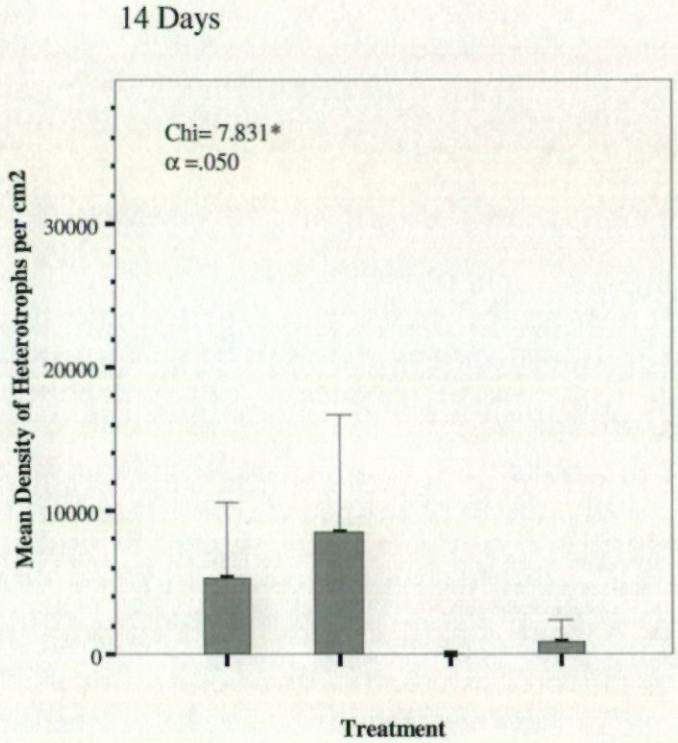


Figure 27.

Figures 26 and 27. Settlement responses of combined heterotrophic fauna. Each bar shows the mean (+/- 2.0 SE) numbers of settlers per given treatment. *Significant at P= 0.05.

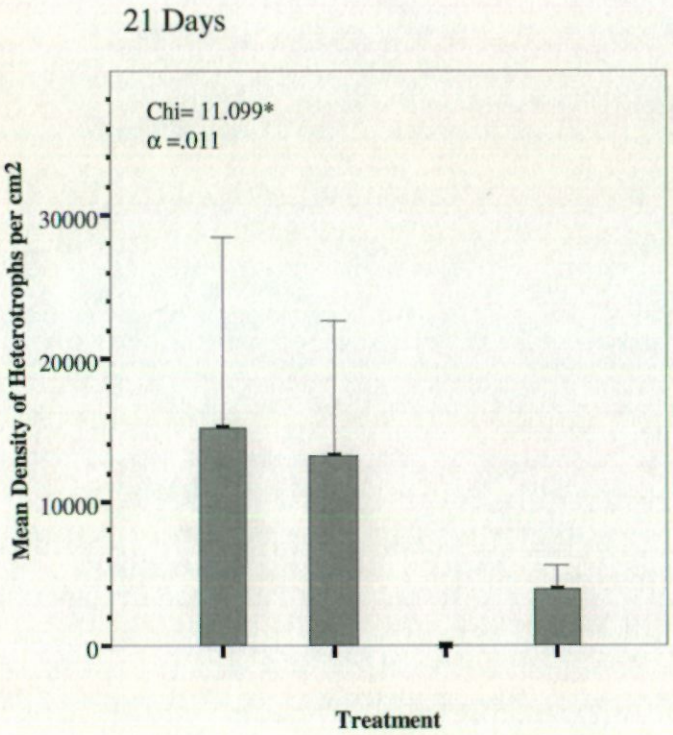


Figure 28.

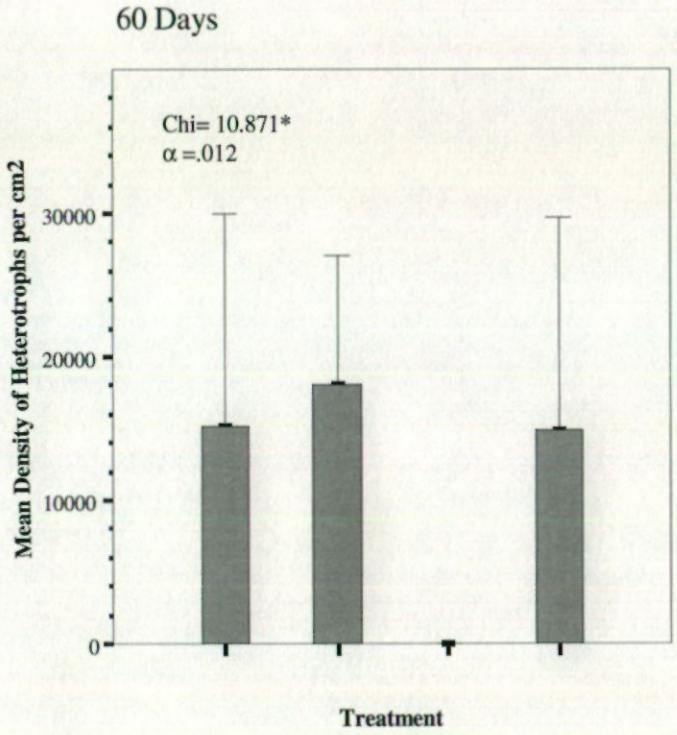


Figure 29.

Figures 28 and 29. Settlement responses of combined heterotrophic fauna. Each bar shows the mean (+/- 2.0 SE) numbers of settlers per given treatment. *Significant at P= 0.05.

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Scientific Presentations

1) Restoration Of A Southeast Florida USA Coral Reef Injured By The Grounding Of A Nuclear Submarine. Coral Reefs. Dodge R.E., R.E. Spieler, D.S. Gilliam, P. Quinn, A. Rogerson, E. Glynn, K. Banks, L. Fisher, D. Stout and W. Jap. 2000.

2) Preliminary Analysis of Initial Microfouling of a Nearshore Artificial Reef in Broward County, Florida. American Society for Limnology and Ocanorgraphy. Judy Robinson and Andrew Rogerson. 2001.

3) Hypothesis-based Restoration Study for Mitigation of a Damaged S.E. Florida Coral Reef: A Work in Progress. Artificial Reef Summit. T.P. Quinn, E.A. Glynn, R.E. Dodge, K. Banks , L. Fisher, R.E. Spieler

4) Growth And Survivorship Of Scleractinian Coral Transplants And Effectiveness Of Plugging Core Hole Sites. International Society for Reef Studies. E.A. Glynn, T.P. Quinn, D.P. Fahy, and R.E. Spieler. 2002.

HYPOTHESIS-BASED RESTORATION STUDY FOR MITIGATION OF A S.E. FLORIDA U.S.A. CORAL REEF DAMAGED BY THE GROUNDING OF A NUCLEAR SUBMARINE

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INTRODUCTION:

The *United States Submarine* Memphis (Figure 1) ran aground in approximately 10 meter depth on a coral reef off southeast Florida (Figure 2) February 25, 1993. Extensive physical damage to the reef substrate and injury to the coral community were attributed to the initial grounding and subsequent attempts to free the submarine from the impacted reef (Figures 3 and 4). The impact of the grounding was assessed, and the area of damage was determined through field and photographic studies.

Figure 1. *United States Submarine Memphis.*

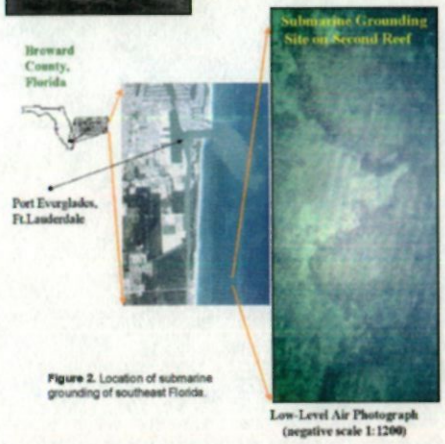

Figure 2. Location of submarine grounding of southeast Florida.

An impacted area of 2,310 m² was assessed with 1,205 m² having been totally destroyed (Figures 3 and 4). In 1997, the State of Florida was awarded a settlement of $750,000 by the Federal government for environmental damages caused by the submarine grounding. A plan to perform hypothesis testing of restoration techniques was developed and initiated.

Using artificial reefs as experimental platforms, we are examining three restoration strategies: 1) the potential of enhancing coral recruitment through the use of coral larval attractants, 2) the effect of reef structure on the associated fish assemblages, and 3) the interaction between fish assemblages and coral recruitment and survival.

Figure 3. Trench hole (3 m depth) excavated by the submarine's propeller during attempts to free itself from the reef.

Figure 4. Entry gouge approximately four years after the grounding.

METHODS:

One hundred and sixty small artificial reef modules (Reef Balls™) will be deployed in 11 m of water on a sand flat between reef tracks adjacent to the *U.S.S. Memphis* grounding site (Figure 5). The Reef Balls will be organized into 40, 4-module reef units (quad) in a square configuration having approximately 4 m sides (Figure 6). The separation of individual Reef Balls (2 m) is judged sufficient to avoid interaction effects between Reef Balls in terms of coral settlement, but close enough for the 4 balls to function as a single reef unit in terms of fish recruitment. Each quad will be located a minimum of 30 m from any hardbottom or other quads.

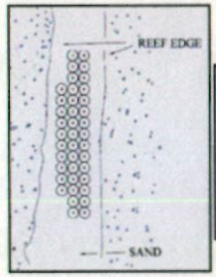

Figure 5. Large scale configuration of Reef Ball deployment. Each circle represents 30 m in diameter. Each X represents one quad of Reef Balls. The Reef Balls will be deployed between two reef tracks.

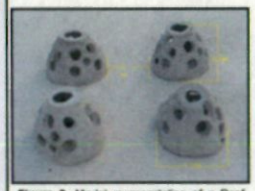

Figure 6. Model representation of a Reef Ball quad. Each individual Reef Ball will have 2 m separation from the other Reef Balls in the quad.

Coral Recruitment:
Settlement plates on each Reef Ball (Figure 7) will be used to test hypotheses on enhancing coral recruitment through the use of larval attractants. The settlement plates attached to each Reef Ball will be treated with a potential attractant (iron, algal extract, coral transplants) and compared with control plates (no attractant). Coral transplants will be 4" cores drilled from large donor colonies (Figures 8 and 9). Eighty coral cores will be transplanted onto the Reef Ball modules (forty cores of each of two different species). Control corals occurring on the natural reef, and of comparable size to the donor corals, will be monitored for comparison of growth and mortality.

Larval Attractants: Each individual Reef Ball in a quad will incorporate one of four different attractants on the settlement plates.
Iron additive ○ ○Algal extract
Coral transplants ○ ○Control

Figure 7. Reef Ball with settlement plates. Each Reef Ball will have two adjoining settlement plates. Settlement plates have smooth and rough surfaces to investigate the effects of surface texture on the preferential settlement of coral recruits. Each individual Reef Ball in a quad will incorporate one of four different attractants on the settlement plates: iron additive, algal extract, coral transplant, or control.

Figure 8. Coring donor corals for transplantation. Eighty coral cores will be transplanted onto the Reef Ball modules (forty cores of each of two different species).

Figure 9. Transplant Reef Balls will hold one core of each species (*Montastrea cavernosa* or *M. faveolata* and *Diploria clivosa*). Coral transplants will be placed in the pre-fabricated transplant holes adjacent to the settlement plates (yellow arrow).

Coral Transplantation and Monitoring:
At quarterly intervals the donor corals, coral transplants, and control corals will be visually assessed to provide information on individual colony health, growth, and mortality.

Fish Recruitment:
The 40 quads will be divided into 4 different levels of structural complexity to test the hypothesis that multiple refuge size and the resultant diverse fish assemblages may affect coral recruitment, survival, and growth. One set of 10 quads will have the void space of all the Reef Balls filled with large refuge structure (Figure 10). One set will have the void spaces of all filled with small refuge structure. Another set will be mixed and have one Reef Ball empty, one with large refuge, and the last two with small refuge. The final set will have the void space of all the Reef Balls empty. The assemblage of fishes (species, number, and size) associated with each quad will be recorded every three months by visual census (Figure 11).

Structural Complexity: Each type of fill will be used for 10 quads.
- Large fill – 4 concrete blocks in each Reef Ball of the quad.
- Small fill – 3/4" plastic mesh in each Reef Ball of the quad.
- Mixed fill – 1 Reef Ball of the quad with blocks, 1 empty and 2 with mesh.
- No fill – all 4 Reef Balls of the quad are empty.

Figure 10. Inside of Reef Ball with large fill complexity.

Figure 11. Diver conducting a fish survey on a Reef Ball displaying structural complexity of large refuge size.

Summary:

Artificial reefs are commonly used to provide structure to damaged reef areas. This project has been designed to use artificial reefs to not only mitigate for lost reef structure but to provide experimental platforms to examine several restoration strategies. The examination of these strategies will aid in making reef restoration decisions that involve: 1) the potential enhancement of coral recruitment through the use of coral larval attractants, 2) the effect of reef structure on fish assemblages, and 3) the interaction between fish assemblages and coral recruitment and survival.

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

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NCRI NATIONAL CORAL REEF INSTITUTE

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.

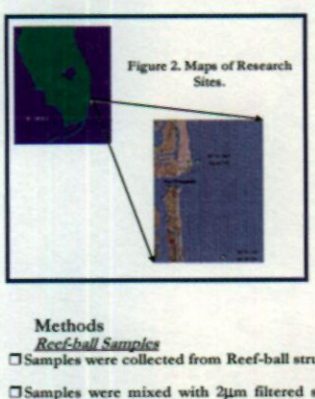

Figure 2. Maps of Research Sites.

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.

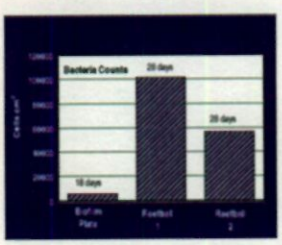

Figure 5a. Number of attached bacteria cm^{-2} determined by epifluorescence microscopy.

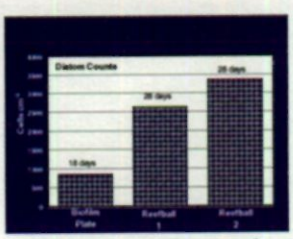

Figure 5b. Number of diatoms cm^{-2} determined by epifluorescence microscopy.

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.

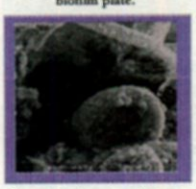

Figure 3c. Scanning electron mircograph of an invertebrate egg found on biofilm plate.

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 epifluorescence microscopy (Fig. 4 a,b, and c).
- Early secondary colonizers on the biofilm plate were mainly composed of copepods, small molluscs, and polychaetes.

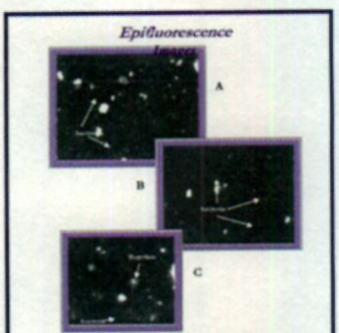

Figure 4 a,b, and c. Micrographs (1000X) of microbiota stained with DNA specific flourochrome DAPI.

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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Gascon, D. and Miller, R. 1981. Colonization of juvenile fish on small artificial reefs in Bodkin Sound, British Columbia. Can. J. Zool. 60: 798-806.

Maki, J., Rittschof, D., Schmidt, A., Snyder, A., and Mitchell, R. 1989. Factors controlling attachment of bryozoan larvae: a comparison of bacterial films and unfiltered seawater. Biol. Bull. 177: 295-302

Acknowledgements

This project is a result of research funded by Broward County Department of Planning and Environmental Protection, Florida Fish and Wildlife Conservation Commission, and the National Oceanic and Atmospheric Administration Coastal Ocean Program under award # NA86OA0390 to Nova Southeastern University for the National Coral Reef Institute.

Figure 5.

HYPOTHESIS-BASED RESTORATION STUDY FOR MITIGATION OF A DAMAGED S.E. FLORIDA CORAL REEF: A WORK IN PROGESS

T.P. Quinn*, E.A. Glynn*, R.E. Dodge*, K. Banks †, L. Fisher†, R.E. Spieler*
*National Coral Reef Institute, Nova Southeastern University Oceanographic Center, 8000 North Ocean Drive, Dania Beach, FL, USA 33004
E-mail address: quinn@nova.edu
†Broward County Department of Planning and Environmental Protection, 218 S.W. 1st Avenue, Ft. Lauderdale, FL 33301

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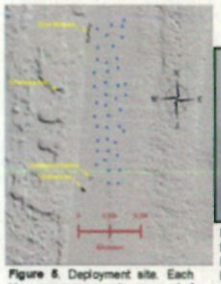

Figure 5. Deployment site. Each blue square represents one quad of Reef Balls.

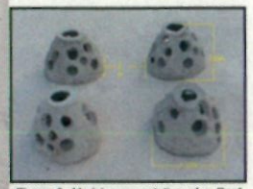

Figure 6. Model representation of a Reef Ball quad. Each individual Reef Ball will have 2 m separation from the other Reef Balls in the quad.

Coral Recruitment:
Settlement plates on each Reef Ball (Figure 7) are being used to test hypotheses on enhancing coral recruitment through the use of larval attractants. The settlement plates attached to each Reef Ball are treated with a potential attractant (iron, $CaCO_3$, coral transplants) and compared with control plates (no attractant). Coral transplants are 4" cores drilled from large donor colonies (Figures 8 and 9). Eighty coral cores have been transplanted onto the Reef Ball modules (forty cores of each of two different species). Control corals occurring on the natural reef, and of comparable size to the donor corals, are being monitored for comparison of growth and mortality.

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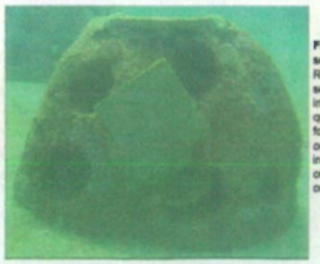

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Fish Recruitment:
The 40 quads are divided into 4 different levels of structural complexity (refuge) to test the hypothesis that multiple refuge size and the resultant diverse fish assemblages may affect coral recruitment, survival, and growth. One set of 10 quads has the void space of all the Reef Balls filled with large refuge structure (Figure 10). One set has the void spaces of all filled with small refuge structure (Figure 11). Another set is mixed having one Reef Ball empty, one with large refuge, and the last two with small refuge. The final set has the void space of all the Reef Balls empty. The assemblage of fishes (species, number, and size) associated with each quad is being recorded every three months by visual census.

Structural Complexity: Each type of refuge is used for 10 quads.
- Large refuge – 4 concrete blocks in each Reef Ball of the quad.
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- Mixed refuge – 1 Reef Ball of the quad with blocks, 1 empty and 2 with mesh.
- No refuge – all 4 Reef Balls of the quad are empty.

Figure 10. Inside of Reef Ball with large refuge.

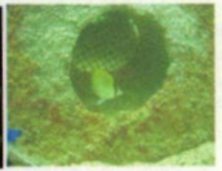

Figure 11. Hole in Reef Ball showing small refuge.

Summary:

Artificial reefs are commonly used to provide structure to damaged reef areas. This project has been designed to use artificial reefs to not only mitigate for lost reef structure but to provide experimental platforms to examine several restoration strategies. The examination of these strategies will aid in making reef restoration decisions that involve: 1) the potential enhancement of coral recruitment through the use of coral larval attractants, 2) the effect of reef structure on fish assemblages, and 3) the interaction between fish assemblages and coral recruitment and survival.

Growth and Survivorship of Stony Coral *Meandrina meandrites* and *Montastrea cavernosa* Transplants to an Artificial Reef Environment: A Work in Progress.

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INTRODUCTION:

Reef Ball Deployment:

In November of 2000, 160 concrete Reef Ball™ modules (1.22m wide x 0.9m high) were deployed, at a depth of approximately 15 meters, between the Second and Third Reef tracts off Dania Beach, FL (Figures 1 and 2). The Reef Balls were grouped into 40 quads, with each quad containing four individual Reef Balls. One modified Reef Ball from each quad was designated as the 'transplant' ball, and was used as the receptacle for the coral transplants. The other three balls in each quad are part of a more comprehensive study. This multifactorial study is examining the effects of reef structure on fish assemblages, the effects of coral larval attractants on coral recruitment, and the interaction between fish assemblages and coral recruitment. The coral transplants are one such 'coral larval attractant' being examined. Coral transplants, and the donor colonies from which they were obtained, are being monitored for growth and survivorship.

Figure 1. Reef Ball construction at Nova Southeastern University Oceanographic Center.

Figure 2. Reef Ball deployment off Dania Beach.

Study sites:

The donor corals and controls of two different sizes are located at a depth of approximately 10 meters on the Second Reef off Dania Beach (natural reef site) (Figure 3). The coral transplants are located on the Reef Balls between the Second and Third Reefs (artificial reef site).

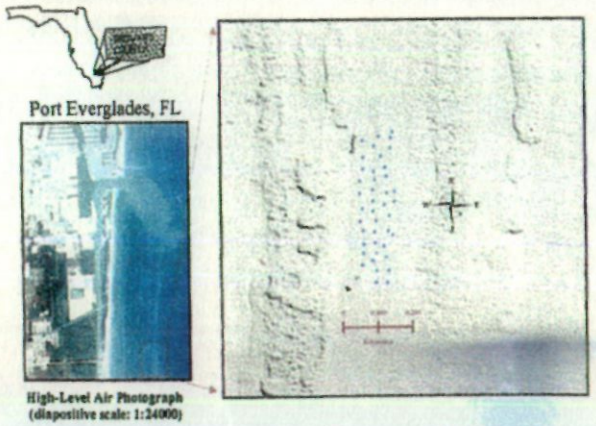

Figure 3. Study site location, including natural reef site (outlined in yellow) and adjacent Reef Ball artificial reef site (blue squares signify quads).

METHODS:

Mapping and Drilling:

Forty colonies of two different species of stony coral, *Meandrina meandrites* and *Montastrea cavernosa*, were tagged and mapped at the natural reef site on the Second Reef (Figure 4). Between March and June 2001, two core hole plugs with living coral tissue (coral transplants) were obtained from each donor coral using a hydraulic drill fitted with a four inch core barrel (Figure 5).

Figure 4. Diver mapping coral colonies.

Figure 5. Diver drilling transplant cores.

Transplantation of coral cores and filling of core holes:

The transplant corals were affixed to the Reef Balls at the artificial reef site using an underwater adhesive marine epoxy (Figure 6). One *Meandrina meandrites* and one *Montastrea cavernosa* transplant were attached to each 'transplant' Reef Ball.

All donor core holes were filled with pre-fabricated, numbered (1-80), concrete plugs to prevent detrimental effects of bioeroders (Figure 7). The concrete plug numbers coincide with the transplant numbers making for ease of comparison of the live tissue surrounding the concrete plug with the live tissue on the transplant.

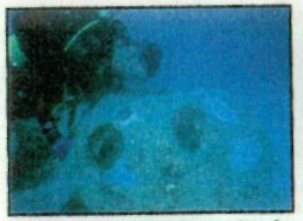

Figure 6. Diver finishing the epoxying of coral transplants onto Reef Ball.

Figure 7. Diver epoxying concrete plug into donor coral.

Acknowledgements: We thank the Broward County Department of Planning and Environmental Protection, the Florida Marine Research Institute, and the numerous NSUOC students who helped with the Reef Ball construction and coral transplantation.

PRELIMINARY RESULTS:

Monitoring of donor corals:

The 40 donor colonies were photographed using a Nikonos V camera with a 20mm lens and a 0.75m² PVC framer marked in 10cm increments, prior to drilling and after plugging the core holes with the concrete plugs (Figure 8). These colonies are now being photographed quarterly for the monitoring of health and survivorship. The donor corals showed 100% survivorship in the second monitoring session. Additionally, 20 large control corals (comparable size to the donors) are being monitored at the natural reef site.

Figure 8. Donor # 2 – Before drilling, post-drilling, and three months after drilling.

Monitoring of transplants and core hole recovery:

The coral transplants and the core holes are being photographed with a 28mm lens and close-up kit for coral skeleton growth (Figures 9 and 10). Coral skeletal growth is defined as an increase in surface area or linear radius and is being measured quarterly. SigmaScan Pro4 image analysis software (Jandel Scientific Corporation) is being used for all of the photographic analysis. Additionally, 20 small control corals (comparable size to the transplants) are being monitored at the natural reef site.

Figure 9. Transplant # 8 – After epoxying onto the Reef Ball, at three months, and at six months.

Figure 10. Tissue surrounding Plug # 7 – After epoxying into coral, at three months, and at six months.

CONCLUSIONS:

This photographic method is suitable for continuous monitoring and causes no harm to the coral colony. The preliminary results obtained during the first quarterly sample session demonstrated 100% survivorship for all coral plug transplants and donor colonies. It was noted that most coral plug transplants, with exposed skeleton around the margins of the plug, experienced tissue advancement over the bare coral skeleton.