EFFECTS OF DRILLING MUD ON SEVEN SPECIES OF REEF-BUILDING CORALS AS MEASURED IN FIELD AND LABORATORY[1]

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ABSTRACT

Eight modified 96 hour bioassays were conducted in the field on seven species of reef-building corals at Carysfort Reef in the Florida Keys. Three concentrations (100 µℓ/ℓ, 316 µℓ/ℓ, and 1,000 µℓ/ℓ) resulting in dilutions of 10,000:1, 3,160:1, and 1,000:1, respectively, of a drilling fluid (mud A) from an offshore rig drilling at a formation depth of 4,200 m was used in each bioassay. Mud A and a second drilling fluid (mud B) from a formation depth of 1,650 m were compared in the laboratory with similar bioassays on *Porites divaricata*. Muds A and B were also used in a clearing rate experiment on *Montastrea annularis*. Behavioral reactions of all coral colonies used in the bioassays were quantified by determining the percentage of polyp retraction measured from macro-underwater photographs taken during the bioassays.

Three of the species tested in the field (*Montastrea annularis*, *Agaricia agaricites*, and *Acropora cervicornis*) were killed after 65 hours of exposure to a 1,000:1 dilution of mud A. No mortality occurred in the other four species (*Porites astreoides*, *P. divaricata*, *P. furcata*, and *Dichocoenia stokesii*) exposed to the same dilution. No mortality resulted in any of the above species exposed to dilutions of 3,160:1 and 10,000:1 of mud A. In laboratory studies mud A was shown to be slightly more toxic than mud B when tested at equal dilutions.

Burial and clearing rate experiments were also conducted in the field. In the burial study some corals recovered from burial in natural carbonate reef sand but suffered significant tissue and/or zooxanthellae loss. Burial under drilling mud A caused mortality sooner than in carbonate reef sand. In clearing rate experiments a thin cover of mud A and natural carbonate reef sand were each removed from the

surface of *Montastrea annularis* within 2 hours, but mesenterial filament extrusion indicated both corals were stressed.

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INTRODUCTION

Exploratory drilling for oil and gas has been taking place near the Flower Garden Coral Reefs (located approximately 110 nautical miles south-southeast of Galveston, Texas) during the last few years, and production facilities are planned for this area in the near future. The Flower Gardens are the only true coral reefs in the northern Gulf of Mexico, and there has been considerable concern over the potential effects of man's increasing activities in their vicinity. During offshore drilling, plumes of turbid water are commonly seen trailing downstream from the drilling platform. Occasionally, the plumes may be visible for more than 3 km.

Drilling fluid or "mud," which is the principle component of these plumes, is routinely discharged into the surrounding water at widely varying rates during normal operations.

One of the most important functions of drilling mud is to remove material (cuttings) being discharged by the drill bit. Although drilling fluids are separated from these cuttings by a shaker table and recirculated, some mud still adheres to the cuttings until they are discarded overboard. When the cuttings enter the water, much of the remaining mud is washed off and carried away by currents.

Drilling mud may also be introduced into the ocean when sand and silt traps are emptied to make room in the mud pits for the addition of new components or when the mud pits are emptied at the end of drilling operations. The last instance may involve disposing of as much as 2,000 bbls of mud in a few hours.

Before the potential effects of these effluents on a coral reef can be calculated, two basic kinds of information are necessary: (1) what dosages (time-concentration) of the various materials in question are required to induce lethal or sublethal effects on the target organisms; and (2) how these materials will be distributed about their source (the drilling rig). If answers to both questions are well understood, then it is possible to make realistic predictions concerning the possible effects of drilling operations at different locations around the community of interest. The

work presented in this paper was designed to provide information useful in answering the first question.

Scleractinian corals have been chosen as the test organisms for several reasons: (1) they are the basic frame builders of a coral reef; without their presence, the reef and its associated organisms would not exist; (2) they are sessile and, therefore, are unable to exhibit avoidance reactions; (3) preliminary investigations have indicated that corals are relatively sensitive to environmental perturbations; and (4) behavioral reactions of the individual animals, or "polyps," of which the coral colonies are composed can be readily quantified.

Experiments were conducted in both laboratory and field. Laboratory efforts permit economical development of technique, screening of test materials and range-finding of concentrations. Field experiments permit exposure of test organisms to test substances under the most realistic environmental conditions.

FIELD STUDY

96 Hour Behavioral Tests

Materials and Methods

Experiment site

The experiments were conducted in 2-3 meters of water on a sand flat at Carysfort Reef off Key Largo, Florida (Figs. 1, 2). Carysfort Reef is a particularly desireable study area as it is one of the most flourishing reefs in the Florida Keys, and the 126 year-old lighthouse built directly on the reef provides an excellent research platform. Although no longer manned, the structure was temporarily occupied for research purposes with permission from the Coast Guard. By conducting the experiments in chambers located on the reef, it was possible to:

1) assure an ample supply of test organisms;
2) transport the specimens with a minimum of handling and stress to the corals;
3) conduct the experiments under natural environmental conditions.

Treatment chambers

Eight experimental chambers were constructed of 22-liter glass aquaria equipped with tightly fitting plexiglass lids (Fig. 3). Foam weather stripping was added to the sealing surfaces to reduce leakage.

Circulation was provided within each tank by a 300 gph submersible bilge pump (Attwood Mini-King). Each of the 12-volt pumps was connected in parallel to a lead acid storage battery located on the lighthouse. A Honda E1500 generator charged the battery. Pump outflow was directed across the back of each aquarium to produce sufficient turbulence to maintain suspension without subjecting the corals to excessively high water velocities which might disturb the polyps. All eight chambers were secured on a metal frame approximately 1 m wide and 2.2 m long. The frame provided a stable, level support for the chambers, which otherwise would have been rocked back and forth by wave surge.

Coral collection

Divers collected corals by hand, using techniques which minimized trauma to living tissue. Generally, it was possible to remove the colonies from their substrate by splitting the non-living base of the head with a chisel. Branching species were removed from the parent 'bush' by pruning with wire cutters. This procedure produced a clean break around which the coral tissue quickly healed. Once collected, the corals were placed in plastic bags and carried under water to the nearby experiment chambers. Seven species were collected: *Acropora cervicornis*, *Montastrea annularis*, *Porites astreoides*, *P. divaricata*, *P. furcata*, *Dichocoenia stokesii*, and *Agaricia agaricites*.

Test mud

Mud A: The drilling mud examined in this series of experiments was a fresh-water ferrochrome lignosulfonate mud, which was obtained from the mud pit of a platform located offshore of Louisiana. At the time of sample collection, drilling was

occurring at a depth of 4,200 m. The daily mud record indicates that mud density was 10.5 lbs/gal. A pH of 8.84 and suspended solids of 476,00 mg/ℓ were measured in our laboratory.

Test dilutions were obtained by first preparing a slurry containing 20 percent whole mud and 80 percent sea water. Appropriate volumes of slurry were then injected into each treatment chamber with large syringes (Fig. 4). This mud was used in all field experiments.

Mud B: A second mud was used only in the laboratory experiments. It was similarly collected from another platform offshore of Louisiana. Drilling had reached a depth of 1,650 m at time of collection. Mud density was also recorded as 10.4 lbs/gal; however, the pH and suspended solids concentration were measured as pH 9.61 and 255,000 mg/ℓ. Test dilutions were prepared for both muds in the laboratory as described above.

Experimental design

In each experiment two colonies of a given species were randomly selected and placed in each of four treatment chambers. The concentration of mud used with a particular chamber was also randomly determined.

Concentrations in the test tanks were 100 µℓ/ℓ, 316 µℓ/ℓ, and 1,000 µℓ/ℓ, plus a control with no mud. These concentrations correspond to dilutions of 10,000:1, 3,160:1 and 1,000:1, respectively. Some of the mud was continuously lost from suspension as particles floculated and settled out or became attached to various surfaces, including the coral and the vertical sides of the glass aquarium. To restore the concentration of suspended mud and to avoid build-up of waste products from the corals, the water in each tank was changed daily and new mud injected. It was also necessary to clean inner and outer surfaces of the glass daily to prevent build-up of algae.

Physical condition of each colony was recorded with close-up photographs taken

with a Nikonos underwater camera equipped with a 35mm lens and extension tube (Fig. 3). A photo sequence of the corals was taken immediately prior to administering the first doses of mud, and at approximately 24 hour intervals thereafter. Additional series of photographs were taken at night, as some species of coral exhibit diurnal behavioral patterns. Photographic documentation was supplemented with direct observations made several times a day. Each experiment was terminated after 96 hours (4 days) and the coral specimens returned to the reef.

Measurement of behavioral response

As a measure of stress in the corals, the number of polyps (1) fully retracted (polyp completely withdrawn into its calice, no tentacles visible), (2) partially retracted, and (3) fully expanded (full whorl of tentacles displayed at maximum or near maximum length) were counted from each of the color slides (see Figs. 5-1, 5-2). Any other stress reactions, such as excessive mucus production or mesenterial filament extrusion, were also noted. The total number of polyps visible on each colony was reasonably uniform between colonies of the same species, but varies from as few as 25 polyps in *Dichocoenia stokesii* to as many as 200 in *Porites furcata*.

Analysis of data

Using measurements taken from each photographic slide, percentage of polyps not fully expanded (i.e., number completely retracted + number partially retracted / total number visible polyps) was calculated. For some species, such as *Montastrea annularis* whose polyps are normally seldom fully expanded, the percentage of polyps completely retracted was also calculated.

An arcsin transformation was performed on these percentages in order to facilitate the use of a 2-way analysis of variance for determination of statistical significance of results. After the analysis of variance was completed, Student-Newman-Keul's multiple mean comparison test was used to compare the amount of polyp retraction which occurred in each concentration of mud. Also, total amount of polyp retraction in all

concentrations was compared for each measurement period. The S-N-K test is a sensitive procedure which permits one to compare every mean to all other means and determine which ones are far enough apart to indicate real differences in their values. In addition, these values were plotted along with their 95 percent confidence intervals as determined by Tukey's test. If such confidence intervals do not overlap, there is only a 5 percent chance in the entire experiment that non-existent differences will be noted. Because the confidence level is determined for all comparisons rather than individual comparisons, Tukey's test is very strict. Therefore, means with slight overlaps in the plotted confidence intervals may still be significantly different when analyzed by the more sensitive S-N-K. The results of analysis of variance are given in the graph beneath the bar graphs showing coral response to the three levels of mud concentration (Figs. 6-12). The results are presented in tabular form in the Appendix.

Field Bioassay Results

Results will be discussed by species and will be presented in the following order: 1) *Porites divaricata*, 2) *P. furcata*, 3) *P. astreoides*, 4) *Montastrea annularis*, 5) *Acropora cervicornis*, 6) *Agaricia agaricites*, and 7) *Dichocoenia stokesii*.

Porites divaricata (Figs. 6a, b, c)

Both control and 100-µℓ/ℓ-test corals showed polyps which were generally well expanded for the entire 96 hour experiment. The 316-µℓ/ℓ-test corals completely retracted their polyp tissue and became covered with a thin layer of mucus within the first 24 hours of exposure. This condition continued for more than 48 hours and drilling mud slowly accumulated. The mucus/mud covering never exceeded an estimated 0.5 mm thickness. Sixty-eight hours after treatment began, the 316-µℓ/ℓ-test corals were observed to be shedding this layer of mucus and sediment, and by 72 hours the two corals were mostly free of their sheath and nearly as expanded as the control corals. The corals in 1,000 µℓ/ℓ mud produced a similar layer of mucus. This was never shed, however, and sediment gradually accumulated during the entire 96 hours. The

final layer of mud was estimated to be 2 mm thick, yet none of the polyps died.

Porites furcata (Figs. 7a, b, c)

Control coral polyps were well expanded prior to experiment initiation, during the first day, and after 96 hours. For more than 24 hours during the middle of the test, however, all polyps retracted and became covered with a thin mucus sheath. The battery powering all recirculation pumps became discharged during the night before this retraction was noticed, and it is presumed that the corals became temporarily stressed by lack of water movement. The corals in 100 µℓ/ℓ mud underwent a similar period of complete retraction, but a small amount of mud became incorporated into the mucus which was produced during that period. More polyps were retracted on the 100-µℓ/ℓ-test corals both before and after the intermediate period of complete retraction. In 316 µℓ/ℓ mud the polyps retracted and became encased in a thin mucus sheath within the first 24 hours of exposure. They remained in that condition for most of the treatment period, but began to expand and shed the mucus and accumulated sediment shortly before the end of the experiment at 96 hours. The corals in 1,000 µℓ/ℓ mud similarly retracted and were covered with a thicker layer of mucus and sediment; however, no recovery was evident at the end of 96 hours. None of the *Porites furcata* specimens exhibited mucus extrusion or lethal effects.

Porites astreoides (Figs. 8a, b, c)

Essentially all polyps within the control were partially expanded during the first test day, but by the end of the second day nearly all were fully expanded and remained expanded for the duration of the experiment. In the 100-µℓ/ℓ-test corals more than 90 percent of the polyps were partially expanded at the onset of treatment, and gradually all became fully retracted after 40 hours of exposure. Corals in 316 µℓ/ℓ mud behaved much like those in the 100 µℓ/ℓ concentration, except that a few polyps remained partially expanded until after 72 hours of exposure. This difference proved not to be statistically significant. In 1,000 µℓ/ℓ suspension

the same pattern was observed, i.e., initial partial expansion with a gradual increase in number of polyps completely retracted during the first 40 hours, and no expanded polyps thereafter. The amount of sediment cover on each coral was roughly proportional to mud concentration. Development of very thin layers of mucus was detected on corals after 48 hours of exposure to mud.

Montastrea annularis (Figs. 9a, b, c)

Polyps of control corals were partially expanded during the entire experiment, except for one period about 24 hours after initial exposure, when most were fully expanded. Colonies exposed to 100 µℓ/ℓ and 316 µℓ/ℓ mud were never fully expanded. After 17 hours of exposure, almost all polyps in these concentrations became fully retracted. At night, the polyps swelled, giving a bloated appearance with some also becoming partially expanded. At 17 hours, the corals in 316 µℓ/ℓ mud temporarily extruded mesenterial filaments. Corals in the 1,000 µℓ/ℓ concentration likewise became fully retracted after 17 hours and exhibited pronounced swelling in the polyp tissue at night. After 65 hours of treatment, one of the corals in the 1,000 µℓ/ℓ mud began to extrude large quantities of mucus, the polyps became very swollen, and tissue began to disintegrate. The second coral only exhibited swollen polyps at this time. Nine hours later, the first coral was dead over an estimated 70 percent of the colony, and the second coral had begun extruding large amounts of mucus. Within a few more hours, both corals were dead.

Acropora cervicornis (Figs. 10a, b, c)

Experiment #1: The control colonies exhibited partially retracted tissue immediately after being placed in their chamber. Within 17 hours polyps on both colonies were well expanded and remained expanded until 52 hours into the experiment, when both colonies suffered massive mortality. Cause of death is thought to be lack of circulation caused by pump failure, which occurred in the control tank at some time during the 11 hours preceding death. Fresh pieces of coral taken from the same branch which supplied the

original controls were substituted at 66 hours. Polyps on the new corals were predominantly expanded during the first few hours of treatment, and remained in that condition for the remainder of the experiment. Colonies in 100 µℓ/ℓ mud were fully expanded at the start of the mud treatment. After 17 hours, a small amount of sediment had accumulated on horizontal surfaces of the colonies. About one-third of the polyps were retracted after 24 hours of exposure. These specimens fluctuated between 39 percent and 85 percent polyp retraction until termination of the experiment at 96 hours. Corals in 316 µℓ/ℓ mud followed a similar pattern but with a significantly higher level of polyp retraction (71 to 100 percent for the same period). In 1,000 µℓ/ℓ drilling mud, none of the polyps was ever fully expanded. Within 30 minutes of mud treatment, mucus strands about 1 cm long were formed on one of the corals. After 41 hours of treatment, both corals in the 1,000 µℓ/ℓ suspension had lost all their zooxanthellae, and all tissue had disintegrated when the 52 hour measurements were taken.

Experiment #2 (Figs. 11a, b, c): Because first-study results were clouded by pump failure in the control treatment chamber, a second experiment was performed with additional branches of *Acropora cervicornis*. Treatments were begun very shortly after placing the corals in their respective chambers; therefore, most polyps in all concentrations and in the control tank were retracted for the first hours of exposure. After 20 hours, nearly half the polyps on the control corals were fully expanded and the remainder were well expanded. Seven hours later (night time), only 4 percent of the control polyps were not fully expanded. Polyps on both control colonies remained well expanded until termination of the experiment.

Corals exposed to 100 µℓ/ℓ drilling mud had a greater number of retracted polyps than the controls after 72 hours of treatment. The proportion of polyps retracted increased slowly with time until 95 percent were at least partially retracted at 96 hours. After 25 hours of depuration, nearly all polyps had re-expanded.

In 316 µℓ/ℓ mud the corals produced some excess mucus after 20 hours of exposure. A small amount of sediment had accumulated at that time as well, and only one polyp was expanded. Polyps on these corals became well expanded during the second

night of treatment (27 hours), and about 30 percent of the polyps were expanded on the third night. No polyp expansion was observed during daytime measurement at 20 hours. Twenty-five hours after mud suspension was replaced with clean water, only 5 percent of the polyps had re-expanded.

Corals treated with 1,000 µℓ/ℓ drilling mud had only 25 percent of their polyps expanded after 20 hours of exposure. Some sediment had also accumulated. None of the polyps was fully expanded for the duration of the experiment, and small amounts of sediment continued to be deposited. After 25 hours of depuration, some sediment remained on horizontal surfaces, and no polyps had become well expanded. No mortality was detected at that time.

Agaricia agaricites

Quantitative measurements were not possible on *Agaricia* because the small polyps were never distinguishable on any of the colonies. Upon addition of mud to each treatment chamber, copious amounts of mucus were produced. Although excess mucus production lasted for only a few minutes, the bolus of mucus generated by one colony in 100 µℓ/ℓ mud remained attached for more than 24 hours. Forty-one hours after beginning treatment, the corals in 1,000 µℓ/ℓ mud began dying and were completely dead less than 24 hours later. No changes were observed in any of the other chambers.

Dichocoenia stokesii (Figs. 12a, b, c)

Both control corals exhibited 100 percent partial polyp expansion during daylight hours with 100 percent expansion at night for the entire experimental period. One colony in 100 µℓ/ℓ mud consistently (i.e., day and night) showed fully expanded polyps, whereas the second colony behaved in the same manner as the control corals. The corals in 316 µℓ/ℓ mud exhibited diurnal behavior similar to the controls; however, during the pretreatment period, one had partially (instead of fully) expanded polyps and the other was fully (instead of partially) expanded during the 96 hour experiment.

Prior to application of mud slurry, 90 percent of the polyps which were to be exposed to 1,000 µℓ/ℓ concentrations were completely expanded. After 20 hours of treatment, some mud had accumulated on non-living (sampled in this condition) areas of the coral heads, but the polyps were in the same partially retracted state as those in other chambers. No change was discernible after 45 hours of exposure, but 5 hours later, a night observation, the polyps in 1,000 µℓ/ℓ mud were less expanded than those in other tanks. After 96 hours of treatment, 36 percent of all polyps were fully retracted. Although equipment malfunction prevented absolute quantitative evaluation of the degree of polyp expansion at 96 hours, direct observations indicate that one of the corals in 1,000 µℓ/ℓ mud had polyps which did not re-expand until treatment was terminated. Twenty-five hours after the mud suspension was replaced with clean water, one coral exhibited all polyps fully expanded and the other all partially expanded.

Burial Experiments

Survival in Carbonate Sand

Materials and methods

Burial was accomplished by placing individual coral specimens in small depressions dug in a sand flat near the test site and gently covering them with 10 to 12 cm of coarse carbonate reef sand. After a predetermined amount of time, the corals were carefully uncovered and observed for: (1) tissue loss; (2) zooxanthellae expulsion; (3) tactile response; (4) polyp expansion; (5) mucus production; and (6) mesenterial filament extrusion. Macro-photographs were taken to document these observations. The corals were then left unburied and additional photographs and observations were made 24 hours later.

Three lengths of burial time were tested: (1) 12.5 hours, (2) 24 hours, and (3) 72 hours. A total of nine coral species were subjected to burial. *Porites astreoides*, *Agaricia agaricites*, and *Montastrea annularis* were used in all three experiments; *Acropora cervicornis* was used in the 12 and 72 hour tests; and *Porites*

furcata was buried for 24 and 72 hours. *Montastrea cavernosa* and *Acropora palmata* were treated for 72 hours only, and *Diploria strigosa* (one colony) and *Siderastrea siderea* were used for the 24 hour experiment. Except as noted above, three specimens of a given species were used in each test.

Results

12.5 Hour Experiment: Immediately after being uncovered, the corals were in the following conditions: (1) *Agaricia agaricites*: tissue loss and zooxanthellae expulsion over an estimated 40 percent of two of the heads, the third piece appeared intact although no polyp expansion or tactile response could be detected; (2) *Acropora cervicornis*: tissue and zooxanthellae loss predominantly on the protruding corallite walls; (3) *Montastrea annularis*: some tissue atrophy, but no major zooxanthellae expulsion or tissue decay; and (4) *Porites astreoides*: one coral appeared completely normal and had partially expanded polyps, the other two heads had lost zooxanthellae from the coenosarc, but one of these also had partially expanded polyps.

24 Hour Experiment: The corals buried for 24 hours were observed to be in the following conditions: (1) *Agaricia agaricites*: complete loss of tissue over 60 percent of the corallum. the remaining tissue was decaying; (2) *Diploria strigosa* (single colony): partial zooxanthellae loss, most tissue remained on skeleton but appeared to be dead and decaying; (3)*Montastrea annularis*: estimated 40 percent of tissue disintegrated, excess mucus on remaining area, and partial zooxanthellae loss in the remaining tissue; (4) *Siderastrea siderea*: substantial zooxanthellae loss over 50 percent of the corallum, atrophied tissue, and no tactile response or polyp expansion; (5) *Porites furcata* and (6) *P. astreoides*: atrophied tissue, complete zooxanthellae loss in 90 percent of the tissue.

Thirteen hours later, the corals were re-examined and photographed, revealing that: (1) *Agaricia agaricites* had lost almost all tissue on two of the colonies and some tissue atrophy had occurred on the third colony; (2) one colony of *Porites*

astreoides had lost more zooxanthellae, whereas the others were essentially unchanged; (3) *Acropora cervicornis* colonies were completely denuded of tissue; and (4) one *Montastrea annularis* head had lost more zooxanthellae, but the remaining two colonies were as previously described.

72 Hour Experiment: Condition of the corals after 72 hours under 10-12 cm of sand was as follows: (1) *Agaricia agaricites*, (2) *Acropora cervicornis*, and (3) *A. palmata*: 100 percent mortality, no tissue remaining on any of the carbonate skeletons; (4) *Porites astreoides* and (5) *P. furcata*: approximately 70 percent tissue loss, some portions of each colony retained intact tissue, and only partial loss of zooxanthellae; no tactile response was detectable; (6) *Montastrea annularis*: estimated 90 percent tissue deterioration, remaining material appeared to be decaying; and (7) *M. cavernosa*: 70 percent of the tissue remained on the skeleton but was in an obvious state of decay; no tactile response could be detected. Forty-one hours after uncovering the corals, they were again observed for changed condition. At that time, *Porites astreoides* exhibited some intact tissue which was colored sufficiently to indicate the presence of a limited number of zooxanthellae. No tissue remained on the skeletons of any other species.

The results of burial experiments described above are summarized in Table 1.

Table 1

Species	Length of Burial and Percent Tissue or Zooxanthellae Loss		
	12.5 Hours	24 Hours	72 Hours
Porites astreoides	*	90✓	70
Porites furcata	--	90✓	70
Agaricia agaricites	40	60	100
Montastrea annularis	†	40	90
Montastrea cavernosa	--	--	70
Acropora cervicornis	†	--	100
Acropora palmata	--	--	100
Diploria strigosa	--	*	--
Siderastrea siderea	--	50✓	--

† some tissue loss but not quantifiable
✓ refers to zooxanthellae (not tissue) loss
* partial zooxanthellae loss
-- not examined

Survival in Carbonate Sand vs. Drilling Mud

Materials and methods

Seven liters of sand from the same flat used in the previously described burial experiments were placed in a 22-liter glass aquarium. Likewise, seven liters of drilling mud A were placed in an identical aquarium. Both were then filled with sea water. Three heads of *Montastrea annularis* and three heads of *Porites astreoides* were placed in each of the tanks. Plexiglass covers were fitted to each aquarium and placed on the bottom near Carysfort Light. Sediment covered all corals in both chambers. After 8 hours, the coral heads were removed from each aquarium, placed on the sand flat, and photographs and observations were made to evaluate their condition.

Results: mud vs. sand burial

Carbonate Sand: After 8 hours when the corals were removed from the aquarium containing sand, *Montastrea annularis* had partially lost zooxanthellae in a few corallites and had many mesenterial filaments extruded. *Porites astreoides* colonies had lost zooxanthellae from their coenosarc only.

Drilling Mud: *Montastrea annularis* heads which had been covered with drilling mud for 8 hours were dying and some tissue had begun to decay and form loose strands. *Porites astreoides* colonies had lost some more zooxanthellae than those from the sand tank, and tissue appeared to have atrophied.

Fifteen hours later, the corals were again examined, revealing that: (1) *Montastrea annularis* colonies which had been buried in sand had lost all zooxanthellae but all were living; (2) *Montastrea* heads which had been buried in drilling mud were completely dead, and only some partially decayed tissue remained on those portions of the coralla which were not bare; (3) *Porites astreoides* colonies from the sand tank had no expanded polyps, and the tissue had atrophied further.

Ten days after the corals were removed from their burial tanks, all of those which had been placed in drilling mud were completely dead. *Porites astreoides* colonies from the sand tank appeared to be completely recovered, whereas *Montastrea annularis* colonies from that tank were regaining color (zooxanthellae) and appeared healthy.

Clearing Rate Experiment

Materials and Methods

Eight *Montastrea annularis* "knobs" of similar size and shape were placed in 18 m of water seaward of Carysfort Reef. Approximately 5 ml of a 50 percent slurry of drilling mud A was applied in a thin uniform layer over four of the heads. A similar amount of sand from the sand flat used in burial experiments was likewise applied to the remaining four corals. Close-up photographs were immediately taken after all corals had been treated with sediment. Two hours later, another series of close-up photographs were taken to document the amount of sediment remaining on any of the corals.

Results: Field Study Clearing Rate Experiment

Within seven minutes after applying sand or drilling mud to the small *Montastrea* heads, vertical and near vertical sides of all colonies exhibited greatly reduced amounts of sediment cover. Horizontal and gently sloping surfaces remained covered with sediment. Overall rate of removal of material was approximately equal between drilling mud and natural sand. Two hours after applying the mud and sand, all corals were at least 95 percent free of sediment cover. Observations on subsequent days revealed no obvious mortality or zooxanthellae loss on any of these corals, although mesenterial filaments were extruded on all heads whether exposed to drilling mud or carbonate sand.

LABORATORY STUDY

Clearing Rates

Materials and Methods

One and one-half inch diameter cores were drilled from a single colony of *Montastrea annularis* on a patch reef approximately 3 km seaward of Elliott Key. Elliott Key is located 32 km south of Miami Beach, Florida (Fig. 1). The cores were placed in racks installed in an insulated box (Coleman cooler) and were transported to Fisher Island by boat. All corals were maintained in aquaria equipped with under-gravel filters for 28 days.

Exposure chambers consisted of 22-liter aquaria equipped with plexiglass stands which held the core plugs of *Montastrea* in an upright position (Fig. 13). Two plugs of coral were placed in each tank in random order. Three chambers were used: one to receive natural fine-grained carbonate mud (collected from Ramshorn Spit in Florida Bay), one to receive drilling mud A, and the last to receive drilling mud B.

Ten ml of appropriate mud was applied to each plug of coral with syringes so that an even cover of sediment resulted on the living surface of every core. Amount of sediment cover and condition of corals were recorded with close-up still photographs (1) prior to applying sediment and after (2) 1 minute, (3) 10 minutes, (4) 1 hour, (5) 2 hours, (6) 4 hours, (7) 6 hours, and (8) 26 hours after treating the corals.

Results

Mud A was the most cohesive and formed a layer of sediment estimated at 5 mm thick. No change in the sediment cover was observed during the first 10 minutes of clearing time. After two hours, the extreme edges of one coral and the edge on the lowest side of the other coral were free of mud. Polyps which were uncovered were "swollen," i.e., the oral disks were elevated above their normal positions, and the remaining polyp tissue appeared 'inflated' above the calices. Very poorly expanded tentacles were visible. Four hours after applying mud, one coral was 40 percent

clear and the polyps were less swollen, but mesenterial filaments had been extruded on a few polyps. The other coral was only 20 percent clear of mud, and polyps remained swollen and had tentacles which were partially expanded. Twenty-six hours after the experiment began, one coral had removed all of mud A, whereas the other remained 80 percent covered. At 27 hours, this remaining mud was removed with a syphon. No polyps were decaying at this time, but the coenosarc just inside the periphery of the mud cover had disintegrated. Three days later, all of the tissue which had been covered for 26 hours was dead. At that time, some zooxanthellae loss was apparent on the other coral exposed to mud A, but no tissue necrosis was detected.

Drilling mud B formed a very thin slurry as it was applied to the coral heads. Although complete cover was effected momentarily, the slurry flowed off gently sloping surfaces of the living coral very easily. Within 1 minute, one coral was free of sediment over an estimated 70 percent of living tissue and the other was cleared of sediment over an estimated 30 percent of the living area. Within 2 hours, both corals covered with mud B were essentially completely clear.

The natural carbonate mud from Ramshorn Spit, which served as control, was slightly cohesive and formed a layer of sediment approximately 5 mm thick over the living coral. One minute after application, the edges of living coral had been largely cleared of sediment, and particles from the remaining mound of mud were being visibly moved to the edges of the core. Within two hours, one coral had only a thin sediment cover, and the other was two-thirds cleared. After 4 hours of cleaning time, both corals were free of all but a few small patches of carbonate mud.

96 Hour Laboratory Exposure Tests

Materials and Methods

Collection of specimens

Branches of *Porites divaricata* were collected in 3 to 6 m of water on a patch reef located approximately 32 km south of Miami Beach and 3 km east of Elliott Key (Fig. 1). Epifauna was carefully removed from dead bases of the branches as they

were collected. Without subaerial exposure, all specimens were placed in a cooler containing ambient sea water and were transported by boat to the laboratory at Fisher Island. After allowing for temperature equilibrium, the corals were placed in 120-liter holding tanks containing Gulf Stream water collected at approximately 10 km off Miami Beach. The tanks were equipped with undergravel filters.

Experimental setup

Twenty-two liter glass aquaria served as treatment chambers (Fig. 14). A 300 gph plastic submersible bilge pump (same as described for field tests) was placed at the back of each aquarium to provide water circualtion sufficient to keep sediment in suspension. Outflow from the pump was directed so the high velocity stream of water did not disturb corals placed near the front glass. Illumination was provided by four 34 watt flourescent tubes placed 61 cm above the aquaria. A timer activated these lights automatically to approximate the natural photoperiod. Temperature was controlled by regulating room temperature and was maintained between 26° and 29°C.

Five fingers of *Porites divaricata* were placed in each of four treatment chambers. Plexiglass stands were used to hold the branches upright. Eighty to 100 polyps were visible on each finger.

Preparation of treatments

Three concentrations of drilling mud A and B were tested simultaneously: (1) 100 µℓ/ℓ; (2) 316 µℓ/ℓ; (3) 1,000 µℓ/ℓ, plus a control with no mud. Desired concentrations were prepared by adding 15 ml, 47.4 ml, and 150 ml of a 10 percent suspension of whole mud to the appropriate chamber (containing 15 liters of sea water). The control chamber was treated exactly as the other tanks, but no mud was added. Every 24 hours, water in each tank was replaced with clean sea water and new mud slurry was added.

Measurement of behavioral responses

Behavior of experimental corals was recorded by two photographic techniques. Time-lapse movies were taken of two adjacent fingers in the control, 100 µℓ/ℓ, and 316 µℓ/ℓ chambers (Fig. 14). Turbidity was too great to permit movies of corals in 1,000 µℓ/ℓ mud. Additionally, still close-up photographic slides were taken of each finger daily after water was changed but before new mud slurry was added.

Analysis of results

Data from time-lapse movies were first quantified by counting each polyp as it became expanded, partially retracted, or fully retracted, and by timing when each event occurred. The average amount of full polyp retraction was then calculated as previously described (Thompson, in preparation). Average full polyp retractions (R-factor) were then compared for each concentration and for each 24 hour period with a two-way analysis of variance. Fraction of polyps fully retracted was also measured from each of the still photographs and that data analyzed as described for the field experiments. Raw data and statistical methods are provided in the appendices.

Results of Laboratory 96 Hour Tests

The value of quantitative analysis of corals in both 96 hour tests (muds A and B) were negated by problems with the controls, which unfortunately were stressed by laboratory conditions and exhibited much higher percentages of polyps retracted than had been observed both in holding tanks and *in situ*. Interesting observations were made, however, concerning the accumulation of sediment on corals exposed to drilling mud and subsequent removal or failure to remove that sediment.

Mud A

Eighteen hours after beginning treatment, corals in 100 µℓ/ℓ mud were partially expanded, but mucus flocs were visible and a small amount of mud was entangled in the mucus. Corals exposed to 316 µℓ/ℓ mud were not expanded and sediment was accumulating on horizontal surfaces. Corals in 1,000 µℓ/ℓ mud could not be observed until

the mud was allowed to settle for three hours. At that time, a thin layer of mud uniformly covered all corals. Sediment continued to build up slowly and at a fairly uniform rate on all corals exposed to mud. The rate was roughly proportional to the concentration of mud in each tank. When mud was removed from each chamber after 96 hours of exposure: (1) control corals had a thin mucus covering but no sediment; (2) corals exposed to 100 µℓ/ℓ mud were completely covered with floculated sediment in the form of short strands estimated to average less than 1 mm in length; strands were longest and thickest between calices; (3) corals in 316 µℓ/ℓ mud were completely covered with strands of mucus about 4 mm long, and no calices were discernible; and (4) corals in 1,000 µℓ/ℓ mud appeared much as those in 316 µℓ/ℓ mud, but their sediment covering was significantly thicker.

There was little change in the appearance of any corals until 66 hours after mud treatment was terminated. At that time, some polyps on two of the five corals which had been in 100 µℓ/ℓ suspension partially expanded and began pushing away the mud/mucus covering. After 114 hours of depuration, one of these corals was 50 percent clear and the other 80 percent clear of sediment. No change was observed in any other corals. Two days later, seven days after ending mud treatments, all corals which had been in 100 µℓ/ℓ mud had removed all mud and mucus from their surfaces. Coral in the 316 µℓ/ℓ mud tanks had not changed, but a few polyps on two corals in 1,000 µℓ/ℓ mud had partially expanded and were in the process of pushing away small patches of mucus and sediment. The control corals were mostly expanded.

After 24 days of depuration, three corals in the 316 µℓ/ℓ tank had begun clearing sediment, and corals in the 1,000 µℓ/ℓ tank had all begun removing mud but only over small areas. The remaining mud was gently washed from each coral by hand, and all were placed in a holding tank. These corals recovered and were kept alive for an additional two weeks before being sacrificed.

Mud B

Corals were not disturbed by addition of the mud suspensions, and no changes

in any of the test specimens were discernible after an hour of treatment. After 44 hours of treatment, very thin layers of mucus and sediment had built up on the corals exposed to 100 and 316 µℓ/ℓ drilling mud. A thicker mud cover was observed on corals in 1,000 µℓ/ℓ mud. Six hours later, three of the five corals in 100 µℓ/ℓ mud began to expand polyps and dislodge mud and mucus which had been encasing them. Shortly thereafter, three corals in the 316 µℓ/ℓ mud and two in 1,000 µℓ/ℓ mud began to remove their mud and mucus coverings. At the end of the experiment, three corals in 100 µℓ/ℓ suspension were 70 percent free of cover, whereas the other two were still completely encased. Two corals in 316 µℓ/ℓ mud were mostly free of the mud covering, and two in the 1,000 µℓ/ℓ suspension were almost completely uncovered. The remaining corals had sediment covers whose thickness was roughly proportional to the concentration of mud to which they had been exposed.

DISCUSSION

Field Bioassays

The 96 hour behavioral bioassays conducted within exposure chambers submerged on Carysfort Reef successfully alleviated problems associated with maintaining natural light levels and photo-period, as well as temperature and salinity. Control corals exhibited stress reactions only in response to failure of the water circulation pumps. Four species, *Porites furcata*, *P. astreoides*, *Montastrea annularis*, and *Acropora cervicornis*, exhibited significant polyp retraction at all three concentrations tested. One species, *Porites divaricata*, had significant polyp retraction at 3,160:1 and 1,000:1 dilutions, whereas another, *Dichocoenia stokesii*, did not exhibit a statistically significant increase in polyp retraction at any of the test concentrations, although there was an indication of response to the 1,000:1 mud, as most polyps were fully expanded both before and after exposure, but no polyps were fully expanded during treatment with 1,000:1 mud. Large fluctuations of polyp retraction masked this effect in the statistical analysis. It is worth noting that individual heads of *Dichocoenia* were used as test specimens; therefore, the experimental units were

not from a single colony and more natural variability is to be expected.

No corals died in 10,000:1 or 3,160:1 dilutions of mud, but all *Agaricia agaricites* and all *Montastrea annularis* exposed to 1,000:1 diluted mud were killed within 75 hours of exposure. *Acropora cervicornis* was tested twice, with mixed results. Although there was good agreement in the behavioral measurements, all coral in 1,000:1 mud died during one experiment but not during the other. It may be that 1,000:1 was very close to the 96 hour LC_{50} for this mud and species, and small differences in the inherent resiliance of the coral colonies from which branches were collected could account for the variability between the two runs. Rapid death in corals similar to the "shut down reaction" described by Antonius (1977) appears to be a threshold type response, and it is entirely possible that the total stress experienced by the corals in the first *Acropora* experiment just barely exceeded that level, while the threshold was not quite reached in the second experiment.

No quantitative behavior measurements are offered for *Agaricia agaricites*, as the small polyps of the colonies tested were not clearly discernible. However, the copious quantities of mucus produced when mud was first added to the tanks indicate that even 10,000:1 mud dilution affected this species. Complete mortality of the colonies in 1,000:1 dilutions also indicates that this is a relatively sensitive species.

Since behavioral effects were detected in several corals at the lowest test concentration, it is not possible to determine accurately a "no effect" concentration for the more sensitive species (except to say that it is less than 10,000:1). As no death occurred at the two lower concentrations, it may be concluded that the threshold lethal dilution of the mud tested was between 3,160:1 and 1,000:1 for *Montastrea annularis* and *Agaricia agaricites*, and was very close to 1,000:1 for *Acropora cervicornis*.

Experience and the laboratory experiments in this paper indicate that toxicity to corals varies from mud to mud, so caution must be used when extrapolating these figures to other drilling fluids. It is reasonable to expect that a lightly treated

mud would be less toxic, whereas heavily treated muds could contain higher concentrations of both particles and chemicals, which have been demonstrated to be relatively toxic by themselves (i.e., thinners, surfactants, biocides, or other special purpose additives).

Application of Results

Another difficulty which must be considered before applying the presented data is that of exposure regime. In the experiments corals were exposed to rather constant concentrations of mud throughout the 96 hour experimental period. Corals near a drilling platform would be exposed to widely varying concentrations of mud for a much longer period of time (several weeks to several months, depending on depth, number of wells, or drilling difficulties).

Factors which affect the actual concentration of mud near a drilling site include: (1) type of mud; (2) rate of discharge; (3) duration of discharge; (4) quantity of "wash down" water used to flush effluents down the discharge pipe (can be responsible for considerable pre-dilution); (5) velocity and direction of water currents; (6) wave regimen; (7) eddies caused by water flow around the rig itself; (8) depth of mixed layer (generally determined by thermocline but could be determined by any pycnocline); (9) density of water beneath mixed layer (light particles potentially could conceivably float along an isopycnal surface and hence be concentrated and transported either beyond or to a target area; (10) fractionation of different phases of the drilling fluid (light and heavy particulate fractions and dissolved fraction); and (11) depth of discharge pipe. Unfortunately, many of these parameters cannot yet be predicted because they are widely variable, both with time and from location to location.

When better information becomes available concerning realistic dilution levels for an area of particular interest, such as the East and West Flower Garden Coral Reefs, it would be instructive to treat several sensitive species of coral to exposure scenarios which might represent ideal, expected, and worse case conditions.

Only a few studies have attempted to quantitatively measure dilution rates and dispersion patterns of drilling fluids discharged from offshore drilling platforms. Such information is essential if the results of experimental studies on corals or other organisms is to have application. The consensus of existing literature on mud dispersal is that rapid dilution occurs near the point of discharge, if not within the discharge pipe (at times, as much as 1,000:1 within 3 meters), but then the rate of dilution generally decreases as the plume travels farther from its source. The data presented in reports from Tanner Bank (Ecomar, Inc., 1978) and the Lower Cook Inlet (Miller and others, 1978) indicate that dilutions of whole mud roughly equivalent to those used in the present experiment may occur anywhere from a few meters from the discharge, i.e., Tanner Bank, to almost a kilometer away, i.e., Lower Cook Inlet. In Cook Inlet dilution rates were determined by "spiking" the mud with known amounts of Rhodamine WT and measuring its concentration in the plume with a flourometer. At Tanner Bank dilutions are calculated from suspended solids, Ba, Cr, and Pb concentrations measured in the whole mud and in water samples from the plume.

The data from Tanner Bank indicate that dilutions of whole mud range from 290:1 to more than 1,000:1 within 3 m of the discharge point, depending on which parameter one uses to calculate the dilution and at what rate the mud is delivered. It is thought that wash down sea water combined with reversing wave surge within the discharge pipe is responsible for a good portion of this high initial dilution. Generally, an additional 10 dilutions (2,900:1-10,000:1) had occurred within 100 m of the discharge source and 10 more dilutions (20,900:1-100,000:1) within 300-500 m. Only one measurement was reported for 1,000 m. It indicated a total dilution of 1,000,000:1. During the sampling periods at Tanner Bank, surface currents ranged from 0.04 kts to 0.88 kts (2.05 cm/sec - 45.2 cm/sec) and the swell ranged from 1.5 m to 2.1 m.

At Cook Inlet the measurement technique necessitated by extreme conditions precluded sampling less than 100 m from the discharge point, and the closest measurement available during bulk discharge was 830 m, where a total dilution of 2.2×10^4:1 was

calculated. From 1.76 km to 11.67 km downstream, dilutions fluctuated from 1.07 x 10^5:1 to 7.52 x 10^5:1. At the most distant sampling point 13.15 km downstream, 2.03 x 10^6 dilutions are calculated to have occurred. Currents at the sampling points varied from 1.57 kts to 2.35 kts (80.7 cm/sec - 120.8 cm/sec). Although wave heights during the sampling periods are not specifically referenced, photographs and wave height summaries for that month indicate that seas were generally calm (modal height 1 ft).

These data indicated that for the two situations studied (and the mud whose toxicity has been investigated), acute lethal effects of a drilling mud plume are unlikely to occur farther than a few hundred meters from a drilling platform. It does appear, however, that under some conditions acute behavioral effects on corals might occur as far as a kilometer from a drilling operation. The potential for effects due to chronic exposure is much more difficult to assess. No studies are yet available to ascertain either what chronic concentrations of drilling fluids are likely to have effects or what concentrations of drilling mud might chronically exist around long term operations, such as production drilling.

Burial Experiments

Survival in Natural Carbonate Sediment

Most corals must be capable of removing considerable amounts of sediment from their surfaces to survive in nature, but occasionally sediment may accumlate so rapidly that burial may occur. The burial experiments were designed to give some preliminary information on the ability of various species to survive, whether it be of natural or man-made causes. The results indicate that 12.5 hours of burial is sufficient to cause damage to many corals but that recovery is possible. After 24 hours of burial, recovery is less likely, and after 48 hours most tissue will already be decayed. It appears that of the species examined, *Porites* spp. are somewhat more tolerant of burial than most. During the 96 hour bioassays, *Porites* spp. were rather inept at sediment rejection. Bak (1977) found that *P. astreoides* became covered

during an influx of sediment from dredging operations, whereas most other corals did not. *Agaricia agaricites*, a coral restricted to outer wave-swept reefs, appeared to be the least tolerant to burial, as considerable tissue loss was noted after only 12.5 hours. This species also was killed by a 1,000:1 dilution of drilling mud A.

Drilling Mud vs. Natural Sediment Burial

The results of this simple experiment indicate that the effects of drilling mud on corals are not solely due to the effects of sedimentation. It must be noted, however, that the grain size of carbonate sand was much larger than that of a drilling fluid, and the larger interstitial spaces could play a part in the survival of *Montastrea annularis* and *Porites astreoides* in carbonate sand but not in an equal volume of drilling mud A.

Clearing Rate Experiment - Field

Under the conditions of this test, *Montastrea annularis* was able to remove dilute drilling fluid at about the same rate as natural carbonate sediment. However, the presence of mesenterial filaments on both corals treated with drilling mud and with carbonate sand indicates that burial under even easily removed sediments causes significant stress to *M. annularis*.

Clearing Rate Experiment - Laboratory

The clearing rate experiment suggests that at equal dilutions mud A was more toxic and difficult to remove than mud B. However, mud A was more concentrated than mud B. It is possible that at the same absolute concentrations, differences between the two samples would not be discernible. While this a point of interest, it is more meaningful to compare equal dilutions rather than equal concentrations, since this is the way the material is discharged into the environment. That is, to effect the same concentration of mud from a mud plume, it would be necessary to move the corals exposed to mud B closer to the mud outfall.

The effective concentrations of muds used to measure clearing rate do not represent levels expected to exist around actual drilling operations, but rather are used to permit a rapid comparison of various muds.

The 96 hour behavioral bioassays also suggested that mud A was more toxic than mud B. Unfortunately, the reliability of laboratory bioassays was not completely satisfactory, as control corals did not exhibit normal behavior. It is suspected that temeprature variations and artificial lighting conditions created sufficient stress to cause substantial polyp retraction.

CONCLUSIONS

1) A 1,000:1 dilution of the mud tested caused significant mortality in three species of coral examined within 65 hours (*Montastrea annularis*, *Agaricia agaricites*, and *Acropora cervicornis*). Four species (*Porites astreoides*, *P. divaricata*, *P. furcata*, and *Dichocoenia stokesii*) suffered no mortality at any dilution tested.

2) A 10,000:1 dilution of the drilling fluid tested caused a statistically significant increase in polyp retraction for five of the seven species tested, whereas 3,160:1 dilution was required for one species (*Porites divaricata*), and no statistically significant effects were detected for another (*Dichocoenia stokesii*). Dilutions greater than 10,000:1 were not studied.

3) Corals may be stressed by even short term (2 hours) cover with either natural carbonate sediment or drilling mud.

4) Burial in drilling mud can cause mortality sooner than similar burial in natural carbonate sand.

5) Some corals can recover from more than 12 hours of burial in carbonate sand, but suffer significant tissue and/or zooxanthellae loss.

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Appendix

R FACTORS AND STATISTICAL ANALYSIS

Porites divaricata LABORATORY BIOASSAY WITH MUD A

Concentration:	Control		100 µℓ/ℓ		316 µℓ/ℓ	
	R Factor	arcsin $\frac{R}{10}$	R Factor	arcsin $\frac{R}{10}$	R Factor	arcsin $\frac{R}{10}$
Time Period (hrs)						
0-24	10.00	90	7.210	46.14	7.777	51.05
24-48	10.00	90	10.000	90	10.00	90
48-72	10.00	90	7.891	52.10	10.00	90
72-96	10.00	90	10.000	90	10.00	90

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	3	5.63	1.88	2.62	>.10
Concentration	2	3.01	1.51	2.10	>.10
Error	6	4.31	.718		

AVERAGE RETRACTIONS

(arcsin transformed data: see text)

Time		Concentration	
0-24	= 62.4	Control	90.0
24-48	= 90.0	100 µℓ/ℓ	69.6
48-72	= 50.7	316 µℓ/ℓ	80.3
72-90	= 90.0		

RAW DATA AND STATISTICAL ANALYSIS

Porites divaricata LABORATORY BIOASSAY WITH MUD B

Concentration:		Control R/T $\bar{x}$ %		100 µl/l R/T $\bar{x}$ %		316 µl/l R/T $\bar{x}$ %		1,000 µl/l R/T $\bar{x}$ %	
Time	Colony								
Pretreatment	1	4/30		4/30		1/85		2/69	
	2	7/52		7/67		4/78		3/47	
	3	19/90	15.8	3/60	8.8	0/65	26.1	1/51	5.7
	4	7/33		0/69		4/95		7/74	
	5	6/60		4/40		14/70		6/78	
48 Hours	1	30/30		30/30		85/85		69/69✓	
	2	59/85		67/67		78/78		47/47✓	
	3	90/90	66.9	60/60	100.0	65/65	100.0	51/51✓	100.0
	4	20/32		69/69		95/95		74/74✓	
	5	8/59		70/70		70/70		78/78✓	
96 Hours	1	30/30		30/30		80/80		60/60	
	2	2/115		67/67		6/85		10/60	
	3	130/130	45.2	77/77	100.0	35/35	67.6	9/77	65.7
	4	14/60		90/90		51/164		85/85	
	5	1/120		65/65		80/80		100/100	

R = # polyps fully retracted
T = total # polyps
✓ estimated from direct observations

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	2	61,253.64	30,626.82	49.116	<.01
Concentration	3	6,061.80	2,020.60	3.24	<.05
Time x Conc.	6	6,043.61	1,007.27	1.62	>.10
Error	48	29,930.51	623.55		

AVERAGE RETRACTIONS
(arcsin transformed data: see text)

Time		Concentration	
Pretreatment =	5.25	Control	61.69
48 =	80.63	100 µl/l	33.54
96 =	61.17	316 µl/l	50.66
		1,000 µl/l	50.17

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time		Concentration (µl/l)			
Pretreat.	96 hrs 48 hrs	100	316	1,000	0

R FACTORS AND STATISTICAL ANALYSIS

Porites divaricata LABORATORY BIOASSAY WITH MUD B

Concentration:	Control		100 µℓ/ℓ		316 µℓ/ℓ	
	R Factor	arcsin $\frac{R}{10}$	R Factor	arcsin $\frac{R}{10}$	R Factor	arcsin $\frac{R}{10}$
Time Period (hrs)						
0-24	7.40	47.73	2.23	12.87	5.29	31.91
24-48	10.0	90.0	0	0	10.0	90.0
48-72	22.21	29.21	9.65	74.80	10.0	90.0
72-96	0	0	10.0	90.0	10.0	90.0

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	3	2,166.16	722.05	.409	>.10
Concentration	2	2,813.55	1,406.77	.796	>.10
Error	6	10.598.0	1,766.35		

AVERAGE RETRACTIONS

(arcsin transformed data: see text)

Time		Concentration	
0-24	= 30.84	Control	41.74
24-48	= 60.00	100 µℓ/ℓ	44.42
48-72	= 64.67	316 µℓ/ℓ	75.48
72-96	= 60.00		

RAW DATA AND STATISTICAL ANALYSIS

Dichocoenia stokesii FIELD BIOASSAY

Concentration:		Control R/T	Control x̄ %	100 µℓ/ℓ R/T	100 µℓ/ℓ x̄ %	316 µℓ/ℓ R/T	316 µℓ/ℓ x̄ %	1,000 µℓ/ℓ R/T	1,000 µℓ/ℓ x̄ %
Time	Colony								
Pretreatment	1	0/24	0	2/24	4.2	30/30	50.0	3/25	9.1
	2	0/42		0/50		0/26		1/16	
20 Hours	1	20/20	100.0	25/25	50.0	27/30	95.0	32/32	100.0
	2	45/45		0/46		37/37		22/22	
27 Hours	1	0/20	0	0/25	0	0/25	0	32/32✓	100.0
	2	0/20		0/46		0/37		22/22✓	
45 Hours	1	20/20	100.0	15/20	37.5	30/30	100.0	12/12	100.0
	2	40/40		0/46		38/38		19/19	
50 Hours	1	0/20	0	0/12	0	3/30	5.0	23/23✓	100.0
	2	0/40		0/20		0/38		27/27✓	
69 Hours	1	28/28	100.0	29/29	50.0	30/30	100.0	23/23	100.0
	2	30/30		0/25		35/35		27/27	
96 Hours	1	16/17	87.8	29/29	50.0	15/19	41.0	23/23	100.0
	2	31/38		0/25		1/30		27/27	
25 Hours (depuration)	1	1/14	3.6	19/21∝	89.7	1/25	2.0	15/19	39.5
	2	0/30		40/45∝		0/30		0/27	

R = # polyps retracted
T = total # polyps

✓ estimated from direct observations

∝ fish in tank disturbing colonies

ANALYSIS OF VARIANCE TABLES

Dichocoenia stokesii ANALYSIS #1

(Day and Night Times - Excludes 1,000 µℓ/ℓ Concentration)

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	5	35,781.3	7,156.2	8.635	<.01
Concentration	2	5,284.2	2,642.1	3.188	>.05
Time x Conc.	10	6,505.1	650.5	0.785	>.10
Error	18	14,916.0	828.7		

AVERAGE RETRACTIONS
(arcsin transformed data: see text)

Time		Concentration	
20	= 70.74	Control	55.59
27	= 0.48	100 µℓ/ℓ	29.93
45	= 68.15	316 µℓ/ℓ	47.94
50	= 1.52		
69	= 75.10		
96	= 44.92		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time						Concentration (µℓ/ℓ)		
27 hrs	50 hrs	96 hrs	45 hrs	20 hrs	69 hrs	100	316	0
(night)		(day)						

Dichocoenia stokesii ANALYSIS #2

(Daytime Measurements Only - Includes 1,000 µℓ/ℓ)

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	5	32,421.8	6,484.4	7.682	<.01
Concentration	3	4,737.5	1,579.1	1.870	>.10
Time x Conc.	15	19,570.2	1,304.7	1.546	>.10
Error	24	20,259.4	844.1		

AVERAGE RETRACTIONS
(arcsin transformed data: see text)

Time		Concentration	
Pretreatment	= 13.38	Control	55.87
20	= 75.56	100 µℓ/ℓ	37.75
45	= 73.61	316 µℓ/ℓ	55.13
69	= 78.82	1,000 µℓ/ℓ	65.26
96	= 56.19		
(depuration) 25	= 23.44		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time						Concentration (µℓ/ℓ)			
Pretreat.	25 hrs Depuration	96 hrs	45 hrs	20 hrs	69 hrs	0	100	316	1,000

RAW DATA AND STATISTICAL ANALYSIS

Acropora cervicornis EXPERIMENT #1 FIELD BIOASSAY

Concentration:		Control R/T	Control x̄ %	100 µℓ/ℓ R/T	100 µℓ/ℓ x̄ %	316 µℓ/ℓ R/T	316 µℓ/ℓ x̄ %	1,000 µℓ/ℓ R/T	1,000 µℓ/ℓ x̄ %
Time	Colony								
0.5 Hours	1	50/50	66.3	28/28	100.0	63/63	100.0	61/61✓	100.0
	2	57/175		55/55		38/38		20/20✓	
17 Hours	1	18/124	10.8	20/56	35.6	43/75	45.3	61/61	100.0
	2	13/182		32/90		26/78		20/20	
24 Hours	1	37/99	23.1	76/76	100.0	72/72	100.0	61/61✓	100.0
	2	16/180		80/80		55/55		20/20✓	
52 Hours (night)	1	7/78	4.5	46/59	84.4	56/75✓	71.0	61/61✓Z	100.0
	2	0/136		118/130		64/95		20/20✓	
65 Hours (pump broken)	1	†	100.0	31/65	39.0	93/96	92.2	1/61	100.0
	2	†		39/129		63/72		20/20	
76 Hours (night)	1	34/80	29.1	40/76	62.1	43/50	78.3	†	100.0
	2	15/95		59/85		48/68		†	
96 Hours	1	30/50	52.7	71/73	82.0	92/92	100.0	†	100.0
	2	45/99		60/90		70/70		†	

R = # polyps retracted
T = total # polyps
✓ estimated from direct observations
Z = zooxanthellae loss
† 100% polyp death

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	5	6,439.8	1,287.9	7.93	<.01
Concentration	3	19,336.7	6,445.6	39.68	<.01
Time x Conc.	15	15,493.9	1,032.9	6.359	<.01
Error	24	3,898.1	162.4		

AVERAGE RETRACTIONS

(arcsin transformed data: see text)

Time		Concentration	
0.5	= 81.13	Control	34.94
24	= 70.88	100 µℓ/ℓ	59.84
(night) 52	= 49.02	316 µℓ/ℓ	72.62
65	= 67.85	1,000 µℓ/ℓ	90.00
(night) 76	= 49.45		
96	= 67.78		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time						Concentration (µℓ/ℓ)			
52 hrs (night)	76 hrs	96 hrs	65 hrs	24 hrs	0.5 hrs	0	100	316	1,000

RAW DATA AND STATISTICAL ANALYSIS

Acropora cervicornis EXPERIMENT #2 FIELD ANALYSIS

Concentration:		Control R/T	Control x̄ %	100 µl/l R/T	100 x̄ %	316 µl/l R/T	316 x̄ %	1,000 µl/l R/T	1,000 x̄ %
Time	Colony								
20 Hours	1	52/64	56.0	12/128	9.1	60/60✓	99.4	36/65	73.8
	2	23/75		12/135		79/80		72/78	
27 Hours (night)	1	4/75	4.3	32/90	58.6	4/60	4.9	65/65✓	100.0
	2	4/124		49/60		2/65		78/78✓	
45 Hours	1	5/130	11.9	17/83	32.4	56/56	100.0	65/65	100.0
	2	20/100		62/140		110/110		50/50	
50 Hours (night)	1	6/60	14.4	37/60	77.9	32/35	68.9	65/65✓	100.0
	2	17/90		64/68		19/41		50/50✓	
69 Hours	1	1/59	7.9	55/68	90.4	24/24	100.0	59/59	100.0
	2	11/78		57/57		38/38		59/59	
96 Hours	1	8/55	13.9	72/80	95.0	24/24	100.0	30/30	100.0
	2	9/68		57/57		30/30		50/50	
25 Hours (depuration)	1	0/55	12.9	9/150	4.7	70/70	95.1	82/86	97.7
	2	18/70		3/90		55/61		74/74	

R = # polyps retracted
T = total # polyps
✓ estimated from direct observations

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	5	6,368.1	1,273.6	3.817	<.01
Concentration	3	30,114.6	10,038.21	30.557	<.01
Time x Conc.	15	22,842.9	1,522.86	4.636	<.01
Error	24	7,884.1	328.51		

AVERAGE RETRACTIONS
(arcsin transformed data: see text)

Time		Concentration	
20	= 44.33	Control	11.04
27	= 33.26	100 µl/l	44.22
45	= 51.48	316 µl/l	67.52
50	= 49.83	1,000 µl/l	83.42
69	= 64.13		
96	= 66.27		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time						Concentration (µl/l)			
27 hrs	20 hrs	50 hrs	45 hrs	69 hrs	96 hrs	0	100	316	1,000

RAW DATA AND STATISTICAL ANALYSIS

Porites furcata FIELD BIOASSAY

R = # polyps retracted
T = total # polyps

Concentration:		Control R/T	x̄ %	100 µl/l R/T	x̄ %	316 µl/l R/T	x̄ %	1,000 µl/l R/T	x̄ %
Time	Colony								
Pretreatment	1	2/30	4.2	0/150	25.3	2/54	13.4	16/135	8.5
	2	2/114		40/82		38/165		12/232	
24 Hours	1	5/100	2.5	181/196	68.2	400/400	100.0	135/135	100.0
	2	0/232		80/182		236/236		232/232	
48 Hours	1	138/138	100.0	114/114	100.0	400/400	100.0	135/135	100.0
	2	223/223		182/182		230/230		232/232	
72 Hours	1	27/27	100.0	39/39	100.0	191/191	100.0	135/135	100.0
	2	159/159		50/50		295/295		232/232	
96 Hours	1	0/200	2.8	33/220	19.0	75/179	41.9	50/50	100.0
	2	9/163		30/130		52/124		50/50	

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	4	42,036.1	10,509.0	152.69	>.001
Concentration	3	6,938.7	2,312.0	33.6	>.001
Time x Conc.	12	13,521.9	1,126.8	16.37	>.001
Error	20	1,376.6	68.8		

AVERAGE RETRACTIONS

(arcsin transformed data: see text)

Time		Concentration	
0	7.41	Control	37.1
24	57.05	100 µl/l	50.4
48	90.00	316 µl/l	60.5
72	90.00	1,000 µl/l	73.0
96	31.75		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time					Concentration (µl/l)			
0 hrs	96 hrs	24 hrs	48 hrs	72 hrs	0	100	316	1,000

RAW DATA AND STATISTICAL ANALYSIS

Porites divaricata FIELD BIOASSAY

R = # polyps retracted
T = total # polyps

Concentration:		Control R/T	Control x̄ %	100 µℓ/ℓ R/T	100 µℓ/ℓ x̄ %	316 µℓ/ℓ R/T	316 µℓ/ℓ x̄ %	1,000 µℓ/ℓ R/T	1,000 µℓ/ℓ x̄ %
Time	Colony								
Pretreatment	1	14/120	39.5	15/165	10.3	25/108	15.5	27/179	10.9
	2	89/132		15/130		23/290		11/166	
24 Hours	1	6/71	10.4	0/165	7.9	110/110	100.0	179/179	100.0
	2	7/57		28/180		250/250		166/166	
48 Hours	1	14/126	7.2	0/165	5.5	96/96	100.0	179/179	100.0
	2	4/119		12/110		235/235		166/166	
72 Hours	1	30/30	62.1	2/165	7.2	5/68	8.5	179/179	100.0
	2	14/58		20/152		16/167		166/166	
96 Hours	1	11/175	9.5	12/203	16.5	75/129	29.9	179/179	100.0
	2	17/33		37/137		3/178		166/166	

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	4	7,037.8	1,759.4	8.078	<.001
Concentration	3	26,666.4	8,888.8	40.81	<.001
Time x Conc.	12	22,902.6	1,908.0	8.76	<.001
Error	20	4,356.2	217.8		

AVERAGE RETRACTIONS

(arcsin transformed data: see text)

Time		Concentration	
0	11.42	Control	18.42
24	47.61	100 µℓ/ℓ	5.44
48	46.82	316 µℓ/ℓ	42.42
72	37.74	1,000 µℓ/ℓ	73.24
96	30.81		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time					Concentration (µℓ/ℓ)			
0 hrs	96 hrs	72 hrs	48 hrs	24 hrs	100	0	316	1,000

RAW DATA AND STATISTICAL ANALYSIS

Montastrea annularis FIELD BIOASSAY

Concentration		Control R/T	x̄ %	100 µℓ/ℓ R/T	x̄ %	316 µℓ/ℓ R/T	x̄ %	1,000 µℓ/ℓ R/T	x̄ %
Time	Colony								
Pretreatment	1	16/70	3.8	60/89	35.6	0/59	3.4	20/107	32.3
	2	2/47		4/108		4/58		23/56	
17 Hours	1	0/68	0	81/83	96.9	60/70	92.9	*	
	2	0/44		104/108		56/56		*	
23 Hours	1	0/67	0	42/50	92.0	26/27	98.1	*	
	2	0/45		73/73		87/87		*	
42 Hours	1	15/49	55.3	51/59	79.3	35/35	95.5	*	
	2	24/30		70/97		51/56		*	
65 Hours	1	0/50	16.7	50/60✓	79.3	30/40	83.9	†	
	2	10/30		73/97✓		65/70		†	
96 Hours	1	20/33	63.6	58/58	100.0	46/46	100.0	†	
		20/30✓		98/98		56/56		†	

R = # polyps fully retracted
T = total # polyps

* no polyps visible in photograph

† 100% polyp death

✓ estimated from direct observations

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	5	12,916.2	2,583.2	13.47	<.01
Concentration	2	17,834.7	8,917.3	46.49	<.01
Time x Conc.	10	6,199.3	619.9	3.23	
Error	18	3,452.4	191.8		

AVERAGE RETRACTIONS
(arcsin transformed data: see text)

Time		Concentration	
0 =	10.7	Control	15.5
17 =	50.2	100 µℓ/ℓ	61.2
23 =	52.0	316 µℓ/ℓ	64.1
42 =	55.4		
65 =	40.3		
96 =	73.2		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time						Concentration (µℓ/ℓ)		
0 hrs	65 hrs	17 hrs	23 hrs	42 hrs	96 hrs	0	100	316

RAW DATA AND STATISTICAL ANALYSIS

Porites astreoides FIELD BIOASSAY

Concentration:		Control R/T	Control x̄ %	100 µℓ/ℓ R/T	100 µℓ/ℓ x̄ %	316 µℓ/ℓ R/T	316 µℓ/ℓ x̄ %	1,000 µℓ/ℓ R/T	1,000 µℓ/ℓ x̄ %
Time	Colony								
Pretreatment	1	3/101	3.2	5/100	5.8	2/139	7.0	2/228	3.9
	2	3/90		7/75		11/88		7/100	
17 Hours	1	0/70	0.9	52/62	47.1	10/76	13.7	11/30	22.4
	2	1/57		10/97		10/76		41/113	
42 Hours	1	3/80	6.5	75/75	100.0	78/90	72.1	145/158	95.9
	2	7/75		110/110		23/40		50/50	
52 Hours	1	0/100	0.0	40/40	100.0	29/35	88.7	158/158	100.0
	2	0/210		47/47		53/56		50/50	
65 Hours	1	2/100	1.7	33/40	91.3	53/60	90.0	158/158	100.0
	2	3/210		47/47		33/36		50/50	
76 Hours	1	0/200	0.0	40/40	100.0	60/60	100.0	158/158✓	100.0
	2	0/150		50/50		36/36		50/50✓	

R = # polyps fully retracted
T = total # polyps
✓ estimated from direct observations

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sums of Squares	Mean Squares	F	Probability of no Significance
Time	5	30,063.55	6,012.71	51.99	<.01
Concentration	3	29,568.56	9,856.19	85.22	<.01
Time x Conc.	15	12,359.95	824.00	7.125	<.01
Error	24	2,775.64	115.65		

AVERAGE RETRACTIONS

(arcsin transformed data: see text)

Time		Concentration	
0 =	2.85	Control	1.21
17 =	13.23	100 µℓ/ℓ	62.93
41.5 =	54.91	316 µℓ/ℓ	46.21
52.5 =	61.92	1,000 µℓ/ℓ	60.60
65 =	57.01		
76 =	67.52		

Results of Student-Newman-Keuls Multiple Mean Comparison Test (values underlined by a common line are not significantly different)

Time						Concentration (µℓ/ℓ)			
0 hrs	17 hrs	42 hrs	65 hrs	52 hrs	76 hrs	0	316	1,000	100

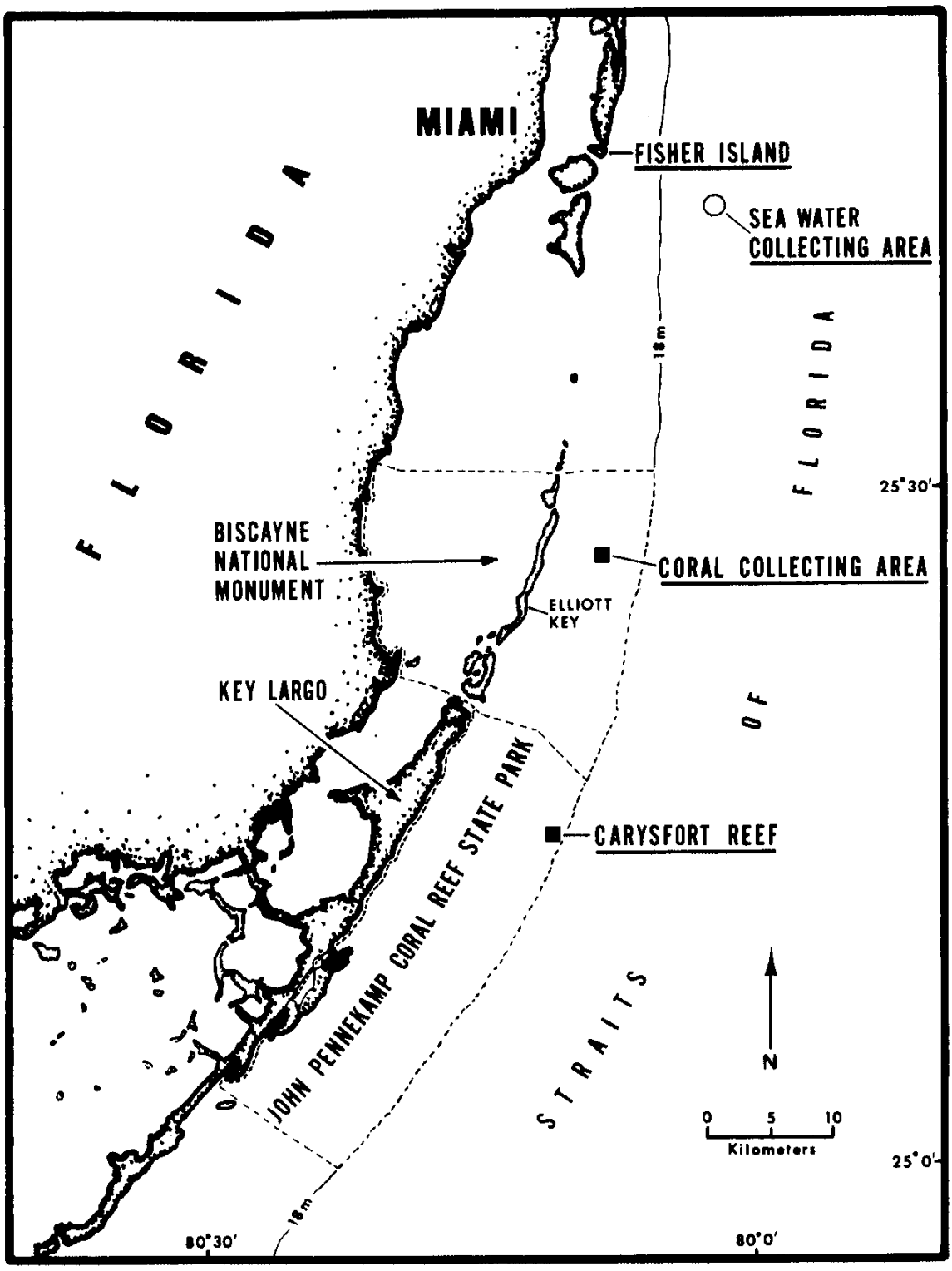


Figure 1. Map of the upper Florida Keys showing locations of Fisher Island Station collecting area off Elliott Key and Carysfort Reef, where field studies were conducted.

Figure 2. Oblique aerial photo of Carysfort Reef and lighthouse, which served as base for month-long field studies. Bioassay chambers were located in sand area next to loading platform (arrow).

Figure 3. Underwater photo of bioassay chambers set up on steel platform. Wires provide 12 V DC to plastic bilge pumps visible in each aquarium. Diver is taking closeup photographs used for counting polyp retractions and other behavioral responses.

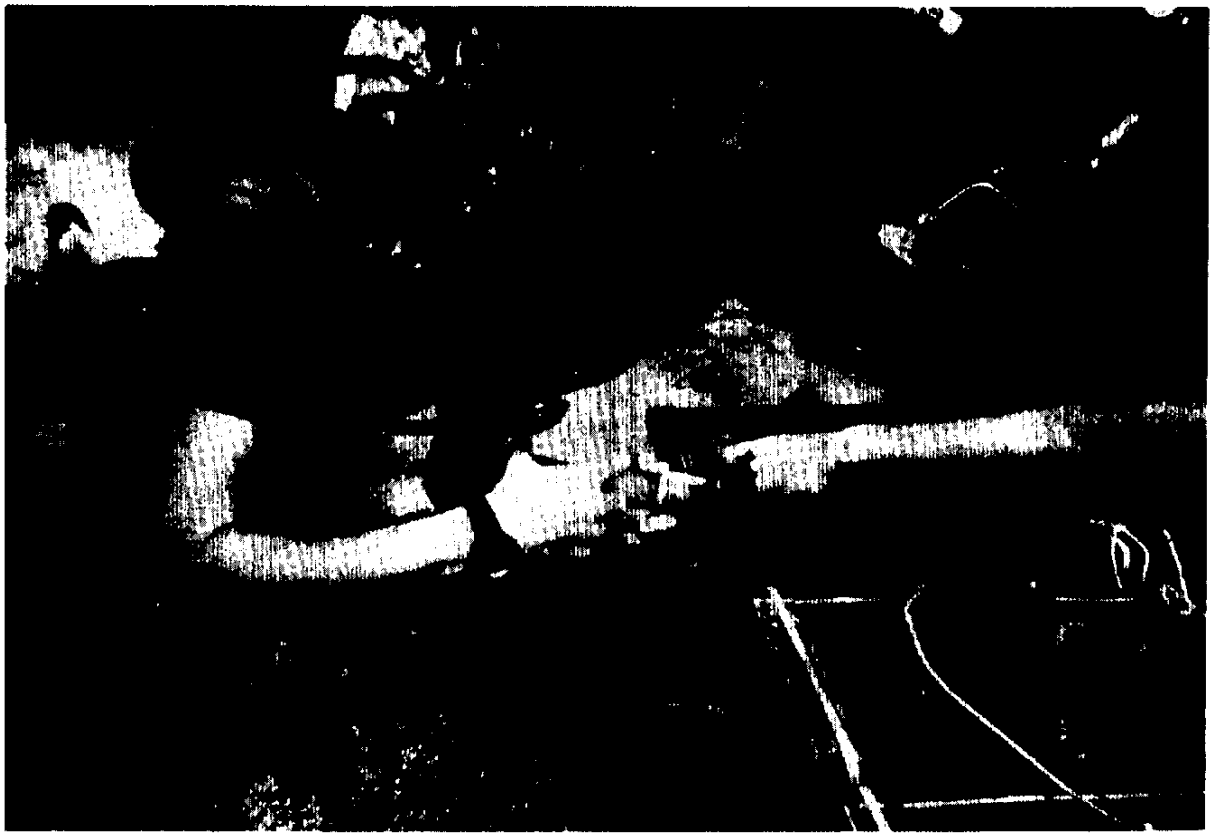


Figure 4. Underwater photo of principal investigator injecting pre-measured amounts of drilling mud A into bioassay chambers. Plastic lid was quickly shut and sealed to prevent loss of mud.

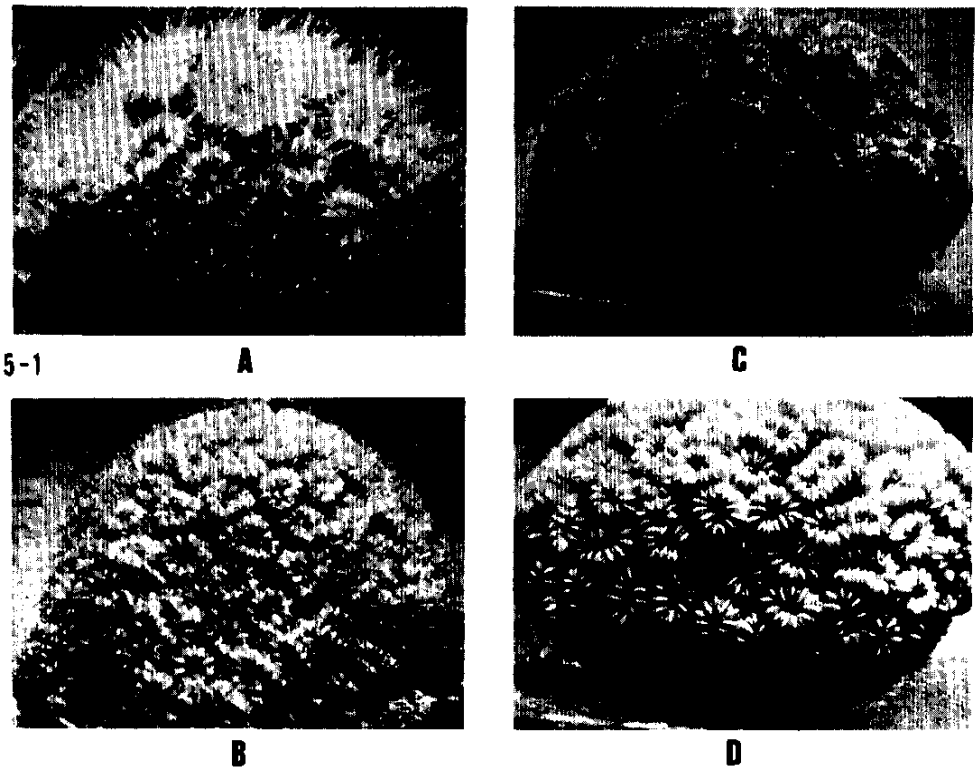


Figure 5-1. Series of photographs of head coral *Dichocoenia stokesii* showing degree of polyp retraction (specimen approximately 5 cm high). (A) Full expansion of polyps. (B) 50 percent retraction. (C) Approximately 80 percent retraction. (D) 100 percent retraction.

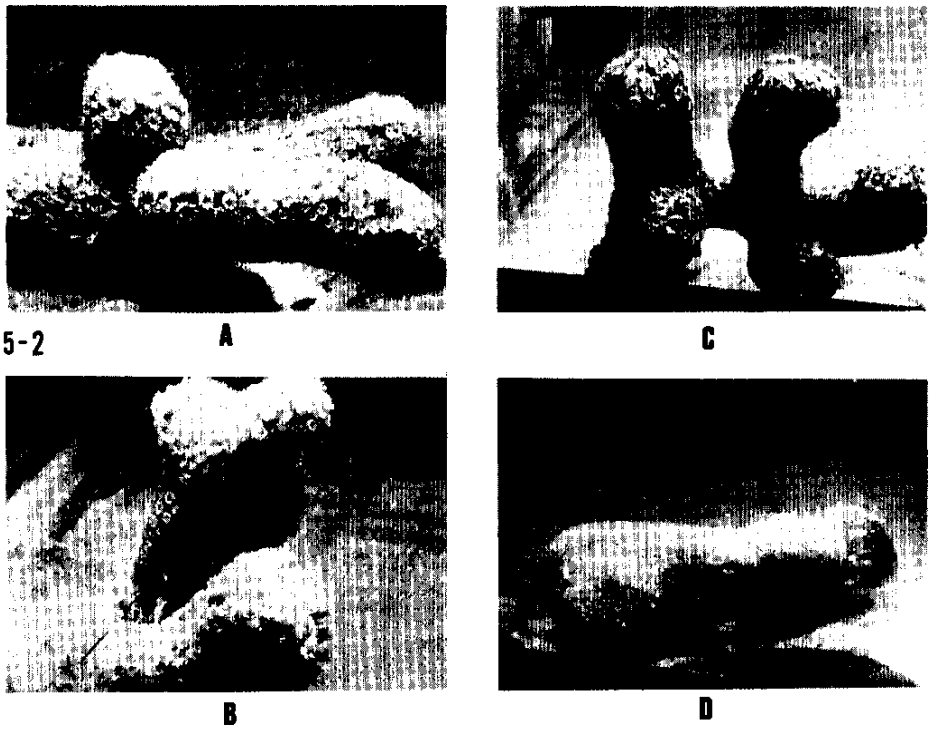
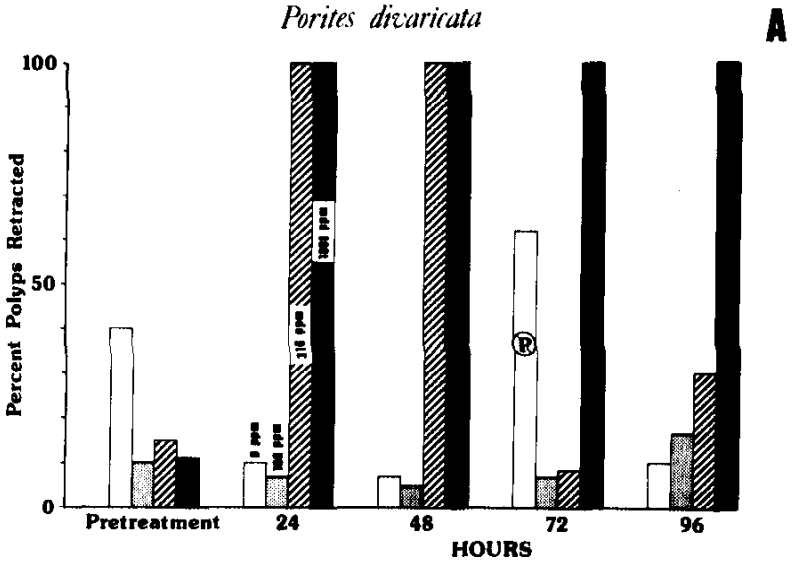


Figure 5-2. Series of photographs of branching coral *Porites divaricata* (fingers approximately 3 cm long). Note difference in method of polyp retraction: in Figure 5-1 all polyps retract synchronously, whereas in Figure 5-2 some polyps retract fully while others do not. (A) Full expansion of all polyps. (B) Approximately 10 percent of polyps retracted. (C) Approximately 50 percent of polyps retracted. (D) All polyps retracted. Polyp retraction data in Figures 6-12 were based on closeup photographs such as these.



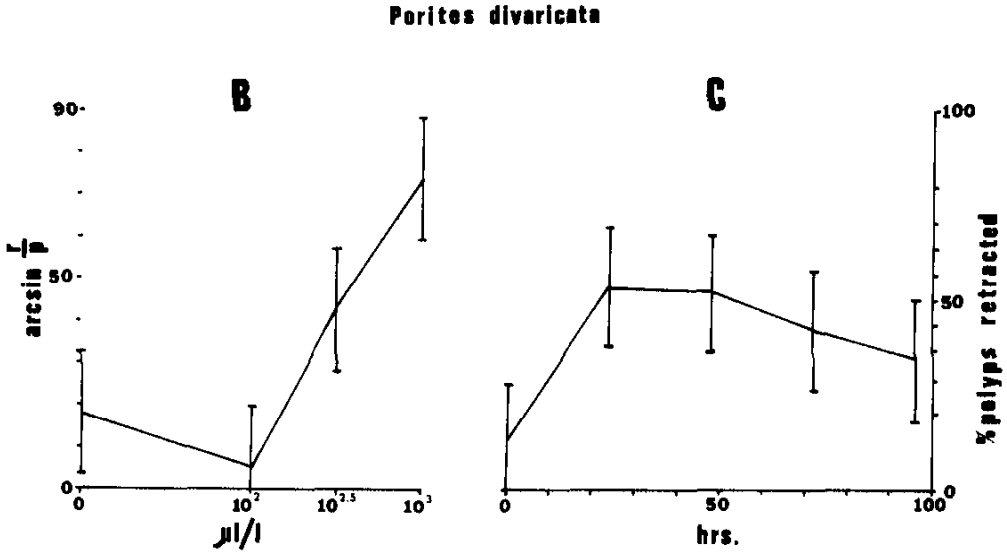
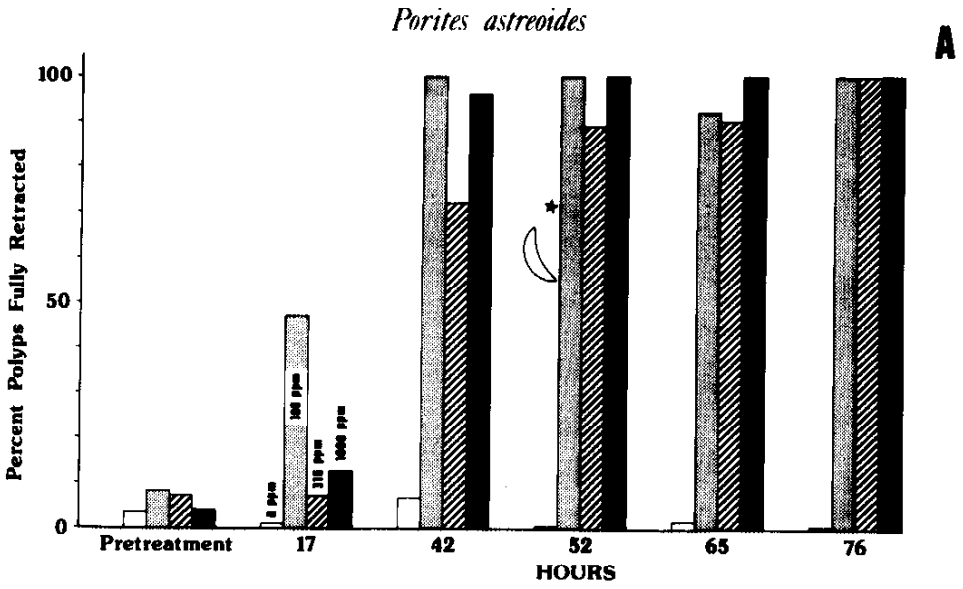


Figure 6. (A) Graphs showing polyp reaction in *Porites divaricata* to the concentrations described in text and on bars in graph. Observation times are shown as hours. Symbol showing circle with "P" for the control at 72 hours indicates pump malfunction. Note fairly uniform reaction to mud concentration at end of 96 hours. Also note that values in bar graph are given as ppm. This is ppm liquid and means the same a µℓ/ℓ of stock suspension. (B) Results of all observations plotted against concentration. Bars indicate 95 percent confidence intervals (see Appendix for raw data). (C) Percent polyps retracted plotted against time with bars showing 95 percent confidence intervals.



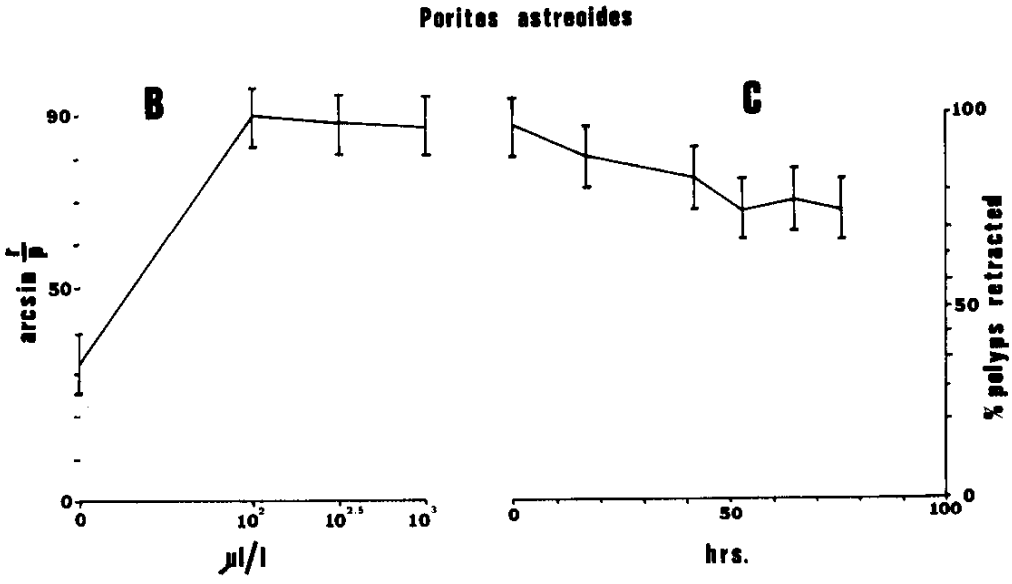


Figure 8A, B, C. Data for *Porites astreoides* (massive head coral), as described for Figure 6. Note half moon and star, indicating night-time observation.

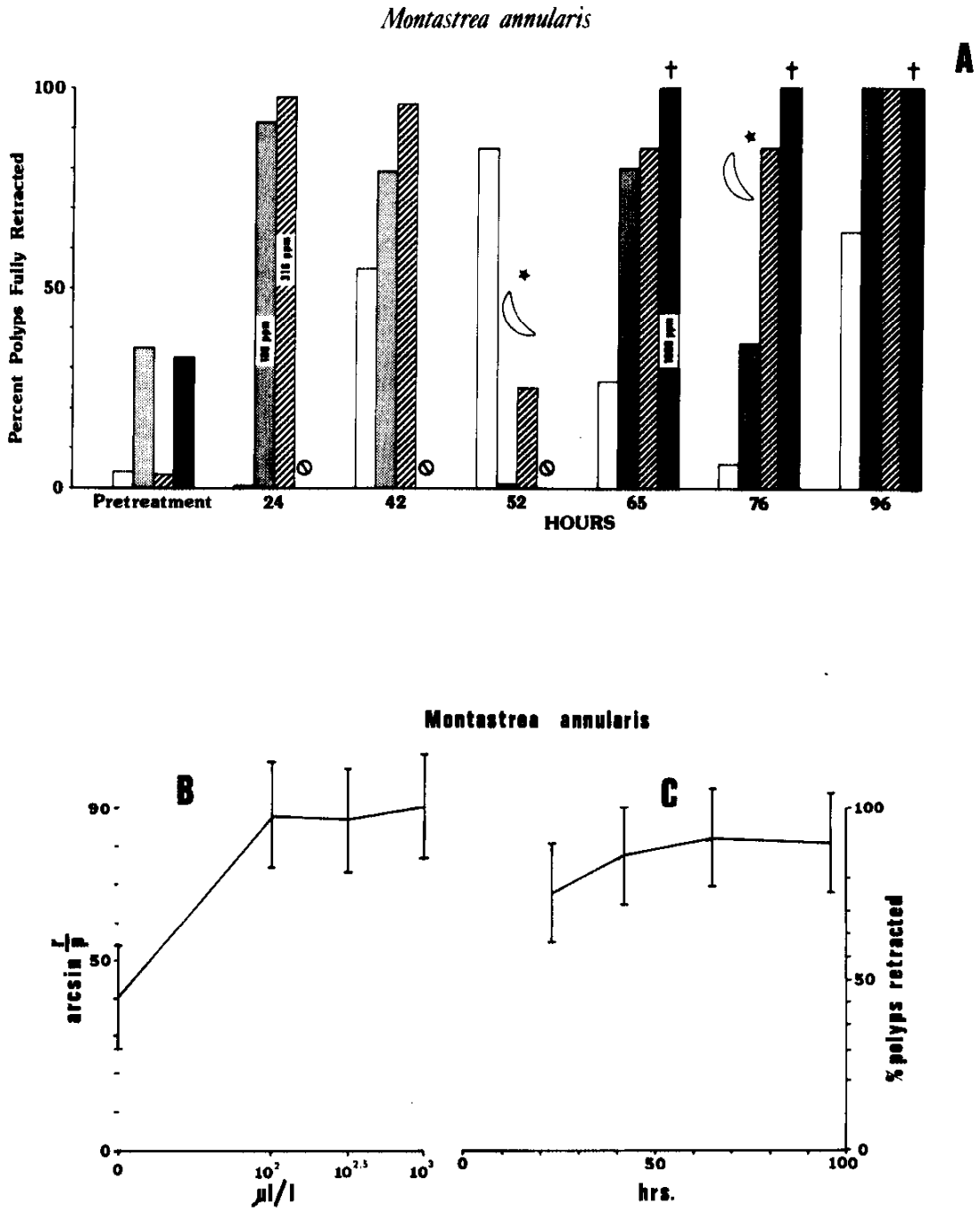


Figure 9A, B, C. Data for *Montastrea annularis* (massive head coral), as described for Figure 6. Note night-time observations and circle with slash (Ø), indicating that photography was not possible due to turbidity of water in chamber treated with 1,000 ppm dilution of drilling mud. At 65 hours, specimen was moved closer to edge of test chamber to allow for photography. Also note cross (†), indicating death of specimens.

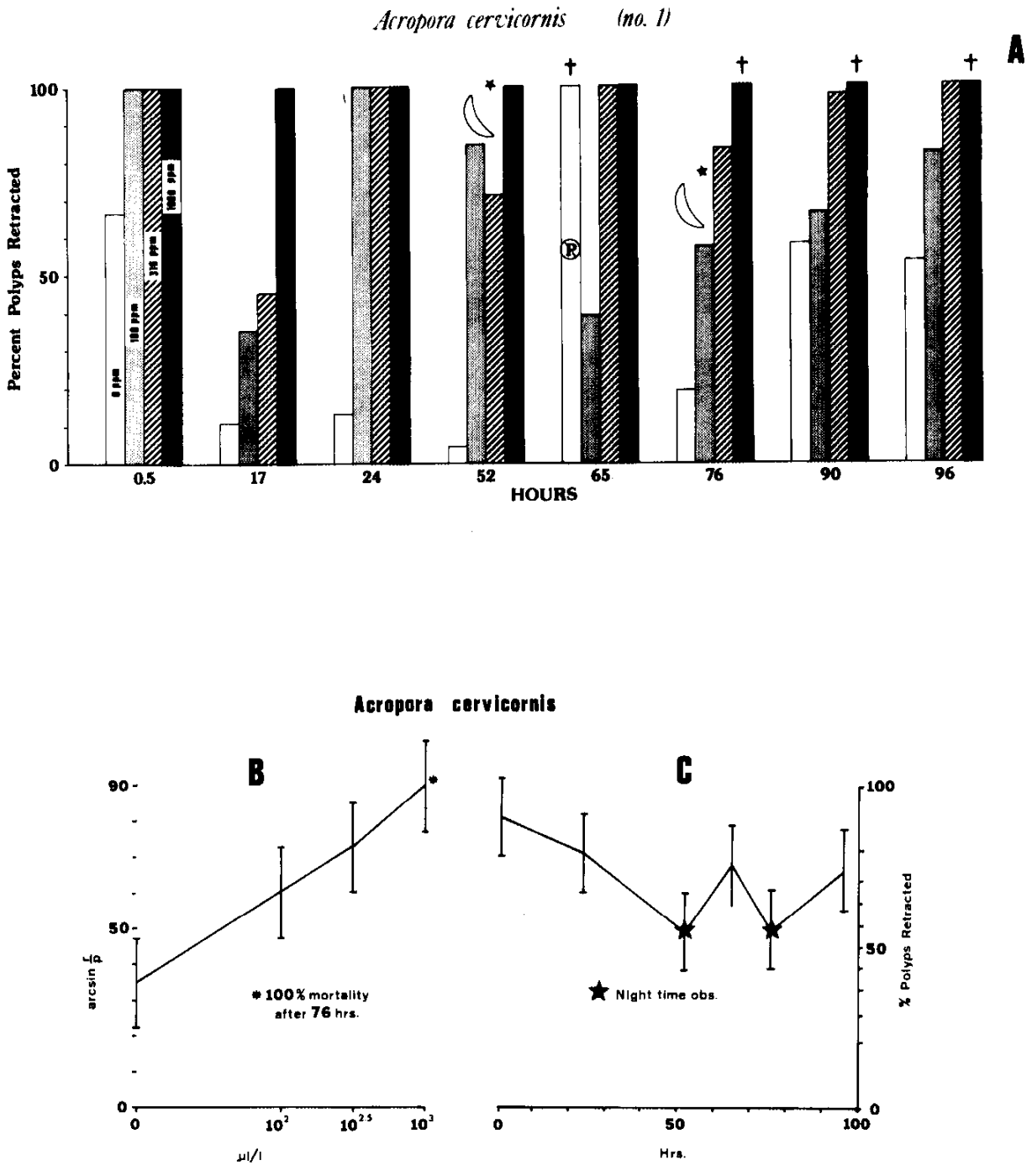
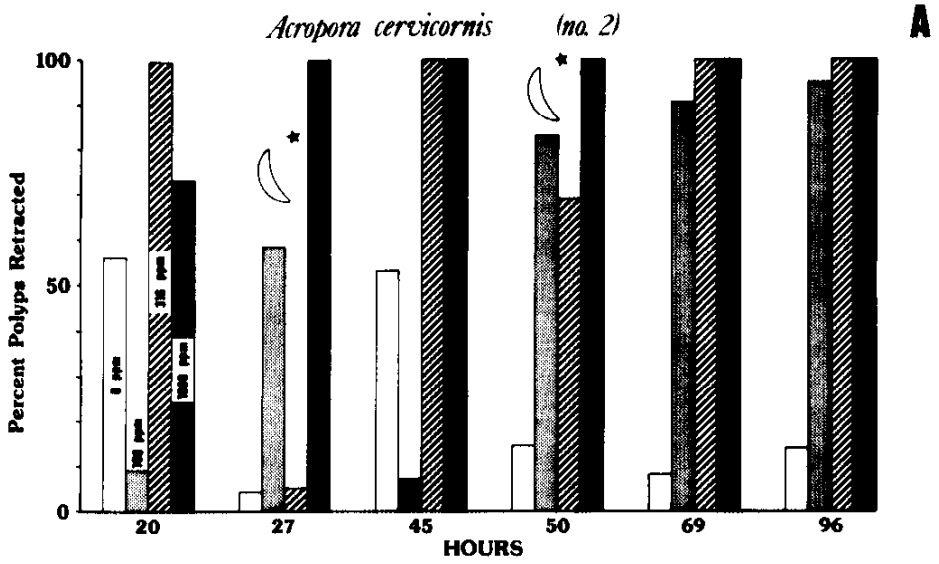


Figure 10A, B, C. Data for *Acropora cervicornis* (branching coral), test no. 1. Data same as previously presented.



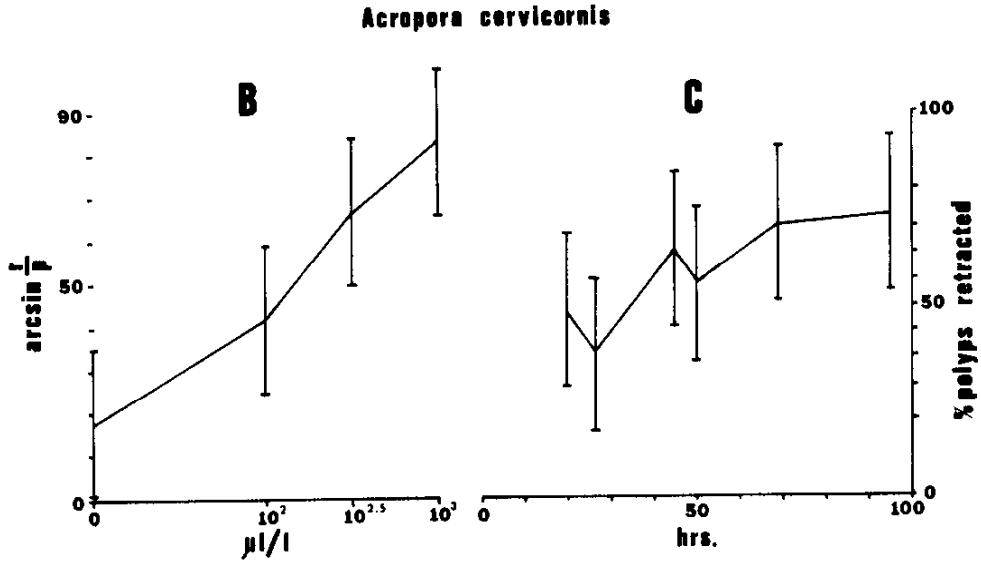


Figure 11A, B, C. Data for *Acropora cervicornis*, test no. 2. Data presented same as in previous Figures. Note that specimens did not die in this test.

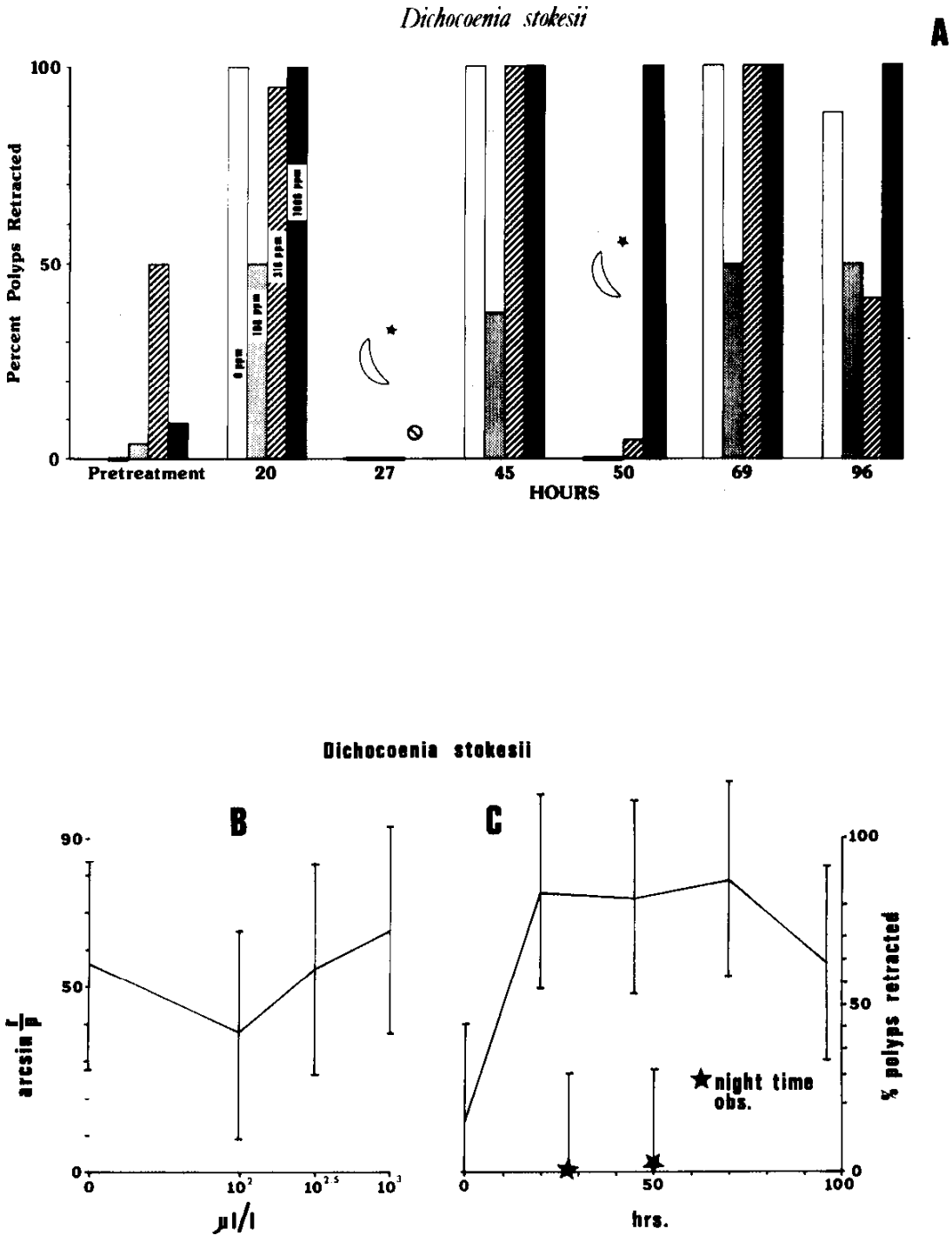


Figure 12A, B, C. Data for *Dichocoenia stokesii* (small, massive coral), as shown in Figure 5-1. Data presented same as in previous Figures. Note diurnal fluctuation in polyp expansion, regardless of drilling mud presence.

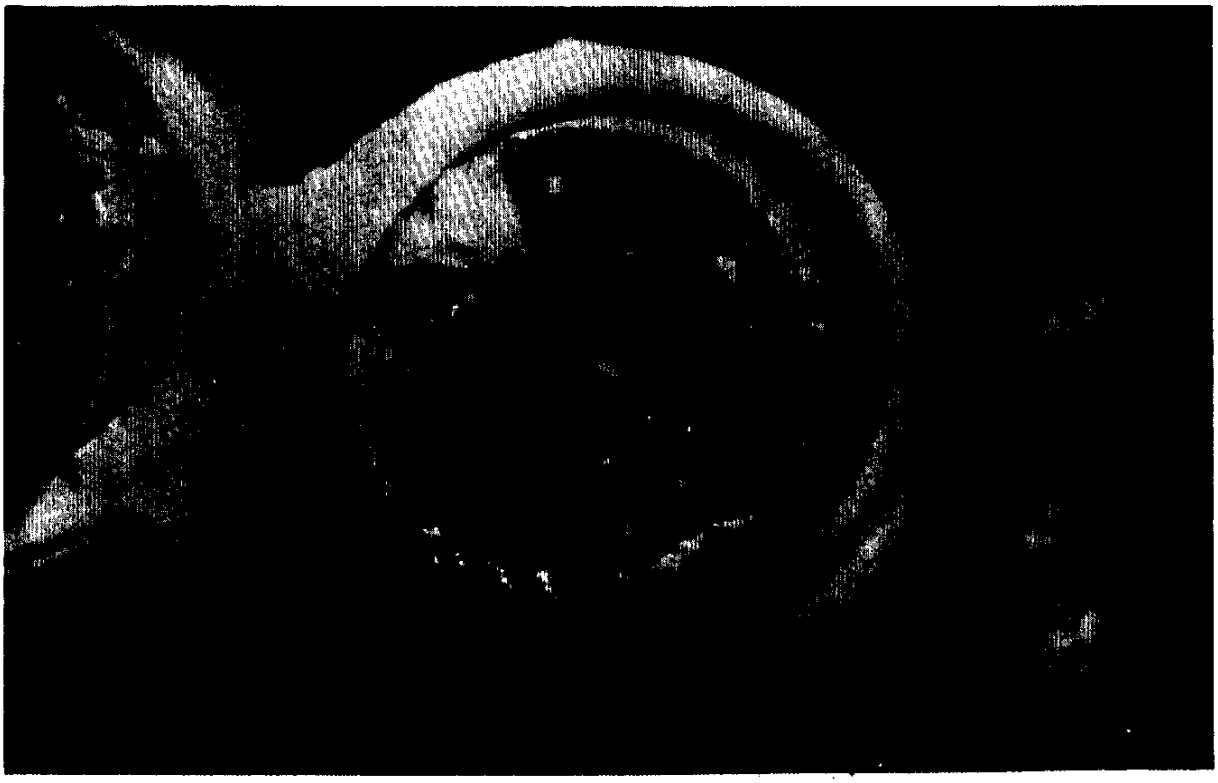


Figure 13. Photo of core plugs of living *Montastrea annularis* in laboratory test chambers.

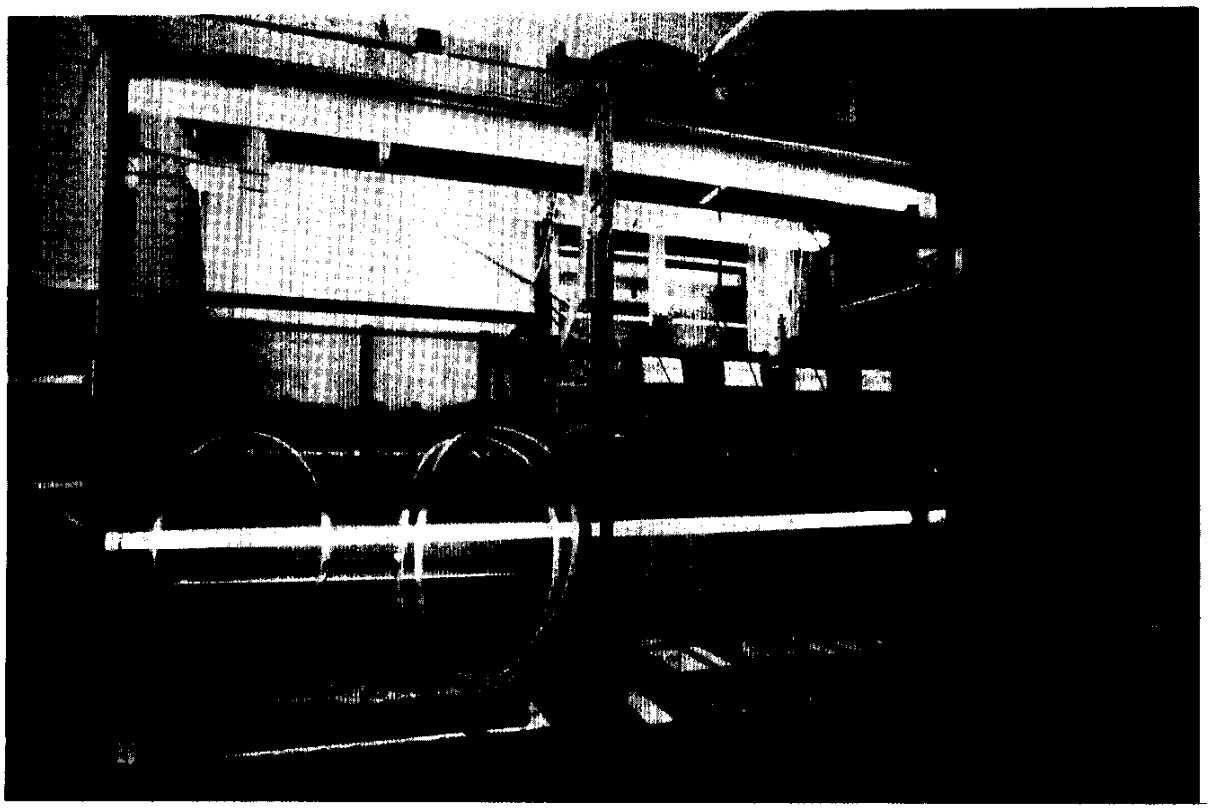


Figure 14. (A) Laboratory setup of test chambers showing lighting and time-lapse movie arrangement. (B) Detail of test chambers and time-lapse photo equipment. Small electronic flash units on top of chambers illuminate specimens for each movie frame exposure. Corals are not affected by the high-speed strobe light.