PHYSIOLOGICAL EFFECTS OF DRILLING MUDS ON REEF CORALS

by

Alina Szmant-Froelich
Department of Oceanography
Florida State University
Tallahassee, Florida 32306

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Project Officer

Thomas W. Duke
Environmental Research Laboratory
Gulf Breeze, Florida 32561

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Agency
Gulf Breeze, Florida 32561

DISCLAIMER

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FOREWORD

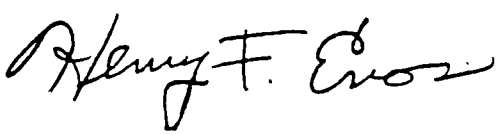
The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities of organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environments.

This report describes the toxicological and several physiological responses of two species of coral, Montastrea annularis and Acropora cervicornis, after exposure to fluids produced by drilling operations for oil exploration. Although these fluids originated from a land-based operation and were not to be disposed at sea, their characteristics closely resembled those that are released in marine waters. The research data when coupled with information related to environmental levels of fluids used in offshore drilling will contribute to a hazard assessment of the impact of drilling fluids on the marine environment.



Henry F. Enos
Director
Environmental Research Laboratory
Gulf Breeze, Florida

ABSTRACT

Discharge of drilling muds, a by-product of oil drilling, could have a detrimental effect on some marine organisms. This research was designed to test the effects of exposure to drilling muds on coral physiology.

Coral from two species, Montastrea annularis and Acropora cervicornis, were exposed in the laboratory to concentrations of 0, 1, 10 and 100 ppm drilling mud for two days to seven weeks. Several physiological functions of the coral animal (calcification rate, respiration rate) and of their zooxanthellae (photosynthesis rate, nutrient uptake rate) were monitored at regular intervals during the exposure periods. In addition, biomass parameters (tissue nitrogen, zooxanthellae cell density, chlorophyll content) were measured at two-week intervals during the long exposure and at the end of each shorter exposure.

Initial long-term exposures of pieces of M. annularis to a series of drill muds (designated JX-2 through JX-7) collected from a Jay (Florida) oil-field well produced a significant reduction in calcification, respiration, and NO_3-uptake rates during the fourth week of exposure to 100 ppm drill mud. Photosynthesis and NH_4-uptake rates also decreased during the fifth week of exposure. Normal feeding behavior was absent from these corals when tested during the sixth and seventh weeks of exposure. Several corals exposed to 100 ppm died during the fifth and sixth weeks.

Short-term (2 to 5 days) exposures of pieces of M. annularis to 100 ppm JX-7 mud (the drill mud used during weeks 5 and 6, which had a much higher Cr and hydrocarbon content than muds used during weeks 1 to 3) caused large reductions in calcification, and, to a lesser degree, in respiration, gross photosynthesis, and NO_3 uptake rates in one of two experiments. A. cervicornis showed a large reduction in calcification after 12 hours of exposure to 100 ppm JX-7, and a decrease in NO_3-uptake within 24 hours. No coral deaths occurred during these short tests.

Implications of the results are discussed, and recommendations are given for future studies.

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ACKNOWLEDGEMENTS

This study is the result of the dedicated effort of many students and technicians who spent many days and nights, often under unpleasant conditions, in order to keep the experiments going twenty-four hours a day. Virginia Johnson and Ted Hoehn did most of the alkalinity titrations and ran the Auto Analyzer; Dr. Roger Adam and Jeff Parker did the TCO_2 analyses, built our experimental facilities and organized efforts the first summer; James Battey, Jason Smith, and Esther Fleischmann, graduate students of Dr. James W. Porter, Univiversity of Georgia, did the oxygen analyses both summers, and constructed the experimental equipment the second summer. J. Parker, T. Hoehn, J. Battey, and E. Fleishmann did the chlorophyll analyses, and T. Hoehn also did many of the zooxanthellae counts and carbohydrate analyses. Gregg Stanton, Les Parker, Dr. James Porter, and J. Parker collected the corals used the first summer. Many staff members and students at the department of Marine Sciences, University of Puerto Rico, made our second summer effort successful and enjoyable; principal among them are Dr. Manuel Hernandez-Avila, the director, who gave us his open cooperation, Dr. Tom Tosteson, Sofia Gil-Turnes, and Linda Riggs.

We also thank EPA personnel Ted Gaetz and Herb Fredickson for their patience and help with logistics during our summer on Stage I.

SECTION 1.

INTRODUCTION

Drilling muds are a necessary by-product of oil-drilling. The muds serve to lubricate the drill-string, remove cuttings, maintain hydrostatic pressure, prevent pipe corrosion, and seal the bore hole in porous formations. Drilling muds are a complex mixture of clay minerals or polymers, barite, and a series of chemical additives which vary to suit the drilling conditions. Many of these additives, such as ferrochrome lignosulfonate, fuel oil, and some proprietary chemical additives are considered toxic and hazardous to living organisms (Richards, 1981).

Disposal of used drilling muds recently has become an environmental concern. A common procedure is to discharge the muds from the drilling rig into surrounding waters. An alternative used in many nearshore areas is to remove the spent muds by barge, either to deeper waters, or to chemical waste burial sites on land. The latter procedure is obviously more expensive and has only been used in selected sites where there was concern that the toxic mud components might enter the human food chain or damage ecologically sensitive marine communities.

Since used muds are generally dumped into the immediate vicinity of the drilling rig, it is important to identify marine communities or organisms that might be adversely affected by exposure to drilling muds.

Drilling activities on the outer continental shelf of the Gulf of Mexico are approaching the East and West Texas Flower Gardens--two unique, submerged coral reefs (Bright and Pequegnat, 1974). These reefs are the only two extensive coral communities in the northern Gulf of Mexico, and have formed on salt domes--formations that often contain gas or oil. There is concern about how the prolonged discharge of large quantities of drilling mud on or near the Texas Flower Gardens will affect the health and viability of these reef ecosystems (Science, 1979). The ecologically dominant reef corals are known to be sensitive to high siltation (Dodge and Vaisnys, 1977; Dodge et al., 1974; Loya, 1976), such as would result from the discharge of muds onto the reef, and also to oil pollution (Loya and Rinkevich, 1980), which might result from an oil additive or contaminant in the drilling muds or from an accidental oil spill. Since reef corals are responsible for reef framework building, as well as for much of the primary production in the reef ecosystem, their survival is essential to the integrity of the reef system as a whole. Previous studies have concentrated on behavioral (polyp expansion), growth rate, and lethal

effects of short-term exposure of corals to drilling muds (Thompson, 1980; Thompson et al., 1980). Those studies showed that concentrations greater than 100 ppm of drilling mud cause reduced polyp expansion in several species, and concentrations greater than 1000 ppm cause death in several species within 65 hours. Other experiments, some of which were non-quantitative and thus difficult to reproduce, indicated a decreased growth rate after direct application of drilling mud slurries to the coral surface.

Building on these results, the present study has focused on several coral physiological and biochemical processes that might be affected by short- and long-term exposure to drilling muds. Calcification and respiration rates were chosen as indicators of animal functions, and nutrient uptake and photosynthesis rates as indicators of zooxanthellae function. (Zooxanthellae are the small, symbiotic algae that live within most reef coral tissues.) Animal and algal biomass were also measured as a function of time to monitor for any deterioration of nutritional status during the exposure period. When coral polyps are fully retracted they cannot feed, and the amount of light that reaches their zooxanthellae is reduced. Therefore, prolonged periods of polyp retraction could gradually starve the corals.

Montastrea annularis was chosen as the primary test species because of its ecological importance in the Texas Flower Gardens (Tresslar, 1974) and throughout the Caribbean (Goreau, 1959). A second species, Acropora cervicornis, was used in later tests to compare our experimental procedures and results with those of EPA-funded investigators studying this species (E. Powell, Texas A & M).

Initially, groups of corals were exposed in the laboratory to four mud concentrations (0 ppm, 1 ppm, 10 ppm, and 100 ppm) for six weeks. The mud-exposed corals were fed during the experiments. Two control groups were used: one control group was fed periodically throughout the experimental period; the second control group was not fed to simulate the starvation effects expected in the exposed groups. Previously listed physiological parameters were measured at biweekly intervals. Respiration and photosynthesis were measured both as changes in O_2 and changes in CO_2 in the media; calcification was measured as the decrease in total-alkalinity (TA) of the media, and nutrient uptake was measured as the disappearance of NO_3^- and NH_4^+ from the media. All methods chosen were non-destructive, which allowed us to test individual corals repeatedly throughout the exposure period. A second set of experiments measuring the same physiological parameters, focused on the short term (2 to 5 days) effects of one of the more toxic muds used in the first experimental series.

The studies were conducted as a cooperative agreement between Florida State University and U.S. EPA Environmental Research Laboratory, ERL, Gulf Breeze Florida, with the additional participation of Dr. James W. Porter and several of his graduate students from the University of Georgia. The first experiments were conducted during July and August, 1980, in a laboratory provided by the EPA. on the U.S. Navy Stage I platform located

12 miles offshore from Panama City, Florida. The site was selected because its clear oceanic seawater was suitable for maintaining corals in a healthy state, and because of its proximity to both to both Florida State University and ERL, Gulf Breeze. The second experiments were conducted a year later at the marine laboratory of the Department of Marine Sciences, University of Puerto Rico, La Parguera, P.R., which offered easy access to freshly collected coral specimens.

SECTION 2.

CONCLUSIONS

The results of the first experiment show that the reef coral Montastrea annularis can be adversely affected by long-term (more than three weeks) exposure to drilling muds. Adverse effects ranged from an 84% reduction in calcification rate and 40% reduction in coral respiration rate after a six-week exposure to 100 ppm drilling mud to lesser effects on photosynthesis by the zooxanthellae of these same corals. Several of the corals exposed to 100 ppm drilling mud died during the fifth and sixth weeks, while none of the corals in the other treatments died. In addition, the corals exposed to 100 ppm drilling mud for six weeks lost normal feeding response and 20% of their zooxanthellae, while those in the other treatments did not.

Since different batches of drilling mud were used during the 6-week experiment (collected from one oil well in Jay, Florida, during an ongoing drilling operation and presented to the corals in the same time-sequence as collected), it was not clear whether the absence of any discernible physiological effect during the first three weeks, and the sudden decay in the corals' physiological functions during the fourth week, were due to a cumulative time effect or to a greater toxicity of the batches of drilling mud used after the third week. The muds used during the last three weeks of exposure (JX-5 and JX-7) contained higher concentrations of chromium and hydrocarbons than mud used earlier in the experiment (JX-2 to JX-4) (Gilbert and Kakareka, New England Aquarium, unpublished).

The second set of experiments, in which we exposed specimens of M. annularis and Acropora cervicornis to mud JX-7 for up to five days, showed that there is a considerable amount of variability in the response of different coral colonies to drilling mud. The first specimens of M. annularis exposed to 100 ppm of JX-7 suffered a 20% decrease in calcification within 24 hours of exposure and a 40% decrease by the fifth day of exposure, with smaller decreases in respiration, photosynthesis, and nutrient uptake rates. A second set of specimens of M. annularis collected from a single large colony (from the same reef where the first specimens were collected), showed no adverse effects after three days of exposure to 100 ppm JX-7. A. cervicornis suffered a 50% decrease in calcification within 12 hours of exposure to 100 ppm of JX-7, and a 40% reduction in NO_3-uptake within 36 hours.

The conclusion from both sets of experiments is that short-term exposures (less than two days) to concentrations of 100 ppm drilling mud may cause a large decrease in calcification rate in some colonies of these coral species. Longer exposures, however, especially when more toxic

drilling mud additives are used, increase the chance that sublethal and lethal effects will occur. Concentrations higher than 100 ppm will probably have an effect much sooner, and concentrations of 10 ppm or less are unlikely to have an effect in exposures as long as one to two months. These results, however, are only indicative of what might occur in a fully developed oil field where corals may be exposed for prolonged periods (six months to several years) to intermittent and variable doses of drilling mud.

SECTION 3.

RECOMMENDATIONS

Initial studies were undertaken and designed with little information on expected exposure concentrations and duration. It appears that a realistic exposure regime for corals on a reef adjacent (within 1 km) to a single drilling rig would be frequent (1 to 3 times per week) exposures of short duration (3 to 12 hours) to concentrations below 100 ppm of whole muds over a period of three to six months. Corals on a reef situated amidst an oil field probably would be subjected to higher concentrations for a longer duration of time. Only corals situated within about 100 meters of a rig should encounter higher concentrations or problems of burial beneath drilling mud. Therefore, any future studies should concentrate on experiments designed to determine the effects of repeated exposures and the factors that might affect recovery between exposure episodes.

A second recommendation is that the composition of the drill muds to be used be determined before the tests are conducted, or that "typical" muds for the drill site in question be used in the tests. Tests with individual additives would also be useful to identify the source of the toxicity. Critics of drilling mud studies contend that the muds used to expose the organisms was "atypical" or "not meant for discharge." The Jay muds used in our study were from a terrestrial, not an offshore well; but we have no information to indicate that the ingredients of these muds, save one, were any different from those of muds used offshore. Present U.S. regulations prohibit the use of fuel oils as a lubricant in drilling muds discharged offshore, and fuel oil was a component of the JX-5 and JX-7 muds. However, in spite of the regulations, hydrocarbon residues indicative of fuel oil have been detected in discharged offshore muds in the Gulf of Mexico (Weichert et al., 1981). While not added as a routine ingredient, fuel oil is used at the discretion of the drilling engineer on an as-needed basis to free stuck drill strings, even in offshore waters. In addition, other countries where drilling is occurring near or on coral reefs, such as the Philippines, Mexico and Trinidad, do not regulate the composition of the drilling muds used as strictly as the United States.

A final recommendation is that future studies should be concerned with dispersal characteristics of different fractions of the mud. Heavy particulates will settle quickly over a small downstream area where corals may be both buried and poisoned. Light particulates and dissolved fractions will disperse over larger areas, but in lower concentrations, and potential effects will be limited to those associated with chemical toxicity. The solubility of many of the biologically active additives gives reason to believe that much of the potential toxic activity will be in the dissolved fraction.

SECTION 4.

MATERIALS AND METHODS

FIRST EXPERIMENTAL SERIES - STAGE I

Coral Collection and Maintenance

At the outset EPA agreed to provide coral specimens from the Texas Flower Gardens for our study, but there were logistical problems in providing them and we were authorized to obtain corals from the Florida Keys. There are no reasons to believe that there are any basic physiological differences between the two populations.

Colonies of Montastrea annularis and Madracis decactis were collected by scuba divers at depths of 4 to 10 m from reefs near Big Pine Key, Florida. Large heads of M. annularis were broken into smaller pieces with a chisel and hammer. The pieces of coral were placed in submerged buckets and transferred to large coolers without exposure to air. The coolers were kept overnight in a running seawater holding tank and air-shipped the next morning to Panama City, Florida. The pieces of coral were put into individual plastic bags to minimize damage from abrasion during shipping. The coolers were immediately transferred to Stage I by boat, where the individual bags were suspended in large aquaria of running seawater. Stage I seawater was admitted to the bags slowly over a 2 h period to minimize shocking from change in temperature and water conditions. The corals appeared to be in good condition, and most were fully expanded within a few hours after transfer to tanks.

The Madracis colonies were used by EPA personnel for behavior studies.

The corals were maintained in five 202-L glass aquaria housed on water tables in an air-conditioned Butler building located on the lower level of the Stage I platform. The aquaria were outfitted with plastic "eggcrate" bottom racks to help support the irregularly shaped pieces in an upright position. Fresh unfiltered seawater, pumped up from a depth of 28 m, was drawn into the aquaria by Little Giant water pumps at a rate of 5.2 L/min, resulting in a turnover time of 40 min. Details of the seawater system are illustrated in Figure 1. A 12-h light/dark cycle was provided with banks of VHO cool-white fluorescent bulbs. The average light level was 100 μEin $m^{-2}s^{-1}$. The water tables housing the aquaria were surrounded with dark, opaque shower curtains to shield the corals from the laboratory lights at night. The aquaria were cleaned as needed to remove algae and other fouling organisms. During the exposure period, drill mud that

settled in the tanks was siphoned out. The exposed skeletons of the coral pieces were also scrubbed with soft bottle brushes to remove algae. Corals in four of the aquaria (Tanks 1 to 4) were fed periodically with freshly collected zooplankton or with brine shrimp nauplii.

Experimental Design

Forty pieces of coral were selected and randomly assigned: eight pieces to each of five aquaria. The aquaria were then randomly assigned to one of five treatments: control unfed, control fed, 1 ppm drill mud, 10 ppm drill mud, and 100 ppm drill mud. Since only eight corals could be tested each day, a scheme was devised to divide the 40 corals among the five incubation days. Four of the eight corals in each treatment (the lowest numbered ones) were designated the "A" subgroup, and the remaining four were designated the "B" subgroup. The ten "A" and "B" subgroups were assigned to the five incubation days accordingly: 1) there were one A subgroup and one B subgroup per day; 2) the A and B subgroups were from different treatments, and 3) there was only one control subgroup per day. The final incubation sequence is summarized in Table 1.

TABLE 1. ALLOCATION OF TREATMENT SUBGROUPS TO 5-DAY INCUBATION SEQUENCE

Sub Group	Week day				
	1	2	3	4	5
A	Control fed	1 ppm	100 ppm	10 ppm	Control unfed
B	100 ppm	10 ppm	Control unfed	Control fed	1 ppm

Oxygen consumption and production rates (respiration and photosynthesis) were measured once for the 24 corals in the 1, 10, and 100 ppm treatments during the two days before exposure to mud. Mud exposure began on July 21, 1980, and continued until September 3, 1980. The 5-day incubation sequence began on July 22, 1980 (Week 1) and was repeated beginning on July 28, (Week 2), August 4 (Week 3), August 11 (Week 4), August 18 (Week 5) and August 24 (Week 6). The corals were exposed to the various mud concentrations continuously except when removed from the exposure tanks for tests. Each coral was tested once per week; each test consisted of a light and a dark incubation.

During Week 1, only ΔO_2 was measured, with incubations lasting two hours. Four chambers with coral and one control chamber without coral could be measured simultaneously; thus, each complete incubation series consisted of two 2-h incubations in the light and two 2-h incubations in the dark. During subsequent weeks, O_2 incubations were shortened to one

hour, since the ΔO_2 rates were found to be constant throughout the 2-h period. The nutrient uptake, calcification, and TCO_2 measurements began the second week and were done during a separate 3-h incubation of all eight corals simultaneously, using seawater supplemented with NH_4Cl and $NaNO_3$ to elevate the initial nutrient concentrations. During Week 2, the initial incubation water concentrations were about 1 to 2 µM NO_3^- and NH_4^+; during subsequent weeks, about 3 to 6 µM NO_3^- and NH_4^+.

At the end of the six-week exposure, the 40 experimental corals were sacrificed and their surface area and biomass measured (see below). In addition, four corals were sacrificed before the exposure to mud began, and three corals from each treatment were sacrificed after two and four weeks of mud exposure to detect any differences in the biochemical composition of the corals with duration of exposure to drill muds.

Mud Delivery System

The mud delivery system consisted of two separatory funnels (36.8 L capacity) to hold diluted mud stock, and two multichannel peristaltic pumps used to deliver the mud at a constant rate from the funnels to the inflowing seawater lines of the treatment aquaria (Figure 1). The separatory funnels were stirred continuously to keep the muds in suspension. Muds were collected from a well in the Jay oil field, Jay, Florida, by EPA personnel. Mud batches were changed in our exposure system to approximate the sequence and timing of collection of these muds. Table 2 summarizes the collection dates of the muds and their use in our experiments.

TABLE 2. DATES OF COLLECTION OF JAY DRILLING MUDS AND THEIR USE IN TEST EXPOSURES.

Date Mud Collected	7-9	7-11	7-22	7-29	8-4
Designation of Mud Used	JX-2	JX-3	JX-4	JX-5	JX-7
Date Exposure Began	7-21	7-27	8-3	8-10	8-24

Incubation Procedures

Oxygen Incubation -- Five 15-cm diameter Plexiglass chambers with O-ring sealed lids were used for respiration and photosynthesis measurements. The lids were fitted with openings to accommodate Orbisphere self-stirring BOD probes. The oxygen probes were calibrated daily against Winkler titrations (Strickland and Parsons, 1972). Four corals from each treatment were run simultaneously, one chamber serving as a control. All incubations were conducted in filtered seawater (Honey-Comb Superfine, 1 µm filters). The

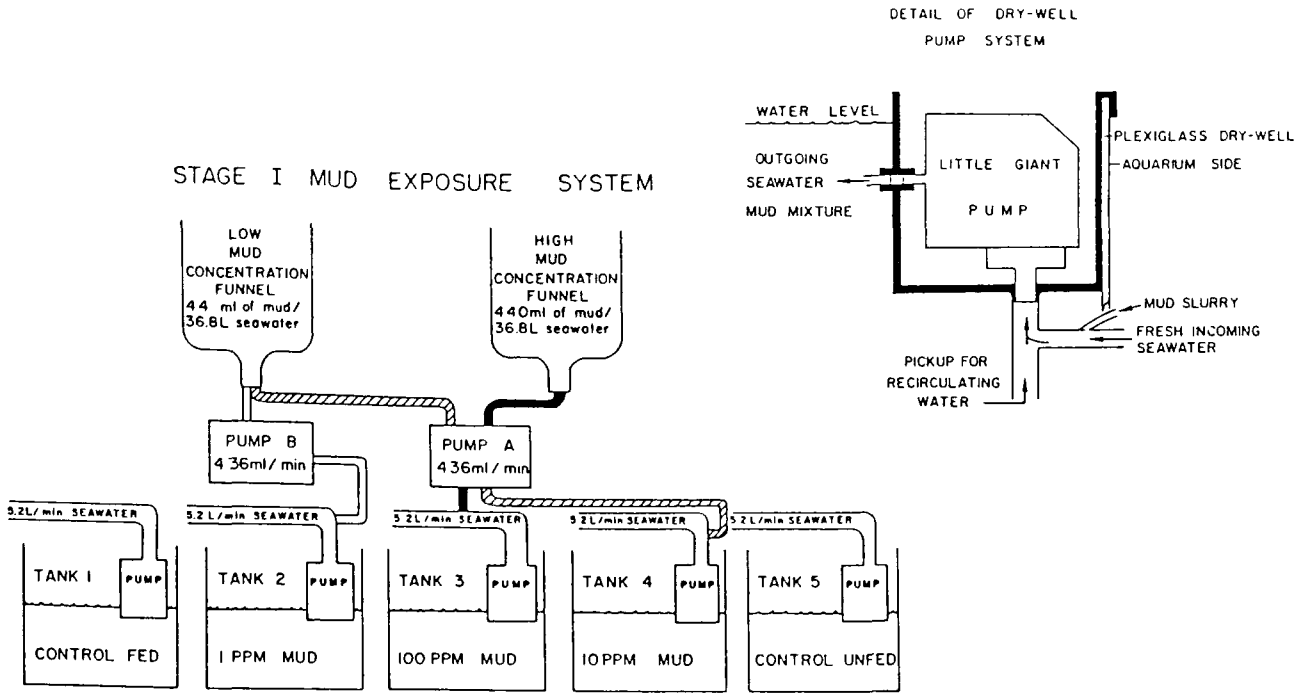

Figure 1. Diagram of the system used for exposing corals to a constant drilling mud concentration.

rack of five chambers was placed in a Plexiglass trough, where a continuous flow of filtered seawater acted as a constant temperature water bath (±1°C). Salinities, measured periodically with a refractometer, remained constant at 35 °/oo. Light incubations were conducted under a bank of two G.E. 1500 watt cool-white fluorescent bulbs, that yielded an average light intensity of 94 μEin $m^{-2}s^{-1}$ inside the incubation chambers.

Nutrients, CO_2 and Calcification Incubation -- Glass chambers with stirring bars and support racks were placed in the same trough and light source as above. A measured amount of $NaNO_3$ and NH_4Cl stock was mixed into the trough before the chambers were put into place. Initial water samples for pH, total alkalinity (TA) and for nutrient analysis were withdrawn from each chamber; pH was measured immediately and the samples were capped to prevent evaporation before TA analysis. Sampling was repeated at the end of the incubation period.

NO_3^- determinations were made by the standard Technicon Auto Analyzer NO_3^- technique for seawater and wastewater. NH_4^+ determinations were made by an adaptation of the automated method of Berg et al. (1977), with the ethanol eliminated from the phenol reagent. pH determinations were made with an Orion model 701 pH meter, using a calomel combination electrode, the slope of which was checked periodically with N.B.S. buffers of known pH. TA was determined by potentiometric titration of duplicate 10-ml aliquots of the seawater used for the pH determinations. The endpoint of

the titration was calculated by the Gran method (Stumm and Morgan, 1970). The TA of the seawater sample was calculated as:

$$TA\ (meq/l) = N \frac{v}{v_2}$$

where: v is the volume of sample titrated in ml, v_2 is the titration equivalence point in ml, and N is the normality of acid in meq/l.

The total inorganic CO_2 concentrations were determined by thermal conductivity detection of CO_2 stripped from acidified seawater samples after gas chromatographic separation. A Shimadzu Model 3BT gas chromatograph (GC) and a Shimadzu integrator-data processor were used for the analyses. The stripping was done in a Swinnerton-type stripping chamber (Swinnerton et al., 1962). The gas stream passed through a 15 cm Drierite column before entering the GC. Gas separation was achieved with a 2m x 1/8" Porapak Q column at 30°C. Two to five replicates were run for each sample. The mean coefficient of variation of the replicates was less than 1%. Initial tests with Na_2CO_3 standards showed that the response of the system was linear up to at least 3 mM Na_2CO_3. Unfortunately, standard Na_2CO_3 solutions were not run routinely during the experimental runs. We later discovered that the response of the machine to a given amount of TCO_2 was affected by the way in which the Drierite columns were packed. We could analyze the results of each run only by calibrating results against the initial TCO_2 concentration calculated from the pH and alkalinity data (see below). We calculated calibration factors (f) for each test run, as:

$$f = \frac{(TCO_2 \text{ conc. of "initial" samples calculated from pH and TA})}{(\text{integrated peak area of } TCO_2 \text{ in "initial" samples from GC})}$$

where "Initial" samples were the samples taken at the beginning of each incubation set. TCO_2 concentrations in the final samples were then calculated as:

$$TCO_2(mM) = f \times (\text{peak area from GC})$$

Biomass Analysis

The surface areas of the living portion of the 40 intact experimental corals were determined by the aluminum foil method (Marsh, 1970). These surface areas were used to normalize the metabolic rates measured in the above incubations.

All coral colonies processed for biomass analysis and biochemical composition were first scrubbed to remove any mud or encrusting organisms adhering to the dead portions of the skeletons. A chisel and hammer were used to break the colonies into smaller pieces to be used for the individual analyses. Surface areas of these pieces were determined as above.

Pieces of each coral were placed into wide-mouth glass jars with 100 ml of a chloroform:methanol:seawater solution (1:2:0.5; Bligh and Dyer, 1959). The caps of the jars were lined with aluminum foil and the jars were pre-washed with Bligh-Dyer solution. These samples were returned to Florida State University for lipid analysis by Dr. D.C. White who will report the results separately.

The remainder of the tissue analyses were conducted on a smaller piece of each coral. Tissues were removed from the skeletons by first breaking up the tissue with a small wire brush powered by an electric hand drill, then blasting the remaining tissue off with a fine jet of filtered seawater from a Water-Pik (Johannes and Wiebe, 1970). The tissue slurry was homogenized in a blender for 3 min and the volume of the homogenate recorded. Duplicate 1-ml aliquots of the homogenate were analyzed for total-nitrogen by the persulfate oxidation method of D'Elia et al. (1977). Subsamples of homogenate were frozen in polyethylene bottles for later carbohydrate analysis (Dubois et al., 1956); 30-50 ml volumes of homogenate were filtered onto glass fiber filters for chlorophyll determinations following the procedures of Strickland and Parsons (1972). Additional subsamples of homogenate were preserved with Lugol's iodine solution for zooxanthellae counts (Szmant-Froelich and Pilson, 1980).

Other Procedures

Histological Fixation--Small pieces of coral from each treatment were fixed with a seawater-Zenkers fixative (Yevich and Barszcz, 1977) for 24 to 48 h. The fixed samples were washed in running seawater for another 24 to 48 h and then preserved in 70% ethanol. All further processing and examination were done by the EPA histopathology unit, at ERL, Narragansett under the direction of Mr. P. Yevich, who will report results separately.

Staining with Alizarin-Red-S--Two days before mud exposures began, twelve large colonies were placed into two ten-gallon aquaria filled with an alizarin/seawater solution (15 mg alizarin/L of seawater). The corals were left in the solution for 8 h before running seawater was restored to the aquaria. A second treatment with alizarin was repeated the following day to ensure that a strong stain mark had been incorporated into the skeleton. Three of the stained colonies were placed into each of the four "fed" treatment tanks (control, 1 ppm, 10 ppm, and 100 ppm) on the first day the exposures to mud began. They remained in these tanks until sacrificed on September 7, 1980 (except for two of the 100 ppm corals which died before the end of the experiment). Tissues were removed by first soaking the corals in buckets of fresh tapwater, and then squirting the corals with a jet of water from a garden hose. The skeletons were shipped for analysis to Dr. R. Dodge (Nova University) (Dodge, Marine Biology, in press).

SECOND EXPERIMENTAL SERIES - PUERTO RICO

Coral Collection and Maintenance

Specimens of Montastrea annularis were collected fom the reef Cabo

de la Raya at a depth of 2 to 5 m. Corals for Test 1 were collected from several adjacent colonies, but those for Test 2 were from a single large colony. The corals were kept in aquaria with running seawater for 48 to 72 hours until used in the experiments.

Specimens of Acropora cervicornis in Test 3 were collected from the lagoon (2 to 3 m depth) of San Cristobal reef the day before the experiment began. Whole colonies were returned to the laboratory, where individual branches 6 to 9 cm long were clipped-off and placed upright into small Plexiglass stands. Thus, we could move the 'fingers' without having to touch live coral tissue.

Experimental Design

Two exposure series were conducted with M. annularis: In Test 1, corals were exposed to 0, 10 ppm, and 100 ppm drilling mud (6 replicate corals each) for five days; In Test 2, 9 replicate corals were exposed to 0 and 100 ppm drilling mud for three days. From the day before mud exposure was to begin (2 days for Test 2), the corals were incubated for two hours in the daytime and for one hour at night. Parameters measured during the daytime incubations were O_2 concentration, TA, NO_3^-, and NH_4^+ concentrations (nutrients not measured during Test 2); only O_2 concentrations were measured at night.

The A. cervicornis experiments consisted of exposing coral fingers (four replicates each) to 0, 10 ppm, and 100 ppm drilling mud for 48 hours. The corals were incubated as above beginning one day before exposure. Concentrations of O_2, TA, NO_3^-, and NH_4^+ were measured as above.

All corals were sacrificed at the end of the experiments to determine their surface area, chlorophyll, zooxanthellae, and tissue-nitrogen content by the methods described above.

Mud Delivery System

The mud delivery system was a scaled-down version of the Stage I system. We used 80-L aquaria, and maintained a flow rate of 1 L/min. The aquaria were kept on a shaded water table and received supplemental light from cool-white fluorescent bulbs. Light levels in the tanks were about 100-150 μEin m^{-2} s^{-1}. Dark curtains around the water table shielded the corals from extraneous lights at night.

The mud tested was JX-7 collected the previous summer from the Jay oil field and preserved by refrigeration.

Incubation Procedures

The same glass chambers used on Stage I were used for the M. annularis incubations. Chambers were filled with seawater, which had been

allowed to sit in cubitainers for one hour to degas, and placed in a through with a continuous flow of seawater to maintain constant temperature. Salinities measured with a refractometer, remained constant at 35 °/oo. Light incubations were conducted under a bank of 40-watt cool-white bulbs, which yielded an average light intensity of 450 μEin $m^{-2}s^{-1}$ inside the incubation chambers. Water samples for O_2, TA, pH, and nutrient analyses were taken from the cubitainers at the beginning of the incubation and from each chamber at the end of the incubation.

A. cervicornis was incubated in 500 ml cylindrical chambers constructed from 3-inch diameter Plexiglass tubing, using the same trough and procedures as above.

DATA ANALYSIS

The changes in concentration of the incubation media were corrected for water volume, incubation duration, and concentration changes in the control chambers, then normalized to the living surface area of the coral to give a rate per surface area of coral (nmol $cm^{-2}h^{-1}$) for each physiological function.

Calcification rates ($\Delta CaCO_3$) from the Stage I experiments was calculated as:

$$\Delta CaCO_3 = 1/2\ [\Delta TA - \Delta NH_4^+ + \Delta(NO_3^- + NO_2^-)]$$

to correct for any changes in TA caused by the uptake of the added nutrients (Brewer and Goldman, 1976; Jacques and Pilson, 1980). Calcification rates from the Puerto Rican experiments were not corrected for nutrient uptake. Total-CO_2 was calculated from the pH and alkalinity data, using the relationships expressed in Riley and Chester (1971). The change in TCO_2 due to respiration and photosynthesis [$\Delta CO_{2P/R}$] was calculated from the equation:

$$[\Delta CO_{2P/R}] = \Delta TCO_2 - \Delta CaCO_3$$

Derivations and discussions of the above equations and their application to metabolic measurements on corals have been previously described (Smith and Kinsey, 1978; Jacques and Pilson, 1980).

One-way ANOVA using SPSS (Nie et al., 1975) was used to analyze the Stage I data. We tested trends over time within each treatment group and differences between treatments within each of the six weekly incubation series. The program also calculated t-tests between specified treatment groups. The 1 ppm coral rates were not significantly different from the controls; therefore the 10 ppm and 100 ppm coral rates were tested against the mean of the two control and 1 ppm groups.

SECTION 5

RESULTS AND DISCUSSION

CORAL SURVIVORSHIP

One of the ~90 pieces of M. annularis collected from the Florida Keys for use in the Stage I experiments died within a week of collection. All the surviving corals appeared healthy at the beginning of the exposure experiment; polyps readily expanded both in the aquaria and the experimental chambers.

Further coral mortality occurred only in the 100 ppm treatment tank. One of the eight experimental corals lost most of its zooxanthellae during week 5 and one-third of its polyps after 34 days of exposure to 100 ppm mud. A white flocculent film covered the dead portion of the coral. Two other colonies from the 100 ppm tank had partially bleached after 34 days of exposure and were dead by 43 days. Portions of several other coral pieces from this tank were dead by the end of the experimental period.

No deaths occurred among the corals used in the short exposures to JX-7 mud in Puerto Rican studies.

PHYSIOLOGICAL RATES - STAGE I.

The mean rates of calcification, respiration, photosynthesis and nutrient uptake for the 6-week exposure period can be found in Appendix A and are plotted in Figures 2 to 8. Results of feeding behavior studies with exposed corals at the end of the exposure period and of the coral and algal biomass determinations are given in Tables 3 and 4 respectively.

Calcification

Daytime and nighttime calcification rates showed the same trends with time and treatment, but daytime rates were two to three times greater than nighttime. Only daytime rates are discussed here.

There were no significant week-to-week differences in the calcification rate for either control or 1 ppm treatments, but for both 10 ppm and 100 ppm treatments the rates decreased with time (Figure 2). Between-treatment comparisons for each week (Appendix B) showed no

statistically significant differences between treatments until the fourth week, when 100 ppm daytime calcification rates dropped to 16% of control and 1 ppm coral rates ($p = 0.005$). During the sixth week, the 10 ppm corals calcified at 67% of the rate of the controls, but the difference was not significant ($p = 0.084$).

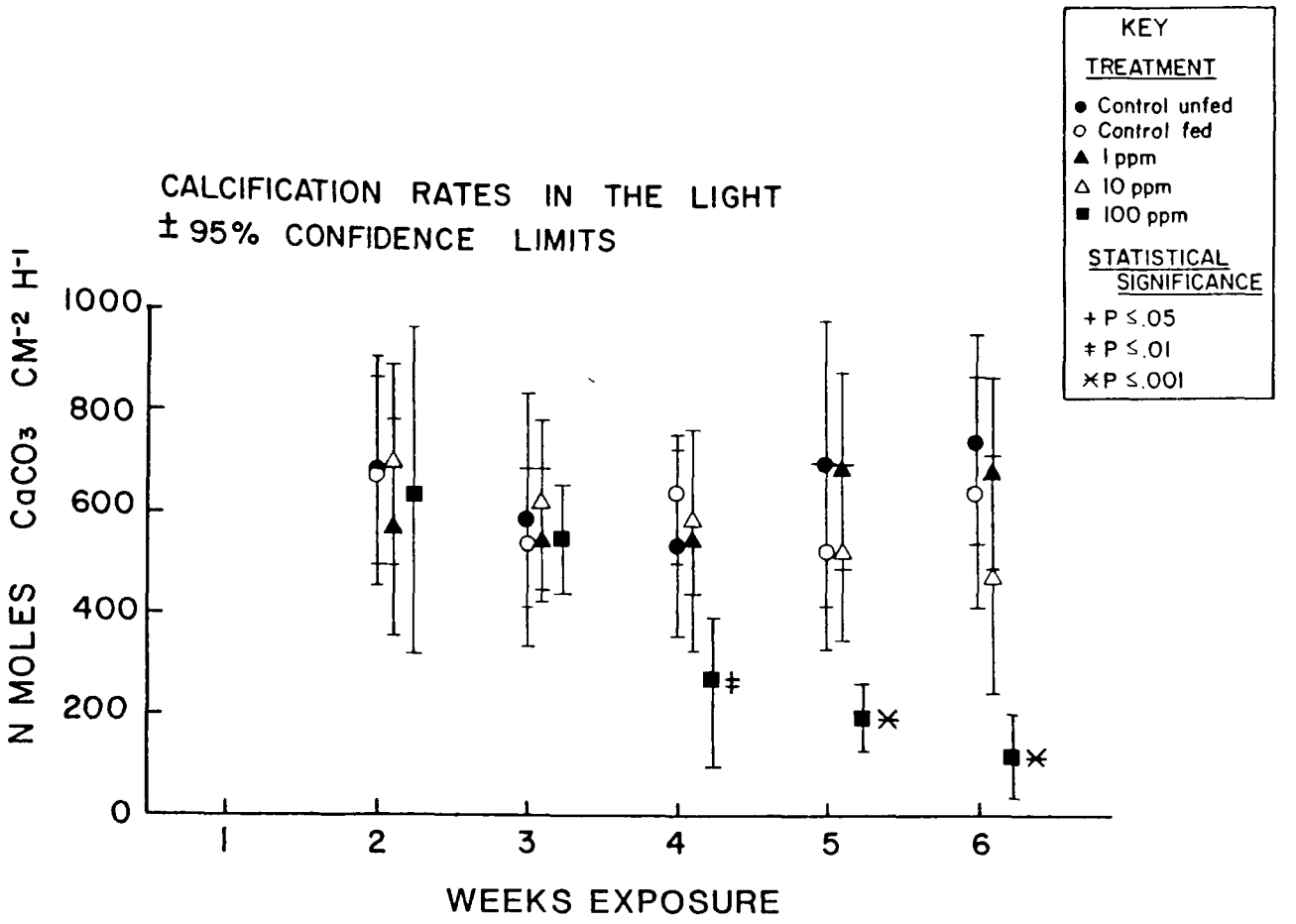

Figure 2. Daytime calcification rates of M. Annularis measured as changes in total alkalinity. n=8. Legend applies to Figures 2 to 8.

Respiration

Respiration rates were measured in two ways: as decreases in O_2 concentration and as increases in CO_2 concentration. The respiratory quotient ($RQ = \Delta CO_2/\Delta O_2$) reflects the degree of reduction of material being catabolized, as well as differences in analytical methodology. The overall CO_2 to O_2 ratio for all the measurements was 0.85 ($r^2 = 0.95$; $n = 39$). There were no obvious changes in RQ over the six weeks, nor any significant differences in RQ between treatments.

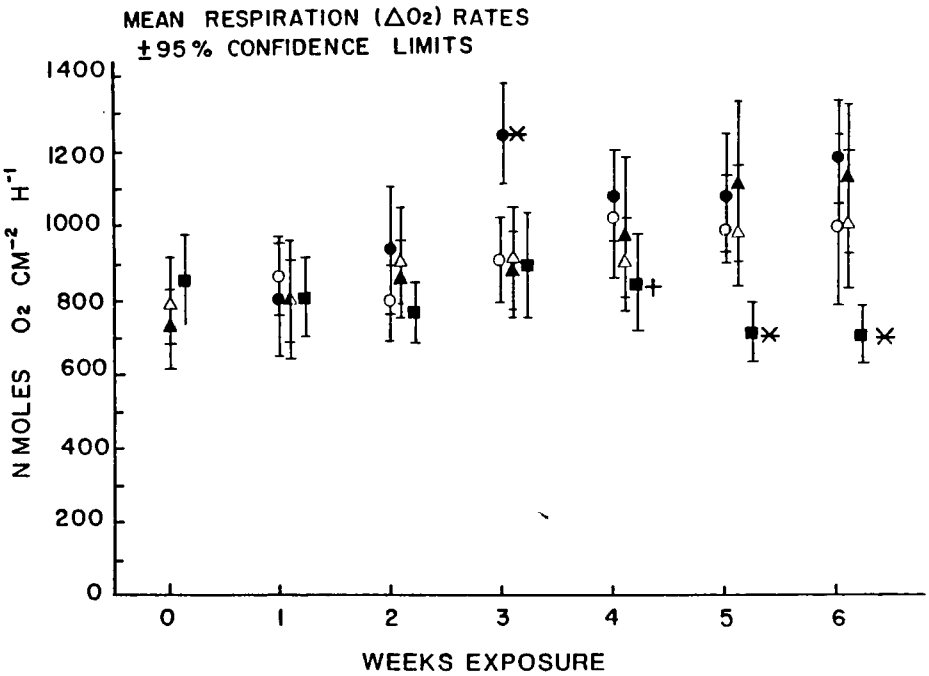

Figure 3. Respiration of M. annularis measured as changes in oxygen concentration. $\bar{n}$=8. For symbols, see key to Figure 2.

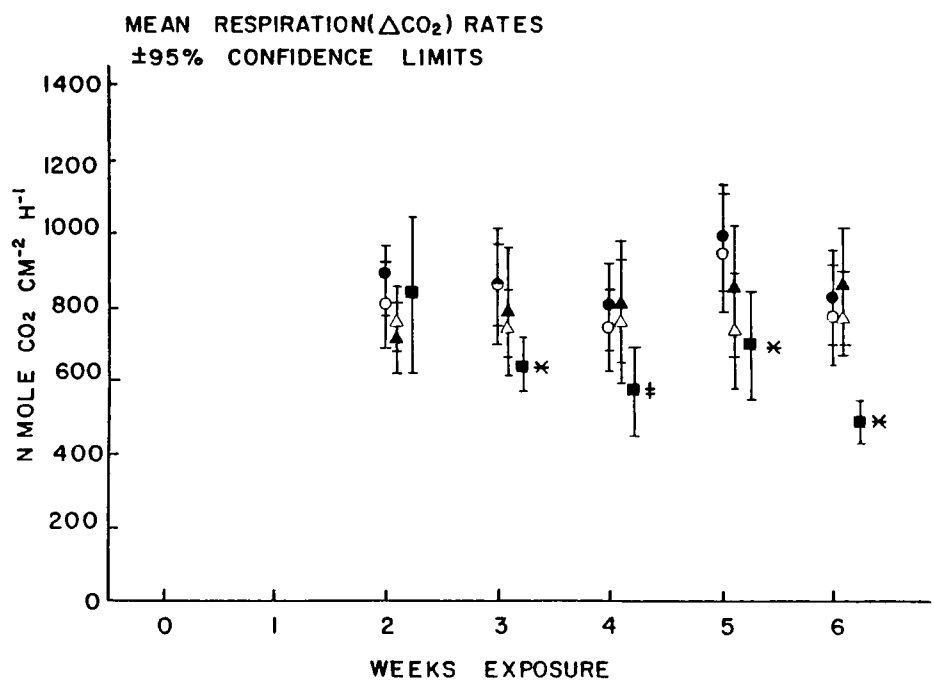

Figure 4. Respiration of M. annularis measured as changes in CO_2 concentration. $\bar{n}$=8. For symbols, see key to Figure 2.

Respiration rates of all except the 100 ppm corals increased gradually with time (Figures 3 and 4). The 100 ppm corals, whose respiration rate decreased over the six-week exposure period, had significantly lower respiration rates than the controls following the second week of mud exposure (Figure 4); by the sixth week, their respiration rate was reduced to 60% of that of the controls ($p < .001$).

Photosynthesis

Gross photosynthesis was measured as both increase in O_2 and decrease in CO_2 concentrations. The overall photosynthetic quotient ($PQ = \Delta O_2/\Delta CO_2$) was 0.98 ($r^2 = .93$; $n = 39$). As with RQ's, there were no trends over time nor differences between treatments.

Photosynthetic rates gradually increased with time for all treatments except the 100 ppm treatment (Figures 5 and 6). O_2 production by the 100 ppm corals decreased to 74% and 83% of the control rate during weeks 5 and 6 respectively, while CO_2 estimates decreased to 75% and 67%. Tissue analyses of corals sacrificed during the seventh week revealed that the zooxanthellae content of the 100 ppm corals was 20% lower than that of the control corals ($p = .05$) (Table 4). Therefore, most of the decrease in photosynthesis rate and a portion of the decrease in respiration rate of the 100 ppm corals during the last two weeks of exposure may have been due to a loss of zooxanthellae biomass.

Nutrient Uptake

The control-unfed corals consistently took up more NH_4^+ than the control-fed corals, and the differences were frequently statistically significant. However, there was no consistent difference in NO_3^- uptake between the two control groups. A possible explanation is that the zooxanthellae of unfed corals had less NH_4^+ available from coral metabolic waste and, therefore, took up more NH_4^+ from the media.

Nutrient uptake rates by zooxanthellae are known to follow Michaelis-Menton kinetics (D'Elia, 1977; Muscatine and D'Elia, 1978). Therefore, net nutrient uptake in these type of experiments will depend on the initial nutrient concentration of the incubation media. Nitrogen uptake rates were lowest for all treatments during week 2 (Figures 7 and 8) because of the lower initial nutrient concentrations and there were no significant differences in that week between the control and the exposed corals. Significant differences between the 100 ppm corals and the controls were first seen during the fourth week of exposure (Figures 7 and 8), and between the 10 ppm corals and the controls, during the fifth week. NO_3^- uptake appeared to be affected slightly more than NH_4^+ uptake. By the sixth week, NO_3^- uptake by the 100 ppm corals had dropped to 42% of the control rate and NH_4^+ uptake had dropped to 51% of the control rate. Since zooxanthellae densities decreased by only 20% (see above), there must have been a decrease in the capacity of the 100 ppm zooxanthellae to take up nutrients.

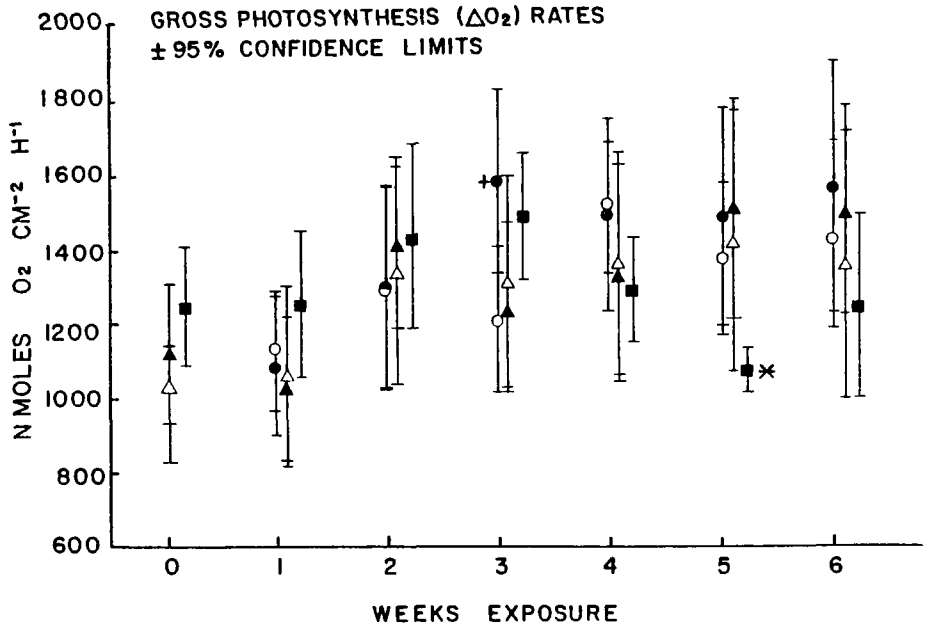

Figure 5. Photosynthesis by M. annularis measured as changes in O_2. n=8. For symbols, see key to Figure 2.

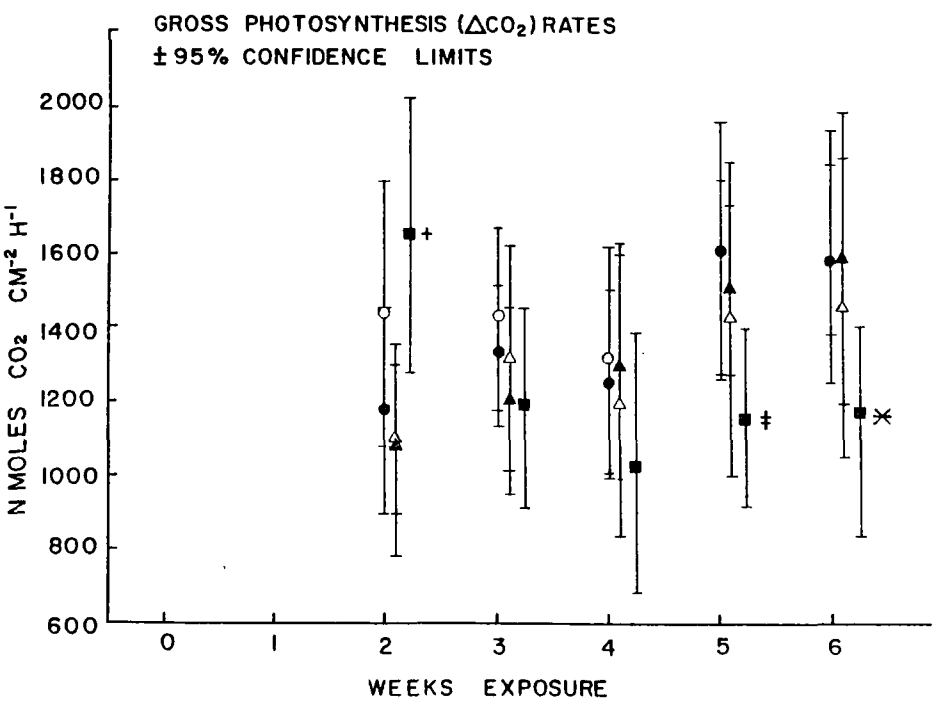

Figure 6. Photosynthesis by M. annularis measured as changes in CO_2. n=8. For symbols, see key to Figure 2.

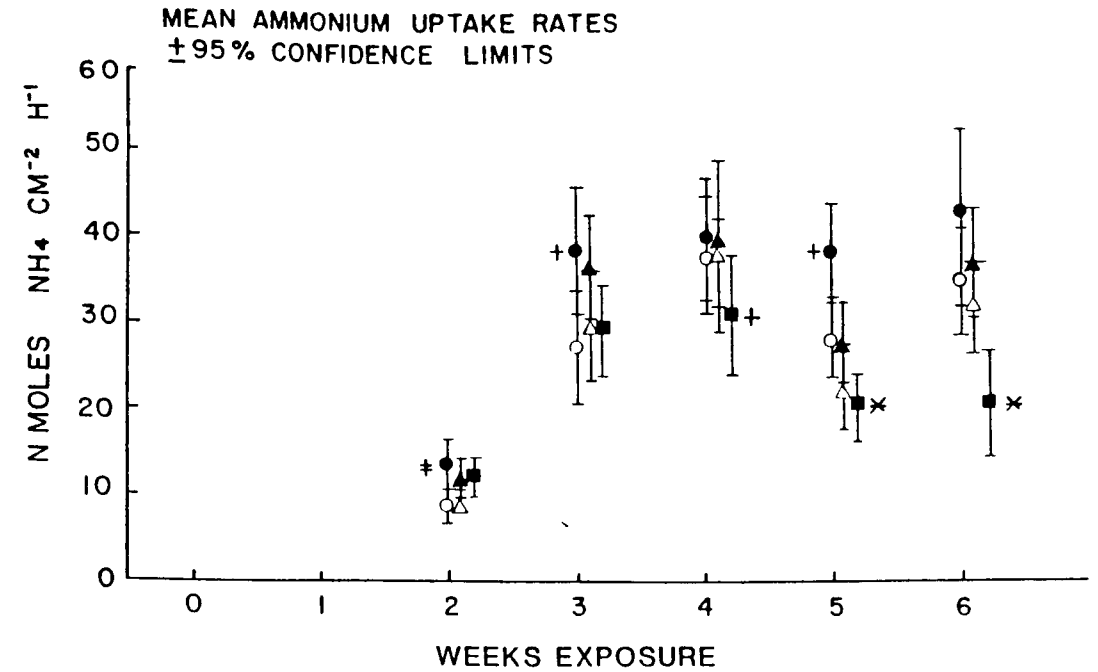

Figure 7. Ammonium uptake by M. annularis during both light and dark incubations. n= 16. For symbols, see key to Figure 2.

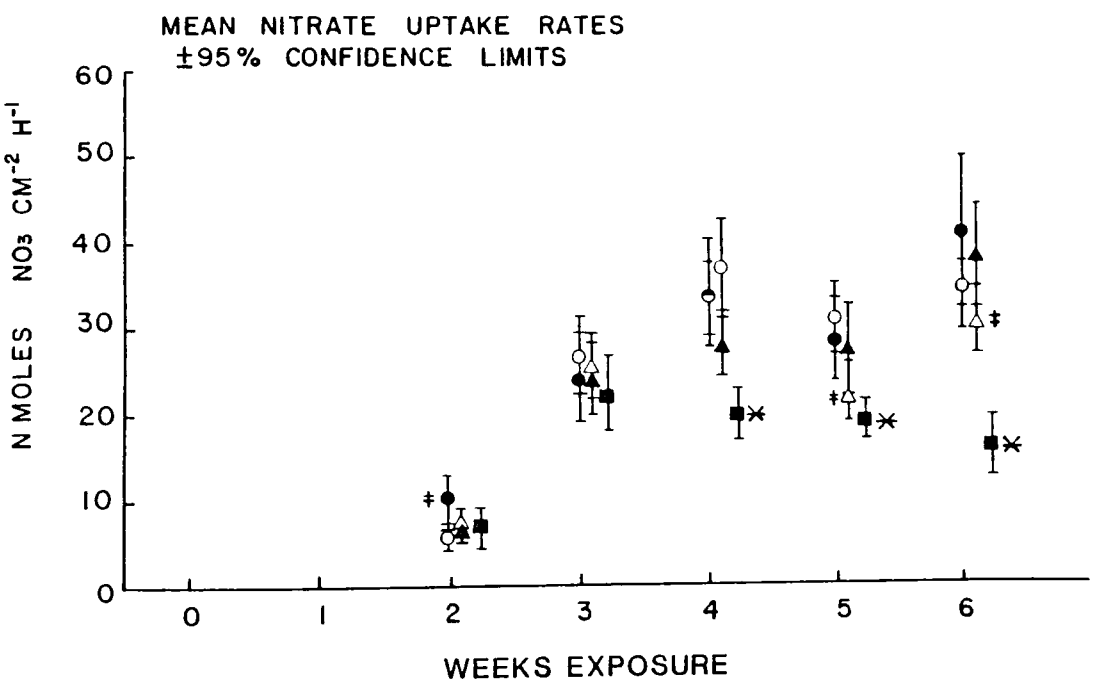

Figure 8. Nitrate uptake by M. annularis during both light and dark incubations. n= 16. For symbols, see key to Figure 2.

Feeding Behavior

During the fourth and fifth week of exposure the polyps of the 100 ppm corals no longer expanded during the incubations. At the end of the 6-week exposure period, several corals from each treatment were placed in finger bowls containing filtered seawater. The two 100 ppm colonies selected appeared the healthiest of those surviving that treatment. Small pieces of filter paper soaked with Artemia nauplii homogenate were presented to five polyps per colony. The individual polyps were observed for normal feeding behavior (Lenhoff, 1968; Mariscal, 1971), the criteria for which were swallowing the papers within 10 min and retaining them for at least 5 min. After initial testing, all the colonies were placed in an aquarium with clean running seawater and retested twice daily for six days (Table 3). The corals previously exposed to 100 ppm did not exhibit normal feeding behavior even after almost a week of relief from the exposure. On the sixth day of testing a few polyps from one of the 100 ppm corals appeared to be trying to capture the papers but were unable to swallow them. One of the three 10 ppm corals tested also exhibited depressed feeding behavior.

TABLE 3. FEEDING BEHAVIOR OF M. ANNULARIS, AFTER SIX-WEEK EXPOSURE TO DRILL MUD, IN RESPONSE TO PIECES OF FILTER PAPER SOAKED IN BRINE SHRIMP HOMOGENATE. †

DAY/TEST No.	TREATMENT			
	CONTROL	1 PPM	10 PPM	100 PPM
0	+ + +		+ + -	- -
1/1	+ + +	+ +	+ + -	- -
1/2	+ + -	+ +	+ + -	- -
2/1	+ + +	+ +	+ + -	- -
2/2	+ ± -		± + -	- -
3/1	+ + +		+ + ±	- -
3/2	- - -		+ + -	- -
4/1	- - -		+ + -	- -
4/2	- - -		- - -	- -
5/1	+ ± -		+ - -	- -
5/2	+ + +		+ + +	- -
6/1	+ - +		+ ± -	+ -
6/2	+ ± -		+ + -	+ -

† Each colony was scored: (+) if papers were captured and swallowed, (±) if papers were captured but not swallowed and (-) if there was no response. Number of symbols represents the number of colonies tested. Corals were tested after six weeks of exposure (day 0), then allowed to recover in clean seawater (days 1 to 6).

TABLE 4. SUMMARY OF CORAL AND ALGAL BIOMASS OF *MONTASTREA ANNULARIS* EXPOSED TO JAY DRILLING MUDS FOR UP TO SEVEN WEEKS. †

TREATMENT	TISSUE-N µg-at. N per cm^2	ZOOX. DENSITY 10^6 cells per cm^2	Chla µg/cm^2	µg Chla per 10^6 zoox.	Chla/Chlc
Freshly Collected (n = 4)	58±10	-	-	-	-
Pre-Exposure (n = 4) (2 weeks on Stage 1)	66±12	3.3±1.6	13.8±3.4	5.0±2.3	1.2±0.1
Two Weeks Exposure (n = 3)					
Control, unfed	67±20	4.5±1.0	14.6±6.1	3.2±0.6	1.3±0.1
Control, fed	60± 3	2.3±0.5	11.0±4.0	5.1±2.9	1.6±0.7
1 ppm	60±35	3.5±0.7	11.7±4.7	3.3±0.8	1.3±0.1
10 ppm	71±24	4.5±0.8	12.2±1.3	2.8±0.7	1.5±0.2
100 ppm	78±21	5.0±1.5	16.0±7.6	3.1±0.8	1.3±0.2
Four Weeks Exposure (n = 3)					
Control, unfed	100±17	5.8±0.7	16.4±3.2	2.8±0.2	1.4±0.1
Control, fed	92± 4	6.6±0.6	17.5±3.0	2.7±0.6	1.3±0.1
1 ppm	72±28	4.8±1.5	15.9±4.6	3.4±0.6	1.4±0.1
10 ppm	95±20	5.5±0.8	19.0±3.3	3.5±0.2	1.4±0.2
100 ppm	73±10	3.9±0.3**	16.4±1.3	4.2±0.5	1.4±0.1
Seven Weeks Exposure (n = 8) (experimental corals)					
Control, unfed	61±16	4.6±0.7	11.6±2.4	2.5±0.3	1.0±0.1
Control, fed	67±20	5.3±1.5	17.2±5.6	3.3±0.7	1.3±0.1
1 ppm	66±20	4.8±1.2	14.0±2.6	3.1±0.8	1.2±0.1
10 ppm	67±11	4.8±1.1	15.2±3.1	3.2±0.6	1.3±0.1
100 ppm	56±14	3.9±0.2**	14.6±3.2	3.7±0.8	1.3±0.2

† Mean ± std. dev. of eight pieces of coral.
**Significantly different from control at $p < .05$

Coral and Algal Biomass

Table 4 summarizes the data on nitrogen and carbohydrate content of coral tissue, and on density and chlorophyll content of zooxanthellae.

Nitrogen content is an indicator of the amount of coral tissue protein, and thus a measure of coral biomass. Earlier studies have shown that coral tissue N and biomass vary with the nutritional state of the animal (Szmant-Froelich and Pilson, 1980). We expected a lower N content in tissues of unfed control corals and corals exposed to 100 ppm that exhibited reduced feeding behavior. Although the mean tissue N of these

two groups was slightly lower than that of the rest, the differences were not statistically significant. There was also no difference in the tissue carbohydrate content.

The zooxanthellae density, but not the chlorophyll content, of the 100 ppm corals was significantly lower than that of the other groups of coral (Table 4). It is not clear whether the 100 ppm corals expelled some of their original symbionts or whether the internal conditions of these corals were unfavorable for the continued growth and survival of the zooxanthellae. It is clear, however, that the zooxanthellae remaining in the 100 ppm corals had a higher chlorophyll concentration per algal cell, presumably an adaptation to the lower light level in the 100 ppm exposure tank.

PHYSIOLOGICAL RATES - PUERTO RICO

It was not clear from the Stage 1 experiments whether the detrimental effects on coral calcification, respiration, nutrient uptake, feeding behavior, and zooxanthellae content observed after the third week of exposure were due to the prolonged exposure to drilling mud, or to the use of more toxic drilling mud during the last three weeks of exposure (see Table 2). Drilling muds JX-5 and JX-7 had much higher chromium and hydrocarbon content than some of the earlier muds (Gilbert and Kakareka, unpublished). Thus we wanted to see whether detrimental effects could be induced in M. annularis by short exposures to the more toxic JX-7 mud. Two tests were conducted with M. annularis and a third test with Acropora cervicornis (shown to be extremely sensitive to Mobile Bay muds, E. Powell, personal communication). Results of the physiological measurements are summarized in Tables 5 to 7 and the biomass analyses in Table 8.

Test 1

Calcification was the most sensitive physiological function to drilling mud stress. Within 12 hours, corals exposed to 100 ppm drilling mud had depressed calcification rates relative to the controls. By the fifth day their calcification rate was only 22% of the control rate, and 26% of their own pre-exposure rate (Table 5). Corals exposed to 10 ppm drilling mud also exhibited a depressed calcification rate beginning the second day of exposure. Results of the respiration measurements were variable (Table 5), those of the control group being more variable than those of the exposed groups. All three groups had depressed respiration rates on day 5, possibly indicating a slowing of metabolism due to reduced nourishment under laboratory conditions. Although respiration rates of 10 ppm and 100 ppm corals were significantly lower than those of controls on day 5, they were not significantly lower than their pe-exposure rates. Tissue nitrogen results (Table 8) indicated no differences in coral biomass among the three groups that might account for the differences in respiration.

TABLE 5. PHYSIOLOGICAL RATES (MEAN ± STD. DEV.) OF MONTASTREA ANNULARIS EXPOSED TO JX-7 DRILLING MUD (TEST 1). †

PARAMETER		DAYS EXPOSURE			
TREATMENT	PRE-EXPOSURE	1	2	3	5
Calcification ($nmol\ CaCO_3.cm^{-2}.h^{-1}$)					
Control	752±326	635±148	811±294	461±496	930±453
10 PPM	644±272	556±200	430± 81*	546±237	538±261
100 PPM	762±139	410±240	308±143**	233± 72	200±140**
Respiration (R) ($nmol\ O_2.cm^{-2}.h^{-1}$)					
Control	1079±338	1302±530	1273±377	1326±396	975±241
10 PPM	852± 64	933±365	927±262	859±268*	666±144*
100 PPM	853±237	1055±319	1024±306	1155±556	759±114
Photosynthesis (P) ($nmol\ O_2.cm^{-2}.h^{-1}$)					
Control	1910±653	1986±833	2064±754	1814±826	1631±613
10 PPM	1508±234	1564±347	1474±230	1385±297	1359±321
100 PPM	1782±251	1536±456	1446±312	1480±472	1197±211
P/R					
Control	0.94±.47	0.75±.16	0.79±.14	0.67±.16	0.82±.23
10 PPM	0.89±.11	0.91±.26	0.82±.11	0.86±.27	1.03±.21
100 PPM	1.08±.19	0.73±.07	0.73±.12	0.70±.17	0.79±.06
NO_3^- Uptake ($nmol.cm^{-2}.h^{-1}$)					
Control	4.99±1.57	6.45±1.59	6.99±1.21	5.39±2.08	7.84±2.16
10 PPM	3.53±1.44	5.14±1.33	4.46±1.18	5.35±1.66	5.07±1.68*
100 PPM	4.35±1.33	4.94±2.74	5.56±2.32	4.24±1.75	4.73±1.53*
NH_4^+ Uptake ($nmol.cm^{-2}.h^{-1}$)					
Control			1.22±1.02		2.03± .92
10 PPM			1.17± .91		1.58± .82
100 PPM			0.81±1.84		1.55±1.12

† n=6 for each treatment
* Statistically different from control at $p < 0.05$
** Statistically different from control at $p < 0.01$

The photosynthesis rates of the control corals were also quite variable: their coefficient of variation was at least twice that of the exposed groups. Therefore, although a trend of decreasing photosynthesis with time exists for the 100 ppm corals, it is not statistically significant. Inspection of the zooxanthellae density and chlorophyll data (Table 8) showed no differences in these parameters among the three groups. Therefore differences in photosynthesis among the groups were due to differences in physiological rate, not differences in algal biomass.

The photosynthesis to respiration ratio (P/R, Table 5) is generally viewed as an index of autotrophic potential. P/R was generally less than one indicating that photosynthesis could not meet the demands of respiration. Although the P/R of the control and 100 ppm corals tended to decrease in time, that of the 10 ppm corals remained relatively constant.

Nitrate and ammonium uptake rates (Table 5) were measured at ambient concentrations (about 1μM NO_3^- and 0.2 μM NH_4^+). Little confidence can be placed on NH_4^+ uptake rates measured at this low initial concentration that approached the sensitivity of the analytical technique; therefore, only the NO_3^- data is discussed. The control and 10 ppm corals showed a definite trend of increasing NO_3^- uptake rate with time ($p < 0.01$ and $p < 0.05$, respectively) while the 100 ppm corals did not. Therefore, by day 5 the NO_3^- uptake rate of the 100 ppm corals was significantly lower than that of the controls ($p < 0.02$), but not significantly different from their own pre-exposure rate. It is possible that the increase in uptake rate of the control and 10 ppm corals was a result of the adaptation of the zooxanthellae to the reduced light levels of our experimental system, which were much lower than ambient light levels where the corals were collected. The light levels in the 100 ppm exposure tank were even lower due to turbidity from the suspended drilling mud; the zooxanthellae may not have been able to adjust to it.

Test 2

The purpose of Test 2 was to replicate the adverse effects of 100 ppm drilling mud observed in Test 1, then stop the stress, and observe the time course of recovery. All specimens were collected from a single large colony chosen from a slightly greater depth and thus adapted to lower light levels. Two treatments (100 ppm JX-7 and control) with nine replicates were used. Also, the physiological rates of interest were monitored for two days prior to initiation of exposure for a more extensive baseline. By exposure day 3, no difference could be observed between the exposed and control corals in any of the parameters measured (Table 6) and both groups showed a significant decrease in photosynthesis. Since this decrease indicated a possible uncontrolled external source of stress, we terminated the experiment. The biomass analyses (Table 8) showed the two groups of corals to be similar in tissue N and algal biomass. Their algal biomass was similar to that of corals used in Test 1 and in the Stage I test (Table 4), but their tissue N was about 20% lower than that of the Test 1 corals. However, the *M. annularis* from both Puerto Rican tests had 20-40% more coral tissue N than the *M. annularis* collected from the Florida Keys for the Stage I tests.

TABLE 6. PHYSIOLOGICAL RATES (MEAN ± STD. DEV.) EXPOSED MONTASTREA ANNULARIS TO 100 PPM JX-7 DRILLING MUD (TEST 2). †

PARAMETER	TREATMENT	DAYS PRE-EXPOSURE		DAYS EXPOSURE		
		2	1	1	2	3
Calcification ($nmol.cm^{-2}.h^{-1}$)						
	Control	597±209	721±140	728±217	478±112	576±255
	100 PPM	426±172	573±192	522±219	490±153	551±201
Respiration (R) ($nmol\ O_2.cm^{-2}.h^{-1}$)						
	Control	791±309	1091±200		1010±282	929±359
	100 PPM	713±372	1010±181		999±224	852±237
Gross Photosynthesis (P) ($nmol\ O_2.cm^{-2}.h^{-1}$)						
	Control	2037±436	2183±302		2053±301	1335±616
	100 PPM	1844±621	2057±306		2074±338	1239±611
P/R						
	Control	1.36±.27	1.01±.07		1.17±.74	0.72±.23
	100 PPM	1.49±.53	1.03±.11		1.07±.21	0.70±.22

† n = 9 for each treatment.

Test 3

As was true for M. annularis, daytime calcification rates of Acropora cervicornis were approximately twice as fast as nighttime rates (Table 7). The calcification process of A. cervicornis also appears to be the more sensitive to drilling mud. Both daytime and nighttime calcification rates of the 100 ppm corals decreased by 40% during the first day of exposure to drilling mud (Table 7). By the second day of exposure, calcification rates had decreased by approximately 60%. The only other physiological function to show a difference was nitrate uptake. Nitrate uptake rates of the control and 10 ppm corals were higher than their pre-exposure rates ($p<0.01$) but those of the 100 ppm corals were not significantly different from their pre-exposure rates. The biomass analyses (Table 8) show no differences in animal or algal biomass among the three groups.

TABLE 7. PHYSIOLOGICAL RATES (MEAN ± STD. DEV.) OF ACROPORA CERVICORNIS EXPOSED TO JX-7 drilling mud for 48 HOURS (TEST 3). †

PARAMETER	PRE-EXPOSURE		DAYS EXPOSURE			
			1		2	
TREATMENT	DAY	NIGHT	Day	Night	Day	Night
Calcification ($nmol.cm^{-2}.h^{-1}$)						
Control	575±143	363± 72	609± 74	382±60	576±181	336±49
10 PPM	597± 86	330±114	548±108	410±90	687±113	357±48
100 PPM	495± 48	303± 57	307± 84**	180±95*	227± 45**	118±55**
Respiration ($nmol\ O_2.cm^{-2}.h^{-1}$)						
Control		446± 63		504±34		497±37
10 PPM		480± 49		570±17		565±20
100 PPM		462± 56		546±62		478±72
Photosynthesis ($nmol\ O_2.cm^{-2}.h^{-1}$)						
Control	833±171		980±105		885± 98	
10 PPM	886±105		969± 94		841±319	
100 PPM	793± 53		891± 44		807± 13	
P/R						
Control	0.93±.09		0.97±.07		0.89±.06	
10 PPM	0.92±.07		0.85±.07		0.74±.27	
100 PPM	0.87±.07		0.82±.09		0.86±.13	
NO_3^- Uptake ($nmol.NO_3.cm^{-2}.h^{-1}$)						
Control	2.64±1.92	3.59±.63	2.59±.33	3.29±.53	5.38±.59	4.65±.60
10 PPM	2.50±1.91	3.38±.23	2.87±.07	3.49±.23	5.22±.65	4.50±.75
100 PPM	2.13± .99	3.36±.88	1.63±.53*	2.62±.59	3.19±.60**	2.97±.61**
NH_4^+ Uptake ($nmol.NH_4.cm^{-2}.h^{-1}$)						
Control				1.44±.15	1.55±.29	1.62±.14
10 PPM				1.52±.42	1.34±.12	1.55±.31
100 PPM				1.50±.34	1.52±.41	1.58±.15

† Controls, n = 4; 10 PPM, n = 3; 100 PPM, n = 4. Mean ± std. dev.
* Statistically different from control at $p < 0.05$
* Statistically different from control at $p < 0.01$
*

TABLE 8. CORAL AND ALGAL BIOMASS (MEAN ± STD. DEV. OF CORAL SPECIMENS EXPOSED TO JX-7 DRILLING MUD. †

TREATMENT	Tissue N µg-at-N per cm^2	Zooxanthellae Density 10^6 cells/cm^2	Chla µg/cm^2	µg Chla per 10^6cells	Chla to Chlc
Test 1 (6)					
Control	91±25	5.2±1.8	17.0±4.9	3.30±0.57	1.1±.2
10 PPM	103±14	5.2±1.2	17.4±2.7	3.49±1.00	1.0±.3
100 PPM	106±22	5.2±1.5	17.8±3.2(3)	3.41±1.28(3)	1.1±.2(5)
Test 2 (9)					
Control	80±11	4.9±0.5	16.0±3.2(8)	3.32±0.78(8)	1.7±.3
100 PPM	76±12(7)	4.8±0.9	18.4±2.3(7)	3.90±0.44	1.4±.1
Test 3					
Control(4)	24± 3	1.8±0.9	8.1±1.5	6.65±6.05	1.5±1.0
10 PPM(3)	25± 2	2.1±1.1	8.4±1.8	3.36±0.08	2.4±1.5
100 PPM(4)	23± 4	2.1±0.5	9.5±2.2	4.48±0.75	1.1±0.2

† ( ) = number of replicates
chl = chlorophyll

Test 1: Montastrea annularis exposed for 5 days.
Test 2: M. annularis exposed for 3 days.
Test 3: Acropora cervicornis exposed for 2 days.

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APPENDIX A

TABLE A-1. MEAN (± 1 s.d.) RESPIRATION AND GROSS PHOTOSYNTHESIS RATES (ΔO_2) OF *MONTASTREA ANNULARIS* EXPOSED TO DRILLING MUD FOR SIX WEEKS ON STAGE I.

TREATMENT	nmol O_2 cm^{-2} h^{-1} WEEK						
	0	1	2	3	4	5	6
RESPIRATION							
Control : Unfed	-	795 ±179	921 ±201	1240 ±164	1073 ±187	1074 ±187	1183 ±116
Control : Fed	-	858 ±124	780 ±119	903† ±139	1024 ±205	981 ±167	1002 ±269
1 PPM:	727 ±127	796 ±183	854 ±138	865 ±137	972 ±241	1106 ±252	1115 ±235
10 PPM:	806 ±138	790 ±129	908 ±169	902 ±166	908 ±130	967 ±158	979 ±190
100 PPM:	860 ±139	800 ±124	753 ±99	887 ±169	843* ±151	704** ±93	693** ±72
GROSS PHOTOSYNTHESIS							
Control : Unfed	-	1095 ±221	1292 ±327	1583 ±279	1486 ±308	1476 ±349	1562 ±399
Control : Fed	-	1127 ±193	1287 ±194	1206† ±233	1506 ±206	1365 ±245	1433 ±294
1 PPM:	1114 ±229	1032 ±229	1400 ±258	1236 ±269	1331 ±347	1498 ±353	1497 ±338
10 PPM:	1030 ±245	1062 ±290	1335 ±364	1303 ±341	1350 ±356	1414 ±423	1347 ±426
100 PPM:	1240 ±194	1256 ±232	1431 ±296	1481 ±207	1278 ±171	1066 ±70	1241 ±266

† Controls significantly different from each other ($p<0.05$)
* Significantly different from controls and 1 ppm corals at $p<0.05$.
** Significantly different from controls and 1 ppm corals at $p<0.001$.

TABLE A-2. MEAN (± 1 s.d.) RESPIRATION AND GROSS PHOTOSYNTHESIS RATES (ΔCO_2) OF MONTASTREA ANNULARIS EXPOSED TO DRILLING MUD FOR SIX WEEKS ON STAGE I.

TREATMENT	nmol CO_2 cm^{-2} h-1								
	Alkalinity and pH method					Gas Chromatography Method			
	WEEK					WEEK			
	2	3	4	5	6	3	4	5	6
RESPIRATION									
Control : Unfed	859 ±112	858 ±134	789 ±143	970 ±174	816 ±144	1003 ±135	801 ±369	925 ±200	931 ±162
Control : Fed	796 ±139	846 ±186	733 ±132	933 ±192	782 ±150	818 ±270	937 ±335	994 ±365	1021 ±298
1 PPM:	703 ±102	782 ±205	805 ±198	851 ±239	863 ±262	861 ±202	786 ±106	833 ±298	937 ±225
10 PPM:	755 ±104	745 ±129	755 ±195	726 ±183	775 ±132	820 ±92	717 ±76	608* ±153	782 ±199
100 PPM:	822 ±257	800 ±124	561* ±137	685* ±173	487** ±60	704** ±93	742 ±295	675* ±86	675* ±216
GROSS PHOTOSYNTHESIS									
Control : Unfed	1138 ±320	1319 ±224	1249 ±297	1595 ±413	1570 ±415	1588 ±317	1351 ±422	1672 ±325	1856 ±478
Control : Fed	1429 ±427	1419 ±291	1301 ±372	1534 ±314	1595 ±280	1710 ±304	1533 ±455	1807 ±356	1841 ±502
1 PPM:	1058 ±312	1196 ±303	1300 ±378	1499 ±270	1581 ±465	1205 ±493	1190 ±131	1537 ±383	1744 ±453
10 PPM:	1083 ±237	1306 ±363	1208 ±363	1414 ±504	1450 ±478	1307 ±426	1129 ±149	1558 ±353	1640 ±495
100 PPM:	1522 ±466	1176 ±321	1033 ±419	1153* ±286	1059** ±250	1285 ±536	1974 ±476	1185* ±388	1358** ±246

* Significantly different from controls at $p<0.01$
** Significantly different from controls at $p<0.001$.

TABLE A-3. MEAN (±1 s.d.) NH_4^+ UPTAKE, NO_3^- UPTAKE AND CALCIFICATION RATES OF MONTASTREA ANNULARIS EXPOSED TO DRILLING MUD FOR SIX WEEKS ON STAGE I. CU = CONTROL UNFED; CF = CONTROL FED; L=LIGHT; D=DARK.

nmol cm^{-2} h^{-1}

TREATMENT	WEEK 2		WEEK 3		WEEK 4		WEEK 5		WEEK 6	
	L	D	L	D	L	D	L	D	L	D
NH_4^+ Uptake										
CU:	13±6	13±6	33±13	45±13	42±16	38±10	39±10	38±13	48±15	41±12
CF:	7±2†	11±4	23±11†	32±14	32±13	45± 9	26±9†	30± 7	32± 7	37±16
1 PPM:	14±5	9±2	39± 9	34±13	44±25	34± 9	26±9	29± 9	34± 9	41±13
10 PPM:	9±2	10±3	35±14	26± 8	38±12	38±11	20±10*	25±10	31±11	32±10
100 PPM:	12±3	13±5	23± 7	36± 8	30±12*	32±15	21±8**	20± 7	26±10**	14±10
NO_3^- Uptake										
CU:	13±6	6±2	17±7	29±7	35±14	33± 9	28±11	29± 8	41±12	41±21
CF:	5±2†	6±2	26±9	27±9	37± 8	29± 5	30± 6	31±10	34± 6	33± 9
1 PPM:	7±3	6±2	25±7	23±9	26± 7	29± 6	30± 9	25±11	39±12	38±11
10 PPM:	8±2	7±3	26±5	25±8	37± 9	37±12	20± 5*	24± 8	31± 8	30± 7
100 PPM:	8±5	6±2	19±9	26±3	18± 8**	21± 3	18± 4**	20± 5	19± 6	13± 6
Calcification										
CU:	682±264	291±166	590±297	387±166	538±225	214±153	695±341	214±154	745±247	261±85
CF:	679±223	335±141	553±166	280±197	624±154	244±107	417±226	229±131	643±277	219±147
1 PPM:	546±255	326±157	555±162	182±106	542±266	158±173	720±299	204±124	739±209	179±139
10 PPM:	689±239	254±165	623±210	331±183	574±160	244±111	518±209	177±156	475±283	188±139
100 PPM:	638±388	211±260	552±129	315±113	**266±213	122± 88	**189± 80	45± 32	116± 91	33± 31

† Controls significantly different from each other ($p<0.05$)
* Significantly different from controls and 1 PPM at $p<0.01$
** Significantly different from controls and 1 PPM at $p<0.001$.

APPENDIX B

TABLE B-1. SUMMARY OF RESULTS OF T-TESTS PERFORMED TO TEST WHETHER DIFFERENCES BETWEEN TREATMENTS FOR EACH WEEK OF DRILLING MUD EXPOSURE DURING STAGE I EXPERIMENT WERE STATISTICALLY DIFFERENT. †

TEST WEEK	STATISTICAL TEST	PARAMETER						
		CALC	R-O_2	R-CO_2	P-O_2	P-CO_2	NH_4^+	NO_3^-
1	CU vs CF	-	.43	-	.76	-	-	
	C'S vs 10 PPM	-	.65	-	.87	-	-	
	C'S vs 100 PPM	-	.77	-	.09	-	-	
2	CU vs CF	.99	.12	.35	.97	.18	.007*	.007*
	C'S vs 10 PPM	.64	.52	.50	.95	.28	.028	.85
	C'S vs 100 PPM	.98	.06	.71	.39	.04±	.53	.73
3	CU vs CF	.76	.000*	.94	.02*	.46	.03*	.36
	C'S vs 10 PPM	.64	.12	.14	.76	.97	.25	.74
	C'S vs 100 PPM	.82	.07	.000	.14	.31	.13	.29
4	CU vs CF	.39	,59	.43	.84	.76	.78	.87
	C'S vs 10 PPM	.94	.08	.80	.54	.68	.77	.07±
	C'S vs 100 PPM	.005	.02	.003	.07	.16	.05	.000
5	CU vs CF	.241	.31	.70	.48	.74	.009*	.46
	C'S vs 10 PPM	.233	.23	.027	.84	.52	.004	.002
	C'S vs 100 PPM	.000	.000	.007	.000	.007	.000	.000
6	CU vs CF	.44	.13	.57	.48	.89	.19	.11
	C'S vs 10 PPM	.08	.16	.50	.39	.50	.06	.008
	C'S vs 100 PPM	.000	.000	.000	.06	.001	.000	.000

† Numbers are the probabilities (P) that the means are the same (P=1.00 when two means are identical; P<0.05 for differences to be significant).

Abbreviations: CU = control unfed; CF = control fed; C's = mean of CU, CF and 1 PPM; * = CU higher rates than CF; ± = exposed higher rates than C's; CALC = calcification in the light; R-O_2 = oxygen respiration; R-CO_2=CO_2 respiration; P-O_2 = oxygen photosynthesis; P-CO_2 = CO_2 photosynthesis; NH_4^+ = ammonium uptake; NO_3^- = nitrate uptake.