United States
Environmental Protection
Agency

Environmental Research
Laboratory
Gulf Breeze FL 32561

Research and Development

EPA-600/S3-83-013 July 1983

EPA

Project Summary

Physiological Effects of Drilling Muds on Reef Corals

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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 to five days and for seven weeks. The drilling muds (designated JX-2 through JX-7) were collected from a Jay (Florida) oil field, and those fluids were not intended to be disposed of on the Outer Continental Shelf. Physiological functions of the coral (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 lengthy exposure and at the end of each shorter exposure. Significant reductions in calcification, respiration and NO_3- uptake rates of *M. annularis* were observed during the fourth week of exposure to 100 ppm drilling mud. Photosynthesis and NH_4+uptake rates also decreased during the fifth week of exposure. Normal feeding was absent from these corals when they were 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 day) exposure of *M. annularis* to 100 ppm JX-7 mud (the drilling mud used during weeks 5 and 6, which had a much higher chromium and hydrocarbon content than muds used during weeks 1 to 3) caused great reductions in calcification and lesser reductions in respiration, gross photosynthesis, and NO_3- uptake rates in one of two experiments. *Acropora cervicornis* showed a great 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 future studies are recommended.

This Project Summary was developed by EPA's Environmental Research Laboratory, Gulf Breeze, FL, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Drilling muds are necessary to oil-drilling operations in that they lubricate the drill string, remove cuttings, maintain hydrostatic pressure, prevent pipe corrosion, and seal the bore hole in porous formations. They 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 are considered toxic and hazardous to organisms.

Disposal of used drilling muds recently has become an environmental concern. Since used drilling muds are generally dumped into waters immediately adjacent to the drilling rig, it is important to identify local marine communities or organisms that might be adversely affected by exposure to them.

Drilling on the outer continental shelf of the Gulf of Mexico is conducted near the East and West Texas Flower Gardens--two unique, submerged coral reefs. 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.

The purpose of this study was to examine several physiological and biochemical processes in coral 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 algae that live symbiotically within most reef coral tissue.) Animal and algal biomass were also measured as a function of time to monitor for any deterioration in nutrition during the exposure period. *Montastrea annularis* was chosen as the primary test species because of its ecological importance in the Texas Flower Gardens and throughout the Caribbean. A second species, *Acropora cervicornis,* was used in later tests to compare the experimental procedures and results of this project with those of other EPA-funded studies of this species.

During the first laboratory experiment, groups of corals were exposed to four drilling mud concentrations (0 ppm, 1 ppm, 10 ppm, and 100 ppm) for seven weeks. The mud-exposed corals were fed during the experiments. Two control groups were used. One control group was fed periodically throughout the experiment; the second was not fed so as to simulate the starvation effects expected in the exposed groups. Previously listed physiological parameters were measured biweekly. Respiration and photosynthesis were measured as changes both in O_2 and 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 during exposure. A second set of experiments measuring the same physiological parameters focused on the short-term (2 to 5 day) effects of one of the more toxic muds used in the first experimental series. The first experiment was conducted during July and August, 1980, on the U.S. Navy Stage I platform, located 12 miles offshore from Panama City, Florida. 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.

Experimental Procedures

First Experimental Series: Stage I -- Coral specimens were obtained from the Florida Keys. They were immediately taken to Stage I by boat, where they were transferred to large aquaria of running sea water. The corals appeared to be in good condition, and most were fully expanded within a few hours after transfer to tanks. Forty pieces of coral were selected and randomly assigned to the five treatments: control unfed, control fed, 1 ppm drilling mud, 10 ppm drilling mud, and 100 ppm drilling mud. Exposed and control-fed corals were fed periodically with freshly collected zooplankton or with brine shrimp nauplii.

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 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. During subsequent weeks, O_2 incubations were shortened to one hour and the nutrient uptake, calcification, and TCO_2 were measured during a separate 3-hour incubation 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, tissue-N, carbohydrate, zooxanthellae density, and zooxanthellae chlorophyll content were measured. 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 drilling muds.

The system for delivering the mud consisted of two funnels to hold diluted mud stock and two multichannel peristaltic pumps to deliver the mud at a constant rate to the inflowing seawater lines of the treatment aquaria. Since drilling mud composition varies with depth of drilling, mud batches used to expose the corals were changed to approximate the sequence and timing of collection of these muds so as to simulate as much as possible the long-term exposure that would have resulted from discharge of these muds. Table 1 summarizes the collection dates of the muds and their use in our experiments.
Second Experimental Series: Puerto Rico-- The mud tested was JX-7 collected the previous summer from the Jay oil field and preserved by refrigeration.

Specimens of *M. annularis* were collected from 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 they were used in the experiments. In Test 1, corals were exposed to 0, 10, and 100 ppm drilling mud (six replicate corals each) for five days; in Test 2, nine replicate corals were exposed to 0 and 100 ppm drilling mud for three days. From the day before mud exposure was to begin (two 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.

Specimens of *A. cervicornis* for Test 3 were collected from the lagoon (2 to 3 m depth) of San Cristobal reef the day before the experiment began. Branch tips were exposed (four replicates each) to 0 ppm, 10 ppm, and 100 ppm drilling mud for 48 hours. The corals were incubated as above beginning one day before exposure. Changes in concentration of O_2, TA, NO_3-, and NH_4+ were measured during both day and night incubations.

Table 1. *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*

**These muds were collected from terrestrial wells near Jay, Florida, and were not intended for disposal on the Outer Continental Shelf.*

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

Data Analysis

The changes in concentration of the incubation media were corrected for water volume, incubation duration, 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 were calculated as: $\Delta CaCO_3 = \frac{1}{2}[\Delta TA - \Delta NH_4 + \Delta(NO_3 + NO_2)]$ to correct for any changes in TA caused by the uptake of the added nutrients. Total-CO_2 (TCO_2) was calculated from the pH and alkalinity data. The change in CO_2 due to respiration and photosynthesis [$\Delta CO_{2\,P/R}$] was calculated from the equation:

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

One-way analysis of variance (ANOVA) was used to analyze the Stage I data. Trends over time were tested 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; thus, the 10 ppm and the 100 ppm coral rates were tested against the mean of the two control and 1 ppm groups.

Results

Coral Survival -- Coral deaths occurred only in the 100 ppm treatment group on Stage I. 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. 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 the Puerto Rican experiments.

Physiological Rates: Stage I -- Drilling mud had the greatest measured detrimental effect on calcification in coral physiology. For the Stage I experiment, no statistically significant differences occurred between treatments until the fourth week, when calcification rates of the 100-ppm-exposed corals dropped to 16% of rates of control and 1 ppm exposed coral (Figure 1). During the sixth week, the coral exposed to 10 ppm calcified at 67% of the rate of the controls, but the difference was not significant ($p = 0.084$).

Respiration rates (measured both as decreases in O_2 concentration and as increases in CO_2 concentration) of all except the corals exposed to 100 ppm mud increased gradually with time (Figure 2). 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 exposure to mud and by the sixth week, their respiration rate was reduced to 60% of that of the controls ($p < .001$).

Photosynthetic rates also increased with time for all treatments except the 100 ppm treatment (Figure 3). 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 ($p = .05$). 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 rates by zooxanthellae are known to follow Michaelis-Menton kinetics and therefore depend on the initial nutrient concentration of the incubation media. Nitrogen uptake rates were lowest for all treatments during week 2 (Figure 4) because of the lower initial nutrient concentrations, but 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 appeared during the fourth week of exposure, and between the 10 ppm corals and the controls, during the fifth week. In both cases, NO_3- uptake was 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

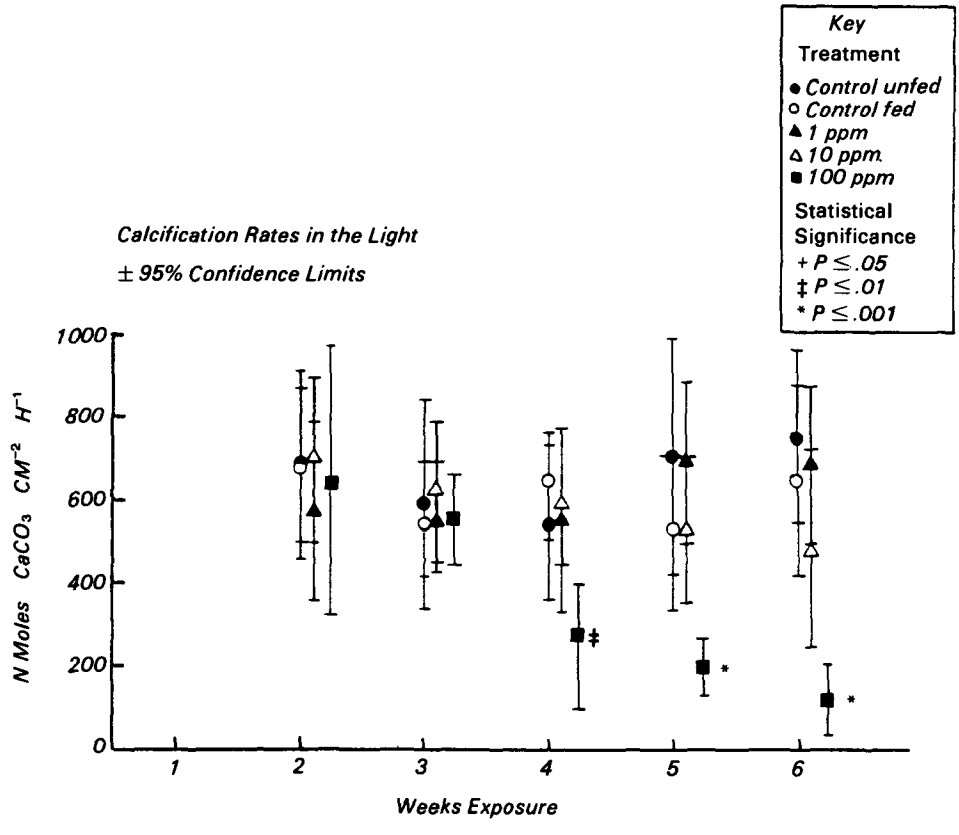

Figure 1. *Daytime calcification rates of* M. annularis *measured as changes in total alkalinity. n = 8.*

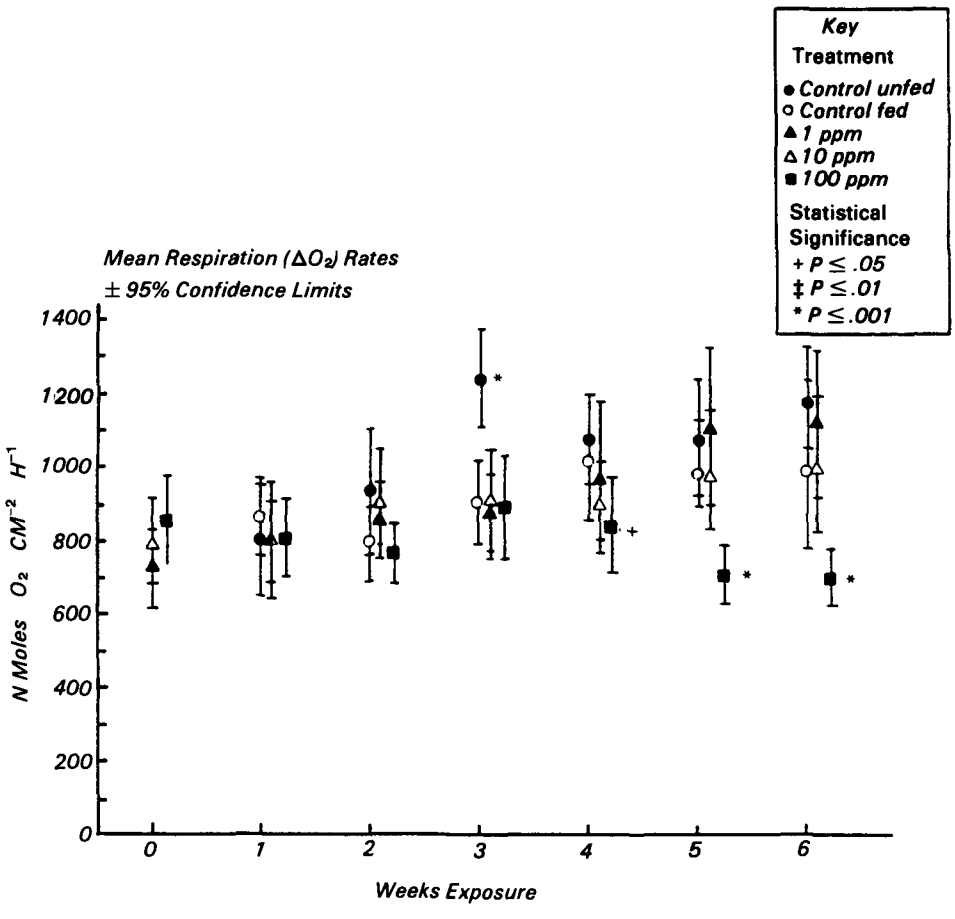

Figure 2. *Respiration of* M. annularis *measured as changes in O_2 concentration. n = 8.*

to 51% of the control rate. Since zooxanthellae densities decreased by only 20%, there must have been a decrease in the capacity of the 100 ppm zooxanthellae to take up nutrients.

Feeding Behavior -- 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 to be the healthiest of those surviving that treatment. Small pieces of filter paper soaked with brine shrimp nauplii homogenate were presented to five polyps per colony. The individual polyps were observed for normal feeding behavior, the criteria for which were swallowing the papers within ten minutes and retaining them for at least five minutes. After initial testing, all the colonies were placed in an aquarium with clean running seawater and retested twice daily for six days. 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.

Coral and Algal Biomass -- 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 biomass varies with the nutritional state of the animal. 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. 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 I 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 1). Drilling muds JX-5 and JX-7 had much higher chromium and hydrocarbon content than some of the earlier muds. Thus we wanted to see whether detrimental effects on corals could be induced by short exposures to the more toxic JX-7 mud. Two tests were conducted with *M. annularis* and a third test with *A. cervicornis.*

The first test with *M. annularis* (five day exposure) again showed calcification to be 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. Corals exposed to 10 ppm drilling mud also exhibited a depressed calcification rate beginning the second day of exposure.

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 own pre-exposure rates.

A trend of decreasing photosynthesis with time was observed for the 100 ppm corals, but it was not statistically significant. Inspection of the zooxanthellae density and chlorophyll data showed no differences in these parameters among the three groups that might account for the differences in photosynthesis.

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

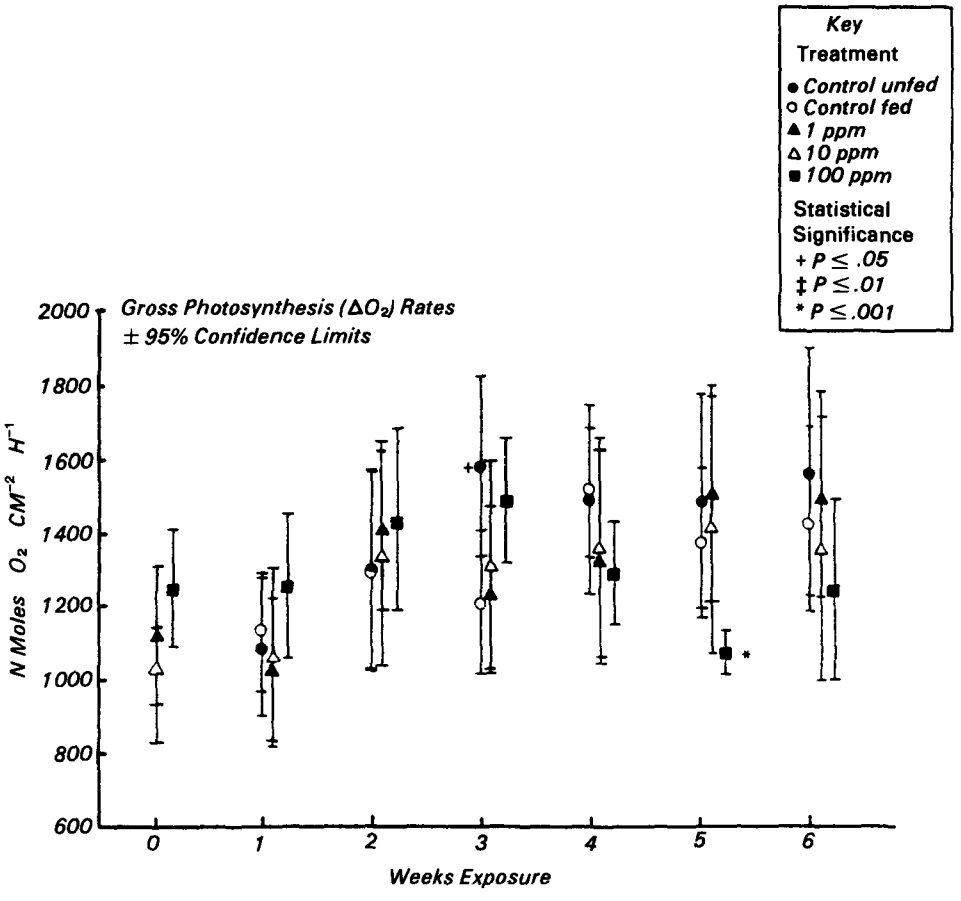

Figure 3. *Photosynthesis by* M. annularis *measured as changes in* O_2. *n = 8.*

controls ($p<0.02$), but not significantly different from their own pre-exposure rate.

The purpose of the second test with *M. annularis* 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. However, by exposure-day 3, no difference could be observed between the exposed and control corals in any of the parameters measured and both groups showed a significant decrease in photosynthesis. Since this decrease indicated a possible uncontrolled external source of stress, we terminated the experiment.

As was true for *M. annularis,* the calcification process of *A. cervicornis* was 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. 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 showed no differences in animal or algal biomass among the three groups.

Conclusions

Based on the results of the first experiment we can conclude that:

- The reef coral *Montastrea annularis* can be adversely affected by long-term (more than three weeks) exposure to 100 ppm drilling mud.
- Adverse effects ranged from an 84% reduction in calcification rate and 40% reduction in coral respiration rate to lesser effects on photosynthesis by the zooxanthellae of these same corals.
- Corals exposed to 100 ppm drilling mud for six weeks lost normal feeding response and 20% of their zooxanthellae, and several of them died during the fifth and sixth weeks.

Since different batches of drilling mud were used during the 6-week experiment, it was not clear whether the absence of any discernible physiological effect during the first three weeks was due to a cumulative time effect or to a greater toxicity of the batches of drilling mud used after the third week. The second set of experiments, in which we exposed specimens of *M. annularis* and *A. 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; specimens of *M. annularis* exposed to 100 ppm of JX-7 during one test suffered a 20% decrease in calcification within 24 hours of exposure and a 40% decrease by the fifth day of exposure, while those used in a second test showed no adverse effects after three days of exposure.
- Some coral species are more sensitive to drilling mud than others: *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 (or greater) may cause a large decrease in calcification rate in some colonies of these coral species.
- Longer exposures, especially when more toxic drilling mud additives are used, increase the chance that sublethal and lethal effects will occur.

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.

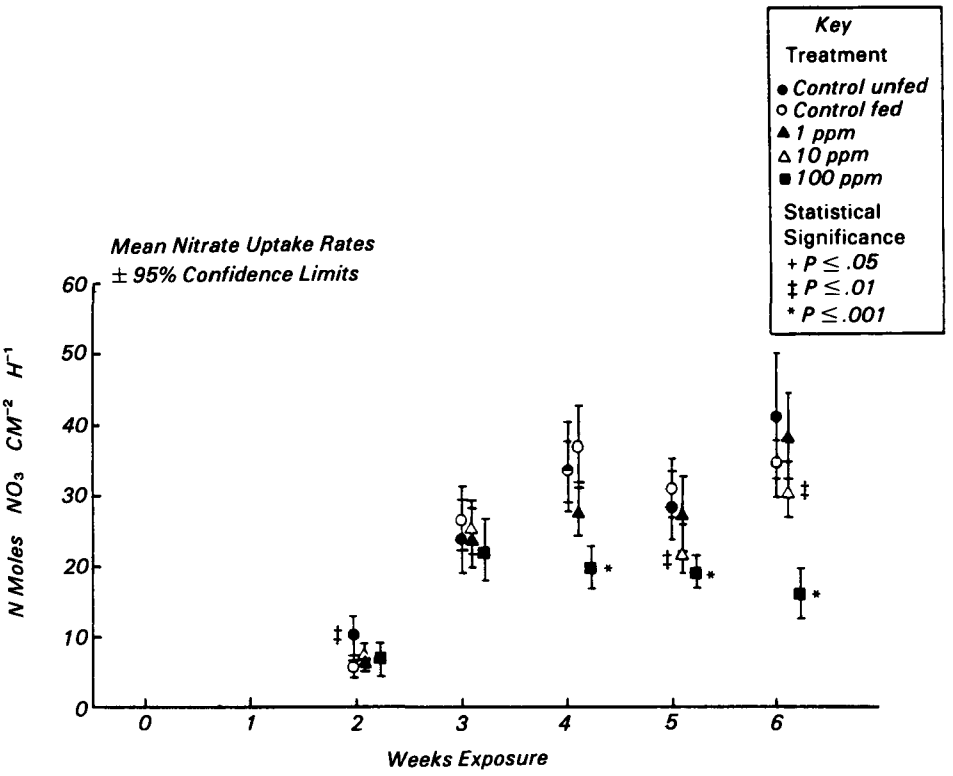

Figure 4. *Nitrate uptake by* M. annularis *during both light and dark incubations. n = 16.*

Recommendations

Initial studies were undertaken and designed with little information on expected exposure concentrations and duration. It appears that real exposures will consist of frequent (several per week) doses of drilling mud of varying concentrations over prolonged periods (three months to a year). Recent field studies indicate that only corals situated within about 100 meters of a rig should encounter concentrations higher than 100 ppm drilling mud 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.

A final recommendation is that future studies should be concerned with dispersal characteristics of different fractions of the mud. Whole muds were used in the present experiments for lack of better information on which fractions were more likely to affect reef areas. However, heavy particulates will settle quickly over a small downstream area, where corals may both be buried and poisoned, while light particulates and dissolved fractions will disperse over larger areas, 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.

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***Thomas W. Duke** is the EPA Project Officer (see below).*
The complete report, entitled "Physiological Effects of Drilling Muds on Reef Corals," (Order No. PB 83-181 560; Cost: $8.50, subject to change) will be available only from:

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