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THE EFFECT OF DISPERSED OIL ON THE CALCIFICATION RATE OF THE REEF-BUILDING CORAL *DIPLORIA STRIGOSA*

CONSEQUENCES DE LA POLLUTION PAR LES HYDROCARBURES SUR LES TAUX DE CALCIFICATION DU CORAIL HERMATYPIQUE *DIPLORIA STRIGOSA*

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ABSTRACT

Hermatypic corals represent environmentally and economically important components of the reef ecosystem. Oil spills and clean-up operations in reef areas are potential sources of pollution impact. This paper presents an evaluation of the calcification rate of specimens of the reef-building coral Diploria strigosa in response to 24 hour treatments of chemically dispersed oil at concentrations of 20 ppm. The concentrations and durations were chosen to represent a scenario of a short-term oil spill treated with dispersant passing over a coral reef.

Calcification rates were determined by the buoyant weight technique at several day intervals for up to 29 days following treatment. Results from laboratory experiments (Winter and Summer) conducted in a flow-through seawater system indicate that treated corals, both in comparison to untreated controls as well as to their pretreatment rates, experienced no depression in calcification.

In contrast, a possible short-term enhancement of calcification for the treated corals was observed.

RESUME

Les coraux hermatypiques réprésentent pour l'environnement et l'économie, une composante importante de l'écosystème récifal. Le déversement d'hydrocarbures et les opérations de vidange des cuves des bateaux dans les zones récifales constituent des sources importantes de pollution. Cette étude présente une estimation de taux de calcification de spécimens d'une espèce de corail hermatypique Diploria strigosa, après une exposition de 24 heures à des hydrocarbures à une concentration de 20 ppm. Les concentrations et durées d'exposition ont été choisies de façon à simuler le scénario d'un déversement d'hydrocarbures sur une courte période, ces hydrocarbures ayant été traités avec un dispersant et s'étendant sur le récif corallien.

Les taux de calcification ont été déterminés par la technique du "poids flottant" à intervalles de plusieurs jours jusqu'à 29 jours après le traitement. Les résultats des expériences de laboratoire (hiver et été), réalisées dans un système à eau de mer courante, indiquent que les coraux traités n'ont pas de ralentissement dans la calcification, par rapport à des coraux non traités, ou par rapport aux taux obtenus avant le traitement.

Au contraire, on a observé une augmentation probable à court terme de la calcification pour les coraux traités.

INTRODUCTION

Coral reefs are subject to petroleum hydrocarbon pollution from a variety of chronic and short-term sources (e.g., refinery operations, oil spills). It is important to evaluate the consequences of such pollution for management and conservation purposes. Hermatypic corals are organisms of interest because of their key roles in the coral reef ecosystem, their contribution to the structure of the reef, and their habitat forming features for other important organisms. Loya and Rinkevich (1980) and Peters et al. (1981) have noted mortality or sub-lethal damage to corals from chronic oil pollution, even at low concentrations. While immediate mortality seems unlikely after short-term pollution events, sub-lethal effects may occur at substantial time periods following the episode (see review by Loya and Rinkevich, 1980).

Chemical dispersants used to treat and break up oil slicks are a possible method of preventing floating oil from impacting environmentally sensitive intertidal and shallow subtidal zones where oil may adhere and pose a chronic and detrimental environmental perturbation. Past work on effects of dispersed oil on reef corals has suggested that increased toxicity may result (Lewis, 1971; Elgerschuizen and de Kruijf, 1976; Eisler, 1975). Such studies may not be applicable to field conditions since workers have used extremely high hydrocarbon concentrations and static water conditions in the experimental protocol (Ray, 1981; Knap et al., 1983; Wyers et al., manuscript submitted; Dodge et al., 1984b). In addition, new generation chemical dispersants are intended to be less toxic in field applications (Wardley-Smith, 1983).

This paper examines the calcification rate of the reef coral Diploria strigosa (Dana) from Bermuda in relation to exposure to chemically dispersed oil at a concentration of 20 ppm for 24 hours in a flow-through sea water system. The concentration tested is assumed to be a near maximum possible under actual field conditions when an oil spill is chemically dispersed (McAuliffe et al., 1981). The skeletal calcification rate of corals has been monitored before, during, and for up to 30 days after dosing to assess effects. Calcification is the product of a variety of physiological processes and changes may be indicative of sub-lethal toxicity. The buoyant weight method has been used for calcification determination in order to perform sequential non-destructive growth measurements on the same corals over a relatively long period. Because of varying seasonal responses by other invertebrates (Anderson et al., 1981), experiments have been conducted both under winter and summer conditions.

METHODS AND MATERIALS

Coral colonies (approx. 12 cm diameter, hemispherical shape, and dry weight range of 350 to 1,000g) of the hermatypic species Diploria strigosa (Dana) were collected for each experiment from 5 m depth on a reef near North Rock, Bermuda. D. strigosa was chosen because it is the dominant coral species on Bermuda reefs (Dodge et al., 1982). Specimens were transported to the Bermuda Biological Station and maintained in aquaria within a filtered flow-through sea-water system (Knap et al., 1983). As much as possible of the associated fauna was removed from the bases of the 36 corals used in each experiment.

Flow rates were adjusted to 3 l/min for each aquarium. Experimental tanks were under overhead cover and received reflected ambient light from the north side which produced a slight light gradient in the laboratory system. Lighting was supplemented by a double bank of fluorescent lights on a dark/light cycle corresponding to the natural regime (Knap et al., 1983; Dodge et al., 1984a).

For each experiment aquaria were divided into two parallel rows, each north-south pair representing a treatment. Prior to treatment with dispersed oil, each group of corals was stained with alizarin red S (Lamberts, 1981) at a concentration of 10 mg/l for a total of 24 hours. This procedure was used to place a red mark in the skeletons as a reference for later measurements (Dodge et al., 1984b). After staining, corals were allowed to recover for 8 to 17 days to avoid possible detrimental effects of the staining procedure (Dodge et al., 1984a).

Treatments consisted of Arabian Light crude oil in combination with the dispersant Corexit 9527 (10:1). Arabian Light is assumed to be a moderately toxic crude oil. Combinations of the dispersant and oil were made according to the manufacturer's specifications and were initially physically mixed in separate chambers followed by introduction at appropriate flow rates into treatment aquaria (Knap et al., 1983). A flow-through system was employed in preference to a static system which may exaggerate the effects of pollution (Cohen et al., 1977; Eisler, 1975).

Table 1: Descriptive information on each experiment.

Season:	Winter	Summer
Experiment Designation:	Exp. C	Exp D
Treatments:	Control Disp. Oil	Control Disp. Oil
Concentration/ Duration of dose:	20ppm/24h	19ppm/24h
# corals/Treatment:	18	18
# corals/Tank:	9	9
# Tanks/Treatment:	2	2
Collection Date:	1/26/82	6/16/82
Stain Date:	2/6-11/82	7/1-2/82
Initial Weighings:	2/27/82	7/10/82
Dose Date:	3/3-4/82	7/14-15/82
Final Weighings:	4/2/82	7/30/82
# Pre-treatments: weighing periods:	1	1
# Post-treatment: weighing periods:	5	4
Water Temperature:	16.5-20°C	27-28°C

To maintain a relatively constant dose, the water accomodated fraction (WAF) of oil (or oil content of water) was measured every 30 minutes in each experimental tank by use of hexane extracts measured on a Perkin-Elmer 650-20s fluoresence spectrometer (excitation 310nm/ slit

10nm, emission 360nm/slit 2.5nm). Concentrations during each experiment were adjusted regularly and maintained as close to 20 ppm as possible. The treatment concentrations were taken as the average of hourly weighted means over the duration of the exposure (Sleeter et al., in preparation).

Table 1 indicates collection, staining, and weighing dates for each experiment. A Sartorious balance (1204 MP, 2 kg range, 0.01 g readability, 0.005 g SD), with a weigh below hook, was used for weighings. Further details are presented in Dodge et al. (1984a). Seawater density for buoyant weight calculations was determined by NOAA calibrated hydrometer in winter experiment C and in summer experiment D by measurement of water temperature and salinity and subsequent reference to standard oceanographic tables (Bailek, 1966).

On each weighing day, weighings of the experimental series covered a 1-3 hour period. On subsequent days, the sequence was the same and weighings were conducted at approximately the same time period. During weighings, the flow-through seawater system for a particular aquarium was shut down for 20-40 minutes to reduce errors associated with water motion.

RESULTS

tank corals (slightly light enhanced) of each Treatment showed elevated calcification values and for the combined Tanks (Fig 1a) where corals exposed to the dispersed oil showed elevated calcification compared to those of the Control treatment.

For summer experiment D, ANOVA indicated a significant Treatment effect ($p<.0005$), a slightly significant Period effect ($p<.05$), and Treatment by Tank interaction ($p<.05$). Fig. 2a,b illustrates the data. Corals of the dispersed oil treatment were consistently elevated relative to those of the Control treatment. The time trend of both data sets was similar with the exception that the second period after dosing for the Treatment corals showed elevated calcification, especially in the north tank.

DISCUSSION

It seems clear that for both summer and winter experiments, there is no suppression of coral calcificaion rates for a period of up to 30 days following dosing with dispersed oil of approximately 20 ppm for 24 hours. This confirms results determined on the same corals for extension rate measured one year after dosing (Dodge et al., 1984b). It is of interest to note

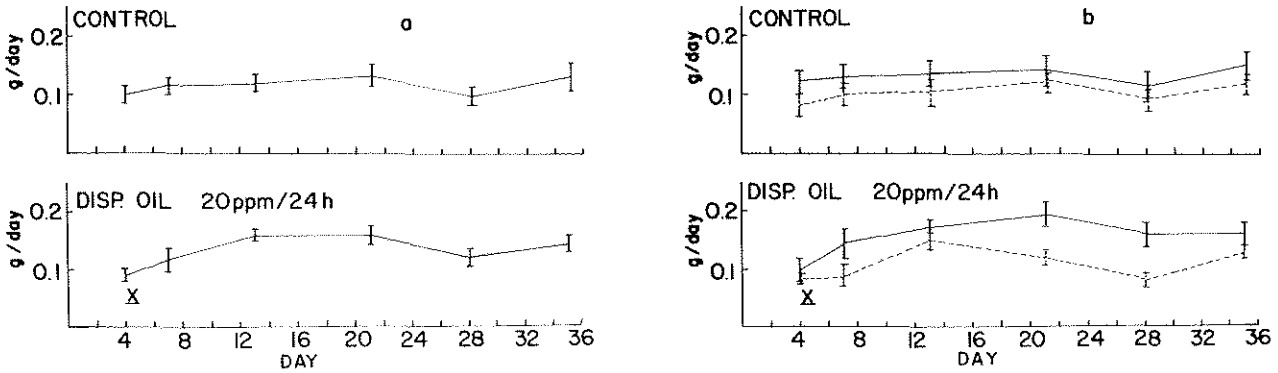

Figure 1: Winter experiment C. Average calcification rates (g/day) for Control and Dispersed Oil treated corals plotted on the last day of weighing interval. X indicates the time interval of the 24 hour dose. Error bars are ± 1 standard error. The (a) portion of the figure contains data averaged over both tanks in each treatment. The (b) portion of the figure presents data averaged over each tank within treatments. Solid lines represent north (slightly light enhanced) tanks. Dashed lines represent south tanks.

Figure 1 and Figure 2 show for each experiment and each treatment the average coral daily weight gain in g/day for each period. Table 2 presents these mean calcification rates and associated coefficients of variation.

A fixed model three-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981) comparing the factors of Treatments, Tanks, and Periods was performed on the post-treatment data of each experiment to evaluate and assess significant differences.

For winter experiment C there was a highly significant Tank ($p<.0005$) effect and a slightly significant Treatment effect ($p<.05$). There were no significant Period or interaction effects. This is illustrated in Fig. 1b where the north

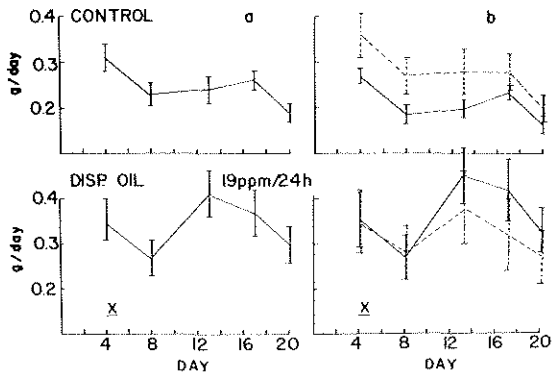

Figure 2: Summer experiment D. Description same as caption for Fig. 1.

that calcification rates of all corals in winter experiment C were significantly lower than in summer experiment D (Fig. 1, 2, Table 2). This is probably because of the well-known suppression of growth by low water temperature.

In each experiment significantly elevated calcification rates are characteristic of dosed corals in comparison to Controls (confirmed by ANOVA which assessed the importance and interaction of the three factors: Treatments, Tanks, and Periods). More clearcut results might also have included a significant three way interaction or significance in the Treatment by Period interaction, e.g., corals showing an increased calcification directly after dosing which subsequently declined to (or below) Control levels. The lack of interaction effects in these results require further experimentation to confirm whether observed enhanced calcification levels in dosed corals are the result of the treatment or, rather, an experimental artifact.

It does not seem likely that the buoyant weight technique (Jokiel et al., 1978) itself was the cause of the observed calcification enhancements. We have successfully used this method before to assess growth changes to alizarin red S (Dodge et al., 1984a).

Other investigators (Neff and Anderson, 1981) have reported apparently elevated calcification rates in some corals following exposure to petroleum hydrocarbons. Species which showed the most pronounced behavioral stresses also showed the most 45-Ca enhancement. Corals of the experiments described in this paper did exhibit pronounced, but short-term, stress symptoms following dosing (Wyers et al., manuscript submitted). Neff and Anderson (1981) postulated that 45-Ca enhancements they observed may have been the result of influence by petroleum hydrocarbons to increase the relative permeability of living coral polyp tissues to Ca++ and thereby to increase passive exchange of 45-Ca between the medium and the skeleton. This mechanism does not satisfactorily explain enhanced weight gain unless increased permeability of Ca also promotes increased $CaCO_3$ formation.

Cook and Knap (1983) found Diploria strigosa corals to exhibit reduced photosynthesis by zooxanthellae after an eight hour exposure to dispersed oil (19 ppm). Recovery was, however, rapid. If the results of the present experiments are taken into account, photosynthesis reduction is not apparently sufficient to reduce calcification rates.

Rinkevich and Loya (1983) found enhanced carbon fixation by zooxanthellae of Stylophora pistillata at low hydrocarbon concentrations as opposed to inhibition at higher concentrations. Stebbing (1976) and Mitchel and Fitt (1984) refer to this as hormesis ("any stimulatory effect of low-level exposure to pollutants"). Given the results of the experiments reported here and those of Neff and Anderson (1981), it seems possible hormesis operates for calcification of certain coral species in the presence of dispersed oil, although more experimentation will be necessary for confirmation.

Table 2: Average daily calcification rate (g/day) for n=18 corals and Coefficient of Variation (C.V.) (%) for each period of each treatment.

Experiment C - Winter						
Weighing Period						
# of Days:	4	3	6	7	7	7
Control Treatment						
Mean (g/day)	.10	.11	.12	.13	.10	.14
C.V. (%)	61	52	56	47	64	46
Dosed Treatment	Pre-	Post-Treatment				
Mean (g/day)	.09	.12	.16	.16	.12	.14
C.V. (%)	55	61	23	37	55	37
Experiment D - Summer						
Weighing Period						
# of Days:	4	4	5	4	3	
Control Treatment						
Mean (g/day)	.31	.23	.24	.26	.19	
C.V. (%)	40	48	47	35	47	
Dosed Treatment	Pre-	Post-Treatment				
Mean (g/day)	.35	.27	.41	.37	.30	
C.V. (%)	52	59	50	59	55	

In an attempt to reduce intercolony variability and to increase resolution of statistical testing, the daily calcification rate of each coral for each post-treatment period was divided by its pre-treatment value. This normalization procedure for each experiment did not cause appreciable reduction in the coefficients of variation of post-treatment period means. For this reason and because other normalization procedures (e.g., mass, minimum radius (Maragos, 1978), or surface area) did not perform well in other experiments with buoyant weights (Dodge et al., 1984a), only raw data were used in statistical testing. Variability among coral replicates is common in coral experimental work (e.g., Barnes, 1981; Barnes and Crossland, 1982). In other experiments we have successfully reduced variability by normalization of experimental growth rates to pre-treatment values (Dodge et al., 1984a) when more than one pre-treatment period was available. For the experiments reported here, normalization might have been more useful had more pre-treatment weighing periods been incorporated into the experimental design.

This experiment only provides results for dispersed oil effects on a single coral species at a single concentration (20 ppm) and for a short duration (24 hr). The experimental conditions were intended to represent a major short term oil spill (chemically dispersed) passing over a coral reef. Future work should consider other possibilities, scenarios, and species.

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