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Bioaccumulation and Histopathological Effects of Oil on a Stony Coral

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Colonies of the shallow-water Caribbean coral *Manicina areolata* incorporated petroleum hydrocarbons into their tissues during exposure to water accommodated fractions of No. 2 fuel oil for three months. This contamination was not removed after depuration periods of up to two weeks. Although these corals remained alive, evidence of pathological responses was found which included impaired development of reproductive tissues, degeneration and loss of symbiotic zooxanthellae, and atrophy of mucous secretory cells and muscle bundles.

Despite growing concern about the effects of crude and refined oils on the marine environment, most studies have centred on short-term exposures of various organisms to determine lethal concentrations of oils or their water soluble or accommodated fractions. This is particularly true in studies on corals, which are important in the geology and ecology of tropical and subtropical seas around the world, and which are increasingly exposed to the hazards of oil pollution (Johannes, 1975). Loya & Rinkevich (1980) reviewed past laboratory and field research demonstrating that oil floating over corals may not cause visible damage, but whole oil or oil-derived water accommodated hydrocarbons in direct contact with coral tissues due to coating or ingestion can impair the colonies' growth rates, damage reproductive systems, reduce the viability of the coral larvae, and cause death. However, there have not been any studies utilizing histopathological techniques in conjunction with chemical analyses to examine the effects of such pollutants on the coral tissue. Histopathology is an increasingly important tool for investigating the sublethal effects of environmental perturbations and diseases in marine organisms and is useful in correlating physicochemical and physiological changes in population and community-level ecological studies.

This report examines the correlation between bioaccumulation of hydrocarbons and histopathological changes occurring in colonies of the shallow-water Caribbean hermatypic coral *Manicina areolata* (Linné, 1758) during exposure to water accommodated No. 2 fuel oil hydro-

carbons in a three month long 'chronic' flow-through bioassay.

Materials and Methods

M. aerolata colonies were collected by divers from 1 to 3 m depth off West Summerland Key, Florida (24° 40′ N, 81° 30′ W) on 19 March 1977) and transported in aerated seawater-filled styrofoam coolers to the University of South Florida, Department of Marine Science flow-through seawater laboratory at St. Petersburg Beach, Florida. Corals were transferred without exposure to air to three 120 l. glass aquaria fitted with 'eggcrate' screens 2.5 cm off the bottom. Two 122 cm long fluorescent Vita-lites placed 66 cm above the sea table provided 12 h light/12 h dark cycle. The specimens were maintained in the flow-through system for 15 days before starting the fuel oil experiments.

Locally available No. 2 fuel oil (Chevron/Pascagoula, Gravity, °API = 33.0–39.0) was used as the toxicant in the bioassay. The oil was pumped through a Gilson peristaltic pump at 10 ml min^{-1} into an epoxy-coated concrete casket (92 × 229 cm inside) and carried over and under five baffles to simulate ocean mixing by the method of Hyland *et al.* (1977). The resulting water accommodated fraction (WAF) from the middle of the last section was carried by gravity through large and small diameter lengths of Tygon Special (oil resistant) tubing to the inflow lines of two aquaria. A third aquarium was used as the control. Seawater and WAF flow rates were adjusted so that the expected hydrocarbon concentrations would be 0.1 ppm in the low oil aquarium and 0.5 ppm in the high oil aquarium. Three corals were randomly sampled for tissue analysis and three for histopathology before the WAF was added and at two week intervals for 12 weeks. To determine depuration, six colonies from both the low and high oil aquaria were rinsed and placed in the control aquarium after 10 weeks and three from each group were removed one and two weeks later for analysis. Water quality analyses were performed on alternate days three times each week on water samples taken through separate siphons in the middle of each aquarium. Total hydrocarbon concentrations were measured by infrared spectroscopy by extracting 800 ml samples with carbon tetrachloride as described in Storet No. 00560 (Environmental Protection Agency, 1976).

Hydrocarbon contents of tissue from natural coral colonies, control colonies and colonies exposed to WAF for different periods were determined and compared. Colonies for this analysis were placed in solvent-rinsed glass jars sealed with aluminium-foil-lined caps and stored at −20°C until analysis. Tissue was removed from the carbonate skeleton by dissolving the intact coral in 3 N HCl and collecting the residue on glass-fibre filters. The tissue was dried to constant weight at 60°C and then saponified by refluxing for 1 h in one part benzene to one part 0.5 N KOH in methanol. Non-saponifiable lipid material was extracted with petroleum ether from this alkaline solution and hydrocarbons were isolated by thin-layer chromatography on silica gel using petroleum ether/diethyl ether/acetic acid, 90/10/1, as the developing solvent system. The hydrocarbon band was scraped from the glass TLC plate and extracted with chloroform. Gas–liquid chromatography was done with a Hewlett-Packard 5831 Dual FID Reporting Chromatograph equipped with 2.2 mm i.d. × 2.6 m stainless steel columns packed with 10% SP-2100 on 80/100 Supelcoport (Supelco, Bellafonte, Pennsylvania). Column temperatures were programmed from 150 to 300°C at 5°C min^{-1}, and nitrogen carrier gas flow rates were adjusted daily to maximize resolution of pristane and *n*-heptadecane peaks. Quantification was achieved by electronic integration of individual hydrocarbon component peak areas and comparison to the peak area of a known amount of *n*-tetradecane (n-C_{14}) added to each sample prior to gas chromatography. Relative retention times (RT) of individual components were obtained by

TABLE 1

Summary of chromatogram data from flow-through bioassay.

	Total hydrocarbons ($\mu g\ g^{-1}$)			
	Control	Low oil		High oil
Range	8310 – 614	5100 – 688		10 700 – 288
Mean (*)	2890 (± 2050)	1850 (± 1450)		2780 (± 2800)
No. indiv.	12	9		15
	Total *n*-alkanes ($\mu g\ g^{-1}$)			
	Control	Low oil		High oil
Range	318 – 17.9	670 – 27.2		1780 – 1.4
Mean (*)	94.6 (± 89.2)	174 (± 203)		360 (± 475)
No. indiv.	12	9		15
	Height of tangent line ratio C_{17}/C_{62}			
	Control	Low oil w/o HCE †	Low oil w/HCE	High oil w/HCE
Range	0.3 – 0.08	0.5	1.0 – 1.5	5.3 – 5.6
Mean (*)	0.2 (± 0.08)	0.5 (± 0.0)	0.7 (± 0.2)	1.6 (± 1.2)
No. indiv.	12	2	7	15
	Height of tangent line ratio C_{20}/C_{27}			
	Control	Low oil w/o HCE	Low oil w/HCE	High oil w/HCE
Range	0.8 – 0.2	0.9	1.7 – 1.1	9.7 – 1.1
Mean (*)	0.6 (± 0.2)	0.9 (± 0.0)	1.2 (± 0.2)	2.5 (± 2.1)
No. indiv.	12	2	7	15

*Standard deviation.
† HCE = hydrocarbon enrichment.

comparison to *n*-alkane retention times of No. 2 fuel oil. This analytical scheme was developed from procedures described by Meyers (1977) and Meyers *et al.* (1978) and additional details are described by these reports.

All corals for histopathological examination were fixed in Zenker-formol (Helly's) solution for 16 h and rinsed in seawater for 24 h. Colonies were decalcified in Cal-Ex (Fisher) and washed for 24 h in running tap water. Tissues were then processed by standard techniques (Yevich & Barszcz, 1977), embedded in Paraplast Plus and cut in 6 μm sections. The slides were stained with Harris' hematoxylin and eosin and Heidenhain's Aniline Blue for connective tissue (Luna, 1968). Tissue sections were examined with a Zeiss Photomicroscope III.

Results

Measured WAF concentrations fluctuated daily because of the operation of the mixing casket and clogging of Tygon lines with silt. A comparison of mean concentrations of the aquaria obtained by pairing daily observations showed no significant difference between the overall hydrocarbon content of the control and low oil aquaria ($t = 0.32 < t_{0.95}$, 33 df), but a definite difference between the low and high oil aquaria ($t = 5.21 > t_{0.99}$, 33 df). The high oil aquarium averaged about twice as much hydrocarbons (0.15 ± 0.10 ppm) as the low oil aquarium (0.07 ± 0.04 ppm). Background concentrations averaged 0.04 ± 0.03 ppm. Seawater temperature increased approximately 8.5°C to 31.0°C and salinity averaged 33.2 ± 0.5‰ from April to June.

Three examples of chromatograms illustrating the typical 'fingerprint' patterns of corals collected from each aquarium during the flow-through bioassay are presented in Fig. 1A–C, and demonstrate the increase in unresolved peak envelopes in the C_{14}–C_{24} region with exposure to No. 2 fuel oil. The total hydrocarbons and total computer-derived *n*-alkanes were highly variable, yet show an increase in the mean *n*-alkanes along with increasing hydrocarbon exposure (Table 1). Oil-exposed corals were divided into additional groups based on the presence of possible hydrocarbon enrichment (HCE) in the C_{14}–C_{24} region. The values for the C_{17}/C_{28} tangent line ratios (Clark & Finley, 1973) were greater than 1.0 for corals suspected of fuel oil uptake in the high oil aquarium, and for the C_{20}/C_{27} ratio among corals in both the high and low oil aquaria with that type of 'fingerprint'. This calculation indicated a possibility of HCE in the tissues of four out of five corals in the low oil aquarium and all of the corals in the high oil aquarium sampled during the experiment (Table 2). Oil hydrocarbons were detected in coral tissues after only two weeks in the high oil aquarium and after six weeks in the low oil aquarium.

Subtotal and total weight percentages of the major peaks at the high temperature ends of the chromatograms are compiled in Table 3. Controls have a greater percentage of the total hydrocarbons contributed by these

TABLE 2

Tissue chromatograms with possible oil contamination, based on tangent line ratios (Table 1).

Exposure	Control	Low oil	High oil
0 weeks	–	–	–
2 weeks	–	–	1†, 1*
4 weeks	–	–	2†
6 weeks	–	1*	1†, 1*
8 weeks	–	1*	1†, 1*
10 weeks	–	1*	1†, 1*
12 weeks	–	2*	1†, 1*
1 week depuration		1*	1*
2 weeks depuration		1*	1†, 1*

Only one coral from the control and low oil aquaria was extracted from 0–10 weeks, two were extracted from each period for the high oil aquaria and at 12 weeks for all aquaria. One from each was extracted after one week of depuration, one from the low oil and two from the high oil after two weeks depuration.

*Ratio of C_{20}/C_{27} is greater than 1.0.

†Ratio of C_{17}/C_{28} is also greater than 1.0.

TABLE 3

Summary of weight percentage contributions of large peaks at the end of the chromatograms of *M. areolata*, flow-through bioassay.

	Control	Low oil w/o HCE	Low oil w/HCE	High oil w/HCE
RT = 44				
Range	2.3 – 0.56	0.65 – 0.39	1.8 – 0.14	14.8 – 0.29
Mean (*)	1.4 (±0.52)	0.52 (±0.13)	1.4 (±1.2)	2.2 (±3.6)
No. indiv.	12	2	7	14
RT = 50				
Range	30.1 – 4.0	22.6 – 7.1	28.4 – 0.73	25.9 – 0.03
Mean (*)	18.6 (±8.5)	14.9 (±7.8)	9.8 (±8.3)	9.5 (±8.2)
No. indiv.	12	2	7	15
RT = 58				
Range	37.7 – 5.3	38.6 – 4.8	33.5 – 1.1	25.9 – 0.11
Mean (*)	19.2 (±10.3)	21.7 (±16.9)	10.6 (±1.0)	11.0 (±8.4)
No. indiv.	12	2	7	15
RT = 70				
Range	18.1 – 0.2	12.0 – 3.1	12.7 – 0.13	12.4 – 0.28
Mean (*)	10.1 (±6.4)	7.5 (±4.5)	4.4 (±4.0)	4.2 (±3.7)
No. indiv.	12	2	7	12
Totals				
Range	78.7 – 10.6	73.6 – 15.7	76.4 – 2.4	58.0 – 0.14
Mean (*)	49.3 (±23.2)	44.6 (±29.0)	26.0 (±22.5)	25.3 (±18.5)
No. indiv.	12	2	7	15

*Standard deviation.

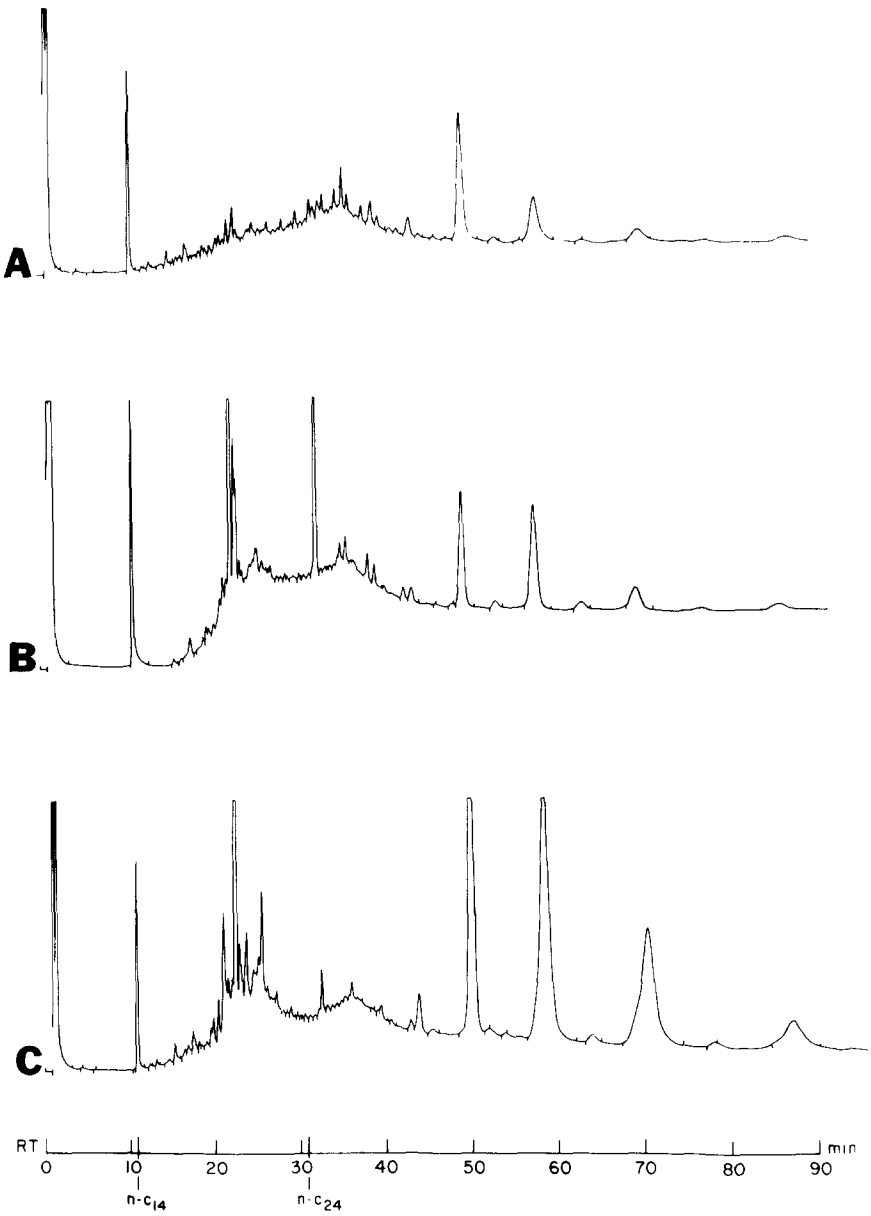

Fig. 1 Chromatograms of corals collected from flow-through bioassay after six weeks exposure to No. 2 fuel oil hydrocarbons. (A) Control, (B) low oil aquarium, (C) high oil aquarium.

compounds. Some chromatograms from the high oil-exposed corals not only have a greater percentage contributed by hydrocarbons in the unresolved peak envelope but also show a reduction in size of these large peaks. All corals which were extracted for analysis after 1–2 weeks of exposure to clean flowing seawater still retained oil hydrocarbons in their tissues, although most were missing the lower boiling fractions which are more volatile and more soluble in seawater.

None of the corals died during the three month period and all appeared to be in good condition; however, gradual histopathological and physiological changes were noted during the course of the exposure. Corals examined after two, four and six weeks showed an extensive increase in mucous secretory cell activity. This was indicated by a proliferation of mucous secretory cells as well as an increase in size of these cells in the epidermis and mesenteries. Many cells had increased to such an extent that the cell walls were broken and huge vacuoles were formed. Many mucous secretory cells were also noted in the tips of the mesenterial filaments where they are not usually present. Also, during the sixth week of exposure to the high oil concentration there was noticeable swelling of the ground substance of the mesogloea throughout the colony.

During the eighth through twelfth weeks of exposure the mucous secretory cells had atrophied and in some places completely disappeared. There was also degeneration and loss of the symbiotic zooxanthellae, not only from the gastrodermis but also the mesenteries. The mesenterial filament tips showed swelling and fragmentation of the nematocysts and degeneration and loss of gland cells, especially those containing basophilic granules. Additionally, the muscle bundles in all areas showed some atrophy and fragmentation (Fig. 2A–D).

While these effects were most prevalent among corals exposed to the higher oil concentration for more than six

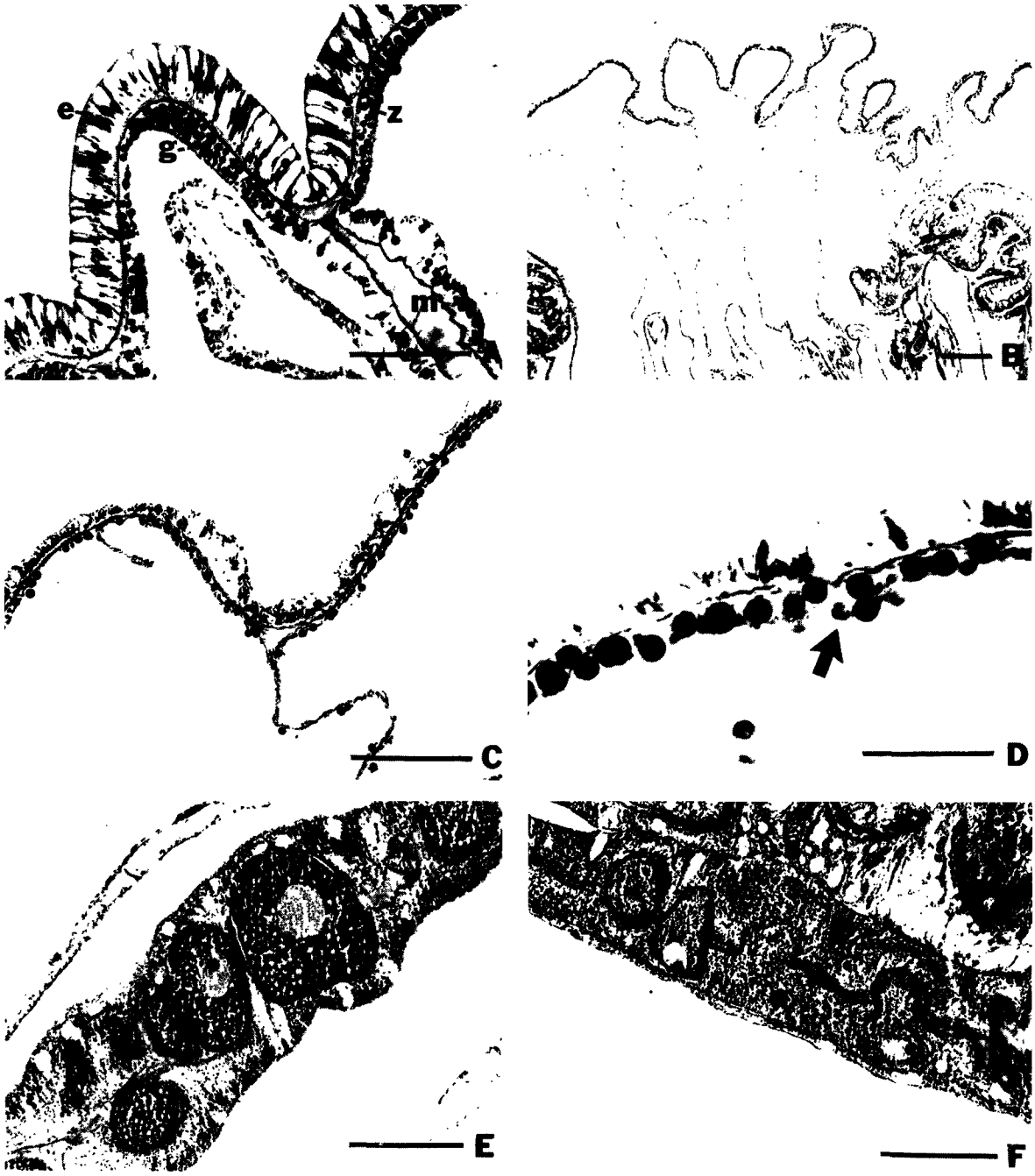

Fig. 2 Effects of oil hydrocarbons on coral tissues. All sections were stained with Heidenhain's Aniline Blue. (A) Healthy coral tissue e = epidermis, g = gastrodermis, m = mesogloea, z = zooxanthellae, bar = 100 μm. (B) Vacuolation and atrophy of coral tissue: bar = 200 μm. (C) Same: bar = 100 μm. (D) Same: note degenerating zooxanthellae (arrow), bar = 50 μm. (E) Developing ova from unexposed coral: bar = 100 μm. (F) Degenerating ova from low oil aquarium coral after twelve weeks exposure: bar = 100 μm.

weeks, areas of atrophied tissue were noted after two weeks exposure in the high oil aquarium and after four weeks in the low oil aquarium (Table 4). Small patches of similar atrophied tissue were observed in two corals sampled at two weeks and in one sampled at eight weeks from the control aquarium; however, none of the other control corals exhibited this condition.

Developing ova were found in most but not all corals collected from the aquaria during the exposure period. Mature ova and testes were observed in only two corals, one each from the control and low oil aquaria, which would be expected at this time in the reproductive cycle of this coral from the Keys (Peters, 1978). Of 27 corals sampled which had not been exposed to oil and 18 corals exposed to the low WAF concentrations, nearly 75% contained distinguishable gonads. One of the three corals taken from the low oil aquarium after 12 weeks exposure had degenerating ova (Fig. 2 E–F). Seven of 18 colonies

TABLE 4

Summaries of histopathological observations on specimens of *M. areolata* collected during the flow-through bioassay, indicating numbers of corals in which the conditions appeared. Three colonies were examined from each aquaria at each sampling period.

	Tissue atrophy		
Exposure (weeks)	Control	Low oil	High oil
0	–	–	–
2	2	–	1
4	–	2	1
6	–	1	2
8	1	1	3
10	–	1	3
12	–	2	3
Totals	3	7	13
	Presence of gonads, ova or sperm*		
Exposure (weeks)	Control	Low oil	High oil
0	3	3	2
2	2 (1*)	2	2
4	1	2 (1*)	2
6	2	3	2
8	2	3	–
10	3	2	1
12	1	1 (degen.)	–
Totals	14	16	9

exposed to the higher WAF concentrations contained ova, but only one coral of the nine sampled after six weeks exposure possessed poorly developing ova.

Discussion and Conclusions

Rinkevich & Loya (1977) surveyed colonies of the branched coral *Stylophora pistillata* from two reef areas in the northern Gulf of Eilat, Red Sea. Corals from the chronically oil polluted reef contained gonads in only 44.6% of the specimens, while those from a 'pollution-free' reef had 75.5% with gonads. The relative maturity of the gonads and the release of larvae from the polyps were also significantly higher at the control reef. More recent studies involving exposure of the same coral species to water soluble fractions of crude oil in a flow-through system also revealed decreases in gonad development after two months exposure (Rinkevich & Loya, 1979). Although Rinkevich & Loya did not perform any histological examinations on their corals other than noting gonad development, they observed that four times as many tagged corals died in the oil polluted area of the Gulf of Eilat during one year. In the flow-through system, no coral mortalities were noted after two months, but after six months mortalities of *S. pistillata* were significantly higher in the oil-exposed tanks (80%) than in controls (10%).

The combined results of both the histopathological and bioaccumulation analyses on oil-exposed *M. areolata* in this study agree with the above observations that longer term 'chronic' exposures to oil hydrocarbons, even in low concentrations, begin a process of cellular degeneration and atrophy of coral tissue as well as reducing the ability of the coral to reproduce. The exact mode of action of oil compounds on invertebrate cells is far from clear. Recently, Hutchinson *et al.* (1979) reported that less soluble hydrocarbons were more toxic on a per mole basis to two species of freshwater unicellular algae and suggested that toxic effects were produced by absorption and lipid partitioning into the lipo-protein membrane of the cells resulting in leakage of cell contents due to interference with the plasmalemma. Because of the high lipid content of coral tissues (Meyers, 1977), the evidence for a host/zooxanthellae cycle in operation for lipid synthesis in these organisms (Patton et al., 1977), and the actual uptake and persistance of hydrocarbons during and after oil exposure in the coral *M. areolata,* it appears that oil hydrocarbons may either partition into the cells or be incorporated into the membrane structure causing disruptions of vital biosynthetic processes as seen in the toxic effects of atrophy of the tissue itself as well as damage to the zooxanthellae.

With increasing exposure to oil hydrocarbons, the hydrocarbons comprising the major peaks near the ends of the chromatograms appear to decrease as the lower boiling unresolved complex envelope hydrocarbons increase. These major peaks seem to be characteristic of corals in general and are probably high boiling polyunsaturated hydrocarbons contributed by the zooxanthellae. Winters *et al.* (1975) found that growth and photosynthesis were inhibited in micro-algae exposed to No. 2 fuel oils. The hydrocarbons taken into the coral tissue may affect the biosynthetic capabilities of the zooxanthellae in a similar manner, resulting in a decrease in the polyunsaturated hydrocarbons which normally contribute up to 75% of the total hydrocarbons. From the microscopical examinations, it appears that oil hydrocarbons may also kill the zooxanthellae, resulting in a decrease in the numbers of algal cells normally found in the gastrodermis which are necessary to maintain the coral in good condition.

Another noteworthy observation from this experiment is the lack of depuration of oil hydrocarbons up to two weeks after removal from oil exposure. The uptake and release of hydrocarbons from an organism's tissues are dependent on the type of oil, water soluble or accommodated fractions and length of time exposed, as well as different lipid-tissue partitioning factors and the ability of the organism to modify or metabolize the various compounds (Anderson *et al.*, 1974; Neff *et al.*, 1976). Hydrocarbons may be relatively stable in marine food webs. The ability of the next trophic level to metabolize these compounds to less toxic products might increase, but these hydrocarbons might be converted into more toxic metabolites and affect the organisms in different ways (Teal, 1977). Because little is actually known about the complex metabolic processes of most marine organisms, future studies of hydrocarbons in the tissues of corals should identify individual compounds in order to determine if there are differences in hydrocarbon uptake, in types of hydrocarbons possibly released, and in hydrocarbons which might be metabolized into different compounds. However, in view of the potential food chain importance of corals (Benson & Muscatine, 1974; Steudler *et al.*, 1977), it appears likely that hydrocarbons and carcinogens from oil polluted areas might be passed along from contaminated corals to higher marine organisms.

Exposing *M. areolata* to sublethal concentrations of No. 2 fuel oil in flow-through bioassay suggests that a complex interaction of processes affects the bioaccumulation and retention of hydrocarbons by corals. However, histopathological examinations performed on these corals provided an early indication of tissue damage which would only be realized later in comparative benthic ecological studies using more traditional methods to determine

percentage mortalities or species recruitment in polluted areas.

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Effect of Oils on Cell Membrane Permeability in *Fucus serratus* and *Laminaria digitata*

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Leakage of electrolytes from discs of treated fronds of *Laminaria digitata* and *Fucus serratus* was the criterion used to study the effect of oils over 24 h on cell membrane permeability. Motor fuel oil increased cell membrane permeability in both algae at 1 h, or longer, after treatment. *Laminaria* changed to a green colour after 3 h, but *Fucus* was unaffected visibly by the oil. White spirit, diesel oil, or aviation fuel did not alter cell membrane permeability or visibly affect either alga over 24 h.

The more volatile constituents of crude oil such as the low boiling aromatic compounds are the most toxic to plant life (Currier, 1951; Van Overbeek & Blondeau, 1954; Crafts & Robbins, 1962; Baker, 1970, 1971; Dodd, 1974; Prendeville & Warren, 1977). Most of the hydrocarbons in crude oil with up to eight carbon atoms can evaporate after 5 h on exposure to air (Dodd, 1974). Fresh crude oil is more toxic than weathered crude oil from which the lower boiling compounds have evaporated (Baker, 1969; Cowell, 1969; Nelson-Smith, 1972).