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REPORT TO GT.BARRIER REEF MARINE PARK AUTHORITY

Grant: Augmentative Research Grant 1987 to N. Khandekar.

Project Title : A comparison of live and dead coral regions with regards to recolonisation of damaged reef areas.

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Sample Collection and Scope of this Report.

Samples were collected in mid-August, 1987 through the courtesy of the Queensland Govt. Marine Park Authority, Cairns, facilitated by Mr. Peter Ottersen of GtBRMPA. Through a set of unforeseeable circumstances, the planned collections with James Cook University researchers for earlier in the year did not eventuate.The consequence of the later collection is that the data reported on here represent the current point to which the data have been refined and interpreted. The project has revealed a novel result in that it appears that polysaccharides may also be used as ecological markers. This finding will require further confirmatory work but would appear most promising.

Introductory Background.

Since the early 1960's the damage to the Great Barrier Reef by the crown-of-thorns starfish, Acanthaster planci, has caused concern as to the future of the reef.[1,2] After extensive damage to the reef, recolonisation occurs and the rates of coral recovery were noted to depend upon the cause of the damage.[3] After coral polyps are removed from their exoskeleton, algal growth rapidly occurs coating the 'dead' coral with a film, which

then gives rise to an ecosystem significantly different from that of the original.[3]

Polysaccharides are ubiquitous components in marine and terrestrial organisms, but have not been as extensively studied in the marine as in the terrestrial geosphere.[4] Polysaccharides are used as a store of energy in organisms[5] and are therefore one of the first classes of compounds to be utilized by other organisms as a substrate.[6]

Because sugars are used rapidly as a food source, they may well be regarded as an indication of nutrient levels present in the environment immediately surrounding the coral, assuming that the environment has reached equilibrium. Hence a comparison of the polysaccharides contained in live coral and dead coral, could give an approximation of the nutrient values of the different systems.

Polysaccharides are present in organisms in detectable concentrations and each type of organism has its own distinctive distribution of monosaccharides, which make up the polysaccharides present in its body.[4] Polysaccharides are therefore a potentially powerful tool in the elucidation of source material, particularly of live organisms. Another tool used for the elucidation of source material is the distribution of components in various lipid classes. Lipid distributions have been used more extensively to collect information concerning biological community structures than have polysaccharides.[7] A comparison of sugar and lipid distributions is therefore in order to observe the suitability of sugars as indicators of the

community structure, particularly in the marine environment.

Hence the aims of this report are to

(i) compare nutrient values of live and dead coral using sugars as a basis, and

(ii) examine the suitability of polysaccharides as indicators of the community structure around both live and dead coral.

Experimental

Preliminary Considerations: All glassware was washed with teepol followed by chromic acid and finally distilled water. Openings were then sealed with aluminium foil to prevent contamination from dust particles. After oven drying, glassware was then rinsed with chloroform followed by the appropriate solvent immediately prior to use.

Organic solvents were AR grade where possible, and were purified by distillation. Solvent purity was tested by gas liquid chromatographic (GC) analysis of concentrated solvent blanks. All equipment coming in contact with samples were prewashed in chloroform:methanol (2:1).

Sample Collection and Preparation: Live and dead coral (Acropora sp.)samples were collected from Arlington Reef, near the channel used by ships (Fig 1). After collection, samples were immediately frozen and stored frozen until prepared for extraction. All samples were freeze-dried prior to extraction. After freeze-drying, the samples of the coral surface were removed using a pre-extracted file until a smooth core remained. The surface of the coral was then ground into a fine powder and extracted. The

entire coral samples were ground into a fine powder and extracted.

Four coral samples prepared as above were studied in this project and are designated as follows :

Whole, live coral = Total Live Coral (TLC) sample

Whole, dead coral = Total Dead Coral (TDC) sample

Surface of the Live coral = SLC

Surface of the dead coral = SDC

Sample Extraction: All samples were extracted with chloroform:methanol (2:1 v/v + 0.05% pyridine) to remove the free lipids. Samples were then extracted with ethanol (80%) to remove oligosaccharides. The residue was in turn extracted with water and finally sodium hydroxide (10%) to obtain the polysaccharide and acidic polysaccharide fractions respectively. All extractions were subjected to ultrasonication to improve extraction efficiency.

Total lipid yields were determined gravimetrically for aliquots of the lipid fraction after feeze-drying to remove traces of residual solovent.

Lipid Fractionation: Aliquots of the lipid fraction were saponified using potassium hydroxide (5 ml, 5% w/v) in methanol:water (80:20 v/v) at 60^{o} on a water bath. Solvent extractable neutral lipids were obtained by partition of the alkaline extract from the saponification into heptane:chloroform (2 x 5 ml, 4:1 v/v). The aqueous layer, after acidification (pH 2) with concentrated hydrochloric acid, was similarly extracted to isolate the solvent extractable acidic components. After

evaporation to near dryness the total acids were esterified by heating with boron trifluoride (14%) in methanol (2-3 ml, 50°) for one hour. Fatty acid methyl esters (FAMEs) were extracted twice with chloroform.

The neutral lipid fraction was separated into hydrocarbons, ketones, alcohols and sterols. The acidic fraction was separated into monocarboxylic-, dicarboxylic-, and hydroxy- FAME fractions by isocratic high performance liquid chromatography using a solvent mixture of heptane:propanol (97:3) at a flow rate of 1 ml/min (Waters M6000 A pump). Samples were introduced onto a spherisorb 5uCN-bonded column (25 cm x 4.6 mm i.d.) using a 25ul loop injector (Specac). Refractive index detection (Varian Pty. Ltd.) was employed.

Derivatisation of Lipid Fractions: Alcohols, sterols and other hydroxy compounds were treated with bistrimethylsilyl-trifluoroacetimide (BSTFA) prior to GC analysis to produce the corresponding trimethylsilyl ethers.

Alditol Preparation: Aliquots of the polysaccharide and acidic polysaccharide extracts were refluxed with hydrochloric acid (1M) under nitrogen (7h, 100°). Inositol, an internal standard was added to the mixture and the mixture was then evaporated to dryness. A solution of sodium borohydride (50 mg in 7ml) was added and the solution was heated (2h, 60°). This solution containing the alditols was applied to the top of a column (70mm x 10mm i.d.) of Dowex 50W-X8 (H^+) cation exchange resin, and the alditols were eluted with water (25 ml). The eluate containing the alditols was evaporated to dryness methanol (10 ml) was added

and then evaporated, to free the solution of borate ions. This process was repeated twice and the sample was freeze-dried.

Alditol Derivitisation: A solution of acetic anhydride:pyridine (1:1) was added to the freeze-dried alditols and the solution was refluxed under nitrogen (2h, 100°) to form the acetylated alditols of the monosaccharides. The mixture was evaporated to dryness and the residue was dissolved in chloroform. An aliquot of this solution was injected onto the GC column.

Gas Liquid Chromatographic Analyses: All samples were analysed using a Varian 3700 GC, using flame ionisation detection. Analyses were performed with a glass support coated open tubular (SCOT) BP-1 column (25 mm x .2 mm i.d., SGE Australia) with hydrogen as a carrier gas. The sample was injected at 40°C and after 2 min the temperature was increased to 120°C and programmed from there to 300°C at 4°C/min followed by an isothermal period of 20 min.

GC peaks were identified by relative retention time and equivalent chain length data. Standards were used to quantify peak areas. Integration and chromatograms were recorded for all samples using a Hewlett-Packard 3390A integrator.

RESULTS AND DISCUSSION

Monosaccharide Distribution Patterns

Neutral Polysaccharide Fraction.

The distribution of monosaccharides in both the neutral polysaccharide and acidic polysaccharide fractions appear diverse

for the surface and entire coral samples (Fig 2), but various trends are apparent in these distributions. The polysaccharides exhibit low rhamnose concentrations except for the sample of the surface of the dead coral(SDC). Live coral shows high concentrations of fucose/ribose. The surface samples (SLC;SDC) of both corals show high concentrations of arabinose. Dead coral shows higher concentrations of xylose than the other samples. The six carbon (6C) sugars: mannose, glucose and galactose, are best examined together as they exhibit the strongest similarity in distribution of all the monosaccharides present. The surface of dead coral (SDC) has the highest concentrations of these 6C sugars. The concentrations of the 6C sugars in the remaining samples is significantly lower than that found in the surface sample from the dead coral(SDC), with the exception of mannose (in sample TLC) which occurs at a magnitude similar to that in the SDC sample.

Acidic Polysaccharide Fraction.

The distribution of monosaccharides in the acidic polysaccharide fraction is entirely different to that in the neutral polysaccharide fraction. The concentration of all the monosaccharides is highest in the surface of dead coral (sample SDC) except for arabinose whereas the total dead coral (TDC) has a significantly higher amount present. The concentration of rhamnose in all the samples is very low. The surface of dead coral (SDC) and the total live coral (TLC) show high levels of arabinose, whereas the total dead (TDC) and surface of live coral (SLC) exhibit lower levels of arabinose. The distribution of

xylose was the reverse of that seen for arabinose. Again the 6C sugars exhibited similar patterns of distribution to each other. Here the surface of the dead coral (SDC) has a higher concentration than the entire dead coral(TDC), as would be expected with a biologically inactive centre to the coral. The surface of live coral (SLC) has a lesser concentration of the 6C sugars than the total live coral(TLC), suggesting that there must be sugars contained in the centre of the live coral. When sugars are used as an indication of the nutrient level it can be seen that the surface of the dead coral (SDC) is the higher source of energy compared to live coral, although the distribution of sugars is very different from the live coral which indicates that a different ecosystem develops and exists around the coral after it has been damaged.

Lipid Analyses and their use as biomarkers.

The distributions of monosaccharides and components in lipid classes can give an indication of some of the organisms present in a marine environment.[4,8] Although sterols are not as specific a biomarker as once thought, they can still strongly suggest their likely biological source.[9] Both live and dead corals exhibited similar distributions of lipids for the Total and Surface corals (Fig 3), hence a comparative discussion of live and dead corals will be considered. The presence of relatively high concentrations of campesterol as well as lower concentrations of stigmasterol and their stanols indicate large amounts of green algae present on the dead coral samples, whereas live corals are lacking in these compounds. The presence and concentration of brassicasterol and also 24-methyl-cholesta-

5,24(28)E-dien-3β-ol, and their stanols are indicative of small amounts of diatoms being present on dead coral. Again these markers are lacking in live coral, thus indicating that live coral has much lower concentrations of diatoms. The presence of Δ7 sterols could be indicative of algae but when considering the even over odd predominance and high carbon numbers in the straight chain fatty acids in dead coral it would appear that sponges are the likely major contributor of this class of compound. Finally the presence of small amounts of the 4-methyl sterol dinosterol is indicative of dinoflagellates present on the dead coral, and these are again absent on the live coral. The live coral has a much smaller range of sterols than the dead coral, the predominant one being cholesterol and all others were detected in much lower concentrations. Some of the sterols present in the live coral were absent in the dead coral, the most notable being desmosterol and 5α-cholesterol-22E-en-3β-ol.

The distribution of straight chain fatty acids (Fig 4) reveals little except marine inputs indicated by the low carbon numbers ($<C_{22}$) and even over odd predominance. The surface of dead coral is an exception; it contains long chain fatty acids ($>C_{22}$) with an even over odd predominance which may be indicative of sponges when the presence of the Δ7 cholesterol is also taken into account.

The major unsaturated fatty acids contained in the dead coral(Figs 5,6) are 16:1ω7, 18:1ω7 and tr18:1ω7, the first of which is a biomarker for diatoms and the latter two are markers for bacteria. Minor unsaturated acids present indicative of

bacteria are i15:0, i17:0, i19:0. Markers for the presence of algae are 16:4ω3, 16:3ω3, 18:2ω6 and 18:3ω3. The isoprenoid acid, 16:0 is also present andch is due to degraded phytol. The acid and tr16:1ω13 is indicative of chloroplast input. 16:3ω4 is present as a marker for diatoms on dead coral.

The live coral does not contain the range of fatty acids that the dead coral does (Figs 7,8) and contains only biomarkers for bacteria. The major marker acids being tr16:ω7 and 18:1ω7 and the minor acids being tr13:1ω7, i14:0, i16:0, a17:0, 18:1ω9,tr18:1ω7 and a19:0.

Polysaccharides as Biomarkers.

Distributions of saccharides from specific biological sources do show a sufficient degree of differentiation to suggest that these distibution patterns could be used as markers of biological input sources to an environment[4]. Using this approach it is possible to suggest some of the likely components contributing to the community structure on the four coral samples examined here.

Algae are known to have high concentrations of 6C sugars; hence the dead coral has a large amount of algae present shown by its high concentrations of 6C sugars. The concentrations of ribose present indicate inputs from bacteria in both live and dead corals. Arabinose can be a marker for bacterial and higher plant inputs. In this instance it is indicative of a bacterial source, since there is no suggestion of a higher plant input. Its presence suggests that the surface of both live and dead corals support bacteria. It can be seen that although polysaccharides are not as specific as lipids in determining their biological

origin they have a potential for use as a semiquantitative tool in the elucidation of source material in marine community structures.

Conlusions

Using polysaccharides as a first approximation in the determination of the nutrient levels it can be seen that the surface of dead coral has the highest concentrations of sugars for any of the samples collected. However, the distribution of sugars in Live and Dead coral are significantly different from each other. Such data would suggest that the ecosystem surrounding the coral has changed significantly on the dead coral. As a source of nutrients the dead coral would appear to be adequate, but because of the changed ecosystem, recolonisation by coral may be difficult.

The use of lipids and polysaccharides as indicators of community structure on dead coral indicates significant inputs from green algae, diatoms, bacteria, sponges and dinoflagellates. In live coral, however, the biomarkers indicate only the presence of bacteria. It can also be seen from this study that polysaccharides have potential as indicators of community structure in a marine environment. Because this is a new observation which will require further development and proving, the polysaccharides can be used only semiquantitatively at present.

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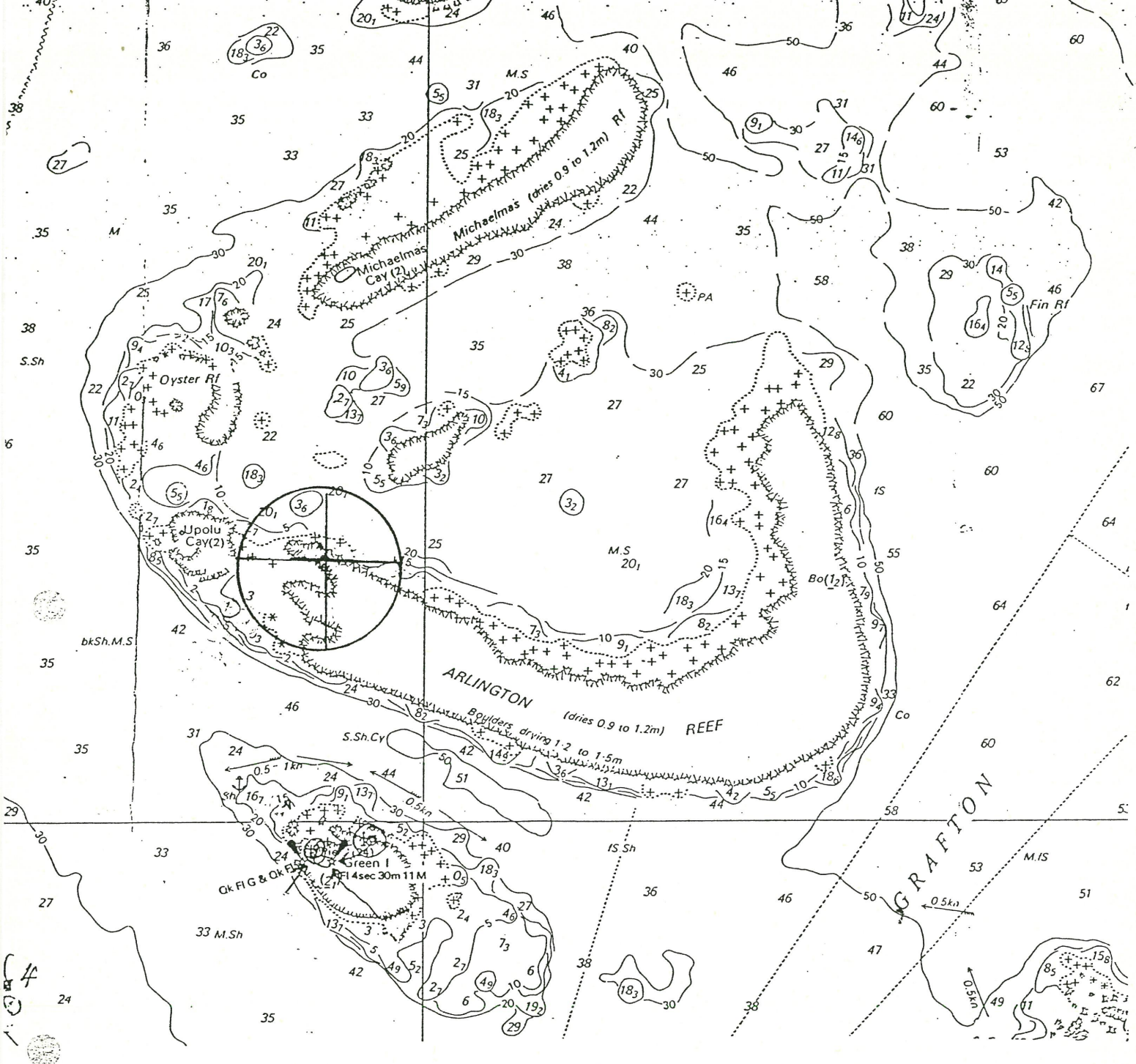


Fig. 1 A Map of Arlington Reef Showing the position of the sampling site

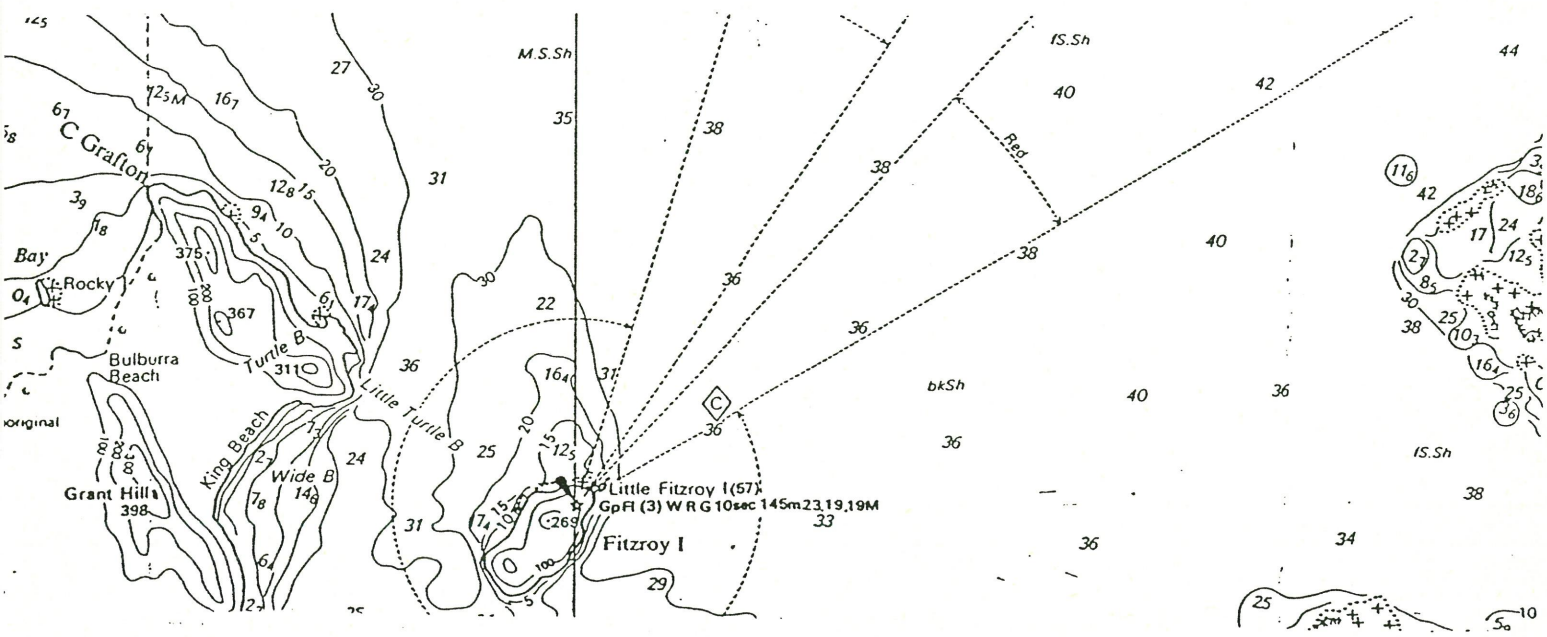
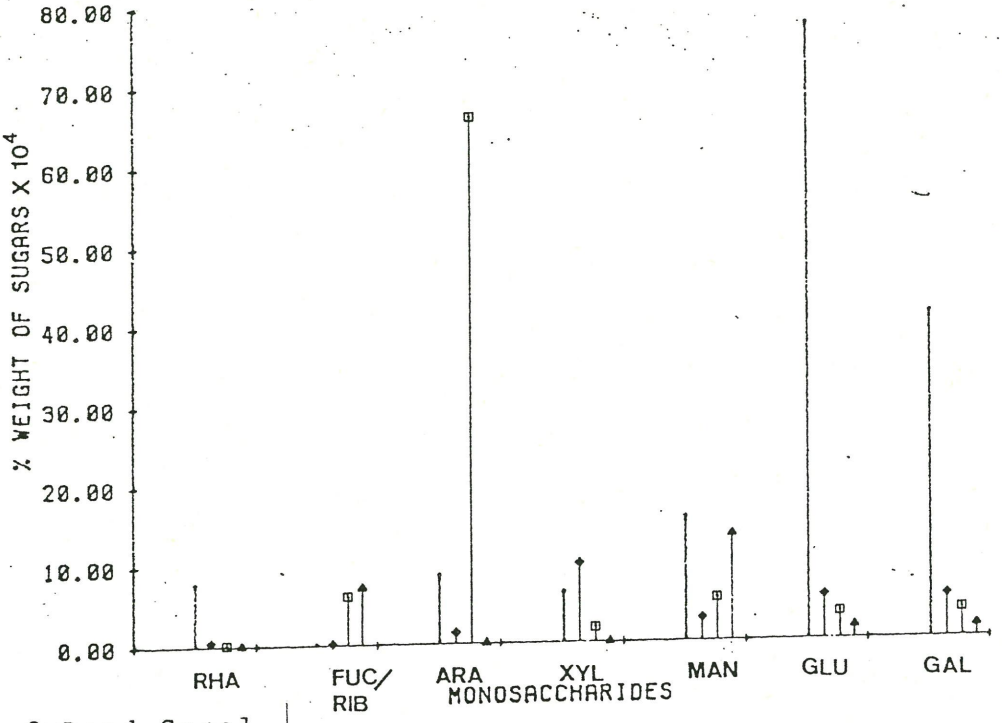


FIG 2

% WEIGHTS OF MONOSACCHARIDES
OF WATER EXTRACTS IN
LIVE AND DEAD CORALS



■ = Surface of Dead Coral

▲ = Total Dead Coral

• = Surface of Live Coral

◆ = Total Live Coral

% WEIGHTS OF MONOSACCHARIDES
OF NaOH EXTRACTS IN
LIVE AND DEAD CORALS

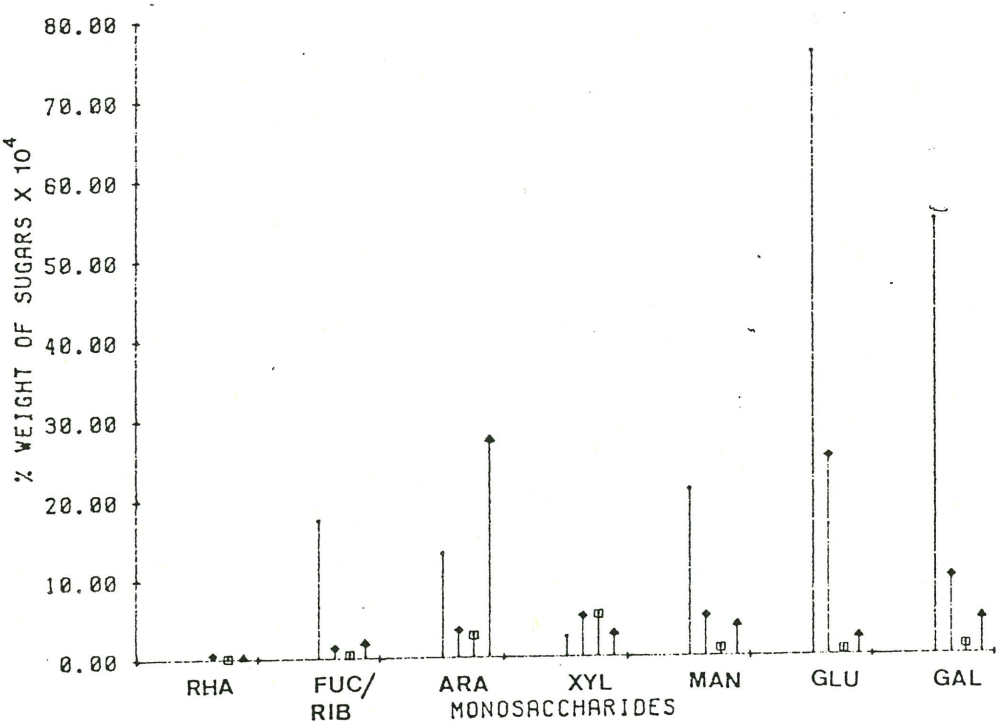


Table I

Percentage Weight of Monosaccharides in Dead Coral X 10 000

Sugar	Surface of Dead Coral		Total Dead Coral	
	Water Extr	NaOH Extr	Water Extr	NaOH Extr
Rhamnose	7.78	-	0.38	0.35
Fucose/Ribose	-	17.44	1.14	5.96
Arabinose	8.50	13.16	1.24	65.88
Xylose	6.22	2.50	9.80	4.94
Mannose	15.42	21.06	2.68	4.95
Glucose	77.12	75.58	5.34	24.92
Galactose	40.74	54.46	5.23	9.76
Total	155.78	130.04	74.01	49.33

Percentage Weight of Sugars in Live Coral X 10 000

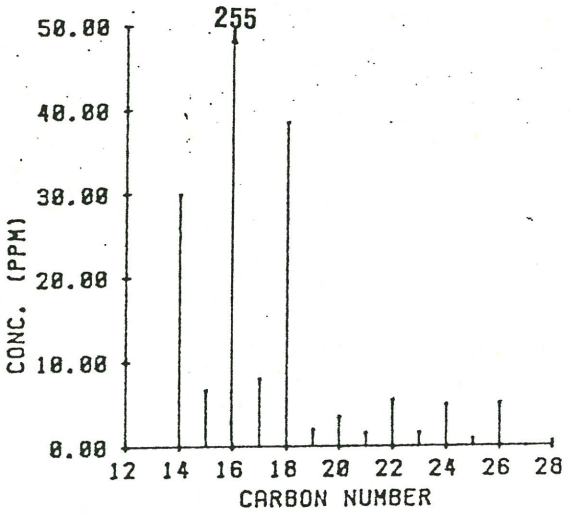
Sugar	Surface of Live Coral		Total Live Coral	
	Water Extr	NaOH Extr	Water Extr	NaOH Extr
Rhamnose	-	-	-	-
Fucose/Ribose	5.96	0.39	7.17	1.80
Arabinose	65.88	2.71	-	27.25
Xylose	1.72	5.23	-	2.77
Mannose	5.26	0.93	13.32	3.79
Glucose	3.26	0.33	1.55	2.18
Galactose	3.42	1.13	1.52	4.49
Total	85.50	11.02	23.56	42.28

Fig. 3

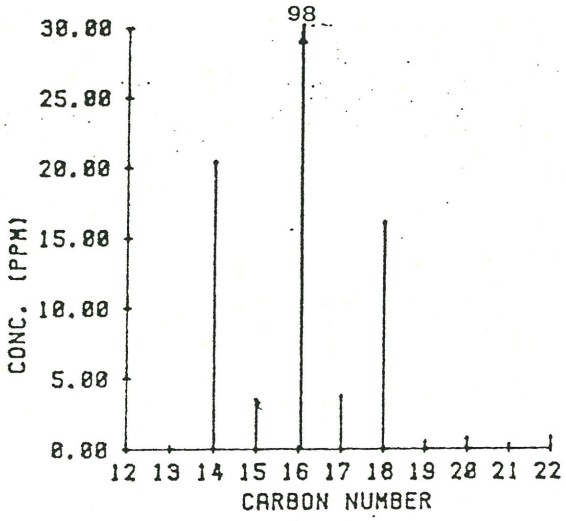
Concentration of Sterols (PPM) in Live and Dead Coral

Sterol	Surf Dead	Tot Dead	Surf Live	Tot Live
24-Norcholesta-5,22E-dien-3β-ol	-	.02	.10	.06
Cholesta-5,22Z-dien-3β-ol	1.06	.05	.63	-
Cholesta-5,22E-dien-3β-ol	.13	.04	.14	-
Cholest-5-en-3β-ol (Cholesterol)	8.04	1.91	133.09	11.77
5α-Cholestan-3β-ol (Cholestanol)	.72	.16	1.58	.18
24-Methylcholesta-5,22E-dien-3β-ol (Brassicasterol)	-	.56	tr	.01
5α-cholest-7-en-3β-ol (Δ7-Cholesterol) 24-Methyl-5α-cholest-22E-en-3β-ol (Brassicastanol)	2.65	.12	.12	tr
24-Methylcholesta-5,24(28)E-dien-3β-ol (24-Methlycholesterol)	.13	.04	.99	-
24-Methylcholest-5-en-3β-ol (Campesterol)	.92	.19	-	.01
24-Methyl-5α-cholest-5-en-3β-ol (Campestanol)	1.74	1.44	-	-
24- Ethylcholesta-5,22E-dien-3β-ol (Stigmasterol)	.03	.05	-	-
24-Ethyl-5α-cholesta-5,22E-en-3β-ol (Stigmastanol)	.72	.31	-	.01
24-Ethyl-cholesta-5-en-3β-ol (Sitosterol)	2.63	1.39	18.29	2.72
24-Ethyl-5α-cholestan-3β-ol (Sitostanol)	.37	.14	1.34	.19
4,23,24-Trimethylcholesta-22E-3β-ol (Dinosterol)	.04	.02	-	-
Cholesta-5,24-dien-3β-ol (Desmosterol)	-	-	1.09	.11
5α-Cholest-22E-en-3β-ol	-	-	.80	.09

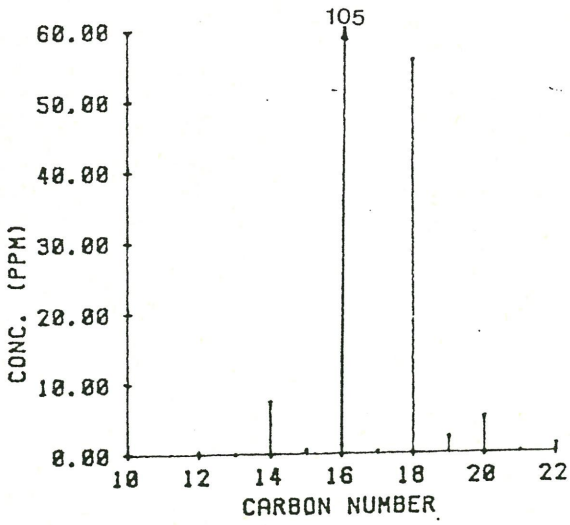
SURFACE DEAD CORAL STRAIGHT CHAIN FAMES



TOTAL DEAD CORAL STRAIGHT CHAIN FAMES



SURFACE LIVE CORAL STRAIGHT CHAIN FAMES



TOTAL LIVE CORAL STRAIGHT CHAIN FAMES

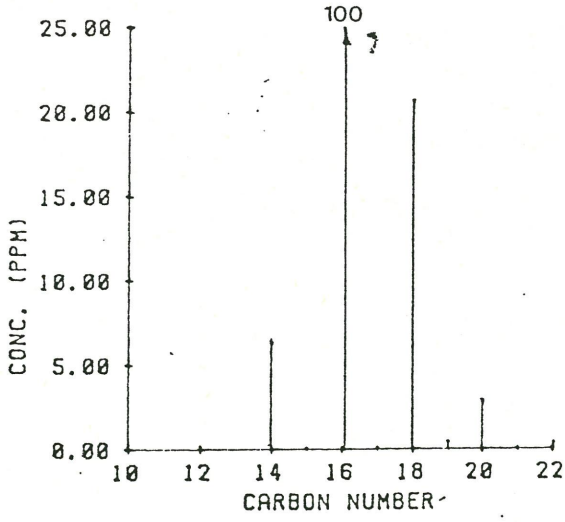


FIG 4

Fig. 5

Major Unsaturated Fatty Acids in the Surface of Dead Coral

Acid	Concentration (PPM)
16:1ω7	27.26
18:1ω9	15.46
18:1ω7	40.15
tr18:1ω7	27.39
20:3ω6	9.04

Minor Unsaturated Fatty Acids Present in the Surface of Dead Coral

Acid	Concentration (PPM)	Acid	Concentration (PPM)
14:1ω5	.32	i17:0	4.02
ip16:0	.79	18:2ω6	2.59
i15:0	4.08	18:3ω3	.92
15:1ω8	1.66	18:1ω5	2.48
16:4ω3	1.51	i19:0	.80
16:3ω4	.89	20:1ω9	2.99
16:2ω6	.31	21:1ω7	2.35
16:3ω3	3.22	22:1ω7	1.14
16:1ω9	.87	24:1ω7	.43
16:1ω5	1.58		
tr16:1ω13	1.55		
br17:1	.59		

Fig. 6

Major Unsaturated Fatty Acids in Total Dead Coral

Acid	Concentration (PPM)
tr16:1ω7	12.16
18:1ω9	10.95
18:1ω7	16.63
tr18:1ω7	15.60

Minor Unsaturated Fatty Acids in Total Dead Coral

Acid	Concentration (PPM)	Acid	Concentration (PPM)
14:1ω5	.27	i17:0	2.46
i15:0	1.76	18:2ω3	1.70
16:4ω3	.60	i19:0	.18
16:3ω4	1.05	20:4ω3	1.12
16:3ω3	1.15	20:2ω6	.32
16:1ω9	3.26	20:1ω1	.31
16:1ω5	.67	20:1ω9	2.22
tr16:1ω13	.72	tr18:1ω7	2.28
br17:1	.15	22:5ω3	.69

Fig. 7

Major Unsaturated Fatty Acids in the Surface of Live Coral

Acid	Concentration (PPM)
tr16:1ω7	7.03
18:2ω6	9.46
18:1ω7	15.86
20:3ω6	53.95
20:1ω7	12.59

Minor Fatty Acids in the Surface of Live Coral

Acid	Concentration (PPM)
tr13:1ω7	.16
i14:0	.18
i16:0	.55
a17:0	.24
18:1ω9	2.72
tr18:1ω7	1.74
a19:0	1.52
20:2ω6	1.6
20:1ω11	.53
20:1ω9	2.36
22:1ω7	.46

Fig. 8

MajorFatty Acids in Total Live Coral Samples

Acid	Concentration
16:1ω7	6.25
18:1ω9	1.28
20:4	7.74
20:5ω3	9.39
20:1ω7	5.59

Minor Fatty Acids in Total Live Coral

Acid	Concentration (PPM)	Acid	Concentration (PPM)
i14:0	.22	18:1ω7	1.04
tr14:1ω7	.17	18:1ω5	.02
14:1ω7	.03	a19:0	.14
16:2ω6	.29	20:5ω6	.02
tr16:1ω7	.08	20:2ω6	1.04
i17:0	.21	20:1ω11	3.36
a17:0	.04	20:1ω9	.73
cy17:0	.12	tr20:1ω7	.59
18:2ω6	3.25	22:1ω7	.51