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Depth Analysis of Fatty Acids in Two Caribbean Reef Corals

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Abstract

Total fatty acid compositions of colonies of two hermatypic, reef-building corals collected during the day-time over a depth range of 21 m were determined to assess the effect of depth-related environmental factors upon the lipid content of these organisms. No systematic changes were found, suggesting a steady-state balance between algal and animal lipogenesis in these symbiotic partnerships. *Stephanocoenia michelinii*, a day and night feeder, contained lipids indicative of external dietary sources such as copepods, whereas *Montastrea annularis*, a night feeder, did not.

Introduction

Hermatypic reef corals are characterized by a mutualistic association between host cnidarians and their dinoflagellate symbionts. A feature of this relationship is the translocation of photosynthetic products from the algae to their animal parters. Carbon fixed by the algae can appear in the host in a variety of forms. To date, synthesis and movement has been demonstrated for sugars, primarily glucose and glycerol (Muscatine, 1967; Muscatine and Cernichiari, 1969; Lewis and Smith, 1971; Trench, 1971a, b); amino acids, for instance alanine or leucine (Muscatine and Cernichiari, 1969; Lewis and Smith, 1971); proteins (Young *et al.*, 1971); chitin (Young *et al.*, 1971); and lipids, primarily triglycerides (Muscatine and Cernichiari, 1969; Young *et al.*, 1971; Patton *et al.*, 1977). Von Holt and Von Holt (1968) demonstrated with the Caribbean coral *Scolymia lacera* (Pallas) that roughly one-half of all CO_2 fixed during photosynthesis and translocated into water-soluble or alcohol-soluble extracts is located in the lipid fraction. This amount fixed into animal lipid represents almost 20% of all the CO_2 fixed during photosynthesis. Muscatine and Cernichiari (1969) found that when this labeled animal-tissue lipid was deacylated, ^{14}C was detected only in the glycerol moiety suggesting animal synthesis of these lipids from simpler components. Recent evidence, however, suggests an algal origin for some lipid synthesis (Patton *et al.*, 1977), and it has been speculated that coral fatty acid compositions are to a large extent controlled by algal biosynthesis, and that much of the fatty acid content of the host animal is derived unaltered from the zooxanthellae (Meyers, 1977; Patton *et al.*, 1977).

Since fatty acids can be photosynthetically derived, whether as direct products of translocation or indirect products of animal synthesis from algal components, their production might be a function of depth-related changes in light intensity or spectral quality. Other parameters show such trends. For instance, with increasing depth, light-controlled, stable carbon isotopic compositions of Caribbean reef corals become progressively lighter in ^{13}C in both tissues (Land *et al.*, 1975) and in the carbonate skeletons (Weber *et al.*, 1976). Further, colonies of the Pacific coral *Pavona praetorta* (Dana) from depths of 10 and 25 m show distinctive oxygen production and consumption rates indicative of physiological adaptations to their individual light regimes (Wethey and Porter, 1976a, b). These photoadaptation patterns reoccur in the Caribbean coral *Montastrea annularis* (Ellis and Solander) from

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Jamaica (Davies, 1977; Wethey and Porter, in preparation). This species also shows a close correlation between ambient light conditions and skeletal morphology (Graus and Macintyre, 1976).

In view of these observed depth-related variations in coral composition, we were interested in determining if there existed changes in coral fatty acid content with increasing depth. If such changes were found, they might indicate variations in the dependence of coral on an algal source of coral lipids, and hence an increasing or decreasing dependence on heterotrophically derived food over depth ranges of decreasing light supply. Differences in total fatty acid composition might also indicate variations in the amount of translocated photosynthates with depth.

Materials and Methods

Coral samples from the seaward edge of the fringing-barrier reef at Discovery Bay, Jamaica, were hand-collected between 10.00 and 13.00 hrs using SCUBA in March, 1976. Small pieces of colonies of two hermatypic species, *Montastrea annularis* and *Stephanocoenia michelinii* (Milne-Edwards and Haime), were obtained at depths ranging from 3 to 24 m. *M. annularis* was one of the first scleractinian corals in which the movement of photosynthetically fixed carbon was experimentally demonstrated (Goreau and Goreau, 1960), and, as cited above, has been the object of a variety of physiological studies, many of them being conducted at Discovery Bay. It is a major frame builder, with colonies at Discovery Bay occurring from sea level to 80 m (Goreau and Wells, 1967). It is a capable plankton feeder, expanding at night and contracting during the day (Porter, 1974). *S. michelinii* is similar in many respects in size, overall shape, and depth range on the reef, but differs in position on the substrate and in activity periods. *S. michelinii* generally grows closer to the substrate, often in the understory layer with numerous branching coral species overtopping it. It too is a capable planktivore, but most colonies are expanded fully both day and night instead of just at night. The result of this behavior is that it is probably feeding continuously on ambient plankton supplies.

The samples were frozen immediately at -20°C and remained frozen until analysis was started in July 1976. Freezing at -30°C has been shown to be a satisfactory method for preservation of zooplankton for lipid analysis for periods up to 9 months (Morris, 1972).

Water temperature was measured at each collection location and was found to be isothermal at 26.0°C over the entire depth range. Salinity was determined by taking samples of water from each depth back to the laboratory, for measurement with an American Optical Company Refractometer Salinometer. A small salinity increase of 2‰ was found in the top 14 m. At depths below this, salinity did not vary from 35‰.

Total fatty acids in the combined coral and algal tissue were prepared for analysis by the procedure of Meyers *et al.* (1974), as modified by Meyers (1977). Extracted fatty acids were converted to their methyl esters as described by Meyers *et al.* (1974) using a procedure adapted from that of Metcalfe *et al.* (1966). Analysis of these esters was by gas-liquid chromatography (Meyers, 1977). Individual components of the total fatty acid composition were identified by comparison of their retention times to those of authentic standards. Replicate analyses yielded coefficients of variation of less than 3%.

Results

Fatty acid compositions for the 9 individual tissue samples of *Montastrea annularis* from various depths are listed in Table 1. Single samples from 4 entirely separate colonies of *M. annularis* were collected at 9 m in order to assess variability between colonies of this species from one depth. The most abundant component in the composition of these 4 replicate samples from 9 m is palmitic acid (16:0) with a mean weight percent of 61% and a coefficient of variation of 11.3% of the mean. The second-most abundant fatty acid is oleic (18:1). Its mean contribution is 11.7% in these 4 samples, and its coefficient of variation is 15.0% of the mean. The other major acids, defined as contributing 1% or more to the total composition, show considerable variability. The data from these 4 samples support the findings of Meyers *et al.* (in preparation) from analysis of 8 replicate samples of *Manicina areolata* (Linnaeus). Acids comprising 20% or more of the total composition have considerably less variability than lesser components, and such acids may be useful in comparative studies of the type reported here.

No systematic change with depth of collection was observed in the total fatty acid composition of the samples of *Montastrea annularis* analyzed in this study. Furthermore, there appeared to be no sig-

Table 1. *Montastrea annularis*. Fatty acid weight percent compositions of colonies from various depths

Major acid	Depth (m)								
	3	6	9	9	9	9	12	18	24
Myristic (14:0)	2.5	3.8	1.0	0.8	2.4	1.6	2.5	0.9	1.8
Palmitic (16:0)	65.4	62.5	59.6	52.0	64.5	67.8	66.4	72.3	70.3
Palmitoleic (16:1)	4.2	5.1	4.3	0	0.9	2.8	4.8	2.6	0
Stearic (18:0)	12.2	8.2	2.7	18.5	5.5	6.6	9.4	12.1	18.8
Oleic (18:1)	8.8	12.4	11.6	12.5	9.3	13.4	10.3	7.8	7.0
Linoleic (18:2)	1.1	1.6	2.5	0.3	3.1	1.9	1.1	0.6	0.6
Arachidic (20:0)	0.1	0.5	0	0.5	0	0	0.5	1.0	0
Eicosenoic (20:1)	0.1	0	0	2.4	0	0	2.0	2.8	1.5
Docosahexaenoic (22:6)	0	2.2	2.6	0	3.1	0	0	0	0

Table 2. *Stephanocoenia michelinii*. Fatty acid weight percent compositions of colonies from various depths

Major acid	Depth (m)						
	3	6	9	12	14	18	24
Myristic (14:0)	0.3	1.9	0.6	2.6	1.0	1.5	1.0
Palmitic (16:0)	6.9	43.2	54.9	68.3	29.3	50.7	52.6
Palmitoleic (16:1)	0.5	3.0	0	2.9	1.8	2.1	2.1
Stearic (18:0)	2.6	9.7	19.1	11.6	7.6	13.9	15.3
Oleic (18:1)	2.1	7.8	15.3	8.4	18.9	17.4	15.8
Linoleic (18:2)	0.4	2.5	1.1	2.1	1.5	3.0	1.9
Arachidic (20:0)	0.6	3.7	4.4	2.0	2.0	5.4	4.5
Eicosenoic (20:1)	0.5	0	4.8	2.1	5.9	5.9	4.0
Docosahexaenoic (22:6)	82.8	16.4	0	0	15.0	0	0

nificant difference between the shallowest and the deepest samples. The mean palmitic acid composition of samples from all depths is 64.5%, with a coefficient of variation of 9.5%. This is essentially the same as the 4 replicate samples from 9 m. Similarly, oleic acid has a mean composition of 10.3% of the mean. Other minor components are more variable, both within depths as well as between depths.

Fatty acid compositions of the 7 samples of *Stephanocoenia michelinii* in Table 2 show more variability than those of *Montastrea annularis*, but also show only minor, non-significant depth-related trends. Palmitic acid is again the major fatty acid in most of the samples, and averages 44% of the total acids versus 64.5% for *M. annularis*. However, the shallowest sample contains a low percentage of this acid. Its composition is dominated (82.8%) instead by the marine-type, polyunsaturated docosahexaenoic acid (22:6), which also appears in sizeable amounts in the samples of this species collected from depths of 6 and 14 m but not from any of the other depths. This acid is present in some of the *M. annularis* samples only as a minor component (<3.1%).

The low level of polyunsaturated fatty acids in most of these samples agrees with other reports of low levels or absence of such acids in stoney corals (Pasby, 1965; Meyers *et al.*, 1974; Patton *et al.*, 1977). However, some corals have been found to contain relatively large amounts of polyunsaturation (Meyers, 1977; Sassen, 1977). It is possible that some loss of polyunsaturated acids may have occurred during desiccation of the coral tissues or during thin-layer chromatography (TLC) isolation of fatty acid methyl esters. Nichaman *et al.* (1963) have observed losses of polyunsaturated acids during TLC visualization by iodine vapors, and Schultz and Quinn (1977) have determined that 19% of the 22:6 fatty acid can be lost by this method. Although iodine was the visualizing agent in the present study, exposure to vapors was kept brief to minimize loss of polyunsaturated components. Furthermore, the overall procedure used here has been able to detect substantial amounts of polyunsaturated acids and hydrocarbons in a previous study (Meyers, 1977). Therefore, while some losses may have been encountered, it is not likely that they would have been complete, and the

low level of polyunsaturated acids found in these samples is probably real.

Because docosahexaenoic acid has been previously detected in substantial amounts in only 8 out of 28 coral species studied, not including *Stephanocoenia michelinii* (Meyers, 1977), we were curious to see the effect of omitting this acid from our tabulated data. When the compositions of *S. michelinii* in Table 2 were recalculated without docosahexaenoic acid, the coefficients of variation of palmitic, stearic (18:0), and oleic acids were reduced by half. This implies that these three acids may comprise the bulk of the coral fatty acid composition, whereas docosahexaenoic acid may be a dietary component still in the process of being digested, or that the presence of this acid is due to some other special circumstances.

Discussion

It is interesting that no systematic depth trend was found in these determinations of total fatty acids in corals. Weber *et al.* (1976) found important depth-related variations in stable carbon isotope ratios to occur in skeletal carbonate of *Montastrea annularis* samples from St. Croix, U.S. Virgin Islands. These changes resulted in a steady decrease in ^{13}C content from surface samples to samples collected at a depth of 18 m. Between 19 and 22 m, a discontinuity in the ^{13}C:^{12}C ratio was found which was associated with morphological changes in *M. annularis* skeleton that also occur at this depth. In contrast, our fatty acid data from this same species show no differences over this critical depth range. This can be interpreted to mean that skeletal morphology has no facile correlation with the total fatty acid composition of hermatypic coral tissue.

However, analysis of ^{13}C:^{12}C ratios in whole tissues of *Montastrea annularis* and of separated extracts of its endosymbiotic algae by Land *et al.* (1975) showed that, while both total tissue and algal isotope ratios were very similar, no depth-related changes occurred above 40 m in samples from the same Jamaican site we sampled. The amount of ^{13}C in tissues decreased in deeper samples, similar to skeletal ^{13}C, but not until a depth of 40 m was reached and exceeded. Therefore, it is possible that changes in fatty acid composition should not be expected until samples from greater depths than the 24 m reached in our collection are analyzed.

One of the objectives of this study was to determine if depth-related changes in translocated lipids could be determined. It is known that lipid materials such as glycerol (Muscatine, 1967; Muscatine and Cernichiari, 1969; Young *et al.*, 1971) and fatty acids (Patton *et al.*, 1977) are transferred from zooxanthellae to their hosts. It is further speculated (Patton *et al.*, 1977) that fatty acids contributed by zooxanthellae are incorporated largely intact into the host-animal tissue. Because of distinctive differences between animal and plant fatty acids (Gunstone, 1967), the total fatty composition of the algal-animal association should show depth-related changes. These could be the result of a decrease in the amount of algal biomass relative to animal material or due to a reduction in algal lipogenesis. Both of these possibilities could accompany the reduced light levels encountered with increasing depth. Either possibility would cause a depth-related shift to decreasing amounts of algal-like saturated fatty acids and a relative increase in amounts of animal-like polyunsaturated acids.

However, fatty acid compositions of *Montastrea annularis* in Table 1 are not affected by depth between 3 and 24 m. Therefore, it must be assumed either that coral metabolism is in fine balance with algal photosynthetic rates and thus decreases with depth, or that the coral animal is altering translocated lipids for its own use. Both these assumptions have interesting implications. The first implies that coral metabolic rates are related to depth. Indirect evidence exists to support this possibility. Davies (1977) found that oxygen consumption rates per unit area of tissue in samples of *M. annularis* from 40 m at Discovery Bay, Jamaica, were half those in samples from 2 m. Therefore, it is possible that metabolism and tissue growth rate also decrease with depth. The second assumption implies that fatty acids synthesized *de novo* or transformed from algal lipids by the coral animal are predominantly saturated, plant-like materials. This means that coral animals have exceptional lipogenesis schemes because most marine animals are characterized by polyunsaturated lipids (Youngken and Shimizu, 1975).

Three of the *Stephanocoenia michelinii* samples in Table 2 contained substantial amounts of the marine-type polyunsaturated docosahexaenoic acid. This acid is found in large percentages in many marine animals, including copepods (Jeffries, 1970; Morris, 1971). It is possible that these samples may have compositions which give chemical evidence of their last prey. As stated, the samples

were collected between 10.00 and 13.00 hrs, a time period when *Montastrea annularis* does not typically feed but *S. michelinii* does. Along this depth transect in Discovery Bay, copepods constitute over 90% of the planktonic individuals available for capture both day and night (Porter, in preparation). These prey are digested in less than 3 h (Porter, 1974), but assimilation rates for the products of digestion are not known. It seems unlikely that recent prey items could account for the high percentage composition of the 22:6 fatty acid (nearly 83%), and it seems necessary to postulate some means of accumulation or manufacture to account for so large an amount. It is possible that the presence of substantial amounts of this polyunsaturated acid in the tissues of *S. michelinii* is due to partial alteration of dietary lipids and accumulation of these products as well as recent evidence of diet. If this is true, then corals that actively feed both night and day should have lipid compositions which are more related to those of their prey than corals which feed only at night.

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