J Comp Physiol B (1995) 165: 183–192

ORIGINAL PAPER

S. Al-Moghrabi · D. Allemand · J. M. Couret
J. Jaubert

Fatty acids of the scleractinian coral *Galaxea fascicularis*: effect of light and feeding

Accepted 23 March 1995

Abstract In order to investigate nutritional interactions in the symbiotic scleractinian coral-zooxanthella association, fatty acids of the coral *Galaxea fascicularis* were analysed in two groups of cultured microcolonies. The first group was fed with *Artemia* sp., while the second group was starved. After an initial 1-month period during which both groups were subjected to the same "normal" light conditions (constant irradiance of 125 $\mu E \cdot cm^{-2} \cdot s^{-1}$ and 14:10 h light:dark), a light cap was used to cover the aquarium and keep all the microcolonies in permanent darkness for 20 days. During the light phase of the experiment it was shown that the nutritional status lead to large variations in the percentage of saturated, mono-unsaturated and polyunsaturated fatty acids. Palmitic acid (C16:0) was the most abundant fatty acid in both groups. Important differences between fed and starved microcolonies occurred during the dark phase of the experiment. In the fed group the dark phase was characterized by a significant increase in polyunsaturated fatty acids. Particularly arachidonic acid (C20:4 n-6) became the most important fatty acid followed by docosatrienoic acid (C22:3 n-3). A slight increase in these two fatty acids was also found in the starved group but the bulk of polyunsaturated fatty acids was significantly decreased. In this group, palmitic acid remained the most important fatty acid while an increased concentration of *cis*-vaccenic acid (C18:1 n-7) was found at the end of the experiment. The increased concentration of *cis*-vaccenic acid might indicate that bacteria serve as a source of energy. While the number of zooxanthellae per milligram of protein and the chlorophyll *a* to protein ratio strongly decreased in the starved microcolonies immediately after the beginning of the dark period, the decrease in fed microcolonies was delayed for about 10 days. Furthermore, after 20 days of dark incubation the chlorophyll *a* to protein ratio was the same as measured at the beginning of the dark period. This suggests that in the dark the metabolic requirements of the zooxanthellae are in part met from the animal host through a heterotrophic mode of nutrition.

Key words Zooxanthellae · Fatty acids · Light · Feeding · Coral, *Galaxea*

Abbreviations *CZ* cultured zooxanthellae · *FAME* fatty acid methylester(s) · *FDM* fed dark microcolonies · *FLM* fed light microcolonies · *MUFA* monounsaturated fatty acid(s) · *PUFA* polyunsaturated fatty acid(s) · *SDM* starved dark microcolonies · *SFA* saturated fatty acids · *SLM* starved-light microcolonies · *SW* sea water · *TFA* total fatty acids

Introduction

The symbiotic association between corals and zooxanthallae is thought to be fundamental to their flourishing in oligotrophic tropical oceans. Such an association provides inorganic nutrients otherwise inaccessible to the dinoflagellate host, while the symbiont helps in the processes of animal excretion, calcification and nutrition. The participation of zooxanthellae in the nutrition of the coral is thought to be through translocation of a portion of their photosynthates (Muscatine 1990).

S. Al-Moghrabi[1] · D. Allemand (✉) · J. Jaubert
Observatoire Océanologique Européen, Centre Scientifique de Monaco, Avenue Saint Martin, MC-98000 Monaco, Principality of Monaco

J. M. Couret
Laboratoire de Chimie Analytique, Université de Nice-Sophia Antipolis, F-06108 Nice-Cedex 2, France

Present address:
[1] Marine Science Station, P.O. Box 195, Aqaba-Jordan

Such nutrients can supply between 20 and 150% of the daily respiratory carbon demand of the host (Muscatine et al. 1981; McCloskey and Muscatine 1984; Steen and Muscatine 1984). The excess photosynthetically fixed carbon is either stored in the host, mainly in the form of wax esters and triglycerides (Patton et al. 1977; Harland et al. 1991, 1992b), or it may be excreted as mucus rich in lipids (Benson and Muscatine 1974; Crossland et al. 1980).

Besides this autotrophic source of carbon, the debate within the literature concentrates on the question of whether heterotrophic feeding is a major source relative to the carbon provided by the symbionts (Johannes et al. 1970; Erez 1990). It is likely that corals inhabiting deep water or living in low light need to fed on plankton or absorb dissolved organic matter (Muscatine and Weis 1992). However, the role of this heterotrophic nutrition is not known for shallow-water corals (Johannes 1974). In the coral *Galaxea fascicularis* a light-regulated amino acid uptake mechanism (Al-Moghrabi et al. 1993) may contribute to the coral feeding.

Lipids are particularly useful biomarkers (Sargent et al. 1987). Since some fatty acids are specific to particular organisms they have been used as chemical tracers of nutrient transfer in reef communities (Meyers 1977, 1979), as well as an index to evaluate the source of organic matter in marine environments (De Baar et al. 1983; Hama 1991; Scribe et al. 1991; Ben-Mlih et al. 1992). Meyers (1979) has suggested that the presence of PUFA in symbiotic corals may indicate an external dietary source such as zooplankton, whereas their absence may indicate a reliance upon algal translocation. High light intensities, low nitrogen concentrations and low temperatures were found to increase the concentrations of (n-3) PUFA in algal total lipids (Sargent et al. 1987, 1990).

The total amount of lipids in symbiotic sea anemones and hermatypic corals is about 10–46% of tissue dry weight (Patton et al. 1977; Stimson 1987; Harland et al. 1992a, 1993). In the sea anemonia such lipid levels are found to depend on light (Harland et al. 1992b) and on feeding frequency (Szmant–Froelich and Pilson 1980; Fitt and Pardy 1981). In scleractinian corals similar experimental data are not yet available. Stimson (1987) maintained corals *in situ* in shaded conditions and showed that this treatment significantly reduced lipid content over a period of 1–2 weeks. Analysis of lipids from corals collected at different depth showed that lipid levels are little affected by light (Harland et al. 1992a), while the range of lipid levels could be quite broad (Harland et al. 1993). Latyshev et al. (1991) demonstrated that the degree of fatty acid unsaturation may be affected by the depth of the coral habitat.

The aim of this study is to experimentally investigate host-symbiont interactions by studying (1) the effects of light and hetero- and autotrophic modes of nutrition on fatty acid composition, chlorophyll content and number of zooxanthellae in the microcolonies of the scleractinian coral *G. fascicularis*, and (2) whether heterotrophic nutrition alone can sustain the symbiotic relationship between the animal host and its symbiont zooxanthellae. To this purpose we compared the effect of feeding of two groups of microcolonies kept under light and dark conditions by measuring the change in fatty acid composition, chlorophyll content and number of zooxanthellae with time. A dark period of 20 days was chosen (Stimson 1987). Lipids and fatty acids were measured in the whole association (Harland et al. 1992, 1993). Fatty acids of CZ isolated from *G. fascicularis* and of *Artemia* sp., used as food, were also identified.

Materials and methods

Sampling sites

Colonies of *Galaxea fascicularis* (Linnaeus 1767) were collected in November 1991 in the Gulf of Aqaba near the Marine Science Station of Aqaba (Jordan) at a depth of 2 m. Colonies were packed in humidified plastic bags and transported to the Musée océanographique de Monaco (14 h transport time). Corals were stored in a 300–1 aquarium supplied with filtered SW from the Mediterranean (exchange rate: $2\% \cdot h^{-1}$) heated to 26 ± 0.1 °C and illuminated with constant irradiance of $175\ \mu E \cdot m^{-2} \cdot s^{-1}$ using metal halide lamps (Philips HQI-TS, 400 W) on a 12:12 photo period.

Experimental protocol

Single microcolonies of *G. fascicularis* were prepared as described previously by Al-Moghrabi et al. (1993). One group of 20 microcolonies was fed for 1 month (FLM), once a day, five times a week, with frozen adult *Artemia* sp., while the second group (20 microcolonies) was not fed (SLM). After this initial period both groups were kept under dark conditions for 20 days, during which both groups continue to be subject to the following diet: the first group was fed (FDM) once every 5 days in order to avoid any contamination by fed *Artemia* of fatty acid composition, while the second was starved (SDM). Every 5 days 6–11 specimens were collected for fatty acid analyses.

Cultured zooxanthellae

Zooxanthellae, isolated from *G. fascicularis*, were cultured as described by C. Goiran et al. (unpublished work) in modified ASP-8A medium (Blank 1987) at pH 8.2 in an incubator at 27 °C under a 14:10 (L:D) regime with an irradiance of $100\ \mu E \cdot m^{-2} \cdot s^{-1}$ and relative humidity of 90%.

Extraction of lipids

It is very difficult to study the compartmental distribution of fatty acids between the animal coral host and its symbionts. The reason is the difficulty in obtaining algal material devoid of animal host cells. Our efforts to separate the animal tissue from zooxanthellae indicated high and variable contamination of zooxanthellae with

Table 1 Fatty acid composition (wt % of total FA) of control microcolonies of *Galaxea fascicularis* (*SLM* starved light microcolonies) and mirocolonies fed for one month (*FLM* fed light microcolonies)

Name	FAME	wt % of total FA	
		Control (Starved)	Fed
Myristic acid	C 14:0	1.92	1.84
Pentadecanoic acid	C 15:0	0.04	0.04
Palmitic acid	C 16:0	17.25	26.31
Margaric acid	C 17:0	0.35	0.31
Stearic acid	C 18:0	6.77	6.70
Σ Saturated fatty acids		26.33	35.20
Palmitoleic acid	C 16:1 *n*-7	2.27	6.61
Heptadecenoic acid	C 17:1	0.18	0.08
Oleic acid	C 18:1 *n*-9	1.34	2.98
cis-Vaccenic acid	C 20:1 *n*-11	0.81	1.76
Eicosenoic acid	C 20:1 *n*-11	0.34	0.22
Σ Mono-unsaturated fatty acids		4.94	11.65
Linoleic acid	C 18:2 *n*-6	0.61	0.90
γ Linolenic acid	C 18:3 *n*-6	5.77	6.64
Linolenic acid	C 18:3 *n*-3	–	0.08
Octadecatetraenoic	C 18:4 *n*-3	7.30	5.00
Eicosatrienoic	C 20:3 *n*-6	1.22	2.86
Archidonic acid	C 20:4 *n*-6	14.50	9.61
Eicosatetraenoic	C 20:4 *n*-3	0.08	0.24
Eicosapentaenoic	C 20:5 *n*-3	10.41	5.81
Docosatrienoic	C 22:3 *n*-3	10.10	7.21
Docosapentaenoic	C 22:5 *n*-3	1.26	1.24
Docosahexaenoic	C 22:6 *n*-3	9.55	8.95
Σ Poly-unsaturated fatty acids		60.80	48.54
Σ Unknown fatty acids		7.93	4.61

animal tissue. Such a contamination modified the fatty acid composition of isolated zooxanthellae in a misleading manner. Therefore, the results of fatty acid analyses from freshly isolated zooxanthellae are not included in this paper. Lipid extraction techniques on the whole tissue were then adapted from Harland et al. (1992a). During extraction, precautions against oxidation were taken by working under N_2 gas. Microcolonies were crushed in 20 ml filtered SW and the tissue was completely separated and homogenized by ultrasonication. The method of Folch et al. (1957) as modified by Bligh and Dyer (1959) was used for extraction and purification of lipids from the coral tissue. In brief, this procedure uses a one-phase alcoholic solvent system composed of chloroform: methanol: water (1:2:0.8, v/v/v) to extract lipids. The monolayer was separated by the addition of chloroform: water (1:0.9, v/v), leaving the lipids free of contaminants in the chloroform phase. Any emulsions formed were broken by a stream of N_2. Total lipid weight was determined gravimetrically using a Mettler balance (AT 261 Delta Range, 0.01 mg).

Measurement of fatty acids

The lipids were first saponified with 0.5 $mol \cdot l^{-1}$ methanolic NaOH and esterified to FAME by boiling for 5 min with boron trifloride (BF_3). FAME were recovered by adding NaCl-saturated water to the mixture and extracting twice with petroleum ether (b.p. 40–65 °C) in a separating funnel (Metcalfe and Schmitz 1961). The aqueous solution was drained off. The organic phase, dried with Na_2SO_4, was used for GC analysis.

FAME (0.5 µl) were analysed with a Carlo Erba (GC 6000) gas chromatograph using a 30-m Supelcowax 10 fused silica capillary column (diameter 0.32 mm) held isothermally at 190 °C for 3 min and then increased to 240 °C at 2 °C · min^{-1}, with a final 10-min hold at 240 °C. The carrier gas (He) flow rate was 0.95 ml · min^{-1}. The signal was treated by a Carlo-Erba DP 700 integrator. The identification of fatty acids was based on their retention time relative to a fish standard (Qualmix, Interchim, Sweden). The identification of unknown fatty acids was made using a gas chromatograph mass spectrometer (GC-MS) Carlo-Erba (VG type Trio-1) with a chromatograph (HR Mega). Quantification of fatty acids was achieved by integration of peaks assuming that the response of the flame ionization detector was proportional to the mass of all measured fatty acids. Known quantities of nonadecanoic acid (C19:0) were added to samples prior to esterification. This internal standard was selected because preliminary studies had shown its absence from lipid samples. The use of this standard allowed us to check the protocol and the quantification of fatty acids.

Measurement of protein and chlorophyll content

The results were standardized by the protein concentration. Protein content was determined by the method of Lowry et al. (1957) using an autoanalyser (Alliance Instrument). The protein standard, albumin fraction V, was obtained from Merck, Germany. Chlorophyll *a* and c_2 were determined for each sample by acetone extraction at 4 °C overnight; their concentrations were calculated using the equation of Jeffrey and Humphrey (1975). The number of zooxanthellae was determined from the mean of four replicate counts made at 40 × magnification using a Neubauer Hemacytometer.

Results

Effect of feeding on fatty acids of microcolonies subjected to "normal" light conditions

A total of 21 fatty acids – 5 saturated, 5 mono-unsaturated, 11 poly-unsaturated – were identified from control colonies (i.e. starved) of *G. fascicularis* (Table 1)

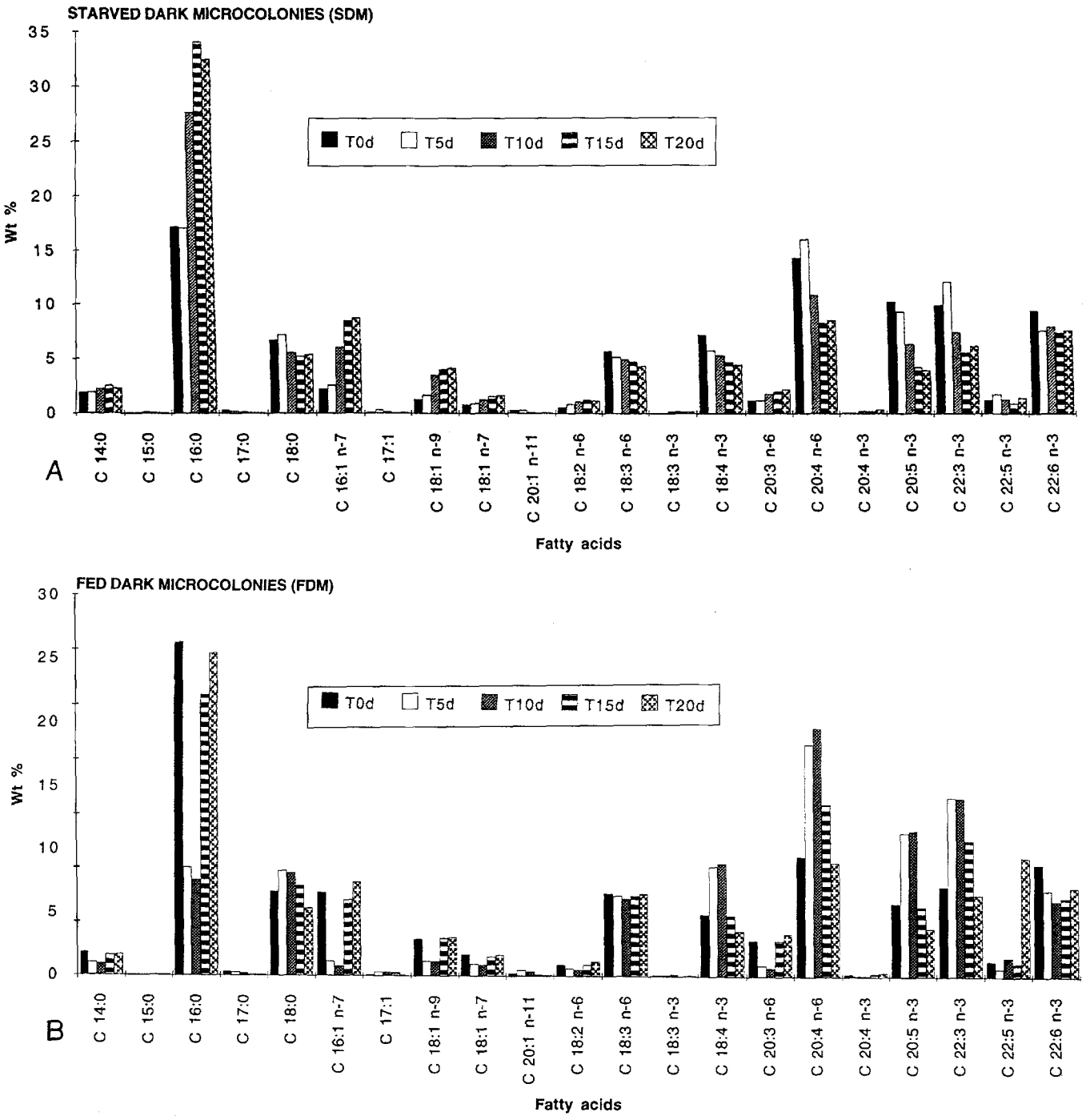

Fig. 1A, B Evolution of fatty acid profile of (**A**) starved and (**B**) fed *Galaxea fascicularis* microcolonies as a function of time of maintenance in permanent darkness. Fatty acid composition is expressed as weight per cent of total fatty acid

with PUFA appearing to be preponderant. By comparison to this control, quantitative (percentage) variations of certain fatty acids were noted after feeding microcolonies under "normal" light conditions for 1 month (FLM). The relative proportions of arachidonic acid (C20:4 n-6) and eicosapentaenoic acid (C20:5 n-3) decreased with feeding while that of palmitic acid (C16:0) increased. The largest quantities of total SFA and MUFA were recorded in FLM, while the highest levels of PUFA were found in SLM.

Effect of incubation in permanent darkness on fatty acids

Figure 1A, B shows the variation in the composition of fatty acids in SDM and FDM, respectively, during 20

Table 2 Fatty acid composition (wt % of total FA) of *Artemia* sp. and cultured zooxanthellae of *Galaxea fascicularis*

Name	FAME	wt % of total FA	
		Artemia	Cultured zooxanthellae
Myristic acid	C 14:0	2.28	6.88
Pentadecanoic acid	C 15:0	0.51	–
Palmitic acid	C 16:0	14.43	44.03
Margaric acid	C 17:0	1.47	–
Stearic acid	C 18:0	6.86	4.12
Σ Saturated fatty acids		25.76	54.53
Palmitoleic acid	C 16:1 *n*-7	18.39	3.24
Heptadecenoic acid	C 17:1	–	–
Oleic acid	C 18:1 *n*-9	15.60	2.95
cis-Vaccenic acid	C 18:1 *n*-7	16.20	–
Eicosenoic acid	C 20:1 *n*-11	–	–
Σ Mono-unsaturated fatty acids		50.19	6.19
Linoelic acid	C 18:2 *n*-6	3.09	1.28
γ Linolenic acid	C 18:3 *n*-6	–	1.66
Linolenic acid	C 18:3 *n*-3	–	–
Octadecatetraenoic	C 18:4 *n*-3	1.34	–
Eicosatrienoic	C 20:3 *n*-6	–	–
Arachidonic acid	C 20:4 *n*-6	4.46	–
Eicosatetracenoic	C 20:4 *n*-3	0.48	–
Eicosapentaenoic	C 20:5 *n*-3	11.50	–
Docosatrienoic	C 22:3 *n*-3	–	–
Docosapentaenoic	C 22:5 *n*-3	–	–
Docosahexaenoic	C 22:6 *n*-3	2.54	21.71
Σ Poly-unsaturated fatty acids		23.41	24.65
Σ Unknown fatty acids		0.65	14.63

days incubation in total darkness. In both groups these variations are only quantitative and limited to certain fatty acids. In SDM, a few fatty acids increased, in contrast to FDM. Palmitic acid (C16:0) doubled in SDM, while it showed a transient decrease in FDM. MUFA, palmitoleic acid (C16:1 n-7), oleic acid (C18:1 n-9), and *cis*-vaccenic acid (C18:1 n-7) increased as a result of dark incubation in SDM, while they remained almost constant in FDM. In addition, the total concentration of MUFA increased in SDM, while it remained constant in FDM, although there was a transient decrease in the first 10 days in permanent darkness.

On the other hand, arachidonic acid (C20:4 n-6) and docosatrienoic acid (C22:3 n-3) increased during the first 10 days of darkness in FDM, while they remained virtually constant during the same period in SDM. Eicosapentaenoic acid (C20:5 n-3) also underwent a transient increase in FDM, while it showed a decrease in SDM. In addition, the total concentration of PUFA increased within FDM while it decreased in SDM during the dark period.

Fatty acids in cultured zooxanthellae and *Artemia* sp.

The fatty acid composition of CZ isolated from *G. fascicularis*, and those of *Artemia* sp. used as the nutritional source, is illustrated in Table 2. Palmitic acid (C16:0) and docosahexaenoic acid (C22:6 n-3) were the two most important fatty acids in CZ. Total SFA formed the bulk of TFA, while MUFA represented only 6.3% of TFA.

In contrast to cultured zooxanthellae, MUFA in *Artemia* sp. formed the bulk (49.79%) of TFA, while SFA and PUFA were almost equally distributed. The largest amounts of fatty acids present in *Artemia* sp. were palmitoleic acid (C16:1 n-7), *cis*-vaccenic acid (C18:1 n-7), oleic acid (C18:1 n-9), palmitic acid (C16:0) and eicosapentaenoic acid (C20:5 n–3). The latter represented 11% of the total lipids of *Artemia* used in our experiments. This led us to classify these *Artemia* sp. in the "marine type" of the classification of Watanabe et al. (1978, in Léger et al. 1986).

Variations of total lipids, chlorophyll and the number of zooxanthellae in FLM, SLM, FDM and SDM

Figure 2 shows the effect of feeding on total lipid concentrations in FLM, SLM, FDM and SDM. After 1 month of feeding the concentration of lipids per mg protein increased in FLM compared with SLM. Under dark conditions, lipid concentration decreased to the same level in both groups before increasing once more after 5 and 10 days for SDM and FDM, respectively. It is noteworthy that the lipid increase in SDM was transient, while it remained stable in FDM during the entire dark incubation period.

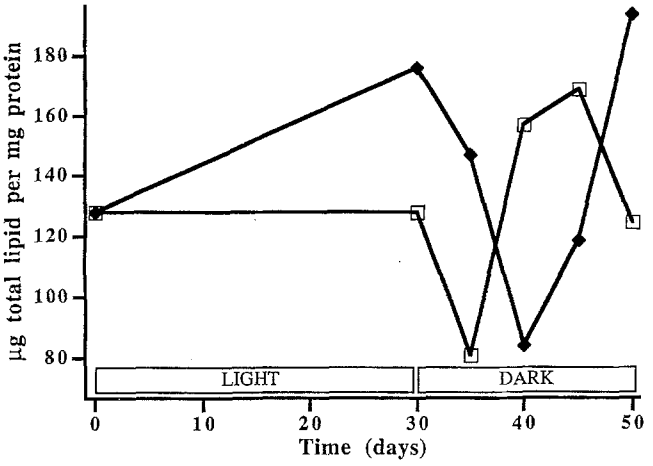

Fig. 2 Lipid concentration (µg lipid per mg protein) in *Galaxea fascicularis* microcolonies subjected to feeding (*filled lozenge*) and starvation (*open squares*) under light and dark conditions

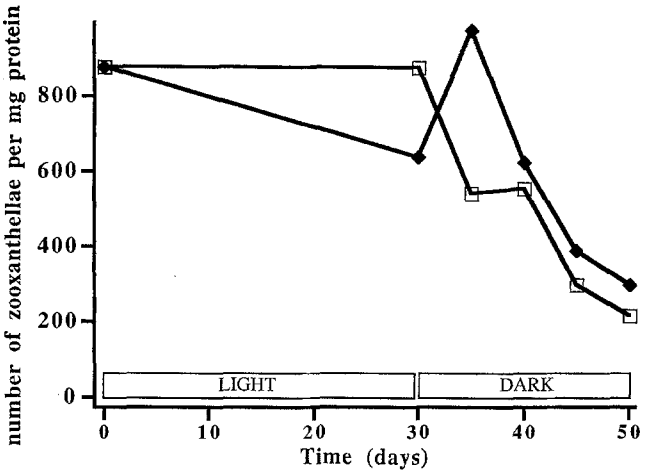

Fig. 4 Number of zooxanthellae (zooxanthella per mg protein) in *Galaxea fascicularis* microcolonies subjected to feeding (*filled lozenge*) and starvation (*open squares*) under light and dark conditions

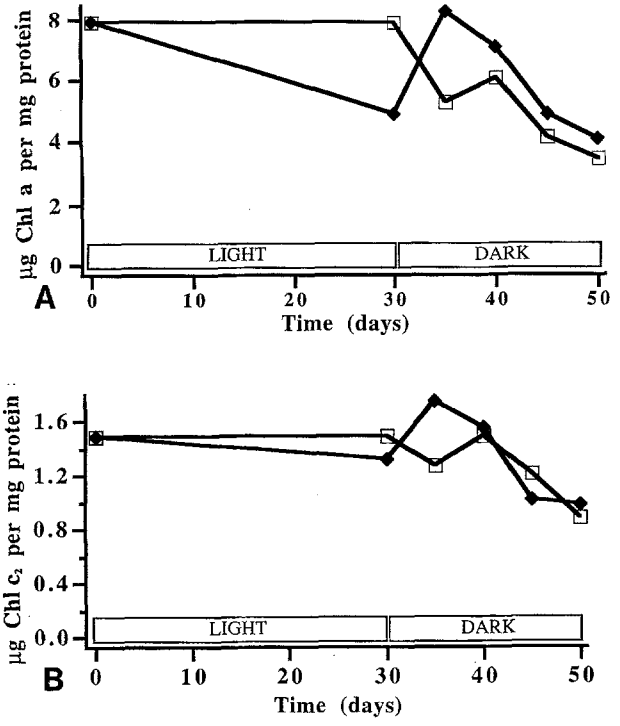

Fig. 3A, B Chlorophyll concentration in *Galaxea fascicularis* microcolonies subjected to feeding (*filled lozenge*) and starvation (*open squares*) under light and dark conditions: **A** Chlorophyll *a* concentration (µg Chl *a* per mg protein); **B** Chlorophyll c_2 concentration (µg Chl c_2 per mg protein)

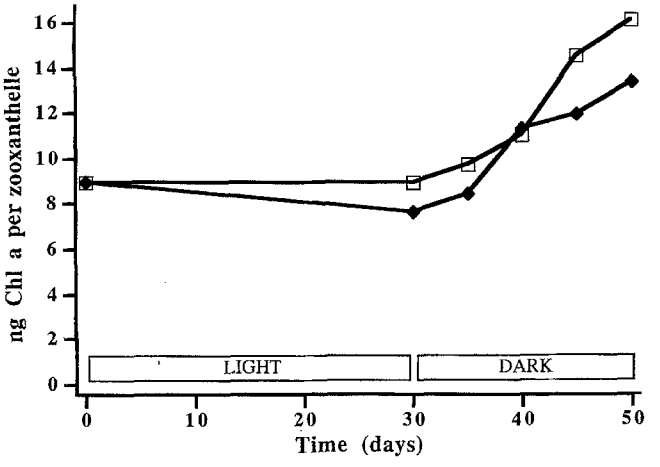

Fig. 5 Chlorophyll *a* concentration (ng Chl *a* per zooxanthella) in *Galaxea fascicularis* microcolonies subjected to feeding (*filled lozenge*) and starvation (*open squares*) under light and dark conditions

Figure 3A, B shows the variations in chlorophyll *a* and chlorophyll c_2 per mg protein, respectively. There was an apparent decrease in the concentration of both chlorophylls after 1 month in FLM. After dark incubation the concentration of both chlorophylls decreased in SDM, while it increased during the first 5 days of dark incubation in FDM before decreasing subsequently.

Figure 4 shows the number of zooxanthellae per mg protein. There was an apparent decrease in the number of cells after 1 month of feeding under light conditions. After dark incubation the number of zooxanthellae decreased in SDM while it increased transiently for the first 5 days of dark incubation in FDM before decreasing subsequently.

Figure 5 shows the effect of feeding and dark incubation on the concentration of chlorophyll *a* per zooxanthella. This concentration slightly decreased in FLM compared with SLM after 1 month of feeding. Dark incubation increased the concentration of chlorophyll *a* per zooxanthella in both SDM and SLM.

Discussion

The problem of standardization

Current standardization parameters include protein concentration, dry weight, chlorophyll *a*, skeletal weight and surface area; the results in this study were standardized by protein concentration. Unfortunately this might introduce an unnecessary ambiguity resulting from the increase in protein concentration due to feeding (i.e. some parameters seemed to have decreased after a given treatment but had actually remained constant or even increased). Such an increase in protein concentration after feeding has been reported by Szmant–Froelich and Pilson (1980), Clayton and Lasker (1984) and Muller–Parker (1985), whereas Fitt and Pardy (1981) did not find any effect of feeding on protein concentration in *Anthopleura elegantissima*. In our experiment, chlorophyll *a* proved to be no better than protein because the responses of zooxanthellae to host feeding are highly variable according to different symbiotic associations (Kevin and Hudson 1979; Szmant-Froelich and Pilson 1980; Fitt et al. 1982; Clayton and Lasker 1984; Muller-Parker 1985).

Skeletal weight – which was not measured during this experiment – is also subject to variations due to feeding. Rasmussen (1988) found that the skeleton of *Acropora formosa* became less dense with high nutrient concentration. This result was confirmed by Risk and Sammarco (1991). Last, but not least, the surface area of *G. fascicularis* microcolonies is a difficult parameter to measure and none of our attempts gave reliable results. Thus, a reliable standardizing parameter is still not available.

Fatty acid composition of cultured zooxanthellae and control *Galaxea fascicularis* microcolonies

The major fatty acids present in CZ are palmitic acid (C16:0) and docosahexaenoic acid (C22:6 n-3). They formed 67% of the total lipid composition. These results are in agreement with reported results on dinoflagellates, characterized by a high percentage of 22:6 (n-3) PUFA (Sargent and Whittle 1981; Loeblich III 1984; Viso and Marty 1993). However, CZ lacked octodecatetraenoic acid (C18:4 n-3) which is abundant in zooxanthellae isolated from *Tridacna maxima* (Bishop et al. 1976) and in free photosynthetic dinoflagellates (Loeblich III 1984). Zooxanthellae freshly isolated from the sea anemone *Anemonia viridis* also lacked this fatty acid (Harland et al. 1991). Its concentration was very low (0.6% TFA) in the dinoflagellate *Amphidinium* sp. (Viso and Marty 1993). This discrepancy in fatty acid profiles has been previously noted and attributed to differences in culture conditions (Viso and Marty 1993).

In control (i.e. starved) microcolonies of *G. fascicularis*, our results show that the five most abundant fatty acids were palmitic acid (C16:0), acrachidonic acid (C20:4 n-6), eicosapentaenoic acid (C20:5 n-3), docosatrienoic acid (C22:3 n-3) and docosahexaenoic acid (C22: 6 n-3). These results are in agreement with Harland et al. (1993). PUFA formed the bulk of TFA.

Published data on PUFA levels in corals are particularly discordant. Although poly-unsaturation is considered characteristic of marine lipids (Lovern 1964; Sargent 1976), Meyers (1977, 1979) and Patton et al. (1977) found low levels of polyunsaturated compounds in corals (<10%). They suggested that this might be explained by the biosynthetic activity of zooxanthellae and concluded that this might be a general property of lipids in symbiotic Cnidaria. On the other hand, Latyshev et al. (1991) and more recently Harland et al. (1993) found a higher level of PUFA (10–60%).

The first possible explanation of this discrepancy is in the experimental conditions used by Meyers (1977, 1979), where the maximum temperature of the gas chromatography was 170 °C. At such a temperature it is very difficult to obtain all the unsaturated fatty acids present. The second possibility involves species differences. Harland et al. (1993), by using the same methodology to study five species, found PUFA to vary from 10% (in *Montastrea annularis*) to 37% (in *Porites porites*). The third possibility is that the zooxanthellae of fed polyps are kept by the host under severe – limitation. This condition has already been associated with a similar increase in the relative abundance of palmitic acid (C16:0) and a similar decrease in the percentage of PUFA (C18:4 n-3) in the unicellular marine algae *Isochrysis galbana* (Sukenik and Wahnon 1991). This severe N limitation practised by the host over its symbiont might be important for maintaining a constant density of zooxanthellae (Porter et al. 1984; Miller and Yellowlees 1989).

Effect of feeding on lipid concentration and fatty acid composition under normal light

The concentration of lipids per mg protein in *G. fascicularis* microcolonies increased in FLM compared with SLM after 1 month of feeding (Fig. 2). This is in agreement with the results of Szmant-Froelich and Pilson (1980) and Clayton and Lasker (1984) who reported an increase in lipid concentration in the temperate coral *Astrangia danae* after zooplankton feeding.

At the end of the first phase of the experiment (normal light), it was shown that the nutritional status leads to some variations in the fatty acid composition of microcolonies (Table 1). Feeding microcolonies with *Artemia* sp. led to an increase of C16:0 and C16:1 n-7, respectively, by a factor 1.52 and 2.9, and a decrease of C20:5 n-3, C20:4 n-6 and C22:3 n-3, respectively, by a factor 1.79, 1.51 and 1.40. Table 2 shows that *Artemia* sp. represent a good source of both C16:0 and C16:1 n-7. Animal fatty acid synthesis pathways are

considered unable to produce n-6 fatty acids (Conway and McDowell Capuzzo 1991). Therefore, the high concentrations of arachidonic acid (C20:4 n-6) and eicosatrienoic acid (C20:3 n-6) in both groups might indicate their origin in the zooxanthellae.

Effect of feeding on lipid concentration and fatty acid composition in total darkness

Dark incubation decreased lipid concentration per mg protein before it started to increase again after 5 and 10 days in SDM and FDM, respectively (Fig. 2). This might be explained by either an initially high lipid metabolic rate, which subsequently fell, or an increase in lipid biosynthesis under dark conditions. Decreased utilization might result from either a slower lipid release rate by coral (Crossland et al. 1980), or decreased coral metabolism (McCloskey and Muscatine 1984). It was shown that the fat and oil content tends to be inversely proportional to the growth rate (Piorreck et al. 1984), and depend on light (Patton et al. 1977; Stimson 1987; Harland et al. 1992) and temperature (Bell et al. 1986). In addition, nutrient deficiencies, especially N deficiency, lead to an increase in the lipid content of most micro-algae (Sargent 1976).

Dark incubation induced variations in the percentage of certain fatty acids in both SDM or FDM (Fig. 1A, B). The increase in palmitic acid (C16:0) after 20 days in dark conditions might be explained by N limitation and the increased need to produce structural rather than storage components. This was confirmed by the increased biosynthesis of chlorophyll *a* as a result of dark incubation (Fig. 5).

It is noteworthy that the total percentage of PUFA increased significantly after 5 days of dark incubation in FDM, while it decreased in SDM. It is most likely that the origin of these PUFA is the external feeding by *Artemia* sp. which represent a good source for this kind of fatty acids (Table 2). This means that feeding maintains or provides high concentrations of PUFA under conditions where zooxanthellae cannot do this job. Meyers et al. (1978) did not find any variations in the fatty acid composition of *Montastrea annularis* with depth and suggested a steady-state balance between zooxanthellae and the animal partner. This steady-state balance might result from the known photoadaptation capacity of zooxanthellae which can compensate for reduced light intensity (Falkowski and Dubinsky 1981).

The ability of symbiotic corals, deprived of zooplankton, to tolerate extended periods of darkness, may be accounted for by postulating that there is a reduction in metabolism in response to substrate depletion, or that an alternative energy source is available to the coral. The known potential sources of energy for corals and their symbionts living under dark conditions may be from bacteria (Sorokin 1973, 1981; Farrant et al. 1987), breakdown of their zooxanthellae (Muscatine 1973), heterotrophic uptake of host-derived organic substrates (Steen 1986, 1987), or uptake of dissolved organic matter (Schlichter 1980; Al-Moghrabi et al. 1993). The simultaneous increase in fatty acids of the n-7 series (C16:1 n-7 and C18:1 n-7) in SDM might indicate a microbial source of nutrition during dark incubation. Although *cis*-vaccenic acid is found in many organisms together with oleic acid, it appears to be present in considerably higher concentrations in bacteria. Indeed, *cis*-vaccenic acid (18:1 n-7) is synthesized by the anaerobic chain elongation pathway found predominantly in bacteria, whereas O_2-dependent desaturases, common to most organisms, produce oleic acid (18:1 n-9). Therefore, it is considered a useful marker for bacteria (Joint and Morris 1982; Wakeham and Canuel 1988; Conway and McDowell Capuzzo 1991; Ben-Mlih et al. 1992). While C16:1 n-7 was not also thought to be strictly bacterial (Sargent et al. 1987), the combination of both n-7 fatty acids suggests that they are of bacterial origin (Ben-Mlih et al. 1992). In the present study, branched chain iso and anteiso C:15 and C:17 fatty acids, which are considered to be good markers for bacteria (Gillan and Johns 1986), were not identified. Since the extent of participation of bacteria, or of zooxanthella digestion and amino acid uptake in our experiments were unknown, additional studies are necessary before considering them further.

Arachidonic acid (C20:4 n-6) increased significantly during the first 10 days of dark incubation in FDM, while it remained constant during the same period in SDM. After 10 days it decreased significantly in both groups. Arachidonic acid in mammals is the immediate precursor of the highly biologically active eicosanoids (prostaglandins and leukotrienes); however, their role in invertebrates is not understood (Stanley-Samuelson 1987). Inhibition of photosynthesis in some unicellular algae is associated with the biosynthesis of eicosapentaenoic acid (20:5 n-3) and the simultaneous increase in the biosynthesis of arachidonic acid (20:4 n-6) (Radwan et al. 1988).

Effect of feeding, light and dark conditions on physiological parameters

The response of symbiotic zooxanthellae to host feeding is highly variable between different symbiotic associations. Three possible responses to host feeding have already been reported: an increase (Szmant-Froelich and Pilson 1980; Clayton and Lasker 1984), no change (Kevin and Hudson 1979; Fitt et al. 1982), and a decrease (Muller-Parker 1985) in symbiont density. In our case, an apparent decrease in chlorophyll *a* and c_2 and in the number of zooxanthellae per mg protein was observed after 1 month of feeding in light. However, it is possible that the absolute concentrations of both chlorophylls and the number of zooxanthellae either

increased or at least remained constant, the apparent decrease in these parameters resulting from a significant increase in protein concentration in that group.

In total darkness, the concentration of both chlorophylls and the number of zooxanthellae per mg protein decreased in SDM and in FDM. However, a transient increase during the first 5 days of dark incubation was found in FDM. Dark incubation increased the concentration of chlorophyll *a* per zooxanthella in both SLM and SDM. Published data are contradictory: Muller-Parker (1984) and Steen and Muscatine (1987) found that under dark conditions and starvation, the sea anemone *Aiptasia pulchella* lost approximately 50% of its zooxanthellae after 10 days. On the other hand, Kevin and Hudson (1979) found that both fed and unfed *Plesiastrea urvillei*, a temperate coral, were able to retain their zooxanthellae in the dark for 48 days. An increase in photosynthetic pigment in zooxanthellae subjected to lower light intensities is one of the reported modes of photoadaptation (Porter et al. 1984).

The results reported in this paper do not agree with previous results considering SFA to be characteristic of Cnidaria. Although dark incubation increased PUFA in the fed group, they decreased in the starved group. On the other hand, our results tend to support the idea that zooxanthellae are N limited. Dark incubation seems to induce several physiological modifications including expulsion of zooxanthellae, increased chlorophyll *a* per zooxanthella and lipid biosynthesis beyond a certain threshold. Understanding the exact role of such fatty acids as palmitic, *cis*-vaccenic and arachidonic acids, and of bacteria as an additional source of nutrition, requires further experimentation.

Acknowledgements The present study is part of the O.O.E. 1991–1995 program implemented with the support of the Council of Europe (Open Partial Agreement on Major Natural and Technological Disasters) and the "Programme National Récifs Coralliens" (PRCO, INSU-ORSTOM). We thank Prof Lucien Elegant for granting access to his laboratory of Analytical Chemistry at the University of Nice, Dr Jacques Cassan for making the mass spectrometry experiments, Drs Danielle Pesando and Catherine Ungar for their suggestions on the manuscript. Thanks are extended to the Marine Science Station of Aqaba (Jordan) for facilitating the collection of corals. S. Al-Moghrabi was the recipient of a grant from the Ministère français des affaires étrangères. The manuscript was greatly improved by comments from the anonymous referees.

References

Al-Moghrabi S, Allemand D, Jaubert J (1993) Valine uptake by the scleractinian coral *Galaxea fascicularis*: characterization and effect of light and nutritional status. J Comp Physiol B 163: 355–362

Baar HJW de, Farrington JW, Wakeham SG (1983) Vertical flux of fatty acids in the north Atlantic Ocean. J Mar Res 41: 9–41

Bell MV, Henderson RJ, Sargent JR (1986) The role of polyunsaturated fatty acids in fish. Comp Biochem Physiol 83B: 711–719

Ben-Mlih F, Marty J-C, Fiala-Médioni A (1992) Fatty acid composition in deep hydrothermal vent symbiotic bivalves. J Lipid Res 33: 1797–1806

Benson AA, Muscatine L (1974) Wax in coral mucus: energy transfer from corals to reef fishes. Limnol Oceanogr 19: 810–814

Bishop DC, Kenrick JR (1980) Fatty acid composition of symbiotic zooxanthellae in relation to their hosts. Lipid 15: 799–804

Blank RJ (1987) Cell architecture of the dinoflagellate *Symbiodinium* sp. inhabiting the Hawaiian stony coral *Montipora verrucosa*. Mar Biol 94: 143–155

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917

Clayton WS, Lasker HR (1984) Host feeding regime and zooxanthellal photosynthesis in the anemone, *Aiptasia pallida* (Verrill). Biol Bull 167: 590–600

Conway N, McDowell Capuzzo J (1991) Incorporation and utilization of bacterial lipids in the *Solemya velum* symbiosis. Mar Biol 108: 277–291

Crossland CJ, Barnes DJ, Borowitzka MA (1980) Dirunal lipid and mucus production in the staghorn coral *Acropora acuminata*. Mar Biol 60: 81–90

Erez J (1990) On the importance of food sources in coral-reef ecosystems. In: Dubinsky Z (ed) Coral reefs, ecosystems of the world 25. Elsevier, Amsterdam, pp 411–418

Falkowski PG, Dubinsky Z (1981) Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature 289: 172–174

Farrant PA, Borowitzka MA, Hinde R, King RJ (1987) Nutrition of the temperate Australian soft coral *Capnella gaboensis*. II. The role of zooxanthellae and feeding. Mar Biol 95: 575–581

Fitt WK, Pardy RL (1981) Effects of starvation, and light and dark on the energy metabolism of symbiotic and aposymbiotic sea anemones, *Anthopleura elegantissima*. Mar Biol 61: 199–205

Fitt WK, Pardy RL, Littler MM (1982) Photosynthesis, respiration, and contribution to community productivity of the symbiotic sea anemone *Anthopleura elegantissima* (Brandt 1835). J Exp Mar Biol Ecol 61: 213–232

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509

Gillan FT, Johns RB (1986) Chemical markers for marine bacteria: fatty acids and pigments. In: Johns RB (ed) Biological markers in the sedimentary environment. Elsevier, Amsterdam, pp 291–309

Hama T (1991) Production and turnover rates of fatty acids in marine particulate matter through phytoplankton photosynthesis. Mar Chem 33: 213–227

Harland AD, Davies PS, Fixter LM (1992a) Lipid content of some Caribbean corals in relation to depth and light. Mar Biol 113: 357–361

Harland AD, Fixter LM, Davies PS, Anderson RA (1991) Distribution of lipids between the zooxanthellae and animal compartment in the symbiotic sea anemone *Anemonia viridis*: wax esters, triglycerides and fatty acids. Mar Biol 110: 13–19

Harland AD, Fixter LM, Davies PS, Anderson RA (1992b). Effect of light on the total lipid content and storage lipids of the symbiotic sea anemone *Anemonia viridis*. Mar Biol 112: 253–258

Harland AD, Navarro JC, Davies PS, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals: total lipids, wax esters, triglycerides and fatty acids. Mar Biol 117: 113–117

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanzen 167: 191–194

Johannes RE (1974) Sources of nutritional energy for reef corals. Proc 2nd Int Coral Reef Symp 1: 133–137

Joint IR, Morris RJ (1982) The role of bacteria in the turnover of organic matter in the sea. Oceanogr Mar Biol Annu Rev 20: 65–118

Kevin KM, Hudson RCL (1979) The role of zooxanthellae in the hermatypic coral *Plesiastrea urvillei* (Milne Edwards and Haime) from cold waters. J Exp Mar Biol Ecol 36: 157–170

Latyshev NA, Naumenko NV, Svetashev VI, Latipov YY (1991) Fatty acids of reef-building corals. Mar Ecol Prog Ser 76: 295–301

Léger P, Bengtson DA, Simpson KL, Sorgeloos P (1986) The use and nutritional value of *Artemia* as a food source. Oceanogr Mar Biol Annu Rev 24: 521–623

Loeblich III AR (1984) Dinoflagellate physiology and biochemistry. In: Spector DL (ed) Dinoflagellates. Academic Press, New York, pp 299–342

Lovern JA (1964) The lipids of marine organisms. Oceanogr Mar Biol Annu Rev 2: 169–191

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275

McCloskey LR, Muscatine L (1984) Production and respiration in the Red Sea coral *Stylophora pistillata* as a function of depth. Proc R Soc Lond B 222: 215–230

Metcalfe LD, Schmitz AA (1961) The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal Chem 33: 363–364

Meyers PA (1977) Fatty acids and hydrocarbons of Caribbean corals. Proc 3rd Int Coral Reef Symp 1: 529–536

Meyers PA (1979) Polyunsaturated fatty acids in coral: indicators of nutritional sources. Mar Biol Letters 1: 69–75

Meyers PA, Porter JW, Chard RL (1978) Depth analysis of fatty acids of two Caribbean reef corals. Mar Biol 49: 197–202

Miller DJ, Yellowlees D (1989) Inorganic nitrogen uptake by symbiotic marine cnidarians: a critical review. Proc R Soc Lond B 237: 109–125

Muller-Parker G (1984) Photosynthesis-irradiance responses and photosynthetic periodicity in the sea anemone *Aiptasia pulchella* and its zooxanthellae. Mar Biol 82: 225–232

Muller-Parker G (1985) Effect of feeding regime and irradiance on the photophysiology of the symbiotic sea anemone *Aiptasia pulchella*. Mar Biol 90: 65–74

Muscatine L (1973) Nutrition of corals. In: Jones OA, Endean R (eds) Biology and geology of coral reefs. II Biology 1. Academic Pres, New York, pp 77–112

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed) Coral Reefs, ecosystems of the World 25, Elsevier, Amsterdam, pp 75–87

Muscatine L, McCloskey LR, Marian ER (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr 26: 601–611

Muscatine L, Weis V (1992) Productivity of zooxanthellae and biogeochemical cycles. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York, pp 257–271

Patton JS, Abraham S, Benson AA (1977) Lipogenesis in the intact coral *Pocillopora capitata* and its isolated zooxanthellae: evidence for a light-driven carbon cycle between symbiont and host. Mar Biol 44: 235–247

Piorreck M, Baasch K-H, Pohl P (1984) Biomass production, total protein, chlorophylls, lipids and fatty acids of fresh water green and blue-green algae under different nitrogen regimes. Phytochemistry 23: 207–216

Porter JW, Muscatine L, Dubinsky Z, Falkowski PG (1984) Primary production and photoadaptation on light and shade adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc Lond B 222: 161–180

Radwan SS, Shaaban AS, Gebreel HM (1988) Arachidonic acid in the lipids of marine algae maintained under blue, white and red light. Z Naturforsch 43C: 15–18

Rasmussen C (1988) Effects of nutrients carried by mainland runoff on reefs of the Cairns area: a research plan and preliminary results. In: Baldwin CL (ed) Nutrients in the Great Barrier Reef region. Great Barrier Reef Marine Park Authority Workshop Series No. 10, pp 66–91

Risk MJ, Sammarco PW (1991) Cross-shelf trends in skeletal density of the massive coral *Porites lobata* from the Great Barrier Reef. Mar Ecol Prog Ser 69: 195–200

Sargent JR (1976) The structure, metabolism and function of lipids in marine organisms. In: Malins DC, Sargent JR (eds) Biochemical and biophysical perspectives in marine biology, vol. 3. Academic Press, New York, pp 149–212

Sargent J, Bell MV, Henderson RJ, Tocher DR (1990) Polyunsaturated fatty acids in marine and terrestrial food webs. In: Mallinger J (ed) Animal nutrition and transport process. 1. Nutrition in wild and domestic animals. Karger, Basel, pp 11–23

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleigh MA (ed) Microbes in the sea. Ellis Horwood, Chichester, pp 119–138

Sargent JR, Whittle KJ (1981) Lipids and hydrocarbons in the marine food web. In: Longhurst AR (ed) Analysis of marine ecosystems. Academic Press, Toronto, pp 491–533

Scribe P, Fillaux J, Laureillard J, Denant V, Saliot A (1991) Fatty acids as biomarkers of planktonic inputs in the stratified estuary of the Krka River, Adriatic Sea: relationship with pigments. Mar Chem 32: 299–312

Schlichter D (1980) Adaptation of cnidarians for integumentary absorption of dissolved organic material. Rev Can Biol 39: 259–282

Sorokin YI (1973) On the feeding of some scleractinian corals with bacteria and dissolved organic matter. Limnol Oceanogr 18: 380–385

Sorokin YI (1981) Aspects of the biomass, feeding and metabolism of common corals of the great barrier reef, Australia. Proc 4th Int Coral Reef Symp 2: 27–32

Stanley-Samuelson DW (1987) Physiological roles of prostaglandines and other eicosanoids in invertebrates. Biol Bull 173: 92–109

Steen RG (1986) Evidence for heterotrophy by zooxanthellae in symbiosis with *Aiptasia pulchella*. Biol Bull 170: 267–278

Steen RG (1987) Evidence for facultative heterotrophy in cultured zooxanthellae. Mar Biol 95: 15–23

Steen RG, Muscatine L (1984) Daily budgets of photosynthetically fixed carbon in symbiotic Zoanthids. Biol Bull 167: 477–487

Steen RG, Muscatine L (1987) Low temperature evokes rapid exocytosis of symbiotic algae by a sea anemone. Biol Bull 172: 246–263

Stimson JS (1987) Location, quantity and rate of change in quantity of lipids in tissue of Hawaiin hermatypic corals. Bull Mar Sci 41: 889–904

Sukenik A, Wahnon R (1991) Biochemical quality of marine unicellular algae with special emphasis on lipid composition. I. *Isochrysis galbana*. Aquaculture 97: 61–72

Szmant-Froelich A, Pilson MEQ (1980) The effects of feeding frequency and symbiosis with zooxanthellae on the biochemical composition of *Astrangia danae* Milne Edwards and Haime. J Exp Mar Biol Ecol 48: 85–97

Viso A-C, Marty J-C (1993) Fatty acids from 28 marine microalgae. Phytochem 34: 1521–1533

Wakeham SG, Canuel EA (1988) Organic geochemistry of particulate matter in the eastern tropical north pacific ocean: Implication for particle dynamics. J Mar Res 46: 183–213

Communicated by H. Huddart