J. Phycol. 24, 328–332 (1988)

EFFECT OF ENVIRONMENTAL CONDITIONS ON FATTY ACID COMPOSITION OF THE RED ALGA *PORPHYRIDIUM CRUENTUM*: CORRELATION TO GROWTH RATE[1]

Zvi Cohen,[2] *Avigad Vonshak and Amos Richmond*

Algal Biotechnology Laboratory, Jacob Blaustein Institute for Desert Research
Ben-Gurion University of the Negev, Sede-Boker Campus 84993, Israel

ABSTRACT

The lipid and fatty acid composition of Porphyridium cruentum *was determined as a function of light intensity, temperature, pH, and salinity. In cultures cultivated at the optimal temperature under non-limiting light conditions, eicosapentaenoic acid was the main polyunsaturated fatty acid. When growth rate was reduced by decreased light intensity, increased cell concentration, suboptimal temperature, suboptimal pH, or increased salinity, the content of eicosapentaenoic acid decreased and that of arachidonic acid increased, the latter becoming the major polyunsaturated fatty acid.*

Key index words: arachidonic acid; eicosapentaenoic acid; environment effects, microalgal mass cultivation; Porphyridium cruentum

The unicellular red marine alga *Porphyridium cruentum* contains arachidonic acid (20:4, n-6) and eicosapentaenoic acid (20:5, n-3) (Pohl and Wagner 1968, Nichols and Appelby 1969, Nyberg 1985), both of which are relatively rare polyunsaturated fatty acids (PUFA). In preliminary experiments, we found that the fatty acid composition of *P. cruentum* changed drastically with temperature and culture density (Cohen et al. 1986). This result was consistent with that reported by Kost et al. (1984) who found variations of 50–100% in the fatty acid composition of this alga and with the recent report of Klyachko-Gurvich et al. (1985) of increased arachidonic acid accumulation in this alga when grown under extreme conditions.

The effect of environmental conditions on the lipids and fatty acids of algae is not fully understood. Similar environmental factors have been shown to exert opposite effects. In *Nitzschia closterium* the content of 20:5 acid decreased when light intensity was increased (Orcutt and Patterson 1974), whereas in *N. palea* it increased (Opute 1973). In higher plants, an increase in irradiance causes an increase in the glycolipids that characterize chloroplast membranes. It is the 18:3 (n-3) acid that generally increases in the glycolipids (Harwood and Russell 1984), but in species lacking this acid, the concentration of other PUFA increases in response to increased light intensity. In *Euglena gracilis,* Pohl and Wagner (1972) found that, when photosynthesis was inhibited by DCMU, the total quantity of glycolipids and their 16:4 acid was drastically decreased. Other studies have also shown that the content of several unsaturated C-16 and C-18 fatty acids, which are prevalent in glycolipids, increased upon illumination (Rosenberg and Pecker 1964, Constantopoulos and Bloch 1967). The results of many studies on the effect of temperature on fatty acid composition (see review by Harwood and Russell 1984) clearly indicate that decreased temperatures result in increased fatty acid unsaturation.

Some PUFA have recently been suggested as having therapeutic value—the 20:5 acid for reducing blood cholesterol (Dyerberg 1986) and the 20:4 acid as a starting material for the synthesis of prostaglandin PGE_2 (Ahern 1984).

The feasibility of outdoor mass production of *P. cruentum* has already been studied (Golueke and Oswald 1962, Vonshak et al. 1985). In this work we studied the effect of light intensity, cell concentration, temperature, pH, and salinity on the fatty acid composition of *P. cruentum.* A clear correlation between growth rate and fatty acid composition was observed.

MATERIALS AND METHODS

Organism. P. cruentum strains 1380 1a–f and B113,80 were obtained from the Gottingen Algal Culture Collection. Stock cultures were maintained according to Vonshak (1986).

Growth conditions. Cultures were grown on Jones' medium (Jones et al. 1963) in glass tubes (80 × 5 cm), incubated in a temperature-regulated water bath (±1° C), illuminated (unless otherwise stated) with four cool-white fluorescent lamps providing 170 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the side of the water bath. The culture was mixed by bubbling an air-CO_2 mixture (99:1) through a sintered glass tube placed at the bottom of each culture tube. Cultures were also grown in Erlenmeyer flasks placed in a New Brunswick incubator shaker model G25 and illuminated from above at a light intensity of 115 $\mu E \cdot m^{-2} \cdot s^{-1}$, under air-$CO_2$ (99:1) atmosphere. For at least three days prior to sampling, the cultures were kept in the exponential stage by daily dilution. The specific growth rate was estimated by measurements of chlorophyll concentration and of turbidity (Vonshak 1986). The pH of the medium was kept constant with a buffer solution (20 mM Tris HCl + 10 mM 2(N-Morpholino)ethanesulfonic Acid (MES) for pHs 7.0–8.5 and 10 mM Tris-HCl + 20 mM MES for pH 5.5–6.5). Final pH was adjusted daily with 1 N NaOH.

Lipid extraction and fractionation. Freeze-dried samples of biomass were extracted with chloroform-methanol-water according to Bligh and Dyer (1959). Lipids were separated by TLC on 20 × 20 cm glass plates coated with silica gel-60 (Merck). Chromatography was carried out in light-protected Teflon-lined jars under an Ar atmosphere. Lipids were eluted with chloroform/ace-

[1] *Accepted: 4 April 1988.*
[2] Address for reprint requests.

TABLE 1. *Effect of light intensity, cell concentration and temperature on fatty acid composition in* P. cruentum.

Temp. (°C)	Light ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Cell conc. (mg chl·L^{-1})	Growth rate (day^{-1})	Fatty acid composition (%)										
				16:0	16:1	18:0	18:1	18:2	18:3	20:2	20:3	20:4	20:5	R[a]
20	170	2	0.65	35.0	3.7	0.2	3.1	8.3	0.2	0.1	0.2	19.3	30.0	0.6
	170	28	S[b]	32.7	1.8	0.5	1.0	8.3	0.3	1.2	1.2	28.8	24.1	1.2
25	170	1.5	1.2	35.0	2.8	0.6	2.0	6.1	0.5	0.3	0.9	15.9	35.8	0.4
	170	12	0.77	36.8	2.3	0.5	0.7	5.8	0.4	0.6	0.6	18.1	34.1	0.5
	170	30	S[b]	33.3	1.4	0.3	1.0	9.0	0.2	0.7	1.7	27.3	25.1	1.1
30	170	1.5	0.98	38.9	2.9	1.1	1.5	11.0	1.0	0.5	0.8	18.6	23.7	0.8
	170	8	0.73	36.3	1.2	1.2	2.7	17.2	1.0	0.6	2.3	28.1	9.4	3.0
	30	3	0.30	33.9	0.1	0.1	2.3	12.0	0.1	0.2	1.5	42.1	7.8	5.4
	170	17	S[b]	31.0	0.5	1.5	7.7	18.5	0.3	1.6	3.3	32.7	2.9	11

[a] Ratio of % 20:4 to % 20:5.
[b] Stationary culture.

tone/methanol/acetic acid/water (100:40:20:20:10) as the developing solvent (Kates 1972). The components were visualized by a brief exposure to I_2 vapors. The lipid-containing bands were scraped off and immediately treated with a methanol-acetyl chloride mixture, as described by Cohen et al. (1987). For analytical purposes, the bands were extracted with chloroform–methanol (9:1), and identity and purity of the lipids were determined by comparison with standard lipids in three different solvent systems and by characteristic color reactions, i.e. α-naphthol for glycolipids and molybdate for phospholipids (Kates 1972). The neutral lipids fraction consisted of all the components of *Rf* values higher than 0.9 and contained tri-, di- and mono-glycerides, free fatty acids, and pigments.

Lipid transmethylation. Freeze-dried samples of *P. cruentum* (100 mg) were transmethylated with 2 mL of methanol/acetyl chloride (95:5), as previously described (Cohen et al. 1987). Heptadecanoic acid was added as an internal standard, and the mixture was sealed in a light-protected Teflon-lined vial under an Ar atmosphere and heated at 80° C for 1 h. The vial contents were then cooled, diluted with 1 mL water, and extracted with 1 mL of hexane containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The hexane layer was dried over Na_2SO_4, evaporated to dryness under vacuum, and redissolved in hexane.

Fatty acid analysis. Gas chromatographic analysis was performed with a SP-2330 fused silica capillary column (30 m × 0.2 mm) at 200° C (injector and flame ionization detector temperatures 230° C, split ratio 1:100). The results were formulated with an HP 3390A integrator. Fatty acid methyl esters were identified by co-chromatography with authentic standards (Sigma) and by gas chromatography-mass spectrometry (GC-MS). GC-MS analyses were performed with a Finnigan 500 mass spectrometer equipped with a carbowax capillary column (30 m × 0.25 mm). Chemical ionization spectra were obtained at 250 eV with isobutane as the reactant gas. The quantity of the fatty acids was determined by comparing their peak areas with that of the internal standard. The data shown are mean values of at least two independent samples, analyzed in duplicate.

RESULTS

The major fatty acids of *P. cruentum* were found to be 16:0, 18:2, 20:4 (n-6) and 20:5 (n-3) under all conditions tested.

An increase in cell concentration resulted in an increase in the proportions of the 18:2 and 20:4 fatty acids accompanied by a decrease in the 20:5 acid (Table 1). Under high light intensity, the calculated ratio R of the % of 20:4 to the % of 20:5 acid of a culture grown at a low cell concentration was 0.78, whereas the R for a culture maintained at a high cell concentration was 3.0. Cultivation under low light intensity produced similar results. The increase in the R value resulted mainly from the decrease in the 20:5 acid and to a lesser extent from the increase in the 20:4 acid (Table 1).

The change in temperature had a more complex effect than light. A temperature shift from the optimum of 25° C (Vonshak et al. 1985) to 20° C or to 30° C at low cell concentration resulted in a predictable reduction in the growth rate (Table 1). The effect of these temperatures on the fatty acids was an increase in R from 0.44 at 25° C to 0.64 at 20° C and to 0.78 at 30° C. The R value correlated with a reduction of about 20% and 46% of the growth rates at 30° C and 20° C, respectively, as compared with the growth rate at 25° C. The largest differences in R were observed at the stationary phase relative to the early exponential phase, i.e. a 14-fold increase at 30° C compared with a 2.5-fold increase at 25° C.

Cultivation of *P. cruentum* at pH values of 5-8.5 resulted in altered growth ratse. Cultivation at suboptimal pH effected an increase in the 20:4 and 18:2 acids and a decrease in the 20:5 acid (Fig. 1). The R value over this pH range increased from 0.64 at pH 7.6 to as high as 1.31 at pH 5.5. In a comparable experiment conducted under lower light intensity, the effect was even greater: at pH 8.5 and 5.0, R was 1.67 and 1.52, respectively. These values were even higher than the corresponding values obtained in a stationary culture at the same temperature and under optimum pH.

Increasing the NaCl concentration in the growth medium from 1M to 2M resulted in retarded growth, reduced fatty acid content and an increased R. NaCl concentrations lower than in the control medium had no effect on the growth rate or on the R value (Table 2).

Lipid fractionation (Table 3) showed the presence of the neutral and polar lipids, monogalactosylacyldiglyceride (MGDG), digalactosylacyldiglyceride (DGDG), and sulfoquinovosylacyldiglyceride (SQ). The phospholipids were phosphatidylcholine (PC), phosphatidylglycerol, phosphatidylethanolamine,

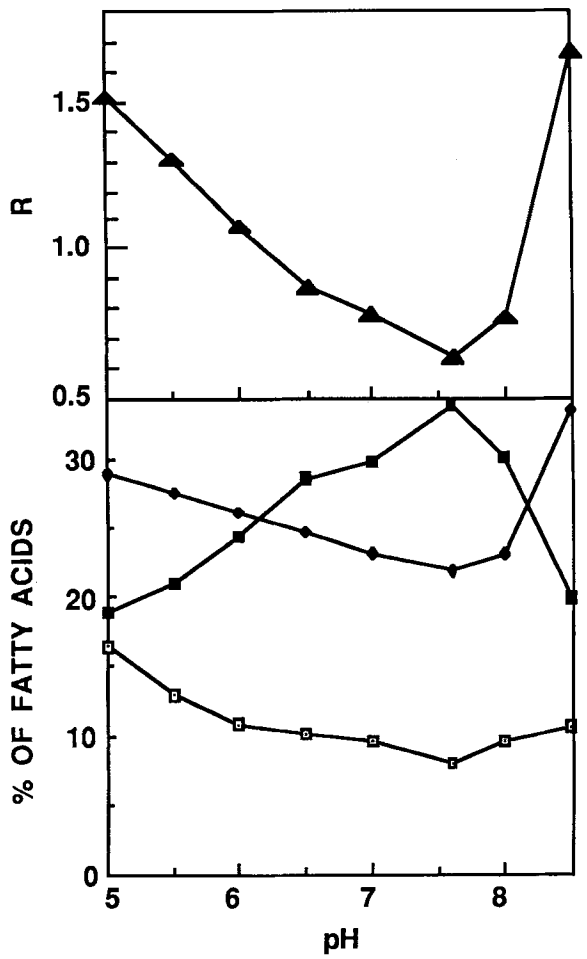

FIG. 1. Effect of culture pH on fatty acid composition of *P. cruentum* and the 20:4/20:5 ratio (R) (▲); 18:2 (□); 20:4 (◆); 20:5 (■). Cultures were cultivated in glass tubes (170 $\mu E \cdot m^{-2} \cdot s^{-1}$).

and phosphatidylinositol. The latter three together comprised only 5–8% of the total lipid content and were not analyzed further. Fatty acid analysis of each lipid class showed that the 20:4 acid is concentrated in the neutral lipids and PC, whereas the 20:5 acid is found mainly in the glycolipids (Table 3).

In cells grown at the optimal temperature (25° C) and low cell concentration, the content of neutral lipids was minimal, and the major lipids were MGDG and DGDG. Increasing the temperature to 30° C resulted in an increase in the neutral lipid content and a decrease in the other lipids at low cell concentration and even more so at high cell concentrations. In the latter case, 58% of total lipids were neutral lipids. A high content of neutral lipids was also found in cultures cultivated at 28° or 30° C under low light intensity (data for 28° C not shown).

Under growth conditions conducive to fast growth rates, (25° C, low cell concentration), the 20:5 acid became the dominant PUFA in every lipid fraction analyzed but PC. In MGDG it represented 49.1% of the fatty acids. Under suboptimal growth conditions of light and temperature, the 20:5 acid content decreased sharply whereas the contents of the 18:2, 18:3, 20:3 and 20:4 acids increased. These effects were maximal during the stationary phase. Similar results were obtained for cultures grown at 28° C (data not shown).

The most significant effect of light and temperature stresses on the fatty acid composition was observed in the lipid composition of cells cultured at 30° C. Increasing the temperature to 30° C resulted in a marked increase of the 16:0 and 18:0 acids in both glycolipids and neutral lipid fractions. This effect was barely observable in the total lipid mixture due to the increase in the proportion of neutral lipids, which were relatively poor in the 16:0 acid.

DISCUSSION

The fatty acid composition of *P. cruentum* was influenced by environmental conditions. Under optimal conditions, which provide the fastest exponential growth, the dominant PUFA was the 20:5 acid. In contrast, under growth-limiting conditions (high cell concentration, low light intensity, nonoptimal temperature and pH, and increased salinity), the 20:4 and 18:2 acids became dominant. The level of this effect was maximal at the stationary phase. The relationships we found between fatty acid composition, environmental conditions and growth rates may explain the quantitative discrepancy between our results and those of Pohl and Wagner (1968), Nichols and Appelby (1969) and Ahern et al. (1983).

The results suggest that the previous reports were based on work conducted with either stationary or

TABLE 2. *Effect of NaCl concentration on growth rate and fatty acid content and composition in* P. cruentum. *Cultures were cultivated (25°C, 115 $\mu E \cdot m^{-2} \cdot s^{-1}$) on an Erlenmeyer shaker.*

NaCl conc. (M)	Specific growth rate (d^{-1})	Total fatty acids (% of AFDW[a])	Fatty acid composition (%)										R[c]
			16:0	16:1	18:0	18:1	18:2	18:3	20:2	20:3	20:4	20:5	
0.25	1.2	4.4	33.5	3.0	0.8	0.7	5.7	0.2	0.6	0.6	17.3	37.5	0.5
0.5	1.2	3.7	33.1	3.9	1.0	1.2	5.9	0.2	0.6	0.5	17.6	36.0	0.5
1	0.88	3.5	31.7	3.9	0.7	0.8	7.5	0.4	0.8	1.0	17.7	35.3	0.5
1.5	0.52	3.0	27.6	2.9	1.0	1.6	13.4	1.1	2.4	1.2	22.6	26.3	0.9
2	0.34	1.2	30.0	4.3	2.2	4.5	15.3	1.2	+[b]	1.4	22.5	18.9	1.2

[a] AFDW = ash-free dry weight.
[b] + = trace amount.
[c] Ratio of % 20:4 to % 20:5.

TABLE 3. *Effect of temperature and cell concentration on fatty acid composition of various lipids*[a] *in* P. cruentum. *Cultures were cultivated at a light intensity of 170* $\mu E \cdot m^{-2} \cdot s^{-1}$.

Lipid	Temp. (°C)	Cell conc. (mg chl·L^{-1})	Fatty acid composition (%)										R[b]	% of total fatty acids
			16:0	16:1	18:0	18:1	18:2	18:3	20:2	20:3	20:4	20:5		
Total	25	1.5	35.1	2.8	0.6	2.0	6.1	0.5	0.3	0.9	15.9	35.8	0.4	
	30	1.5	38.9	2.9	1.1	1.5	11.0	1.0	0.5	0.8	18.6	23.7	0.8	
	30	8	36.3	1.2	1.2	2.7	17.2	1.0	0.6	2.3	28.1	9.4	3.0	
Neutral	25	1.5	21.8	5.3	9.2	7.6	15.5	0.6	0.4	0.8	16.9	21.9	0.8	17
	30	1.5	20.8	1.5	8.3	4.8	20.6	1.7	+[c]	3.6	31.3	7.4	4.2	34
	30	8	25.7	1.1	6.0	4.5	22.9	2.1	0.6	3.2	29.8	4.2	7.1	58
MGDG	25	1.5	31.5	3.6	1.0	3.1	8.3	0.2	0.1	0.1	2.9	49.1	0.06	26
	30	1.5	47.3	2.2	2.8	2.1	9.8	+	0.9	+	6.5	28.4	0.2	18
	30	8	51.0	2.9	4.2	4.9	10.2	1.8	2.4	0.4	13.8	8.4	1.6	14
DGDG	25	1.5	46.9	8.6	0.8	2.2	4.2	0.4	0.2	0.1	1.4	35.2	0.04	26
	30	1.5	64.0	3.6	0.9	1.4	4.0	+	0.2	0.3	2.7	22.9	0.1	15
	30	8	69.5	2.6	1.9	2.6	5.2	1.3	0.1	0.1	8.5	8.3	1.0	9
SQ	25	1.5	55.7	3.0	2.9	8.8	4.7	+	1.2	+	2.5	21.2	0.1	13
	30	1.5	65.9	0.6	2.0	3.6	3.7	0.8	2.7	1.2	8.0	11.5	0.7	10
	30	8	68.8	0.8	5.8	5.1	9.1	0.7	2.7	1.0	3.3	2.6	1.3	8
PC	25	1.5	30.9	6.0	2.6	3.6	6.7	2.0	2.3	3.0	38.9	3.9	10	9
	30	1.5	31.7	2.0	2.1	3.1	9.5	0.9	+	2.0	42.3	6.4	6.6	8
	30	8	26.0	+	4.4	5.2	9.2	1.5	0.6	3.5	46.6	2.9	16	6

[a] MGDG, monogalactosylacyldiglyceride; DGDG, digalactosylacyldiglyceride; SQ, sulfoquinovosylacyldiglyceride; PC, phosphatidylcholine.

[b] Ratio of % 20:4 to % 20:5.

[c] + = trace amount.

severely light limited cultures. The R values which we calculated for their data were in the range of 2.1 to 2.9, and the proportion of the 18:2 acid was 15–16% of total fatty acids. The rise in the content of the 18:2 acid was essentially a marker of the proportion of neutral lipids, since large quantities of the 18:2 acid were found only in this lipid class. High levels of the 18:2 acid can thus be correlated with high levels (>50%) of neutral lipids, which accumulate only under slow growth conditions. The relationship of fatty acid composition to growth rate also explains the variations of 50 to 100% in the fatty acid composition found by Kost et al. (1984) for *P. cruentum* cultures grown under apparently identical conditions. We found that the fatty acid composition changed as batch cultures became dense and light became growth limiting. In contrast, in a chemostat culture in which an exponential growth rate was kept constant, the fatty acid composition also remained constant (data not shown).

It appears that the relative variations described here in the proportions of the 20:4 and 20:5 acids in response to environmental factors are not limited to *P. cruentum*. An analysis of the data of Pohl and Zurheide (1982) and of Kayama et al. (1986) reveals similar variations in the R value of various algae in response to changes in light or temperature. A controversy exists regarding the fatty acid composition of the red macroalga *Gracilaria confervoides*. Pohl and Wagner (1968) and Araki et al. (1986) reported that the 20:4 acid was the main PUFA, whereas Sato (1975) and Hayashi et al. (1974) claimed the 20:5 acid to be the main PUFA. We suggest that this disagreement could possibly stem from the different environmental conditions that govern the growth rate of the alga.

The increase of the 20:5 acid in response to increased light intensity is analogous with the previously mentioned increase in the 18:3 (n-3) acid in plants and algae in response to enhanced illumination (Rosenberg and Pecker 1964, Constantopoulos and Bloch 1967). Kates and Volcani (1966) suggested that in diatoms the 20:5 acid fulfills a role in photosynthesis similar to that of the 18:3 acid (n-3) in other plants and algae. *P. cruentum* does not contain a significant quantity of the 18:3 (n-3) acid, and the 20:5 acid may have an analogous role to that of the 18:3 acid. When growth is retarded, less 20:5 acid and glycolipids are produced. It appears, however, that in *P. cruentum* this effect relates to the growth rate rather than directly to the light intensity, since it occurs not only as a result of light limitation but also in response to any factor limiting growth, such as nonoptimal pH and salinity (Fig. 2), which affect an overlapping R curve.

The temperature effect on the fatty acid composition of *P. cruentum* is similar to the effects of other factors affecting growth. The effect of cultivation at 30° C on fatty acid composition in terms of increased R and 18:2 acid was, however, more intense than could be expected on the basis of growth rate retardation alone. The increase in unsaturation at the lower growth temperatures was explained by the need to compensate for the decrease in membrane fluidity at low temperatures (Marr 1962). The influence of temperature on the content of the 20:5

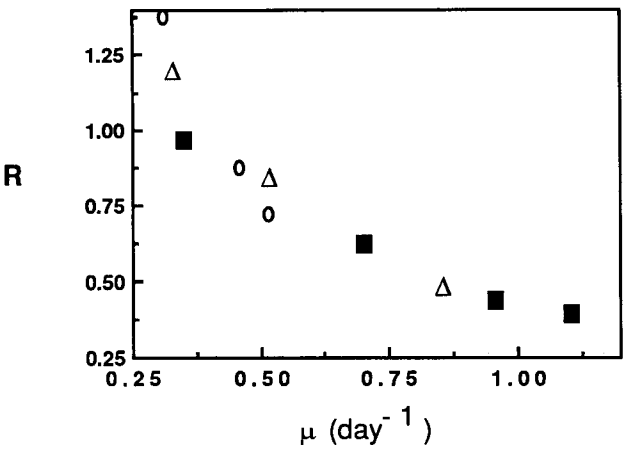

FIG. 2. Correlation between the specific growth rate (μ) and the 20:4/20:5 ratio (R) in *P. cruentum* as affected by the environmental variables, cell concentration (■), salinity (△) and pH (○).

acid seems to be more intense than on that of the 20:4 acid, since most of the 20:5 acid was found in glycolipids, which are the main membrane lipids. This may result in preferential desaturation towards the 20:5 acid. We suggest that temperature exerts a two fold effect on the composition and content of the fatty acids. First, any deviation from the optimum leads to reduction in the growth rate and to an accumulation of the 18:2 and the 20:4 acids as well as a decrease in the 20:5 acid. Second, the direct effect of high temperature (30° C) is expressed in *P. cruentum* by a rise in saturated fatty acids (16:0 and 18:0) and in a reduction in the 20:5 acid. Thus, at supraoptimal temperatures, the two effects will be similar, whereas at suboptimal temperatures the contents of the 16:0 and 18:0 acids are reduced and that of the 20:5 acid increases. The direct effect of temperature on fatty acid composition and the temperature effect on the growth rate and thus on the fatty acids oppose each other and as a result the increases in the 20:4 acid and in the value of R at 20° C are rather modest.

We are grateful to Dr. Sammy Boussiba for helpful discussions, to Ms. Dorot Imber for editing the manuscript and to Ms. Shoshana Didi for dedicated technical assistance. This study was supported by a grant from the Israeli National Council for Research and Development. Contribution No. 31 from the Algal Biotechnology Laboratory, Jacob Blaustein Institute for Desert Research.

Ahern, T. J. 1984. Plant-derived catalysts and precursors for use in prostaglandins synthesis. *J. Am. Oil Chem. Soc.* 61:1754–7.

Ahern, T. J., Katoh, S. & Sada, E. 1983. Arachidonic acid production by the red alga *Porphyridium cruentum. Biotech. Bioeng.* 25:1057–70.

Araki, S., Sakurai, T., Oohusha, T. & Kayama, M. 1986. Component fatty acids of lipid from *Gracilaria verrucosa. Bull. Jap. Soc. Sci. Fish.* 52:1871.

Bligh, E. G. & Dyer, W. J. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911–7.

Cohen, Z., Vonshak, A. & Richmond, A. 1986. The effect of environmental conditions on fatty acid composition of *Porphyridium cruentum. In* Stumpf, P. K., Mudd, J. B. & Ness, W. D. [Eds.] *The Metabolism, Structure, and Function of Plant Lipids.* Plenum Press, New York, pp. 641–4.

——— 1987. Fatty acid composition of *Spirulina* strains grown under various environmental conditions. *Phytochemistry* 26: 2255–8.

Constantopoulos, G. & Bloch, K. 1967. Effect of light intensity on the lipid composition of *Euglena gracilis. J. Biol. Chem.* 242: 3538–51.

Dyerberg, J. 1986. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutr. Rev.* 44:125–34.

Golueke, C. G. & Oswald, W. J. 1962. The mass culture of *Porphyridum cruentum. Appl. Microbiol.* 10:102–7.

Harwood, J. L. & Russell, N. J. 1984. *Lipids in Plants and Microbes.* George Allen & Unwin, London, pp. 64–5.

Hayashi, K., Kida, S., Kato, K. & Yamada, M. 1974. Component fatty acids of acetone-soluble lipids of 17 species of marine benthic algae. *Bull. Jap. Soc. Sci. Fish.* 40:609–17.

Jones, R. E., Speer, L. & Kury, W. 1963. Studies on the growth of the red alga *Porphyridium cruentum. Physiol. Plant.* 16:636–43.

Kates, M. 1972. *Techniques of Lipidology.* American Elsevier, New York, pp. 363–469.

Kates, M. & Volcani, B. E. 1966. Lipid components of diatoms. *Biochim. Biophys. Acta* 116:264–78.

Kayama, M., Iijima, N., Sado, T., Mankura, M., Araki, S. & Sakurai, T. 1986. De novo syntheses and conversion of fatty acids in *Porphyra. Bull. Jap. Soc. Sci. Fish.* 52:575.

Klyachko-Gurvich, G. L., Yureva, M. I. & Semenenko, V. E. 1985. Specificity of the fatty acid composition of acyl-containing lipids in the unicellular red alga *Porphyridium cruentum. Fiziologiya Rastenii* 32:115–23.

Kost, H. P., Senser, M. & Wanner, G. 1984. Effect of nitrate and sulfate starvation on *Porphyridium cruentum* cells. *Z. Pflanzenphysiol.* 113 suppl.:231–49.

Marr, A. G. 1962. Effect of temperature on the composition of fatty acids in *E. coli. J. Bacteriol.* 84:1260–8.

Nichols, B. W. & Appelby, R. S. 1969. The distribution of arachidonic acid in algae. *Phytochemistry* 8:1907–15.

Nyberg, H. 1985. The influence of ionic detergents on the phospholipid fatty acid compositions of *Porphyridium purpureum. Phytochemistry* 24:435–40.

Opute, F. I. 1973. Lipid and fatty acid composition of diatoms. *J. Exp. Bot.* 25:823–35.

Orcutt, D. M. & Patterson, G. W. 1974. Effect of light intensity upon lipid composition of *Nitzchia closterium* (*Cylindrotheca fusiformis*). *Lipids* 9:1000–3.

Pohl, P. & Wagner, H. 1968. Inhaltsstoffe von algen—II. Über die unterschiedliche fettsaurezusammensetzung von salz und susswasseralgen. *Phytochemistry* 7:1565–72.

——— 1972. Control of fatty acid and lipid biosynthesis in *Euglena gracilis* by ammonia, light and DCMU. *Z. Naturforsch.* 27:53–61.

Pohl, P. & Zurheide, F. 1982. Fat production in freshwater and marine algae. *In* Hoppe, H. A., Levring, T. & Tanaka, Y. [Eds.] *Marine Algae in Pharmaceutical Science.* Walter de Gruyter & Co., Berlin, pp. 65–80.

Rosenberg, A. & Pecker, M. 1964. Lipid alterations in *Euglena gracilis* cells during light-induced greening. *Biochemistry* 3: 254.

Sato, S. 1975. Effects of environmental factors on the lipid components of *Porphyra* species. *Bull. Jap. Soc. Sci. Fish.* 41:1173–83.

Vonshak, A. 1986. Laboratory techniques for the cultivation of microalgae. *In* Richmond, A. [Ed.] *Handbook of Microalgal Mass Culture.* CRC Press, Boca Raton, Florida, pp. 117–45.

Vonshak, A., Cohen, Z. & Richmond, A. 1985. The feasibility of mass cultivation of *Porphyridium. Biomass* 8: 13–25.