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Lipids, and their constituent fatty acids, of *Phaeocystis* sp. from the Arabian Gulf

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Abstract

This paper reports on the first *Phaeocystis* sp. (hereafter *Phaeocystis*) bloom in the Arabian Gulf. Total lipids from three *Phaeocystis* colonies sampled in November 1987 and in March and May 1988, comprised ca 11.0% of the dry biomass. Triacylglycerols (TG) and/or free fatty acids (FA) predominated in the lipids of all three samples. Polar lipids consisted of mixtures of phospholipids and glycolipids. Diacylglycerotrimethylhomoserines (DGTH) were present in all extracts, whilst conventional phospholipids only occurred in low concentrations. The predominant glycolipids were monogalactosyldiacylglycerols (MGDG) digalactosyldiacylglycerols (DGDG), sulfoquinovosyldiacylglycerols (SQDG) and steryl glycosides (SG). The predominant fatty acids in the total lipids of all three samples were palmitic (16:0) and oleic (18:1) acids. Total lipids of the November sample contained the smallest proportion of palmitic and the largest of polyunsaturated fatty acids (PUFA) – especially C_{16}. The proportion of PUFA was considerable in individual polar lipids. C_{16} PUFA were more common in individual polar lipid classes of the November and March samples than that of May. The concentration of constituent C_{18} PUFA was relatively low. PUFA with chains longer than 18 C atoms only occurred in very low concentrations, and were confined to certain polar and nonpolar lipid classes of the November sample only.

Introduction

Phaeocystis sp. (hereafter *Phaeocystis*) is distributed worldwide (Kashkin 1963) both in warm (Lohmann 1920, Gaarder 1954) and cold (Weiße 1983, Bätje and Michaelis 1986) waters. Blooms have been repeatedly observed directly after the breakdown of diatom blooms associated with the enrichment of water with soluble inorganic nitrogenous compounds (Jones and Haq 1963, Boalch 1984, Bätje and Michaelis 1986). However, Lancelot et al. (1987) believed that *Phaeocystis* only proliferated in large abundance in its colonial form – utilizing the gelatinous polysaccharide material, after the diatoms have initiated the process of nutrient depletion. *Phaeocystis* blooms are interesting from both environmental and economical points of view. On the one hand, during their breakdown, strong blooms liberate large amounts of proteins and carbohydrate material (Guillard and Hellebust 1971, Lancelot 1983, Eberlein et al. 1985) which normally produces nuisance, massive foam-banks that cover whole beaches (Bätje and Michaelis 1986, Lancelot et al. 1987). On the other hand, it has been found that cultured colonies constitute an important food source for aquatic animals such as copepods (Weiße 1983). However, Verity and Smayda (1989) found that *Phaeocystis*, both in the colonial and in the solitary-cell form, was not as satisfactory a food source as chain-forming diatoms for two *Acartia* species, i.e. *A. hudsonica* and *A. tonsa*. Natural *Phaeocystis* colonies don't seem to be efficiently grazed by zooplankton, and ungrazed colonies lead to the production of foam on beaches (Lancelot et al. 1987). These facts underline the need to study the precise chemical composition of this organism in different seasons. This is especially true in view of the fact that the polyunsaturated fatty acid (PUFA) content is one of the factors affecting its value as a food source. As far as *Phaeocystis* lipids are concerned, there is only one publication in the available literature which solely describes the fatty acid pattern of total lipids from one sample of this alga (Sargent et al. 1985).

In recent years we have observed the start of *Phaeocystis* blooms in Kuwaiti coastal waters of the Arabian Gulf. This observation is interesting in view of the extreme climate of this region – summer temperatures frequently exceed 50 °C with surface-water temperatures over 36 °C. There are no earlier reports of such blooms in this area, although Jacob and Zarba (1979) recorded unidentified 1 to 3 cm brownish algal bladders (257 m^{-3}). In the present study we describe a spring bloom of *Phaeocystis* in the Arabian Gulf and report

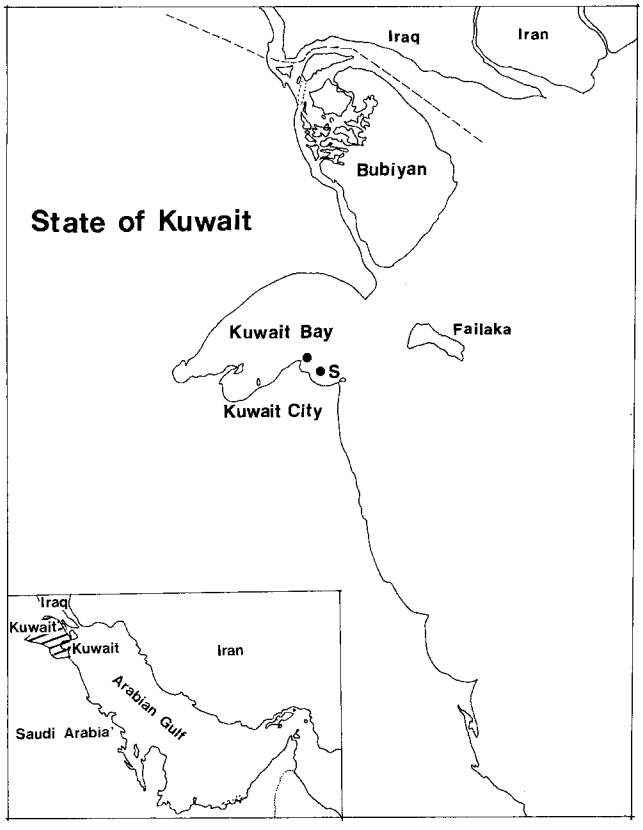

Fig. 1. Map of Kuwait Bay indicating coastal locations (•) of sampling and observation sites. Samples from location S

fully on the lipids and fatty acid composition of samples collected in three different seasons.

Materials and methods

Field observations and sampling

Observations on *Phaeocystis* were recorded between October 1987 and December 1988 at two coastal locations in Kuwait Bay (Fig. 1), and surface-water samples were collected monthly for phytoplankton analysis. *Phaeocystis* colonies were counted and total cell numbers calculated by microscopically counting the mean cell number per colony. Cells of other phytoplankton were counted using the inverted microscope method (Lund et al. 1958). Total chlorophyll contents were determined spectrophotometrically (Parsons and Strickland 1965) after extraction with 90% acetone in water (Hansmann 1973). Surface-water temperature, salinity, and pH were measured using an in situ water checker (Hydrolab Company). Ammonium- and Nitrate-nitrogen (NH_3-N and NO_3-N) were determined following established procedures (Grashoof 1976).

Three samples of *Phaeocystis* were harvested for lipid extraction and analysis in mid November 1987, and in March and May 1988. Large *Phaeocystis* colonies (1 to 3 cm diam) were collected individually and immediately dipped in boiling isopropanol under gaseous nitrogen to kill cells and deactivate lipases (Kates 1972, Radwan 1984). Isopropanol samples, ca 300 colonies for each analysis, were then transferred to the laboratory for further study.

Extraction and purification of total lipids

All solvents were freshly redistilled and, as far as feasible, all steps were performed under oxygen-free nitrogen. Lipids were extracted three times with isopropanol:chloroform (1:2 by vol.; Nichols 1964) and purified according to Folch et al. (1957).

Analysis of lipids

Lipid extracts were analyzed by thin layer chromatography (TLC) on silica-gel plates. Nonpolar lipid classes were fractionated using the solvent hexane:diethyl ether:acetic acid (90:10:1 by vol.; Mangold and Malins 1960). Ionic and other polar lipids were resolved by two-dimensional chromatography (Nichols 1964) using chloroform:methanol:7*N* ammonium hydroxide (65:25:4 by vol.) in the first direction and chloroform:methanol:acetic acid:water (170:25:25:4 by vol.) in the second. Glycolipids were resolved from phospholipids by unidimensional chromatography using acetone:benzene:water (91:30:8 by vol.; Pohl et al. 1970). Lipid fractions were detected by charring after spraying plates with 50% H_2SO_4 and were quantified densitometrically, using an LKB Ultroscan XL laser densitometer. Individual lipid classes were identified by their chromatographic behaviour using standards and specific spray reagents (Dittmer and Lester 1964, Siakotos and Rouser 1965, Stahl 1967, Vioque 1984).

Analysis of constituent fatty acids

Samples of total lipids and individual lipid classes resolved by TLC (see Radwan 1978) were subjected to methanolysis (Chlavardijian 1964) by heating samples with 2% H_2SO_4 in absolute methanol under nitrogen for 90 min at 90 °C. The resulting methyl esters of fatty acids were extracted with hexane, purified by preparative TLC and analyzed by argentation TLC and gas-liquid chromatography (GLC). Argentation TLC was performed on silica-gel plates impregnated with 5% silver nitrate using hexane:diethyl ether (87:13 by vol.). GLC was performed using a Pye-Unicam 204 chromatograph equipped with a glass column (1.83 m × 4 mm i.d.) packed with 15% (by wt) diethylene glycol succinate (DEGS) on a Anakrom D (100 to 120 mesh, operated isothermally at 180 °C with nitrogen as the carrier gas). For confirmation, the same analyses were repeated using a nonpolar column (10% Silar 5 CP on Gas-Chrom Q). Individual fatty acids were identified by comparing their retention times with those of standard samples (16:0, 16:1, 18:0, 18:1, 18:2, 18:3 and 20:4) as well as by using the "equivalent chain method" (16:2, 16:3, 18:4 and 18:5) of Miwa et al. (1960).

Results

Ecological observations

Seasonal variations in the total phytoplankton and *Phaeocystis* populations as well as the total chlorophyll, NH_3-N and NO_3-N contents in Kuwaiti coastal waters between October 1987 and December 1988 are presented in Fig. 2. These variations were marked with autumnal (November) maxima, with the exceptions of *Phaeocystis* numbers which exhibited a peak in spring (March), and NH_3-N content which was highest in May and October. The phytoplankton population consisted, predominantly, of diatoms (>85%); minor constituents were dinoflagellates (<10%) and cyanobacteria (<3%). *Phaeocystis* colonies commenced their appearance in November and exhibitited an extensive bloom in March; colonies (1 to 3 cm diam) washed onto the beach formed a thick brown, jelly layer ca 5 cm. The surface-water temperature increased from 10° in November to 18° in March and 28 °C in June, and the corresponding salt contents were 37, 36 and 38%.

Lipid composition

The lipid content of *Phaeocystis* samples collected in November 1987, and in March and May 1988 was ca 11% of the dry biomass. In all extracts nonpolar lipids predominated over polar, although extracts from March and May samples contained much more polar lipids than those from November (Table 1). In the November sample the most dominant class was triacylglycerols (TG); in March, free fatty acids (FA) and sterols; and in May TG free FA were the predominant classes. In addition, trace amounts of steryl esters (SE) and hydrocarbons (HC) were present in all extracts.

Polar lipids consisted of mixtures of phospholipids and glycolipids. Diacylglycerophosphocholines (PC), diacylglycerophosphoethanolamines (PE), diacylglycerophosphoseries (PS) and diacylglycerophosphoglycerols (PG) occurred in low concentrations. Glycolipids were those found in other photosynthetic systems, namely monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG) and sulfoquinovosyldiacylglycerols (SQDG). In addition considerable amounts of sterylglycosides (SG) were present and were most abundant in November and March samples. Diacylglycerotrimethylhomoserines (DGTH) were detected in small amounts in the different extracts – this lipid was only tentatively identified by its chromatographic behaviour, its positive reaction to Dragendorff's reagent and its lack of phosphorus and sugar moieties. A few, other unidentified polar lipids occurred in trace amounts (Fig. 3).

Fatty acid patterns

The results in Table 2 show that the fatty acids of total lipids from *Phaeocystis* consisted predominantly of palmitic (16:0) and oleic (18:1) acids, a result confirmed by argentation TLC which indicated that saturated and monoenoic fatty acids predominated (Fig. 4). The data in Table 2 also illustrate that small amounts of C_{18} PUFA with 2 to 5 double bonds were present and that eicosatetraenoic acid (20:4) occurred only in trace amounts. Lipids from the November sample contained smaller proportions of palmitic acid (16:0)

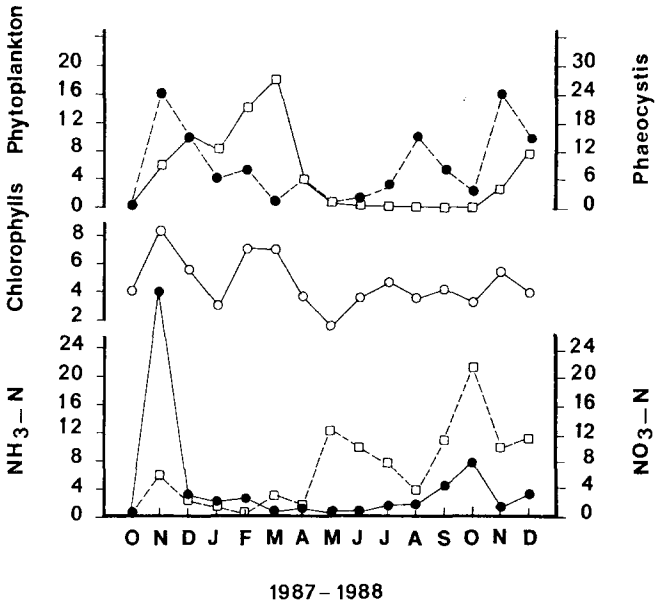

Fig. 2. Seasonal variations in phytoplankton and nutrients in Kuwait Bay. ●—●: Phytoplankton cell no. ($\times 10^4\ l^{-1}$); □—□: *Phaeocystis* sp. cell no. ($\times 10^5\ l^{-1}$); ○—○: total chlorophyll ($mg\ m^{-3}$); □---□: ammonium nitrogen ($NH_4^- - N$; $\mu g\text{-at}\ l^{-1}$); ●—●: nitrate nitrogen ($NO_3^- - N$; $\mu g\text{-at}\ l^{-1}$)

Table 1. *Phaeocystis* sp. Lipid composition of samples collected in November 1987, and March and May 1988. Data expressed as % of total lipids

Lipid class	November	March	May
Hydrocarbons	Trace	Trace	Trace
Steryl esters	Trace	Trace	Trace
Triacylglycerols	62.4	4.2	24.0
Free fatty acids	16.0	42.9	37.9
Sterols	11.1	20.9	6.4
Polar lipids	9.6	32.0	31.7

Table 2. *Phaeocystis* sp. Constituent fatty acids of total lipids from samples collected in 1987 and 1988. Data expressed in % wt

Fatty acid	November	March	May
16:0	22.0	46.0	43.9
16:1	3.9	Trace	0.2
16:2	1.8	Trace	Trace
16:3	Trace	Trace	Trace
18:0	7.7	Trace	Trace
18:1	40.3	47.7	48.8
18:2	5.3	Trace	Trace
18:3	6.0	0.3	0.3
18:4	5.2	1.2	1.2
18:5	7.8	4.8	5.4
20:4	Trace	Trace	Trace

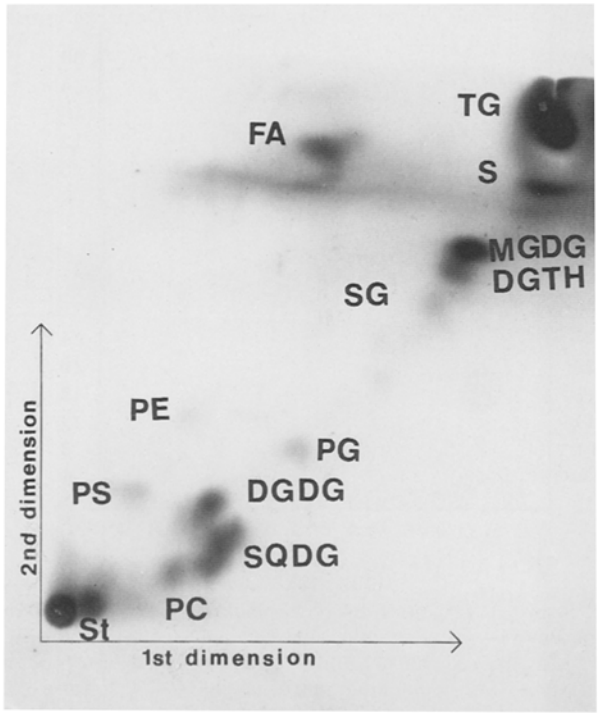

Fig. 3. *Phaeocystis* sp. Typical two dimensional thin layer chromatogram (TLC) showing lipid composition of March sample [TLC performed using silica gel G-plates and the following solvent systems chloroform:methanol:7*M*-ammonium hydroxide (65:25:4, by vol.), in the first dimension and chloroform:methanol:acetic acid:water (170:25:25:2, by vol.), in the second dimension. Lipid fractions visualized by charring after spraying with 50% H_2SO_4.] TG: triacylglycerols; S: sterols; MGDG: monogalactosyldiacylglycerols; DGTH: diacylglycerotrimethylhomoserines; PG: diacylglycerophosphoglycerols; PE: diacylglycerophosphoethanolamines; DGDG: digalactosyldiacylglycerols; SQDG: sulphoquinovosyldiacylglycerols; SG: sterylglycosides; PC: diacylglycerophosphocholines; PS: diacylglycerophosphoserines; St: start; FA: fatty acids

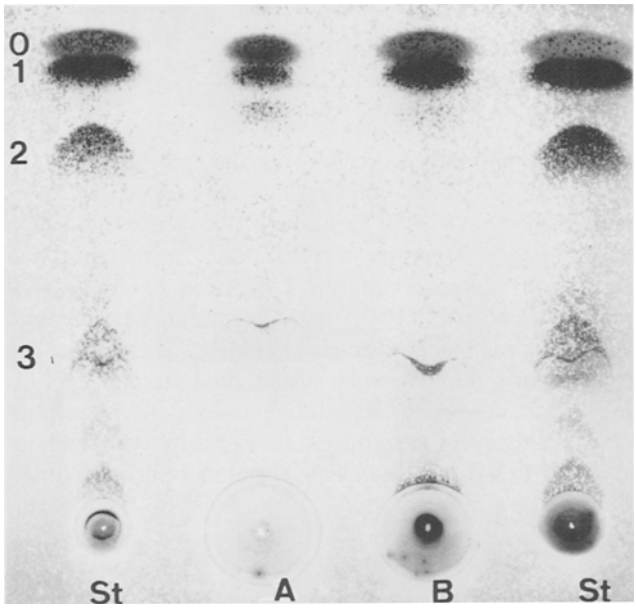

Fig. 4. *Phaeocystis* sp. Argentation TLC of the fatty acid methyl esters of the total lipids [TLC performed using silica gel G containing 5% $AgNO_3$ and hexane:diethyl ether (87:13, by vol.), as solvent. Fatty acid methyl esters visualized by charring after spraying with 50% H_2SO_4.] St: standard methyl stearate, i.e. methyl oleate, methyl linoleate and methyl linolenate. Samples A: May; B: March. Fatty acids are 0: saturated; 1: monoenoic; 2: dienoic; 3: trienoic

but higher concentrations of stearic (18:0) and PUFA than lipids from the March and May samples.

Table 3 shows the fatty acid patterns of individual lipid classes, palmitic (16:0) and oleic (18:1) acids predominated in TG and free FA. These two major lipid classes contained only small proportions of PUFA. There were only slight differences in the fatty acid patterns between March and May samples, whilst November samples contained more stearic (18:0) and C_{16} PUFA (16:2 and 16:3) but less oleic acid (18:1).

Polar lipids contained larger proportions of C_{16} and C_{18} PUFA than nonpolar lipids. In additon differences in the fatty acid patterns of the three samples were more obvious. C_{16} PUFA (16:2 and 16:3) in DGDG, SQDG and total phospholipids of November and March samples were concentrated more than in May. Furthermore, MGDG of the November sample were richer in 16:2 than March and May samples. Linolenic acid (18:3) was most predominant C_{18} PUFA in phospholipids, DGDG and SQDG in March, and in MGDG from the November sample. In the various lipid classes of the November and March samples the proportion of octadecatetraeroic acid (18:4) was often small, but was considerably higher in phospholipids and DGDG of the May sample. In all polar lipid classes, octadecapentaenoic acid 18:5 occurred in varying proportions, its highest concentration being in MGDG of the May sample. Eicosatetraenoic acid (20:4) was only found in considerable but again low concentrations in some lipid classes of the November sample.

Discussion and conclusions

Our study presents the first report on a *Phaeocystis* bloom in the Arabian Gulf. Confirming earlier observations on remote regions, e.g. the East Frisian coastal waters of the North Sea (Bätje and Michaelis 1986), this bloom was subsequent to a diatom-bloom peak. There is reason to believe that the appearance of *Phaeocystis* blooms in the Arabian Gulf is a new phenomenon. Earlier studies on the phytoplankton of this region (e.g. Jacob et al. 1979) did not mention any such blooms, which are too obvious to be overlooked. It is not improbable that recent urban developments by the rich oil-countrics and the associated increase in the amount of treated sewage disposed into the Gulf (leading to inorganic nitrogen enrichment of the water) are among the causative agents of this phenomenon.

Our results on the lipids and fatty acids are basically different from those of the only other study performed on *Phaeocystis* (Sargent et al. 1985). Contradicting Sargent et al.'s results, we found that nonpolar lipids, especially TG and free FA, predominate in lipid extracts of *Phaeocystis* collected in different seasons. In this context it should be noted that TG are frequently predominant in lipids of other

Table 3. *Phaeocystis* sp. Constituent fatty acids of individual lipid classes from samples collected in 1987 and 1988. Data expressed % wt

Fatty acid	November	March	May	Fatty acid	November	March	May
Triacylglycerols				Monogalactosyldiacylglycerols			
16:0	42.7	37.8	45.7	16:0	30.5	47.6	39.2
16:1	0.6	Trace	Trace	16:1	Trace	Trace	Trace
16:2	2.6	Trace	Trace	16:2	18.2	Trace	Trace
16:3	1.9	8.3	2.1	16:3	0.5	Trace	0.1
18:0	7.9	Trace	Trace	18:0	17.4	Trace	Trace
18:1	38.3	41.3	43.8	18:1	16.5	49.1	27.1
18:2	3.9	Trace	Trace	18:2	3.0	Trace	Trace
18:3	0.5	6.9	4.3	18:3	8.0	2.0	Trace
18:4	0.7	6.4	4.1	18:4	Trace	0.9	1.9
18:5	Trace	Trace	Trace	18:5	1.4	Trace	31.5
20:4	0.9	Trace	Trace	20:4	4.5	Trace	Trace
Free fatty acids				Digalactosyldiacylglycerols			
16:0	51.8	39.5	34.3	16:0	28.8	2.9	28.5
16:1	Trace	Trace	Trace	16:1	Trace	18.9	13.8
16:2	4.7	Trace	Trace	16:2	18.7	Trace	Trace
16:3	1.0	Trace	3.5	16:3	Trace	23.9	Trace
18:0	13.4	Trace	Trace	18:0	17.5	Trace	Trace
18:1	23.8	56.0	54.4	18:1	13.3	23.2	36.2
18:2	2.2	Trace	Trace	18:2	10.5	Trace	Trace
18:3	Trace	2.7	4.6	18:3	0.7	11.0	Trace
18:4	0.3	0.8	2.6	18:4	Trace	Trace	10.1
18:5	4.5	1.1	3.1	18:5	3.0	12.1	11.4
20:4	0.3	Trace	Trace	20:4	7.2	Trace	Trace
Total phospholipids				Sulfoquinovosyldiacylglycerols			
16:0	29.2	10.3	52.6	16:0	28.0	0.8	36.5
16:1	Trace	19.2	Trace	16:1	Trace	21.8	Trace
16:2	28.0	Trace	Trace	16:2	19.9	Trace	Trace
16:3	16.0	22.5	Trace	16:3	Trace	20.5	Trace
18:0	0.1	Trace	Trace	18:0	15.5	Trace	Trace
18:1	4.1	24.4	22.9	18:1	18.8	23.5	29.2
18:2	14.2	Trace	Trace	18:2	16.2	Trace	Trace
18:3	8.4	15.6	Trace	18:3	0.6	21.5	25.6
18:4	Trace	1.6	11.7	18:4	1.4	Trace	Trace
18:5	Trace	7.1	8.0	18:5	Trace	11.9	4.1
20:4	Trace	Trace	Trace	20:4	0.8	Trace	Trace

phytoplankton blooms (e.g. Morris et al. 1983). Furthermore, we found much lower concentrations of the PUFA octadecatetraenoic acid (18:4), and did not find any significant amounts of C_{20} and C_{22} PUFA – which reportedly contribute 25% of total fatty acids. Certainly, variations in environmental conditions and the colony age at the time of sampling are responsible for many of these differences, for it is well known that biological systems accumulate TG during ageing and unsaturated fatty acids during chilling (for review see Radwan and Mangold 1976). The major difference in the fatty acid composition between *Phaeocystis* blooms in the Arabian Gulf and those in very cold waters (i.e. the relatively low PUFA concentrations in the Arabian Gulf blooms), is apparently due to variation in the habitat temperature.

One of the factors affecting the value of a phytoplankton as a food source is its PUFA content, consequently *Phaeocystis* colonies in the Arabian Gulf should be only of a limited value. The fatty acid patterns of total lipids (especially from March and May samples) were similar and lacked PUFA, although there are obvious differences in the PUFA contents of individual polar-lipids which are rather minor components of the total lipids. It is interesting to note that none of the *Phaeocystis* polar lipids revealed a fatty acid pattern typical of higher photosynthetic eukaryotes (for review see Radwan and Mangold 1980; Quinn and Williams 1983), e.g. MGDG and DGDG of algae and leaves are rich in linolenic acid (18:3); *Phaeocystis* is not. Similarly, SQDG of leaves and algae are particularly rich in palmitic acid (16:0); *Phaeocystis* is not.

The higher content of C_{16} PUFA polar lipids from November and March, than May samples, may be attributed to the lower water temperature and higher oxygen content at these times. Such factors are known to favour fatty acid desaturation (for review see Radwan and Mangold 1976). However, it remains unclear why such factors do not also favour the desaturation of C_{18} fatty acids in *Phaeocystis*.

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