Pergamon

0305-1978(94)E00074-3

Biochemical Systematics and Ecology, Vol. 23, No. 1, pp. 65–69, 1995

0305-1978/95 $9.50+0.00

Fatty Acid Composition of Freshwater Phytoplankton During a Red Tide Event

GUILLERMO E. NAPOLITANO,* HORACIO HERAS†‡ and ARTHUR J. STEWART*

*Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6351, U.S.A.;
†Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, Halifax, N.S., Canada B3J 2X4

Key Word Index—Octadecapentaenoic acid; dinoflagellate; fatty acids; freshwater; spring bloom; red tide.

Abstract—The fatty acid composition of phytoplankton in a freshwater reservoir in east Tennessee was investigated during a short-lived algal bloom. Phytoplankton were dominated by the euplankter dinoflagellate *Peridiniopsis penardii*. The major fatty acids were (in decreasing order of abundance), 16:1ω7, 16:0, 22:6ω3, 14:0, 20:5ω3, 24:1ω9, 16:4ω1 and 16:3ω4. The phytoplankton lipids contain relatively low proportions of some of the typically major fatty acids of dinoflagellates (e.g. 18:1ω9 and 18:4ω3). Furthermore, the phytoplankton lipids exhibited only trace amounts of 3,6,9,12,15-octadecapentaenoic acid. Octadecapentaenoic acid (18:5ω3) is a predominant and potentially toxic fatty acid characteristic of dinoflagellates. This fatty acid profile differed substantially from those reported for marine and cultivated dinoflagellate species.

Introduction

Capillary gas chromotography (GC) has allowed analysis of fatty acids to be increasingly useful in microbial taxonomy and ecology. Unsaturated fatty acids, and especially some polyunsaturated fatty acids (PUFAs) of phytoplanktonic origin, have been the subject of a number of geochemical and food web studies (Prahl and Muehlhausen, 1989; Sargent, 1986). These studies cover a wide geographical range, but are limited almost exclusively to the marine environment.

Octadecapentaenoic acid (all-*cis*-3,6,9,12,15-18:5) is a novel PUFA, whose existence was unsuspected despite considerable efforts in the identification of fatty acids from aquatic organisms (Ackman *et al.*, 1968; Chuecas and Riley, 1969; McIntyre *et al.*, 1969). The occurrence of 18:5ω3 in a marine dinoflagellate was first deducted from quantitative chromatographic data by Ackman *et al.* (1974). Subsequently, the existence of 18:5ω3 was confirmed by Joseph (1975) in *Prorocentrum micans* and eleven other marine dinoflagellates. Most of the work on phytoplankton, and in particular on dinoflagellate fatty acids, has involved the use of laboratory cultures of marine species. Fatty acid analyses from various marine dinoflagellates consistently show the presence of high levels of 18:1ω9, 18:4ω3, 18:5ω3 and 22:6ω3 (Withers and Nevenzel, 1977; Pillsbury, 1985; Parrish *et al.*, 1993). The proportion of 18:5ω3 in the total lipids is variable, but typically fluctuates between 4 and 23% of the total lipid fatty acids. More recently, 18:5ω3 was reported in Primnesiophytes at approximately the same range of concentrations as originally found in dinoflagellates (Volkmann *et al.*, 1981; Napolitano *et al.*, 1988, 1990; Al-Hasan *et al.*, 1990; Okuyama *et al.*, 1992). An examination of the lipid class distribution of 18:5ω3 has shown that this fatty acid is concentrated in the glycolipids (Okuyama *et al.*, 1992), which in turn form a major part of the chloroplast membranes. Interestingly, 18:5ω3 was not detected in the lipids of the dinoflagellate *Crypthecodinium cohnii*, a marine heterotrophic species (Henderson *et al.*, 1988). Early detection and monitoring of the occurrence of dinoflagellates that

‡Present address: INIBIOLP, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, La Plata 1900, Argentina.

(*Received* 7 *June* 1994)

cause "red tides" is a major concern for the fish and shellfish industry, as well as in health and environmental spheres. Octadecapentaenoic acid was suggested as a potential biomarker for the detection of toxic marine dinoflagellates (Joseph, 1993). Moreover, 18:5ω3 itself has been implicated in fish kills caused by *Gyrodinium aureolum* and *Chryschomulina polylepsis* (Prymnesiophyceae) in Norwegian coastal waters (Yasumoto *et al.*, 1990). Here we report the fatty acid composition of a dinoflagellate bloom, and seek to address the problem of the occurrence of 18:5ω3 in freshwater dinoflagellates.

Materials and Methods

A sample of surface water was collected from Melton Hill Reservoir at the Bearden Creek Embayment (eastern Tennessee) in April 1993. Surface water temperature was 11.5°C. The concentration of major inorganic nutrients were: NH_3+NH_4, 0.010 mg l^{-1}; NO_2+NO_3, 0.55 mg l^{-1}; total P, 0.008 mg l^{-1}. The algae were concentrated by centrifugation 30 min after collection, and subsampled for taxonomic identification and fatty acid determinations.

Phytoplankton lipids were extracted with a mixture of chloroform:methanol (Blight and Dyer, 1959). After extraction, the lipid extracts in the chloroform layer were washed with water, dried over sodium sulfate, concentrated and stored in chloroform in screw-cap (Teflon-lined) glass vials at −35°C under a nitrogen atmosphere. Fatty acids were converted to their respective methyl esters using 10% BF_3–methanol. For this purpose the total lipid extract was redissolved in benzene (1 ml) in a 10 ml screw-capped centrifuge tube. Then 1 ml of 10% BF_3–methanol was added to the tube, which was flushed with nitrogen and capped. This was then shaken thoroughly and heated at 100°C in a heating block for 1 h. After cooling the sample to room temp, distilled water (2 ml) was added, the mixture was shaken vigorously, and the top layer containing the methyl esters removed. The remaining mixture was again extracted with benzene (2×2 ml). The combined benzene extracts were concentrated under a stream of nitrogen and dried over anhydrous Na_2SO_4. The solution was filtered and evaporated to dryness under a stream of nitrogen. The esters were redissolved in hexane, which was the standard solvent for injection into the gas chromatograph. FAME analysis was carried out in a Perkin–Elmer Autosystem (Perkin–Elmer, Norwalk, CT, U.S.A.) equipped with FID (flame ionization detector) and a bonded flexible fused silica capillary column (30 m in length×0.25 mm i.d., DB-23, J & W Scientific, Folsom, CA, U.S.A.). Helium was the carrier gas at 20 psi. The oven temperature was programmed as follows: an initial temperature of 153°C was maintained for 2 min, then the temperature was increased to 174 at a rate of 2.3°C min^{-1}, and held for 0.2 min. Finally the temperature was increased to 220°C at a rate of 2.5°C min^{-1}. Retention times and area percentages were recorded on a computer interfaced with the GC using the software PE Nelson 7.0. FAME were identified by their characteristic retention times and by co-injecting the sample with authentic standards, or a FAME mixture of established composition.

Results and Discussion

The occurrence of a short-lived "red tide" dinoflagellate bloom in Melton Hill Reservoir provided an opportunity to investigate the fatty acid composition of a natural phytoplankton assemblage dominated by a single dinoflagellate species. The sample contained high quantities of the euplanktonic dinoflagellate *Peridiniopsis penardii* (Lemmermann) Bourrelly 1968. A few unidentified pennate diatoms were also present in the sample. *Peridiniopsis penardii* is commonly found in lakes and ponds, and until now has not been known to cause toxic red tides (Ingrid Kelley, personal communication).

More than 50 fatty acids present in the phytoplankton lipids were separated and identified by capillary GC (Fig. 1). Major fatty acids in decreasing concentration were (% of total fatty acids), 16:1ω7 (13.5), 22:6ω3 (10.9), 16:0 (10.3), 20:5ω3 (6.7), 14:0 (4.7), 16:3ω4 (3.9), 16:4ω1 (3.8) 16:3ω6 (3.1) 18:3ω3 (2.8) and 18:4ω3 (2.4). Although this fatty acid profile contained most of the fatty acids typically found in dinoflagellates (i.e. 18:1ω9, 18:4ω3 and 22:6ω3), their concentrations were lower than expected (Table 1). The relatively high concentration of 16-carbon PUFA, and especially of 20:5ω3 served as evidence for the presence of diatoms in the sample. The most interesting finding was that the lipids contained only trace amounts of the dinoflagellate fatty acid marker 18:5ω3.

We are aware of only two previous reports on the occurrence of 18:5ω3 in freshwaters. The analysis of the seasonal variation of the fatty acid composition of natural

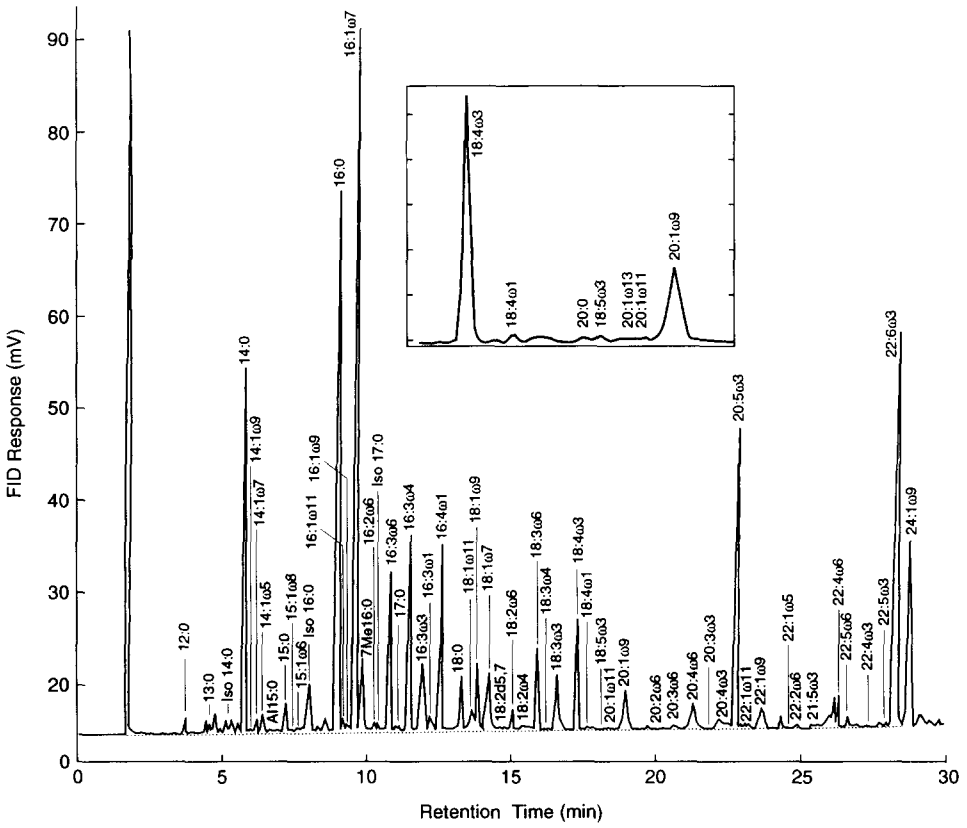

FIG. 1. GAS CHROMATOGRAM OF THE FATTY ACID METHYL ESTERS IN THE TOTAL LIPIDS OF PHYTOPLANKTON FROM MELTON HILL RESERVOIR DURING A DINOFLAGELLATE (*Peridiniopsis penardii*) BLOOM. Insert shows a detail of the chromatogram at the 18:5ω3 region.

phytoplankton in Lake Biwa (Japan) revealed the sporadic presence of 18:5ω3 (Hama *et al.*, 1992). In that study, the concentration of 18:5ω3 ranged from 0.6 to 2.5% of total fatty acids in April and May, respectively. Although the study by Hama *et al.* (1992) did not provide detailed information about phytoplankton species composition, it is apparent that dinoflagellates were not dominant at that time of the year. The fatty acid and taxonomic composition of the phytoplankton in Lake Pavin (France) (Bourdier *et al.*, 1987) showed that 18:5ω3 was among the major fatty acids of phytoplankton collected from May to July (ranging from approximately 2 to 12%). These high concentrations of 18:5ω3 corresponded to a maximum in dinoflagellate biomass, at a depth of 10 m, where *Peridinium willei* predominated. Samples taken during a different time of the year or at different depth showed both lower concentration of 18:5ω3 and smaller numbers of dinoflagellates. In contrast to the results of these analyses of natural phytoplankton, 18:5ω3 was not reported by Ahlgren *et al.* (1992) in analyses of fatty acids in cultures of two freshwater dinoflagellates (*Peridinium cinctum* and *Peridiniopsis borgei*) harvested at different growth phases. These dinoflagellates contained high proportions of all the typical dinoflagellate fatty acids but apparently lacked 18:5ω3.

The limited information that is available suggests that the presence of 18:5ω3 is less consistent and predictable in freshwater dinoflagellates than in marine dinoflagellates. If so, the irregular distribution of 18:5ω3 suggest limitations on its use as a marker for dinoflagellate and as an ichthyotoxon. Considering the role of PUFAs in

TABLE 1. FATTY ACID COMPOSITION (weight %) OF PHYTOPLANKTON FROM MELTON HILL RESERVOIR (TR = trace amounts $<0.1\%$, $n=2$)

Fatty acids	Weight %	Fatty acids	Weight %
12:0	0.26	18:2	0.42
13:0	0.18	18:2ω6	0.55
iso 14:0	0.35	18:2ω4	0.62
14:0	4.77	18:3ω6	1.8
14:1ω9	0.23	18:3ω4	0.13
14:1ω7	0.44	18:3ω3	2.77
14:1ω5	0.71	18:4ω3	2.39
antiso 15:0	0.44	18:4ω1	TR
15:0	0.65	20:0	0.11
15:1ω8	0.39	18:5ω3	TR
15:1ω6	0.13	20:1ω11	0.18
iso 16:0	1.70	20:1ω9	2.06
16:0	10.33	20:1ω7	TR
16:1ω11	0.54	20:2ω6	0.21
16:1ω9	0.43	20:3ω6	0.30
16:1ω7	13.53	20:4ω6	1.80
7Me16:0	2.74	20:3ω3	TR
16:2ω6	0.27	20:4ω3	0.27
iso 17:0	0.33	20:5ω3	6.68
16:3ω6	3.09	22:1ω11	0.17
17:0	0.26	22:1ω9	1.29
16:3ω4	3.95	22:1ω5	TR
16:3ω3	2.94	22:2ω6	0.17
16:3ω1	0.71	21:5ω3	0.14
16:4ω1	3.77	22:4ω6	0.85
18:0	1.77	22:5ω6	0.30
18:1ω11	0.90	22:4ω3	TR
18:1ω9	1.62	22:5ω3	TR
18:1ω7	0.26	22:6ω3	10.94
		Unknown	4.36

maintaining the fluidity of the cellular membranes at low temperatures, the observed large variations the concentration of 18:5ω3 may reflect different biochemical adaptations to differing environments. The relationship between 18:5ω3 and temperature could be manipulated under culture conditions and it could be useful for the prediction of the levels of this fatty acid in bloom situations.

Acknowledgements—We thank Dr Ingrid Kelley for the identification of *P. penardii*, the Tennessee Valley Authority for providing temperature and nutrients data, and professor R. G. Ackman for valuable comments on the manuscript. This work was supported in part by an appointment to the ORNL Postdoctoral Research Associates Program administered by ORNL and the Oak Ridge Institute for Science and Education. Oak Ridge National Laboratory is managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract No. DE-AC05-84OR21500. Environmental Sciences Division publication number 4374.

References

Ackman, R. G., Tocher, C. S. and MacLachlan, J. (1968) Marine phytoplankter fatty acids. *J. Fish. Res. Bd. Can.* **25**, 1603–1620.

Ackman, R. G., Manzer, A. and Joseph, J. D. (1974). Tentative identification of an unusual naturally-occurring polyenoic fatty acid by calculations from precision open-tubular GLC, and element retention data. *Chromatographia* **7**, 107–114.

Al-Hasan, R. H., Ali, A. M. and Radwan, S. S. (1990) Lipids, and their constituent fatty acids, of *Phaeocystis*, sp. from the Arabian Gulf. *Mar. Biol.* **105**, 9–14.

Ahlgren, G., Gustafsson, I.-B. and Boberg, M. (1992) Fatty acid content and chemical composition of freshwater microalgae. *J. Phycol.* **28**, 37–50.

Bligh, E. G. and Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917.

Bourdier, G. and Amblard, C. (1987) Evolution de la composition en acides gras d'un phytoplankton lacustre (Lac Pavin, France). *Int. Rev. Ges. Hydrobiol.* **72**, 81–95.

Chuecas, L. and Riley, J. P. (1969) Component fatty acids of the total lipids of some marine phytoplankton. *J. Mar. Biol. Assoc. U.K.* **49**, 97–116.

Hama, T., Matsunaga, K., Handa, N. and Takahashi, M. (1992) Fatty acid composition in photosynthetic products of natural phytoplankton population in Lake Biwa, Japan. *J. Plankton Res.* **14**, 1055–1065.

Henderson, R. J., Leftley, J. W. and Sargent, J. R. (1988) Lipid composition and biosynthesis in the marine dinoflagellate *Crypthecodinium cohnii*. *Phytochemistry* **27**, 1679–1683.

Joseph, J. D. (1975) Identification of 3,6,9,12,15-Octadecapentaenoic acid in laboratory-cultured photosynthetic dinoflagellates. *Lipids* **10**, 395–403.

Joseph, J. D. (1993) Octadecapentaenoic acid: A biomarker for toxic marine phytoplankton? *Int. News Fats, Oils Related Mat.* **4**, 534.

McIntyre, C. D., Tinsley, I. J. and Lowry, R. R. (1969) Fatty acids in lotic periphyton: Another measure of community structure. *J. Phycol.* **5**, 26–32.

Napolitano, G. E., Ratnayake, W. M. N. and Ackman, R. G. (1988) All-*cis*-3,6,9,12,15-octadecapentaenoic acid: a problem of resolution in the GC analysis of marine fatty acids. *Phytochemistry* **27**, 1751–1755.

Napolitano, G. E., Ackman, R. G. and Ratnayake, W. M. N. (1990) Fatty acid composition of three cultured algal species (*Isochrisis galbana, Chaetoceros gracilis* and *C. calcitrans*) used as food for bivalve larvae. *J. World Aquacult. Soc.* **21**, 122–130.

Okuyama, H., Morita, N. and Kogame, K. (1992) Occurrence of octadecapentaenoic acid in lipids of a cold stenothermic algae, Prymnesiophyte strain B. *J. Phycol.* **28**, 465–472.

Parrish, C. C., Bodennec, G., Sebedio, J.-L. and Gentien, P. (1993) Intra- and extracellular lipids in culture of the toxic dinoflagellate, *Gyrodinium aureolum*. *Phytochemistry* **32**, 291–295.

Pillsbury, K. S. (1985) The relative food value and biochemical composition of five phytoplankton diets for Queen Conch, *Strombus gigas* (Linne), larvae. *J. Exp. Mar. Biol. Ecol.* **90**, 221–231.

Prahl, F. G. and Muehlhausen, L. A. (1989) Lipid biomarkers as biochemical tools for paleoceanographic study. In *Productivity of the Oceans: Present and Past* (Berger, W. H., Smetacek, V. S. and Wefer, G., eds), pp. 271–289, John Wiley & Sons, New York.

Sargent, J. R. (1986) The structure, metabolism and function of lipids in marine organisms. In *Biochemical and Biophysical Perspectives in Marine Biology* (Malins, D. and Sargent, J. R., eds), pp. 149–212, Academic Press, New York.

Volkman, J. K., Smith, D. J., Eglinton, G., Forsberg, T. E. and Corner, E. D. S. (1981) Sterol and fatty acid composition of four marine Haptophycean algae. *J. Mar. Biol. Assoc. U.K.* **61**, 509–517.

Withers, N. W. and Nevenzel, J. (1977) Phytyl esters in a marine dinoflagellate. *Lipids* **12**, 989–993.

Yasumoto, T., Unerdal, B., Aune, T., Hormazabal, V., Sculberg, O. M. and Oshima, Y. (1990) Screening for hemolitic and ichthyiotoxic components of *Chryoschromulina polylepis* and *Gyrodinium aureolum* from Norwegian coastal waters. In *Toxic Marine Phytoplankton* (Graneli, E., Sundstrom, B., Edler, L. and Anderson, D. M., eds), Elsevier, New York.