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FATTY ACID COMPOSITION OF 15 SPECIES OF MARINE MICROALGAE

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Abstract—The fatty acid composition of 15 marine microalgal species, belonging to the Chlorophyceae, Prasinophyceae, Bacillariophyceae, Prymnesiophyceae, Dinophyceae, Eustigmatophyceae, Cryptophyceae, Rhodophyceae, and cultured under comparable conditions has been studied. It is shown that each class of microalgae is characterized by a specific fatty acid profile. It is proposed that uncommon acids, some typical acids and the ratio of acids are useful chemotaxonomic markers. The reliable markers of Bacillariophyceae were a preference of 16:1(n-7) over 16:0, high levels of 14:0, 20:5(n-3), C_{16} polyunsaturated fatty acids with double bonds at (n-4) and (n-1), and insignificant amounts of C_{18} acids and 22:6(n-3). The fatty acid profiles of the different microalgae were compared with previously published data.

INTRODUCTION

The use of fatty acids as indicators of the presence of various organisms is expanding. It is a convenient method for the study of the food webs in marine ecosystems [1, 2]. Microalgae are the main suppliers of organic matter and energy in marine ecosystems, a source of essential fatty acids, and a food for sea animals. In studies of trophic relations initiated by our laboratory, we needed to analyse in detail the fatty acid composition of the microalgae in the phytoplankton of the temperate zone of the Pacific coastal waters.

There is extensive information about the lipids and fatty acids of microalgae. This information was mainly obtained for evaluating the biological value of microalgae used in mariculture, although some was used for chemotaxonomic purposes [3, 4]. However, the data are often contradictory. Discrepancies observed in the fatty acid composition of cultured marine microalgae originate from (a) the uncertainties in the taxonomic classification of species and (b) the growth of the cultures under different conditions. In addition, they could also arise from the use of inappropriate GC procedures. The main aim of this work was to perform detailed analyses of the fatty acid composition of microalgal species cultured under comparable conditions and to determine which fatty acids are most useful as chemotaxonomic indicators of microalgae.

RESULTS AND DISCUSSION

Fatty acids of Chlorophyceae and Prasinophyceae

The microalgal species studied are listed in Table 1. The better chromatographic methods of analysis used in the study have resulted in a revision of the data on the fatty acids of green microalgae. Particular attention has been paid to positional isomers and the presence or otherwise of 20:5(n-3) and 22:6(n-3) in Chlorophyta [5, 6].

Although variations have been reported in the fatty acid composition of some representatives of the two

Table 1. List of the algae studied and cell counts at harvest

Class and species	Culture medium	Cell count (10^6 ml^{-1})
Chlorophyceae		
1. *Dunaliella tertiolecta*	G*	3.00
2. *D. maritima*	G	2.70
3. *D. salina*	G	2.20
4. *Chlorella* sp.	G	6.25
Prasinophyceae		
5. *Tetraselmis viridis*	G	4.30
6. *T.* sp.	G	3.81
Bacillariophyceae		
7. *Phaeodactylum tricornutum*	G	9.90
8. *Skeletonema costatum*	f†	0.94
9. *Chaetoceros muelleri*	f	4.20
10. *C. constrictus*	G	0.11
Prymnesiophyceae		
11. *Pavlova salina*	G	2.68
Dinophyceae		
12. *Gymnodinium kowalevskii*	G	0.13
Eustigmatophyceae		
13. *Nannochloropsis oculata*	G	5.75
Cryptophyceae		
14. *Chroomonas salina*	f	0.70
Rhodophyceae		
15. *Porphyridium cruentum*	G	5.00

*'Goldberg' medium.
†Medium f.

classes, the most abundant fatty acids were the C_{16} and C_{18} polyunsaturated fatty acid (PUFAs) isomers n-3 and n-6. The main fatty acids Chlorophyceae examined in this study, *Dunaliella* and *Chlorella* species, were 16:0, 16:2(n-6), 16:3(n-3), 16:4(n-3), 18:2(n-6) and 18:3(n-3), whereas the 20:5(n-3) and 22:6(n-3) were practically absent (Table 2).

The fatty acid composition of three species of the genus *Dunaliella* was very similar. This is to be expected for the known systematic relationships between the species studied. The main PUFA, 18:3(n-3), comprised almost 40% of the total fatty acids and the content of 16:4(n-3) was somewhat lower (18.2–23.9%). Our results are in good agreement with the data reported for *D. tertiolecta* [5]. A similar fatty acid profile has been reported for this species [7, 8], but the percentage of the total unsaturation of fatty acids reported here was somewhat higher.

Another representative of this class, *Chlorella* sp. (Table 2), and other similar *Chlorella* species [6], exhibited a high concentration of 16:3(n-3). It differs, additionally, from other algal species examined in being particularly rich in 18:2(n-6).

Based on the results from the analysis of 10 species of Chlorophyta and the literature values for green algae, Dunstan *et al.* [6] suggested that significant amounts of 20:5(n-3) and 22:6(n-3) are not characteristic for green algae. A "marine" *Chlorella*, rich in 20:5(n-3) was correctly identified as one of the species of Eustigmatophyceae, *Nannochloropsis oculata* [9]. Thus the presence of a high concentration of 20:5(n-3) in lipids of Chlorophyceae is never found.

Both species of *Tetraselmis* (Prasinophyceae) generally showed a pattern of fatty acid distribution similar to green algae but they had some peculiarities (Table 2). In contrast to members of Chlorophyceae, the *Tetraselmis* species examined, as other representatives of this class [6], had a high concentration of 18:4(n-3) and considerable amounts of 20:5(n-3) (up to 6.7%). Other main components were 16:0, 16:4(n-3) and 18:3(n-3). The literature affords contradictory information on the fatty acid profile of the Prasinophyceae. Uncommon components were reported for this class of algae. A rare acid 16:3(n-6) was identified in *Tetraselmis suecica* [5]. In the same species, the acid 16:1(n-9) was described as a main acid, whereas 16:4(n-3) was lacking [10]. An unusual acid, 18:5(n-3), was tentatively identified in five of the six prasinophytes and the concentration of this acid in *Micromonas pusilla* made up 16.7% of the total acids [6]. This acid has not previously been reported as a constituent of the lipids of green microalgae. Also, the authors unexpectedly detected considerable amounts of 22:6(n-3) (4.5–8.5%) [6].

Thus, the specific features of Chlorophyceae are high concentrations of C_{16} PUFAs, 16:2(n-6), 16:3(n-3) and 16:4(n-3), and C_{18} PUFAs, 18:2(n-6) and 18:3(n-3). None of the species contained 22:6(n-3) and significant amounts of 20:5(n-3). In Prasinophyceae, the major acids were 16:4(n-3), 18:3(n-3) and 18:4(n-3). The acid 20:5(n-3) was present in an appreciable quantity. In addition, green algae were characterized by an elevated amount of 16:1(n-13) tr.

Fatty acids of Bacillariophyceae

The fatty acids of the Bacillariophyceae have been studied more extensively than other microalgal classes. This interest is connected with the wide use of diatoms in mariculture. Moreover, diatoms have a worldwide distribution. The characteristic features of the fatty acids of Bacillariophyceae, which often represent the main food source in marine ecosystems, are of particular interest.

The four species examined had a similar fatty acid composition. However, comparison of the component fatty acid revealed some differences. The most abundant fatty acids were 14:0, 16:0, 16:1(n-7) and 20:5(n-3) (Table 2), which accounted for 67–77% of the total fatty acids. The concentration of 16:1(n-7) was higher than that of 16:0 for all species analysed and the ratio of 16:1(n-7)/16:0 varied from 1.3 to 2.0. The predominance of 16:1(n-7) over 16:0 has been established in previous studies [4, 5, 8]. The species from other classes examined had 16:1(n-7) as a minor component (Table 2); the exception was *Pavlova salina* (Prymnesiophyceae). Both acids in *P. salina* were of similar abundance, although Volkman *et al.* [11] reported somewhat lower concentrations of 16:1(n-7) for *Pavlova* species.

The acid 20:5(n-3) was most abundant among PUFAs; its content varied from 12.8 (*Chaetoceros constrictus*) to 28.4% (*Phaeodactylum tricornutum*). A wide range of relative amounts of 20:5(n-3) have been reported for different diatom species [5, 12]. Several factors cause this variability in diatoms. Variation of the nutritional and physical factors results in a change in 20:5(n-3) production by *Phaeodactylum tricornutum* from several per cent to 30–40% of the total fatty acids [13]. The variation in fatty acid composition and especially 20:5(n-3) content is a function of silicate availability, light and temperature [14]. Indeed, in our experiments the level of 20:5(n-3) in *Chaetoceros muelleri* decreased from 12.8 to 6.3% under silicate limitation. In addition, the proportion of 20:5(n-3) appears to be a function of culture age. A decrease in 20:5(n-3) with concomitant increase in 14:0 was observed in *Skeletonema costatum* [15] and in *Biddulphia sinensis* [16] as the culture aged.

Diatoms are rich in C_{16} PUFAs (Table 2). Based on the ECL values and the R_t data, the main components were identified as 16:2(n-4), 16:3(n-4) and, additionally, 16:4(n-1) for *S. costatum*, whereas in green algae the n-3 and n-6 positional isomers of C_{16} PUFAs are dominant. Hence, C_{16} PUFAs isomers n-4 and n-1 may be used as chemotaxonomic markers of Bacillariophyceae.

In addition to the attributes of the class in general, features of the fatty acid composition peculiar to individual species were apparent. The percentage of 16:4(n-1) for *S. costatum* (7.5%) was very close to that previously found for this species (6.6%) [8], whereas in the other species examined it was a minor component. It might be expected that 16:4(n-1) is an indicator of this species.

Table 2. Fatty acid composition (wt %) of marine microalgae

Fatty acid	*Dunaliella tertiolecta*	*Dunaliella maritima*	*Dunaliella salina*	*Chlorella* sp.	*viridis*	*Tetraselmis* sp.	*Phaeodactylum tricornutum*	*Skeletonema costatum*	*Chaetoceros muelleri*	*constrictus*	*Pavlova salina*	*Gymnodinium kowalevskii*	*Nannochloropsis oculata*	*Chroomonas salina*	*Porphyridium cruentum*
14:0	0.3	0.4	0.5	2.0	0.8	0.6	7.4	12.7	15.0	14.0	13.1	12.4	3.9	5.0	0.5
14:1(n-7)	0.1	0.1	0.1	0.5	0.2	0.1	0.1	0.4	0.0	0.6	0.0	0.0	0.2	0.3	0.0
iso-15:0	0.0	0.0	1.0	0.0	0.0	0.2	0.0	1.5	0.7	1.5	0.0	0.0	1.2	1.2	0.0
anteiso-15:0	0.9	0.9	0.9	1.6	3.1	1.7	0.7	0.2	0.3	0.4	0.9	0.4	0.1	0.2	1.1
15:0	0.1	0.1	0.2	0.8	0.2	0.1	0.3	1.0	0.9	1.2	0.6	0.0	0.5	0.6	0.3
16:0	10.3	11.8	17.8	19.6	15.9	16.2	11.3	9.4	17.3	16.4	15.1	26.7	20.5	13.5	28.6
16:1(n-7)	4.0	2.7	0.8	6.2	3.3	3.8	21.5	19.0	30.0	14.3	30.4	1.8	25.2	2.0	1.1
16:1(n-13)tr	1.2	1.5	1.7	2.6	2.3	2.4	0.9	0.6	0.4	0.3	0.0	0.2	0.0	0.0	1.8
iso-17:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.6	0.5	0.0	0.0	0.7	1.2	0.0
16:2(n-6)	2.0	1.1	1.5	3.6	0.3	1.1	1.0	0.9	1.6	1.0	0.6	0.0	0.6	0.0	0.0
16:2(n-4)	0.0	0.0	0.0	0.4	0.7	2.2	4.4	4.7	3.2	1.9	0.4	0.0	0.2	0.0	0.1
17:0	0.0	0.0	0.0	0.3	0.0	0.0	0.1	0.3	0.1	0.5	0.1	0.0	0.4	0.2	0.1
16:3(n-6)	1.5	1.1	1.7	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0
16:3(n-4)	0.0	0.0	0.3	0.0	0.0	0.0	12.3	10.2	7.8	7.9	0.4	0.0	0.4	0.0	0.0
16:3(n-3)	3.2	2.7	2.1	12.0	1.2	1.3	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2
16:4(n-3)	23.9	22.6	18.2	0.0	19.9	18.3	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
16:4(n-1)	0.0	0.0	0.0	0.3	0.0	0.0	1.3	7.5	0.4	1.7	0.0	0.0	0.0	0.0	0.0
18:0	0.3	0.4	1.5	3.3	0.8	0.9	0.4	2.2	0.8	4.8	1.0	8.5	1.8	3.0	0.8
18:1(n-9)	1.7	2.1	2.8	5.7	4.6	4.7	2.3	2.6	1.4	4.7	3.1	6.5	3.6	2.3	1.3
18:1(n-7)	0.5	0.4	0.6	1.6	3.1	2.3	0.5	0.0	0.5	1.5	0.7	0.0	0.5	2.9	0.7
18:2(n-6)	5.2	4.1	6.1	11.8	3.1	7.0	1.5	1.6	0.7	1.8	1.5	3.7	2.2	1.2	8.2
18:3(n-6)	3.2	3.2	2.5	0.3	0.2	0.3	0.5	0.0	1.1	0.3	2.2	0.0	0.7	0.0	0.3
18:3(n-3)	38.7	42.6	36.9	22.3	15.0	15.5	0.9	0.2	0.3	0.2	0.0	7.2	0.2	10.8	0.4
18:4(n-3)	1.3	1.3	0.7	0.1	13.3	12.1	0.5	2.9	0.8	0.3	4.2	15.6	0.1	30.3	0.0
18:5(n-3)	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0
20:1(n-9)	0.0	0.0	0.0	0.1	1.2	0.9	0.0	0.0	0.1	0.2	0.0	0.2	0.0	0.0	0.1
20:2(n-6)	0.0	0.0	0.1	0.2	1.2	0.7	0.2	0.2	0.0	0.0	0.3	3.7	0.1	0.0	1.9
20:3(n-6)	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.3	0.0	0.0	0.3	0.0	0.8
20:4(n-6)	0.3	0.0	0.0	0.5	0.2	0.3	0.3	0.2	1.7	3.3	3.7	0.0	5.3	2.8	27.6
20:4(n-3)	0.0	0.0	0.0	0.0	0.5	0.3	0.3	0.0	0.1	0.1	0.0	0.0	0.0	0.0	1.6
20:5(n-3)	0.4	0.0	0.1	1.3	6.7	5.6	28.4	15.4	12.8	18.8	19.1	0.1	29.7	12.9	21.1
22:6(n-3)	0.0	0.0	0.0	0.0	0.0	0.0	0.7	2.3	0.8	0.6	1.5	9.5	0.0	7.1	0.0
Others	0.9	0.9	1.9	2.6	2.2	1.4	2.0	2.3	0.3	0.9	1.0	2.1	1.4	2.5	1.4

However, a large proportion of this acid was recently determined in a toxic marine diatom *Nitzschhia pungens* [17] grown in batch culture. It accounted for 11% of the total fatty acids in nutrient-replete *N. pungens*. The authors suggested that 16:4(n-1) may be a useful marker of *N. pungens*. The presence of a significant concentration of this acid in *S. costatum* decreases this possibility. Comparison of the diatoms examined showed that *Chaetoceros* species are especially rich in 20:4(n-6). Similar results were reported for *C. gracilis* [5, 18] and for *C. calcitrans* [5].

Thus, reliable indicators of Bacillariophyceae are the prevalence of 16:1(n-7) over 16:0, the high levels of 20:5(n-3) and 14:0, and the insignificant amounts of C_{18} acids and 22:6(n-3). The additional markers of diatoms are 16:2(n-4), 16:3(n-4) and 16:4(n-1) which are abundant compared with other classes.

Fatty acids of Prymnesiophyceae

This class is divided into four orders, which have essential biochemical differences. Much attention has recently been given to the fatty acid composition of Pavlovales [3, 11] and Isochrysidales [3, 19]. The differences of fatty acid compositions among individual orders are so appreciable that there is no possibility of delineating a set of marker fatty acids for this class as a whole. Presumably, it would be correct to determine the specificity of the fatty acid composition of orders. A revision of the taxonomy of this class is necessary and the biochemical characteristics such as the fatty acid profile seem to be useful for a more accurate resolution of the problems of the systematics of Prymnesiophyceae.

Only *Pavlova salina* was acceptable for analysis. The fatty acid composition of *P. salina* was very similar to that previously reported for *P. lutheri* [7, 8]. However, *P. salina* differed from *Pavlova* species [11] in its concentration of (n-3) PUFAs. This difference probably arises from the culture conditions and depressed state of the sample studied. A decrease in the proportion of (n-3) PUFAs, 18:4(n-3), 20:5(n-3) and 22:6(n-3), was accompanied by an increase in (n-6) PUFAs, 18:3(n-6) and 20:4(n-6). Although, the results of laboratory culture of this species suggested that no change in the production of 18:4(n-3) and 20:5(n-3) occurred in different light conditions and culture media. Differences in fatty acid content could also be explained by genetic differences among the strains of the same species [11, 19]. Nevertheless, the attributes of this order remain. The major fatty acids in *P. salina* were identified as 14:0, 16:0, 16:1(n-7), 20:5(n-3), 18:4(n-3) and 22:6(n-3). Unlike the other algae analysed, in *P. salina* the rare acid 22:5(n-3) accounted for 1.8% of the total fatty acids. Similar values were previously detected for other *Pavlova* species [11].

Thus, the Pavlovales are similar to diatoms and contain 14:0, 16:0, 16:1(n-7) and 20:5(n-3) as major components. But the distinguishing features are the high content of 18:4(n-3) and the presence of 22:6(n-3). These components are proposed as biochemical indicators of one of the orders of Prymnesiophyceae, Pavlovales.

Fatty acids of Dinophyceae

Autotrophic dinophytes are widely distributed in the oceans and often represent a major part of marine phytoplankton.

The fatty acid composition of the only acceptable species *Gymnodinium kowalevskii* was mainly similar to that found in the other species of Dinophyceae [20, 21], except that the sample examined contained a somewhat lower concentration of 18:5(n-3) and 20:5(n-3). The more prominent components were 14:0, 16:0, 18:4(n-3) and 22:6(n-3). One of the properties of dinoflagellates is the high concentration of 22:6(n-3) which is rare among other microalgae. Only some representatives of Prymnesiophyceae, *Pavlova* species [11] and *Isochrysis galbana* [19], are rich in this acid. Dinophyceae are known to be rich in an unusual acid, 18:5(n-3) [20]. This acid is considered to be a useful signature compound of photosynthetic dinoflagellates, but is now known to be present in other algal classes [3, 6]. Its content varied from 3.8 to 23.2% of the total fatty acids among 11 species of this class analysed by Joseph [20]. In our study, *G. kowalevskii* produced a smaller amount of this acid. Similar amounts of 18:5(n-3) were detected in a toxic species *G. catenatum* [22].

The relative proportion of 20:5(n-3) in the species analysed was insignificant, whereas other researchers identified it as a major constituent [20–22]. This discrepancy may be due to the culture conditions being suboptimal leading to a depression of the biosynthesis of both PUFAs, which are considered to be biogenetically related [20]. Variations of growth temperature, culture medium, and age at harvesting were used in our experiments. None of these parameters had any significant effect on the fatty acid profile of the algae.

Based on our results and the literature data, the main indicators of photosynthetic dinoflagellates are the presence of the unusual 18:5(n-3), the high content of 22:6(n-3), an acid which is rare among microalgae, and C_{18} PUFAs.

Fatty acids of Eustigmatophyceae

Representatives of Eustigmatophyceae make a significant contribution to the organic matter of the coastal waters in the Northern and Southern hemispheres.

The lipids of *Nannochloropsis oculata* exhibited a relatively simple fatty acid profile (Table 2) similar to other species of this class [23, 24]. The fatty acids were dominated by three components 16:0, 16:1(n-7) and 20:5(n-3), which together accounted for 74.8% of the total fatty acids. A significant concentration of 20:4(n-6) was also detected. Fatty acids with C_{18} chain length were present as relatively minor components.

Earlier, this species was incorrectly identified as *Chlorella minutissima*. In order to distinguish this species rich in 20:5(n-3) from other typical green algae, it was named "marine" *Chlorella*. The question about whether the "marine" *Chlorella* are Eustigmatophyceae was resolved [9].

Some morphological resemblance of Eustigmatophyceae species to coccoid green algae sometimes leads to errors in the identification of species. In this case, the fatty acid composition may be a valuable chemotaxonomic indicator. Eustigmatophyceae species principally differ from green algae in the high amount of 20:5(n-3) (nearly one-third of the total fatty acids) and near total absence of C_{16} and C_{18} PUFAs, which are the markers of green algae.

Thus, the high abundance of the acids 16:0, 16:1(n-7) and 20:5(n-3), and the insignificant contribution of other fatty acid components may be considered to be a chemotaxonomic indicator of this microalgal class.

Fatty acids of Cryptophyceae

Cryptomonads are small flagellates which are abundant in some seasons and, hence, play an important role as food for invertebrates. In spite of the importance of cryptophytes in the marine ecosystems as food sources, little attention has been given to their lipids.

Chroomonas salina showed a fatty acid pattern typical of the Cryptophyceae. Among saturated fatty acids, 16:0 was a main one (15%), whereas the concentration of 18:0 was very low (0.9%). In marine cryptomonads, the content of 18:0 does not usually exceed 1% of the total acids [5, 8, 25]. The lipids were especially rich in (n-3) PUFAs, with 18:4(n-3), 18:3(n-3), 20:5(n-3) and 22:6(n-3) being the principle ones (Table 2). The acid 22:6(n-3) exhibited a broad range of values from "trace constituent" [8] to 10% of the total acids [5, 25]. The C_{16} PUFAs were almost totally absent in this culture. This is consistent with the reports for other cryptomonads [5, 8].

The acid 20:1 detected in large amounts (10.3–18.0%) for four species of cryptomonads [26] was not found to be present in *C. salina* (Table 2). It was also not detected earlier for other species [5, 25].

The existing information on the fatty acid composition of Cryptophyceae is controversial and detailed analysis of more species using modern methods of GC analysis will be valuable. Nevertheless, the literature and own data suggest that the Cryptophyceae are rich in 16:0, C_{18} PUFAs, and 20:5(n-3) and have barely detectable amounts of 18:0 and C_{16} PUFAs.

Fatty acids of Rhodophyceae

A representative of the red microalgae, *Porphyridium cruentum*, has been characterized as a warm-water taxon and analysed for comparison with the other algal classes.

The fatty acid composition of *P. cruentum* was dominated by three major fatty acids, 16:0, 20:4(n-6) and 20:5(n-3), which together accounted for 77.3% of the total fatty acids. The concentration of 18:2(n-6) was also significant (8.2%), whereas other C_{16} and C_{18} PUFAs were found only in trace quantity. This pattern closely resembles the fatty acid profile previously reported for this species [27, 28]. Among the species examined only *P. cruentum* showed an appreciable concentration of 20:4(n-6), which is relatively rare in microalgae. Its concentration in this species was an order of magnitude higher compared with the other algae analysed (Table 2).

The Rhodophyceae exhibited a characteristic fatty acid profile dominated by 16:0, 20:4(n-6) and 20:5(n-3). A distinctive feature of this class was the high content of 20:4(n-6), which is a minor component in other classes.

The presence of iso- and anteiso-branched fatty acids indicate bacterial contamination of most of the cultures studied. Most bacteria have a specific fatty acid profile, which is distinctive from those of algae. However, the bacterial contribution of fatty acids was insignificant, because the concentration of branched fatty acids was low (0.4–3.3%).

In conclusion, the taxonomic differences in the fatty acid composition of microalgal classes are supported by our study. Uncommon acids or groups of acids may serve as useful biochemical indicators. In spite of the variability of the fatty acid composition of microalgae under different culture conditions, their specific features are retained.

EXPERIMENTAL

Microalgae (Table 1) were maintained in the Culture collection of the Institute of Marine Biology. Some species were originally isolated from Amursky Bay and Vostok Bay (the Sea of Japan). Experimental cultures were grown in filtered seawater, salinity 32 ‰, enriched with medium f [29] or with "Goldberg" medium [30], in 150 ml of culture medium in 250 ml flasks at 20°. Light was supplied by 4000 lux daylight fluorescent tubes. The photoperiod used was 12 hr light to 12 hr dark. Cultures were not aerated and were harvested towards the end of the log phase of growth. The cultures were not axenic. Cell counts at harvest are given in Table 1.

Lipid extraction and fatty acid analysis. The cells were harvested by centrifugation at 3000 g for 5 min or by filtration through a filter. Total lipids were extracted from wet cells by the method of ref. [31]. The residue was reextracted 2–3 times with small portions of $CHCl_3$–MeOH (2:1). Fatty acids were converted to Me esters using 1% Na in MeOH and then 5% HCl in MeOH according to ref. [32] and purified by TCL using C_6H_6 as solvent. The esters were eluted with $CHCl_3$ and redissolved in $CHCl_3$. The FAMEs were analysed by FID-GC. Both a polar Supelcowax-10 fused-silica column (30 m × 0.25 mm i.d.) at 210° and a nonpolar SPB-5 fused-silica column (30 m × 0.25 mm i.d.) at 240° were used for analysis. Individual peaks of FAMEs were identified by comparison of R_ts with those of the authentic standards of fatty acids and by ECL measurements [33]. For identification of PUFAs, Ag^+-TLC was used. FAMEs were sepd by prep. Ag^+-TLC by double-development with hexane–Et_2O–HOAc (94:4:3) and the fractions analysed by GC.

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