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POLAR LIPIDS AND FATTY ACIDS OF SOME MARINE MACROPHYTES FROM THE YELLOW SEA

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Abstract—Polar lipid and fatty acid compositions of 26 species of marine macrophytes collected in the Yellow Sea during winter were determined. Each division of seaweeds and seagrasses have distinguishing lipid and fatty acid profiles which have a chemotaxonomic value for marine plants. Algal habitat conditions affect quantitative characteristics of the fatty acids but its influence was not the same for different species. The content of polyunsaturated fatty acids in Chinese algae, in comparison with the same or related species from other regions, was found to be noticeably higher for most of the algal species examined. Members of the genera *Rhodomela*, *Gracilaria*, *Sargassum*, *Ulva*, *Enteromorpha* (except *E. linza*) and *Zostera* had the same ratios of the principal fatty acids as those for related species from other regions. The draining period during low tides affected lipid content and ratio of polyunsaturated fatty acids in algae but it did not influence the polar lipid and fatty acid profiles.

INTRODUCTION

In recent years, polar lipids and fatty acids of marine macrophytes have attracted increasing attention [1–28]. However, up to now, only a small number of marine macrophytes have been collected from rather a few sites in the World Oceans. We found no data on marine plant lipids from the Yellow Sea, although this area is rich in seaweeds, both in biomass and algal species [29]. Previously, it was found that the distribution of both fatty acids [30–33] and polar lipids [3, 8] in marine plants were closely linked with their taxonomic position. Yet, there are discrepancies in the literature on lipid composition even for the same or related algal species. Hence, we analysed for polar lipids and fatty acids in 26 species belonging to four main divisions of marine macrophytes collected from the Yellow Sea. The results obtained are discussed together with recent literature data to verify previously proposed lipid distributions for marine macrophytes and to formulate future targets in the field.

The region of the Yellow Sea near Qingdao is a zone of high tides [34], hence, we also decided to check how the tidal regime influences algal lipid composition.

RESULTS AND DISCUSSION

Polar lipids

The lipid extracts of 24 species of marine algae and two species of seagrasses were examined by two-dimensional HPTLC. Polar lipids were detected by spraying the plates with specific reagents. Although quantitative analyses have not been carried out, an estimation could be obtained from the intensity of spots.

Like all photosynthetic plants, marine algae and sea grasses from the Yellow Sea contained both glycolipids and phospholipids. In addition, green and brown algae had compounds without glycoses or phosphorus diacylglyceryl-(*N,N,N*-trimethyl)homoserine (DGTS) and diacylglyceryl-hydroxymethyl-(*N,N,N*-trimethyl)-alanine (DGTA) as essential polar lipid components. Glycolipids (monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol) were present in all investigated species as major lipid components. Distribution of phospholipids and unusual lipids without glycoses and phosphorus among marine plants was distinctive for each macrophyte division. Although we determined all polar lipids in samples, in Table 1 we show the results only for those lipids whose distribution provide information on plant taxonomy.

A characteristic feature of polar lipid composition in all the 11 Rhodophyta species was high phosphatidylcholine (PC) levels, very low concentrations of phosphatidylethanolamine (PE) and the presence of an unsaponifiable phospholipid, tentatively identified earlier as ceramidephosphorylinositol (CPI) [10]. Generally speaking, the phospholipid composition of red algae from the Yellow Sea was the same as obtained previously for red algae from the Sea of Japan [10] and from the Black Sea [7]. PC was the main phospholipid and PE the minor one in *Chondrus crispus* and *Poly-*

siphonia lanosa collected on the coast of South Wales [4]. The authors did not provide information on the presence of the unsaponifiable phospholipid, but they found that, besides PC, red algae contained phosphatidylsulphocholine (PSC).

However, one difference between the present and previous results [4, 7, 10] was the lesser quantities of PE in algae from the Yellow Sea. We did not find PE in two species and detected traces of this lipid in four species. This showed that the PE content may vary in red algae. There were differences in PE content for *C. crispus* and *P. lanosa*, analysed by other authors at different times [4, 35].

Brown algae were divided into two groups in accordance with their polar lipid composition. The first group was with PC and without DGTA and it included algae of the orders Scytosiphonales and Laminariales. The second group was without PC but contained DGTA as one of the main polar lipids; it consisted of representatives of the order Fucales (Table 1). These results are in agreement with our previous data for 20 species of brown algae from the Sea of Japan [10] and they support the assumption that the distribution of PC and DGTA are connected with taxonomic position of brown algae. However, information on polar lipid composition of brown algae from other studies showed that the PC and DGTA distribution was much more complicated [8, 12, 20, 23, 36]. In investigating 19 species of brown algae from the Black Sea, Dembitsky and Rozentsvet [8] failed to find PC in Sphacelariales, Dictyotales and Fucales species. Their paper did not contain information on DGTA distribution. Jones and Harwood [20] found DGTA as one of the major polar lipid in *Fucus vesiculosus* and *Ascophyllum nodosum*, but both species contained PC as well. Araki *et al.* [12] found high levels of DGTA in representatives of the Dictyotales and Fucales and found this lipid to be a minor component only in one algal species from other orders. Except for *Sargassum thunbergii*, PC was absent in all species from the orders Dictyotales and Fucales. However, we did not detect PC in *S. thunbergii* from the Yellow Sea.

The results of the most intensive investigation of brown algal polar lipids was published recently; more than 100 species from all Phaeophyta orders were examined [23]. The authors showed that algae of the order Fucales contained DGTA, but PC was absent. In contrast, representatives of the orders Scytosiphonales and Laminariales were without DGTA, but PC was their characteristic lipid component. PC was absent in Fucales species, but it was a characteristic lipid of algae from the Scytosiphonales and Laminariales.

An interesting question is whether or not brown algae contain phosphatidylserine (PS). We failed to find PS in the Yellow Sea brown algae or in those from the Sea of Japan [10]. We found a ninhydrin-positive phospholipid in brown algae, but it does not correspond to PS in its chromatographic behaviour. PS was not described as a component of brown algae from the Black Sea [8] or the coast of South Wales [20]. But some authors [12] identified it as a minor phospholipid in members of the Phaeophyta.

Previously we showed the presence of PS and DGTS to be characteristic of green macrophytic algae, because these lipids were absent in red and brown ones. The green algae studied were divided into two groups based on the presence or absence of PC [10]. Analyses of polar lipids of green algae from the Yellow Sea showed similar, together with new results (Table 1). DGTS was found in all species, except two representatives from *Bryopsis* genera. Earlier, we found a low level of DGTS in *B. Plumosa* from the Sea of Japan [10]. Rather high concentrations of this lipid was reported in two species of *Bryopsis* from the Black Sea [3].

Algae belonging to the orders Siphonocladales and Codiales contained PC as the main lipid component but members of Ulvales did not produce it at all, except *E. compressa*, where PC was present. This lipid was not found in other *Enteromorpha* species collected from other regions [3, 10].

Some unusual results were obtained for the common green alga, *Ulva pertusa*, because two out of the five samples of alga contained neither PE nor PS, or traces of PE only. Possibly such differences in phospholipid composition may be due to the development stage of the samples of *Ulva*; samples were taken at different times over a 2-month period. However, as a rule there are no such differences for algal lipids. For example, two other pairs of algae from the Yellow Sea, distinguished morphologically as young and more mature, *viz. Undaria pinnatifida* and *Enteromorpha linza*, showed no differences in their polar lipid composition.

There were no differences in the polar lipids of the two species of seagrasses examined, *Zostera marina* and *Phyllospadix iwatensis*, from the Yellow Sea (Table 1) in comparison with those of seagrasses from the Sea of Japan [25] and the Black Sea [13]. PC was the main phospholipid and PE the second most abundant. As in Chlorophyta, PS was a usual lipid component of seagrasses. Comparison of the polar lipid composition of seagrasses with those of seaweeds (Table 1) shows that seagrasses were distinguished from Rhodophyta, Phaeophyta and Chlorophyta.

A survey of results obtained for the distribution of taxonomically distinguishing polar lipids in macrophytes from the Yellow Sea (Table 1), those of our previous studies and other literature data, showed definite features in polar lipid patterns for each division of marine macrophytes and, moreover, for some groups among the Phaeophyta and Chlorophyta. However, there are some exceptions to this conclusion for several species of green algae. This indicates the need for further work to understand the causes for differences in polar lipid distributions for given seaweeds.

The distribution of other phospholipids was not related so clearly to taxonomy. We found phosphatidylglycerol (PG) as one of the principal polar lipids and phosphatidylinositol (PI) in all species of macrophytes from the Yellow Sea. The latter phospholipid was present as a minor component and in some red algae

Table 1. Distribution of polar lipids in algae and seagrasses from the Yellow Sea

DIVISION CLASS **Order** Family *Species*		Polar lipids*					
		PC	PE	PS	CPI	DGTA	DGTS
RHODOPHYCOTA							
RHODOPHYCEAE							
Cryptonemiales							
Dumontiacea							
1. *Hyalosiphonia caespitosa* Okam. Halymeniaceae		+++	+	–	+	–	–
2. *Grateloupia licina* (Lamx.) C. Ag.		+++	+	–	+	–	–
3. *Halymenia sinensis* Tseng et C. F. Chang		+++	+		+	–	–
Endocladiacea							
4. *Gloiopeltis furcata* (Post. et Rupr.) J. Ag.		+++	tr		+	–	–
Gigartinales							
Gracilariaceae							
5. *Gracilaria asiatica* Zhang et Xia	a)	+++	–	–	+	–	–
	b)	+++	–	–	+	–	–
6. *Gracilaria textorii* (Sur.) DeToni		+++	+	–	+	–	–
Ahnfeltiales							
Phyllophoraceae							
7. *Ahnfeltiopsis flabellifois* Harv. in Kuetzing		+++	+	–	+	–	–
Gigartinaceae							
8. *Chondrus sinensis*		+++	tr	–	+	–	–
Plocamiaceae							
9. *Plocamium telfairiae* Harv.		+++	–	–	+	–	–
Ceremiales							
Rhodomelaceae							
10. *Rhodomela confervoides* (Huds.) Silva		+++	tr	–	tr	–	–
11. *Chondria tenuissima* (Good. et Wood.) C. Ag.		+++	tr	–	tr	–	–
CHROMOPHYCOTA							
PHAEOPHYCEAE							
Scytosiphonales							
Scytosiphonaceae							
12. *Colpomenia sinuosa* (Mertens ex Roth)		++	++	–	–	–	–
Derbes et Solier in Castagne							
Laminariales							
Laminariaceae							
13. *Laminaria japonica* Aresch. Alariaceae		++	++	–	–	–	–
14. *Undaria pinnatifida* (Harv.) Sur. Sargassaceae		++	++	–	–	–	–
15. *Sargassum miyabei* Yendo (S. muticum)		–	++	–	–	+	–
16. *Sargassum thunbergii* (Mertens ex Roth)		–	++	–	–	+	–
O'Kuntze		–	++	–	–	+	–
CHLOROPHYCOTA							
CHLOROPHYCEAE							
Ulvales							
Ulvaceae							
17. *Ulva pertusa* Kjellm.							
	a)†	–	–	–	–	–	++
	b)	–	tr	–	–	–	++
	c)	–	+	+	–	–	++
	d)	–	+	+	–	–	++
	e)	–	+	+	–	–	++

Table 1. *Continued*

DIVISION CLASS **Order** Family *Species*	Polar lipids*					
	PC	PE	PS	CPI	DGTA	DGTS
18. *Enteromorpha linza* (L.) J. Ag.	−	++	+	−	−	++
19. *Enteromorpha compressa* (L.) Nees	−+	++	+	−	−	++
20. *Enteromorpha intestinalis* (L.) Nees	−	++	+	−	−	++
Siphonocladales						
Cladophoraceae						
21. *Cladophora rudolphiana* (Ag.) Kutzing	−+	++	+	−	−	++
Codiales						
Bryopsidaceae						
22. *Bryopsis hypnoides* Lamx.	−+	++	tr	−	−	−
23. *Bryopsis corticulans* Setch. Codiaceae	−+	++	+	−	−	−
24. *Codium fragile* (Sur.) Heriot	−+	+	+	−	−	++
MAGNOLIAPHYTA						
LILIOPSIDA						
Zosteraceae						
25. *Zostera marina*	+++	++	+	−	−	−
26. *Phyllospadix iwatensis* Makino	+++	++	+	−	−	−

*Quantity of lipid determined by visual evaluation of spot size and density: +++, a major spot; ++, a big spot; +, a small spot; tr, traces; −, the lipid not detected.

†Samples collected at different times and from different localities: 5a) sample taken from a small water pool; 5b) sample taken from a draining sandy beach; 17c) sample taken from the upper-tide horizon; 17d) sample taken from the low-tide mark.

Abbreviations: PC, phosphatidylcholine, PE, phosphatidylethanolamine, PS, phosphatidylserine; CPI, ceramidephosphorylinositol; DGTS, diacylglyceryl(*N*,*N*,*N*-trimethyl)homoserine; DGTA, diacylglycerylhydroxymethyl(*N*,*N*,*N*-trimethyl)-β-alanine.

was detected at trace levels only. Compared to literature data, PI was absent in some algae, for instance in the red algae, *C. crispus* and *P. lanosa* [4, 35] and in the brown alga, *Dictyota dichotoma* [8, 12]. We detected PI in the latter alga previously [10]. The difference between our results with those of others was possibly caused by the use of a more sensitive reagent to detect phospholipids [37].

The distribution of diphosphatidylglycerol (DPG) and phosphatidic acid (PA) was much more irregular. The same picture was revealed in previous reports from our and other laboratories [3, 4, 7, 8, 10, 35]. Quantities of DPG and PA may vary in the same or related species. For example, both phospholipids were absent in seagrasses from the Black Sea [3]; however, seagrasses from the Sea of Japan contained high levels of DPG and traces of PA [25].

Fatty acids

We examined the fatty acid (FA) composition of 11 Yellow Sea red algal species belonging to the orders Cryptonemiales, Gigartinales and Ceramiales. Red algae from this region were rich in C_{20} polyunsaturated fatty acids (PUFAs) (Table 2); however, their 20:4(n-6)/20:5(n-3) ratios varied between species. The majority of algae were most abundant in 20:5(n-3), except for two *Gracilaria* species. The algae from the order Cryptonemiales (*Gloiopeltis furcata*, *Grateloupia filicina*, *Halymenia sinensis*, and *Hyalosiphonia caespitosa*) had the highest percentage of 20:5(n-3); the highest content was found in *G. furcata* (48.2%). Red algae typically have high contents of PUFAs with 20 carbon atoms, chiefly arachidonic and eicosapentaenoic acids. 20:5(n-3) prevails in most red algae from different regions of the World Oceans [1, 5, 9, 13].

Only two *Gracilaria* species from the Yellow Sea, (*G. asiatica* and *G. textorii*) were rich in 20:4(n-6) (54.5% and 50.2%, respectively). Previously, it was shown that high levels of 20:4(n-6) was typical of some algal species from the genus *Gracilaria* [6, 15, 21, 38].

Plocamium telfairiae accumulated 5.3% of the C_{20} PUFA, 20:3(n-6). Its content greatly exceed that in all the red algal species examined to date [9, 21, 30, 39]. Previously, fatty acids of one other *Plocamium* species, *P. vulgare*, were analysed but this species did not contain appreciable amounts of 20:3(n-6) [5]. Although the need to examine further members of the genus is required, we propose that the high content of this acid is characteristic of *P. telfairiae* rather than of the genus.

Table 2. Fatty acid composition of Rhodophyta (% total FA content)

Fatty acid	Algae*											
	1	2	3	4	5a)	5b)	6	7	8	9	10	11
14:0	3.3	2.5	1.7	2.8	3.3	2.5	3.4	2.8	2.8	5.0	2.3	2.8
i-15:0	0.6	0.7	1.3	0.9	0.8	0.7	0.4	0.6	0.7	1.3	1.3	1.2
15:0	0.2	0.3	0.4	0.3	0.5	0.4	0.5	0.3	0.3	0.3	0.4	0.4
16:0	28.5	24.6	26.1	25.6	26.4	28.1	29.4	26.2	28.1	29.2	26.2	27.7
16:1	0.8	0.8	0.7	1.8	0.8	0.7	1.0	2.0	0.9	1.2	2.7	5.6
16:1*trans*	0.6	1.0	1.1	0.5	0.3	0.5	0.8	0.3	0.6	0.7	0.6	0.4
16:2(n-6)	0.0	tr	tr	0.5	0.2	0.2	0.3	0.8	0.1	0.2	2.1	0.3
18:0	0.8	1.5	1.1	1.0	0.9	0.7	1.2	1.2	1.0	1.7	0.8	0.6
18:1(n-9)	3.6	5.3	4.9	5.2	3.2	3.2	4.1	10.7	6.2	1.5	3.6	6.4
18:1(n-7)	1.3	1.9	1.8	2.2	2.2	2.1	1.6	2.4	2.0	1.5	7.5	3.6
18:2(n-6)	1.6	1.3	1.8	2.1	0.6	0.6	0.9	0.1	1.1	1.1	1.0	0.6
18:3(n-6)	1.0	1.5	1.5	0.4	0.4	0.4	0.7	0.4	0.8	1.6	0.4	0.1
18:3(n-3)	0.1	tr	tr	0.3	tr	0.1	0.1	0.2	0.6	1.0	1.1	0.1
20:3(n-6)	0.4	0.7	0.9	0.7	2.2	2.1	1.4	0.6	0.4	5.3	0.8	0.4
20:4(n-6)	15.9	11.4	11.1	3.0	54.3	54.3	50.2	13.7	15.8	13.9	7.0	4.5
20:5(n-3)	38.0	43.7	42.2	48.2	0.8	0.5	0.3	30.4	35.1	26.8	38.0	41.2
Other†	3.3	2.8	3.4	4.5	3.1	2.9	2.7	7.3	3.5	7.7	4.2	4.1
20:5/20:4	2.4	3.8	3.8	16.1	0	0	0	2.2	2.2	1.9	5.4	9.1

*Numbers designate algal species given in Table 1.

†Other: 17:0, 17:1, 18:4(n-3), 20:0, 20:2(n-6), 20:3(n-3), 20:4(n-3).

5a) Sample taken from a small water pool; 5b) sample taken from a draining sandy beach.

We have previously studied the fatty acids of *Gymnogongrus flabelliformis*, *Gloiopeltis furcata* and *Hyalosiphonia caespitosa*, collected from the Sea of Japan in summer. Eicosapentaenoic acid predominated among their PUFAs; the former and 20:4n6 were in the ratios of 1.1, 9.7 and 8.4, respectively [9]. *Gymnogongrus flabelliformis* and *Gloiopeltis furcata* collected from the Yellow Sea were richer in 20:5 and the 20:5/20:4 ratios were: 2.2 and 16.1, respectively. *Hyalosiphonia caespitosa* on the contrary, had a higher 20:4 content than that from the Sea of Japan.

The fatty acid compositions of *Chondria tenuissima*, *Grateloupia filicina*, *Halymenia sinensis* and *Rhodomela confervoides* were investigated for the first time. A comparison of fatty acid patterns of the first three species with those of species belonging to the same genera [9, 13, 40] showed the algae from the Yellow Sea were richer in PUFAs, mainly in 20:5(n-3). The FA composition of *Rhodomela confervoides* was similar to that of *Rhodomela* species from other regions [29, 40, 41].

We examined the fatty acid composition of five brown algal species abounding in the Yellow Sea (Table 3). *Undaria pinnatifida* and *Laminaria japonica* are particularly interesting because they are popular in Chinese mariculture as edible marine plants. C_{18} and C_{20} PUFAs were the major fatty acids of all species and made up more than half of the total FAs. This is typical of all brown algae [18, 30, 31]. The ratios of C_{18} and C_{20} PUFAs were not the same for *Undaria pinnatifida* and *L. japonica*. The first alga had a high content of 18:4(n-3) (25.0%), whereas *L. japonica* contained only 4.4% of this acid. *Undaria pinnatifida* contained more n-3 PUFAs (20:5 and 18:3) than *L. japonica*. The latter species was richer in the (n-6) PUFAs 18:2, 20:4 and in 18:1(n-9).

Published data on the fatty acids of *Undaria pinnatifida* differ within broad limits. Several researchers found an unusually high content of 18:4(n-3) [38, 40]; others did not detect this acid [42, 43] or indicated its presence only in low amounts (less than 10%) [44, 45]. The reasons for these differences are not clear. Besides environmental factors, age may possibly affect the levels of FAs. Hence, we compared fatty acid composition of young and mature *Undaria pinnatifida*. The profiles were identical (Table 3). We propose that age does not affect fatty acid composition in this species.

Laminaria japonica from the Sea of Japan [38, 44] was richer in saturated (14:0, 16:0) and monounsaturated FAs [16:1, 18:1(n-9)], and poorer in PUFAs, chiefly in 18:3(n-3) and 20:4(n-6), when compared with the same species from the Yellow Sea. However, a high concentration of 18:4(n-3) (39.9%) and 20:4 (20.9%) have been reported for this species from the Sea of Japan [42].

A survey of published data on fatty acids of algal species, belonging to the genus *Laminaria*, shows significant differences between studies [30, 31, 38–40, 42, 44, 46]. Hence, further research is needed to elucidate the causes of these differences, which could be due to environmental factors or to confusion in the botanical identification of certain algal species. With respect to environmental factors, it is known that different species from the same genus, collected from the same localities, may be different in the ratios of their main fatty acids [14, 15].

Table 3. Fatty acid composition of Phaeophyta (% total fatty acid content)

Fatty acid	Algae*					
	12	13	14a)	14b)	15	16
14:0	6.1	5.4	3.0	2.4	2.3	3.3
i-15:0	1.3	0.9	1.6	1.2	1.3	1.4
15:0	0.2	2.7	0.2	0.2	0.3	0.3
16:0	14.2	15.1	13.4	13.2	21.5	19.5
16:1(n-7)	1.4	2.6	0.6	0.3	2.6	6.8
16:1*trans*	1.2	0.3	1.7	1.7	1.0	0.5
16:2(n-6)	0.6	0.6	0.0	0.2	0.4	0.3
18:0	0.8	0.7	1.3	0.9	1.4	1.3
18:1(n-9)	7.7	12.6	7.4	6.3	7.4	6.1
18:2(n-6)	4.8	11.9	4.9	4.6	4.3	5.4
18:3(n-6)	1.1	3.5	1.2	0.7	1.0	0.7
18:3(n-3)	6.5	9.5	10.7	10.6	9.3	8.1
18:4(n-3)	15.6	5.3	25.0	25.3	12.9	6.9
20:1(n-11)	0.3	tr	0.0	0.0	1.2	1.2
20:1(n-9)	0.0	tr	0.0	0.0	1.0	1.1
20:2NMI	0.0	0.0	0.0	0.0	0.1	0.3
20:2(n-6)	0.3	0.5	0.3	0.2	0.3	0.5
20:3(n-6)	0.6	0.6	0.8	0.6	0.9	0.5
20:4(n-6)	8.3	14.3	11.6	11.7	13.1	12.7
20:4(n-3)	0.6	0.4	1.0	0.6	0.8	0.6
20:5(n-3)	21.9	8.2	13.5	13.0	13.3	9.1
Other†	6.5	4.9	1.8	6.3	3.6	13.4

*Numbers designate algal species given in Table 1.

†Other: 16:1(n-9), 17:0, 17:1, 18:1(n-7), 20:3(n-3), 20:4(n-3).

tr, traces.

14a) Sample of young alga; 14b) sample of mature alga.

Fatty acids of *Colpomenia sinuosa* were examined in different laboratories, but all specimens were collected in warm waters (Kuwait, Pakistan and Senegal) [11, 16, 18]. We found that the qualitative fatty acid composition of *C. sinuosa* taken in the temperate waters of the Yellow Sea in winter (Table 3) to be generally similar to that for the same species collected along the coast of Senegal [18]. Fatty acid ratios were different. *C. sinuosa* from warmer waters had 16:0 (35.1%) and 18:1(n-9) (22.0%) as the main acids, but the same alga from the Yellow Sea contained 14.2% of 16:0 and 7.7% of 18:1(n-9) and PUFAs, especially 18:4(n-3) and 20:5(n-3), were dominant. Al-Hasan *et al.* [11] investigated the influence of water temperature on fatty acid composition for *C. sinuosa* and found that at lower temperature (5°) the content of PUFAs, mainly 20:5, was higher and 16:0, 18:0 and 18:1 were lower than at 24°. The differences in the ratios of FAs for *C. sinuosa* collected from warmer waters and from the Yellow Sea in winter are the result of the influence of environmental factors, primarily, the water temperature.

The two *Sargassum* species, *S. miyabei* and *S. thunbergii*, have similar fatty acid compositions. C_{18} and C_{20} PUFAs were the main FAs (after 16:0); 20:1 and 22:1 fatty acids also were present in appreciable amounts in these algae and 20:2 non-methylene interrupted fatty acid was found as a minor component. The two *Sargassum* species had different 20:4 (n-6) to 20:5 (n-3) ratios. The arachidonic acid content in *S. thunbergii* exceeded that of 20:5n3, and approximately equal amounts of these acids were found in *S. miyabei* (Table 3). Our results correlate with those found for the same species from the Sea of Japan [14, 40]. The fatty acid composition of algae from the genus *Sargassum* does not vary between the two regions.

Green algae have the high concentrations of C_{16} and C_{18} PUFAs [13, 30, 31]. This feature was observed for eight green algae belonging to the two orders, Ulvales and Siphonocladales, examined in our study (Table 4). The algae from the Ulvales (*Ulva pertusa*, *Enteromorpha intestinalis*, *E. linza* and *E. compressa*) had the following features in their fatty acid composition. They contained 16:3(n-3) and even more 16:4(n-3), were rich in C_{18} PUFAs [18:3(n-3) and 18:4(n-3)] and had 18:1(n-7)/18:1(n-9) ratios higher than 1. Fatty acid profiles of algae from the Ulvales agreed with literature data [1, 11, 17, 19, 24, 27, 30, 38, 40, 47]. The three *Enteromorpha* species (*E. intestinalis*, *E. linza*, and *E. compressa*) had a similar fatty acid profile (Table 4). However, *E. compressa* was distinguished by a lower content of (n-3) C_{16} and C_{18} PUFAs. Jamieson and Reid [30] also indicated the same feature for *E. compressa* in comparison with *E. intestinalis*. Hence, the lower content of (n-3) PUFAs is believed to be typical of *E. compressa* from different habitats.

Algal species belonging to the Siphonocladales (*Cladophora rudolphiana*, *Bryopsis hypnoides*, *B. corticulans* and *Codium fragile*) had fatty acid compositions different from those of the Ulvales (Table 4). Algae from the Siphonocladales contained high levels of only one C_{16} PUFA – 16:3(n-3) (*B. hypnoides*, *B. corticulans* and *Codium fragile*) or 16:4(n-3) (*C. rudolphiana*). All algae were poor in 18:4(n-3) (0.5–2.0%) in comparison with species from the Ulvales (7.8–17.4%). Two species, *Codium fragile* and *Cladophora rudolphiana*, had 18:1(n-7)/18:1(n-9) ratios less than 1, the same as in other algal species belonging to this order [1, 19, 24]. Conversely, the two Yellow Sea species of the genus *Bryopsis* showed a ratio of these acids higher than 1. The ratio was not so high as for algae from the Ulvales. As *cis*-vaccenic acid was the main isomer among 18:1 FAs in *B. hypnoides* growing in the Black Sea [1] and in *B. plumosa* near the Senegal coast [19], we believed that the prevalence of 18:1n7 among the 18:1 isomers may be typical of *Bryopsis* species.

All the green algae examined contained C_{20} PUFAs [20:4(n-6) and 20:5(n-3)] but their contents were lower than in red and brown algae, amounting to 0.2–8.4%. Algae belonging to the order Siphonocladales are richer in C_{20} PUFAs (1.1–8.4%) than species from the order Ulvales (0.2–4.4%). Two C_{22} PUFAs (22:5(n-3) and 22:6(n-3)] were found in Ulvales species only. Conversely, *Cladophora rudolphiana* contained only 22:5(n-3), while *B. hypnoides*, *B. corticulans* and *Codium fragile* had no C_{22} PUFAs. Our results correlate with data of other authors, who

Table 4. Fatty acid composition of green alga (% total fatty acid content)

Fatty acid	Algae*								
	17c)	17d)	18	19	20	21	22	23	24
14:0	0.5	0.4	0.7	0.5	0.6	4.9	0.5	0.9	1.3
16:0	21.7	20.3	22.3	24.1	20.7	17.5	19.4	19.1	22.4
16:1n7	0.9	0.6	0.3	4.2	0.4	4.4	0.8	3.3	1.9
16:1*trans*	1.6	1.8	1.6	0.7	1.9	0.8	2.1	1.7	0.7
16:2(n-6)	0.3	0.4	0.2	0.3	0.5	1.9	0.9	1.2	1.0
16:3(n-3)	2.9	3.5	2.0	1.0	3.4	0.1	17.7	15.3	12.6
16:4(n-3)	14.4	16.2	15.1	19.2	15.2	13.9	0.0	0.0	0.0
18:0	0.3	0.3	0.4	0.6	0.4	0.8	0.7	0.4	0.9
18:1(n-9)	0.8	0.9	0.4	1.2	0.6	6.3	2.5	2.7	5.2
18:1(n-7)	8.8	8.8	6.0	7.6	7.4	5.3	7.5	7.5	1.1
18:2(n-6)	2.5	2.4	3.4	5.8	3.7	6.8	5.5	4.8	5.4
18:3(n-6)	0.2	0.5	0.5	1.0	0.7	0.2	1.9	1.6	2.6
18:3(n-3)	20.1	20.4	20.4	16.5	18.6	19.5	23.5	19.7	19.1
18:4(n-3)	12.3	16.4	17.7	7.8	15.3	0.5	1.3	1.3	2.0
20:4(n-6)	0.3	0.0	0.2	0.8	0.3	1.1	4.6	4.4	5.9
20:4(n-3)	0.8	0.6	0.6	0.4	0.0	0.3	0.3	0.2	0.5
20:5(n-3)	2.3	0.3	1.1	4.4	0.9	5.2	3.1	6.9	8.4
22:0	0.5	0.0	tr	0.4	0.0	0.0	0.0	2.4	0.2
22:5(n-3)	3.2	2.1	2.5	2.2	2.4	2.5	0.0	0.0	0.0
22:6(n-3)	0.0	0.0	0.6	0.5	0.3	0.0	0.0	0.0	0.0
Other†	5.6	4.1	4.0	0.8	6.7	8.0	7.7	6.6	8.8
18:1(n-7)/(n-9)	11.0	9.8	15.0	6.3	12.3	0.8	3.0	2.8	0.2

*Numbers designate algal species given in Table 1.

†Other: 14:1, 15:0, 16:1(n-9), 17:0, 20:0, 20:2(n-6), 20:3(n-6), 20:3(n-3).

17c) Sample taken from the upper tide horizon; 17d) sample taken from the low-tide mark.

failed to find C_{22} PUFAs in species from the genera *Bryopsis* and *Codium* [19, 43] or found less than 1% 22:5(n-3) [1, 11, 19].

Fatty acids of some algal species belonging to the Ulvales and Siphonocladales from different areas have been investigated [1, 13, 19, 24, 27, 30, 38–40, 47]. A comparison of the results for algae from different regions and from the Yellow Sea showed that, in general, most species had similar fatty acid compositions that did not depend on geographical location. However, slight quantitative differences were noted for *E. linza*, *Codium fragile* and *B. hypnoides*.

The fatty acid composition of seagrasses differed from that of seaweeds (Table 5). The main difference was a higher C_{18} PUFA content, primarily that of 18:3(n-3). The latter made up 49.3% of the total FAs in *Z. marina* and 60.6% in *Phyllosphadix iwatensis*. The level of α-linolenic acid was the highest among all seagrasses so far examined [25, 40, 48–50]. Both *Z. marina* and *P. iwatensis* contained 16:3(n-3) as one of the main acids. Earlier it was found that 16:3(n-3) is typical of seagrasses belonging to Zosteraceae which also include *Z. marina* and *P. iwatensis* [25, 48]. Seagrasses from the Yellow Sea contained saturated FAs with chain lengths longer than C_{20}; 22:0 was prevalent among them (2.1–2.5%), as was found for seagrasses from the Sea of Japan [25].

Earlier, we studied FAs of *Z. marina* and *P. iwatensis* from the Sea of Japan in summer [25]. The fatty acid composition of the former was very similar to that of the same species from the Yellow Sea. However, *P. iwatensis* from Yellow Sea waters was richer in (n-3) PUFAs (16:3 and 18:3) and poorer in 16:0 and 18:2, than the specimen from the Sea of Japan. These differences were probably caused by environmental factors, primarily water temperature.

Table 5. Fatty acid composition of seagrasses (% total fatty acid content)

Fatty acid	Seagrasses*	
	25	26
16:0	16.5	12.4
16:1(n-7)	0.2	0.3
16:1*trans*	0.8	0.9
16:3(n-3)	7.1	8.4
18:0	1.0	0.8
18:1(n-9)	1.8	0.7
18:2(n-6)	16.1	3.0
18:3(n-3)	49.3	60.6
20:0	0.9	1.0
21:0	0.2	0.3
20:5(n-3)	0.1	0.2
22:0	2.1	2.5
23:0	0.1	0.2
24:0	0.6	1.6
Other†	3.2	7.1

*Numbers designate seagrass species given in Table 1.

†Other: 14:0, 14:1, i-15:0, 15:0, 16:1(n-9), 16:2, 17:0, 17:1, 18:1(n-7), 18:3(n-6), 18:4(n-3), 20:3(n-3), 25:0.

Studies of marine macrophytes growing in the Yellow Sea showed these plants to have fatty acid patterns typical of red, brown, green seaweeds and seagrasses. Habitat conditions affect the quantitative characteristics of the FA profiles, but the influence is not the same for each algal species. The more notable increases of PUFA contents in Yellow Sea algae in comparison with published data were found for the following species: brown algae (*Undaria pinnatifida*, *Laminaria japonica*, and *Colpomenia sinuosa*); red algae (*Gymnogongrus flabelliformis*, *Gloiopeltis furcata*, *Chondria tenuissima*, *Grateloupia filicina*, *Halymenia sinensis*, *Chondrus sinensis*); and green algae (*E. linza*, *Codium fragile* and *B. hypnoides*); and for the seagrass *Phyllospadix iwatensis*. We found no significant influence of habitat conditions on the fatty acids for *Sargassum* and *Gracilaria* species and also *Rhodomela confervoides*, *Ulva pertusa*, *Enteromorpha intestinalis*, *E. compressa*, and *Zostera marina*. These marine plants had the same ratios of main FAs as the same or related species collected from other regions.

The results obtained are partly of applied value. Marine macrophytes are rich in PUFAs of the (n-3) and (n-6) series, which are considered essential fatty acids for humans and animals. Some of these fatty acids [20:4(n-6), 20:5(n-3), 20:3(n − 6)] have high biological activity and serve as precursors for eicosanoids [51]. Both *Gracilaria* species (*G. asiatica* and *G. textorii*) may be considered as good potential sources of 20:4(n-6), with *G. asiatica* being richer in lipids than *G. textorii*. Algae belonging to the Cryptonemiales may be used as sources of 20:5(n-3). *Undaria pinnatifida* and *Laminaria japonica* presently used for food have different (n-3)/(n-6) ratio of PUFAs. *Undaria pinnatifida* has a higher content of (n-3) PUFAs than *L. japonica*, but the latter is richer in (n-6) fatty acids. Hence, their use in food products may be expected to produce different biological effects.

Possible influence of tides on algal lipid composition

We could not find any reliable data on the influence of tides on algal lipid composition in the literature. The descriptions of tidal characteristics for algae collected for lipid analysis are usually absent in the papers. Publications by Dembitsky and coworkers [3, 7, 8, 22] indicate that algae were collected from depths of 0–10 m or 2–3 m. Samples from low tide zones were collected by Harwood's group [4, 20]. All algal species investigated previously in our Laboratory were collected in the Sea of Japan near Vladivostok from the low tide zone. Tidal fluctuation of a much higher amplitude in comparison with the Sea of Japan is typical of the Yellow Sea near Qingdao [34].

To check the influence of duration of draining period during low tides on polar lipid and fatty acid compositions of algae, we collected pairs of samples of *Gracilaria asiatica* and *Ulva pertusa*, which differed in their habitat conditions. The first specimen of *G. asiatica* (No 5a, Table 1) was gathered from a small water pool among rocks, second (No 5b) from a draining sandy beach. One sample of *Ulva pertusa* (No 17c, Table 1) was taken from the upper horizon of dwelling of this species, the other (No 17d) grew at the level of the low-tide mark.

The results obtained showed that both samples of *Ulva pertusa* and *G. asiatica* had no difference in their polar lipid composition. Algae from drained locations were richer in lipids; *Ulva pertusa* contained 22.2 mg g^{-1} wet weight and *G. asiatica* 8.2 mg g^{-1} in comparison with algae taken from water 17.9 and 5.8 mg g^{-1}, respectively. The fatty acid composition of both *G. asiatica* samples were the same. *Ulva pertusa* from the upper and lower horizons had some more differences in contents of some fatty acids (Table 4). These data indicate that tidal regime may have only a minor influence on lipid content and ratio of PUFAs in some algal species.

CONCLUSION

Our results suggest that each division of seaweeds and seagrasses have distinctive features in the composition of their phospholipids and betaine polar lipids – DGTA and DGTS. Hence, polar lipid composition may have chemotaxonomic value for marine plants. Further study of the distribution of lipids in several seaweeds is required, for example, the presence of DGTA and PC in brown algae, DGTS in green algae from the genus *Bryopsis*, PC in algae from the genus *Enteromorpha* and to understand the reasons affecting the synthesis of PS and PE in *Ulva pertusa*. Marine macrophytes from the Yellow Sea have fatty acid patterns typical of red, brown, green seaweeds and seagrasses from other regions. Algal habitat conditions may affect the quantitative content of PUFAs, but the influence is not the same for different algal species.

Tidal regime did not influence the qualitative composition of polar lipids and fatty acids of algae, but it changed the lipid content and ratio of selected PUFAs. These results showed that the ecology, and more importantly taxonomy, determine the lipid characteristics of marine macrophytes.

EXPERIMENTAL

Algae and seagrasses were collected from November 1992 to January 1993 in the Yellow Sea near Qingdao. Fresh plants were thoroughly cleaned to remove epiphytes, small invertebrates and sand particles, and then heated for 2 min in boiling H_2O to inactivate enzymes [52]. Lipids were extracted by homogenization in $CHCl_3$–MeOH (1:2) [53]. Polar lipids were sepd in solvent systems for plant lipids [54] and phospholipids [55]. To identify polar lipids on chromatographic plates, the following specific spray-reagents were used: a molybdate spray for phospholipids [56], anthrone for glycolipids [57], 0.5% ninhydrin in Me_2CO for aminophospholipids and Dragendorff's reagent for choline-

containing lipids. Glyco- and phospholipids were also identified from their R_f values.

Fatty acids were converted to Me esters using 1% Na in MeOH, followed by 5% HCl in MeOH [58] and then purified by TLC. Analyses of Me esters were carried out by GC-FID using fused silica Carbowax 20 M and SPB-5 capillary columns (25 m × 0.25 mm) at 210° and at 220°, respectively. Carrier gas was He. Individual Me esters were identified by comparison of R_t data with those of authentic standards and by ECL measurements [59]. For identification of FAs, $AgNO_3$-TLC was also used [60].

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