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Seasonal changes of fatty acid composition and thermotropic behavior of polar lipids from marine macrophytes

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Abstract

Major glyco- and phospholipids as well as betaine lipid 1,2-diacylglycero-*O*-4′-(*N,N,N*-tri-methyl)-homoserine (DGTS) were isolated from five species of marine macrophytes harvested in the Sea of Japan in summer and winter at seawater temperatures of 20–23 and 3 °C, respectively. GC and DSC analysis of lipids revealed a common increase of ratio between *n*−3 and *n*−6 polyunsaturated fatty acids (PUFAs) of polar lipids from summer to winter despite their chemotaxonomically different fatty acid (FA) composition. Especially, high level of different *n*−3 PUFAs was observed in galactolipids in winter. However, the rise in FA unsaturation did not result in the lowering of peak maximum temperature of phase transition of photosynthetic lipids (glycolipids and phosphatidylglycerol (PG)) in contrast to non-photosynthetic ones [phosphatidylcholine (PC) and phosphatidylethanolamine (PE)]. Different thermotropic behavior of these lipid groups was accompanied by higher content of *n*−6 PUFAs from the sum of *n*−6 and *n*−3 PUFAs in PC and PE compared with glycolipids and PG in both seasons. Seasonal changes of DSC transitions and FA composition of DGTS studied for the first time were similar to PC and PE. Thermograms of all polar lipids were characterized by complex profiles and located in a wide temperature range between −130 and 80 °C, while the most evident phase separation occurred in PGs in both seasons. Polarizing microscopy combined with DSC has shown that the liquid crystal – isotropic melt transitions of polar lipids from marine macrophytes began from 10 to 30 °C mostly, which can cause the thermal sensitivity of plants to superoptimal temperatures in their environment.

Keywords: *Ahnfeltia tobuchiensis*; *Laminaria japonica*; *Sargassum pallidum*; *Ulva fenestrata*; *Zostera marina*; Algae; Seagrass; Fatty acids; Thermotropic behavior; Glycolipids; Phospholipids; Betaine lipid DGTS

1. Introduction

Our previous study (Sanina et al., 2004) revealed that fatty acid (FA) compositions of individual polar lipids like total lipids (Khotimchenko, 2003) of marine macrophytes are chemotaxonomic characteristics of these ecologically and commercially important plants. Moreover, some important differences were shown between FA compositions of photosynthetic and non-photosynthetic lipids of marine macroalgae and seagrass. In particular, the *n*−6 polyunsaturated FAs (PUFAs) shared the most part of the sum of *n*−6 and *n*−3 PUFAs in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) compared with glycolipids and phosphatidylglycerol (PG).

It was suggested that *n*−6 PUFAs are more functionally important in the content of the major lipids of plasma membrane than photosynthetic membrane because the main initiating process arises in the former (Tarchevsky, 2002). Therefore, plasma membranes may need more potent mediators participating in the signal transduction. Similarly to eicosanoids of animals, plant mediators, derived from *n*−6 PUFAs, are believed to be more potent than those derived from *n*−3 PUFAs (Lauritzen et al., 2001; Calder, 2006; Bagga et al., 2003).

The importance of *n*−6 PUFAs was also assumed to increase in the most active seasonal period for marine macrophytes analogously to other marine ectothermic organisms–invertebrates (Sanina and Kostetsky, 2002).

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Present work is aimed at clarifying the character of seasonal changes in FA composition of polar lipids isolated from five species, which present four divisions of marine macrophytes, to check our assumption.

In common with other ectothermic organisms, marine macrophytes should adjust FA composition of their membrane lipids to the environmental temperature. It is generally assumed that compensatory adjustments of FA composition are directed toward maintaining a lamellar liquid crystalline state of membrane lipid matrix (Gombos et al., 1994; Morein et al., 1996). This mechanism is triggered by changes in physical state of membrane lipid matrix, which performs the role of thermosensor (Samples et al., 1999; Logue et al., 2000; Török et al., 2003; Vigh et al., 2007). However, data on thermotropic behavior of plant individual lipids, especially glycolipids, are limited. Therefore, thermal transitions from crystalline to liquid crystalline state and from liquid crystalline to isotropic phase of polar lipids were studied to value the efficiency of seasonal adjustments of marine macrophytes to the low and high environmental temperature, respectively.

2. Results and discussion

2.1. Fatty acid composition of polar lipids of marine macrophytes

FA composition of both phospho- and glycolipids was studied for the three species of marine macrophytes: *Ahnfeltia tobuchiensis, Laminaria japonica,* and *Zostera marina*, while glycolipids alone were analyzed in *Sargassum pallidum* and *Ulva fenestrata*. Betaine lipid 1,2-diacylglycero-*O*-4′-(*N,N,N*-tri-methyl) homoserine (DGTS), which is suggested to substitute PC in green algae (Eichenberger and Araki, 1993; Arisz et al., 2000), was also isolated from *U. fenestrate* to characterize its FA composition.

2.1.1. Fatty acid composition of phospho- and glycolipids of *A. tobuchiensis*

The remarkable level of the major FAs (16:0, 18:0, 18:1*n*−9, 18:1*n*−7, 20:4*n*−6 and 20:5*n*−3) remained in polar lipids of red algae *A. tobuchiensis* in both seasons (Table 1). In addition, the percentage of 16:1 was also significant in winter. The content of major FAs 16:0, 20:4*n*−6 and 20:5*n*−3 in contrast to 18:0, 18:1*n*−9 and 18:1*n*−7 in glyco- and/or phospholipids was altered similarly. Percentage of 16:0 decreased in winter, which was the main reason for the total saturated FA level decrease, as well as the rise of UFA/SFA ratio and unsaturation index (UI). The sharpest changes in UI occurred in dominated lipids of *A. tobuchiensis* (Kostetsky et al., 2004) – PC and digalactosyldiacylglycerol (DGDG), whereas UFA/SFA ratio increased in photosynthetic lipids (glycolipids and PG) to a greater extent compared with non-photosynthetic ones (PC and PE). The ratios of 20:5*n*−3/20:4*n*−6 and *n*−3/*n*−6 PUFAs as a whole increased from summer to winter in all polar lipids, while the resulting prevalence of 20:5*n*−3 over 20:4*n*−6 in winter was more pronounced in glycolipids compared with PC and PE. Moreover, the values of both ratios of 20:5*n*−3/20:4*n*−6 (except PC) and *n*−3/*n*−6 PUFAs (except PG) of polar lipids of *A. tobuchiensis* were reversed at season change. The enhanced need of *n*−3 PUFAs in the functioning of photosynthetic membranes in winter may be connected with the defense role of these PUFAs against low-temperature photoinhibition of photosynthesis (Blankenship, 2002). Our assumption is confirmed by the data on cyanobacterium *Synechocystis* PCC 6803, where unsaturation of glycerolipids stabilized the photosynthetic apparatus against low-temperature photoinhibition (Gombos et al., 1994). On the contrary, the swap of 18:3*n*−3 by 18:2*n*−6 was shown to improve the rate of photosynthesis growth at moderately high-temperatures (Murakami et al., 2000). It is interesting to note that the level of 20:5*n*−3 increased significantly in PC and PE in contrast to glycolipids and PG of *A. tobuchiensis*. As a result, the difference between the levels of 20:4*n*−6 and 20:5*n*−3 in non-photosynthetic lipids became negligible in winter. In spite of the common rise in unsaturation of FAs in winter compared with summer, UI and UFA/SFA decreased in the lines MGDG → DGDG → SQDG and PC → PE → PG as in summer (Sanina et al., 2004). The low-temperature adaptation of this red algae was also accompanied with the decrease in the ratio between C_{20} and C_{18} PUFAs in all lipids, especially in MGDG (Table 1).

2.1.2. Fatty acid composition of phospho- and glycolipids of *L. japonica*

The list of common dominated FAs (14:0, 16:0, 16:1, 18:1*n*−9, 18:2*n*−6, 18:3*n*−3, 20:4*n*−6 and 20:5*n*−3) also remained in polar lipids of *L. japonica* in both seasons (Table 2), while the content of major SFAs, MUFAs and *n*−6 PUFAs in most of the lipids decreased greatly in winter. Besides, the level of 18:0 in PG dropped from 23.0% to 0.3%, while this FA did not reach 4% in other lipids in both seasons. The percentage of γ-linolenic acid 18:3*n*−6 dropped two times in MGDG in winter, whereas its content remained low in other lipids in both seasons. On the contrary, the high level of stearidonic acid 18:4*n*−3 in MGDG in summer became higher in winter. Else sharper enrichment in 18:4*n*−3 occurred in other glycolipids, whereas phospholipids maintained a low level of this PUFA in both seasons. The seasonal change in the percentage of 18:3*n*−3, which was the major PUFA of photosynthetic lipids, was significant in SQDG and PG only. The inverse ratio between 20:5*n*−3 and 20:4*n*−6 in phospholipids compared with glycolipids remained in both seasons, while an increased 20:5*n*−3/20:4*n*−6 ratio as well as 18:3*n*−3/18:2*n*−6 and 18:4*n*−3/ 18:3*n*−6, occurred in all lipids in winter. Similarly to *A. tobuchiensis* (Table 1), the prevalence of 20:5*n*−3 over 20:4*n*−6 ratio became more pronounced in glycolipids, mainly due to the significant increase of 20:5*n*−3 level in their content except MGDG, where this role seems to belong to 18:4*n*−3. As a whole,

Table 1
Fatty acid composition of major polar lipids of red algae *Ahnfeltia tobuchiensis,* harvested in summer (S) and winter (W) (% of the sum of fatty acids)

Fatty acids	Lipid classes											
	MGDG		DGDG		SQDG		PC		PE		PG	
	S[a]	W	S[a]	W	S[a]	W	S[a]	W	S[a]	W	S[a]	W
14:0	0.4	0.7	0.6	1.0	4.2	3.2	0.6	1.5	0.7	1.7	0.2	2.7
15:0	n.d.	0.3	0.2	0.5	1.5	1.8	0.3	1.1	0.6	0.7	0.2	1.3
16:0	10.5	8.1	31.5	26.0	32.3	23.8	12.5	9.8	15.4	11.1	41.2	27.6
16:1	1.4	4.6	0.9	3.8	3.4	8.7	0.9	4.4	2.7	11.5	0.6	9.7
16:2*n*−6	n.d.	1.5	0.1	0.4	n.d.	n.d.	n.d.	0.2	0.5	0.3	1.5	0.2
17:0	1.0	0.1	0.3	0.1	0.5	0.5	0.7	0.2	0.5	0.2	0.3	0.5
18:0	0.9	1.1	4.1	1.9	8.7	3.9	4.1	2.8	6.0	8.4	4.8	6.1
18:1*n*−9	8.1	8.3	25.4	19.6	10.0	12.9	21.6	8.0	21.0	12.3	20.7	16.1
18:1*n*−7	1.5	1.2	n.d.	2.8	2.5	6.3	14.3	7.0	6.6	9.2	4.7	8.9
18:2*n*−6	0.6	1.4	1.0	2.1	1.2	3.0	1.1	1.6	1.1	3.3	0.7	3.6
18:3*n*−3	0.9	1.1	0.5	0.8	0.3	0.9	n.d.	0.9	n.d.	0.9	n.d.	0.7
18:4*n*−3	0.9	1.6	0.8	0.7	0.7	0.5	n.d.	0.8	n.d.	0.5	n.d.	0.2
20:2*n*−6	n.d.	0.1	n.d.	0.2	n.d.	0.8	0.8	0.3	1.9	0.5	n.d.	n.d.
20:3*n*−6	0.6	0.7	1.8	2.4	n.d.	1.5	2.7	1.6	9.9	1.8	n.d.	0.4
20:4*n*−6	36.8	22.2	19.7	13.9	20.1	9.5	28.3	22.0	20.6	10.8	14.5	7.8
20:5*n*−3	36.8	41.4	13.2	19.0	13.0	14.3	6.7	20.1	6.7	12.9	8.5	9.1
22:6*n*−3	n.d.	0.5	0.4	1.8	0.7	3.0	0.8	11.2	n.d.	6.5	n.d.	0.8
SFA	13.0	10.6	37.0	29.6	48.0	35.9	18.2	16.5	25.0	22.9	47.8	39.0
MUFA	11.0	15.0	27.0	26.8	16.0	30.0	40.0	21.6	33.0	37.4	27.0	33.8
PUFA	76.0	74.4	36.0	43.6	35.0	34.1	41.8	61.9	42.0	39.7	25.2	27.2
Unsaturated/saturated	6.7	8.4	1.7	2.4	1.1	1.8	4.5	5.1	3.0	3.4	1.1	1.6
UI	347	346	186	216	164	176	199	305	188	211	132	141
n−3 PUFA	36.8	46.4	13.2	23.1	13.0	18.8	6.7	35.1	6.7	21.6	8.5	11.8
n−6 PUFA	38.0	28.5	22.5	20.7	21.3	15.3	32.9	26.8	33.5	18.1	15.2	15.4
n−3/*n*−6 PUFA	0.97	1.63	0.57	1.12	0.61	1.23	0.20	1.31	0.28	1.19	0.56	0.77
C_{18} PUFA	0.6	4.6	1.0	3.8	1.2	4.4	1.1	3.3	1.1	4.7	0.7	4.8
C_{20} PUFA	74.2	64.7	34.7	35.5	33.1	26.1	38.5	45.2	39.1	26	23.7	17.2
C_{20}/C_{18} PUFA	123.7	14.07	34.7	9.3	27.6	5.9	35.0	13.7	35.5	5.5	33.9	3.6
20:5*n*−3/20:4*n*−6	1	1.9	0.7	1.4	0.6	1.5	0.2	0.9	0.3	1.2	0.6	1.2

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.
n.d. – not detected, tr. – traces (content less than 0.1%).
[a] Sanina et al. (2004).

the significant acceleration of the *n*−3 PUFA content against the background of ambiguous changes of the *n*−6 PUFA level resulted in the rise of *n*−3/*n*−6 ratio from summer to winter in all polar lipids especially in glycolipids and PG. UI and the ratio of UFA/SFA also increased in all studied lipids except MGDG and PC, respectively. It is necessary to point out that the increasing lipid unsaturation due to the swap of *n*−6 for *n*−3 PUFAs can cause the rise of T_{max} of respective polar lipids (Huang and Li, 1999; Kostetsky et al., 1992).

n−3 PUFAs quantitatively prevailed over *n*−6 PUFAs in glycolipids and PG in winter only, except for MGDG, where this trait occurred in both seasons. To the contrary, the ratio of *n*−3/*n*−6 PUFAs in PC and PE was less than one in both seasons. Photosynthetic lipids differed from non-photosynthetic ones by a ratio of C_{18}/C_{20} PUFAs also. C_{18} prevailed over C_{20} PUFAs in the former lipids, while the inverse ratio occurred in later lipids independently of the season. The increase of the C_{18} PUFAs level in glycolipids and PG in contrast to PC and PE promoted this difference in winter. However, the ratio of C_{18}/C_{20} PUFAs decreased in most lipids of *L. japonica* at the seasonal acclimatization to the low seawater temperature (Table 2).

The values of UI and/or UFA/SFA increased classically from summer to winter in all lipids of *L. japonica* as of *A. tobuchiensis* (Table 1), while the dependence of FA saturation from polar groups of glyco- and phospholipids differed somewhat in winter compared with summer. So, DGDG and PE were the most saturated neutral glyco- and phospholipids, respectively, in winter, as opposed to MGDG and PC in summer, while acid lipids SQDG and PG remained the most saturated lipids in both seasons.

2.1.3. Fatty acid composition of phospho- and glycolipids of *Z. marina*

FA composition of polar lipids from *Z. marina* (Table 3) essentially differed from FA composition of polar lipids of *L. japonica* and *A. tobuchiensis* (Tables 1 and 2, respectively) by very low content of C_{20} PUFAs. Therefore, the greatest seasonal changes occurred in two dominated C_{18} PUFAs, 18:2*n*−6 and 18:3*n*−3, as well as in saturated FA 16:0. Interestingly, the level of 18:2*n*−6 was highest

Table 2
Fatty acid composition of major polar lipids of brown algae *Laminaria japonica,* harvested in summer (S) and winter (W) (% of the sum of fatty acids)

Fatty acids	Lipid classes											
	MGDG		DGDG		SQDG		PC		PE		PG	
	S[a]	W	S[a]	W	S[a]	W	S[a]	W	S[a]	W	S[a]	W
14:0	5.0	5.8	9.0	3.4	3.6	1.1	12.7	18.5	4.4	3.0	0.8	1.5
16:0	5.5	2.9	20.0	1.9	45.2	25.5	12.4	12.4	29.3	9.5	14.4	27.0
16:1	4.0	1.1	20.5	7.1	4.3	2.6	4.4	2.6	6.1	4.6	1.7	12.1
18:0	0.5	0.9	3.3	0.6	3.6	1.5	0.7	0.4	3.8	1.9	23.0	0.3
18:1*n*−9	9.6	4.2	14.1	1.2	21.9	17.0	12.1	2.6	9.2	3.9	15.7	13.1
18:2*n*−6	11.1	6.3	10.9	1.1	7.7	5.1	12.4	8.3	5.4	2.8	13.7	9.5
18:3*n*−6	8.0	3.3	1.8	0.1	0.9	1.4	0.4	0.7	0.7	0.2	1.2	0.2
18:3*n*−3	8.7	5.4	5.2	4.5	3.0	11.4	1.3	2.7	2.8	3.0	8.7	29.0
18:4*n*−3	20.3	54.3	3.2	38.0	0.9	9.3	0.4	0.8	0.8	1.6	0.3	0.9
20:4*n*−6	9.9	1.5	2.1	2.6	3.0	2.0	29.1	25.0	26.9	44.8	4.5	3.2
20:5*n*−3	15.9	10.9	3.4	33.2	1.4	9.9	7.5	17.7	2.3	18.5	0.3	1.4
SFA	11.5	9.9	35.0	6.1	53.1	29.8	27.6	32.3	40.0	16.1	46.9	29.6
MUFA	14.0	6.4	37.7	12.4	30.0	21.6	17.6	5.5	19.9	9.8	20.6	25.6
PUFA	74.5	83.7	27.3	81.5	16.9	48.6	54.8	62.2	40.1	74.1	32.5	44.8
UI	387	327	120	362	79	182	214	246	169	311	110	157
Unsaturated/saturated	7.7	9.1	1.9	15.4	0.9	2.4	2.6	2.1	1.5	5.2	1.1	2.4
C_{18} PUFA	48.1	69.3	21.1	43.6	12.5	28.9	14.5	12.5	9.7	7.6	23.9	39.6
C_{20} PUFA	25.8	13.2	5.5	36.3	4.4	14.8	36.6	49.0	29.2	64.8	4.8	4.9
C_{18}/C_{20} PUFA	1.9	5.2	3.8	1.2	2.8	2	0.4	0.3	0.3	0.1	5	8
20:5*n*−3/20:4*n*−6	1.6	7.3	1.6	12.8	0.5	4.9	0.3	0.7	0.1	0.4	0.1	0.4
18:3*n*−3/18:2*n*−6	0.8	0.9	0.5	4.1	0.4	2.2	0.1	0.3	0.5	1.1	0.6	2.2
18:4*n*−3/18:3*n*−6	2.5	16.5	1.8	380	1	6.6	1	1.1	1.1	8	0.25	4.5
n−3 PUFA	44.9	71.5	11.8	75.7	5.3	32.3	9.2	24.1	5.9	24.6	9.3	31.8
n−6 PUFA	29	11.4	14.8	5.4	11.6	12.6	41.9	37.6	33	48.7	19.4	12.9
n−3/*n*−6 PUFA	1.5	6.3	0.8	13.3	0.5	2.6	0.2	0.6	0.2	0.5	0.5	2.5
$\sum(n-3+n-6)$	73.9	82.9	26.6	81.1	16.9	44.9	51.1	61.7	38.9	73.3	28.7	44.7

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; Fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.
n.d. – not detected, tr. – traces (content less than 0.1%).
[a] Sanina et al. (2004).

in PC and PE of *Z. marina* similar to 20:4*n*−6 in the same lipids of *L. japonica*. The percentage of 18:2*n*−6 increased practically in all lipids of *Z. marina* from winter to summer. To the contrary, the percentage of 18:3*n*−3 was 1.5–2 times higher in galactolipids compared with phospholipids of *Z. marina* and became lower in summer. Probably, the resulting decrease of the 18:3*n*−3/18:2*n*−6 ratio is a typical adaptive reaction of plants to the elevation of environmental temperature (Klyachko-Gurvich et al., 1997; Zhu et al., 1997). These C_{18} PUFAs seem to perform a similar role with 20:5*n*−3 and 20:4*n*−6 in polar lipids of macroalgae *L. japonica* and *A. tobuchiensis* at thermal adaptation. Then, both PUFA pairs, 18:3*n*−3 ↔ 18:2*n*−6 and 20:5*n*−3 ↔ 20:4*n*−6, take part in thermal adaptation of *L. japonica,* unlike *A. tobuchiensis* and *Z. marina*. Such partial substitution of *n*−3 by *n*−6 PUFAs and *vice versa* can allow regulation of both membrane viscosity and power of second messengers. The seasonal swap of *n*−3 ↔ *n*−6 PUFAs, discovered in marine invertebrates (Sanina and Kostetsky, 2001, 2002), suggests the universal importance of this mechanism for thermal adaptation of eukaryotic ectotherms. However, *n*−3 PUFAs are less effective than even more saturated *n*−6 PUFAs in regulating lipid fluidity (Huang and Li, 1999; Kostetsky et al., 1992). Then, the increase of *n*−3/*n*−6 PUFAs ratio in the seagrass galactolipids, already enriched in *n*−3 PUFAs, cannot only be inefficient to fluidize these lipids, but even able to increase their viscosity.

FA composition of MGDG and especially DGDG of *Z. marina* became more homogeneous in winter (Table 3). In this season, 18:3*n*−3 alone remained a common major FA of galactolipids instead of the three FAs in summer. However, three common major FAs (16:0, 18:2*n*−6 and 18:3*n*−3) remained in PC, PE and PG in both seasons. One more major FA, 16:1 esterifies PG independently of season. So, the difference between FA composition of galacto- and phospholipids became more observable in winter compared with summer (Table 3). The content of 16:0 became 4–16 times higher in phospholipids compared with glycolipids in winter against 2–3 times in summer. The level of 18:2*n*−6 remained high in phospholipids only despite its about 1.5-fold decrease in PC and PE. The percentage of linoleic acid was strongly reduced in glycolipids, where it dropped from about 10% to 0.8% and 3.1% of the sum of FAs. However, the increase in 18:3*n*−3 percentage promoted domination of this FA in phospholipids and

Table 3
Fatty acid composition of major polar lipids of seagrass *Zostera marina,* harvested in summer (S) and winter (W) (% of the sum of fatty acids)

Fatty acids	Lipid classes									
	MGDG		DGDG		PC		PE		PG	
	S[a]	W	S[a]	W	S[a]	W	S[a]	W	S[a]	W
16:0	5.8	1.7	10.8	4.1	20.4	21.0	33.1	16.9	28.1	28.1
16:1	1.6	0.8	1.1	0.6	0.5	1.1	0.3	0.5	14.7	10.0
16:2	2.5	1.0	1.4	tr.	0.1	n.d.	0.2	n.d.	0.1	n.d.
16:3*n*−3	15.0	30.5	1.8	5.4	0.2	0.2	n.d.	0.2	0.1	0.2
18:0	1.1	0.2	1.3	0.5	1.6	0.9	0.8	0.5	1.5	0.5
18:1*n*−9	3.1	0.4	1.9	1.4	1.6	1.1	0.9	0.5	2.9	0.6
18:1*n*−7	n.d.	0.2	n.d.	0.7	n.d.	0.7	n.d.	0.6	2.8	0.7
18:2*n*−6	10.0	0.8	10.3	3.1	37.4	23.3	32.8	26.6	4.1	9.6
18:4*n*−3	0.8	1.8	0.3	1.1	0.6	1.1	0.2	0.8	0.8	0.4
18:3*n*−3	58.8	56.7	70.0	78.3	35.6	42.3	30.8	45.9	34.1	39.8
20:5*n*−3	1.2	2.7	1.4	3.3	1.9	4.9	n.d.	1.7	3.4	3.1
SFA	7.7	2.9	12.1	5.2	22.5	24.4	34.9	20.7	33.9	30.3
MUFA	4.7	2.2	3.0	2.7	2.3	3.1	1.3	1.9	22.0	13.8
PUFA	87.6	94.9	84.9	92.1	75.2	72.5	63.8	77.4	44.1	55.9
UI	258	292	252	283	197	208	161	212	155	174
Unsaturated/saturated	12.0	33.5	7.3	18.2	3.4	3.1	1.9	3.8	1.9	2.3
C_{18} PUFA	68.8	59.3	80.3	82.5	73.0	66.7	63.6	73.3	39.1	50.0
C_{20} PUFA	1.2	2.7	1.4	4.2	1.9	5.4	n.d.	3.0	4.1	3.5
C_{18}/C_{20} PUFA	57.3	22	57.3	19.6	38.4	12.3	∞	24.4	9.5	14.3
n−3 PUFA	75	92.7	73.2	88.1	37.7	48.5	30.8	49.1	38.6	43.7
n−6 PUFA	10	0.8	10.3	4	37.4	23.8	32.8	27.9	4.6	11.1
n−3/*n*−6	7.5	115.9	7.1	22	1	2	0.9	1.8	8.4	3.9
$\sum(n-3+n-6)$	85	93.5	83.5	92.1	75.1	72.3	63.6	77.0	43.2	54.8
18:3*n*−3/18:2*n*−6	5.9	70.9	6.8	25.3	1.0	1.8	0.9	1.7	8.3	4.1

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; Fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.
n.d. – not detected, tr. – traces (content less than 0.1%).
[a] Sanina et al. (2004).

DGDG especially. The content of 16:3*n*−3 also increased at least two times in glycolipids, while it was minor in phospholipids in both seasons.

As a whole, phospholipids of *Z. marina* remained more saturated than glycolipids, in spite of a common increase in both UI and UFA/SFA ratio of polar lipids in winter compared with summer. The saturation accelerated in the lines of MGDG → DGDG and PE → PC → PG. So, PC was more saturated than PE in winter in contrast to summer. However, more detailed analysis revealed that glycolipids and PG have common peculiarities, which distinguish them from PC and PE. The levels of 18:2*n*−6 and total *n*−6 PUFAs as well as *n*−6/*n*−3 PUFAs were essentially higher in PC and PE compared with glycolipids and PG in both seasons. In winter, *n*−3/*n*−6 ratio increased, but C_{18}/C_{20} reduced in all lipids of Z. *marina* in contrast to lipids from *L. japonica* and *A. tobuchiensis*.

2.1.4. Fatty acid composition of glycolipids of S. pallidum and U. fenestrata

A similar trend (the rise of unsaturation and *n*−3/*n*−6 ratio) occurred in FA composition of glycolipids from *S. pallidum* and *U. fenestrata* under season change from summer to winter (Tables 4 and 5, respectively). The content of most FAs from galactolipids of *S. pallidum* altered moderately compared with lipids of other macrophytic species. SQDG was the more saturated glycolipid compared with galactolipids independent of season. However, MGDG became more saturated than DGDG in winter in contrast to summer.

Moderate seasonal changes were also detected in MGDG of *U. fenestrata*, the content of which reaches 60–70% of total glycolipids in this seaweed (Kostetsky et al., 2004). More remarkable impact of season occurred in SQDG, where the content of 16:0 and 18:1*n*−7 changed the most. However, profound quantitative alterations were shown in the major FAs of DGDG. Interestingly, the changes in the content of *n*−3 PUFAs 16:3*n*−3, 18:3*n*−3 and 18:4*n*−3 were contrary, while total percentage of *n*−3 PUFAs as well as *n*−3/*n*−6, UFA/SFA ratios and UI of DGDG from *U. fenestrata,* increased from summer to winter, as with other polar lipids of this and other studied species.

2.2. Thermotropic behavior of polar lipids of marine macrophytes

As ectothermic organisms, marine macrophytes should maintain a lamellar liquid crystalline state of membrane lipids by a modulation in FA composition at environmental temperature alterations. The resulted changes in the crystal-liquid crystal-isotropic melt phase transitions were

Table 4
Fatty acid composition of major polar lipids of brown alga *Sargassum pallidum,* harvested in summer (S) and winter (W) (% of the sum of fatty acids)

Fatty acids	Lipid classes					
	MGDG		DGDG		SQDG	
	S[a]	W	S[a]	W	S[a]	W
14:0	3.7	4.0	2.7	1.5	3.2	3.0
16:0	13.2	9.4	12.5	10.6	16.6	33.4
16:1	3.2	4.0	4.7	2.0	4.9	6.2
16:2	0.2	0.2	1.1	0.4	n.d.	0.5
17:0	1.4	0.7	5.0	4.3	13.4	10.6
16:4*n*−3	0.3	0.7	0.4	1.0	8.8	0.7
18:0	1.6	0.6	1.7	2.9	5.4	4.4
18:1*n*−7	n.d.	0.2	0.8	0.3	1.1	0.5
18:1*n*−9	6.0	3.1	3.5	1.7	7.0	8.2
18:2*n*−6	16.3	14.4	7.0	3.2	2.9	5.4
18:3*n*−6	1.6	2.8	1.0	1.0	n.d.	0.4
18:3*n*−3	5.1	7.0	11.2	6.0	5.1	5.1
18:4*n*−3	7.1	16.5	14.6	26.4	3.2	1.3
20:1*n*−9	0.9	0.5	0.3	n.d.	1.3	2.6
20:1*n*−7	n.d.	1.2	0.3	0.6	1.2	2.3
20:3*n*−6	6.0	8.6	0.9	0.8	1.3	1.1
20:4*n*−6	15.6	15.0	10.0	10.3	1.0	3.7
20:5*n*−3	12.6	10.5	14.2	21.4	0.4	2.7
22:0	0.4	0.3	0.3	1.0	1.5	1.5
22:2*n*−6	0.3	n.d.	0.1	0.3	1.4	0.6
22:3*n*−6	n.d.	0.2	0.2	0.3	2.7	0.6
SFA	21.0	15.1	24.1	22.7	50.5	52.6
MUFA	11.3	9.0	13.5	5.2	17.4	23.6
PUFA	67.7	75.9	62.4	72.1	32.1	23.8
Unsaturated/saturated	0.27	0.18	0.32		1.02	1.1
UI	248	275	247	315	125	101
n−3 PUFA	25.9	34.7	40.8	54.9	20.8	10.1
n−6 PUFA	40.2	41.2	19.3	16.8	10.7	13.2
n−3/*n*−6 PUFA	0.6	0.84	2.1	3.3	1.9	0.8
$\sum(n-3+n-6)$	66.1	75.9	60.1	71.7	31.5	23.3
C_{18} PUFA	30.1	40.7	33.8	36.1	13.3	12.2
C_{20} PUFA	35.4	34.1	25.6	33.4	5.3	9.7

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; Fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.
n.d. – not detected, tr. – traces (content less than 0.1%).
[a] Sanina et al. (2004).

Table 5
Fatty acid composition of major polar lipids of green alga *Ulva fenestrata,* harvested in summer (S) and winter (W) (% of the sum of fatty acids)

Fatty acids	Lipid classes							
	MGDG		DGDG		SQDG		DGTS	
	S[a]	W	S[a]	W	S[a]	W	S[a]	W
16:0	1.1	0.2	24.8	11.5	58.7	50.9	35.5	24.6
16:1	0.6	0.4	3.4	1.5	1.5	1.7	2.0	1.7
16:3*n*−3	1.0	0.2	3.6	24.6	n.d.	0.2	0.6	0.8
16:4*n*−3	43.7	48.8	2.6	3.5	n.d.	0.1	0.3	n.d.
18:0	0.2	0.4	0.9	0.2	1.6	0.2	0.4	0.2
18:1*n*−7	1.8	0.3	5.0	0.2	15.4	19.0	8.4	16.3
18:1*n*−9	0.4	n.d.	1.6	n.d.	0.2	0.4	1.2	0.5
18:2*n*−6	2.1	0.2	14.2	9.1	2.1	2.6	6.9	3.5
18:3*n*−3	24.1	23.1	13.8	38.6	18.6	21.0	14.6	8.9
18:3*n*−6	0.3	n.d.	n.d.	n.d.	n.d.	n.d.	5.5	2.4
18:4*n*−3	24.3	26.2	23.8	2.7	1.6	0.7	15.8	27.5
20:4*n*−6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.9	0.6
20:5*n*−3	tr.	n.d.	n.d.	n.d.	n.d.	n.d.	3.5	6.7
SFA	1.3	0.6	26.0	12.9	60.6	52.3	36.3	26.4
MUFA	2.5	0.9	7.5	3.9	17.1	21.8	11.6	19.7
PUFA	96.2	98.5	66.5	83.2	22.3	25.9	52.1	53.9
UI	357	371	217	246	84	95	189	216
Unsaturated/ saturated	75.9	165.6	2.8	6.8	0.6	0.9	1.8	2.8
C_{18} PUFA	50.8	49.5	51.8	50.4	22.3	24.3	42.8	42.3
C_{20} PUFA	n.d.	n.d.	n.d.	0.1	n.d.	0.3	8.4	10.3
n−3 PUFA	93.1	98.5	43.8	69.7	20.2	22.3	34.8	46.9
n−6 PUFA	2.4	0.2	14.2	9.5	2.1	2.6	17.3	6.5
n−3/*n*−6	38.8	492.5	3.1	7.3	9.6	8.6	2.0	7.2
$\sum(n-3+n-6)$	95.5	98.7	58.0	79.2	22.3	24.9	52.1	53.4

SFA, UFA, MUFA, PUFA, saturated, unsaturated, monounsaturated, polyunsaturated fatty acids, respectively; UI, unsaturation index; Fatty acids with content below 1% are excluded. Standard deviations were less than 0.5% for three replicates.
n.d. – not detected, tr. – traces (content less than 0.1%).
[a] Sanina et al. (2004).

shown to underlie the low- and high-temperature adaptation of marine invertebrates (Sanina and Kostetsky, 2001, 2002). These phase transitions were studied in phospho- and glycolipids of marine macrophytes by DSC combined with polarizing microscopy to detect whether the same regulation exists in marine macrophytes.

2.2.1. DSC study

Differential scanning calorimetry (DSC) is the most appropriate method for a detection of the lipid phase transitions, which are attributed fundamentally to the thermally induced *trans-gauche* rotational isomerization of methylene groups about the single C–C bonds along lipid acyl chains. When phospholipids are subjected to heating, the heat flow difference between a sample and a reference is scanned in some temperature range. Phase transition from crystalline to liquid crystalline state of phospholipids is an endothermic process, which is visualized by DSC as an integral curve (thermogram) of dependence between a heat capacity and temperature. This phase transition, unlike mesophase or isotropic transitions, is characterized by the high heat capacity. Therefore it is named as the main phase transition, in which peak maximum temperature (T_{max}) is characteristic for a lipid sample of a definite chemical structure (Huang and Li, 1999). The transition enthalpy corresponds to the integrated area under the calorimetric curve. Both crystalline and liquid crystalline phases co-exist at temperatures, which correspond to a temperature range of the main phase transition. Crystalline phase only exists at temperatures lower than minimal temperature of this range, whereas liquid crystalline state only exists at temperatures higher than maximal temperature of a temperature range of the main phase transition. The width and profile of lipid's thermograms depend on heterogeneity of fatty acid composition. Heterogeneity of a fatty acid pool promotes the widening of a temperature range and *vice versa*. Essential differences of fatty acid structures in the content of individual lipids can lead to the phase

separation, which is resulted in the discrete or poorly-resolved peaks on thermograms. Broad phase transitions are also a common feature of highly unsaturated lipids (Williams, 1998).

Thermograms of polar lipids from four marine macrophytic species (*L. japonica, S. pallidum*, *U. fenestrata* and *Z. marina*), harvested in summer and winter were characterized by complex profiles and located in the wide temperature interval between −130 and 80 °C (Figs. 1–4). Endothermic peaks occurred at the low and high-temperature ranges and can arise due to the phase separation of lipids (Mouritsen and Kinnunen, 1996). PGs were characterized by the most resolved peaks (Figs. 1 and 2C) that can reflect a difference of FA composition of PG compared with PE and PC in both seasons (Tables 1 and 2). Phase separation of PG can promote the physical segregation of slow PSI and rapid PSII, which is one of the common principles found in nature. Phenomena of segregation and its motive forces are far from clear understanding, while just membrane lipids alone were shown to induce segregation of PSI and PSII (Borodich et al., 2003), and PG is needed to support their superstructures (Kruse et al., 2000; Jordan et al., 2001). The refractory high-temperature domains of PG or other polar lipids can also play the role of low-temperature sensors in respective membranes of marine macrophytes.

Thermal transitions of glycolipids (MGDG, DGDG and SQDG) of all four species are shown in Figs. 1–4, while thermotropic behavior of phospholipids (PC, PE and PG) was studied for two species, *L. japonica* and *Z. marina* (Figs. 1 and 2), where all three lipids dominate irrespective of season (Kostetsky et al., 2004). The values of T_{max} for all lipids are presented in Table 6. The differences marked in the seasonal changes of FA composition of photosynthetic and non-photosynthetic lipids seem to underlie the different season impact on DSC transitions of these lipid groups isolated from *Z. marina* and *L. japonica.* The increased FA unsaturation in winter similarly influenced thermotropic behavior of PC and PE of these taxonomically distant species. T_{max} dropped by 10–36 °C (Table 6), which promoted the location of the most parts of thermograms at temperatures below 3 °C (seawater temperature in winter). Therefore, the most part of respective lipid sample will exist in liquid crystalline state at this temperature. The shift of T_{max} was stronger in PE compared with PC (by 20–36 °C against 10–15 °C, respectively).

However, T_{max} of PG and glycolipids from *Z. marina* and *L. japonica* did not decrease, which is contrary to the conception of homeoviscous adaptation (Sinensky, 1974). So, the position of two endothermic peaks, occurred on thermograms of PG from *Z. marina,* little depended on season (Fig. 2C). No essential changes were also detected in T_{max} of DGDG from *Z. marina* (Fig. 2E; Table 6). Thermograms of PG from *L. japonica* were characterized by the low- and high-temperature peaks of the same intensity (Fig. 1C; Table 6). The change of season resulted in the increase of the distance between these peaks mainly due

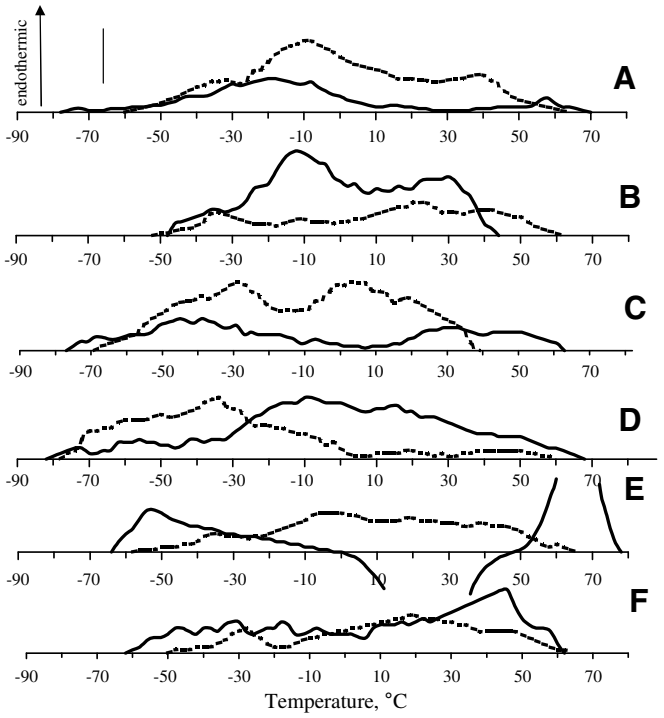

Fig. 1. DSC thermograms of major polar lipids of *Laminaria japonica*, harvested in winter (solid curve) and summer (dotted curve). PC (A), PE (B), PG (C), MGDG (D), DGDG (E) and SQDG (F). Vertical bar represents 1 mW. Scanning rate, 16 °C/min. Sample weight, 10 mg. Each lipid sample was scanned at least three times.

to the sharp elevation of T_{max} of the high-temperature peak by about 40 °C. T_{max} of the low-temperature peak decreased by 8 °C. The seasonal changes in thermal transitions of DGDG from *L. japonica* and MGDG from *Z. marina* were even more ambiguous (Figs. 1E and 2D, respectively). The lowering of seawater temperature resulted in the appearance of two endothermic and one exothermic peaks at −52, 68 and 26 °C, respectively, instead of one wide peak at −3 °C on thermogram of DGDG from *L. japonica* in summer. The opposite effect was shown in MGDG of *Z. marina.* The seasonal elevation of seawater temperature promoted the split of the main peak at −10 °C, which was detected on thermogram of the winter sample, into two weak-resolved peaks at −34 and 38 °C (Table 6). T_{max} of MGDG and SQDG from *L. japonica* increased by 25 and 28 °C, respectively, from summer to winter (Fig. 1D and F, respectively; Table 6). So, there was no classic decrease of T_{max} of photosynthetic lipids in contrast to non-photosynthetic PC and PE from *L. japonica* and *Z. marina* at their acclimatization to the low seawater temperature. The same conclusion should be done in respect to thermal transitions of glycolipids from *S. pallidum* and *U. fenestrata* (Figs. 3 and 4; Table 6). However, non-decreasing T_{max} of photosynthetic lipids can cause the increased viscosity of these lipids and thereby the delayed photosynthetic activity of marine macrophytes in winter (Kizevetter et al., 1981) despite the classic increase of UI occurring in FAs of these lipids.

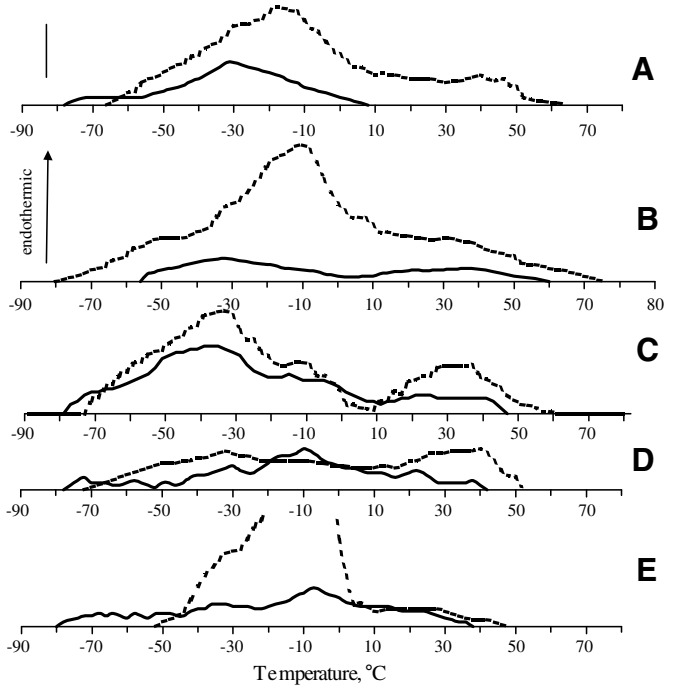

Fig. 2. DSC thermograms of major polar lipids of *Zostera marina*, harvested in summer (dotted curve) and winter (solid curve). PC (A), PE (B), PG (C), MGDG (D) and DGDG (E). Vertical bar represents 2 mW. Scanning rate, 16 °C/min. Sample weight, 10 mg. Each lipid sample was scanned at least three times.

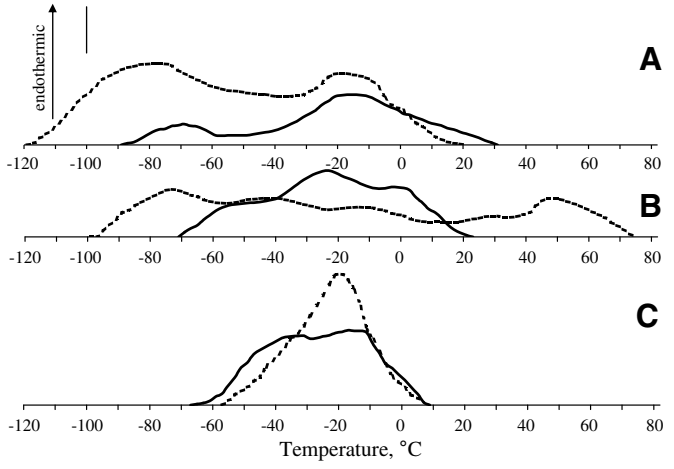

Fig. 3. DSC thermograms of glycolipids of *Sargassum pallidum*, harvested in summer (dotted curve) and winter (solid curve). MGDG (A), DGDG (B) and SQDG (C). Vertical bar represents 2 mW. Scanning rate, 16 °C/min. Sample weight, 10 mg. Each lipid sample was scanned at least three times.

Calorimetric transition of betaine lipid DGTS of *U. fenestrata*, harvested in summer, was characterized by the cooperative single peak maximum at −21 °C (Fig. 4D, Table 6). It was more typical to PC and PE of *L. japonica* and *Z. marina* than glycolipids of *U. fenestrata.* Seasonal change of thermotropic behavior of DGTS was also similar to PC and PE of *L. japonica* and *Z. marina*: T_{max} sharply decreased from −21 to −38 °C. Moreover, temperature

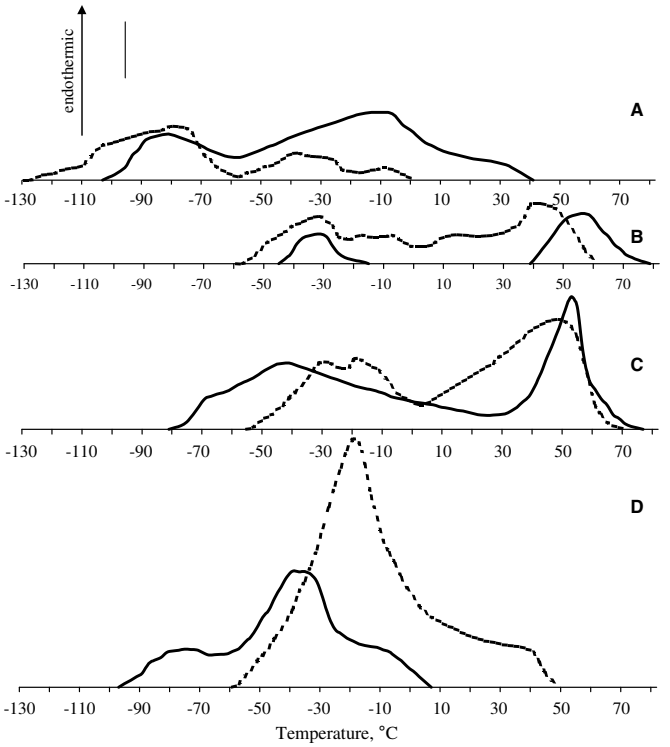

Fig. 4. DSC thermograms of glyco- and betaine lipids of *Ulva fenestrata*, harvested in summer (dotted curve) and winter (solid curve). MGDG (A), DGDG (B), SQDG (C) and DGTS (D). Vertical bar represents 2 mW. Scanning rate, 16 °C/min. Sample weight, 10 mg. Each lipid sample was scanned at least three times.

Table 6
Peak maximum temperatures (T_{max}, °C) of DSC transitions of polar lipids from marine macrophytes, harvested in summer and winter

Lipids	T_{max}, °C[a]	
	Summer	Winter
Laminaria japonica		
PC	−9	−19
PE	24	−12
PG	−34; −5	−42; 32
MGDG	−34	−9
DGDG	−3	−52; 26[b], 68
SQDG	18	46
Zostera marina		
PC	−16	−31
PE	−12	−32
PG	−35	−36
MGDG	−34; 38	−10
DGDG	−5	−6
Sargassum pallidum		
MGDG	−80	−16
DGDG	−74; 45	−24
SQDG	−20	−16
Ulva fenestrata		
MGDG	−75	−10
DGDG	−30; 41	−32; 56
SQDG	49	52
DGTS	−21	−38

[a] Standard deviations were less than 1 °C for three replicates.
[b] Exothermic peak.

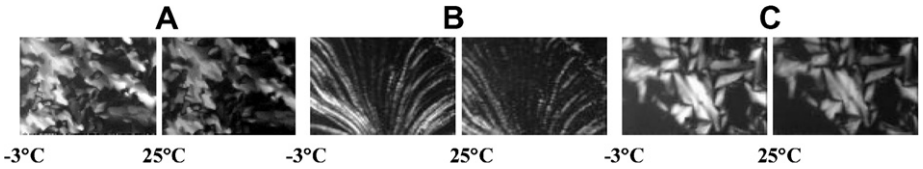

Fig. 5. Textures of polar lipids (MGDG – A, DGDG – B, DGTS - C) of *Laminaria japonica* (A) and *Ulva fenestrata* (B, C), harvested in winter (A, C) and summer (B), during isotropic melting. Polarizing microscope POLAM-P-312 (Russia), ×100.

range of calorimetric transition shifted to the side of low-temperatures (from −60–50 to −98–6 °C).

A common effect of increased unsaturation of the most polar lipids of marine macrophytes was the lower intensity of their calorimetric peaks in winter compared with summer.

2.2.2. Polarizing microscopy

Phospho- and glycolipids belong to mesogens whose physical state can be transformed from crystal (gel) to liquid crystal and then from liquid crystal to isotropic phase. The first thermal transition, defining low-temperature adaptation, is extensively studied in contrast to the second one, which refers to high-temperature adaptation of ectotherms. DSC does not always allow registration of isotropic melting because of low heat absorption. Therefore, polarizing microscopy was also applied to detect isotropic melting of lipid samples. Fig. 5 shows the most representative textures of major polar lipids from marine macrophytes during isotropic melting which began from real environmental temperatures of these plants (10–30 °C) and mainly corresponded to high-temperature calorimetric peaks. However, most lipid preparations did not melt completely until reaching 60–70 °C. It is known that 25 °C is a critical temperature for the growth of macrophytes (Kizevetter et al., 1981). Temperatures above 35 °C provoke irreversible changes in the photosynthetic and mitochondrial apparatus of marine macrophytes and higher plants (Kizevetter et al., 1981; Nagasato et al., 1999; Raven et al., 2002; Sharkey, 2000). Therefore, it is possible that an anomalous increase in seawater temperatures can result in the deterioration of physiological functions or death of plants owing to fluidization of liquid crystalline membrane lipids or the destruction of liquid crystalline matrix.

3. Conclusions

The present study has shown that chemotaxonomic traits of FA compositions remain in the individual polar lipids in both seasons despite the classic increase of their unsaturation (UI and UFA/SFA) during acclimatization of marine macrophytes from summer to winter. FA unsaturation rose due to the increase in the ratio between $n-3$ and $n-6$ PUFAs. Plant mediators, derived from $n-6$ PUFAs, are believed to be more potent than those derived from $n-3$ PUFAs, similarly to eicosanoids of animals (Lauritzen et al., 2001; Calder, 2006; Bagga et al., 2003). So, the need of $n-6$ PUFAs, which must be the precursors of more potent messengers, than $n-3$ derivatives, became higher in both photosynthetic and especially non-photosynthetic membranes at the most active period for marine macrophytes. However, T_{max} of just photosynthetic lipids did not decrease in contrast to the classic homeoviscous answer of non-photosynthetic PC and PE. It can be connected with the different balance of $n-3$ and $n-6$ PUFAs in these two groups of polar lipids. As shown, the share of $n-3$ PUFAs is higher in photosynthetic lipids compared with non-photosynthetic ones in summer (Sanina et al., 2004). However, its further increase in winter can turn out to be inefficient for the T_{max} decrease in photosynthetic lipids, because the rise in unsaturation in account of the substitution of $n-6$ PUFAs for $n-3$ ones can increase T_{max} (Huang and Li, 1999). Hence, the need of especially high content of $n-3$ PUFAs in photosynthetic lipids rather may be connected with some other function(s) than a fluidization of thylakoid membranes *per se*. Probably, the big number of double bonds in the content of $n-3$ PUFAs is important for electron-transport activity of photosystems, especially in winter. On the one hand, the lower capacity of $n-3$ PUFAs than $n-6$ PUFAs to decrease T_{max} of lipids (Kostetsky et al., 1992) can result in the low-temperature photoinhibition of photosynthesis in this season. On the other hand, $n-3$ PUFAs of photosynthetic lipids can facilitate electron transfer between large protein complexes of energy transducing membranes of thylakoids-like mobile electron carriers such as quinines (Jones, 2007).

Change in season influenced thermotropic behavior and FA composition of DGTS similarly to PC and PE of *L. japonica* and *Z. marina.* So, these traits confirm the idea that DGTS really can perform the role of PC in green algae (Dembitsky, 1996).

In fact, studied lipids were the mixture of different molecular species, which undergoes phase separation. The most obvious phase separation of PGs is suggested to promote the physical segregation of slow PSI and rapid PSII in thylakoid membranes of marine macrophytes.

Polarizing microscopy has shown that liquid crystalline membrane lipids began to be fluidized at physiological temperatures that can be the reason of the high sensitivity of marine macrophytes to superoptimal environmental temperatures.

The dependence of FA saturation from polar head groups of polar lipids in summer (MGDG → DGDG → SQDG and PC → PE → PG) transformed to electroneutral phospho-/glycolipids → acid lipids (PG/SQDG) in winter, because the dependence of saturation from the head group

of electroneutral polar lipids became fuzzy. The substitution of $n-6$ by $n-3$ PUFAs, occurring at the change in season from summer to winter, was accompanied by the partial substitution of C_{20} by C_{18} PUFAs in glycolipids and PG in contrast to PC and PE from *L. japonica*, where both series of PUFAs are presented.

4. Experimental

4.1. Algal material

Five species of marine macrophytes: *Anfeltia tobuchiensis* (Rodophyta), *L. japonica* and *S. pallidum* (Phaeophyta), *Ulva fenestrata* (Chlorophyta), *Z. marina* (Embriophyta) were harvested in the Sea of Japan in summer and winter at seawater temperatures of 20–23 °C and 3 °C, respectively. Freshly collected algae or seagrass were thoroughly cleaned to remove epiphytes, small invertebrates and sand particles and then heated for 2 min in boiling H_2O to inactivate enzymes.

4.2. Lipid extraction and isolation

Total lipid extracts from about 10 kg of algae or seagrasses were obtained according to the method of Folch et al. (1957). Crude glyco- and phospholipids were isolated from total lipid extract by column chromatography on silica gel by elution with Me_2CO, Me_2CO/benzene/EtCOOH/H_2O (200:30:3:10, by vol.) and a gradient of $CHCl_3$ and MeOH. Then, lipids were purified by preparative silica TLC using Me_2CO/benzene/EtCOOH/H_2O (200:30:3:10, by vol.) and $CHCl_3$/MeOH/H_2O (65:25:4, by vol.). Purity of lipids was checked by two-dimensional silica TLC (Vaskovsky and Terekhova, 1979; Vaskovsky and Khotimchenko, 1982). Silica gel for TLC was prepared according to the method of Svetashev and Vaskovsky (1972).

4.3. GC analysis of fatty acid composition

Esterification of lipids was accomplished by the addition of freshly prepared acetylchloride–methanol (1:20) and leaving the reaction to take place at 95 °C for 1 h in the heating module Block-Therm (MTA KUTESZ, Hungary). Fatty acid Me esters were extracted with *n*-hexane and purified by TLC. Analysis of fatty acid Me esters was carried out using a gas–liquid chromatograph Agilent GC6898, equipped with a flame-ionization detector, a silica capillary column (25 m × 0.25 mm) with Carbowax 20 M. The carrier gas was He. Individual peaks of fatty acid Me esters were identified by comparison of GC R_tS with those of authentic standards of fatty acid Me esters and by ECL (equivalent chain length) (Kramer et al., 1985; Christie, 1988).

4.4. DSC analysis of thermal transitions

Chromatographically pure phospholipids were solubilized in chloroform and introduced into standard aluminium pans. Vacuum dried samples of approximately 10 mg were sealed into pans and placed in a DSC-2M differential scanning calorimeter (Puschino, Russia). Samples were either heated or cooled at 16 °C min between −120 and 80 °C at a sensitivity of 5 mW. Position of the maximum of heat capacity *vs.* temperature plot was recorded as the phase transition temperature, T_{max}. The temperature range was calibrated by using naphthalene, mercury and indium.

4.5. Polarizing microscopic analysis of isotropic melting

Temperature ranges for the isotropic transitions of the same phospholipids were defined by means of polarizing microscopy POLAM-P-312 (Russia) employing heating stage, at ×100. Anhydrous phospholipid preparations were placed between glass slides and investigated microscopically. Observation of textures was carried out in linear-polarized light between crossed polarizers in heating and cooling modes. The beginning of a liquid crystalline to isotropic phase transition was determined as the temperature where the observed field began to darken; the end of phase transition was defined as the point where the field turned black.

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