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Phytochemistry 66 (2005) 73–79
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance

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Received 18 August 2004; received in revised form 27 October 2004
Available online 9 December 2004

Abstract

We examined variations in the lipid composition of the marine red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance: 70–80% and 8–10% of the incident photosynthetically active radiation (PAR). The content of storage and structural lipids was significantly affected by the light intensity. Exposure of *T. crinitus* to low light conditions induced an increase in the abundance of structural components of the cell membranes, especially sulfoquinovosyldiacylglycerol, phosphatidylglycerol (PG) and phosphatidylcholine, while growth of algae at high light intensity resulted in a 1.5-folder increase in the level of storage lipids, i.e. triacylglycerols. There were no significant differences in the fatty acid composition of the total lipid pool in algae grown under different light conditions. However, the content of the most unsaturated acid, 20:5$n-3$, was slightly higher in *T. crinitus* under 8–10% PAR compared to those at 70–80% PAR. Each lipid class was found to have a characteristic fatty acid composition. The relative proportions of fatty acids esterifying monogalactosyldiacylglycerol (MGDG) and PG were significantly affected by irradiance conditions. Exposure of algae to low light resulted in increase in the content of 20:5$n-3$ in MGDG and in decrease in the level of this acid in PG. The concentration of *trans*-16:1 acid in PG increased in algae grown under high light intensity. Light conditions influenced on total lipid content, which made up 4.2 ± 0.5 and 3.4 ± 0.3 mg g^{-1} fresh weight in algae exposed to 8–10% PAR and 70–80% PAR, respectively. We suggest that variations in the lipid composition of *T. crinitus* exposed to different levels of light intensity may be a response of alga to light conditions and it can be considered as one of the mechanisms of adaptation of *T. crinitus* to varying incident light intensity.

Keywords: *Tichocarpus crinitus*; Rhodophyta; Algae; Light; Solar irradiance; Fatty acids; Glycolipids; Lipids; Eicosapentaenoic acid

1. Introduction

It has been recognized that environmental factors (temperature, light, nutrients, etc.) affect lipid composition of algae (Harwood, 1984; Thompson, 1996). Much attention has been given to study of the influence of temperature on the fatty acid (FA) composition. It was found that the lowering of growth temperature induced, as a rule, an increase in FA unsaturation in marine algae and this is one of the generally accepted mechanisms of low-temperature acclimation (Harwood and Russell, 1984; Al-Hasan et al., 1991; Dawes et al., 1993). In contrast, the change in FA composition in response to the alteration of light intensity is a less studied process. Most of the studies were focused on microalgae. As to seaweeds, the effect of light on FA composition of these plants has been examined only for a few algal species and these studies yielded contradictory results. In *Gracilaria* sp., the content of the most unsaturated FA, 20:5$n-3$, showed an increase with increasing photon flux density (Levy et al., 1992), whereas in *Gracilaria*

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doi:10.1016/j.phytochem.2004.10.024

verrucosa, proportion of the main unsaturated FA, 20:4*n* − 6, decreased under high light (Levy et al., 1992; Floreto et al., 1993; Imbs et al., 2001). In *Gracilaria tikvahiae* and *Grateloupia sparsa*, FA composition was not affected by light (Dawes et al., 1993; Floreto and Teshima, 1998). All the above studies were undertaken under laboratory-based experimental conditions and addressed light-dependent changes only in composition of FAs. However, benthic marine algae have a complex lipid composition and contain both neutral and polar lipids, structural components of which are FAs. It may be supposed that lipid composition of macroalgae may show changes during alteration of light conditions because there is a close connection between lipids and photosystem subcomplexes anchored within the thylakoid membranes through lipids (Thompson, 1996; Klyachko-Gurvich et al., 1999), and production of photosynthates of benthic algae varies with light intensity (Titlyanov et al., 1990). Therefore, the objective of this study was to compare the content and composition of lipid classes and FAs of the red alga *Tichocarpus crinitus* growing under differing solar irradiances in the field experiments. This information may be important not only for clarifying the dependence of lipid composition of algae on light intensity but can also give a better insight of the role of lipids in the adaptation of algae to different irradiance conditions.

2. Results

2.1. Lipid content and lipid classes

The red algae *T. crinitus* were grown for three weeks at different irradiance conditions. One algal group grew under large flow of solar irradiance at 70–80% photosynthetically active radiation (PAR), and the other algae were cultivated in temperate shade at 8–10% of PAR. We found that samples of algae differed in total lipid content depending on light intensity. Lipids made up 3.4 ± 0.3 mg g^{-1} fresh weight in *T. crinitus* exposed to 70–80% PAR and 4.2 ± 0.5 mg g^{-1}in those at 8–10% of PAR.

An analysis of lipid classes showed that *T. crinitus* contained glycolipids, phospholipids and neutral lipids. Glycolipids were main lipid group regardless of the light environment and made up 57.5–62.7% of the total lipids and were comprised of three constituents – monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG). The major phospholipids in *T. crinitus* were phosphatidylcholine (PC) and phosphatidylglycerol (PG). Additionally, the algal extracts contained small amounts of inositolphosphoceramide, phosphatidylinositol and traces of phosphatidylethanolamine. The major neutral lipid components were triacylglycerols (TG). Other species of red algae had similar lipid composition and it is typical of members of Rhodophyta (Pettitt et al., 1989; Dembitsky and Rozentsvet, 1990; Khotimchenko et al., 1990).

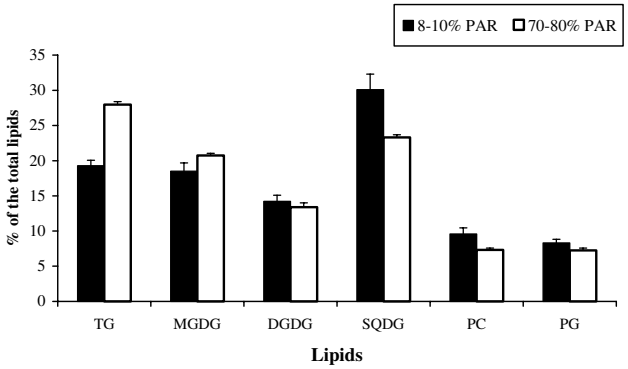

Fig. 1. Content of individual lipids (% of the total lipids) in *Tichocarpus crinitus* grown under different level of photosynthetically active radiation (PAR). Vertical bars indicate ±SD. *Abbreviations*: TG, triacylglycerols; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine.

The light environment affected the concentration of lipid classes of the *T. crinitus* (Fig. 1). The TG content in algae exposed to 70–80% PAR was 1.5 times higher ($P = 0.003$) than in those grown at 8–10% PAR. Changes in the relative proportion of MGDG caused as a response to light treatments followed the same trend and were not significantly but statistically authentic ($P = 0.03$). On the contrary, low irradiance led to a strong increase ($P < 0.001$) in the content of other structural lipids: SQDG, PC and PG, whereas the level of DGDG did not vary significantly under the different irradiances.

2.2. Fatty acids

Tichocarpus crinitus had FA composition typical of the red algae with a dominance of C20 polyunsaturated fatty acids (PUFAs). Three acids, 16:0, 20:4*n* − 6 and 20:5*n* − 3, made up more than 80% of the total FAs in this alga regardless of the light conditions (Table 1). The ratio of the individual FAs did not differ significantly in *T. crinitus* grown at different levels of incident light. However, the relative proportion of 20:5*n* − 3 was though not significantly but authentically ($P = 0.014$) higher in shade-grown algae than in those exposed to high light intensity. The content of other quantitatively less important FAs, 14:0 and 18:1, was slightly but authentically greater in *T. crinitus* grown under 70–80% PAR.

Benthic algae typically contain FAs as the structural components of the more complex lipids. Each lipid class of this alga differed in FA composition and response to different irradiance conditions. Table 2 shows the differ-

Table 1
Fatty acid composition (% of total fatty acids) of *Tichocarpus crinitus* grown at different levels of photosynthetically active radiation

Fatty acids	Photosynthetically active radiation		*P* value
	8–10%	70–80%	
14:0	3.00 ± 0.36	3.60 ± 0.20	0.07
16:0	37.77 ± 1.65	40.37 ± 2.65	0.22
16:1	4.97 ± 0.31	4.63 ± 0.35	0.283
16:1-*trans*	0.17 ± 0.06	0.40 ± 0.10	0.025
18:0	0.70 ± 0.10	0.75 ± 0.05	0.482
18:1	4.60 ± 0.20	5.40 ± 0.30	0.019
18:2$n-6$	0.93 ± 0.15	0.97 ± 0.06	0.742
20:4$n-6$	13.80 ± 0.60	14.0 ± 0.20	0.613
20:5$n-3$	33.80 ± 1.60	29.33 ± 0.91	0.014
Saturated FAs	41.47 ± 1.86	44.93 ± 2.43	0.119
PUFAs	48.53 ± 1.15	44.30 ± 0.82	0.007

ences in the relative content of FAs between lipid classes of *T. crinitus* exposed to high and low light intensities. The ratio of saturated and monounsaturated FAs involved in the formation of TG in *T. crinitus* differed significantly between light treatments. This alga grown at 70–80% PAR had lower content of saturated and higher level of monounsaturated FAs than those exposed to 8–10% PAR. Light intensity did not influence the proportion of PUFAs. The relative content of FAs, esterifying MGDG (the most unsaturated glycolipid), was significantly affected by irradiance conditions. The concentration of 16:0, 16:1, 18:1 and 20:4$n-6$ acids in MGDG increased, while the relative content of 20:5$n-3$ decreased in this glycolipid of *T. crinitus* exposed to 70–80% PAR compared to those cultivated at 8–10% PAR. Thus, the level of the MGDG unsaturation was significantly greater in *T. crinitus* grown under low light intensity.

Fatty acid composition of chloroplast phospholipid, PG, was strongly related to irradiance conditions. In *T. crinitus* similar to all photosynthetic eukaryotic organisms, *trans*-3-16:1 acid was concentrated in the PG, though the level of this acid was small. The relative content of *trans*-16:1 acid showed a twofold increase in *T. crinitus* exposed to high light intensity compared to those grown at low light 8–10% PAR. The concentrations of 18:1, 20:4$n-6$ and 20:5$n-3$ in PG were significantly ($P < 0.05$) increased, whereas the level of 14:0, 16:0 and 18:0 acids in this phosholipid was decreased in algae at 70–80% PAR compared to *T. crinitus* grown under 8–10% PAR. In the most saturated lipid, SQDG, the content of main acids, 14:0 and 16:0, also was higher in algae grown at high light intensity than in those under low light conditions. Fatty acid composition of DGDG slightly depended on light intensity. The highest concentration of 20:4$n-6$ (29.0-33.2% of total FAs) was found in PC (data not shown). Also this phospholipid had a high level of 20:5$n-3$ acid (22.3–30.3%). The content of both these PUFAs in PC was increased in *T. crinitus* exposed to high light intensity.

3. Discussion

Previous studies on the physiological response of marine benthic algae during acclimation to light environment in the natural habitats showed the differences in the content of structural and storage substances among deep-water or shaded species and those grown in the intertidal zone or the areas of the bottom that are open to direct sunlight (Yadikin and Titlyanov, 1980; Titlyanov et al., 1990). These investigators have reported changes in the content of proteins, starch and other sugars in benthic algae exposed to different photon irradiances. But lipids are also functioning as storage and structural components in plant, and alterations in their composition and content might take place during light adaptation of seaweeds as it was shown for micro-

Table 2
Fatty acid composition of lipid classes (% of total fatty acids) of *Tichocarpus crinitus* grown at different levels of photosynthetically active radiation (PAR)

Fatty acids	TG		MGDG		DGDG		SQDG		PG	
	8–10% PAR	70–90% PAR	8–10% PAR	70–80% PAR	8–10% PAR	70–80% PAR	8–10% PAR	70–80% PAR	8–10% PAR	70–80% PAR
14:0	5.5	3.9*	2.2	1.5	3.3	1.9	11.2	14.7	4.1	2.1*
16:0	40.8	36.6	44.1	49.3	61.7	63.4	60.3	67.3	48.5	37.6*
16:1	4.0	5.5	2.7	3.2	3.6	3.1	11.2	0.5*	2.5	1.1*
16:1*trans*	–	–	–	–	–	–	–	–	3.4	7.2*
18:0	8.2	5.3*	1.7	1.3	2.2	1.1	1.5	0.6*	2.4	1.5*
18:1	9.2	14.6*	6.3	8.9*	8.3	10.3	2.0	1.1	2.1	5.1*
18:2$n-6$	3.0	2.8	1.4	1.3	2.1	1.2	0.0	0.0	2.7	1.8*
20:4$n-6$	13.6	13.3	8.4	11.6*	2.2	1.2	1.4	0.8*	8.4	9.7*
20:5$n-3$	15.6	17.7	33.1	22.6*	16.6	17.7	12.3	14.7	25.5	33.9*
Saturated FAs	54.5	46.0*	48.0	52.1	67.2	66.4	73.0	82.6*	55.0	41.2*
PUFAs	32.2	33.8	42.0	35.5*	20.9	20.1	13.7	15.5	36.6	45.4*

Mean values marked by * are significantly different ($P < 0.05$); unmarked mean values are not significantly different ($P > 0.05$).

algae (Harwood and Jones, 1989; Sewon et al., 1997; Klyachko-Gurvich et al., 1999). Our study clearly showed that total lipid content of the red alga *T. crinitus* was affected by light. Algae grown at low solar irradiance had a relatively higher content of the total lipids compared to those exposed to high light intensity. Similar results were reported for the red algae *Grateloupia turuturu* (Khotimchenko, 2002) and for red microalga *Porphyridium cruentum* (Klyachko-Gurvich et al., 1999).

It is well known that lipids are a group of substances that have different functions depending on their chemical structure (Thompson, 1996). Neutral lipids, such as TG, are storage substance and energy sources, while polar lipids, glycolipids and phospholipids are the structural components of cellular membranes as well as modulators of photosystem efficiency and regulators of energy flow (Harwood and Russell, 1984; Thompson, 1996; Mock and Kroon, 2002). Our results showed that ratio of storage and structural lipids in *T. crinitus* was considerably affected by light conditions. The content of structural lipids (SQDG, PG and PC), commonly associated with photosynthetic and cellular membranes increased in shade-grown algae, while *T. crinitus* exposed to high light intensity had a high abundance of storage lipids (TG). In available literature we did not find any information on the light-dependent changes in the content of neutral and polar lipids in marine macroalgae. However, increased levels of TG under high, photoinhibitory light intensity, when sunlight exceeds the cellular capacity for energy utilization, were reported for different species of microalgae (Roessler, 1990; Napolitano, 1994; Mock and Gradinger, 2000). Triacylglycerols synthesis requires large amounts of photosynthetically produced ATP and NAD(P)H and, therefore, may helps in the dissipation of excess light energy and prevent photochemical damage of algal cells until other protective mechanisms have been implemented (Roessler, 1990). *T. crinitus* has a narrow range of irradiance tolerance, and light intensity of 90% PAR depressed growth of this alga and sometimes induced bleaching and tissue necrosis (Yakovleva et al., 2001). Since irradiance level of 70–80% PAR in the high light intensity treatment of the present experiments was high enough to induce photoinhibition in *T. crinitus*, the ability for the rapid accumulation of TG under such conditions might be important for this alga in terms of decreasing the susceptibility to high light stress and survival under unfavorable conditions.

Low light conditions stimulated the increase in abundance of structural lipids in *T. crinitus*. It is similar to that seen in the microalga *P. cruentum* (Klyachko-Gurvich et al., 1999) and Arctic-ace algae (Mock and Gradinger, 2000). Like the several species of microalgae (Klyachko-Gurvich et al., 1999, 2000) the low light intensity caused an increase in the content of MGDG in *T. crinitus* (as mg/g weight). The largest variations among different irradiance were observed in the content of SQDG and PG. The concentration of these lipids increased in shade-grown algae. Glycolipids and PG are dominant lipid classes of the thylakoid membranes as well as outer and inner chloroplast membranes (Douce et al., 1984; Ohnishi and Thompson, 1991), which are strongly developed in the shade adapted marine macroalgae (Yadikin and Titlyanov, 1980; Titlyanov et al., 1990). Thus, production of these lipids under low light conditions might be attributed to a response of *T. crinitus* to decreasing irradiance. The high levels of structural lipids in the shade-grown *T. crinitus* coincides with the fact that light optimum for the growth of this alga is 10–15% of the incident PAR (Yakovleva et al., 2001) and support the previous findings that active algal growth favors the production of structural components (Mock and Gradinger, 2000).

Klyachko-Gurvich et al. (1999) have demonstrated that in microalgae under high light intensity the $(n-3)$ desaturation of FAs esterifying the MGDG is an adaptive response of algal cells to the alterations in light conditions, and optimization of photosynthetical processes correlates with increase in the relative content of the FAs of the highest degree of unsaturation. As a rule, MGDG is mostly composed of $(n-3)$ PUFAs in the majority of plant organisms, including algae (Dembitsky, 1996; Khotimchenko, 2003). In our study, the proportion of the most unsaturated $(n-3)$ acid, $20{:}5n-3$, in MGDG increased in *T. crinitus* exposed to low light conditions compared to those grown at high solar irradiance. Eicosapentaenoic acid accumulated in MGDG of this alga with simultaneous decrease in the proportion of the main $(n-6)$ fatty acid, $20{:}4n-6$, that led to the changes in the $(n-3)$FAs/$(n-6)$FAs ratio. It is possible that some part of $20{:}5n-3$ in *T. crinitus* forms via desaturation of $20{:}4n-6$ in chloroplasts as it was shown for the red microalgae *P. cruentum* (Adlerstein et al., 1997). Thus, the alteration of FA composition in MGDG may be one of the adaptive responses of *T. crinitus* to varying light intensity.

Significant differences in the composition of FAs acylating PG of *T. crinitus* between thalli exposed to low and high solar irradiance were also observed. In shade-grown algae the concentration of saturated FAs in PG increased while in *T. crinitus* grown at high photon irradiance this phospholipid was enriched in unsaturated FAs, especially $20{:}5n-3$. Light-dependent variations in the proportions of *trans*-16:1 and 16:0 acids in PG exhibited an opposite tendency. It has been proposed that *trans*-16:1 acid is formed through the insertion of double bond into 16:0 acid (Ohnishi and Thompson, 1991). Our results support the observation that the conversation of 16:0 to *trans*-16:1 can be regulated by certain light treatment (Harwood, 1984). Thus, the alteration of FA composition of MGDG and PG of

T. crinitus exposed to different solar irradiance might be explained by the fact that these lipids, being the principle components of chloroplast membranes, play a key role in the formation of photosynthetic apparatus of the algae (Thompson, 1996; Hagio et al., 2000; Klyachko-Gurvich et al., 2000), and that light-dependent changes in the composition of FAs esterified to MGDG and PG are an adaptive response of *T. crinitus* to the changes in light environment.

The changes of lipid class content and the composition of acyl groups of individual lipids determined the variations in the FA composition of total lipid pool of *T. crinitus* caused by different light conditions. The relative proportion of the most unsaturated FA, 20:5$n-3$, slightly increased in shade-grown algae, while the level of main saturated FA, 16:0, decreased. However, these results differ from those reported in other studies performed on the red macroalgae grown under differing levels of light intensity. *G. turuturu* (Khotimchenko, 2002) and *Gracilariopsis* sp. (Levy et al., 1992) showed the alterations of 20:5 content in total lipids opposite to those seen in *T. crinius* – increasing light intensity resulted in an increase in the proportion of this acid. In *G. tikvahiae* (Dawes et al., 1993), *G. sparsa* (Floreto and Teshima, 1998) and *Gelidium pristoides* (Levy et al., 1992), fatty acid composition was unaffected by light conditions. There are a few possible explanations for the differences in the changes of PUFA content among red seaweeds during light adaptation. First, these different responses may be attributed to the differences in the intensity of light and time of exposure used in experiments. For example, both *T. crinitus* and *G. turuturu* grew in the field conditions but showed the opposite changes in the 20:5$n-3$ content during acclimation to high and low light. This may be related to the different time of exposure: three weeks and several months, respectively. Second, it might be of species-specific and genetic origin (Araki et al., 1990) and related to the differences in light tolerance of the photosynthetic apparatus of the different algal species because the degree of unsaturation of the glycolipids is known to affect photosynthetic capacity, the chlorophyll content and photosynthetic complexes (McConn and Browse, 1998). For example, *G. turuturu* is a light-requiring plants and capable for rapid acclimation to changes in light conditions in the range of 5–100% PAR (Yakovleva, 1999). Therefore, a significant increase in the relative content of highly unsaturated ($n-3$) FAs, localized primarily in MGDG of this alga might be a protective mechanism enhancing the tolerance to high light stress. In contrast, *T. crinitus* is a shade-requiring alga with a narrow range of irradiance tolerance, 10–30% PAR (Yakovleva et al., 2001) and light level of 70–80% is excessive for this algal species, leading to irreversible photodamage of the photosynthetic apparatus (Yakovleva, 1999). It is possible that the low content of 20:5$n-3$ under high light affects the photosynthetic capacity of this alga and determines its susceptibility to high light. But in any case, all these results support the finding of Araki et al. (1990) and Levy et al. (1992) that FA composition of total lipids of red seaweeds is slightly affected by environmental conditions.

4. Conclusion

Thus, photon irradiance conditions in the natural habitats of *T. crinitus* did affect the lipids in this alga. Our results evidences that *T. crinitus* is capable of regulating lipid metabolism depending on the irradiance conditions. This alga is able to change the content of storage and structural lipids and the composition of FAs both in total lipid pool and individual lipids. High light intensity stimulates the accumulation of the storage lipids (TG), while low light induces an increase in the abundance of structural lipids (DGDG, SQDG, PG, PC). Small light-dependent changes take place in the composition of FAs in the total lipid pool, affecting only two FAs: 16:0 and 20:5$n-3$. The composition of acyl group esterifying MGDG and PG, which are localized in chloroplasts, was strongly affected by irradiance conditions. This may indicate a high degree of control of structure of the cellular membranes in algae, because the degree of FA unsaturation has been considered as one of the most important factors controlling membrane fluidity and functionality. Also the differences in lipid composition of *T. crinitus* grown under high and low solar irradiance may be considered as one of the adaptive responses of the algal cells to the varying growth conditions. Therefore, the survival of algae under changed environmental conditions might be attributed to the functioning of the membranes. Since glycolipids and PG are the structural components of the chloroplast membranes where photosynthetic apparatus is formed, and it is possible to assume that changes in the lipid content and their ratio are necessary for readjustment of the structure of chloroplast membranes of *T. crinitus* to provide the efficient photosynthesis under varying irradiance conditions.

5. Experimental

5.1. Plant material

Specimens of *Tichocarpus crinitus* (Gmel.) Rupr. were collected from natural habitats in Sivuchya Bay (Sea of Japan) at 1.5–2 m depth. Plants were narrow, flattened and cartilaginous, dark-brown or brown-red in color and 10–15 cm in length. Healthy-looking thalli without bleached parts were selected. Plants were in the sterile state without any reproductive organs throughout the period of our investigations.

5.2. Culture experiment

The experiments were conducted during July–August 2001. Whole thalli of *T. crinitus* were sampled from natural beds as described above, cleaned of epiphytes and attached to the bottom of two open plastic chambers (50×50 cm). The chambers containing the algae were equipped with sinkers and foam rubber buoys and placed in the sea under the open sky at Sivuchya Bay. The plants were about 15–20 cm below the water surface at a constant tidal height. The different irradiances were obtained by covering the chambers with floating filters of plastic film of different optical density that resulted in 70–80% and 8–10% of the incident photosynthetically active radiation. Algae were grown during 3 weeks under different irradiance conditions before harvesting. Irradiance levels were measured with a "LI-189" quantum meter (Li-Cor, USA).

5.3. Lipid analysis

Prior to analysis of lipid classes, fresh thalli were washed with distilled water to eliminate salt and solid particles. Three plants of algal species were taken and combined together in a single extract and three extracts of algae grown under both light intensities were analyzed. Lipids were extracted with $CHCl_3$–MeOH (1:2) using the modifying procedure of Bligh and Dyer described by Kates (1986). Fatty acids were converted to methyl esters using 1% Na in MeOH, followed by 5% HCl in MeOH (Carreau and Dubacq, 1978) and purified by silica gel TLC in C_6H_6. The resulting fatty acid methyl esters (FAMEs) were analyzed by FID-GC (Shimadzu GC-17A) equipped with a flame ionization detector and fused quartz capillary column(30 $m \times 0.25$ mm), coated with Supelcowax 10M. Column and injector temperatures were 210 and 240 °C, respectively. The carrier gas was helium, split ratio 1:30. Identification of FAME was confirmed by chromatographic comparison with authentic standards and calculation of equivalent chain length values (Christie, 1988).

Lipid classes were separated by silica gel TLC with $CHCl_3$–CH_3COCH_3–CH_3OH–CH_3COOH–H_2O (100:40:20:20:8 v/v) in first direction and CH_3COCH_3–C_6H_6–CH_3COOH–H_2O (200:30:3:10) in second direction. To identify lipids on chromatographic plates the specific spray-reagents were used as described earlier (Khotimchenko and Kulikova, 2000). To estimate the amount of lipid classes the separated lipids were subjected to methanolysis and the resultant methyl esters were analyzed by GLC as described above. The quantity of lipids was obtained via estimated amounts of FAs with using the 15:0 acid as an internal standard (coefficients of calculation were 1.47, 1.78, 1.59 and 1.2 for MGDG, DGDG, SQDG and TG, respectively, and 1.45 for phospholipids).

5.4. Statistical analysis

The data were assessed statistically by one-way ANOVA and Tukey HSD-test for a posteriori comparisons. Differences $P < 0.05$ were taken as significant. All tests were performed using Statistica 6.0 statistical software.

Acknowledgements

Financial support was provided by the Far Eastern Branch of Russian Academy of Sciences ("Response of marine biota to the environmental and climate changes" Grant No. 04-1-П13-O43).

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