

Influence of Extremely Low Temperatures of the Pole of Cold on the Lipid and Fatty Acid Composition of Aerial Parts of the Horsetail Family (*Equisetaceae*)

Vasiliy V. Nokhsorov ^{1,*}, Lyubov V. Dudareva ², Svetlana V. Senik ³, Nadezhda K. Chirikova¹ and Klim A. Petrov ⁴

¹ North-Eastern Federal University, 48 Kulakovskogo str., Yakutsk 677000, Russia; vv.nokhsorov@s-vfu.ru (N.V.V.); hofnung@mail.ru (N.K.C.)

² Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of Russian Academy of Sciences, 132 Lermontova str., Irkutsk 664033, Russia; laser@sifibr.irk.ru

³ Komarov Botanical Institute, Russian Academy of Sciences, 2 Professor Popov str., St. Petersburg 197376, Russia; senik@binran.ru

⁴ Institute for Biological Problems of Cryolithozone of Siberian Branch of the Russian Academy of Sciences, 41 Lenina av., Yakutsk 677000, Russia; kap_75@bk.ru

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Abstract: The lipid composition of 2 species of vascular plants *Equisetum variegatum* Schleich. ex Web. and *E. scirpoides* Michx. growing in the permafrost zone (North-Eastern Yakutia, the Poles of Cold of the Northern Hemisphere) with an average daily air temperature in summer - 17.8°C, autumn -0.6°C, and winter -46.7°C has been comparatively studied. Betaine lipids (BLs) O-(1,2-diacylglycero)-N, N, N-trimethylhomoserine (DGTS) were identified in the lipid profile of the horsetail species. DGTS is assumed to play the role of the main plant phospholipid – phosphatidylcholine (PC) – under various abiotic stresses, including low-temperature ones. For the first time, the effect of extremely low air temperatures (–46.7°C) on the composition and content of membrane lipids and fatty acids (FAs) of vascular plants growing in especially severe conditions has been shown. The level of the absolute amounts of unsaturated FAs was found to increase in the autumn-winter period due to an increase in the proportion of linoleic C18:3 (n-6) and α-linolenic C18:3 (n-3) acids. According to the data obtained, it can be concluded that a high level of accumulation of membrane lipids, polyunsaturated FAs (PUFAs), and the presence of Δ5 FAs in lipids are apparently one of the features of the cold hardening of perennial herbaceous plants in the cryolithozone.

Keywords: *Equisetum variegatum*; *Equisetum scirpoides*; horsetails; low temperatures; hardening; phospholipids; glycolipids; betaine lipids; DGTS; fatty acids; seasonal dynamics; the Pole of Cold

1. Introduction

The study of the organism adaptation to extreme environmental conditions is one of the important problems of modern biology. North-Eastern Yakutia is a physico-geographical region with severe and very diverse living conditions. The Pole of Cold of the Northern Hemisphere is located here. The entire region lies in the permafrost zone, the thickness of which does not exceed 700 m. Many plant species grow at their distribution limit.

The specificity of the seasonal growth of the bulk of the herbaceous vegetation of North-Eastern Yakutia is that it develops intensively in the first half of summer in order to have time to go through the complete vegetation cycle and give full-fledged seeds. However, horsetail meadows, especially those consisting of variegated horsetail (*E. variegatum*) (Fig. 1), are often subjected to long-term hardening by floodwaters. Under these conditions, they do not have time to go through the complete vegetation cycle. When the winter season begins, autumn-vegetating plants get covered with snow with their shoots

green and acquire the ability to accumulate relatively more storage lipids and other nutrients [1, 2].

At first glance, it seems incredible that herbaceous plants at the Pole of Cold hardened by autumn low positive temperatures (10–0°C) are enriched with a large amount of the most energy- and material-intensive substances, such as proteins, carbohydrates, and lipids. In autumn, winter, and early spring, they are an adaptive fattening feed for both non-hibernating and hibernating herbivorous animals [3, 4]. Horsetails are characterized by some specific phytochemical characteristics. Thus, we previously determined that variegated horsetail (as *E. arvense* and *E. scirpoides*) belongs to 16:3 plants in terms of the content of triene FAs. Δ -5 acids, including 5,11,14,17-eicosatetraenoic (uniperonic) one, which are characteristic of evolutionarily ancient taxa, were identified in the FA composition of the aerial parts of horsetails [5]. It was also found that in the autumn, when adapting to low ambient temperatures of the Pole of Cold in the Northern Hemisphere, horsetails synthesize a secondary keto carotenoid – rhodoxanthin [6].

According to modern concepts, an increase in the concentration of unsaturated FAs of membrane lipids, and characteristic changes in the composition of some membrane lipids play an important role in the process of plant adaptation to low-temperature stress. Thus, it is known that the ability of cells to adapt to low temperatures is determined by their ability to synthesize unsaturated FAs de novo. In particular, linolenic acid, which increases the phase separation of lipids under the influence of low temperatures, is synthesized [7–9].

The purpose of this paper was a comparative analysis of the seasonal (summer, autumn, and winter) dynamics of the quantitative content of membrane lipids and the FA composition of total lipids in the aerial parts of *E. variegatum* and *E. scirpoides* growing in the cryolithozone of North-Eastern Yakutia (the Pole of Cold).

2. Results

Fig. 3 and 4 show the results of studies of the seasonal variability of polar lipids in the aerial parts of *E. variegatum* and *E. scirpoides*. The content of phospholipids (PLs) was characterized by individual seasonal dynamics of changes. Phosphatidic acid (PA) in *E. variegatum* and phosphatidylcholine (PC) in *E. scirpoides* were the main PLs among the objects studied. In summer, the studied plants had a relatively low PA content – 1.8 ± 0.7 mg/g dry weight. An increase in the PA content began in September with a decrease in the mean daily air temperature and a decrease in the photoperiod (Fig. 2). The PC content in *E. variegatum* increased in winter. The most noticeable increase in this lipid content was observed in *E. scirpoides* – 1.4 times in the autumn, compared with the summer-growing shoots of this species. A sharp increase in the content of phosphatidylethanolamines (PE) was observed in variegated horsetail in autumn by 0.4 mg/g dry weight. As compared to the summer values, in the winter period when the ambient temperature was extremely low (–46.7°C), a decrease in the PE content of 0.7 mg/g dry weight in reed horsetail was found. The content of DPG (diphosphatidylglycerols) in *E. variegatum* gradually increased with the beginning of hardening stages I and II. On the contrary, the DPG content decreased in *E. scirpoides*, which was apparently related to the species specificity. The level of phosphatidylglycerols (PG) in *E. variegatum* in the autumn-winter period remained unchanged and was about 0.4 mg/g dry weight. In *E. scirpoides*, the PG content sharply decreased from summer to the autumn-winter period and amounted to 0.4 ± 0.1 mg/g dry weight and up to 0.1 mg/g dry weight, respectively. After the end of the horsetail summer growing season and with a sharp break of low positive and extremely low negative air temperatures, the content of glycolipids (GL) in the studied species changed differently. Thus, with the beginning of the autumn period, the content of monogalactosyldiglycerides (MGDG) in *E. variegatum* decreased by 2.1 times. In *E. scirpoides*, in comparison with the summer values, the decrease in the content of this lipid was noticeably pronounced in the autumn and winter period when it decreased by 3.9 and 5.4 times, respectively. In the second half of September, with the onset of steady negative mean daily temperatures and

precipitation in the form of snow, the rate of increase in digalactosyldiglycerides (DGDG) rose. Thus, in autumn, the level of DGDG in *E. variegatum* was 1.8 ± 0.4 mg/g dry weight; in winter, it was about 1.4 ± 0.1 mg/g dry weight. In *E. scirpoides*, decreased with the beginning of the winter period by 0.9 mg/g dry weight compared with the summer values. The content of sulfoquinovosyldiacylglycerols (SQDG) in *E. scirpoides* decreased 2 times in autumn compared to the summer values, while in *E. variegatum* the maximum SQDG content was observed in autumn, when it exceeded the summer values twice.

The DGTS content in both horsetail species increased in the cold season. In *E. variegatum*, the DGTS accumulation in shoots was noted earlier (just after the onset of low positive hardening temperatures) to a much greater extent (by 8.8 times, up to 1.7 mg/g dry weight in September), while in *E. scirpoides*, a twofold increase in the DGTS content was observed only after the onset of extremely low temperatures in November and reached only 0.9 mg/g dry weight.

Glycoceramides (GlcCer) were found in the content of sphingolipids. A twofold increase in the GlcCer content was observed in winter in *E. scirpoides* compared with the summer period. The GlcCer content in *E. variegatum* remained practically the same in the summer-autumn periods and increased in winter.

When analyzing seasonal changes in the FA composition of horsetail shoots, a rather wide variety of both saturated and unsaturated mono-, di-, tri- and tetraene FAs with a cis-configuration of double bonds was found (Tables 1, 2). In the FA profile of *E. variegatum*, 18 and 15 FAs were identified during summer and autumn, respectively; 16 FAs were identified in the winter period. Among saturated FA, palmitic acid C16:0 prevailed in all seasons. In the autumn-winter period, its content was 4–6% higher than in the summer period.

In another representative of fattening horsetails, *E. scirpoides*, the qualitative composition of FA lipids of summer and autumn vegetative shoots was also quite different from winter plants of the same species (Table 2). Thus, 19 individual FAs were identified in summer and autumn; 15 FAs were identified in winter. *E. scirpoides* plants that got covered with the snow in the frozen state differed significantly from summer non-vegetating plants in terms of the absolute FA content level. Thus, the total FA content increased by 4.6 mg/g in autumn and by 6.3 mg/g dry weight in winter, as compared to the summer values. Acids with an odd number of carbon atoms (C15:0 and C17:0) were found in small quantities in both horsetail species. Monounsaturated FAs with 16, 18, and 20 carbon atoms were also found in the species studied. The relative content of palmitoleic C16:1 acid in summer-vegetating *E. variegatum* was more than twice higher than that in autumn and winter plants. In general, in the species studied, the content of monoenoic acids was significantly lower than the content of polyeneic ones and did not exceed 8.4% of the total FA content.

PUFAs in horsetail tissues are represented by $\Delta 12$, $\Delta 15$, $\Delta 5$ diene, triene and tetraene acids. Linoleic acid C18:2 was the major diene FA in all seasons. In *E. variegatum*, the content of this acid in autumn reached $23.3 \pm 1.5\%$ of the total FA, which is 12% higher than in summer and 8.1% higher than in winter. In *E. scirpoides*, the C18:2 content in the summer-autumn period varied within narrow limits: 2–2.3 mg/g dry weight. In winter, its content increased to 18% of the total FA. Small amount of hexadecadienoic acid C16:2 ($\Delta 7,10$), which is a precursor in the biosynthesis of hexadecatrienoic acid C16:3 ($\Delta 7,10,13$), was identified in the tissues of the studied plants. Hexadecatrienoic acid was found in all seasons in both species studied. *E. variegatum* had a higher content in summer (6.2%) than in autumn (1.3%). Then, with the onset of extreme frosts, the content of C16:3 ($\Delta 7,10,13$) increased again and reached 4% of the total FAs. In *E. scirpoides*, during the entire autumn-winter period, the content of C16:3 ($\Delta 7,10,13$) was at a consistently high level (5–6%). Plants containing a significant amount of C16:3 ($\Delta 7,10,13$) in lipids of photosynthetic tissues, mainly in the MGDG composition, belong to the 16:3 type [9].

In all seasons, the main PUFA in the two studied species was α -linolenic acid. In *E. variegatum*, its content was maximum (6.6 mg/g dry weight) in the winter period

(November, 22). In *E. scirpoides*, its content was maximum (6.5 mg/g dry weight) in autumn (September 25).

In the tissues of *E. scirpoides*, $\Delta 5$ FAs were found: C18:2 ($\Delta 5,9$) taxoleic and C20:4 ($\Delta 5,11,14,17$) uniperonic. In *E. variegatum*, only uniperonic acid was found. With the onset of winter extreme air temperatures, the content of uniperonic acid in *E. variegatum* increased to 3% of the total FA, which was 0.6 mg/g dry weight. The content of this acid in *E. scirpoides* doubled (up to 0.6 mg/g dry weight) with the onset of autumn hardening cold weather, snow, and a decrease in the length of daylight hours. With the onset of winter frosts, i.e. critically low air temperatures characteristic of the Pole of Cold in the Northern Hemisphere, the content of uniperonic acid in *E. scirpoides* decreases to 1.7% of the total FA. In the studied species, C20:3 acid ($\Delta 11,14,17$), a necessary precursor in the biosynthesis of C20:4 ($\Delta 5,11,14,17$) uniperonic acid, was identified [10]. The relative content of C20:3 ($\Delta 11,14,17$) in both types of horsetails increases with the break of unfavorable living conditions in the autumn period. The most pronounced increase in this acid content was noted in *E. variegatum*: 3.1 times higher, compared with the summer values. In *E. scirpoides*, it was 0.9 times higher. Seasonal changes in the absolute and relative FA content of total lipids and the calculated activities of the corresponding desaturases are reflected in the changes in the desaturation ratios (SDR, ODR, LDR). This section may be divided by sub-headings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

2.1. Figures, Tables and Schemes



(A)



(B)

Figure 1. Summer-growing shoots of *Equisetum variegatum* (A) and *Equisetum scirpoides* (B) growing at the Pole of Cold of the Northern Hemisphere.

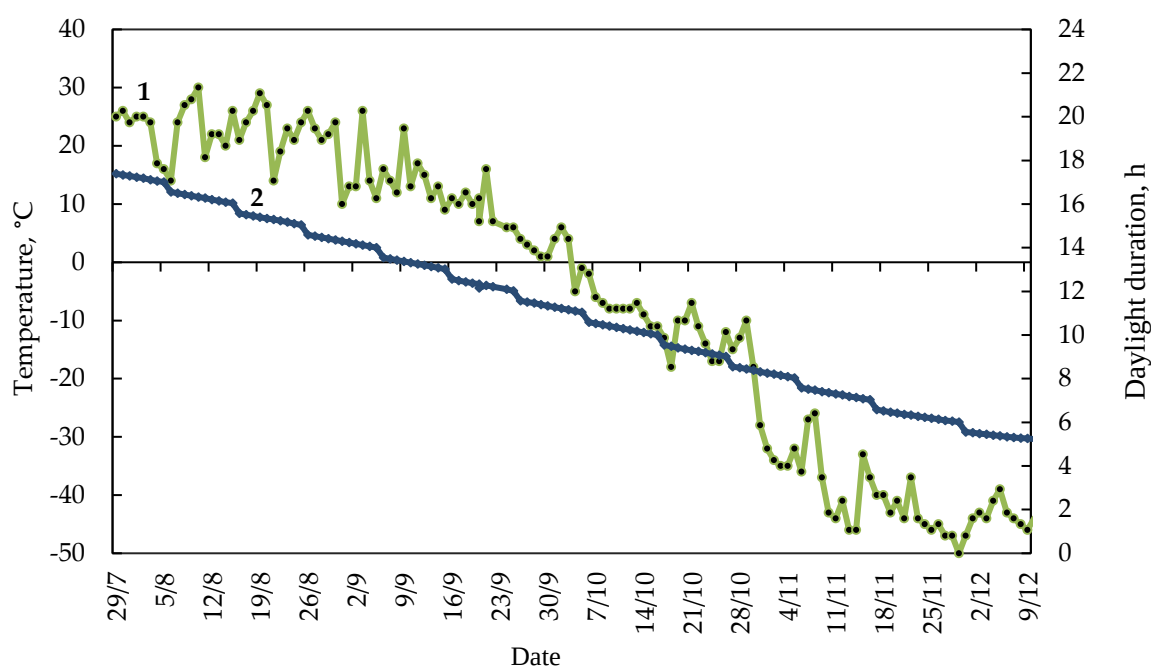


Figure 2. Seasonal changes in air temperature (1) and daylight duration (2) in North-Eastern Yakutia (the Cold Pole of the Northern Hemisphere) in 2019. Air temperature is represented by mean daily values.

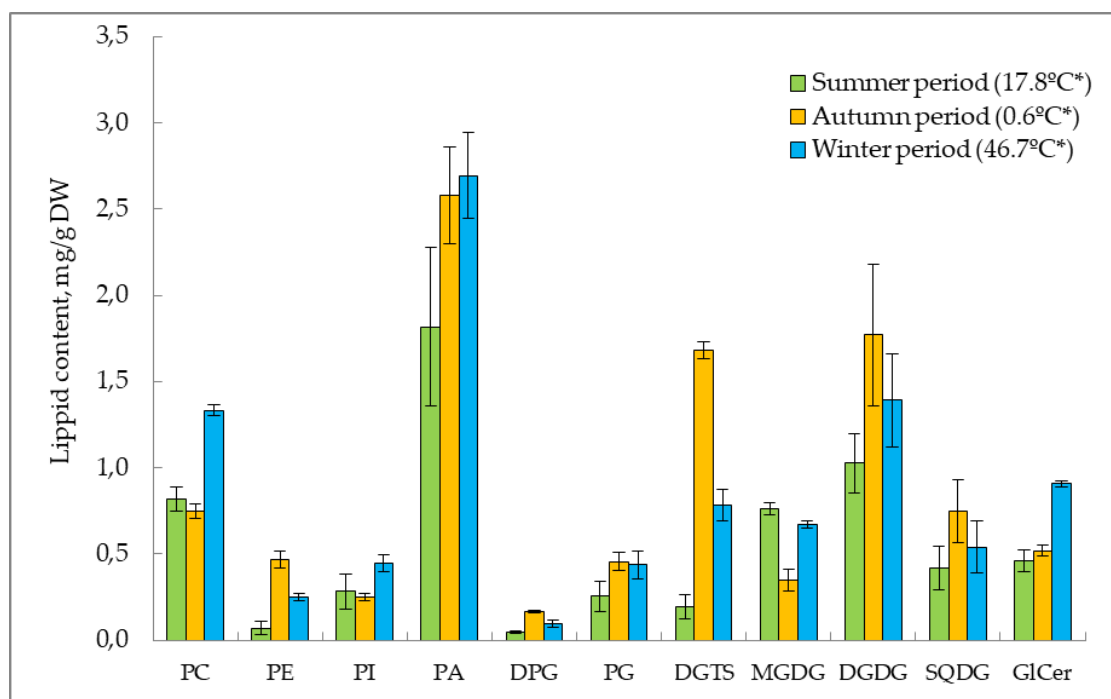


Figure 3. Seasonal changes in the content of individual lipid classes in *E. variegatum* shoots. PC – phosphatidylcholines; PI – phosphatidylinositols; PE – phosphatidylethanolamines; PG – phosphatidylglycerols; PA – phosphatidic acid; DPG – diphosphatidylglycerols; GlCer – glycosceramides, DGDG – digalactosyldiglycerides, DGTS – O-(1,2- diacylglycero)-N,N,N-trimethylhomoserine, MGDG – monogalactosyldiglycerides, SQDG – sulfoquinovosyldiacylglycerols. Data are shown as mean $\pm$ SD (n = 3).

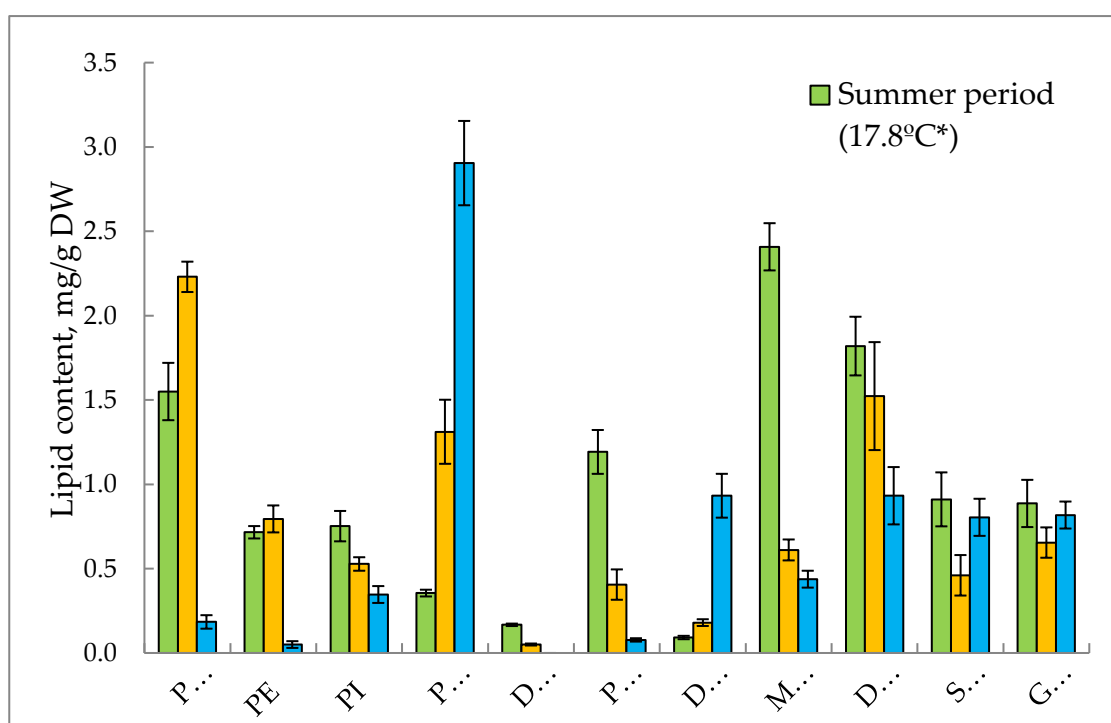


Figure 4. Seasonal changes in the content of individual lipid classes in *E. scirpoides* shoots. PC – phosphatidylcholines; PI – phosphatidylinositols; PE – phosphatidylethanolamines; PG – phosphatidylglycerols; PA – phosphatidic acid; DPG – diphosphatidylglycerols; GICer – glycosceramides, DGDG – digalactosyldiglycerides, DGTS – O-(1,2- diacylglycerol)-N,N,N-trimethylhomoserine, MGDG – monogalactosyldiglycerides, SQDG – sulfoquinovosyldiacylglycerols. Data are shown as mean $\pm$ SD (n = 3).

Table 1. Seasonal changes in the FA composition of lipids in shoots of *Equisetum variegatum* growing at the Pole of Cold.

Fatty acids	Summer period (17.8°C*)		Autumn period (0.6°C*)		Winter period (46.7°C*)	
	mg/g DW	%	mg/g DW	%	mg/g DW	%
C12:0	0,0	0.2 $\pm$ 0.0	–	–	–	–
C14:0	0.1 $\pm$ 0.0	1.2 $\pm$ 0.4	0.1 $\pm$ 0.0	0.9 $\pm$ 0.1	0.2 $\pm$ 0.0	0.9 $\pm$ 0.2
C15:0	следы	0.4 $\pm$ 0.0	0.1 $\pm$ 0.0	0.5 $\pm$ 0.2	следы	0.2 $\pm$ 0.0
C16:0	2.9 $\pm$ 0.7	24.7 $\pm$ 1.1	4.2 $\pm$ 0.7	26.7 $\pm$ 2.1	6.5 $\pm$ 1.2	30.6 $\pm$ 1.3
C16:1	0.3 $\pm$ 0.1	2.4 $\pm$ 0.8	0.2 $\pm$ 0.0	1 $\pm$ 0.2	0.1 $\pm$ 0.0	0.6 $\pm$ 0.1
C16:2(n-6)	0.1 $\pm$ 0.0	1 $\pm$ 0.3	0.1 $\pm$ 0.0	0.7 $\pm$ 0.1	0.1 $\pm$ 0.0	0.7 $\pm$ 0.1
C16:3(Δ 7,11,14)	0.7 $\pm$ 0.2	6.2 $\pm$ 1.2	0.2 $\pm$ 0.0	1.3 $\pm$ 0.3	0.8 $\pm$ 0.1	4 $\pm$ 0.9
C17:0	следы	0.3 $\pm$ 0.0	0.1 $\pm$ 0.0	0.6 $\pm$ 0.1	0.1 $\pm$ 0.0	0.5 $\pm$ 0.1
C18:0	0.2 $\pm$ 0.0	2.1 $\pm$ 0.9	0.5 $\pm$ 0.1	3.3 $\pm$ 0.8	0.5 $\pm$ 0.1	2.3 $\pm$ 0.8
C18:1(n-9)	0.6 $\pm$ 0.1	5.2 $\pm$ 1.2	0.9 $\pm$ 0.3	5.5 $\pm$ 1.4	1 $\pm$ 0.2	4.6 $\pm$ 0.9
C18:1(n-7)	0.1 $\pm$ 0.0	0.5 $\pm$ 0.1	–	–	0.1 $\pm$ 0.0	0.5 $\pm$ 0.0
C18:2(n-6)	1.3 $\pm$ 0.3	11.3 $\pm$ 1.2	3.7 $\pm$ 1.2	23.3 $\pm$ 1.5	3.2 $\pm$ 0.7	15.2 $\pm$ 1.3
C18:3(n-3)	4.6 $\pm$ 0.9	39.5 $\pm$ 2.2	4.1 $\pm$ 1.1	25.7 $\pm$ 2.2	6.6 $\pm$ 1.3	31.5 $\pm$ 2.3
C20:0	следы	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	0.9 $\pm$ 0.1	0.2 $\pm$ 0.0	0.8 $\pm$ 0.1
C20:1(n-9)	следы	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0	1.2 $\pm$ 0.3	0.1 $\pm$ 0.0	0.6 $\pm$ 0.0
C20:3(Δ 11,14,17)	0.2 $\pm$ 0.0	1.9 $\pm$ 0.9	0.9 $\pm$ 0.1	6 $\pm$ 1.3	0.8 $\pm$ 0.2	3.6 $\pm$ 1.1
C20:4(Δ 5,11,14,17)	0.3 $\pm$ 0.0	2.4 $\pm$ 0.8	0.4 $\pm$ 0.0	2.5 $\pm$ 0.9	0.6 $\pm$ 0.1	3 $\pm$ 0.9
C22:0	следы	0.3 $\pm$ 0.0	–	–	0.1 $\pm$ 0.0	0.4 $\pm$ 0.0
Total FAs	11,6 $\pm$ 1.1	100	15.8 $\pm$ 1.2	100	21.1 $\pm$ 1.6	100

$\Sigma_{\text{saturated}}$	3,4 ± 0.8	29.4 ± 2.1	5.2 ± 0.7	32.9 ± 2.2	7.5 ± 0.8	35.7 ± 1.9
$\Sigma_{\text{unsaturated}}$	8,2 ± 0.9	70.7 ± 1.9	10.6 ± 1.1	67.2 ± 2.4	13.6 ± 1.1	64.3 ± 2.1
k		2.4		2		1.8
DBI		1.9		1.6		1.7
SDR		0.7		0.6		0.7
ODR		0.9		0.9		0.9
LDR		0.8		0.5		0.7

Note: “–” – acid not found; * – mean daily air temperature; $\Sigma_{\text{saturated}}$ – the sum of saturated FAs; $\Sigma_{\text{unsaturated}}$ – the sum of unsaturated FAs; k – unsaturation coefficient; DBI – FA double bond index. The table shows the average values from 3-6 biological replicates and their standard deviations.

Table 2. Seasonal changes in the FA composition of lipids in shoots of *Equisetum scirpoides* growing at the Pole of Cold.

Fatty acids	Summer period (17.8°C*)		Autumn period (0.6°C*)		Winter period (46.7°C*)	
	mg/g DW	%	mg/g DW	%	mg/g DW	%
C12:0	следы	0.3 ± 0.0	следы	0.2 ± 0.0	–	–
C14:0	0.2 ± 0.0	1.3 ± 0.2	0.1 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	0.7 ± 0.1
C15:0	следы	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	–	–
C16:0	2.7 ± 0.9	21.1 ± 1.2	3.9 ± 0.9	22.6 ± 1.6	5.9 ± 1.1	31.3 ± 2.3
C16:1	0.1 ± 0.0	1 ± 0.2	0.2 ± 0.0	1.4 ± 0.6	следы	0.2 ± 0.0
C16:2(n-6)	0.1 ± 0.0	0.7 ± 0.1	0.2 ± 0.0	1.2 ± 0.5	–	–
C16:3(Δ7,11,14)	0.3 ± 0.0	2.6 ± 0.9	1.0 ± 0.2	5.7 ± 1.1	1 ± 0.4	5.2 ± 1.4
C17:0	0.1 ± 0.0	0.7 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.6 ± 0.2
C18:0	0.7 ± 0.1	5.2 ± 1.1	0.3 ± 0.0	1.8 ± 0.3	0.3 ± 0.0	1.8 ± 0.4
C18:1(n-9)	0.8 ± 0.1	6.1 ± 1.4	0.6 ± 0.0	3.7 ± 1.3	0.9 ± 0.2	4.8 ± 1.1
C18:1(n-7)	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
C18:2 (Δ5,9)	0.7 ± 0.2	5.8 ± 0.9	0.5 ± 0.1	3.1 ± 0.8	0.5 ± 0.0	2.4 ± 0.6
C18:2(n-6)	2.0 ± 0.4	16.1 ± 1.4	2.3 ± 0.6	13.1 ± 1.1	3.6 ± 0.8	18.8 ± 1.4
C18:3(n-3)	4.0 ± 0.9	31.4 ± 1.6	6.5 ± 1.0	37.5 ± 2.7	5.9 ± 1.1	31 ± 1.9
C20:0	0.1 ± 0.0	0.8 ± 0.1	следы	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.0
C20:1(n-9)	следы	0.1 ± 0.0	следы	0.1 ± 0.0	–	–
C20:3(Δ11,14,17)	0.4 ± 0.1	2.8 ± 0.7	0.6 ± 0.1	3.7 ± 1.1	следы	0.2 ± 0.0
C20:4(Δ5,11,14,17)	0.3 ± 0.0	2.2 ± 0.6	0.6 ± 0.1	3.4 ± 1.3	0.3 ± 0.0	1.7 ± 0.3
C22:0	0.1 ± 0.0	0.8 ± 0.2	0.1 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.5 ± 0.1
Total FAs	12.6 ± 1.2	100	17.2 ± 1.4	100	18.9 ± 1.2	100
$\Sigma_{\text{saturated}}$	3.8 ± 0.8	30.5 ± 1.3	4.6 ± 0.9	26.7 ± 1.9	6.7 ± 1.2	35.4 ± 3.2
$\Sigma_{\text{unsaturated}}$	8.7 ± 0.9	69.4 ± 1.9	12.6 ± 1.3	73.2 ± 2.2	12.2 ± 2.3	64.6 ± 3.4
k		2.3		2.7		1.8
DBI		1.8		1.8		1.6
SDR		0.7		0.6		0.7
ODR		0.9		0.9		0.9
LDR		0.7		0.7		0.6

Note: “–” – acid not found; * – mean daily air temperature; $\Sigma_{\text{saturated}}$ – the sum of saturated FAs; $\Sigma_{\text{unsaturated}}$ – the sum of unsaturated FAs; k – unsaturation coefficient; DBI – FA double bond index. The table shows the average values from 3-6 biological replicates and their standard deviations.

3. Discussion

Having analyzed the data obtained, we can note certain trends in changes in the content of lipids and FAs in the studied horsetail species taken for analysis during the

growing season (in summer, autumn, and winter) at different temperatures of the cryolithozone. All the evidence now suggests that the sharp continental climate contributed to the fact that, in the process of long evolution, vascular plants of permafrost ecosystems developed complex mechanisms of biochemical adaptation to extremely low temperatures. Among the mechanisms, lipid metabolism and their special FA composition play a significant role. According to the data, the content of polar lipids PL and GL, which play the most important structural and functional role in cell membranes, has seasonal differences in horsetail shoots in the summer and autumn-winter periods. Thus, the content of PC, PE, and PC in the tissues of the two studied horsetail species in the autumn-winter period was higher than in summer. PC and PE in plants, as well as in animals, are known to be the main lipids of plasma membranes, mitochondrial membranes, and microsomes. In addition, PCs are included in the structure of the outer shell of chloroplasts (20–30% of the total polar lipids) and, in small amounts, as part of chloroplast lamellae [11]. PGs are the main PLs of chloroplast membranes. They are known to be involved in the stabilization of PS II and light-harvesting complex II. This is probably why the increased level of PG in *E. variegatum* in autumn, as compared with summer, correlated with an increase in the content of pigments in the same period [6].

The presence of betaine lipids (BL) in the lipid profile of both horsetail species, in particular O-(1,2-diacylglycero)-N,N,N-trimethylhomoserine (DGTS) is of particular interest. BLs are believed to be evolutionarily ancient classes of lipids and are characteristic of cryptogamic plants. Under the influence of various types of stress (osmotic, water, phosphorus deficiency, etc.), the DGTS content can increase [12]. The increase in the DGTS content in the composition of membrane lipids is considered as one of the ancient mechanisms of biochemical adaptation of plants with the participation of lipid molecules [13]. In the autumn period, the DGTS content in *E. variegatum* was significantly higher than the PC content (Fig. 3). This lipid is possible to functionally replace PC in *E. variegatum* tissues since the DGTS chemical structure is close to the structure of this most widespread phospholipid. Thus, the authors of [14] emphasize that DGTS, an evolutionarily ancient lipid, plays the same role in the cells of lower plants as PCs play in higher plants. In *E. scirpoides*, however, the high PC content in its tissues during all vegetation periods does not apparently need compensation due to the high DGTS content. The BL content in this species remains low (0.6 mg/g dry weight in the winter period). DGTS was first discovered in *Ochromonas danica* (Chrysophyceae) unicellular seaweed [15]. According to the further studies, BLs are quantitatively significant and widespread in plants and algae. In addition, they are present in fungi and in protozoan tissues. BLs are also synthesized by higher vascular plants such as horsetails, ferns, and mosses. The amount of BL in cell membranes can vary both within a species and among different species. In many cases, BL is not synthesized under optimal cultivation conditions. However, under stress conditions, for example, in response to phosphorus starvation, it can accumulate in noticeable amounts, [12, 15, 16, 17]. A limited phosphorus intake is a physiologically stressful situation of frequent occurrence in free-living organisms. Since phosphorus starvation, among other negative effects, causes the degradation of phospholipids, it was suggested that an increase in the BL content could smooth out the decrease in the phospholipid content. The increase could thereby redistribute the use of phosphorus from the synthesis of membrane lipids to other metabolic pathways.

Depending on the season, the content of individual GL classes in the studied plants showed a common pattern. Since MGDG are known to be lipids of thylakoid membranes, their content apparently correlated with the photosynthetic activity of horsetails. They are associated with PS I, II and are able to restore their activity. The MGDG content in horsetails in the summer period was high compared to the autumn-winter period. During a short growing season at high air temperatures, horsetails are known to grow actively and accumulate biomass. Most of them mature spores. During the period of active growth, the processes of photosynthesis are intensified and there is a need for a large amount of MGDG. The MGDG content decreases in September with the onset of autumn cold

weather and a decrease in the photoperiod in this season. It is known that DGDG are the main lipids of both thylakoid membranes and the outer shell of chloroplasts. They play an important role in the membrane structure and stabilization of proteins in the membrane. SQDG are located mainly in chloroplast lamellae [18]. For the normal plant development, DGDG are required in large amounts during the period of active growth. In *E. variegatum*, the DGDG level increased in the autumn period. The higher SQDG content in summer in *E. scirpoides* coincides with the increased level of chlorophylls and photosynthetic activity of this horsetail tissues in the summer months [6].

After detecting the increase in the content of polar lipids PC, DGTS, PE and PC, the analysis of the changes in the FA composition of the lipids of horsetail tissues when hardened by low positive autumn temperatures in September (0.6°C) and extremely low temperatures in winter (−46.7°C) was carried out. The ratio of saturated and unsaturated FAs in lipids during the hardening was shown to change. The sum of the absolute amounts of unsaturated FAs increased (Tables 1, 2). Adaptive changes in the FA lipid composition occurred due to an increase in the proportion of triene fatty acids (hexadecadienoic C16:3 (Δ7,10,13) and α-linolenic C18:3 (n-3)) in *Equisetum scirpoides*. The functional properties and state of membranes largely depend on the FA composition in the lipid bilayer. The amount of unsaturated FAs is known to significantly affects the membrane permeability and the activity of many membrane-bound enzymes [19]. The high degree of the FA unsaturation is believed to determine the ability of organisms to adapt to low temperatures due to the less dense packing of molecules in the membrane bilayer.

In horsetail lipids, we identified Δ5 FAs. As a rule, they are present in lipids of gymnosperms in varying amounts. Their physiological role is poorly understood but it is assumed that their presence in lipids of gymnosperms is associated with plant resistance to low ambient temperatures. For instance, it is confirmed by the absence of Δ5 FAs in the leaf lipids of *Welwitschia mirabilis*, an inhabitant of tropical water bodies [20], and their high content in frost-hardy conifers [21]. Δ5 FAs are found in lipid compositions of other resistant to low temperatures organisms, for example, in some lichens [22]. The results of the analysis of changes in the content of Δ5 acids in the tissues of horsetails during the growing season do not allow us to draw an unambiguous conclusion about their participation in the formation of these species resistance to hypothermia.

The comparative qualitative and quantitative analysis of the lipid composition of the two horsetail species with the dependence on the season showed that the composition and content of membrane lipids and FA of total lipids are naturally associated with the formation of resistance of these species to extreme temperature environmental factors. We observed species-specific differences in the analyzed parameters. The most significant lipid components of membranes, the content of which noticeably increased following seasonal temperature changes, were: DGTS, DGDG, DPG, PE, and PG for *E. variegatum* species; PC, PC, and PE for *E. scirpoides*. When the temperatures decreased, the DGTS content in *E. scirpoides* increased. However, the proportion of this lipid in the total lipid pool of the species was much lower than in *E. variegatum*. Adaptive changes in the FA composition of lipids in both horsetail species in the autumn and winter periods were expressed in an increase in the absolute content of linoleic C18: 2 (n-6) and α-linolenic C18: 3 (n-3) acids in general pool of polyene FAs during this period.

4. Materials and Methods

Plant material and growing conditions. The objects of the study were the aerial parts of variegated horsetail (*E. variegatum*) and reed horsetail (*E. scirpoides*) (Fig. 1). Time and place of sampling: summer vegetation – early August, autumn vegetation – late September, winter vegetation – late November, North-Eastern Yakutia, 67°N, 137°E (the Pole Cold of the Northern Hemisphere). Data on air temperature at the habitat of perennial herbaceous plants (the Pole Cold) were taken from the Internet resource “Weather Underground” (<http://www.wunderground.com>). Weather conditions during the years of the experiment were typical for North-Eastern Yakutia. The onset of frosts was noted in the

middle of the second decade of September, a steady transition of the night temperature through 0°C was noted in late September and early October (Fig. 2).

Extraction and analysis of lipids. For lipid extraction, a weighed portion of plant material (0.5 g) was fixed in liquid nitrogen and ground until a homogeneous mass was obtained. Cooled laboratory glassware and reagents were used. Then, 10 ml of a 1:2 chloroform: methanol mixture was added. Ionol was added to the mixture as an antioxidant (0.00125 g per 100 ml of the mixture). Everything was thoroughly mixed and left for 30 min until the complete diffusion of lipids into the solvent. The solution was transferred quantitatively into a separatory funnel through a filter. The mortar and filter were washed three times with the same solvent mixture. To separate the non-lipid components, water was added.

For the analysis of total lipids, the lower chloroform fraction was separated. Chloroform (high purity grade, stabilized with 0.005% amylene) was removed from the lipid extract under vacuum using the RVO-64 rotary evaporator (Czech Republic). To control the lipid extractability (%), the known amount of nonadecanoic acid (C19:0) was added at the homogenization stage. FA methyl esters (FAME) were obtained by the Christie method [23]. The additional FAME purification was carried out by thin layer chromatography (TLC) on glass plates with KSK silica gel (Russia). Benzene was used as the mobile phase. To visualize the FAME zone ($R_f = 0.71\text{--}0.73$), the plates were sprayed with 10% H₂SO₄ in MeOH and heated in an oven at 100°C. The FAME zone was removed from the plate with a spatula and eluted from silica gel with (n)-hexane. The FAME analysis was performed by GLC using the 5973/6890N MSD/DS gas chromatography – mass spectrometer (Agilent Technologies, United States). The detector was a quadrupole mass spectrometer. The ionization method was an electron impact with the ionization energy 70 eV. The analysis was performed in the mode of the total ion current recording.

An HP-INNOWAX capillary column (30 m × 250 µm × 0.50 µm) with a stationary phase (PEG) was used to separate the FAME mixture. The carrier gas was helium, the gas flow rate was 1 ml/min. The evaporator temperature was 250°C, ion source – 230°C, detector 150°C. Temperature of the line connecting the chromatograph with the mass spectrometer was 280°C. The scanning range was 41–450 amu. The volume of the injected sample was 1 µL, the flow divider was 5: 1. The separation of the FAME mixture was carried out in an isothermal mode at 200°C. To identify FAs, the NIST 08 mass spectra library and the Christie FAME mass spectra archive were used [24]. The relative FA content was determined by the method of internal normalization – in weight percent (wt.%) of their total content in the test sample taking into account the FA response coefficient.

To characterize the degree of lipid unsaturation, the unsaturation coefficient (k) and the double bond index (DBI) were used [25]:

$$k = \sum P_{\text{unsaturated}} / \sum P_{\text{saturated}},$$

$$\text{DBI} = \sum P_j n_j / 100,$$

where P – the acid content,%; P_j – the acid content,%; n_j – the number of double bonds in each acid.

The activity of acyl-lipid membrane ω₉-, ω₆-, and ω₃-desaturases responsible for the introduction of double bonds into hydrocarbon chains of oleic (C18:1 (n-9)), linoleic (C18:2 (n-6)) and α-linolenic (C18:3 (n-3)) fatty acids were calculated as stearoyl- (SDR), oleyl- (ODR) and linoleyl- (LDR) desaturase ratios [26] by the formulas:

$$\text{SDR} = (\% \text{C18:1}) / (\% \text{C18:0} + \% \text{C18:1}),$$

$$\text{ODR} = (\% \text{C18:2} + \% \text{C18:3}) / (\% \text{C18:1} + \% \text{C18:2} + \% \text{C18:3}),$$

$$\text{LDR} = (\% \text{C18:3}) / (\% \text{C18:2} + \% \text{C18:3}),$$

Individual classes of polar lipids were analyzed by two-dimensional TLC on silica gel plates 60 10 × 10 cm (Merck, Germany) in a system of solvents: chloroform – methanol

– water (65: 25: 4) in the first case and chloroform – acetone – methanol – acetic acid – water (50: 20: 10: 10: 5) in the second case [16].

The lipids were identified using standards for target components and specific reagents for individual functional groups [27].

The amount of phospho-, glyco-, sphingo- and betaine lipids was determined densitometrically using the Denskan (Lenkhrom, Russia). For this purpose, chromatograms were developed in 10% sulfuric acid in methanol followed by heating at 140°C. The calculation of the content of individual classes of lipids in chromatograms was carried out using the DENS-14-12-03 program in the linear approximation mode on calibration curves constructed using standard PC solutions (Larodan, Sweden), bovine cerebroside, MGDG (Sigma, USA).

Statistical processing. The tables show the average data from 3-6 biological replicates and their standard deviations. The experimental data were statistically processed using the statistical analysis package in the Microsoft Office Excel 2017 environment. The statistical significance of the differences between the compared mean values was assessed using the t-test ($P < 0.05$).

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Abbreviations

BLs	Betaine lipids
DBI	Double bond index
DGDG	Digalactosyldiglycerides
DGTS	O-(1:2-diacylglycero)-N, N, N-trimethylhomoserine
DPG	Diphosphatidylglycerols
FA	Fatty acid
GI	Glycolipids
GlCer	Glycoceramides
LDR	Linoleyl desaturase ratios
MGDG	Monogalactosyldiglycerides
ODR	Olelyl desaturase ratios
PA	Phosphatidic acid
PC	Phosphatidylcholines
PE	Phosphatidylethanolamines
PG	Phosphatidylglycerols
PI	Phosphatidylinositols
PLs	Phospholipids
SDR	Stearoyl desaturase ratios

SQDG Sulfoquinovosyldiacylglycerols

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