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Seasonal dynamics of fatty acid composition of *Artemia* cysts lipids from lakes of Pavlodar Region, Kazakhstan

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ABSTRACT

The purpose of this research was to study the fatty acid composition of total lipids of Artemia cysts, *Artemia* sp., taken from four lakes in the Pavlodar region of Kazakhstan in different seasons. Extraction of total lipids was carried out with a binary mixture of organic solvents chloroform-ethanol (2:1). Gas chromatographic separation of fatty acids was carried out after methanolysis of lipids to obtain methyl esters of fatty acids. Quantification was based on the internal standard method using a calibration curve. The seasonal dynamics in the content of both the main groups of fatty acids and individual fractions was shown since a change in ambient temperature induced a modification of the fatty acid composition of lipids. In particular, by autumn, a decrease in the synthesis of monoenoic acids and an elevation in the amount of saturated fatty acids were established. Alterations in the qualitative composition of saturated fatty acids were also noted, in particular, in the autumn samples. There were no such acids as C6:0 caproic, C8:0 caprylic, C10:0 capric, C11:0 undecanoic, C12:0 lauric, C13:0 tridecanoic.

Keywords: Cysts, Artemia, *Artemia* sp., Fatty acid composition, Seasonal dynamics.

INTRODUCTION

Environment and ecosystems have land and water, medicinal plants as well as terrestrial and aquatic organisms. Environment is a habitat in which living organisms can live. In the environment, every living organism is related to other living and non-living factors around it (Yamin *et al.* 2020; Rafiee *et al.* 2020; Nimroodi *et al.* 2023; Shahsavari *et al.* 2023; Hosseini *et al.* 2023; Abangah *et al.* 2024; Fakhri *et al.* 2024). One of the important organisms of the environment and ecosystem is Artemia (Crustacea, Anostraca, *Artemia sp.*). It is a planktonic crustacean that lives in natural salt lakes. Their larvae (nauplii) are the most commonly used live food in aquaculture (Fisheries and Aquaculture Resources Use and Conservation Division 2017). In addition to practical application, *Artemia* is an interesting object for studying the biological mechanisms of adaptation to stressful conditions at various stages of ontogeny. It is well known that *Artemia* nauplii are able to regulate the ionic composition of the hemolymph in a wide range of salinity due to their specialized epithelium. The crustaceans have the ability to adapt and survive in conditions of extreme salinity (up to 25%), while in a hyperosmotic environment, the intestine absorbs ions and water molecules, and the gills release ions. In freshwater, gills absorb ions, and excess water is excreted in the urine (Triantaphyllidis *et al.* 1998).

Most of the previous publications on Artemia lipids are related to the analysis of their nutritional value for aquaculture, because this species is used as live food (Abatzopoulos *et al.* 2002). The fatty acid composition of

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Artemia has been studied. It has been found that lipids are characterized by a high content of triacylglycerides, a low content of long-chain polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic acid (20:5n-3) and especially docosahexaenoic acid (22:6n-3), which are essential fatty acids for the normal development of fish larvae (Sargent *et al*. 1999). Many studies have considered the influence of the lipid composition of *Artemia* as a live food on the physiological, biochemical, and other parameters of the growth and development of aquaculture objects (Webster *et al*. 1990; Van Stappen, 1996; Reis *et al*. 2017). Watanabe *et al.* found significant differences in n3 polyunsaturated fatty acid (PUFA) content within several Artemia strains from Japanese populations. These strains were classified into two types: a "marine" type rich in C20:5 n3 and a "freshwater" type low in this compound (Watanabe *et al*. 1980). Recent studies are related to the lipidome of *A. franciscana* cysts, which has been studied in parallel with the lipid composition of their mitochondria, in order to obtain a basic knowledge of the active proteins of late embryogenesis (LEA proteins - Late Embryogenesis Abundant) associated with lipid membranes (Chen *et al.* 2016). It has been shown that LEA proteins protect mitochondrial membranes from damage during dehydration (Tolleter *et al*. 2010). The work of Chen *et al*. (2016) is devoted to the study of the lipid composition of *A. franciscana* eggs, which provides detailed information on lipid classes, intraclass diversity, and their ratio. Despite this, in the modern scientific literature, there is clearly not enough information on the fatty acid composition of total lipids of Artemia cysts (*Artemia* sp.) of Kazakh populations in seasonal dynamics. This study presents new data on the fatty acid profile of total lipids isolated from Artemia cysts in the conditions of salt lakes in the Pavlodar region in late spring and early autumn 2021.

MATERIALS AND METHODS

Artemia cysts, selected in the spring and autumn 2021 from 4 reservoirs of the Pavlodar region: Kazy, Kyzyltuz, Seiten, Sharbakty, served as the object of research. The geographic location and specification of sampling points are presented in Table 1.

Table 1. Hydrography of bitter-salt lakes in Pavlodar region.

Name of the reservoir	Reservoir coordinates	Altitude above sea level (mBS)	Lake area (km^2)	Mineralization (g L^{-1})	
				May 2021	September 2021
Kazy	51°41'993"N 078°04'838"E	116.3	6.0	209	243
Kyzyltuz	51°41'994"N 078°04'837"E	136.0	8.5	254	289
Seiten	54°45'201"N 068°22'868"E	136.8	15.8	130	139
Sharbakty	51°23'.627"N 078°14'278"E	131.0	6.6	109	123

Fig. 1 shows the sampling points for research. The samples were preliminarily cleaned of impurities and, in the presence of excess water, dried with filter paper, and thoroughly ground in a porcelain mortar to a homogeneous, mushy state. Extraction of total lipids was carried out with a binary mixture of organic solvents chloroform-ethanol (2:1), separation of solvents, and weight determination of lipid mass.

The method for determining fatty acids was carried out in accordance with the state industry standard of the Republic of Kazakhstan MPM.MN 1364-2000 (Method for performing measurements of the content of metronidazole) method for the gas chromatographic determination of fatty acids and cholesterol in food and blood serum. The principle of the method is based on the isolation of lipids by extraction with organic solvents, lipid methanolysis to obtain fatty acid methyl esters, gas chromatographic separation of the latter, and quantitative determination by the internal standard method using a calibration curve expressing the dependence of the ratios of the peak areas of fatty acid methyl esters to the internal standard on the concentration of the corresponding fatty acids.

A portion of 5 g was ground in a porcelain mortar with anhydrous sodium sulfate (20 g) until a crumbly homogeneous mass was obtained. The mixture was transferred quantitatively with 30 mL of extractant (chloroform-ethanol 2:1) and extracted for 20 min. The extract was filtered off; the solvent was distilled off to dryness on a rotary evaporator at 40 °C. The extraction was repeated 3 times.

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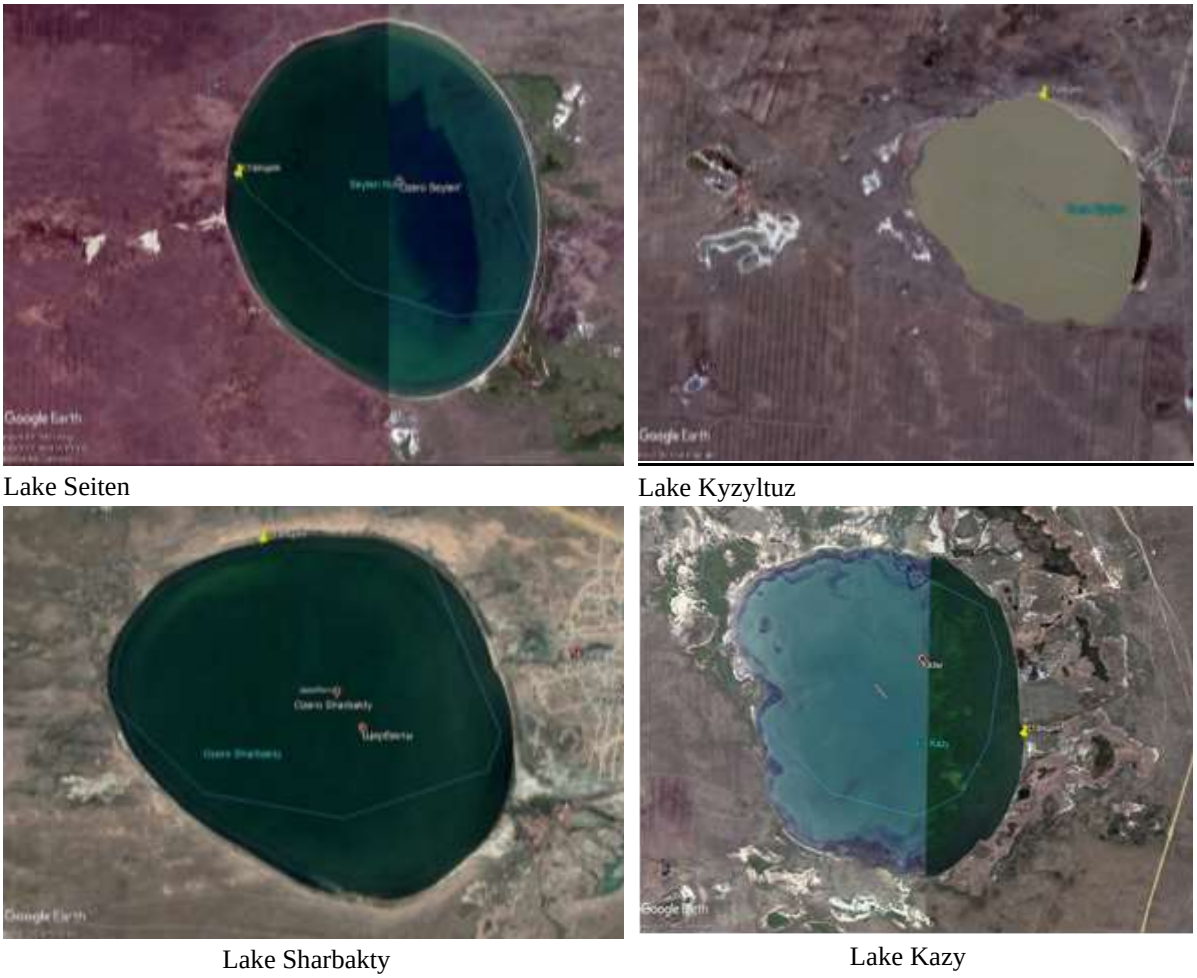

Fig. 1. Points of sampling material for research.

A weighed portion of the fat was dissolved in 2-mL hexane solution of internal standards of methyl esters of pentadecanoic and margaric acids with the addition of a methanolic solution of sodium methoxide and shaken for 2 min. Immediately before analysis, 0.5 mL distilled water was added to the tube, shaken for 30 s, and left until the phases separated. An aliquot 5 μL of the upper hexane layer was chromatographed 2 times. The desired components were identified by the retention times of fatty acid methyl esters.

The fatty acid composition of total lipids was determined using gas-liquid chromatography on a Kristall-5000 capillary chromatograph with a flame ionization detector. A capillary column with an internal diameter of 0.32 mm and a length of 50 m with a stationary phase FFAP, 0.50 μm thick, was used. The analysis was carried out in isothermal mode at 225 °C. The concentration of the components was calculated using the Chromatech-analyst software. Fatty acids were identified by comparing the retention times of available markers and by matching the calculated equivalent chains of molecules with tabular data. The significance of differences was assessed in accordance with the requirements of the normalizing standards.

RESULTS AND DISCUSSION

Table 2 depicts the fatty acid composition of lipids isolated from spring and autumn samples of *Artemia* cysts from lakes in Pavlodar region. It has been established that cyst lipids are rich in polyunsaturated fatty acids (PUFAs), where their content ranged from 39.70% to 60.57%, a relatively low content is characteristic of saturated fatty acids (SFAs) from 11.16% to 23.10%, and the content of monounsaturated fatty acids (MUFA) ranged from 19.07% to 37.20%.

The content of the main groups of higher fatty acids in the total lipids of *Artemia* cysts varied seasonally. In autumn, in the studied samples, compared with summer, there was a decrease in the content of monounsaturated fatty acids by 2-18% and a slight increase in saturated and polyenoic acids (Table 2). These variations were reflected in the value of the unsaturation coefficient (the ratio of the sum of polyunsaturated to the sum of saturated

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higher fatty acids). The values of this ratio were higher in the spring samples of *Artemia* cysts by 15-45% for the populations of lakes Kyzyltuz, Kazy, and Seyten. The elevation in the proportion of unsaturated fatty acids and the decrease in the amount of saturated fatty acids are apparently caused, first of all, by the seasonal increase in water temperature and the need to maintain the "liquid-crystalline" state of membrane structures at the proper level (Kolomiytseva 2011).

Table 2. Comparative content of saturated, monounsaturated and polyunsaturated fatty acids in Artemia cyst lipids from populations of lakes in Pavlodar region, % of the total amount.

Reservoir	Saturated fatty acids		Monounsaturated fatty acids		Polyunsaturated fatty acids		Σ PUFAs / Σ SFAs	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Kyzyltuz	13.18	17.24	30.08	28.02	56.75	54.74	4.31	3.18
Kazy	15.14	18.63	31.67	24.32	53.19	57.05	3.51	3.06
Seiten	11.16	15.62	27.93	25.91	60.43	58.47	5.41	3.74
Sharbakty	20.36	23.10	37.20	19.07	39.70	60.57	1.72	2.97

Among SFAs, the highest content was found for 16:0 palmitic acid from 7.44% to 10.74%. Among the MUFAs, a high content was typical for 16:1 (cis-9) palmitoleic acid (from 2.37% to 15.71%) and for 18:1 oleic acid (from 5.015% to 15.85%). Among PUFAs, the profile can be expressed as follows: 18:3n3 linoleic acid (from 10.34% to 45.67%) > 18:2n6t linoleidic acid (from 1.90% to 15.54%) > 20:5n3 eicosapentaenoic acid (from 0.74% to 27.12%). In seasonal dynamics, changes in the qualitative composition of SFAs can be noted, in particular, in autumn samples there are no acids such as 6:0 caproic, 8:0 caprylic, 10:0 capric, 11:0 undecanoic, 12:0 lauric, 13:0 tridecane (Table 3).

Table 3. Fatty acid composition of lipids extracted from samples of Artemia cysts in the conditions of lakes in Pavlodar region in May and September 2021.

Fatty acid	Lake Sharbakty		Lake Kyzyltuz		Lake Kazy		Lake Seiten	
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
Saturated fatty acids								
C14:0 (%)	1.83 ± 0.09	1.23 ± 0.06	1.29 ± 0.07	1.12 ± 0.06	1.32 ± 0.07	0.86 ± 0.04	0.81 ± 0.04	0.78 ± 0.04
C15:0 (%)	0.53 ± 0.03	0.08 ± 0.01	0.09 ± 0.01	0.65 ± 0.01	0.27 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.36 ± 0.02
C16:0 (%)	9.79 ± 0.49	10.74 ± 0.50	10.01 ± 0.50	8.19 ± 0.42	10.64 ± 0.53	10.61 ± 0.50	7.44 ± 0.35	7.92 ± 0.40
C17:0 (%)	1.24 ± 0.06	0.32 ± 0.02	0.79 ± 0.04	1.44 ± 0.07	0.35 ± 0.02	1.45 ± 0.72	0.26 ± 0.01	1.04 ± 0.05
C18:0 (%)	4.11 ± 0.21	7.07 ± 0.35	0.10 ± 0.01	5.19 ± 0.26	0.04 ± 0.01	5.03 ± 0.25	0.01 ± 0.01	4.93 ± 0.25
C20:0 (%)	-	0.24 ± 0.01	0.16 ± 0.01	0.16 ± 0.08	2.04 ± 0.10	0.18 ± 0.01	2.27 ± 0.11	0.16 ± 0.01
C22:0 (%)	4.63 ± 0.23	0.68 ± 0.034	0.41 ± 0.02	0.22 ± 0.01	0.17 ± 0.01	0.27 ± 0.01	0.16 ± 0.01	0.12 ± 0.01
Monounsaturated fatty acids								
C14:1 (cis-9) (%)	0.24 ± 0.01	0.14 ± 0.01	0.07 ± 0.01	0.17 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.31 ± 0.02	0.14 ± 0.01
C15:1 (cis-10) (%)	1.49 ± 0.08	0.43 ± 0.02	1.31 ± 0.07	0.53 ± 0.03	0.05 ± 0.01	0.10 ± 0.01	0.03 ± 0.01	0.60 ± 0.03
C16:1 (cis-9) (%)	15.71 ± 0.74	2.37 ± 0.12	4.46 ± 0.22	13.69 ± 0.68	9.20 ± 0.46	5.03 ± 0.25	5.68 ± 0.30	6.69 ± 0.33
C17:1 (cis-10) (%)	2.62 ± 0.13	1.35 ± 0.07	1.52 ± 0.08	2.26 ± 0.11	2.87 ± 0.14	3.51 ± 0.18	0.08 ± 0.01	1.85 ± 0.09
C20:1 (cis-11) (%)	0.27 ± 0.01	0.99 ± 0.05	0.57 ± 0.03	0.51 ± 0.03	0.38 ± 0.01	0.53 ± 0.03	0.69 ± 0.04	0.63 ± 0.03
Polyunsaturated fatty acids								
C18:3n3 (%)	17.22 ± 0.87	45.67 ± 2.29	38.93 ± 2.45	10.34 ± 0.50	24.93 ± 1.25	37.05 ± 1.87	32.50 ± 1.63	28.91 ± 1.45
C20:5n3 (%)	-	0.74 ± 0.04	1.30 ± 0.07	27.12 ± 1.36	14.20 ± 0.71	4.70 ± 0.24	13.64 ± 0.68	15.93 ± 0.80
Σ n3 of acids	**17.22**	**46.41**	**40.59**	**37.46**	**39.26**	**41.75**	**46.20**	**44.84**
C18:2n6t (%)	15.54 ± 0.73	1.90 ± 0.10	5.01 ± 0.03	10.42 ± 0.50	7.31 ± 0.37	6.28 ± 0.31	4.84 ± 0.24	4.39 ± 0.22
C18:2n6c (%)	4.11 ± 0.21	8.39 ± 0.42	6.74 ± 0.35	2.77 ± 0.14	4.42 ± 0.22	6.85 ± 0.34	4.50 ± 0.23	4.26 ± 0.21
C18:3n6 (%)	0.711 ± 0.04	2.05 ± 0.10	1.58 ± 0.08	0.53 ± 0.03	0.52 ± 0.03	0.82 ± 0.04	0.77 ± 0.04	0.49 ± 0.02
C20:2n6 (%)	0.31 ± 0.02	0.98 ± 0.05	0.39 ± 0.03	-	0.08 ± 0.01	0.26 ± 0.01	0.02 ± 0.01	0.16 ± 0.01
C20:4n6 (%)	1.82 ± 0.09	0.42 ± 0.02	2.43 ± 0.12	3.32 ± 0.17	1.54 ± 0.08	0.73 ± 0.04	3.97 ± 0.20	4.33 ± 0.22
Σ n6 of acids	**22.49**	**14.17**	**16.15**	**17.29**	**13.94**	**14.94**	**14.10**	**13.63**
Σ n3 / Σ n6	**0.77**	**3.28**	**2.51**	**2.17**	**2.82**	**2.80**	**3.28**	**3.29**

Polyenoic higher fatty acids of the linoleic series (n6 type) have a higher melting point in comparison with acids of the linolenic series (n3 type). As a result, an indicator of changes in the microviscosity of membrane lipids is the ratio of the sum of n3 acids to the sum of n6 fatty acids. In our studies, the value of this coefficient was higher in the population of Lake Seiten, where the salinity of the habitat increased slightly, amounting to 3.28 and 3.29

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in May and September, respectively. The elevation in salt content in the water, which was observed in spring and autumn on the lakes Kazy and Kyzyltuz, did not affect the coefficient, which can be explained by the significant mineralization of these reservoirs. An upraise in the value of n3/n6, and, consequently, a drop in the viscosity of membrane lipids is typical for the Artemia population of Lake Sharbakty were also observed. Palmitoleic acid can be used as a marker for the presence of diatoms in an animal's diet. In temperate waters, the content of this acid in diatoms is affected by environmental conditions (Kharlamenko 1995). It was found that the content of palmitoleic acid varies depending on seasonal variations in the number of diatoms in water and sediment. It can be assumed that the change in the amount of this acid is associated with a change in its content in the Artemia food.

Fatty acids 16:0, 18:1 n9, 18:2 n6, 18:3 n3, and 20:5 n3 have been found to account for up to 80% of the fatty acid profile in Artemia strains (Mura & Fancello 2005). In Artemia cysts from Kazakh populations, these fatty acids accounted for 12.98 to 88.52% of the fatty acid profile (Table 4).

Table 4. The content of 33 fatty acids in lipids of *Artemia* cysts from the populations of lakes in the Pavlodar region (% of the total).

Brief designation of fatty acid	Systematic name of the fatty acid	Fatty acid content, %	
		min	max
Saturated fatty acids			
C6:0 (%)	Caproic	0.01	0.02
C8:0 (%)	Caprylic	0.02	0.09
C12:0 (%)	Lauric	0.05	0.20
C13:0 (%)	Tridecanoic	0.03	0.05
C14:0 (%)	Myristic	0.78	1.83
C15:0 (%)	Pentadecanoic	0.08	0.53
C16:0 (%)	Palmitic	7.44	10.64
C17:0 (%)	Margaric	0.26	1.45
C18:0 (%)	Stearic	0.01	7.07
C20:0 (%)	Arachidic	0.18	2.27
C21:0 (%)	Geneucosanic	0.15	0.77
C22:0 (%)	Behenic	0.12	4.63
C24:0 (%)	Lignoceric	0.06	0.12
Monounsaturated fatty acids			
C14:1 (cis-9) (%)	Myristoleic	0,07	0,31
C15:1 (cis-10) (%)	Pentadecenoic	0,03	1,49
C16:1 (cis-9) (%)	Palmitoleic	2,37	15,71
C17:1 (cis-10) (%)	Margarinoleic	0,08	3,51
C18:1n9t (%)	Octadecenoic	0,01	0,19
C18:1 (trans-9) (%)	Oleic	5,01	15,85
C18:1 (cis-9) (%)	Oleic	10,87	15,02
C20:1 (cis-11) (%)	Eicosenoic	0,27	0,99
C22:1 (cis-13) (%)	Erucic	0,04	0,34
C24:1 (cis-15) (%)	Selaholic	0,16	0,82
Polyunsaturated fatty acids			
C18:2n6t (%)	Linoleladic	1,90	15,54
C18:2n6c (%)	Linoleic	4,11	8,39
C18:3n6 (%)	Y-Linolenic	0,49	2,05
C18:3n3 (%)	Linolenic	10,34	45,67
C20:2n6 (%)	Eicosadienoic	0,02	0,98
C20:3n6c (cis-8,11,14) (%)	Eicosatrienoic	0,02	0,44
C20:3n3c (cis-11,14,17) (%)	Eicosatrienoic	0,06	0,36
C20:4n6 (%)	Arachidonic	0,42	4,33
C20:5n3 (%)	Eicosapentaenoic	0,74	27,12
C22:2n6 (%)	Docosadienoic	0,01	0,25

Three populations were found to have compounds of great aquaculture interest, such as 20:5n3 (eicosapentaenoic acid) at 0.74 to 27.12%, so these populations can be assessed as "marine" type using the Watanabe *et al.* (1978) classification and suggest the presence of mechanisms that promote accumulation, which deserves further study.

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Navarro *et al.* (1992) suggested that in addition to the phenotypic effect of nutrition on fatty acid transport, genotype and bioconversion capacity in Artemia are also factors that should be studied. The marker of brown algae consumption is the total content of 18:2n6, 18:3n3, 18:3n6, 20:2n6, and 20:4n6 acids in the fatty acid composition of the consumer organism (Kharlamenko 1995). According to our data, in the studied species, the amount of these fatty acids was quite high (from 13.17 to 68.57%).

CONCLUSION

For the first time, a study was performed about the fatty acid composition of *Artemia* cysts of Kazakh populations in the conditions of lakes in the Pavlodar region. The results of the study showed the presence of seasonal dynamics in the content of both the main groups of fatty acids and individual fractions. In particular, by autumn, the synthesis of monoenoic acids decreased, while the amount of saturated fatty acids upraised, which is reflected in the value of the unsaturation coefficient. The values of this ratio were higher in the spring samples of *Artemia* cysts by 15-45% for the populations of lakes Kyzyltuz, Kazy, and Seiten. Quantitative variations of polyunsaturated fatty acids in *Artemia* cysts are due to seasonal changes in the species and fatty acid composition of their food. Based on the data obtained, it can be assumed that in the diet of crustaceans in the summer period, a significant proportion is detritus, and in the autumn brown algae. Differences in the values of the n3/n6 ratio of fatty acids depending on the salinity of the habitat were revealed in *Artemia* cysts.

Studying the seasonal dynamics of the fatty acid composition in ectothermic animals, it is difficult to single out the effect of a changing thermal regime, because other accompanying abiotic and biotic factors also fluctuate throughout the season. Thus, temperature adaptations at the lipid level are very closely associated with qualitative and quantitative changes in food composition, species characteristics of ecology, and life cycles of aquatic organisms (Tkach 2007). Among saturated fatty acids, the highest content was found for 16:0 palmitic acid from 7.44% to 10.74%. This acid is the most commonly occurring saturated fatty acid (about 15-50%). Its main role is as an energy store and a substrate in the biosynthesis of fatty acids. Among monounsaturated fatty acids, a high content is typical for 16:1 (cis-9) palmitoleic acid (from 2.37% to 15.71%) and for 18:1 oleic acid (from 5.015% to 15.85%). Oleic acid is the main substrate in the biosynthesis of polyunsaturated fatty acids. Among PUFAs, the profile can be expressed as follows: 18:3n3 linoleic acid (from 10.34% to 45.67%) > 18:2n6t linoleidic acid (from 1.90% to 15.54%) > 20:5n3 eicosapentaenoic acid (from 0.74% to 27.12%).

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