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FATTY ACID COMPOSITION IN TROCHOPHORES OF MUSSEL *MYTILUS GALLOPROVINCIALIS* GROWN UNDER CONTAMINATION WITH POLYCHLORINATED BIPHENYLS

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Status of *Mytilus galloprovincialis* populations in the natural habitat is known to directly depend on development of Black Sea mussel at all its stages, including initial stages of larval ontogenesis, which are very sensitive to environmental pollution. Organic pollutants adversely affect mussel larvae by inhibiting their growth and development. Patterns of mussel reproduction are well studied, which makes it possible to obtain larvae from artificially fertilized eggs of this mollusc species in controlled laboratory conditions. In this work, the fatty acid composition of *M. galloprovincialis* larvae at the trochophore stage on the 3rd day in the control experiment and under artificial contamination with polychlorinated biphenyls (PCBs) in different concentrations is studied for the first time. The fatty acid composition of total lipids in the biomass of larvae obtained on the 3rd day of the experiment was studied by means of gas chromatography – mass spectrometry. Totally, 14 fatty acids were identified in the samples; 59 % of them were saturated fatty acids, 24 % were monounsaturated fatty acids, and 17 % were polyunsaturated fatty acids. Statistical analysis was performed using Statistical Toolbox of MATLAB software (version 8.2). The totals of monounsaturated and polyunsaturated fatty acids significantly differed in lipids of *M. galloprovincialis* trochophores in the experiment with different PCB concentrations. The totals of saturated fatty acids did not significantly differ. The major saturated fatty acids in all mussel trochophores studied were palmitic (C16:0) and stearic (C18:0) acids. Their concentration did not significantly change under the exposure to PCBs. The main monounsaturated fatty acids were oleic (C18:1ω9), palmitoleic (C16:1ω7), and vaccenic (C18:1ω7) acids. The fraction of monounsaturated fatty acids was twice as low when exposed to the PCB concentrations 0.1 and 1.0 μg·L^{-1}. However, when the PCB concentration was 10 μg·L^{-1}, the total of these acids did not differ from the control. Among polyunsaturated fatty acids having biological essentiality, it was possible to identify arachidonic (C20:4ω6), eicosapentaenoic (C20:5ω3), and docosahexaenoic (C22:6ω3) acids. The total fraction of omega-3 and omega-6 acids in mussel larvae in the control did not exceed 12.8 %. With an increase of the PCB concentration in the growth medium 0.1 to 1.0 μg·L^{-1}, the fraction of polyunsaturated fatty acids increased 2.5-fold. At the PCB concentration 10 μg·L^{-1} and in the sample with pure acetone added, the total fraction of polyunsaturated fatty acids was comparable with that in the control. The results of the study indicate that fatty acid response is the highest when the medium is exposed to the PCB concentrations ranging 0.1 to 1.0 μg·L^{-1}. At the PCB concentrations equal to 10 μg·L^{-1} or higher, biochemical processes in larvae seem to slow down. The results of this study will contribute to a better understanding of biochemical rearrangements that allow molluscs at larval developmental stages to adapt to environmental pollution with organic xenobiotics.

Keywords: polychlorinated biphenyls, fatty acids, larvae, trochophore, mussel *Mytilus galloprovincialis*, Black Sea

The study of the effect of contamination of *Mytilus galloprovincialis* living in the natural conditions of Sevastopol marine area and being cultivated in coastal marine farms has been the subject of a number of works, focused mainly on adult mature individuals [11 ; 12 ; 19 ; 24 ; 31], as well as their gametes [7].

Mussels are known to be resistant to various types of pollution. Being filtrators, these molluscs actively accumulate pollutants in the organism. One of the most toxic environmental pollutants is organochlorine compounds (hereinafter OCC). The widespread prevalence of OCC in Black Sea water determined the pollution of natural mollusc populations in many marine areas off Sevastopol, since mussels accumulate hydrophobic OCC even with their relatively low concentration in seawater. In mussels from Martynova, Karantinnaya, and Golubaya bays, the total concentration of polychlorinated biphenyls ($\sum PCB_6$) varied from 3.8 ng·g^{-1} (hereinafter on wet weight) in gills to 459 ng·g^{-1} in hepatopancreas [6]. In Laspi Bay, where the anthropogenic impact is not so pronounced, OCC concentration was lower and ranged from 0.21 ng·g^{-1} in gills to 10.3 ng·g^{-1} in gonads [6]. OCC accumulation in mussel organs positively correlated with the content of total lipids in them [6]. Since embryos and larvae are the most sensitive stages of mussel ontogenesis, the influence of pollutants can lead to inhibition and arrest of their growth [8]. Under experimental conditions, chromosomal aberrations in cells under the influence of solutions of toxicants, such as surfactants, on fertilized eggs have already been established [9]. A few years ago, we showed *in vivo* a positive correlation of OCC concentration in water with pelagic eggs mortality and a negative correlation with the number of fish larvae at early stages of postembryonic development [23].

The aim of this work was to determine fatty acid composition of trophophores of the cultivated mussel *M. galloprovincialis*, grown under experimental contamination with polychlorinated biphenyls.

MATERIAL AND METHODS

The object of research was the bivalve mollusc *Mytilus galloprovincialis* Lamarck, 1819, taken in spring 2019 from collectors of a mussel-and-oyster farm located in Karantinnaya Bay water area (Sevastopol, Crimean Peninsula). To prepare for the study, 150 spec. of mussel with a shell length of 7–10 cm were selected. During that season, the molluscs were mainly at the spawning stage of development.

Larvae were obtained in laboratory conditions; the laboratory did not contain toxic fumes and gases. The ambient temperature in the laboratory was (20 ± 2) °C. The indoor lighting was combined [5]. To clear the digestive tract, 150 spec. of mussel were kept for 4 hours in filtered seawater, collected by a bathometer in Karantinnaya Bay water area. In the mussel-and-oyster farm area, the total polychlorinated biphenyls (hereinafter PCBs) concentration in water did not exceed 3 ng·L^{-1}, which corresponded to the average value for the open areas of the Black Sea [6 ; 10].

Each mollusc was placed the umbo down in a 0.5-L glass beaker. The glassware for the experiment was chemically clean. The seawater filtered through a membrane filter (with the pore size 3–5 μm) and heated to +25 °C was poured into each glass beaker, so as to cover the upper edge of the mussel cusps, thereby stimulating spawning [3]. The seawater, in which the larvae spawning and rearing took place, had the following physiochemical characteristics: temperature +23…+25 °C; pH 8.1–8.3; Ca^{2+} concentration 210–290 mg·L^{-1}; Mg^{2+} concentration 460–640 mg·L^{-1}; salinity 18 ‰; dissolved oxygen saturation in the surface water layer 100–110 %.

During the mollusc spawning, which occurred 4 hours after the stimulation, the eggs deposited on the bottom in the form of bright orange sediment, and sperm was released into the water in the form of a white cloud. After the isolation of gametes, the molluscs were removed from the glass beaker. The resulting solutions with eggs were joined and transferred to a 3-L container. The solutions with sperm were

collected in another 3-L container. Then, 10 mL of the mussel sperm solution was added to the egg containing solution. Since the fertilization process is fast [16], the solution with fertilized eggs was dispensed, 3 minutes after joining the solutions, in five separate 1-dm^3 reactors. A solution of PCB mixture in acetone (Aroclor 1254, Supelco, USA) was added to 3 reactors. PCB concentrations in water of the reactors affecting the larvae were 0.1, 1, and 10 μg·L^{-1}. Acetone was added to the 4th reactor in the same amount as in the reactors with the PCBs. The 5th reactor was a control one. The experiment was carried out in triplicate.

The temperature in the reactors for larvae growing was (20 ± 2) °C. The light in the reactors was both artificial (fluorescent lamps) and natural. The combined illuminance measured with a Yu-116 lux meter did not exceed 750 lx. Mussel larvae were grown for 3 days when they were fed endogenously. At this stage, the development of the digestive system and the increase of the body cavity were only at the very beginning [16].

Lipid extraction and production of fatty acid methyl esters. The fatty acid composition was studied in total lipids isolated from the biomass of larvae obtained on the 3rd day of the experiment (*in vitro*). To obtain fatty acids, the larvae were separated from water by filtration through a filter with a pore size of 84 μm. The larvae were thoroughly washed from the filter with several 5-ml portions of a mixture of ethanol : chloroform (1 : 1). The resulting solution with 20 mL in volume was centrifuged for 10 minutes at 1500 rpm with a double volume of distilled water. The lower chloroform layer was collected with a capillary. The chloroform fraction was triply washed with water and evaporated on a rotary evaporator. After evaporation of chloroform to saponify lipid residues, 5 mL of an alkaline methanolic solution (10 mL of a 3 N solution of NaOH mixed with 90 mL of 90 % methanol) were added into the flask. The resulting solution was refluxed until complete saponification for two hours. After cooling, a few drops of a 1 % solution of phenolphthalein were added into the solution, and the extraction of unsaporifiable lipids was performed three times with hexane. The water-alcohol phase was acidified with hydrochloric acid by adding 300 μL of 6 N HCl. Then the repeated extraction of fatty acids was carried out with 3–4 portions of hexane, 5 mL each. The hexane fraction was evaporated to dryness on a rotary evaporator at a temperature +30…+35 °C; 5 mL of a 3 % solution of hydrogen chloride in methanol was added to the residue for methylation. The mixture was refluxed for 2 hours; after cooling, it was subjected to triple extraction with hexane (5 mL each portion). The hexane layer was filtered using an ashless filter. Before the determination of fatty acid methyl esters (hereinafter FAME), the hexane fraction was stored for no longer than a day at a temperature +5 °C [4].

Identification of fatty acid methyl esters. FAME identification was performed at IBSS "Spectrometry and Chromatography" core facility using a Crystal 5000.2 gas chromatograph (SKB "Chromatek",Yoshkar-Ola, Russia) with a quadrupole mass detector and a capillary column DB-5ms ("Agilent Technologies") 30 m long, with the inner diameter of 0.25 mm and the film phase thickness of 0.25 μm. The measurements were carried out in the electron impact ionization mode with a potential of 70 eV. The carrier gas was helium; the flow rate was 1 ml·min^{-1}. The sample injection was carried out in the splitless mode. The injector temperature was +280 °C. The column temperature was as follows: initial temperature +60 °C; delay for 1 minute; temperature ramp 5 °C·min^{-1} to +180 °C; temperature ramp 5 °C·min^{-1} to +290 °C; final temperature +325 °C maintained for 10 minutes. The volume of the sample injected was 1.0 μl. The FAME identification was carried out in the total ion current. The FAME identification was carried out by comparing the relative retention time of the experimental chromatograms with that of the standard FAME mixture chromatogram (Supelco 37 component FAME mix) and by matching the obtained mass spectra of FAME – against the NIST 14 library counterparts with a degree of agreement exceeding 92 %. The calculation of FAME percentage in the sample was carried out by the normalization to the sum of the peak areas. The standard deviation of the output signal of the chromatograph did not exceed 6 % [15].

Statistical data processing. For the statistical analysis, we used Statistical Toolbox package embedded integrated in MATLAB software (version 8.2). Statistically significant differences between the samples were determined using one-way ANOVA and the Tukey – Kramer post-hoc test.

RESULTS AND DISCUSSION

It is known that the level of PCB accumulation by mussels depends on many factors such as tissue fat, mollusc size, and sexual maturation stage. It is individual differences of these factors that determine wide ranges of variation in the concentration of organochlorine toxicants in individuals collected in one region. Thus, PCB concentration in soft tissues of mussels in the sampling area varied 14 to 162 ng·g^{-1} and averaged 68 ng·g^{-1} ($n = 24$). A comparison of PCB levels in the mussels with the maximum permissible concentration (2000 ng·g^{-1} for the PCB according to the Technical Regulation of the Customs Union [13]), shows that there is no hazard for humans in case of consumption of the cultivated mussels from the farm. Moreover, according to the criteria established in the EU countries, the quality of mussels is ranked as very high, since the values do not exceed the established threshold for PCB concentration of 250 ng·g^{-1} [16].

In the experiments on the effect of environmentally significant PCB doses on mussel larvae, their response to this kind of pollution was revealed, which manifested itself in the variation of the fatty acid composition (Tables 1 and 2).

Table 1. Fatty acid fractions (% of the total) in trochophore lipids of mussel *M. galloprovincialis* grown in a medium with different concentrations of polychlorinated biphenyls

Identified fatty acid	Control	PCBs concentration, μg·L^{-1}			Acetone
		0.1	1	10	
Lauric (dodecanoic) (C12:0)	1.4 ± 0.6	0.6 ± 0.3	1.3 ± 0.4	1.0 ± 0.3	1.4 ± 0.5
Myristic (tetradecanoic) (C14:0)	6.2 ± 0.2	5.7 ± 0.6	7.7 ± 0.7	6.4 ± 0.4	6.5 ± 0.6
Pentadecanoic (C15:0)	4.4 ± 0.8	5.3 ± 1.1	8.5 ± 0.5	5.8 ± 0.2	4.7 ± 0.7
Palmitoleic (*cis*-9-hexadecenoic) (C16:1ω7)	11.0 ± 0.6	6.8 ± 0.6	7.0 ± 0.2	9.6 ± 0.4	10.8 ± 0.8
Palmitic (hexadecanoic) (C16:0)	29.3 ± 4.3	33.8 ± 0.4	30.1 ± 0.3	34.7 ± 0.6	31.7 ± 2.3
cis-10-heptadecenoic (C17:1ω7)	3.9 ± 1.5	1.2 ± 0.4	1.2 ± 0.2	1.4 ± 0.4	2.4 ± 1.5
14-methylhexadecanoic (*anteiso*-C17:0)	2.4 ± 0.5	1.8 ± 0.3	2.3 ± 0.2	2.0 ± 0.1	2.6 ± 0.8
Arachidonic (*cis*,*cis*,*cis*,*cis*-5,8,11,14-eicosatetraenic) (C20:4ω6)	1.3 ± 0.2	20.5 ± 1.5	12.8 ± 0.6	3.3 ± 0.3	1.0 ± 0.4
Linoleic (*cis*,*cis*-9,12-octadecadienoic) (C18:2ω6)	2,2 ± 0,6	3.0 ± 0.3	4.8 ± 0.6	1.3 ± 0.4	1.6 ± 0.7
Oleic (*cis*-9-octadecenoic) (C18:1ω9)	14.4 ± 1.5	1.5 ± 0.2	1.2 ± 0.2	15.3 ± 0.4	14.6 ± 1.1
Vaccenic (*cis*-11-octadecenoic) (C18:1ω7)	2.4 ± 0.3	5.8 ± 0.3	5.7 ± 0.2	2.4 ± 0.3	2.5 ± 0.7
Sum of two isomers of octadecenoic acids	16.8 ± 1.8	7.3 ± 0.5	6.9 ± 0.4	17.7 ± 0.7	17.1 ± 1.8
Stearic (octadecanoic) (C18:0)	14.4 ± 0.5	8.1 ± 0.4	10.3 ± 0.3	9.2 ± 0.3	13.3 ± 0.5
Eicosapentaenoic (*cis*,*cis*,*cis*,*cis*,*cis*-5,8,11,14,17-eicosapentaenoic, EPA) (C20:5ω3)	4.2 ± 0.6	3.0 ± 0.2	4.0 ± 0.1	4.6 ± 0.3	4.3 ± 0.3
Docosahexaenoic (*cis*,*cis*,*cis*,*cis*,*cis*,*cis*-4,7,10,13,16,19-docosahexaenoic, DHA) (C22:6ω3)	2.4 ± 1.0	2.9 ± 0.2	3.1 ± 0.2	2.8 ± 0.4	2.5 ± 0.5
Total saturated fatty acids (SFA)	58.2	55.3	60.2	59.1	60.3
Total monounsaturated fatty acids (MUFA)	31.6	15.3	15.1	28.8	30.3
Total polyunsaturated fatty acids (PUFA)	10.2	29.4	24.7	12.1	9.5
Total unsaturated fatty acids (UFA)	41.8	44.7	39.8	40.9	39.8
Total SFA / total UFA ratio	1.4	1.2	1.5	1.4	1.5

Table 2. Significant differences (denoted by pluses) in fatty acid composition of mussel *M. galloprovincialis* trochophores as found from one-way ANOVA (df = 4) and Tukey – Kramer post-hoc test

Fatty acid	Pollutant concentration, μg·L^{-1}	Control	Pollutant concentration, μg·L^{-1}			F	p
			0.1	1	10		
Lauric	0.1	–		–	–	0.620	0.66
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
Myristic	0.1	–		–	–	1.81	0.20
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
Pentadecanoic	0.1	–		–	–	5.40	$1.4\cdot10^{-2}$
	1	+	–		–		
	10	–	–	–			
	acetone	–	+	–	–		
Palmitoleic	0.1	+		–	–	13.32	$5.1\cdot10^{-4}$
	1	+	–		–		
	10	–	–	–			
	acetone	–	+	+	–		
Palmitic	0.1	–		–	–	1.11	0.41
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
cis-10-heptadecenoic	0.1	–		–	–	1.48	0.28
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
14-methylhexadecanoic	0.1	–		–	–	0.58	0.68
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
Arachidonic	0.1	+		–	+	124	$1.8\cdot10^{-8}$
	1	+	–		+		
	10	–	+	+			
	acetone	–	+	+	–		
Linoleic	0.1	–		–	–	6.80	$6.6\cdot10^{-3}$
	1	–	–		+		
	10	–	–	+			
	acetone	–	–	+	–		
Oleic	0.1	+		–	+	71.3	$2.6\cdot10^{-7}$
	1	+	–		+		
	10	–	+	+			
	acetone	–	+	+	–		

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Fatty acid	Pollutant concentration, μg·L^{-1}	Control	Pollutant concentration, μg·L^{-1}			*F*	*p*
			0.1	1	10		
Vaccenic	0.1	+		–	+	23.1	$4.9 \cdot 10^{-5}$
	1	+	–		+		
	10	–	+	+			
	acetone	–	+	+	–		
Stearic	0.1	+		–	–	41.2	$3.5 \cdot 10^{-6}$
	1	+	–		–		
	10	+	–	–			
	acetone	–	+	+	+		
Eicosapentaenoic	0.1	–		–	–	3.52	$5.0 \cdot 10^{-2}$
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
Docosahexaenoic	0.1	–		–	–	0.344	0.84
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
Total SFA	0.1	–		–	–	1.00	0.45
	1	–	–		–		
	10	–	–	–			
	acetone	–	–	–	–		
Total MUFA	0.1	+		–	+	37.3	$5.6 \cdot 10^{-3}$
	1	+	–		+		
	10	–	+	+			
	acetone	–	+	+	–		
Total PUFA	0.1	+		–	+	65.5	$3.9 \cdot 10^{-7}$
	1	+	–		+		
	10	–	+	+			
	acetone	–	+	+	–		

Note: "+" indicates significant differences ($\alpha = 0.05$; $n = 3$); "–" indicates lack of significant differences ($\alpha = 0.05$; $n = 3$); *F* indicates Fisher's *F*-test; *p* indicates probability. The components with significant differences are in bold.

It was found that in lipids of *M. galloprovincialis* trochophores the totals of monounsaturated fatty acids (hereinafter MUFA) and polyunsaturated fatty acids (hereinafter PUFA) significantly differed. The total of saturated fatty acids (hereinafter SFA) did not statistically change. The main SFA were palmitic (C16:0) (35–39 %) and stearic (C18:0) (8–14 %) acids. Saturated acids with a carbon number of 14 and 15 ranged 4 to 7 %. A relatively high level of SFA in trochophores is associated with high metabolic activity of molluscs during the spring spawning [30]. For example, when studying a seasonal fatty acid composition of the pearl oyster *Pinctada fucata martensii*, it was determined that the major SFA were myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids. Myristic (C14:0) acid in animals is rarely the main component. In our studies, its percentage varied 5.7–7.7 %.

The most common MUFA are represented by palmitoleic (C16:1ω7), oleic (C18:1ω9), and vaccenic (C18:1ω7) acids. Palmitoleic (C16:1ω7) and oleic (C18:1ω9) acids are derivatives of palmitic (C16:0) and stearic (C18:0) acids [14]. Vaccenic (C18:1ω7) acid is an isomer of oleic (C18:1ω9) acid,

which is synthesized in animal cells (endoplasmic reticulum and mitochondria) from stearic (C18:0) acid by the double bond formation. The presence of *cis*-vaccenic (C18:1ω7) acid, which is characteristic of anaerobic bacteria [18], in trochophore samples indicates non-sterile experimental conditions.

The monounsaturated oleic (*cis*-9-octadecenoic) (C18:1ω9) acid, found by us in mussel trochophores, has two possible origins: exogenous (*via* the digestion of diatoms) and endogenous (*via* the conversion of palmitic (C16:0) and stearic (C18:0) acids) [20]. The increased content of irreplaceable oleic (C18:1ω9) acid in mollusc trochophores may be due to its additional synthesis under the toxic effects of pollutants in order to bind and detoxify xenobiotics [22]. An increase in the level of isomers of octadecenoic (C18:1) acids may indicate an enhanced metabolism in larval cells [14].

The following PUFA were identified in trochophore lipids: arachidonic (C20:4ω6), eicosapentaenoic (C20:5ω3), and docosahexaenoic (C22:6ω3) acids. The total fraction of omega-3 and omega-6 acids in mussel larvae of the control experiment did not exceed 12.8 %. The concentration of the essential arachidonic (C20:4ω6) acid in trochophores was not constant and varied over a wide range 1 to 21 %. For comparison, the concentration of arachidonic (C20:4ω6) acid in gastropods reached 5.73 % [25]. As is known, living organisms can synthesize arachidonic (C20:4ω6) acid from the essential omega-6-unsaturated linoleic acid [1]. The biosynthesis of linoleic (C18:2ω6) acid can be carried out only in plants. Then, it is transferred to animals through food chains. Since linoleic (C18:2ω6) acid was found in mussel larvae in almost every sample, it can be assumed that it is necessary for the biosynthesis of arachidonic (C20:4ω6) acid at further stages of the mollusc development. Arachidonic (C20:4ω6) acid is also a major component of membrane phospholipids in animals. In addition, it is necessary for prostaglandin biosynthesis [29]. Probably, higher levels of this fatty acid in trochophores are related to more intense synthesis of prostaglandins [21].

PUFA are involved in the adaptation of the organism to the environment. Most invertebrates are not able to synthesize PUFA and get them with food, satisfying their needs for these essential components to maintain normal functioning of the organism [28]. For example, docosahexaenoic (C22:6ω3) acid can affect the activity of Na^+/K^+-ATPase, an enzyme of cell membranes that selectively pumps out sodium ions from a cell and accumulates potassium ions in it. The difference in concentrations of monovalent cations created by the enzyme is used for key reactions of vital activity: excitation of a nerve impulse and water-salt metabolism and for the regulation of cellular metabolism [26]. In our study, the fraction of eicosapentaenoic (C20:5ω3) acid in all samples was low and did not exceed 4.5 %, and the percentage of docosahexaenoic (C22:6ω3) acid did not exceed 3.1 %. Since eicosapentaenoic (C20:5ω3) and docosahexaenoic (C22:6ω3) acids are produced by phytoplankton [27 ; 29 ; 30], their low levels can most likely be explained by the endogenous feeding of the larvae at the trochophore stage.

It is known that the environmental pollution by PCBs affects fatty acid composition [14]. Our experiments showed that the total fraction of SFA in larvae exposed to the PCBs varied in a rather narrow range 52.2 to 65.3 %. Accumulation of these acids indicates their participation in maintaining membrane structure integrity [14]. The lowest percentage of stearic (C18:0) acid in the larvae was observed when they were exposed to the PCB concentration 0.1 $\mu g \cdot L^{-1}$. Under the effect of the PCB concentrations 1 and 10 $\mu g \cdot L^{-1}$, the fractions of stearic (C18:0) acid practically did not differ, but became lower than in the control and in the sample with acetone. This fact suggests that larvae reaction to the PCB appearance in the medium was manifested in a decrease of the plasma membranes permeability, which could reduce the toxic effect of the PCBs.

MUFA fraction decreased about 2-fold at the PCB concentrations 0.1 and 1 $\mu g \cdot L^{-1}$, while PUFA fraction increased about 2.5–3-fold at the PCB concentrations 1 and 0.1 $\mu g \cdot L^{-1}$ and 1.3-fold at the PCB concentration 10 $\mu g \cdot L^{-1}$.

With a low PCB concentration 0.1 and 1 μg·L^{-1}, the proportion of octadecenoic acids (C18:1) decreased more than 2-fold compared to the control experiment; at 10 μg·L^{-1}, their percentage was equal to the proportions in the control. It is possible that at low PCB concentrations, a change in the MUFA proportion is caused by several catalytic mechanisms, including peroxidation, in addition to the P450 cytochrome monooxygenase pathway. Enzymes of cytochrome P450 system hydroxylate C-H bonds of substrates and catalyze omega-oxidation of saturated fatty acids and peroxidation of unsaturated fatty acids [14]. Prior to the development of digestive organs, trochophores are fed endogenously, while fatty acids are used mainly to form biomembranes and storage lipids [14 ; 16 ; 17 ; 25].

The change in MUFA and PUFA proportions with an almost constant fraction of SFA under the effect of PCBs is associated with the protective function of unsaturated fatty acids in the larval organism. It is explained by the fact that the synthesis of unsaturated fatty acids proceeds from SFA. PUFA, for example, have lower melting points compared to saturated acids and form a looser lipid bilayer structure. The asymmetric structure and melting point are two characteristics of polyenes, which increase fluidity of biological membranes and determine high metabolic activity of membrane enzymes [14]. The effect of pollutants can affect resistance and tolerance of cultivated organisms directly, especially at an early stage of ontogenesis, or indirectly, through changes in material and energy flows in the ecosystem [2].

An increase in the concentration of arachidonic (C20:4ω6) acid from 1.3 % in the control to 20.5 % when exposed to the PCB concentration 0.1 μg·L^{-1} is also explained by its ability to act as a hormone, activating cell receptors, while playing an important role in immune response. At higher PCB concentrations (1 and 10 μg·L^{-1}), the fraction of arachidonic (C20:4ω6) acid decreases, which indicates its intensive use in enzymatic processes [14].

Conclusion. The data obtained allow us to conclude that the type and composition of fatty acids in mussel trochophores changed depending on the level of PCB pollution of mollusc habitat. The fraction of SFA (for example, stearic (C18:0) acid) and octadecenoic (C18:1) acid isomers decreased sharply when exposed even to the PCB concentration 0.1 μg·L^{-1}, although the total SFA fraction was practically unchanged at the PCB concentrations 0 to 10 μg·L^{-1}, and the fraction of octadecenoic (C18:1) acid isomers increased almost 3-fold with an increase in PCB concentration to 10 μg·L^{-1}. This tendency is associated with structural features of both larvae cell membranes and SFA and MUFA molecules. On the contrary, the fraction of PUFA, for example, arachidonic (C20:4ω6) acid, increased under the effect of the PCB concentration 0.1 μg·L^{-1}, which is probably due to its ability to act as a hormone in immune response.

The results of the work can be used in the management of production processes in mollusc farms. A study of the dose-dependent effect of the PCBs on the ratio of SFA, MUFA, and PUFA in mussel larvae tissues can contribute to a better understanding of biochemical rearrangements that allow molluscs to adapt to the effects of adverse environmental factors.

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СОСТАВ ЖИРНЫХ КИСЛОТ В ТРОХОФОРАХ МИДИЙ *MYTILUS GALLOPROVINCIALIS*, ВЫРАЩЕННЫХ В УСЛОВИЯХ ЗАГРЯЗНЁННОСТИ ПОЛИХЛОРБИФЕНИЛАМИ

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Состояние черноморских популяций *Mytilus galloprovincialis* в естественной среде обитания напрямую зависит от развития мидии на всех стадиях, в том числе на начальных стадиях личиночных форм, наиболее чувствительных к загрязнению окружающей среды. Поллютанты органического происхождения оказывают негативное влияние на личинки моллюска, проявляющееся в торможении их роста и развития. Закономерности размножения мидии хорошо изучены, что даёт возможность получать в контролируемых лабораторных условиях личинки из искусственно оплодотворённых яйцеклеток этого вида моллюсков. В работе впервые исследован жирнокислотный состав общих липидов, выделенных из биомассы тканей личинок *M. galloprovincialis* на стадии трохофоры в контроле и после их трёхдневной экспозиции в среде с добавлением различных концентраций полихлорбифенилов. Жирнокислотный состав суммарных липидов в биомассе личинок, полученных на третьи сутки эксперимента, исследовали методом хромато-масс-спектрометрии. Всего идентифицировано 14 жирных кислот: 59 % из них относились к насыщенным, 24 % — к моноеновым, 17 % — к полиеновым. Для статистического анализа использовали программу MATLAB (версия 8.2). В условиях проведённого эксперимента в липидах трохофор *M. galloprovincialis* достоверно отличались значения суммы мононенасыщенных и полиненасыщенных жирных кислот. Сумма насыщенных жирных кислот статистически значимо не изменялась. Основными насыщенными жирными кислотами во всех исследуемых трохофорах мидий являлись пальмитиновая (C16:0) и стеариновая (C18:0). Их концентрации значительно не изменялись под действием полихлорбифенилов. Наиболее значимые мононенасыщенные жирные кислоты — олеиновая (C18:1ω9), пальмитолеиновая (C16:1ω7) и вакценовая (C18:1ω7). Содержание мононенасыщенных жирных кислот понижалось вдвое при действии полихлорбифенилов с концентрациями 0,1 и 1 мкг·л$^{-1}$; при концентрации полихлорбифенилов 10 мкг·л$^{-1}$ суммарное содержание этих кислот было равно таковому в контроле. Среди полиненасыщенных жирных кислот, обладающих положительной биологической активностью, были идентифицированы арахидоновая (C20:4ω6), эйкозапентаеновая (C20:5ω3) и докозагексаеновая (C22:6ω3). Суммарное содержание Омега-3 и Омега-6 кислот в личинках мидий в контрольном опыте не превышало 12,8 %. С увеличением концентрации полихлорбифенилов

в среде выращивания трохофор с 0,1 до 1 мкг·л$^{-1}$ концентрация полиненасыщенных жирных кислот повышалась в 2,5 раза. При концентрации полихлорбифенилов 10 мкг·л$^{-1}$ и в пробе с ацетоном суммарное содержание полиненасыщенных жирных кислот было сопоставимо с таковым в контрольном опыте. Результаты исследования свидетельствуют о том, что жирнокислотный отклик трохофор мидий *M. galloprovincialis* максимален при воздействии концентраций полихлорбифенилов от 0,1 до 1 мкг·л$^{-1}$. При концентрации загрязнителей 10 мкг·л$^{-1}$ и выше биохимические процессы в личинках, по-видимому, замедляются. Результаты данного исследования могут способствовать лучшему пониманию перестроек, позволяющих моллюскам на личиночных стадиях развития адаптироваться к условиям загрязнения среды обитания органическими поллютантами.

Ключевые слова: полихлорбифенилы, жирные кислоты, личинки, трохофора, мидия *Mytilus galloprovincialis*, Чёрное море