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Physiological–biochemical properties of blue mussel *Mytilus edulis* adaptation to oil contamination

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Abstract Bivalves have a known ability to accumulate different contaminants from ambient water and can therefore serve as bioindicators. The paper analyses certain biochemical and physiological parameters of blue mussels in response to varying oil product concentrations. The heart rate (HR) of blue mussels from the sublittoral zone exposed to different levels of oil products was investigated in a long-term experiment using non-invasive monitoring. A sharp rise in HR was observed at oil concentrations of 8.0 and 38.0 mg/l. A decreasing in mussel HR under the effect of lower concentrations (0.4 and 1.9 mg/l) was significant on the fourth day. Strong fluctuations of the cardiac activity were noted under all concentrations. After 6 days of oil treatment, tissues of the mussels were sampled to determine the total lipid composition. Low concentrations of oil products produced no reliable changes in the lipid composition whereas high concentrations induced significant changes in the ratio of lipid components (cholesterol and phospholipids).

Keywords Blue mussel · Heart rate · Lipids · *Mytilus edulis* L. · Oil products · Oil pollution · Phospholipids

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Introduction

The problem of oil contamination in the aquatic environment is now very acute due to intensive offshore oil and gas development. Coastal contamination is especially important at present due to development of new oil and gas fields in coastal and shelf zones (Stockman, Sakhalin-1, Sakhalin-2). It is known that massive hydrocarbon contamination of the coast is often the result of large oil spills, whereas the most important sources of the pollution in the estuarine environment are chronic inputs, including sewage effluent and losses from refinery and shipping operations. Oil is a mixture of over 1,000 components, including polycyclic aromatic hydrocarbons (PAH). Low molecular weight PAH's are among the most toxic components, while some of the high molecular weight PAHs are potent carcinogens (Choiseul et al. 1998; Dyrynda et al. 2000).

Using *Mytilus edulis* as an environmental indicator has been a popular biomonitoring tool for over 30 years due to the ability of blue mussels

to accumulate many of the pollutants in concentrations exceeding those in ambient seawater by thousands or even hundreds of thousands of times (Farrington et al. 1983; Choiseul et al. 1998; Awad 1979; NAS 1980; Widdows and Donkin 1992; Widdows et al. 1997; Shigenaka and Henry 1993).

Biomonitoring of environmental pollution employs a diverse assortment of methods (Capuzzo 1981; Wedderburn et al. 2000. Physiology–biochemical methods help to both reveal the toxic effects of pollutants and explain the mechanism of the organism's response to them. With mussels, however, this is rather difficult due to technical problems (Depledge et al. 1992; Taylor et al. 2000). For instance, the wide application of such a highly informative parameter as cardiac activity was hindered by artifacts evoked by the implantation of electrodes and/or other injuring impacts (Segal 1961; Bayne 1973). Recently, this drawback was resolved with the development of a technique for remote monitoring (Depledge and Andersen 1990; Marshall and McQuaid 1993). Experiments have proved that changes and variation in mussel HR correlate significantly with various natural factors (Marshall and McQuaid 1993, 1994; Santini et al. 2000; Bakhmet et al. 2005). Some other studies revealed high sensitivity of mussel HR to heavy metals and ammonia (Marchan et al. 1999; Bloxham et al. 1999; Curtis et al. 2000). In this context, the absence of publications regarding the effect of oil products on the heart rate of a popular model species such as blue mussel is surprising.

It is well known that the initial response of an organism to contamination takes place at the molecular and cellular levels, i.e. it precedes physiological and morphological deviations (Bertoli et al. 2001; Nemova and Vysotskaya 2004). Changes in biochemical indices reflect the metabolic status and often evidence a compensatory response developing under the impact of pollutants. Lipids in cell membranes play an important role in the organism adaptation to changing environmental conditions (Kreps 1981). Stress factors influence the composition of membrane lipids and, hence, the physical properties of the membrane, mainly microviscosity, so as to support its optimal structure (Nechev et al. 2006). Changes in some lipids indices under the effect of pollutants have been demonstrated for blue mussels (Capuzzo and Leavitt 1988; Leavitt et al. 1990), but these studies were made with whole animals. Yet, the response of a whole organism to different pollutants is integral, representing a sum of responses of different organs. At the same time, the cells of each specific organ may exhibit a different strategy of response to pollution.

Thus, the objective of the present study has been to assess the effect of oil pollution in different concentrations on the cardiac activity and lipid composition in blue mussels from the White Sea with view to further application of these methods in biomonitoring of coastal environment.

Material and methods

Mollusk collection and maintenance

Experiments were carried out at the White Sea Biological Station (Chupa Bay, Gulf of Kandalaksha, White Sea) of the Zoological Institute of the Russian Academy of Sciences. Sublittoral mussels were collected from artificial mussel-rearing substrates installed in Kruglaya Bay at a depth of 2.0 m. After sorting the animals by size and age (retaining those within 58–73 mm and 6–7 years old) and cleaning encrusted organisms off the shells, the animals were acclimatized to laboratory conditions for 7 days. Earlier studies have shown that 1 week was sufficient for blue mussels to acclimatize to the new conditions. (Bakhmet et al. 2005). The mollusks were kept in plexiglas tanks with aerated seawater with a salinity of 25% under constant light and at a temperature of 10°C. The water was partially replaced on a daily basis. The animals were kept without feeding to avoid specific dynamic action.

One day prior to the experiment, optical sensors (CNY-70) were glued to the shells and the experimental animals were placed in five aerated 15 l seawater tanks (15 individuals per tank).

Determination of the hydrocarbon concentration

The oil product used was diesel fuel. No light fractions were used as they are quick to evaporate

Table 1 Concentration of oil products used in the experiment

	1	2	3	4	Control
Counted (ml/l)	1.0	0.3	0.1	0	0
Counted (mg/l)	700	210	70	0	0
True (mg/l)	38.80	8.41	1.88	0.35	0.02

and their effect in a long-term experiment would be minimal. To avoid gravitational stratification, 100 ml of diesel fuel was first added to 900 ml of sea water and then stirred energetically for 10 min. After that, 15, 50 and 150 ml of the resultant mixture were added into each of the five containers/aquaria. Thus, several different concentrations of the fuel were established (Table 1). The concentrations were selected so as to cover the whole range from minimal to sublethal. The lethal effect of diluted hydrocarbons depends on the species of marine animals and ranges from 1 to 100 ppm (GESAMP 1993). For instance, the lethal concentration for some molluscs is 50–70 mg/l (Zambachidze 1975). The maximal hydrocarbon concentration selected for the present study was 40 mg/l. Oil products were added daily, immediately after changing water in the tank. Two controls were used: no oil products were added to the forth and fifth tanks, but the latter was also placed in a separate room to exclude the possibility of oil absorption from the air. It should be noted that part of the oil products added to the tank evaporated, part settled on tank walls and shells of the mussels, and part was absorbed in mussel tissues. Therefore, water samples were taken before every change of the water to determine the true concentration of oil products. Expiration-photometry method with Al_2O_3 column chromatography followed by spectroscopy was employed to this end (RD 52.24.476-95 1995).

HR monitoring and data processing

HR was recorded every 2 h for 1 day before adding the oil products and for 6 days afterwards using remote monitoring of the cardiac muscle volume (plethysmogram) based on IR radiation of the heart region and reflected light recording (Depledge and Andersen 1990). Optical sensors CNY-70 were used. A specially developed amplifier with a system of filters was employed and a portable digital oscillograph (Fluke 125) transmitted the signal to a personal computer to be recorded as consecutive HR waves and processed using the FlukeView 3.0 software (De Pirro et al. 1999; Santini et al. 2000). The time of a single heart contraction, the number of contractions per minute and standard error were calculated then the average value for 24 h was estimated.

Biochemical treatment

After 6 days of continuous exposure to oil products the foot and the distal and sagittal parts of the mantle were taken from the blue mussels for biochemical analysis. Lipids were extracted in a chloroform: methanol mixture (2:1 in volume; Folch et al. 1957). Total lipids were separated into fractions on "Silufol" plates. The content of phospholipids, triacylglycerols, cholesterol esters was determined after Sidorov et al. (1972, p. 151); cholesterol after Endelbrecht et al. (1974, p. 251). The composition of individual phospholipid fractions was determined by high performance liquid chromatography (HPLC) by the method of Arduini et al. (1996, p. 685) on a Nucleosil 100-7 column with the liquid phase acetonitrile: hexane: methanol: phosphorus acid (918:30:30:17.5 by volume); the UV-spectrophotometer had a wave length of 206 nm.

The fatty acids composition was determined by gas–liquid chromatography. Obtained methylesters were identified on apparatus "Chromatek Kristall-5000.1" (Russia) with flame-ionization detector, with Zebron ZB-FFAP columns. Mobile phase was helium; speed of currency was 50 ml per minute. The regime of identification was isotherm by the temperature of columns thermostat of 210°C; temperature of detector of 250°C; temperature of evaporator of 240°C.

Changes in the lipid composition were estimated by nonparametric Wilkokson–Mann–Witney test (Gubler and Genkin 1969).

Results and discussion

The plethysmograms recorded from the mussels allowed a clear discrimination between auricular contractions (short and high peak) and ventricular

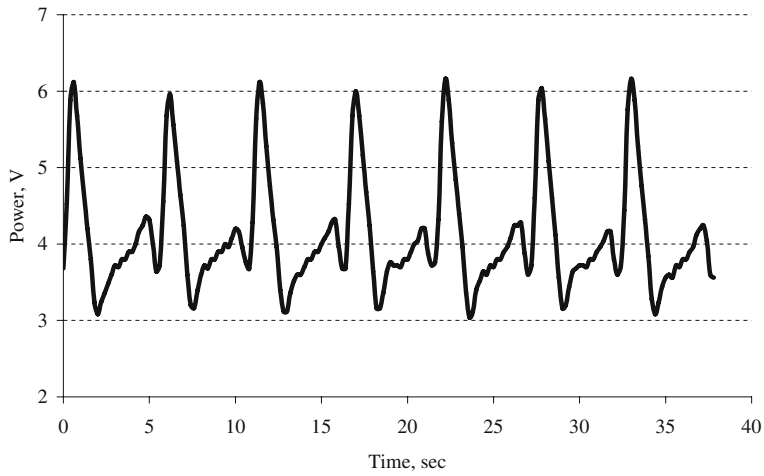

Fig. 1 The typical pletizmogram (cardiogram) of mussel

contractions (a longer and flattened peak; Fig. 1). The signal intensity (as recorded by an oscillograph) was up to 7 V. HR varied during the observation period from 0 to 14.8 beats per minute (bpm). The mean HR was 11.3 ± 0.2 bpm. Before exposure to oil products, the five groups of mussels showed no significant differences in cardiac activity. It is worth mentioning that the HR of mussels in the control was decreasing but not significantly over the 7 days of the experiment (Figs. 2, 3).

The results of two-way ANOVA are shown in Table 2. The HR of *M. edulis* was significantly influenced by oil and time. There was significant interaction among the factors. Thus, we conjecture that cardiac activity significantly responded to oil contamination and that responses significantly change over the course of experiment (see below).

After oil products in a high concentration (38.0 mg/l) were added, the cardiac activity of the mussels increased abruptly (Fig. 2). On the second day of the experiment however, HR went down and rose over the control again on the fourth day. After that, cardiac activity remained reliably higher than in the control until the end of the experiment. Thus, we see a cyclic change in the mussels' cardiac activity with a period of 2 days over the course of the first 4 days of the experiment (Fig. 2). When the concentration was 8.0 mg/l, a similar pattern of change was observed, but the period of fluctuations was about 1 day early in the experiment (Fig. 2).

When lower concentrations of oil products (0.4 and 1.9 mg/l) were applied, mussel HR decreased significantly only on the fourth to fifth day of the experiment. By the end of the experiment how-

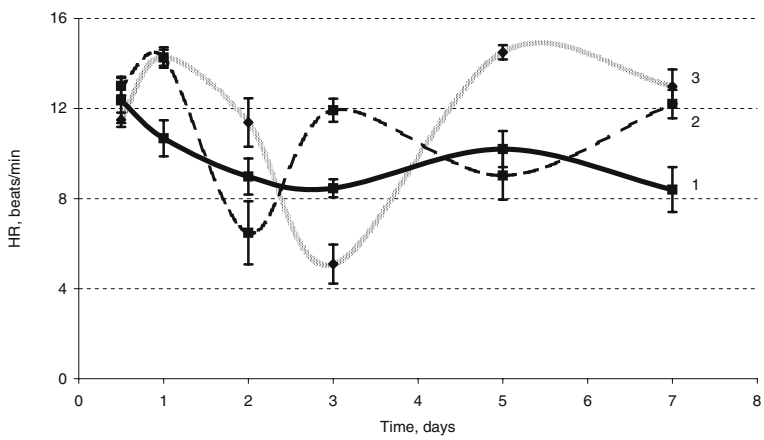

Fig. 2 The change of mussel heart rate under oil products exposure: 1 - control, 2 - concentration of 38.8 mg/l; 3 - concentration of 8.41 mg/l

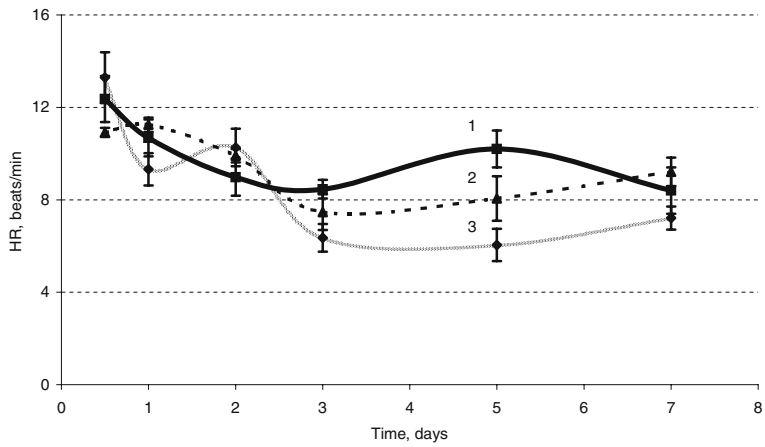

Fig. 3 The change of mussel heart rate under oil products exposure: 1 - control, 2 - concentration 1.88 mg/l, 3 - concentration of 0.35 mg/l

ever, cardiac activity returned to the control level (Fig. 3). Interestingly, HR fluctuations under low oil product concentrations were similar to those under high concentrations (8.0 and 38.0 mg/l).

Average HR indices in mussels are determined by counting the time periods including both actual heartbeats and "periods of silence" (cardiac arrest; Bakhmet et al. 2005). In our case, the number of these periods was the same in all experimental groups. Hence, changes in mussel HR were due to a decrease or increase in the time interval between heart contractions.

Accelerated HR under the impact of high oil concentrations points to a higher oxygen consumption and, consequently, a higher rate of basic metabolism. Earlier studies have in fact shown that the presence of even small quantities of oil increases respiration (Gilfillan 1975). By contrast, a recent study recorded a decrease in the heart rate and basic level of metabolism in the crab *Carcinus maenas* exposed to chronic pollution (Bamber and Depledge 1997). However, the crabs were kept under such conditions for more than 1 year. It is quite likely that in a longer experiment we would have also observed a reduction in cardiac activity. In other words, mussels need more time to complete the process of adaptation to the new environmental conditions. One should remember also that the mussel is an active filter and increased active respiration may be connected with removal of oil from tissues. Other studies support the view that *Mytilus edulis* actively release hydrocarbons after exposure in oil contaminated areas (Dunn and Stich 1976; Stainken 1975, 1977; Farrington et al. 1982). Additional oxygen is needed also to oxidize the pollutant. Oxidation of PAHs in different tissues of mussels with subsequent excretion was reported elsewhere (Dyrynda et al. 2000). In addition, it is known that crude oil induces increased activity of both oxygenase and lysozyme in *Mytilus edulis* blood cells for the oxidative biotransformation of lipophilic xenobiotics (Moore et al. 1980; Stegeman 1985; Ordás et al. 2007). It also has to be mentioned that mussels and other bivalves appear to be quite resistant to oil pollution, and often survive where other organisms die (Goldberg 1986). Blue mussels have demonstrated high adaptive capacity to the impact of heavy metals (Nechev et al. 2006), lipophilic contamination (McDowell et al. 1999) and PAHs (Dyrynda et al. 2000).

It is now common knowledge that adaptation process is oscillatory by nature due to inertia of adaptation mechanisms (Berger 1986; Berger and Kharazova 1997). Perhaps, the oscillatory nature of HR under the effect of oil pollution in our experiment indicates undergoing acclimatiza-

Table 2 ANOVA: effects of oil and time course of experiment on HR of blue mussels

Source	*df*	*SS*	*F*-ratio	*P*
Oil	4	777.74	8.33	< 0.0001
Time	23	1,104.93	2.06	0.0026
Oil × time	1	43,647.33	1,870.82	< 0.0001
Error	639	14,908.26		

Degrees of freedom (*df*), sum of squares (*SS*), *F* ratio and probability level (*P*) are shown

tion of the mussels to the exposure. The main argument for this hypothesis is that the same effect was observed at different oil concentrations. A less prominent effect at concentrations of 1.9 and 0.4 mg/l is logically explained by lower concentrations. It is obvious that survival under such conditions requires certain adaptation, and the course of such adaptation can be traced in our experiment. A similar assumption was previously expressed in relation to the effect of iron and lipophilic contamination on mussels (McDowell et al. 1999; Nechev et al. 2006).

Changes in the mussel HR can be additionally interpreted through the influence of some neurotransmitters. In our case, release of such neurotransmitters as 5-GT, FMFR-amides and/or cardioactive peptides (McMahon et al. 1997) could take place. In the future, special attention should be paid to the biogenic amines FMFR since concentrations of these neurotransmitters grow under stress conditions (Yamagishi et al. 2004). Also, one cannot exclude a contribution of acetylcholine to controlling cardiac activity, as it is one of the main inhibitory neurotransmitters (Deaton et al. 2001). At least, heavy metals have influenced external cholinergic control of heart activity in invertebrates, resulting in a decrease in hemolymph circulation (Bini et al. 2006). However, a recent study shows lower values of AChE activity under the influence of PAHs that might lead to increase of HR (Moreira et al. 2004). The situation in our case may be similar, given a reduction in cholesterol content under oil product pollution (see below).

It is known that adaptation/acclimatization processes involve changes in cell metabolism and an important part of this process is changes in lipid composition (Kreps 1981; Nemova and Vysotskaya 2004). Lipid metabolism changes in response to environmental stress-factors. Eventually, the lipid composition in the organism is affected.

Our investigations of the lipid composition of blue mussel tissues after 6 days of exposure to oil contamination showed no changes to occur in lipid composition under an oil concentration of 1.9 mg/l (Table 3). This data correlates with the recorded values of blue mussel HR under oil concentrations of 0.4 and 1.9 mg/l in the end of the experiment. Moreover, fatty acid compositions did not change under all experimental treatments.

However, hydrocarbon concentrations of 8.0 and 38.0 mg/l caused a threefold reduction in the cholesterol/phospholipids ratio in membranes distal mantle parts due to a decrease in the level of cholesterol and increase in the level of phospholipids (PL), including their individual fractions [phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl inozitol (PI) and an unidentified lipid (Table 2)]. The cholesterol/phospholipids ratio is one of the main parameters characterizing microviscosity of biomembranes (Bell et al. 1986; Elyakov and Stonik 1988; Hall et al. 2002). The decrease in the index in the distal part of the mantle points to a decrease in microviscosity of biomembranes and, at the same time, to changes in the activity of membrane-bound enzymes in the blue mussels (Elyakov and Stonik 1988; Kolomiytseva et al. 2003). Exposure to a high oil concentration (38.0 mg/l) resulted in a rise in the content of phosphatidyl inozitol—a minor component of cell membranes participating in important physiological processes such as signal transduction on the cell surface, regulation of membrane transport and membrane permeability (Kucherenko and Bluym 1986; Di Paolo and de Camilli 2006). The rise in the PI level in the distal part of the mantle suggests the phospholipid takes part in the compensatory response of the mussels to the impact of oil products.

In the sagittal part of the blue mussel mantle, response to oil pollution was detected only under the impact of the highest oil concentration (38.0 mg/l)—the cholesterol/phospholipids ratio decreased 2.7 times, mainly due to a decrease in cholesterol. Apparently, in the case of heavy oil contamination cholesterol plays an important part in regulation of the adaptation processes by changing biomembrane viscosity in both parts of mantle. Compared with other investigated organs, the sagittal part of the mantle demonstrated a higher level of neutral lipids [triacylglycerols (TG) and cholesterol esters]. In some studies on whole organisms of *Mytilus edulis*, TG accumulation and an increase in the neutral/polar lipids ratio were recorded (Capuzzo and Leavitt 1988). Presumably, pollution may slow down the TG to PL

Table 3 The influence of oil contamination on lipid composition in sublittoral mussels *Mytilus edulis* L

	The distal part of mantle				Sagittal part of mantle				Foot			
Lipids in % dry weight	0.4 mg/l	1.9 mg/l	84 mg/l	38.8 mg/l	0.4 mg/l	1.9 mg/l	8.4 mg/l	38.8 mg/l	0.4 mg/l	1.9 mg/l	8.4 mg/l	38.8 mg/l
Total lipids	9.9	10.1	9.7	9.3	10.1	9.0	11.1	8.5	11.9	12.9	9.7*	9.2*
Phospholipids	2.9	2.9	5.3*	5.2*	4.0	3.9	4.3	4.8	6.2	6.4	4.9*	5.9
Triacylglycerols	0.03	0.4	0.0	0.0	2.1	1.8	2.8	2.0	0.0	0.0	0.0	0.0
Either cholesterol	0.07	0.0	0.0	0.0	0.03	0.02	0.2	0.0	0.0	0.2	0.0	0.0
Cholesterol	6.9	6.8	4.4*	4.1*	4.1	3.2	3.8	1.6*	5.7	6.3	4.8	3.3*
Cholesterol/ Phospholipids	2.5	2.8	0.9*	0.8*	1.1	0.9	0.9	0.4*	0.9	1.1	0.9	0.6
PI	0.03	0.05	0.03	0.3*	0.03	0.02	0.07	0.04	0.02	0.08*	0.04*	0.08*
PS	0.2	0.2	0.3*	0.3*	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.4
PE	0.3	0.3	0.5*	0.4*	0.5	0.3	0.5	0.5	0.7	0.7	0.4*	0.6
PC	1.8	1.9	3.5*	3.1*	2.5	2.6	2.7	2.9	4.4	4.9	3.5*	3.8*
Non- identified phospholipid	0.6	0.5	0.9*	1.0*	0.7	0.7	0.7	0.9	0.7	0.6*	0.6	0.9
LPC	0.1	0.1	0.15	0.15	0.1	0.2	0.1	0.1	0.07	0.07	0.1	0.1
SM	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
PC/PE	6.0	6.3	7.0	7.8	5.0	8.7	5.4	5.8	6.3	7.0	8.8	6.3

0.4 mg/l—relative control

*$p < 0.05$, differences are significant in comparison with a control

transformation, and this process, in turn, tells on the structure and function of membranes (Kato et al. 1982; Capuzzo and Leavitt 1988).

In the foot—the main muscular organ of the blue mussel—a significant decrease in total lipids (mainly at the expense of their structural fractions—phospholipids and cholesterol) was observed under the action of high hydrocarbon concentrations (8.0 and 38.0 mg/l). The decrease in the content of phospholipids, in turn, was the result of a decrease in their fractions—PE and PC. Also, these oil concentrations led to a rise in PI, like in the distal part of the mantle.

The changes in the quantitative composition of structural lipids appear to influence the activity of membrane-bound enzymes. They also trigger a series of shifts in cell metabolism in general, enabling mussels to acclimatize to high hydrocarbon concentrations in seawater.

Conclusions

As a summary we would like to say that our studies have demonstrated high sensitivity of cardiac activity and lipid composition in mussels to the presence of oil products. This sensitivity manifests itself not only in an increase or decrease of HR, but also in the pattern of response of cardiac activity in the course of the experiment. Distinct HR variability (HR waves) was observed at all experimental concentrations of oil products. Thus, mussel HR is a reasonable and promising biomonitoring instrument. This method allows monitoring of the situation with pollution in the real time mode for as long as is necessary. It is especially important as the majority of investigations of the oil influences on physiology/biochemistry of marine organisms are carried out after the contamination has occurred.

Correlations between changes in lipid composition and hydrocarbon concentrations were shown. Concentrations of 38.0 and 8.0 mg/l induced a response at the level of structural lipids (PE, PC, PS, PI) and the cholesterol/phospholipids ratio. Changes in the content of PL (mainly PE and PC) had different directivity in the foot and the distal part of the mantle, whereas changes in cholesterol content were similar in all investigated organs. No changes under the effect of the oil concentrations were detected in the content of neutral lipids (TG and cholesterol esters). The combined effect of changes in the content of structural lipids in different organs appears to be a compensatory response of the lipid composition to changes in physical properties (mainly—microviscosity) of membranes induced by high hydrocarbon concentrations. With a low hydrocarbon concentration (1.9 mg/l), no changes were observed in the lipid composition.

Further studies are needed to determine how compensatory responses to oil pollution develop in mussels. For this purpose first of all it is necessary to evaluate the lipid composition over the course of all experiments. Second, we need to estimate the hydrocarbon concentration in the tissues of mussels. In addition, the plans for the next stage of our research are to assess not only changes in the lipid composition, but also the response of enzymatic activity in carbohydrate and protein metabolism. It is assumed that by assessing changes in enzymatic activity one can reach a better understanding of the structural transformations in cell metabolism intended to compensate for adverse environmental impacts.

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