Research Article

An Integrated Approach to Assessing the Effects of Environmental Factors on Mussels in the Black Sea

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Abstract. Metabolic modifications, including in lipid profiles, and physiological and morphological disorders, can be used as indicators of the health status of an organism and its adaptive strategy, such as tolerance and survival under environmental impacts. An integrative approach to studying the effects of environmental factors on an organism must take into account the seasonality and differences in the organism's habitat. Mussels in the bivalve genus *Mytilus* are used worldwide as marine sentinel organisms in biomonitoring programmes. Seasonal changes in the lipid composition of mussels reflect the effect of a wide range of environmental factors: temperature, salinity, quality and availability of food sources, and stages of the reproductive cycle. This study compared the lipid profiles of mussels from different habitats in the Black Sea depending on the stage of their reproductive cycle. The lipid profile of the gills and digestive glands of Black Sea mussels (*Mytilus galloprovincialis*) depends on environmental factors specific to these locations and is determined by reproductive processes. This indicates the important role of lipid molecules in the life cycle of mussels, as well as during their adaptation to environmental factors. These findings can be used for integrated assessment of the impact of environmental factors on the sentinel organisms used in biomonitoring research.

Keywords: lipids, phospholipids, mussels, season, environmental factors, habitat

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1. Introduction

The mussel genus *Mytilus* (family Mytilidae) is widespread in the Arctic, the North and Southeast Pacific, and the North and Southwest Atlantic [1]. Due to the differences in climatic conditions and hydrological regimes, the geographical location of mussel populations is reflected in the timing of the reproductive cycle and the subsequent accumulation and utilisation of metabolic substrates (mainly proteins, carbohydrates, and lipids) for gametogenesis and other stages of the reproductive cycle [1–4]. Lipids, especially the membrane components (phospholipids and cholesterol), play an important role in the metabolism of mussels, providing fluidity necessary for the function of membrane-bound

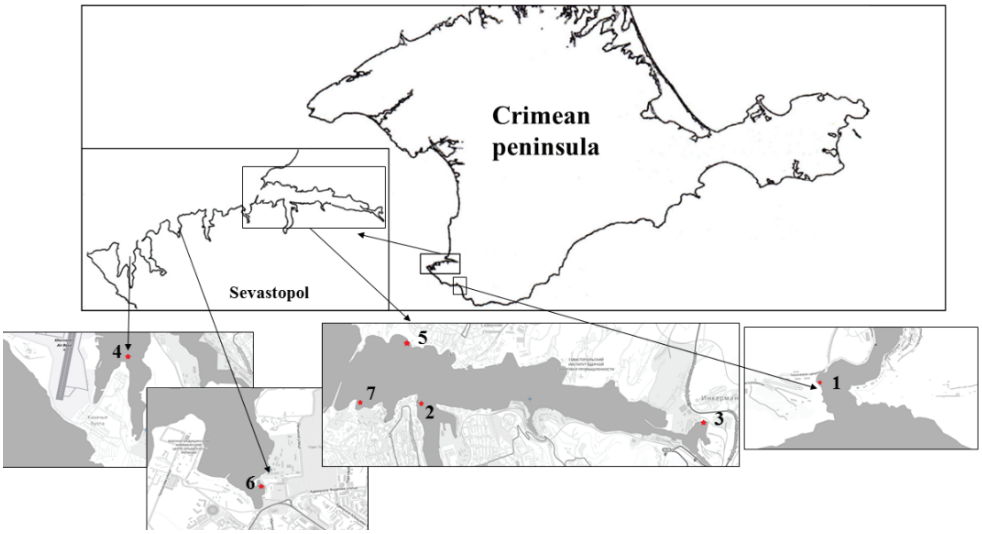

Figure 1: Schematic map of the study locations in the Crimean Peninsula (Black Sea). **(1) - Balaklava Bay (44.495116, 33.592691), the depth of mussel sampling was 1--3 metres. The water transparency was 1.5--2.0 metres; (2) Grafskaya Pier (44.616727, 33.526854), the depth of mussel sampling was 0.5 metres. The water transparency was about 1.5--2.0 metres; (3) Inkerman (44.613618, 33.601390), the depth of mussel sampling was 0.5--1.0 metres. The water transparency is about 0.5 metres; (4) Kazachya Bay (44.579081, 33.409535), the depth of mussel sampling was 1--5 metres. The water transparency was about 1 metre; (5) Matyushenko Bay (44.629388, 33.522752), the depth of mussel sampling was 0.5 metres. The water transparency was about 3 metres; (6) Round Bay (44.597430, 33.448286), the depth of mussel sampling was 0.5 metres. The water transparency was about 0.5 metres; (7) Crystal Beach (44.617626, 33.511528), the depth of mussel sampling was 0.5 metres. The water transparency was 1.5--2.0 metres.**

proteins (enzymes, ion channels, and receptors) [5]. Moreover, sterols are precursors for the synthesis of steroid hormones, which are involved not only in gonad maturation but also in the utilisation of metabolic reserves to stimulate gametogenesis [6]. Esterified cholesterol is known to be the metabolic pool of cholesterol and polyunsaturated fatty acids [7, 8]. Triacylglycerols are high-energy lipids, and their redistribution between the mantle and the digestive gland of bivalves occurs during the reproductive cycle [6]. Moreover, changes at the level of membrane lipids (phospholipids and sterols) reflect seasonal fluctuations in environmental factors (including temperature and salinity) [9]. Thus, this study of seasonal changes in the gill and digestive gland lipid content of mussels from different habitats in the Black Sea (*M. galloprovincialis*) aimed at revealing the peculiarities of the lipid composition due to the influence of environmental factors, depending on the reproductive cycle stage.

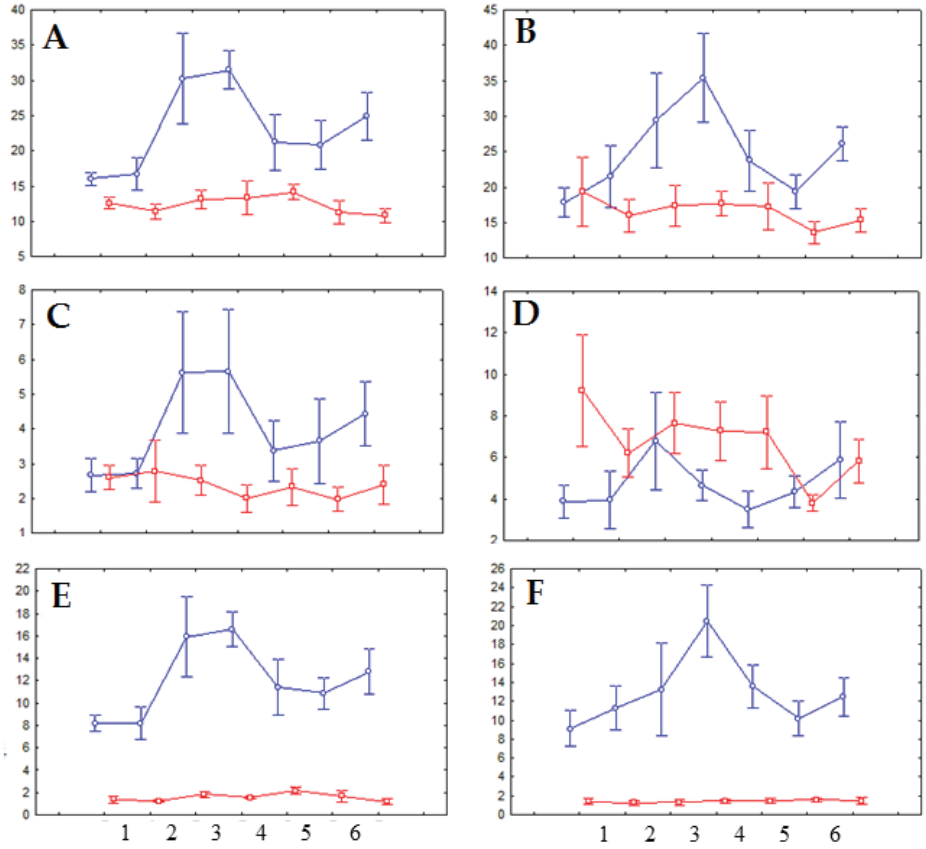

Figure 2: Content of total lipids (A, B), triacylglycerols (C, D), and phospholipids (D, E) (% dry weight) in the gills (A, C, E) and digestive glands (B, D, F) of *M. galloprovincialis* collected from different locations in the Black Sea: (1) Balaklava Bay, (2) Grafskaya Pier, (3) Inkerman, (4) Kazachya Bay, (5) Matyushenko Bay, (6) Round Bay, and (7) Crystal Beach. **Blue lines -- September 2018; red lines -- May–June 2019.**

2. Methods and Equipment

2.1. Mussel sampling

Mussels (*M. galloprovincialis*) were collected in September 2018 and May and June 2019 in various locations on the Black Sea coast of the Crimean Peninsula. The sampling locations are shown in Figure 1.

2.2. Lipid analysis

The lipid composition in the gills and digestive glands of mussels was determined using the equipment of the Core Facility at the Karelian Research Centre, Russian Academy of Sciences (Petrozavodsk). Lipids were extracted by the Folch method [10] and separated into the main lipid classes (phospholipids, triacylglycerols, and sterols) on thin-layer chromatography Silica gel 60 F254 plates (Merck, Germany) in a solvent

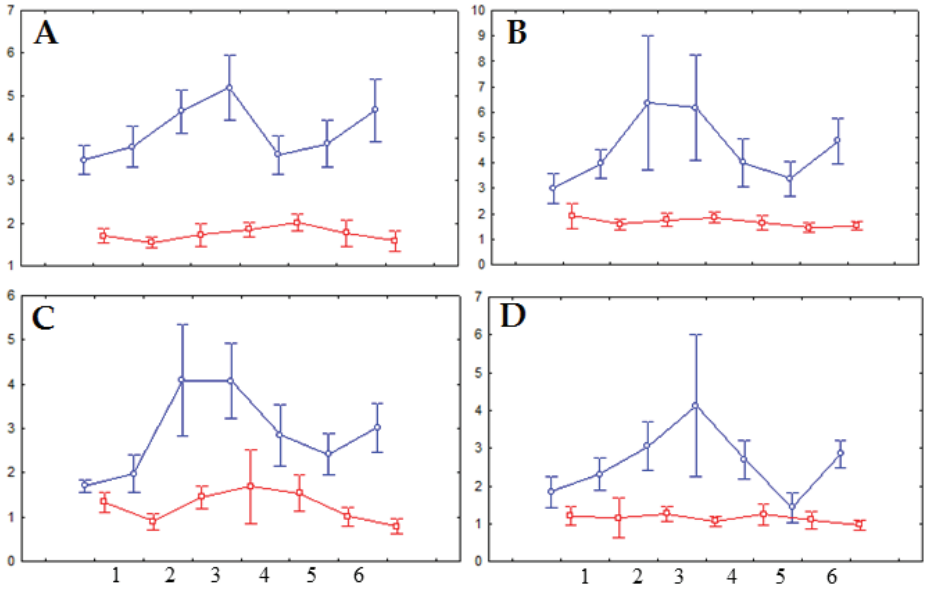

Figure 3: Content of sterols (A, B) and their esters (C, D) (% dry weight) in the gills (A, C) and digestive glands (B, D) of *M. galloprovincialis* collected from different locations in the Black Sea: (1) Balaklava Bay, (2) Grafskaya Pier, (3) Inkerman, (4) Kazachya Bay, (5) Matyushenko Bay, (6) Round Bay, and (7) Crystal Beach. **Blue lines -- September 2018; red lines -- May–June 2019**.

system of petroleum ether, sulphuric ether and acetic acid (90: 10: 1). Quantification of the isolated fractions of total lipids (% dry weight) was performed by various methods described earlier [11, 15-18]. Phospholipids were separated into individual fractions by high-performance liquid chromatography (Stayer, Akvilon, Russia) [12].

The data was statistically processed using nonparametric tests (Mann–Whitney U, Kruskal–Wallis and Tukey's tests). A p-value of 0.05 was accepted as indicating statistical significance.

3. Results and Discussion

Seasonal changes in the lipid composition of bivalves reflect the effect of a wide range of environmental factors – temperature, salinity, quality and availability of food sources – as well as stages of the reproductive cycle [13]. Unlike the lipid composition of the mantle and digestive gland, which undergoes significant modifications associated with reproductive processes [9], the gill lipid composition primarily depends on ambient environmental factors [14–16]. Previously, we noted changes in the gill lipid composition in mussels from the White Sea (*M. edulis*) in response to different temperatures [17], salinity [14], and different types of pollutants [11, 18].

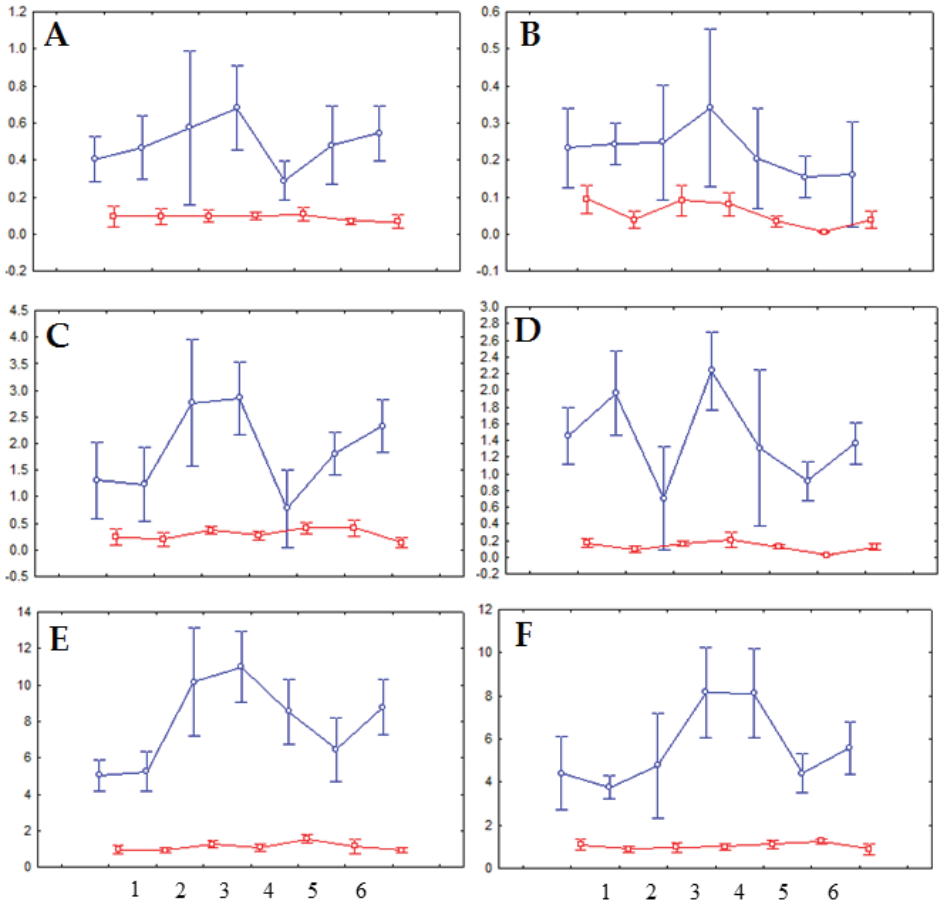

Figure 4: Content of phosphatidylserine (A, B), phosphatidylethanolamine (C, D), and phosphatidylcholine (D, E) (% dry weight) in the gills (A, C, E) and digestive glands (B, D, F) of *M. galloprovincialis* collected from different locations in the Black Sea: (1) Balaklava Bay, (2) Grafskaya Pier, (3) Inkerman, (4) Kazachya Bay, (5) Matyushenko Bay, (6) Round Bay, and (7) Crystal Beach. **Blue lines -- September 2018; red lines -- May–June 2019.**

Black Sea mussels (*M. galloprovincialis*) collected in autumn 2018 and spring 2019 were in different stages of the reproductive cycle: the stages of maturation and spawning, respectively. Firstly, the seasonal features in the lipid composition of the gills and digestive glands of mussels reflect the stage of the reproductive cycle. To wit, the increased content of total lipids and their main classes (phospholipids, triacylglycerols, sterols and their esters) in the gills and digestive glands of the mussels collected in autumn (Fig. 2–4) indicates the accumulation of lipids at the gonadal maturation stage. Further, the variation of the lipid composition among the sampling locations reflects the influence of various environmental factors, including low salinity.

Conversely, the relatively low lipid level in mussels collected from the same habitats in spring was relatively stable under the influence of the ambient factors typical for these sampling locations. This indicates the importance of spawning processes, which determine the physiological state of *M. galloprovincialis*. There were no significant

differences in the lipid composition of the gills and digestive glands of Black Sea mussels among the sampling locations.

Thus, the lipid profile of the gills and digestive glands of Black Sea mussels (*M. galloprovincialis*) depends on environmental factors and reproductive processes. This indicates the important role of lipid molecules in the life cycle of mussels, as well as during their adaptation to environmental impacts.

4. Funding

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5. Conflict of Interest

The authors declare no conflicts of interest.

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