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Original paper

Density Dynamics of Mussel Larvae *Mytilus galloprovincialis* Lamarck, 1819 and Hydrological and Hydrochemical Parameters on a Marine Farm in the Waters of Sevastopol (the Black Sea)

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Abstract

In 2023, a comprehensive study was performed near the mussel and oyster farm in the outer roadstead of Sevastopol to monitor the hydrological, hydrochemical and hydrobiological characteristics of seawater. The study aims to investigate the seasonal dynamics of hydrological and hydrochemical parameters of seawater and the density of *Mytilus galloprovincialis* Lamarck, 1819 mussel larvae in the plankton. Meroplankton samples were collected monthly from the 0–10 m layer using a Juday net. The live material was processed by counting pelagic larvae of benthic invertebrates in a Bogorov chamber under MBS-9 and Micmed-5 light microscopes. At the same time, water samples were taken in the surface water layer to determine the temperature, salinity, dissolved oxygen concentration, BOD5, permanganate index in an alkaline medium, concentrations of nitrites, nitrates, phosphates and ammonium. In 2023, the surface water temperature was minimum in February (8.1 °C) and maximum in August (26.5 °C). The water salinity was 17.70–18.50‰ with an average value of 18.23‰. The oxygen saturation of the waters varied from 93 to 126.6%, and hypoxia was not observed throughout the year. During the study period, the maximum density of mussel larvae (82 ind.$\cdot$m^{-3}) was recorded in March at a water temperature of 9°C, whereas the autumn peak was not observed. The results of the monitoring showed moderate correlation (0.51) between water temperature and the number of bivalve larvae in the plankton. The effect of nutrients on the density of *Mytilus galloprovincialis* larvae was not established. The results confirm that the conditions in the farm's waters are favourable for shellfish cultivation and underscore the need for continued monitoring.

Keywords: mariculture, nutrients, meroplankton, Bivalvia, Black Sea

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Динамика плотности личинок мидии *Mytilus galloprovincialis* Lamarck, 1819 и гидролого-гидрохимических показателей на морской ферме в акватории Севастополя (Черное море)

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Аннотация

Комплексный мониторинг гидрологических, гидрохимических и гидробиологических показателей был проведен в 2023 г. в акватории мидийно-устричной фермы, расположенной на внешнем рейде г. Севастополя. Цель работы – изучение сезонной динамики гидролого-гидрохимических показателей морской воды и плотности личинок мидии *Mytilus galloprovincialis* Lamarck, 1819 в планктоне. Пробы меропланктона отбирали ежемесячно в слое 0–10 м сетью Джеди. Материал обрабатывали в живом виде путем тотального подсчета пелагических личинок донных беспозвоночных в камере Богорова под световыми микроскопами МБС-9 и «Микмед-5». Одновременно отбирали пробы воды в поверхностном слое для определения температуры, солености, содержания растворенного кислорода, биохимического потребления кислорода за пять суток, перманганатной окисляемости в щелочной среде, концентрации нитритов, нитратов, фосфатов, аммония. В 2023 г. температура воды на поверхности была минимальной в феврале (8.1 °C) и максимальной – в августе (26.5 °C). Соленость воды составляла 17.70–18.50 ‰ при среднем значении 18.23 ‰. Насыщаемость вод кислородом изменялась от 93 до 126.6 %, гипоксию в течение всего года не наблюдали. В 2023 г. максимальная плотность личинок мидий (82 экз.·м$^{-3}$) зарегистрирована в марте при температуре воды 9 °C, осенний пик не отмечен. Результаты комплексных исследований показали умеренную корреляцию (0.51) между температурой воды и количеством личинок двустворчатых моллюсков в планктоне. Прямого влияния биогенных элементов на плотность личинок мидии *Mytilus galloprovincialis* не установлено. Результаты подтверждают, что условия в акватории фермы благоприятны для выращивания моллюсков и подчеркивают необходимость продолжения мониторинга.

Ключевые слова: марикультура, биогенные элементы, меропланктон, Bivalvia, Черное море

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Introduction

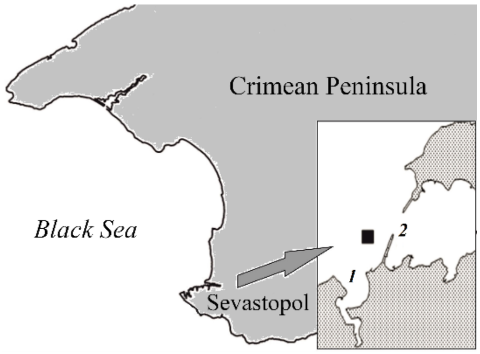
Artificial reproduction of aquatic biological resources (aquaculture) provides food for millions of people worldwide [1]. To ensure the sustainable development of aquaculture, it is important to assess the consequences of environmental changes. In order to reduce the risk of shellfish mortality on farms and obtain high yields, it is necessary to comprehensively study the factors affecting the functioning of benthic and planktonic communities [1, 2]. Currently, mariculture of bivalves is intensively developing in the coastal areas of Crimea and the Caucasus. One of the main cultivated species is mussel *Mytilus galloprovincialis* Lamarck, 1819. The biotechnology of mussel cultivation in the Black Sea is based on the natural settlement of pelagic larvae onto artificial substrates. The foundation of the future harvest has already been established at the initial stage, during spat collection [3], which requires taking into account material and surface quality of collectors, depth of their immersion, timing of their placement in the sea, water temperature and turbulence to be successful.

Concentration of mussel larvae in the plankton at the veliconcha stage ("with an eye spot") is one of the main factors influencing the intensity of shellfish settlement on collectors. Seasonal and interannual variations in many abiotic and biotic factors, as well as food availability, affect shellfish spawning timing and larval density dynamics [3, 4]. Thus, commercial shellfish cultivation is impossible without monitoring water quality and state of biota in the waters of marine farms. Comprehensive environmental monitoring enables tracking seasonal dynamics of mussel larvae density as well as hydrological and hydrochemical parameters [5–7].

The present study aims to investigate the seasonal dynamics of the density of *Mytilus galloprovincialis* mussel larvae and to analyse its relationship with hydrological and hydrochemical parameters in the waters of a marine farm cultivating bivalves.

Material and methods

Comprehensive studies were carried out monthly throughout 2023 in the waters of a mussel and oyster farm located in the outer roadstead of Sevastopol, between Karantinnaya Bay and the south pier (Fig. 1). The 4-hectare farm is located at depths of 10–16 m. Meroplankton samples were collected 1–2 times per month on average in the 10–0 m layer using a Juday net. A total of 69 samples were collected. The live material was processed through a complete count of pelagic larvae of benthic invertebrates using a Bogorov chamber under MBS-9 and Micmed-5 light microscopes [5, 6, 8]. Mussel larvae and total benthic invertebrate larvae densities



F i g . 1 . South-western region of the Crimean peninsula. The inset shows the western part of Sevastopol Bay: *1* – Karantinnaya Bay, *2* – south pier. The research area is marked with a black rectangle

were counted separately. Literature data [1)] were used to identify shellfish larvae [9].

Simultaneously, water samples were taken in the surface layer to determine salinity, temperature, dissolved oxygen concentration, biochemical oxygen demand over five days (BOD5), permanganate index in an alkaline medium as well as concentrations of ammonium, nitrites, nitrates, phosphates and silicon using standard techniques [2), 3)]. Dissolved oxygen content was determined by the Winkler method, and nutrients were measured photometrically. Phosphates were measured using the Murphy–Riley method, nitrites using the Bendschneider–Robinson method (with a "single" colour reagent), nitrates by reduction of nitrates to nitrites using copper-coated cadmium, ammonium nitrogen using the Grasshoff-Johansen method and silicon using the Korolev method. A total of 475 hydrological and hydrochemical analyses were performed.

Results and discussion

Hydrological and hydrochemical characteristics

In 2023, new data on the hydrological and hydrochemical characteristics of seawater in the mussel and oyster farm waters were obtained (Table 1). Surface water temperature ranged from a minimum in February (8.1 °C) to a maximum in August (26.5 °C). Dissolved oxygen concentration varied from 7.51 mg/L in August to 10.42 mg/L in June and oxygen saturation of the water ranged from 93 to 126.6%. Hypoxia was not recorded throughout the year. High oxygen concentration in the farm waters in March and April coincided with the spring phytoplankton bloom [10].

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[3)] *On the Approval of Water Quality Standards for Water Bodies of Fishery Importance, Including Standards for Maximum Permissible Concentrations of Harmful Substances in the Waters of Water Bodies of Fishery Importance*: Order of the Federal Agency for Fisheries (Rosrybolovstvo) No. 20 of January 18, 2010 (in Russian).

T a b l e 1 . Thermohaline and hydrochemical parameters in the surface layer of the water area of the mussel and oyster farm in 2023

Month	*T*, °C	*S*, ‰	O_2		Content, µg/L					Permanganate index, mgO/L
			mg/L	%	NO_2^-	NO_3^-	NH_4^+	PO_4^{3-}	Si	
January	9.5	18.5	10.14	99.2	2.9	16.1	5.9	4.2	83.8	3.62
February	8.1	18.5	9.71	93.0	2.2	21.5	3.1	7.0	43.5	3.19
March	9.0	18.3	10.14	98.3	0.8	0.4	8.7	2.4	34.1	2.64
April	11.8	18.3	10.28	107.2	0.9	16.6	9.4	2.7	76.2	3.61
May	15.2	18.2	9.71	107.5	1.0	7.7	7.0	3.0	85.6	3.62
June	19.6	17.7	10.42	126.6	1.1	42.7	24.0	2.1	174.4	4.43
July	24.0	18.2	8.71	103.7	1.4	56.0	31.0	6.4	158.3	4.93
August	26.5	17.7	7.51	101.2	0.5	22.6	10.5	3.8	35.1	4.13
September	24.2	17.7	8.01	105.9	2.4	219.8	4.7	1.3	89.4	5.97
October	18.2	18.5	9.31	110.4	0.4	4.2	6.8	1.3	60.9	2.77
November	16.3	18.4	8.45	96.53	2.2	8.1	60.9	4.4	87.4	3.01
December	12.1	18.4	9.34	97.57	3.0	87.9	20.5	6.3	160.3	7.86

The water salinity throughout the year ranged from 17.70 to 18.50‰. The decrease in salinity observed in summer was likely caused by the influence of municipal wastewater due to increased anthropogenic load.

Concentrations of mineral nitrogen forms in the water were low. Nitrite content varied from 0.4 µg/L in October to 3.0 µg/L in December, and nitrate content ranged from 0.4 µg/L in March to 219.8 µg/L in September. The reduced nitrate concentrations in March were apparently due to their consumption during the spring phytoplankton vegetation [11]. Elevated nitrate concentrations during the summer were caused by seasonal intensification of anthropogenic load on the waters [4].

Ammonium nitrogen concentration ranged from 3.1 µg/L in February to 60.9 µg/L in November, which characterizes the studied waters as unpolluted. Mineral phosphorus content ranged from 1.3 to 7.0 µg/L. Maximum permissible concentrations (MPC) of nutrients in water are as follows: NO_2^- – 20 µg/L, NO_3^- – 9000 µg/L, NH_4^+ – 390 µg/L, permanganate index shows 4.0 mgO/L. All the indicated nutrient concentrations were significantly below the MPC (Table 1). Exceedances of the permanganate index standard were recorded during the summer and, unexpectedly, in December. Thus, all the thermohaline and hydrochemical parameters of seawater presented in the table were typical for the area and close to long-term seasonal average values [4].

Meroplankton

Pelagic larvae of benthic invertebrates constitute meroplankton, which is a temporary component of zooplankton. In the waters of the marine farm, larvae of bivalves (Bivalvia) are one of the permanent components of meroplankton. These larvae are present in the plankton year-round though their species composition and abundance vary seasonally. During the hydrological winter, larvae of bivalves accounted for 25 to 69% of the total meroplankton density. When the water temperature reached 15.2 °C, the density of these larvae did not exceed 5 ind.·m^{-3} and they accounted for 1% of the total meroplankton only. In the summer–autumn period, the proportion of Bivalvia larvae was 20–30% of the total meroplankton, reaching 80% only in September at a water temperature of 24.2 °C. The most common bivalves in the plankton belong to the family Mytilidae, which includes the mussel *M. galloprovincialis*. Since this mussel is one of the main species cultivated on the marine farm, we investigated the density dynamics of its pelagic larvae in relation to season and water temperature (Fig. 2). Mussel larvae dominated the plankton from autumn to spring and were practically absent in summer.

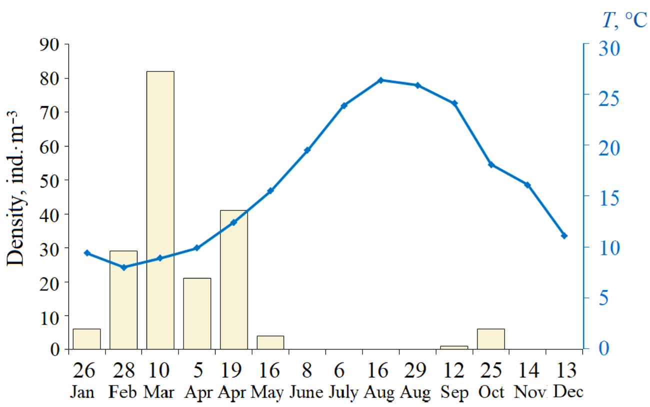

Fig. 2. Dynamics of the density of *Mytilus galloprovincialis* mussel larvae depending on the water temperature

During the winter, the abundance of mussel larvae did not exceed 29 ind.·m^{-3}; all of them were at the veliconcha stage ("with an eye spot"). Since the eye spot in veliconchas appears only on the 30th day [9] and that they remain in the plankton for a long time, it is reasonable to assume that these were the autumn generation larvae. The maximum density of mussel larvae (82 ind.·m^{-3}) was recorded in March at a water temperature of 9 °C. It was during this period that the minimum content of nitrites and nitrates was noted (Table 1). In terms of hydrological characteristics, March can be attributed to the winter season. At the veliconcha stage, the larval density was 15 ind.·m^{-3}, while at the later veliconcha stage "with eye spot" it reached 67 ind.·m^{-3}. In April, when the water temperature reached 11.8°C, the larvae density did not exceed 41 ind.·m^{-3}, and in May, at a water temperature of 15.2°C, it was only 4 ind.·m^{-3}. During the summer months, *M. galloprovincialis* larvae were not found in the plankton. The autumn peak in mussel larval abundance was not recorded, with a maximum of 6 ind.·m^{-3}.

The density of mussel larvae varies by season and year. Thus, in January 2014, the density of veliconchas reached 100 ind.·m^{-3} at a water temperature of 8.9 °C. An autumn peak in larval abundance was recorded at the end of September 2016 (more than 500 ind.·m^{-3}) [8]. However, our results in 2023 align well with long-term research data, which show that the abundance of mussel larvae in the coastal waters of Crimea is on average higher in spring than in autumn, with the maximum density typically occurring in April [12]. Changes in water hydrological and hydrochemical conditions, as well as the state of the food base, can affect larval density in the plankton significantly. Thus, monitoring the state of the ecosystem and trends in its variability is necessary for the marine farm to function optimally [5, 7].

To identify the relationship between hydrological and hydrochemical parameters and the abundance of Bivalvia larvae at the mussel and oyster farm, we calculated the Pearson's pairwise correlation coefficient (Table 2). The results show that water temperature influenced the density of bivalves larvae to a certain extent (correlation coefficient 0.51). Water temperature is known to determine the timing of shellfish reproduction and consequently the appearance of their larvae in the plankton [3, 9]. In the waters of the marine farm, an increase in water temperature was recorded from June to August (Table 1). At these temperatures, *M. galloprovincialis* mussels do not reproduce [9], which explains the absence of their larvae in the plankton.

Significant negative correlation coefficients were found for the abiotic parameters of water temperature and salinity (−0.77), as well as for dissolved oxygen content (−0.73). These results reflect an inverse statistical relationship. As temperature rises, the concentration of dissolved oxygen in water typically decreases. The relationship between water temperature and salinity is a rather complex process influenced by numerous factors. In 2023, in the waters of the marine farm, the minimum salinity values (less than 17.7‰) were recorded in June, August and September (Table 1). According to literature data, salinity values in the summer period were also lower, which was explained by the inflow of less saline waters from the north-western part of the Black Sea, as well as increased runoff from local rivers due to higher precipitation [7]. The variability range of thermohaline parameters

T a b l e 2 . Pearson's pairwise correlation coefficient matrix

Parameter	*T*, °C	*S*, ‰	O_2, mg/L	Content, µg/L				Bivalvia, ind.·m^{-3}	Perman-ganate index, mgO/L
				NO_2^-	NO_3^-	PO_4^{3-}	Si		
T, °C		−0.77	−0.73	−0.31	0.46	−0.28	0.15	0.51	0.25
S, ‰	−0.77		0.39	0.30	−0.45	−0.45	−0.13	−0.51	−0.24
O_2, mg/L	−0.73	0.39		−0.03	−0.43	−0.07	0.15	−0.61	−0.61
NO_2^-, µg/L	−0.31	0.30	−0.03		0.44	0.18	0.32	−0.42	0.52
NO_3^-, µg/L	0.46	−0.45	−0.45	0.44		−0.29	−0.29	−0.07	0.71
PO_4^{3-}, µg/L	−0.28	0.65	−0.07	0.18	−0.29		0.06	0	0.04
Si, µg/L	0.15	−0.13	0.15	0.32	−0.29	0.06		−0.29	0.65
Bivalvia, ind.·m^{-3}	0.51	−0.51	−0.61	−0.42	−0.07	0	−0.29		0
Permanganate index, mgO/L	0.25	−0.24	−0.61	0.52	0.71	0.04	0.65	0	

in the waters of the marine farm was optimal for bivalves cultivation for most of the year [4, 7], which is also confirmed by our studies. High correlation between permanganate index and nitrate content (0.71) can probably be explained by anthropogenic influence: the higher the pollution of waters (e. g., from stormwater runoff or emergency discharge of municipal wastewater), the higher the nitrate content and oxidizability. According to the 2023 data, no direct relationship was found between the shellfish larvae density and the content of nutrients; Pearson's correlation coefficients did not exceed −0.42 (Table 2). The minimum nitrate content recorded in March (Table 1) can limit the growth of microalgae, which serve as the food source for mussels, during the spring.

Conclusion

The obtained data show that the meroplankton structure in the mussel and oyster farm waters changed significantly throughout the year. In winter, Bivalvia larvae accounted for up to 70% of the total meroplankton, while in summer and autumn, their share reached up to 30%. Larvae *Mytilus galloprovincialis* dominated during the winter and spring. The maximum density of mussel larvae was recorded in March (82 ind.·m^{-3}), with no autumn peak observed. We analyzed the relationship between the density of mussel larvae *Mytilus galloprovincialis* and the hydrological and hydrochemical parameters in the waters of the marine farm. A moderate positive correlation (0.51) was found between water temperature and the number of larvae in the plankton. Based on the 2023 research results, no direct influence of nutrients on the density of larvae *Mytilus galloprovincialis* was revealed. These results confirm the optimal location for the mussel and oyster farm, since nutrient concentrations never exceeded the maximum permissible concentrations (MPC) during the study period. The minimum recorded nitrate content in March is not directly related to the density of mussel larvae. Because hydrological, hydrochemical and hydrobiological conditions in the waters of the marine farm can vary by season and year, comprehensive environmental monitoring of shellfish cultivation areas is necessary.

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Elena V. Lisitskaya – processing of meroplankton samples, analysis of the meroplankton composition and abundance, formation of the article.

Vitaly I. Ryabushko – setting the research task, editing the manuscript.

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