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FISHING UNDER CLIMATE CHANGE: DYNAMICS OF COMPOSITION AND STRUCTURE OF CATCHES IN THE RUSSIAN BLACK SEA IN THE XXI CENTURY

© 2021 **P. A. Balykin[1], D. N. Kutsyn[2], and A. V. Startsev[1,3]**

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Currently, the Black Sea ecosystem is undergoing significant changes, and that affects the dynamics of the species composition of catches. The main reasons for the change in the Black Sea ichthyofauna are considered to be: fishing, anthropogenic water pollution, invasion of new species, and the effect of large-scale climatic and related oceanological and hydrological processes on the productivity characteristics of commercial Black Sea fish. With no understanding the patterns of the effect of these factors on ichthyofauna, it is impossible to rationally use bioresources and protect nature. The aim of the work was to study the effect of climate warming on the species composition and structure of catches in the Russian Black Sea in the XXI century. The authors had used: the results of own ichthyological observations; data published on the composition of the Black Sea catches; and the information available on the official websites of the Ministry of Agriculture of the Russian Federation, the Federal Agency for Fishery, and its Sea of Azov – Black Sea Territorial Administration. The analysis of the possible effect of global warming on the results of fishing in the Russian Black Sea was carried out. It is shown that in the XXI century, the ratio of thermophilic fish, which spawn in the summer, increased; those species are European anchovy *Engraulis encrasicolus* (Linnaeus, 1758), red mullet *Mullus barbatus* Linnaeus, 1758, and horse mackerel *Trachurus mediterraneus* (Steindachner, 1868). As established, the dynamics of the ichthyofauna in the area is determined by at least four processes related to water warming: improvement of reproduction conditions and increased survival of eggs and juveniles of thermophilic species; change in the intraspecific structure; invasion and naturalization of Mediterranean species with the subsequent change in the food relationships within the ecosystem; and concentration of psychrophilic species at greater depths. The need for a thorough study of this issue is concluded, aimed at long-term forecasting of the raw material base state of the fishing industry in the Russian Black Sea.

Keywords: global warming, fishery, Black Sea, European anchovy, *Engraulis encrasicolus*, European sprat, *Sprattus sprattus*, red mullet, *Mullus barbatus*, horse mackerel, *Trachurus mediterraneus*

The territory of the Russian Federation is washed by 13 seas; out of them, the Arctic ones only, except for the Barents and White seas, are not fishing areas. The Black Sea is not highly productive: benthic organisms inhabit only 20 % of its bottom area due to the hydrogen sulfide zone. The Black Sea biological productivity is low: about 300 kg·km^{-2} (Kuranova & Moiseev, 1973). However, fishing in the Black Sea area has been known since ancient times (Zaika, 2008) and has experienced ups and downs in its history. In the second half of the XX century, the total catch of all the Black Sea countries reached 600 thousand tons; out of that, the USSR accounted for 200–250 thousand tons (State of Biological Resources of the Black and Azov Seas, 1995).

In the Black Sea, the catch of aquatic biological resources peaked in the 1980s: during that period, the total annual catch exceeded 850 thousand tons (Fig. 1). Then, there was a steady decline in catches: by 1996, those amounted to 396 thousand tons. Thus, over a decade, the catch in the Black Sea decreased by more than 2 times.

Fig. 1. Total catch of aquatic biological resources in the Black Sea in 1970–2010 (Shlyakhov et al., 2018)

The species composition of the catches changed as well. Until the 1950s–1960s, catches mostly consisted of nutritionally valuable species: Atlantic mackerel *Scomber scombrus* Linnaeus, 1758, Atlantic bonito *Sarda sarda* (Bloch, 1793), mullets of the family Mugilidae, horse mackerel *Trachurus mediterraneus* (Steindachner, 1868), Black Sea turbot *Scophthalmus maeoticus* (Pallas, 1814), herrings *Alosa* spp., and sturgeons of the family Acipenseridae. Later (until the 1990s and up to the present), catches consisted mostly of European anchovy *Engraulis encrasicolus* (Linnaeus, 1758) and European sprat *Sprattus sprattus* (Linnaeus, 1758). The catch of the Black Sea fish in the USSR was maximum in the 1970s–1980s, averaging 57 thousand tons; it included mostly European anchovy and European sprat, with their ratios in the total catch reaching 81 and 12 %, respectively (Balykin, 2014).

Over the entire history of observations in the Black Sea, 180 fish species have been recorded; out of them, 110 are of Atlantic origin, 2 are of Indo-Pacific origin, 2 are of Pacific origin, 33 are the Black Sea endemics, 21 are endemics of the Mediterranean basin, 11 are cosmopolitans, and 1 species (sterlet *Acipenser ruthenus* Linnaeus, 1758) is common in European freshwater and brackish water (Black Sea Fish Check List, 2020). Among the invaders currently actively spreading in the Black Sea area, *Lithognathus mormyrus* (Linnaeus, 1758), *Sarpa salpa* (Linnaeus, 1758), and *Gobius xanthocephalus* Heymer & Zander, 1992 are to be noted (Yankova et al., 2013). Out of the total number of species, about 20 % are fishing objects (State of Biological Resources of the Black and Azov Seas, 1995). At present, the fishing species are as follows: European sprat, European anchovy, whiting *Merlangius merlangus* (Linnaeus, 1758), Black Sea turbot, flathead grey mullet *Mugil cephalus* Linnaeus, 1758, golden grey mullet *Chelon auratus* (Risso, 1810), red mullet, horse mackerel, spiny dogfish *Squalus acanthias* Linnaeus, 1758, thornback ray *Raja clavata* Linnaeus, 1758, common stingray *Dasyatis pastinaca* (Linnaeus, 1758), garfish *Belone belone* (Linnaeus, 1758), bluefish *Pomatomus saltatrix* (Linnaeus, 1758), Atlantic bonito, blotched picarel *Spicara maena* (Linnaeus, 1758), Atlantic mackerel, and *Atherina* spp. In recent decades, the commercial part of the Black Sea ichthyocene was replenished by the Far Eastern so-iuy mullet *Planiliza haematocheila* (Temminck & Schlegel, 1845), which successfully acclimatized in the Sea of Azov – Black Sea basin (Balykin & Startsev, 2017).

The Mediterranean and Black seas are the areas where, according to the United Nations Convention on the Law of the Sea (Konventsiya OON, 2020), coastal countries are to cooperate on aquatic biological resources management and scientific research. In the Mediterranean basin, regional fishery management is carried out by the General Fisheries Commission for the Mediterranean (GFCM). Its activities extend to the Black Sea. However, in this water area, there is no regional fishery regulation, since only three coastal countries – Bulgaria, Romania, and Turkey – have GFCM membership, and there is no agreement on fishery between all the Black Sea countries. The leading position in terms of the annual catch volume of aquatic biological resources is held by Turkey: it accounts for more than 75 % of the catch. The second and third places are occupied by Russia (Shlyakhov et al., 2018) and Ukraine, respectively. Ratio of each of the other countries in the area is about 1 % (Drozdov, 2011).

The main reasons for the change in the Black Sea ichthyofauna are considered to be as follows: fishing, invasion of new species, anthropogenic pollution, and the effect of large-scale climatic and related oceanological and hydrological processes on the productivity characteristics of commercial Black Sea fish (Drozdov, 2011 ; Tsikliras et al., 2015 ; Zaitsev et al., 2002). One of the best known and most widely discussed global climatic processes is warming; according to instrumental observations, it is most pronounced in the last 35 years (Toropov et al., 2018 ; Sakalli & Sakalli, 2018). Global warming is evident in the Black Sea area as well (Rybak & Rybak, 2013). As established, warming of the Black Sea surface in 1982–2009 averaged 0.06 $°C \cdot year^{-1}$ (Ginsburg et al., 2011). Climate change in the Black Sea area is manifested mainly in the summer season, during which a statistically significant positive temperature trend is recorded throughout its territory, reaching 1 °C in 10 years (Toropov et al., 2018). Thus, in the Sukhumi area, the mean temperature of the Black Sea water in July increased by 2.2 °C in the period 1994 to 2016 (Dbar et al., 2018).

The work was aimed on studying possible effect of climate warming on the species composition and structure of catches in the Russian Black Sea in the XXI century.

MATERIAL AND METHODS

The data published on the composition of the Black Sea catches (Kozhurin et al., 2018 ; Kumantsov et al., 2012 ; Luts et al., 2004 ; Cardinale et al., 2017) was used, as well as the information available on the official websites of the Ministry of Agriculture of the Russian Federation (2020), the Federal Agency for Fishery (2020), and its Sea of Azov – Black Sea Territorial Administration (2020).

The obtained results of studying possible changes in the ichthyofauna components based on the example of catches do not claim to be completely reliable, primarily due to the inaccuracy of fishing reports (Balykin & Boltnev, 2014). Therefore, the authors additionally used for the analysis the results of ichthyological observations carried out by IBSS researchers. Fish were caught April to October in 2012 and 2018, with BS-3 trap nets with a 12-mm mesh; the trap nets were installed on sand sediments both in the Karantinnaya Bay mouth at a depth of 10–12 m and in the Sevastopol Bay water area.

RESULTS

The state authorities of Russia – the Ministry of Agriculture and the Federal Agency for Fishery – summarize the data on the catch of aquatic biological resources for the fishery basins (Makoedov, 2014). In 2001–2018, the catch in the Sea of Azov – Black Sea basin was of 25–103.4 thousand tons (Fig. 2). In 2018, the total Russian catch exceeded 5 million tons; so, this area does not play a significant role in domestic fishery. Nevertheless, fishing in the Sea of Azov – Black Sea basin significantly contributes to the development of the regional economy.

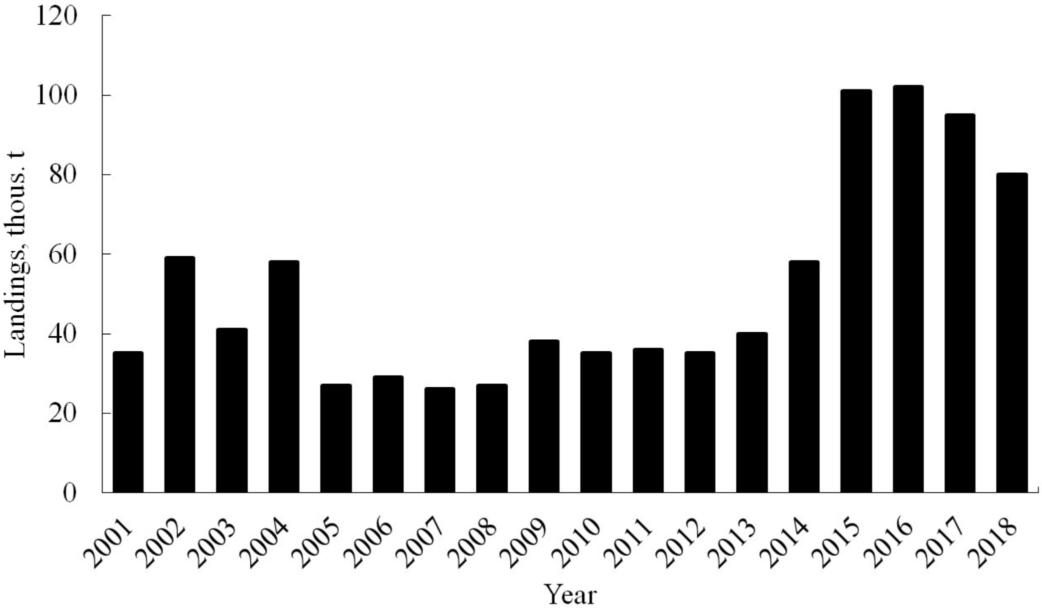

Fig. 2. Russian catches in the Sea of Azov – Black Sea basin

The water area studied can be divided into two parts: the waters of the Caucasian coast (northeastern area) and the waters of the Crimean Peninsula.

In the **northeastern Black Sea**, 102 fish species were recorded in the catches of commercial fishing gear in 1993–2002 (Nadolinskii, 2004). The state of commercial fish stocks is characterized as unstable. In this area, fishing with all fishing gear is multi-species; however, statistics takes into account the main species alone, whereas by-catch goes under the main species name at best or, at worst, is thrown overboard. The use of blocked and balanced quotas can contribute to a more complete development of marine biological resources and a balance in fishing efforts (Kumantsov et al., 2012 ; Nadolinskii, 2004).

In the northeastern Black Sea, the main fishing objects are two species accounting for about 90 % in the total catch: European anchovy (> 60 %) and European sprat (> 30 %). Other species account for less than 10 % (Balykin, 2014). In the total stock of explored biological resources (about 300 thousand tons), a significant part (about 42 % of all stock and more than 53 % of the volume of possible catch) consists of the objects, which are not currently used due to the lack of the technological base required for processing: small crustaceans, molluscs, algae, and seagrass (Dudkin et al., 2011).

Significant variations in the catch can result from the dynamics of its composition. To confirm this assumption, the authors used the data published on the composition of the Black Sea catches in 2001–2003 (Luts et al., 2004) and 2009–2011 (Kumantsov et al., 2012), as well as the material for recent years, 2016–2018, available on the official websites of the Federal Agency for Fishery and its Sea of Azov – Black Sea Territorial Administration. The species composition of catches, averaged over the periods indicated, is shown in Fig. 3.

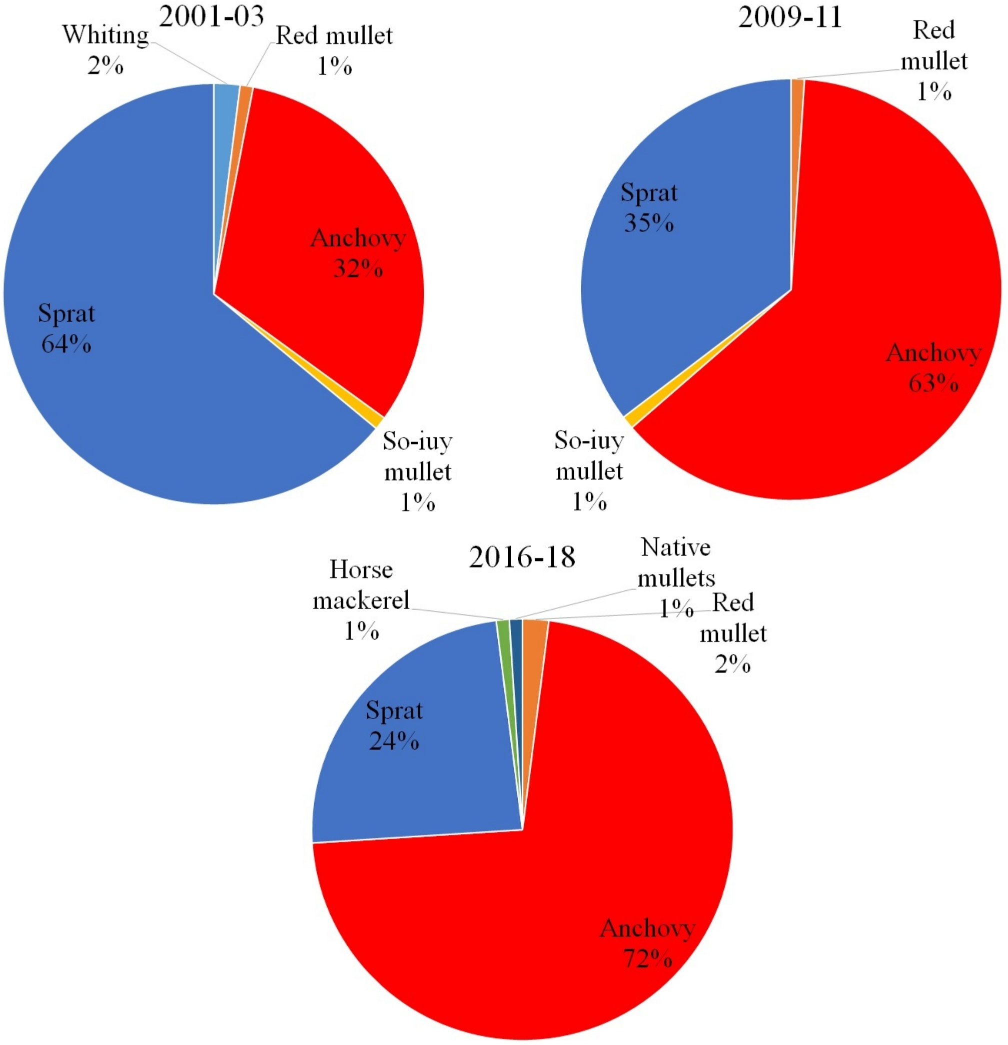

Fig. 3. Contribution of the most common fish species to the structure of Russian catches in the northeastern Black Sea in different periods of the XXI century

The main phenomenon to focus on is the change of dominant species in the composition of catches (Fig. 3). During the study period, the ratio of European sprat decreased by more than 2.5 times, and the contribution of European anchovy increased by the same value. Among other important processes, it is worth noting a twofold increase in red mullet catches (up to 300–560 tons *per* year), an almost complete disappearance of whiting and so-iuy mullet (less than 1 ton), and a significant increase in horse mackerel catches (130–330 tons *per* year) and Black Sea mullet catches (90–270 tons) in 2016–2018 (Fig. 3).

The dynamics of catches in **the waters of Crimea** (Crimean – Black Sea fishing area) is given according to data published (Kozhurin et al., 2018) and is supplemented by the information for 2018 from the website of the Federal Agency for Fishery. In this water area in 2001–2018, from 18.9 thousand tons (2007) to 72.9 thousand tons of fish (2016) were caught. To study possible variations in the species composition, the authors chose the same time intervals as for the northeastern Black Sea: 2001–2003, 2009–2011, and 2016–2018 (Fig. 4).

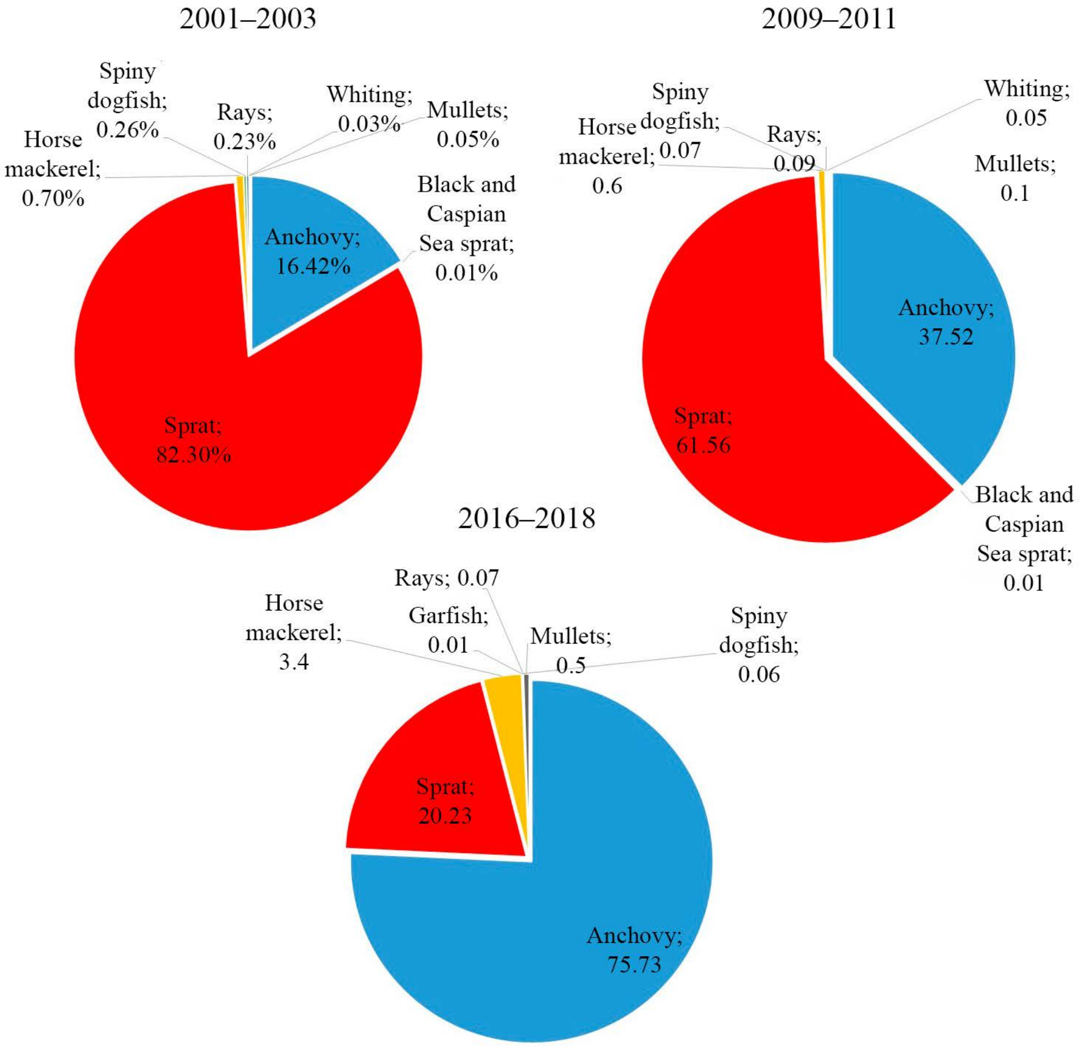

Fig. 4. Contribution of the most common fish species to the structure of Russian catches in Crimean – Black Sea area in different periods of the XXI century

The species composition of catches in the waters of Crimea changed similar to that in the waters of the Caucasian coast. During the study period, the ratio of European sprat decreased by 4 times (80 to 20 %), while the ratio of European anchovy increased approximately in the same proportion (16.5 to 75 %). Other changes include the complete disappearance of so-iuy mullet (< 0.3 tons), more than 10-fold increase of red mullet catches (7 to 300–571 tons *per* year), 10-fold increase of Black Sea mullet catches (270–275 tons in 2016–2018), 4-fold increase in herring catches (20–34 tons in 2016–2018), and almost 4-fold increase in horse mackerel catches (about 2 thousand tons in 2016–2018) (Fig. 4).

The data published, which are presented above, are in good agreement with the results of our own ichthyological observations carried out in the coastal water of the southwestern Crimea using trap nets. In 2012, 1,258 specimens of different fish species were examined; in 2018, 861 specimens were examined. The ratio of thermophilic species in the catches increased. Thus, during the study period, the ratio of greater weever increased more than 3 times, and horse mackerel – almost twice; at the same time, whiting almost disappeared from coastal catches (Fig. 5).

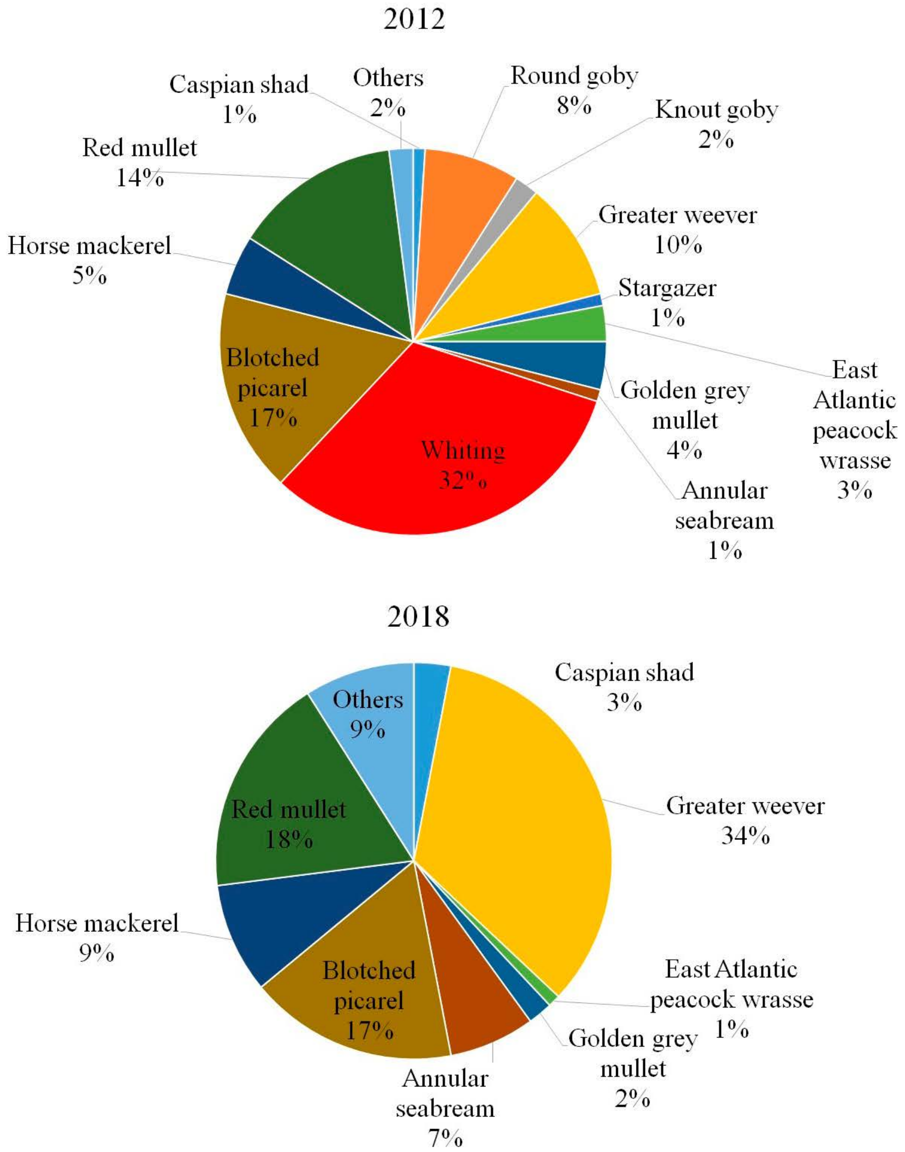

Fig. 5. Fish species composition in Sevastopol coastal waters in April – October 2012 and 2018

DISCUSSION

To date, it has been established that climate warming has a profound effect on the dynamics of ichthyofauna and fishing on a global scale (Cheung et al., 2013).

The most noticeable trend is the increase in the thermophilic species catch, which is clearly seen on the example of Crimean – Black Sea fishing area. For catches of European anchovy (Fig. 6a), horse mackerel (Fig. 6b), and red mullet (Fig. 6c), a pronounced correlation was revealed between the logarithm of catch values and the sea surface temperature shifted by two years (approximate age at maturity). Thus, European anchovy had a linear correlation coefficient $r = 0.52$ at $p = 0.027$; horse mackerel had $r = 0.46$ at $p = 0.057$; and red mullet had $r = 0.57$ at $p = 0.013$. An increase in the ratio of thermophilic species in catches *amid* global climate warming may be related to the improvement of reproduction conditions and an increase in the duration of spawning and feeding periods. Thus, at present, a change is recorded in the species composition and abundance of ichthyoplankton, which is characterized by an increase in the ratio of early stages of summer-spawning thermophilic fish: red mullet, horse mackerel, and European anchovy (Nadolinskii & Nadolinskii, 2018). An increase in the European anchovy productivity in the Russian Black Sea is registered by other researchers as well (Dudkin et al., 2011 ; Zuyev, 2019).

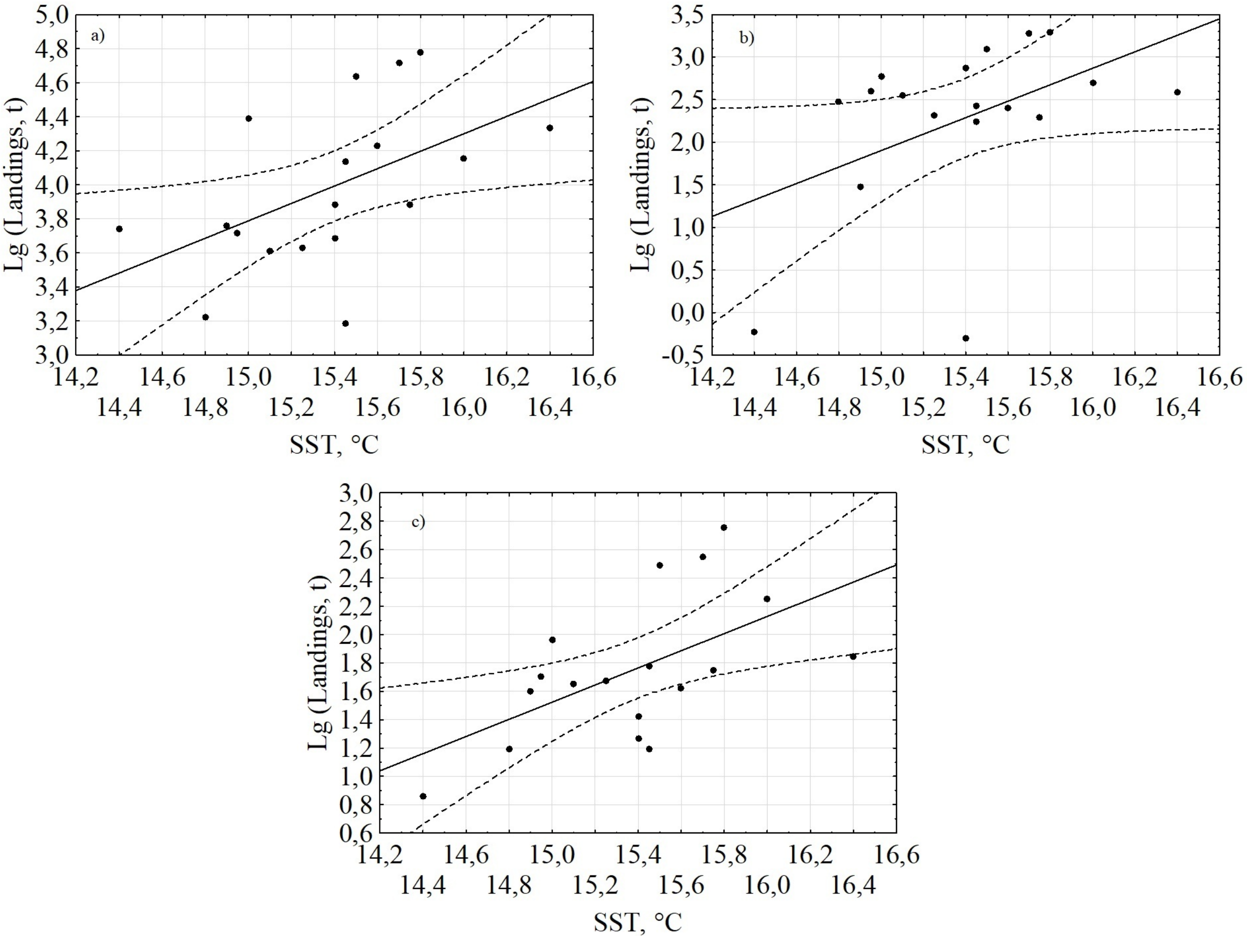

Fig. 6. Correlation between the logarithm of catch values of European anchovy (a), horse mackerel (b), and red mullet (c) and sea surface temperature (SST) (Federal'noe agentstvo po rybolovstvu, 2020) shifted by two years

Along with an increase in the abundance of various fish species, one can observe a change in the intraspecific structure of the ichthyofauna, namely an increase in the ratio of the Azov form of European anchovy (Kozhurin et al., 2018). This may be due to the expansion of its range under increasing salinity of the Sea of Azov, which results from climate changes as well.

Another manifestation of water warming effect is the expansion and displacement of the ranges of the tropical species to high latitudes. Within the Black Sea, those processes result in the intensive invasion of species from the Mediterranean Sea. To date, the number of invasive species and subspecies of the ichthyofauna is 21 (Yankova et al., 2013); taking into account climatic trends, we can conclude that the list will expand.

Climate changes are bound to affect all the ecosystem components, which naturally affects the ichthyofauna composition and structure. A good example is the invasion of the predatory ctenophore *Beroe ovata* Bruguière, 1789 in the Black Sea. As a result of its invasion, the abundance of the ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 decreased significantly, and this led to the restoration of the zooplankton and pelagophilic fish abundance (Shiganova et al., 2003). Other changes, less noticeable ones, in the peculiarities of food relationships are likely as well, *inter alia* among native species.

Climate warming effect on the dynamics of the abundance and distribution of psychrophilic species can also be observed on the example of whiting. With a decrease in catches in coastal water area, its commercial catch with trawls at a depth of 50 m and deeper increases (Kozhurin et al., 2018), which may indicate a vertical redistribution of its population. This is most likely related to global climate changes as well, which is confirmed by hydrological observations. Thus, a long-term increase in the depth of the upper quasi-homogeneous layer in the warm season was established. Until the 2000s, its thickness in the summer period was of 6–8 m; after 2010, the values on average exceeded 10 m (Kubryakov et al., 2019).

Conclusion. The dynamics of ichthyofauna and commercial catch in the Russian Black Sea in the XXI century are determined by at least four processes directly or indirectly related to water warming:

1) an improvement of reproduction conditions and increased survival of eggs and juveniles of thermophilic species, such as European anchovy, horse mackerel, and red mullet;
2) a change in the intraspecific structure observed on the example of European anchovy;
3) an invasion and naturalization of Mediterranean species with the subsequent change in the food relationships within the ecosystem;
4) a concentration of psychrophilic species at greater depths.

In order to assess consequences of climatic processes for the resource base of the Black Sea and ichthyofauna in total, the entire available dataset of scientific observations should be analyzed: hydrological, hydrobiological, and ichthyological ones.

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РЫБОЛОВСТВО В УСЛОВИЯХ КЛИМАТИЧЕСКИХ ИЗМЕНЕНИЙ: ДИНАМИКА СОСТАВА И СТРУКТУРЫ УЛОВОВ В РОССИЙСКОЙ ЧАСТИ ЧЁРНОГО МОРЯ В XXI ВЕКЕ

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В настоящее время экосистема Чёрного моря претерпевает существенные изменения, что закономерно отражается на динамике элементов уловов рыб. Главными причинами изменения ихтиофауны Чёрного моря считаются: рыболовство, антропогенное загрязнение вод, вселение новых видов, влияние крупномасштабных климатических и связанных с ними океанологических и гидрологических процессов на показатели урожайности промысловых рыб. Без понимания закономерностей воздействия этих факторов на ихтиофауну невозможны рациональное использование биоресурсов и охрана природы. Целью работы было изучить влияние потепления климата на состав и структуру уловов в российской части Чёрного моря в XXI веке. Авторы использовали: результаты собственных ихтиологических наблюдений; опубликованные данные о составе черноморских уловов; информацию, доступную на официальных сайтах Минсельхоза РФ, Росрыболовства и его Азово-Черноморского территориального управления. Выполнен анализ влияния процессов глобального потепления на результаты рыболовства в российской части Чёрного моря. Показано, что в XXI веке возросла доля теплолюбивых рыб, нерест которых происходит летом: хамсы *Engraulis encrasicolus* (Linnaeus, 1758), барабули *Mullus barbatus* Linnaeus, 1758 и ставриды *Trachurus mediterraneus* (Steindachner, 1868). Установлено, что динамика ихтиофауны в регионе определяется как минимум четырьмя процессами, сопряжёнными с потеплением вод: улучшением условий воспроизводства и роста выживаемости икры и молоди теплолюбивых видов; изменением внутривидовой структуры; вселением и натурализацией видов из Средиземного моря с последующим изменением характера пищевых взаимоотношений внутри экосисемы; концентрацией холодолюбивых видов на больших глубинах. Сделан вывод о необходимости тщательного изучения влияния потепления вод Чёрного моря в целях долгосрочного прогнозирования состояния сырьевой базы рыбной промышленности черноморского бассейна России.

Ключевые слова: глобальное потепление, рыболовство, Чёрное море, хамса, *Engraulis encrasicolus*, шпрот, *Sprattus sprattus*, барабуля, *Mullus barbatus*, ставрида, *Trachurus mediterraneus*

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BIOINDICATION OF THE WATER SALINITY DYNAMICS BY THE MICROALGAE COMMUNITIES IN THE LENA RIVER DELTA, LAPTEV SEA, RUSSIAN ARCTIC

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The Lena River in the Laptev Sea forms a vast delta, one of the largest in the world. The Ust-Lensky State Nature Reserve saves biodiversity on the Lena Delta territory beyond the Arctic Circle, in the zone of continuous permafrost. In recent years, large-scale plans for the development of extractive industries are implemented in this Russian Arctic sector. In this regard, the study of biodiversity and bioindication properties of aquatic organisms in the Lena River estuary area is becoming more and more relevant. This study aims to identify the species composition of microalgae in lotic and lentic water bodies of the Lena River Delta and use their indicator property for water salinity. It was a trace indicator of species distribution over the delta and their dynamics along the delta main watercourses to assess the impact of river waters on the Laptev Sea coastal areas. For this, all previously published materials on algae and chemical composition of the region waters as well as data obtained in recent years for the waters of the lower Lena reach were involved. In total, 700 species considered to 10 phyla were analyzed: Cyanobacteria (83), Euglenozoa (13), Ochrophyta (Chrysophyta, Xanthophyta) (41), Eustigmatophyta (4), Bacillariophyta (297), Miozoa (20), Cryptophyta (3), Rhodophyta (1), Chlorophyta (125), and Charophyta (111). The available materials of the field and reference observations were analyzed using several statistical methods. The study results indicate that hydrological conditions are the main factor regulating the spatial structure of the species composition of the microalgae communities in the Lena River Delta. The distribution of groups of salinity indicators across flowing water bodies reflects the effect of water salinity, and this allows suggesting possible sources of this effect. The mechanism of tracking the distribution of environmental indicators itself is a sensitive method, that reveals even their subtle changes in them; therefore, as an integral method, it can be helpful for further monitoring.

Keywords: large river, delta, algae, bioindication, statistical mapping, Lena River, Russian Arctic

In terms of their role as natural "recorders" of global environmental changes (Bianchi & Allison, 2009), the ecosystems of the deltas of large rivers, such as the Lena, are recognized important for study. In the estuary, the Lena River forms a delta with an area of 30 thousand km^2, the third among the most significant river deltas globally and the first on the territory of Russia (Bol'shiyanov et al., 2013). The Lena River lower reaches and the Laptev Sea coastal areas are protected by the Ust-Lensky State Nature Reserve and the most significant Russian biosphere reserve Lena Delta with more than 60 thousand km^2. The regional water bodies are located beyond the Arctic Circle, north of 71°N, in the zone of continuous occurrence of permafrost soils, where ephemeral water bodies are widespread in the absence of drainage: streams and hollows. The region is rich in marshes and lakes typical of the Arctic tundra.

In the Lena River lower reaches, as in the lower reaches of other large Arctic rivers, large mixing of freshwater and salt water occurs, where so-called marginal zones can form. Recently, an increase in the activity of industrial development of the region has been noted. In 2017, "Rosneft" oil company started seismic exploration on the Laptev Sea shelf. Therefore, the anthropogenic load here increased. Under these conditions, the urgency of maintaining the biodiversity of aquatic ecosystems in the region rises, especially in the marginal zone, where the role of ecotones is very significant, as proven by several researchers (Schilthuizen, 2000 ; Smith et al., 1977). Large rivers, such as the Lena, flowing in the meridional direction, are natural channels for advancing southern species to the North. Such invasive species can thus replenish the flora of the Lena River Delta and coastal areas. Besides, estuarine areas of large rivers are not only biodiversity reserves but also areas of speciation processes (Popa et al., 2016).

Data on algae in water bodies in the Lena River estuary were published in several works. The deposited manuscript of I. Vasil'eva and P. Remigailo contains the first species list for the Lena River lower reaches, comparative floristic analysis of algae, and information on saprobiological state of water bodies in the region (Vasil'eva & Remigailo, 1986). Some data on the species composition of the region algae are presented in the reports of the Tiksin Territorial Administration for Hydrometeorology and Environmental Monitoring (Pavlyukova, 1993a, b), as well as in the references on algae of the Laptev Sea (Druzhkova & Makarevich, 2013 ; Gogorev, 1994 ; Okolodkov, 1998, 1992 ; Sukhanova et al., 2017 ; Timofeev, 1998 ; Zernova et al., 2000). The information available concerns mainly plankton and to a small extent moss pomace. Despite a long history of algological studies, there is no generalization and analysis of the material accumulated. The available data were summarized to analyze the taxonomic composition and spatial structure for the microalgae communities in the lower Lena (Gabyshev et al., 2019a). A species list of algae was published (Gabyshev et al., 2019b) as part of a regional analysis of the climatic gradient effect on algae communities (Barinova et al., 2015, 2014). Data on the physicochemical characteristics of the lakes in the Lena River lower reaches are given in the works of T. Trofimova (2013a ; 2013b).

The work is aimed at assessing the impact of river waters on the coastal sea areas of the Laptev Sea. For this, the species composition of the microalgae communities in lotic and lentic water bodies of the Lena River Delta was identified, in order to determine the species – indicators of water salinity, as well as to trace their spatial distribution over the delta flowing and standing waters and their dynamics along the delta main waterways.

MATERIAL AND METHODS

The material comes from phytoplankton samples collected in September 2009 in the Lena River (area of Tit-Ary Island) and in August – September 2014 in the Tiksi Bay and Neelova Gulf of the Laptev Sea, as well as in the lakes of the Tiksi Bay and Neelova Gulf and in the Olenyokskaya delta channel, at 41 stations in total. We also included floristic study data from recent references, where species of algae and cyanobacteria were revealed in the delta as mentioned above. Phytoplankton samples were taken using the Apstein plankton net (SEFAR NITEX gas, mesh size 30 μm) in the littoral and pelagic zones of water bodies from the surface water horizon (0–0.3 m). A total of 100 planktonic and 3 hydrochemical samples were collected; their processing was carried out at the Institute for Biological Problems of Cryolithozone of the Siberian Branch of the Russian Academy of Sciences. For the identification of diatoms, 70 permanent preparations were made by calcining the valves and placing them in a Bio Mount synthetic resin. Microscopic examination of preparations was carried out under microscopes "Laboval" and "Olympus BH-2" according to standard methods. Water chemical analyses followed the methods set out in (Alekin et al., 1973 ; Rukovodstvo po khimicheskomu analizu, 1977).

Ionic constituents. Sulfate anions were determined by turbidimetry; chlorides, by mercurometry; hydrocarbonates, by back titration; water hardness, by complexometric titration using eriochrome black; calcium, titrometrically with trilon B; and potassium and sodium cations, by flame photometry.

Other chemicals. Water pH was evaluated electrometrically using a Multitest IPL-101 titrator; phenols, by fluorimetric method using a Flyuorat-02 fluorimeter.

The investigated territory was conditionally divided into five hydrologically homogeneous areas in accordance with their position on the land and hydrological and morphometric features of water bodies (Fig. 1). The Lena River lower reach (st. 1–6) (LowLenaRiv) included a stretch of the Lena River from Chekurovka settlement to Stolb Island. The Lena River Delta (st. 7–14) (LenaDelta) united the main distributary channels (Olenyokskaya, Bol'shaya Trofimovskaya, Saardakhskaya, Arangastakhskaya, Bykovskaya, and Gusinka), as well as the Lena River near the Tyllakh River estuary and near Chay-Tumus location. The near-shore zone (st. 15–23) represented the Laptev Sea coastal areas (Neelova Gulf, Tiksi Bay, and Buor-Khaya Gulf) and the freshened part adjacent to the delta. A separate group (Lakes, st. 24–32) included nine lakes of glacial, water-erosion, erosion-thermokarst, and river origin, located along the shores of the Tiksi Bay, Neelova Gulf, and Olenyokskaya distributary channel. Samples from moss pomace, tundra swamps, mountain streams, and hollows of the shores of the Tiksi Bay, Kosistyy Cape, Bykov Peninsula, spurs of the Kharaulakh Range, Tit-Ary Island, Danube (Danube Island), and Erga-Muora-Sise (Erge-Muora-Sise) were united in the swampy area group (st. 33–41) (SwampAreas). Five waterways were identified for the passage of the river along the Lena Delta, indicated by colored lines in Fig. 1.

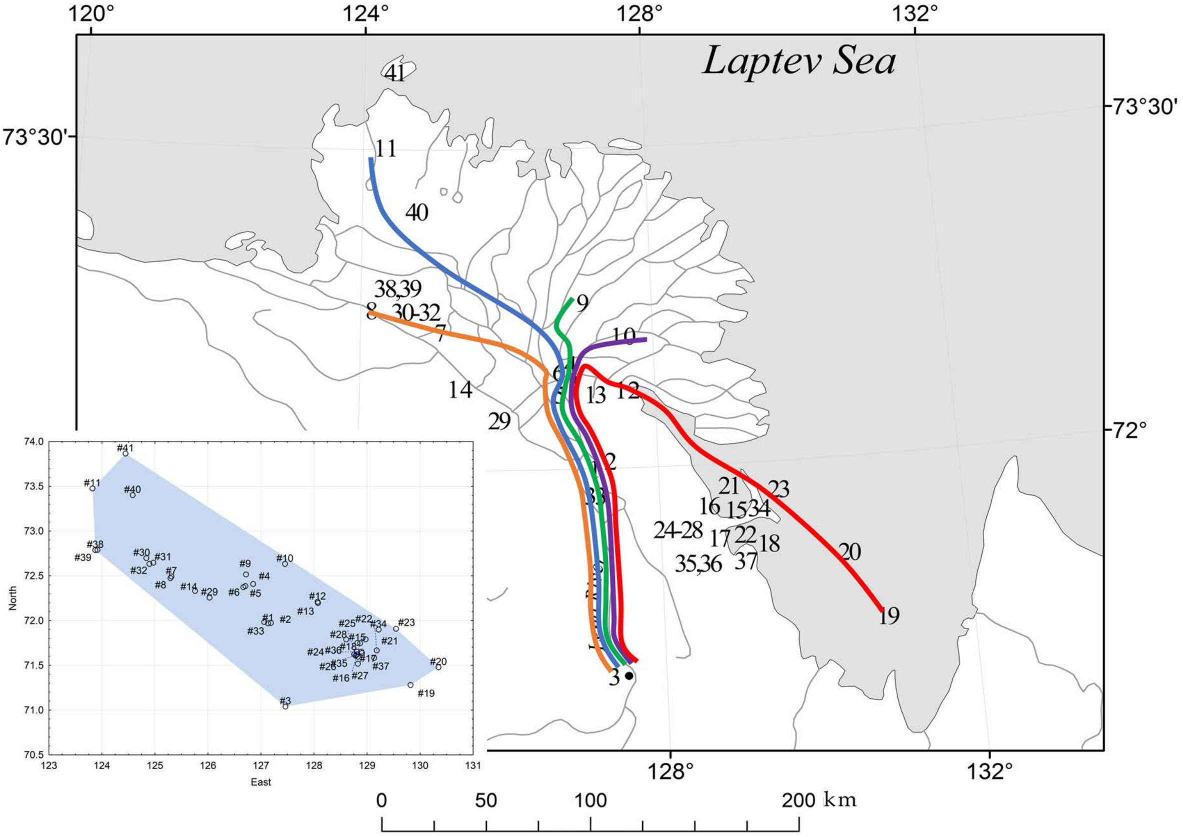

Fig. 1. Location of sampling stations on the Lena River Delta. Waterways are marked with colored lines. Sampling points are shown on the statistical map below in coordinates

The ecological preferences of the identified species based on the indication of water salinity (Barinova, 2017a) were determined using a database of algae indicators compiled at the Institute of Evolution, University of Haifa (Barinova et al., 2019, 2006).

Environmental mapping was carried out in the Statistica 12.0 program according to each site parameter values and geographic coordinates. Calculation of similarity was made as the network analysis in JASP (significant only) on the botnet package in R Statistica package of (Love et al., 2019). A similarity tree was constructed with the help of BioDiversity Pro 9.0 program.

RESULTS

According to the results of our research for the period 2009–2014 at 41 stations and the reference data, 700 species and varieties of microalgae belonging to 10 taxonomic divisions were identified: Cyanobacteria (83), Euglenozoa (13), Ochrophyta (Chrysophyta, Xanthophyta) (41), Eustigmatophyta (4), Bacillariophyta (297), Miozoa (20), Cryptophyta (3), Rhodophyta (1), Chlorophyta (125), and Charophyta (111). All of them turned out to be salinity indicators belonging to five ecological groups (Table 1).

Table 1. Sampling stations on the Lena River Delta with their numbers, GPS coordinates, and the numbers of salinity indicators in ecological groups. Abbreviations: hb – halophobes; i – oligohalobes-indifferents; hl – oligohalobes-halophiles; mh – mesohalobes; eh – euhalobes (marine)

Station	No.	North	East	hb	i	hl	mh	eh
Left bank of a river near Tit-Ary Island	1	71°58′8.2305″	127°8′8.0607″	0	39	7	0	0
Right bank of a river across Tit-Ary Island	2	71°58′27.8234″	127°11′23.6213″	2	41	5	2	0
Left bank of a river near Chekurovka settlement	3	71°02′20.36″	127°27′41.28″	0	10	2	0	0
Near Stolb Island	4	72°24′30.19″	126°51′15.45″	1	37	5	0	0
Near Khokhochu location	5	72°22′22.23″	126°40′09.45″	0	9	1	0	0
Near Stolb Station	6	72°23′6.1677″	126°42′30.0957″	9	128	20	3	0
Gusinka distributary channel	7	72°29′32.30″	125°18′43.96″	0	9	0	0	0
Olenyokskaya distributary channel	8	72°28′26.80″	125°17′44.05″	0	26	6	0	0
Bol’shaya Trofimovskaya distributary channel	9	72°30′51.21″	126°43′03.84″	1	42	9	0	0
Saardakhskaya distributary channel	10	72°37′56.57″	127°27′12.84″	1	37	9	1	0
Arangastakhskaya distributary channel	11	73°28′30.07″	123°49′30.61″	0	19	5	0	0
Bykovskaya distributary channel	12	72°12′33.89″	128°04′05.64″	2	46	11	0	0
Near Tyllakh River estuary	13	72°11′45.26″	128°04′32.04″	1	16	2	1	0
Chay-Tumus location	14	72°19′53.36″	125°45′25.84″	0	5	1	0	0
Neelova Gulf. Area of the abandoned water intake, 16–17 km from Tiksi-1	15	71°44′57.51″	128°49′6.89″	5	58	7	1	0
Neelova Gulf. Oil depot area, 16–17 km from Tiksi-1	16	71°44′48.0754″	128°52′12.1512″	2	47	5	1	0
Tiksi Bay. River port area	17	71°39′06.92″	128°53′34.82″	0	26	8	1	0
Tiksi Bay. District 400 m southwest of River port	18	71°38′15.23″	128°54′20.21″	2	22	5	0	0
Littoral part of Buor-Khaya Gulf	19	71°16′49.03″	129°49′13.20″	0	11	1	1	0
Seaward part of Buor-Khaya Gulf	20	71°28′40.08″	130°20′59.69″	1	20	4	1	0
Neelova Gulf	21	71°47′26.36″	128°58′25.53″	0	31	5	1	0

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Station	No.	North	East	hb	i	hl	mh	eh
Tiksi Bay	22	71°39′56.63″	129°11′01.62″	11	145	20	13	2
Fresh part of the Laptev Sea	23	71°54′27.78″	129°32′51.89″	0	2	1	0	0
Melkoye (in the vicinity of Tiksi Bay)	24	71°39′4.46″	128°48′27.57″	2	21	5	0	0
Dirin-Kyuyel (vicinity of Tiksi Bay)	25	71°36′23.43″	128°47′2.29″	0	15	5	0	0
Vtoroye (vicinity of Tiksi Bay)	26	71°37′10.75″	128°48′24.48″	0	9	2	1	0
Sevastyan (vicinity of Tiksi Bay)	27	71°31′3.03″	128°49′37.40″	1	8	1	0	0
Ladannakh (vicinity of Neelova Gulf)	28	71°47′21.09″	128°36′28.86″	2	28	0	0	0
Vulkan (Olenyokskaya distributary channel)	29	72°15′20.97″	126°1′51.73″	1	15	0	0	0
Lyglay (Olenyokskaya distributary channel)	30	72°41′50.23″	124°50′18.33″	1	15	1	0	0
Kuogastaah (Olenyokskaya distributary channel)	31	72°37′57.75″	124°53′49.93″	3	11	1	0	0
Batyyalaakh (Olenyokskaya distributary channel)	32	72°38′42.93″	124°58′15.08″	3	14	2	0	0
Tit-Ary Island	33	71°59′00.76″	127°03′43.15″	0	0	0	0	0
Bykov Peninsula	34	71°53′53.59″	129°13′06.90″	11	32	3	0	0
Kharaulakh Range	35	71°37′18.61″	128°45′33.75″	1	8	0	1	0
Depression of the tundra surface by a tracked vehicle	36	71°35′46.39″	128°49′34.14″	1	6	0	0	0
Kosistyy Cape	37	71°34′57.56″	129°08′06.93″	3	13	0	0	0
Flarks	38	72°47′14.33″	123°52′38.34″	3	17	1	3	0
Water from mosses	39	72°47′25.77″	123°55′04.31″	0	13	0	0	0
Erge-Muora-Sise Island	40	73°24′05.74″	124°34′53.14″	0	1	1	2	0
Danube Island	41	73°51′55.51″	124°26′50.24″	1	6	0	0	0

In order to reveal the internal connections in the microalgae communities studied, we applied a statistical approach to calculating the correlation in the analysis of JASP at a similarity level of more than 50 %. The network graph, for which we divided the communities by hydrological similarity into five groups, showed that the most similar were the communities of flowing waters in the river itself and its delta (Fig. 2). This group also included the community of the near-shore zone. The communities of lakes and swamps, on the other hand, formed two separate clusters, which had little resemblance both to flowing waters and to each other.

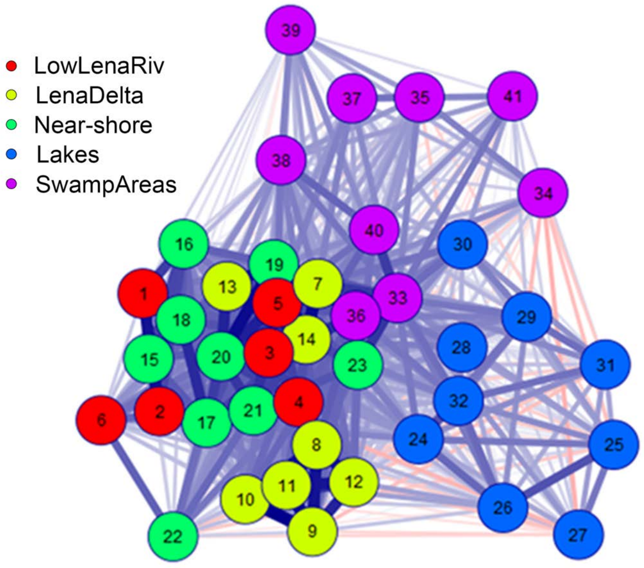

Fig. 2. Correlation graph of JASP for the species composition of communities at the stations of the Lena River Delta. Hydrologically homogeneous stations of five groups are marked with different colors in the legend

The similarity tree of the composition of salinity indicators, according to Bray – Curtis (Fig. 3), showed a high percentage of similarity for the entire dataset. However, the analysis made it possible to identify the communities of st. 40, where mesohalobes made up 50 %, as well as st. 6 and 22, where the species richness in general and, respectively, the number of indicator species were the highest since the communities of these stations were the most well studied. This result requires further expansion of the analysis of the indicator species composition.

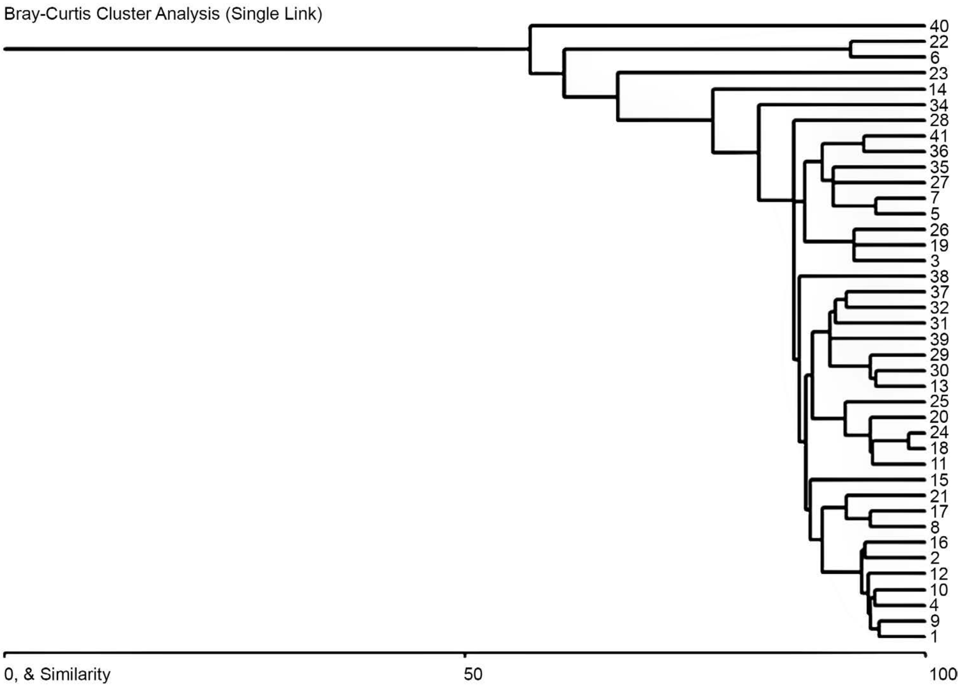

Fig. 3. Similarity tree of the composition of salinity indicators according to Bray – Curtis at the stations of the Lena River Delta

Statistical maps were constructed to clarify the distribution of indicator species and environmental variables (Figs 4 and 5). Fig. 4a shows that the distribution of stations located in the flowing area of the delta only, which have a specific gradient in their altitude above the sea level, turned out to be adequate to the river relief in the investigated area and its delta. Statistical maps reflect the accurate distribution of variables on our material and can be used for further analysis. The distribution of pH values (Fig. 4b) shows increasing, when the stations are located near the coast or on the desalinated sea area in the Tiksi Bay vicinity. This distribution indicates a significant effect of the river water on the sea since seawater is always alkaline.

The distribution of total dissolved solids reveals increased concentrations in the delta lakes in the east and west, while the central part of the delta appears fresher on the map (Fig. 4c). Phenols are a natural component of freshwaters, and they are usually associated with the least saline waters (Barinova, 2017b). The distribution map of phenol concentrations turns out to be similar to the distribution map of the total mineralization, only in the opposite sense. The most phenol-saturated waters are located at the extension of the main river channel (Fig. 4d).

The distribution of taxonomic richness in the communities of the stations of lotic (Fig. 4e) and lentic waters (Fig. 4f) of the study area shows a significant homogeneity of distribution with protruding points of the most accessible and, accordingly, the most well studied st. 6 and 22.

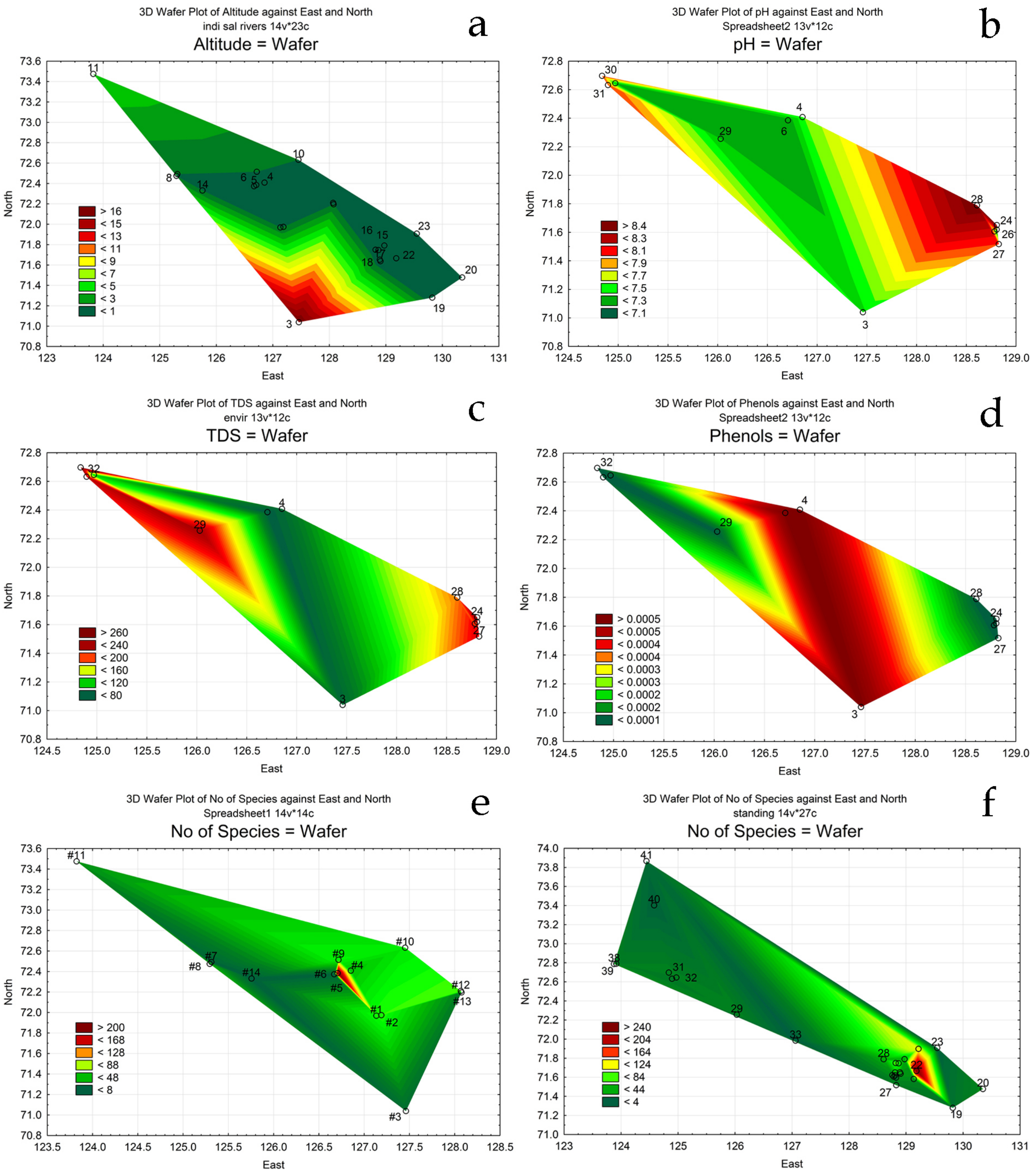

Fig. 4. Statistical maps of the distribution of variables of the environment and microalgae communities over the area of the Lena River Delta: a – distribution of stations altitude only for watercourses in the delta; b – distribution of water pH; c – distribution of total dissolved solids; d – distribution of phenols; e – distribution of the number of algal species in the communities of lotic stations; f – distribution of the number

The distribution of the percentage composition of salinity indicators over the delta area is shown in Fig. 5. Since the importance of hydrology for the distribution of the microalgae communities in the study area was previously revealed, we divided the data on the composition of salinity indicators for 41 sites into groups related to rivers and channels (st. 1–22) and to lakes and swamps (st. 23–41).

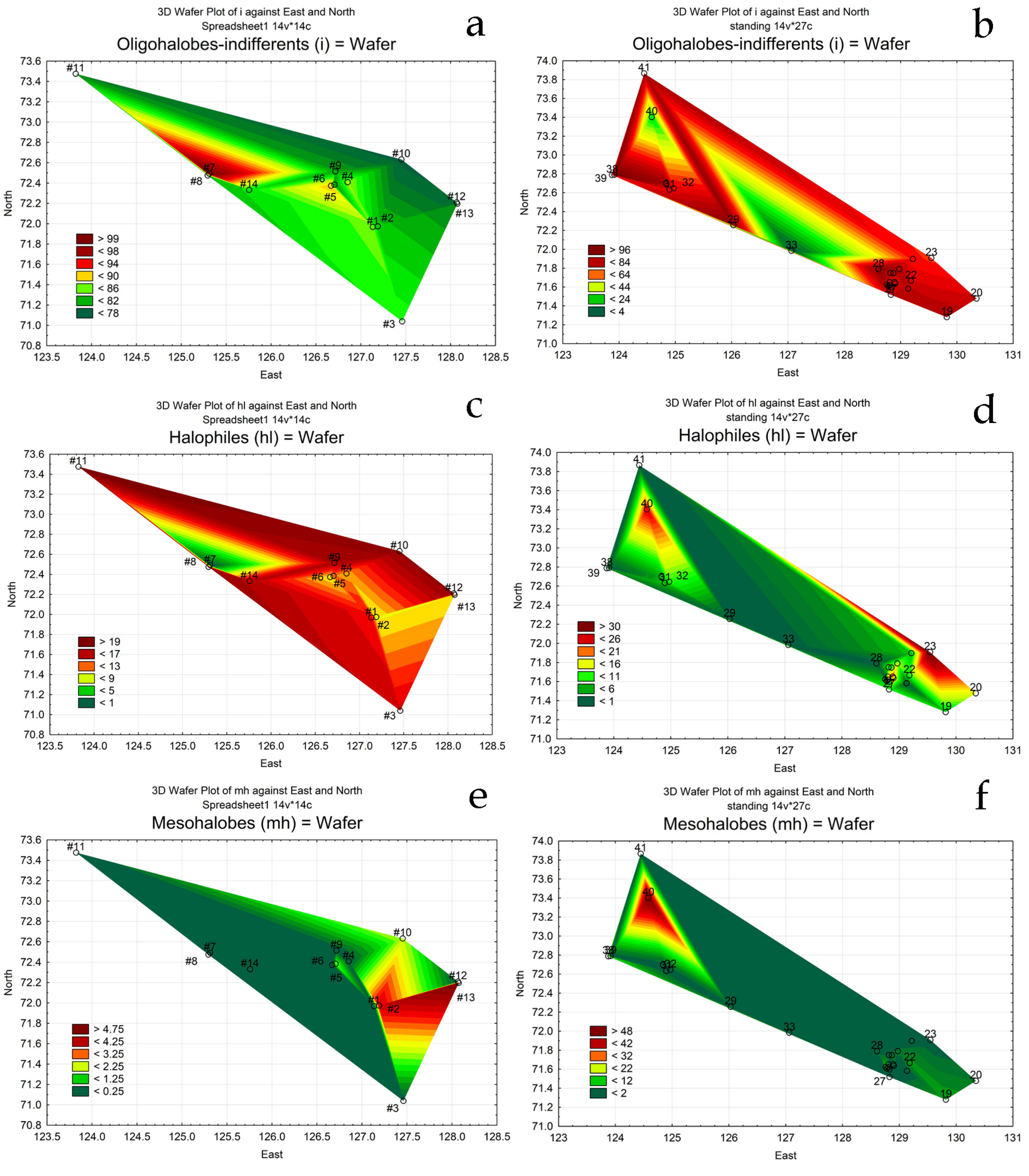

Fig. 5. Statistical maps of the distribution of the number of salinity indicator species over the Lena River Delta area: a, c, e – for lotic waters; b, d, f – for lentic waters

Fig. 5 shows the pairwise distribution of the same groups of indicators in lotic and lentic waters. Thus, oligohalobes-indifferents in lotic waters were better represented in the river itself and its western channels (Fig. 5a). Simultaneously, this indicator taxa group was more noticeable in lakes and swamps along the delta margins but not near flowing waters (Fig. 5b). Halophiles in river waters were concentrated on the east coast (Fig. 5c). In contrast, two different points were noted for lentic waters in the west and east, where this group of salt-tolerant species predominated in the communities (Fig. 5e). Mesohalobes were concentrated in flowing waters in the Tiksi area (Fig. 5f). The distribution of these indicators of increased salinity for lentic waters coincided with the distribution of halophiles west of the delta (Fig. 5f).

The distribution of groups of salinity indicators along waterways in the Lena River Delta was studied in the dynamics of indicator species of various ecological groups, as was done earlier for the Nile Delta (Salem et al., 2017). Thus, stations were located in the order of water flow, starting from st. 3 on the river itself, the southern point, then, on each of the paths along their channel, and further, continuing along the desalinated sea area as the stations move away if any (Fig. 6). The scale of salinity indicators on each graph is located as the indicated variable (salinity) increases. It includes all groups, regardless of whether indicators are present in waterway communities or not.

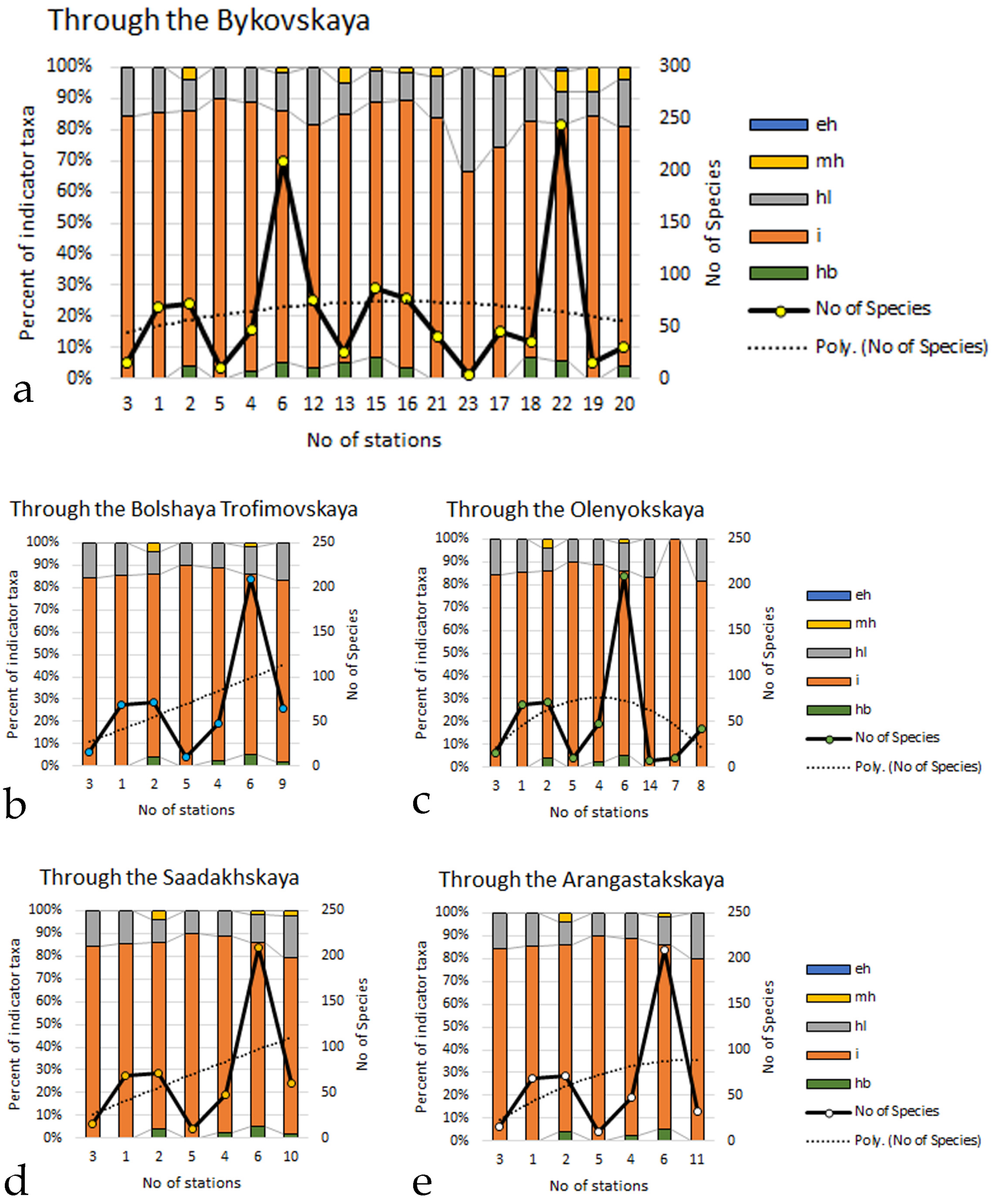

Fig. 6. Histograms of the distribution of salinity indicator species along waterways of the Lena River Delta (see explanation in the text)

The waterway through the Bykovskaya channel is the most saturated with stations (17) and the most well studied (Fig. 6a). The number of species has two peaks – at st. 6 (Near Stolb Station) and st. 22 (Tiksi Bay) – where the microalgae communities are most explored due to sampling stations availability. The trend line shows first an increase in species richness along the river and then a decrease in the number of species as it approaches the coast and at sea. The trend in the number of species is maximum where the percentage of halophiles + mesohalobes decreases: at st. 15 and 16 in the Neelova Gulf. There is a poorly expressed participation of euhalobes and a noticeable increase in mesohalobes closer to the coast and in the desalinated sea area. The sum of the percentages of halophiles + mesohalobes in the community has a noticeable rise towards the coast reflecting the sea effect.

In the communities of waters flowing through the Bol'shaya Trofimovskaya channel, euhalobes, marine species, are not identified (Fig. 6b). Mesohalobes are weakly involved. There is a noticeable increase in the percentage of oligohalobes-halophiles towards the coast. The number of species has the same peak at st. 6, and the trend in the number of species shows a rise in species richness towards the coast.

In the communities of waters flowing through the Olenyokskaya channel, euhalobes do not participate, mesohalobes are weakly involved, and an increase in the percentage of oligohalobes-halophiles is also weakly expressed (Fig. 6c). The number of species is the same as at Bykovskaya, the maximum is at st. 6, but it significantly decreases towards the coast and the sea. In the communities of waters flowing through the Saardakhskaya channel, euhalobes also do not participate, mesohalobes are weakly involved, and there is a noticeably stable increase in the percentage of oligohalobes-halophiles on approaching the coast (Fig. 6d). The number of species follows the distribution along the riverbed. The trend in the number of species shows an increase when approaching the coast.

There are no euhalobes in the waters flowing through the Arangastakhskaya channel, mesohalobes are weakly involved, and a noticeable increase in the percentage of oligohalobes-halophiles is recorded (Fig. 6e). In other waterways, most of which are represented by the riverbed, st. 6 has maximum species richness. However, the trend in the number of species indicates its increasing towards the coast.

DISCUSSION

A statistical analysis of the similarities revealed hydrology as a regulating factor for the composition of the microalgae communities in the Lena River Delta habitats studied. The species composition of algae in the main channel of the Lena River has a high degree of similarity with the composition of the communities of the lower river delta and the coastal area of the sea into which the river flows. This result is understandable since, along with the river runoff, the river microflora is also carried to the coastal sea area, which significantly affects the formation of the microalgae communities in the Neelova Gulf, Tiksi Bay, and Buor-Khaya Gulf.

The identified low-mineralized area of the delta (see Fig. 4c) is a continuation of the river main channel. It can be assumed that the freshwater outflow shows the paleochannel of the river since the sea level fluctuation in this area reaches 1.5 meters, and the elevation gradient on the delta is 0–4 m. Simultaneously, salinity is higher in the west and northwest (10–14 and 8–10 ‰, respectively) (Bol'shiyanov et al., 2013).

Even though the concentrations of natural phenols in the delta waters have a gradient at the lowest level, practically at the boundary of determination, this gradient does exist (Fig. 4d). It can be suggested that, along with the distribution of total dissolved solids gradient, it may indicate the direction of river water outflow.

The statistical map, as a method tested on communities of continental and coastal marine ecosystems (Barinova, 2017c) and recommended for use in EU countries (Dedić et al., 2020), allows revealing hidden trends by the dynamics of the mapped indicators. Maps can reveal otherwise undetectable connections in ecosystems. Thus, statistical maps of the distribution of salinity indicator groups in the Lena River Delta allowed us to identify two trends. The communities of flowing waters react to approaching the coast by increasing the proportion of indicators of chloride increased concentrations (Barinova, 2017b). Salinity indicators in lakes and swamps show that they have local natural sources of chlorides in the west of the delta, on the one hand, and one can assume the effect of salt mists, on the other (Barinova & Stenina, 2013).

We identified waterways through the channels, which begin from the Lena River main channel, to trace salinity effect on the flowing water communities.

The different degree of floristic study, which reveals the maximum number of species on the sites most accessible for research, was the problem for calculation. There, severe climatic conditions, undeveloped landscape, and the lack of infrastructure affect the number of indicator taxa. We tried to remove this effect by calculating the percentage of participation of different group indicators for each community. It turned out that, despite the differences in the total number of species, the indicators show a rise in the proportion of groups of increased salinity as the river approaches the coast in each waterway, as it was revealed for the Nile River Delta waterways (Salem et al., 2017). The most well studied Bykovskaya channel and its surroundings have marine species in their communities since we continued the line of stations towards the sea.

Nevertheless, communities even in the desalinated coastal area contain most freshwater indicators found higher in this waterway. This result indicates the real effect of the channel water on the desalinated sea area. However, other waterways did not have marine species in the communities but showed an increase in the percentage of halophilic species, although their terminal stations (st. 9, 10, and 11) were at some distance from the seacoast. It can be attributed to both the effect of tides, which reach 1.5 meters in this area of the Laptev Sea (Bol'shiyanov et al., 2013), and extremely low delta elevation gradient. Possible influence of salt mists was revealed in another Arctic coastal reserve (Barinova & Stenina, 2013) by indicator species of algae.

Conclusion. The study shows that hydrology is a factor regulating the species composition of the microalgae communities in the Lena Delta. The distribution of groups of salinity indicators along flowing streams shows the effect of water salinity and suggests possible sources of this effect. The mechanism of tracking the dynamics of salinity by distributing algae-indicators became a sensitive method that reveals even subtle changes in environmental variables. Therefore, as an integral method, it can be helpful in further monitoring.

Highlights:

1. Correlations revealed hydrology as the primary regulator factor for the microalgae communities in the Lena River Delta area.
2. Indicators of water quality in the Lena Delta communities are represented by 700 taxa.
3. Gradient of salinity in the delta is revealed by statistical mapping and dynamics of salinity indicators across the main river flows.

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БИОИНДИКАЦИЯ ДИНАМИКИ СОЛЁНОСТИ ВОД ПО СООБЩЕСТВАМ ВОДОРОСЛЕЙ В ДЕЛЬТЕ РЕКИ ЛЕНА, МОРЕ ЛАПТЕВЫХ, РОССИЙСКАЯ АРКТИКА

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Река Лена, впадая в море Лаптевых, образует обширную дельту, одну из крупнейших в мире. Весь эстуарий, включая приморский участок, входит в состав Усть-Ленского государственного природного заповедника. Территория расположена за Полярным кругом, в зоне сплошной вечной мерзлоты. В дельте есть многочисленные русла и множество мелких водоемов — озёр, ручьёв, котловин, болот. Изучение экосистем дельт крупных рек имеет особое значение из-за их роли в качестве естественных «регистраторов» глобальных изменений окружающей среды. Когда большие реки впадают в море, в зоне смешения морской и пресной воды образуются особые экотонические сообщества водных организмов, которые могут играть важную роль в поддержании биоразнообразия регионов. Несколько лет назад в этом российском секторе Арктики началась реализация масштабных планов по развитию добывающих отраслей. В связи с этим всё более актуальными становятся исследование биоразнообразия и изучение биоиндикационных свойств водных организмов в районе устья реки Лена. Целью данной работы было определить видовой состав водорослей проточных и непроточных водоёмов дельты реки Лена и использовать их свойства как индикаторов солёности воды. Чтобы оценить влияние речных вод на прибрежные участки моря Лаптевых, необходимо проследить распространение видов-индикаторов по дельте и их динамику по основным её водотокам. Для этого были использованы все ранее опубликованные сведения по водорослям и химическому составу вод региона, а также данные, полученные для вод нижнего течения Лены за последние годы. Имеющиеся материалы натурных наблюдений проанализированы с применением нескольких статистических методов. Результаты исследования свидетельствуют о том, что гидрологические условия являются основным фактором, регулирующим пространственную структуру видового состава водорослевых сообществ дельты реки Лена. Распределение групп индикаторов солёности по проточным водоёмам отражает влияние солёности воды, что позволяет предположить, каковы возможные источники этого влияния. Сам механизм отслеживания распределения показателей среды — это чувствительный метод, который выявляет даже незначительные их изменения, поэтому он, как интегральный метод, может быть полезен для дальнейшего мониторинга.

Ключевые слова: большая река, дельта, водоросли, биоиндикация, статистическое картографирование, река Лена, Российская Арктика

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ON THE MAXIMUM LENGTH FOR *SARDINELLA AURITA* (OSTEICHTHYES: CLUPEIDAE) FROM THE MEDITERRANEAN SEA

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Two specimens of round sardinella, *Sardinella aurita* Valenciennes, 1847, with a total length of 39.1 and 36.6 cm and a weight of 359.01 and 293.48 g, respectively, were caught by a purse seine net in Bouzedjar Bay, Algeria, on 8 August, 2019. These sizes of both specimens are the maximum recorded for this species in the Mediterranean Sea.

Keywords: round sardinella, *Sardinella aurita*, maximum size, measurements, Bouzedjar Bay, Algeria, Mediterranean Sea

Sardinella aurita Valenciennes, 1847 is a coastal, pelagic species preferring clear saline water with a maximum temperature below +24 °C (Bianchi et al., 1999). The species is distributed from inshore and near surface to the shelf edge and down to 350 m in the Eastern Atlantic along African coast from Gibraltar to South Africa; in the Mediterranean Sea, but is rare in the Black Sea; and in Western Atlantic (Cape Cod to Argentina) including Bahamas, Antilles, Gulf of Mexico, and the Caribbean coast (Whitehead, 1985). It mainly feeds on zooplankton, especially copepods. Juveniles feed on phytoplankton (Bianchi et al., 1999). Gregarious; forms schools that migrate seasonally related to water temperature and richness in plankton. Growth is rapid, and young individuals can reach about 11 cm during their first year of life (Dahel et al., 2016). It breeds perhaps all year round; the breeding pattern is extremely complex, with two principal spawning periods in some areas linked to upwelling regimes off West Africa (Whitehead, 1985), while in Algerian waters one single period was noted June to October (Bouaziz, 2007). In the Black Sea, there is no spawning (Whitehead, 1984). Maturity occurs during the second year; batch fecundity is estimated at 9,397–176,712 eggs in Eastern Atlantic (Baali et al., 2021) and 11,680–40,348 eggs in central Algerian waters (Bouaziz, 2007). Maximum age of *S. aurita* is estimated in the range from 4.5 years in Congolese waters (Ghéno, 1975) to 8 years on Brazilian coast (Richardson et al., 1960). It can be caught with purse and beach seines, lamparos, bottom gillnets, and bottom and pelagic trawls.

Herrings, sardines, and anchovies make up a major part of the total fish species landed in the Mediterranean Sea (44.3 %) and Black Sea (73.2 %) fisheries (FAO, 2020). For Algerian waters, *S. pilchardus* accounts for 59 %, and *S. aurita* accounts for 22 % of the total Algerian catch (DPRH, 2019). We have to mention that the third species, *Sadinella maderensis*, occurs in the fishing area and can easily be confused with two other sardines. Tous et al. (2015) noticed that landing statistics are not comprehensive due to inconsistent reporting of landings, and *S. aurita* catches are often mixed with *S. maderensis* ones.

MATERIAL AND METHODS

On 8 August, 2019, two large Clupeidae specimens were caught by a seiner at 100-m depth in Bouzedjar Bay (35°34′24.0″N, 1°09′58.9″W) on the western Algerian coast. FAO identification sheets (Whitehead, 1985) were used to identify the specimens as *Sardinella aurita* (Fig. 1). Counts and proportional measurements were carried out using a caliper to the nearest 0.01 mm. Twelve morphometric characteristics were measured (Table 1).

Fig. 1. General view of two *Sardinella aurita* specimens, total length of 39.10 cm and 36.57 cm, caught in Bouzedjar Bay (the Mediterranean Sea)

RESULTS AND DISCUSSION

Data on morphometric measurements of the specimens studied are shown in Table 1.

The maximal length ever recorded for *S. aurita* was 41 cm for fish caught on the Mauritanian shelf of the Eastern Atlantic (Thuoc & Szypula, 1973) (Table 2). For the Mediterranean basin, it was stated as follows: this Clupeidae could attain 33 cm, with a common length between 15 and 25 cm (Fischer et al., 1987). The largest known *S. aurita* specimen in the Mediterranean Sea, measuring 36 cm, was caught in Greek waters (Moutopoulos et al., 2013). We found two *S. aurita* specimens in purse seine catches from Algerian waters, making this observation a new record for both Algerian and Mediterranean waters and the second one after reported from Eastern Atlantic.

Maximal length of *S. aurita* varied 19.7 to 27.0 cm in the Western Atlantic, 30 to 41 cm in the Eastern Atlantic, and 25.5 to 39.1 cm in the Mediterranean basin. The differences in the maximum length observed can be attributed to environmental conditions and fishing pressure. Helfman et al. (2009) stated that individuals from populations exposed to high fisheries mortality/pressure will respond by reproducing at reduced average sizes and ages. Our specimens demonstrated that this species can grow beyond maximum length data available in literature despite the fact that the specimens were caught in such an important fishing activity area as Bouzedjar Bay (Bensahla-Talet et al., 2019).

Table 1. Morphometric measurements of *Sardinella aurita* caught in Bouzedjar Bay (the Mediterranean Sea)

Morphometric characteristic, cm	Specimen 1	% of TL	Specimen 2	% of TL
Total length (TL)	39.10	100.00	36.57	100.00
Fork length	34.51	88.27	31.88	87.18
Standard length	33.68	86.13	31.13	85.12
Pre-orbital length	1.67	4.26	1.62	4.43
Eye diameter	1.53	3.92	1.42	3.88
Post-orbital length	3.99	10.20	3.45	9.43
Head length	7.16	18.31	6.45	17.64
Dorsal fin length	4.92	12.58	4.24	11.59
Anal fin length	3.77	9.65	3.66	10.01
Pectoral fin length	5.09	13.02	4.78	13.07
Minimum body height	2.27	5.81	2.20	6.01
Maximum body height	6.81	17.42	6.72	18.38
Total weight, g	359.01	–	293.48	–

Table 2. Maximum length of *Sardinella aurita* in the Western and Eastern Atlantic and Mediterranean Sea given by several authors

Location			TL, cm	References
Western Atlantic	USA	Gulf of Mexico	19.7	Johnson & Johnson, 1986
	Venezuela	Northeastern	26.6	Barrios et al., 2010
		–	27	Fréon & Mendoza, 2003
Eastern Atlantic	Morocco	Dakhla	35.5	Amenzoui & Baali, 2018
	Mauritania	Rio de Oro – Cap Verde	41	Thuoc & Szypula, 1973
	Senegal	Port Kafoutine – St Louis	37	Baldé, 2019
	Côte d'Ivoire	Côte d'Ivoire shelf	30	Marchal, 1993
Mediterranean Sea	Mediterranean	–	33	Fischer et al., 1987
	Egypt	Alexandria	26	Akel, 2009
	Turkey	Izmir Bay	28.5	Bayhan & Kara, 2012
	Greece	Corinthians Gulf	36	Moutopoulos et al., 2013
	Algeria	Bou Ismaïl Bay	31	Bouaziz, 2007
		Eastern Algeria	25.5	Dahel et al., 2016
		Bouzedjar Bay	39.1	Present study

Environmental factors, such as temperature, food availability, nutrient availability, light regime, oxygen, salinity, pollutants, current speed, predator density, intraspecific social interactions, and genetics, are also known to affect growth rates and maximum size (Helfman et al., 2009), but in our case two specimens sampled are insufficient to link these environmental factors to the largest length observed. Nevertheless, it is to be added that producing large specimens is due to the richness of Algerian waters generated by Algerian current which is considered to be the most energetic in the Mediterranean basin (Salas et al., 2001). The entrance of Atlantic waters through the Strait of Gibraltar generates important upwellings, enhancing the development of plankton first link of the aquatic food web (Bensahla-Talet et al., 2017), and primary production reveals a west–east decreasing trend (Bosc et al., 2004 ; Siokou-Frangou et al., 2010).

Maximum length, weight, and age are the key indicators used in fisheries science. These data are applied in most models for stock assessment. Therefore, updating maximum length of an exploited species is important, and our results could constitute a starting point for further studies on factors that affect growth and/or longevity of other species.

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О МАКСИМАЛЬНОЙ ДЛИНЕ *SARDINELLA AURITA* (OSTEICHTHYES: CLUPEIDAE) ИЗ СРЕДИЗЕМНОГО МОРЯ

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Два экземпляра круглой сардины *Sardinella aurita* Valenciennes, 1847, общей длиной 39,1 и 36,6 см и весом 359,01 и 293,48 г соответственно, были пойманы кошельковой неводной сетью в заливе Бузеджар (Алжир) 08 августа 2019 г. Данные размеры обоих экземпляров являются максимальными для этого вида в Средиземном море.

Ключевые слова: круглая сардина, *Sardinella aurita*, максимальный размер, измерения, залив Бузеджар, Алжир, Средиземное море

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THE ROLE OF ALGAE MACROPHYTE IN BIOREMEDIATION OF PETROLEUM PRODUCTS OF THE KOLA BAY OF THE BARENTS SEA

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The contribution of macroalgae to the removal process of diesel fuel from the Kola Bay of the Barents Sea was estimated. The calculations were based on the results of: 1) recent expeditionary observations of the reserves, spreading, and biomass of algae macrophyte of the phyla Chlorophyta and Rhodophyta, as well as the class Phaeophyceae of the phylum Ochrophyta, inhabiting three bay areas; 2) laboratory research of the ability of macroalgae *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. distichus*, *F. serratus*, *Saccharina latissima*, *Palmaria palmata*, and *Ulvaria obscura* to neutralize the toxic effect of diesel fuel. As shown, the total contribution of the algae studied into bioremediation of diesel fuel in the bay was of 312 $\text{kg}\cdot\text{day}^{-1}$. The differences in the absorption capacity of algae macrophyte were revealed. This process was most efficiently carried out by *S. latissima*; the minimum efficiency of participation in bioremediation was determined for *U. obscura*. It was concluded that the existing littoral and sublittoral thickets of marine macroalgae of the Kola Bay can be considered as the key element in the implementation of the preventive, daily cleaning of coastal water from the petroleum products. The inclusion in the calculations of data on the ability of other representatives of the bay phytobenthos to neutralize diesel fuel may increase the role of algae macrophyte in cleaning the coastal marine areas from the petroleum products. As concluded, the thickets of brown algae can be considered as the key component of repair and homeostasis in coastal ecosystems. The destruction of even a part of algae natural communities can change the ecosystem balance.

Keywords: macrophytes, ecosystem of the Kola Bay, sustainability, diesel fuel, bioremediation

The Kola Bay of the Barents Sea has a length of 57 km and occupies one of the leading places in the Arctic for the transportation and transshipment of petroleum products (hereinafter PPs). The complexes for the processing of hydrocarbon raw materials have already been built, and several ones are under construction on the bay coast. The bay is considered one of the most contaminated sea water bodies on the Kola Peninsula. The contamination problem is aggravated by the fact that most of the year the water temperature in the bay is slightly above 0 °C; at a given temperature, the natural decomposition of PPs is greatly slowed down. Microorganisms, *inter alia* microalgae, play an important role in water purification from PPs (Il'inskii, 1995 ; Peretrukhina et al., 2006 ; Semenova et al., 2009 ; Atlas, 1978 ; Wrabel & Peckol, 2000), but their contribution to bioremediation decreases during the polar night.

In the Kola Bay, the content of PPs in water (background concentration) varies from 0.04 mg·L^{-1} (about 1 threshold limit value, TLV) to 2.3 mg·L^{-1} (46 TLV), with 1 TLV = 0.05 mg·L^{-1}. The background content of PPs in the bays of the Murmansk coast in the summer-autumn period ranges 3 to 8 TLV. There were no bays completely clean of PPs. In a number of bays in winter and spring, the content of PPs decreased down to 1–2 TLV since the traffic of ships, mainly fishing, increases in spring and decreases in late autumn (Voskoboinikov et al., 2017 ; Kola Bay and Oil, 2018). The highest concentration of PPs in the bay was noted in its southern area (in the area of an oil terminal, a commercial port, and Atomflot piers), as well as in the middle area (in the area of transshipment complexes). This is due to the existing sources of contamination: operating piers, shipping, refueling of ships, and refueling from tankers on the roadstead. During oil spills, concentration of PPs in water reaches 25–50 mg·L^{-1} (500–1000 TLV) and higher values.

In recent years, we have proposed to use algae macrophyte in the remediation of the Barents Sea water from PPs. It has been shown that many species of macrophytes are resistant to the effect of PPs. The tolerance range differs even in species with similar taxonomy and structure; however, all macroalgae studied by us and other authors earlier (12 species) demonstrated the ability to accumulate PPs from seawater on the thallus surface, to destruct them using epiphytic hydrocarbon-oxidizing bacteria, and to absorb and neutralize PPs subsequently in plant tissues. As revealed, a decrease in the content of PPs in water goes parallel with its increase in algae thalli (Voskoboinikov et al., 2017, 2018).

The studies carried out made it possible to form an evidence base confirming the ability of macroalgae, which differ in structure and taxonomy, to absorb and destruct PPs (Voskoboinikov et al., 2017, 2018, 2020 ; Pilatti et al., 2016 ; Pugovkin et al., 2016 ; Ryzhik et al., 2019). On the basis of the symbiotic association of macroalgae and hydrocarbon-oxidizing bacteria, biotechnologies of plantations-biofilters have been created for purifying coastal water areas from PPs. Assumptions were repeatedly put forward about the role of coastal phytocenoses in the seawater bioremediation of various toxicants (Mironov, 1985 ; Patin, 2017b); however, neither specific data nor calculations based on field or laboratory experiments were provided.

To a great extent, this can be explained by the lack of work on assessing macroalgae reserve and species diversity in the water areas described. Thus, studies of algae reserves on the Murmansk coast, which were carried out in 1980s–1990s (Makarov, 1998 ; Peltikhina, 2005), with attention paid to the Kola Bay phytobenthos, concerned kelps only. The work presented is the first one, where calculations of the participation of macrophytobenthos in bioremediation of PPs for the bay are carried out. The calculations are based on the results of recent expeditionary observations of the distribution and biomass of the dominant phytobenthos representatives – *Fucus vesiculosus*, *F. distichus*, *F. serratus*, *Ascophyllum nodosum*, *Saccharina latissima*, *Palmaria palmata*, and *Ulvaria obscura* – in three Kola Bay areas. Preliminary studies have shown that the species analyzed have a fairly wide tolerance range to PPs and a relatively high coefficient of absorption of the toxicant (Voskoboinikov et al., 2017, 2018, 2020 ; Ryzhik et al., 2019). In parallel with field observations, laboratory analysis of the neutralization capacity of diesel fuel (hereinafter DF) in the above-mentioned algae species was carried out, and their total role in bioremediation of DF for the Kola Bay was assessed.

The authors of the work presented hope that this research will contribute to understanding the role of coastal phytocenoses in the seawater bioremediation of petroleum products not only in the Kola Bay of the Barents Sea, but in other coastal areas of the World Ocean as well.

MATERIAL AND METHODS

Laboratory experiments on the effect of PPs on algae. The objects of study were brown algae (Phaeophyceae) (fucoids *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. distichus*, and *F. serratus*, as well as kelp *Saccharina latissima*), green alga (Chlorophyta) *Ulvaria obscura*, and red alga (Rhodophyta) *Palmaria palmata*. Thalli approximately equal in size and mass were sampled in Zelenetskaya Bay (69°07′09″N, 36°05′35″E) of the Barents Sea (Fig. 1a). *S. latissima* was sampled in the sublittoral zone from a depth of approximately 3 m, and other species – in the littoral zone. Algae were cleared of fouling and placed in 1.3-L glass containers with seawater, except for *S. latissima*: the experiments with this species were carried out in 3-L vessels. Seawater with a salinity of 33 ‰ was taken from the spots algae inhabit and filtered through a cotton-gauze filter; then, summer diesel fuel was added: 6.5 mg·L^{-1}, which is 130 TLV for water in terms of the gross content of PPs. Seawater and DF were not sterilized. The experiment was carried out in a temperature-controlled box at +7…+8 °C, irradiance of 16–18 W·m^{-2}, and constant water aeration.

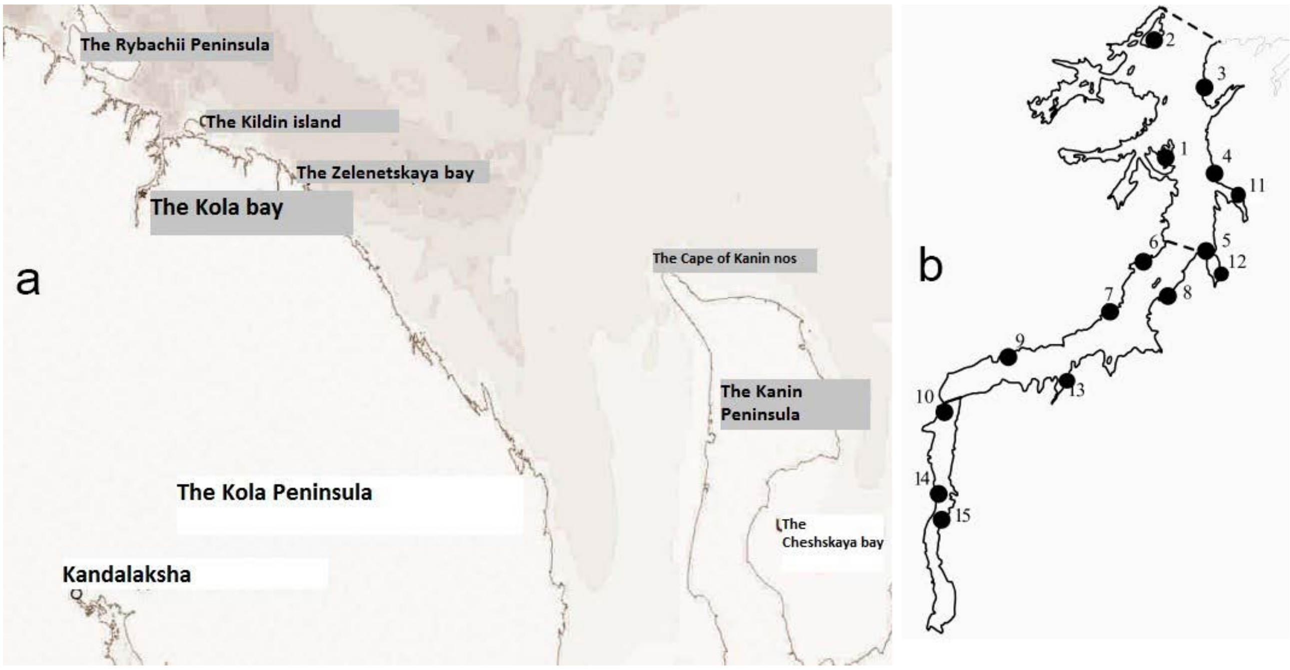

Fig. 1. Work area: a – the Kola Bay and Zelenetskaya Bay of the Barents Sea; b – sampling stations (1–15) in the Kola Bay

The experiment with the determination of algae morphological and functional state lasted for 10 days. The gross content of PPs in water and algae was detected by gas chromatography – mass spectrometry at the beginning and after 5 days of the experiment. Sample preparation and instrumental analysis were carried out by the previously described method (Voskoboinikov et al., 2018). To assess the background content of PPs, samples of water and algae from Zelenetskaya Bay were analyzed. Calculations of the content of DF in algae were carried out on a dry weight basis, taking into account the coefficient of the ratio of wet/dry mass: for *Saccharina* – 7/1; fucoids – 5/1; and *Palmaria* and *Ulvaria* – 4/1. During the experiment, changes in the state and morphology of the algae thallus were monitored visually and by light-optical methods under a Mikmed-6 microscope.

Assessment of algae biomass and reserves in the Kola Bay. Macrophyte reserves were accessed by the traditional method (Peltikhina, 2005). The material was sampled during MMBI RAS expeditions in 2009–2019 (with partial financial support of the Russian Geographical Society). Inspection of a significant part of the bay coastline made it possible to describe macrophytobenthos distribution and to determine the size of the fucoid zone for assessing the reserves.

To calculate the biomass of macrophytobenthos and separate algae species, quantitative samples were taken by the method of sample plots' layout along the transect perpendicular to the water edge. In total, 15 transects were laid along the entire bay; in the southern and middle areas, work was carried out every two years (Fig. 1b). The area of the frame during sampling in the littoral zone was 0.25 m^2, in the sublittoral zone – 1.0 m^2. On each section, stations were performed on the upper, middle, and lower horizons of the littoral zone (about 3.0, 1.5, and 0.5 m above sea level, respectively); in the sublittoral zone – at depths of 5, 10, and 15 m. Three samples were taken at each station. The width of the species zone was estimated on average for an area of relatively uniform vegetation of at least 500 m in length. The dominant species and the projective bottom cover with algae were assessed visually. Sampling in the sublittoral zone was carried out using scuba-diving equipment. The extent of littoral areas with certain types of communities was estimated using the MapViewer 8.0 software. The topographic base was the contour of the coastline in the nautical charts for the Kola Bay, digitized by MMBI RAS specialists.

RESULTS

Macrophyte distribution, reserves, and biomass in the Kola Bay. Four fucoid species investigated are unevenly distributed in the Kola Bay littoral zone.

Fucus vesiculosus was found everywhere from the Tuloma River estuary to the bay mouth; the species uses various substrates for attachment, *inter alia* hydraulic structures. The maximum reserves were recorded in the littoral zone of the middle area. In most of the littoral zone, *F. vesiculosus* biomass was about 1 $kg{\cdot}m^{-2}$, but due to the large area occupied by this species its reserves were very large (Table 1). In a number of spots of the littoral zone of the middle area, the biomass reached (9.4 ± 0.3) $kg{\cdot}m^{-2}$. The total reserves in the bay are 2315.0 tons of wet weight, which is 463.0 tons of dry weight.

Fucus distichus grows in the middle and lower horizons of the littoral zone of the entire Kola Bay. The highest biomass – up to (6.9 ± 3.4) $kg{\cdot}m^{-2}$ – was recorded on boulder beaches in the middle and northern bay areas, but its reserves are concentrated in the southern area, where the species covers vast sandy beaches. On steep cliffs, the biomass is not more than 1 $kg{\cdot}m^{-2}$. The total reserves in the bay are 2017.5 tons of wet weight, which is 403.5 tons in terms of dry weight.

Ascophyllum nodosum lives mainly on boulder beaches, which are protected or weakly protected from wave effect; its thickets achieve significant development on the islands on the leeward side. In the northern area, the biomass reaches (4.2 ± 0.8) $kg{\cdot}m^{-2}$. The total reserves in the bay are 371.4 tons of wet weight, which corresponds to 74 tons in terms of dry weight.

Fucus serratus is distributed mainly in the northern bay area; on Ekaterininsky Island, a high biomass – (6.1 ± 1.5) $kg{\cdot}m^{-2}$ – was recorded. *F. serratus* total reserves are 114 tons of wet weight only, which is 22.7 tons in terms of dry mass.

Saccharina latissima forms thickets in the sublittoral at a depth of 4–6 m in the northern area and is found in small numbers in the middle area. The reserves are 450 tons of wet weight, which corresponds to 64.3 tons of dry weight.

Palmaria palmata is confined to a coarse substrate; it is recorded mainly in the middle and lower horizons of the littoral in the middle and southern bay areas. The reserves are 155.3 tons of wet weight, which is 38.8 tons of dry weight.

Ulvaria obscura is present fragmentarily in the littoral zone in three bay areas. The species occupies a variety of substrates: from stones to wooden structures, metal boards, and ships abandoned on the littoral. The reserves amount to 5.38 tons of wet weight, which corresponds to 1.35 tons of dry weight.

Table 1. Specific rate of diesel fuel absorption by algae macrophyte and estimated volumes of diesel fuel absorption by algae in different areas of the Kola Bay

Species	Specific rate of diesel fuel absorption, µg·g^{-1}·day^{-1}	Volume of diesel fuel absorption, kg·day^{-1}			
		Northern bay area	Middle bay area	Southern bay area	The bay
F. vesiculosus	202.0	12.4	64.2	17.2	93.8
F. distichus	169.0	13.8	22.8	31.6	68.2
A. nodosum	44.0	0.8	2.2	0.8	3.8
F. serratus	123.0	3.0	0	0	3.0
Fucoids in total	538.0	30.0	89.2	49.6	168.8
S. latissima	1752.0	138.0			138.0
P. palmata	146.4	4.4			4.4
U. obscura	25.4	0.17			0.17
In total		168.6	92	51.9	312.5

Potential contribution of macrophytes to the sorption of PPs: result of the experiments. The content of PPs in macrophytes in the habitat (at a concentration of PPs in water of 0.2 mg·L^{-1}, which is 4 TLV) was as follows: *F. vesiculosus* – 25 µg·g^{-1}, *F. distichus* – 24, *F. serratus* – 28, *A. nodosum* – 18, *S. latissima* – 1980, *P. palmata* – 124, and *U. obscura* – 1.4 µg·g^{-1}. Visual and light-optical observations revealed no changes in *Fucus* thalli after 5 and 10 days in seawater with diesel fuel at a concentration of 6.5 mg·L^{-1} (130 TLV). In the thallus color, there were no changes; in the cells, there were no plasmolysis, changes in the plastid color, and vacuolization, which were described earlier when studying the effect of other damaging factors on algae (Ryzhik et al., 2019).

After 5 days of the experiment, the content of DF in 4 fucoid species, *Saccharina*, *Palmaria*, and *Ulvaria* was as follows: in *F. vesiculosus* – 1036 µg·g^{-1}, *F. distichus* – 870, *F. serratus* – 641, *A. nodosum* – 236, *S. latissima* – 10,740, *P. palmata* – 856, and *U. obscura* – 128 µg·g^{-1} of dry weight. Table 1 shows the mean data on DF absorption by the algae species studied *per* 24 hours and the volume of DF absorbed in different Kola Bay areas. The results of calculations of the total daily contribution of the algae investigated to the bay remediation of PPs are shown as well, taking into account their total reserves determined.

DISCUSSION

In the experiments carried out, all macrophytes showed resistance to DF at a concentration of 6.5 mg·L^{-1} (130 TLV). The algae had no signs of damage after 10 days under experimental conditions.

Analysis of the content of PPs in macrophytes sampled for experiments (at a concentration of PPs in the water in the habitat of 0.2 mg·L^{-1}, which is 4 TLV) showed that algae differ in their ability to absorb PPs. Among three *Fucus* species, these differences are insignificant: from 24 $\mu g \cdot g^{-1}$ in *F. distichus* to 28 $\mu g \cdot g^{-1}$ in *F. serratus*; *U. obscura* absorbed PPs 6 times less (1.4 $\mu g \cdot g^{-1}$), and *Palmaria*, on the contrary, 5 times more. *Saccharina* accumulated DF at a given concentration in water 80 times more than fucoids. At higher concentration of PPs in water, the level of DF absorption by algae changes. The maximum absorption level is shown by *Saccharina*: 1752 $\mu g \cdot g^{-1} \cdot day^{-1}$; the species absorbs 3.3 times more than all fucoids algae in total. At relatively high concentrations of DF in water, *Palmaria* is inferior in terms of DF absorption not only to *Saccharina*, but also to two *Fucus* species (Table 1).

The main sources of contamination in the Kola Bay are concentrated in its southern and middle areas. There, handling of hydrocarbon raw materials is carried out; partially, the works are carried out in the middle area from large-capacity tankers on the roadstead. Moreover, ship-repair enterprises, docks, and oil terminals operate. PPs, that got into the water because of spills, mainly end up in the littoral zone (Kola Bay and Oil, 2018 ; Patin, 2017b), *i. e.* in the spots of *Fucus* and *Palmaria* growth, which are a natural biofilter in this situation. Out of algae studied, *Fucus* is the most resistant to PPs. Previously, we noted the ability of *F. vesiculosus* to survive for a long time under conditions of constant contamination: on the littoral zone on stones in a layer of fuel oil (“chocolate mousse”) and near oil terminals and ports. The algae retained their vital functions, but the indicators of their functional activity were at a lower level compared to those of *F. vesiculosus* specimens from clean habitats (Voskoboinikov et al., 2017). Other *Fucus* species demonstrate a lower resistance to contamination; nevertheless, they also survive for a long time when the surface is covered with a film of PPs and are involved in the detoxification (Stepanyan & Voskoboinikov, 2006). A significant ability to accumulate and neutralize PPs was recorded in *P. palmata* as well.

Comparing the volumes of DF potentially absorbed by algae, it should be noted that the primary role in bioremediation is played by fucoids. Out of species studied, *Ulvaria obscura* has the lowest values in terms of reserves and absorption capacity: 5.38 tons of wet weight and 25.4 $\mu g \cdot g^{-1} \cdot day^{-1}$, respectively.

Thus, the algae macrophyte studied are capable of neutralizing 312 kg of DF *per* 24 hours. According to the modern classification, this volume of PPs corresponds to the mean local oil spill in seawater (Patin, 2017a). The efficiency of algae participation in bioremediation is affected by several factors: 1) temperature, light, hydrodynamics, and salinity, determining algae physiological state, quantitative and qualitative composition, and hydrocarbon-oxidizing activity of microorganisms – algae epiphytes; 2) the composition of PPs. In our work, we were unable to take into account the contribution of a large group of algae – the bay inhabitants, representatives of various taxonomic groups – to the bioremediation of the Kola Bay due to the lack of data on their ability to neutralize PPs. The inclusion of this data can significantly increase the importance of algae macrophyte in the bioremediation of coastal waters, although it is undoubted even now.

Conclusion. The ecosystem of the Kola Bay has withstood chronic petroleum contamination for over a hundred years due to a combination of several circumstances. In the bay, most of the coastline is occupied by boulder beaches, where communities of *Fucus* algae with a very high biomass have formed. These communities provide constant water purification from petroleum products, take on the main burden of neutralizing not only PPs dissolved in water, but also “film” forms of toxicants. In the sublittoral zone, kelps develop on separate boulders and purify water from dispersed PPs. The efficiency of the kelp participation in bioremediation is largely ensured by the significant area of contact surface with PPs and, possibly, by the high abundance of epiphytic hydrocarbon-oxidizing bacteria on the thallus surface.

In the Kola Bay water, hydrocarbon-oxidizing microorganisms are constantly present, which process PPs. It can be considered established that there is a natural mechanism for the utilization of petroleum and PPs in natural coastal ecosystems. Undoubtedly, there are lethal doses of PPs for macrophytes, which make this system sensitive to large oil spills or to the effect of its refined products. Taking into account the long period of formation of petroleum hydrocarbons in the Earth's crust, we can say that the use by bacteria of such a high-calorie product as hydrocarbons as a source of energy was a very likely event. The revealed ability of brown, red, and green algae to absorb hydrocarbons is surprising since it is unusual for them, unlike for other autotrophs, to use carbon sources alternative to carbon dioxide in the metabolism. This is based on the ability of macrophytes and hydrocarbon-oxidizing bacteria to form symbiotic associations.

The study presented has shown that the role of algae macrophyte is not limited to the creation of primary production and to regulation of the content of carbon dioxide and oxygen. The thickets of brown algae can be considered as the key component of the repair and homeostasis system in coastal ecosystems, the existence of which was not previously suspected. It should be understood that the destruction of even a part of algae natural communities can change the ecosystem balance. In this regard, the designers and builders of new enterprises on the Kola Bay coast should be warned against backfilling the littoral zone with soil in order to expand the coastal area. The littoral zone is the main habitat of *Fucus* algae, which are a natural biofilter largely ensuring the purity of the Kola Bay water.

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РОЛЬ ВОДОРОСЛЕЙ-МАКРОФИТОВ В БИОРЕМЕДИАЦИИ ОТ НЕФТЕПРОДУКТОВ КОЛЬСКОГО ЗАЛИВА БАРЕНЦЕВА МОРЯ

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Выполнена оценка вклада макроводорослей в очистку от дизельного топлива Кольского залива Баренцева моря. В основу расчётов положены результаты: 1) экспедиционных наблюдений последних лет о запасах, распределении и биомассе водорослей-макрофитов отделов

Chlorophyta и Rhodophyta и класса Phaeophyceae из отдела Ochrophyta, обитающих в трёх частях залива; 2) лабораторных исследований по способности макроводорослей *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. distichus*, *F. serratus*, *Saccharina latissima*, *Palmaria palmata* и *Ulvaria obscura* к нейтрализации токсического действия дизельного топлива. Показано, что общий вклад у исследованных водорослей в биоремедиацию от дизельного топлива в заливе составляет 312 кг в сутки. Выявлены различия в поглощающей способности у водорослей-макрофитов. Так, наиболее эффективно процесс осуществляет *S. latissima*, минимальная эффективность участия в биоремедиации определена у *U. obscura*. Сделан вывод о том, что имеющиеся литоральные и сублиторальные заросли морских макроводорослей Кольского залива являются важным элементом в процессе профилактической, повседневной очистки вод залива от нефтепродуктов. Включение в расчёты данных о способности к нейтрализации дизельного топлива у других представителей фитобентоса залива может увеличить роль водорослей-макрофитов в очистке прибрежных морских акваторий от нефтепродуктов. Сделан вывод, что заросли бурых водорослей — важная составляющая системы репарации и гомеостаза в прибрежных экосистемах. Уничтожение даже части природных сообществ водорослей может изменить баланс, существующий в экосистеме.

Ключевые слова: макрофиты, экосистема Кольского залива, устойчивость, дизельное топливо, биоремедиация

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NEW INFORMATION ON THE BAR-TAILED GODWIT *LIMOSA LAPPONICA* (LINNAEUS, 1758) AND RED-THROATED DIVER *GAVIA STELLATA* (PONTOPPIDAN, 1763) ON THE CRIMEAN PENINSULA (THE BLACK SEA)

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New data on the distribution of the bar-tailed godwit *Limosa lapponica* (Linnaeus, 1758) and red-throated diver *Gavia stellata* (Pontoppidan, 1763) on the Crimean Peninsula are presented. Against the backdrop of aquatic and coastal ecosystems' transformation under natural and anthropogenic impact, even single finds of hydrophilic avifauna representatives in atypical spatiotemporal conditions are of interest for the analysis of emerging trends in migration phenology and abundance of both separate species and taxonomic and ecological groups of birds. The aim of the study was to clarify the distribution boundaries and terms of stay on the Crimean Peninsula of two hydrophilic bird species: semiaquatic species, bar-tailed godwit, and true water bird, red-throated diver. Bird observations were carried out during scheduled accountings on the Isthmus of Ak-Monay (March 2012) and in the Kruglaya Bay (Sevastopol) (July 2019). The bar-tailed godwit is registered in Crimea during spring and autumn migration periods. For the first time, it was found in the north of the Crimean Peninsula in 1972; later, there were an increase in its abundance and expansion of a distribution area. Recent records of this species in southern Crimea significantly expanded the known boundaries of its distribution on the peninsula during the migration period. The observation of the bar-tailed godwit in eastern Crimea on 14 March, 2012, specified the date its spring migration begins; the observation in Sevastopol (western foothill) on 27 July, 2019, was the first one in Mountain Crimea. Off the Crimean coast, the red-throated diver is a rare overwintering and migratory bird. The find of the red-throated diver individual in the Kruglaya Bay on 19 January, 2020, was the third reliable record of this species overwintering in Crimea and the first one in Sevastopol area. The registration of the red-throated diver at a considerable distance from its usual overwintering areas (off the southwestern coast of the Black Sea), along with numerous facts of the expansion of nesting or winter ranges of different bird species northward, confirms the ongoing climatic and ecological changes. Particular attention should be focused on the problem, associated with low ecological culture of the use and disposal of fishing tackles, which have a detrimental effect on hydrophilic birds. Measures have to be developed to regulate fishing with nets, hooks, and line tackles in the areas of bird mass overwintering and seasonal migrations.

Keywords: bar-tailed godwit, red-throated diver, Crimea, distribution, migrations, terms of stay

Fundamental changes in the hydrological regime related to the functioning of the North Crimean Canal since the 1960s and its damming in 2014, as well as to changes in the natural resource management on a large territory of Crimea in recent decades (Sovga et al., 2018), significantly affected the habitat conditions of hydrophilic birds. In this regard, the study of the abundance dynamics, distribution boundaries, and ecology peculiarities of these species is of particular relevance. The finds of even single individuals in atypical areas are of interest for identifying trends in changes in the migration routes and wintering areas of hydrophilic birds on the Crimean Peninsula and in its coastal water area.

The study is aimed at clarifying the distribution boundaries and terms of stay on the Crimean Peninsula of two hydrophilic bird species: semiaquatic species, bar-tailed godwit *Limosa lapponica* (Linnaeus, 1758), and true water bird, red-throated diver *Gavia stellata* (Pontoppidan, 1763).

MATERIAL AND METHODS

Bird observations were carried out during accountings on the Isthmus of Ak-Monay (March 2012) and in the Kruglaya Bay (July 2019) – the areas of the year-round ornithological research, along with other bays of the Heracles Peninsula (Sevastopol). Observations were carried out using binoculars with a magnification 10×. Photos were taken using Canon PowerShot SX60 HS and Canon 400D cameras.

RESULTS AND DISCUSSION

Bar-tailed godwit *Limosa lapponica* (Linnaeus, 1758). The species is common for nesting in the Palearctic tundra zone; in Crimea, it was first found during a migration in the north of the peninsula, in the vicinity of the Portovoe village, in 1972 (Kostin, 1983). In subsequent years, a significant increase in its abundance and expansion of a distribution area during migration periods occurred (ROM Bulletin, 2005 ; Chernichko, 2010 ; Chernichko et al., 2011).

During spring migration, the bar-tailed godwit was first registered in Crimea on 21 May, 1974 (Kostin, 1983). The earliest spring observation date was 25 March, 1999 (Chernichko, 2010). The abundance increases in April and reaches its maximum (up to 1500 ind.) in May. The main area of migratory bird concentration is the Eastern Syvash (Chernichko, 2010). We recorded a single bird on 14 March, 2012, on the central Isthmus of Ak-Monay – near the southern outskirts of the Vladislavovka village (the Kirovsky district). The bird kept on a steppe area not far from a reservoir, in the flock of ruffs *Philomachus pugnax* (Linnaeus, 1758) (Fig. 1A). In the east of Crimea, this spot is the southernmost, but it should be noted that forage biotopes suitable for this species are available to the south as well – in the coastal zone of the Isthmus of Ak-Monay and Kerch Peninsula (Adzhigol, Kuchuk-Adzhigol, and Koyashskoe salt lakes).

The earliest record date of the bar-tailed godwit during autumn migration is 27 July, 1979 (Kostin, 1983), and the latest date is 06 November, 1998 (Chernichko, 2010). The abundance peaks in August; the main areas of bird concentration in autumn are Syvash, the Karkinitsky Bay coast, and salt lakes of the Kerch Peninsula (Chernichko, 2010). We registered an autumn-migrating individual on 27 July, 2019, at the Kruglaya Bay top (Sevastopol) (Fig. 1B). The bird fed on the coast and in shallow water, letting the observer at a distance of 10–15 m. This is the southernmost spot of the species registration on the Crimean Peninsula.

Fig. 1. Bar-tailed godwit *Limosa lapponica*: A – steppe near the Vladislavovka village, in the flock of ruffs, 14 March, 2012, photo by M. Beskaravayny; B – Sevastopol, the Kruglaya Bay, 27 July, 2019, photo by V. Giragosov

Red-throated diver *Gavia stellata* (Pontoppidan, 1763). Off the Crimean coast, it is a rare, occasionally wintering bird (Kostin, 1983). The earliest records of this species date back to the XIX century (Nikol'skii, 1891 ; Blakiston, 1857 ; Nordmann, 1840). There are references to the registration of the red-throated diver in Crimea [in winter, with no dates specified; the article was published in the first third of the XX century (Pusanow, 1933)] and to the record of one individual in the Steppe Crimea [on 03 January, 1907 (Kostin, 1983)]. In subsequent years, single red-throated divers were recorded: near Alushta – on 09 April, 1959, and 25 October, 1961 (Kostin, 1983); near the Lebyazhy Islands – on 04 October, 1964, and 16 November, 1966 (Kostin, 1983); in the interfluve of Kacha and Belbek rivers, Sevastopol area, in the period of 1987–1994 – in March, with no year specified (Klestov & Tsvelykh, 1999); in the Feodosiya Gulf – on 26 January, 2008 (Beskaravayny, 2008); and off the coast of Yalta – on 08 and 10 May, 2017 (Kuzikov, 2017).

Thus, most observations of the red-throated diver are in spring and autumn; winter finds are rare. In Sevastopol area, there were no reliable records of this species at wintering earlier; the cited data on registration "in large quantities" in the XIX century (Blakiston, 1857) most likely have to be attributed to the common wintering black-throated diver *Gavia arctica* (Linnaeus, 1758). We found a red-throated diver at the Kruglaya Bay top on 19 January, 2020 (Fig. 2).

The bird (total length – 59 cm, wing length – 28.7, wingspan – 105, tarsus length – 7.6, beak length – 5.8 cm; body weight – 1.1 kg) got entangled with its beak, wings, and legs in scraps of line fishing net. Therefore, it behaved unusually: it soon got ashore, without fear of the presence of people. It was not possible to free the bird from the net on the shore. The red-throated diver was obviously too weakened, and the attempt to shelter it at home (to treat the wounds on the wings inflicted by the fishing line) and then to release it into the natural environment, was unsuccessful; the bird died.

Moreover, a young spring-migrating red-throated diver was registered on 13 May, 2021, at the Kamyshovaya Bay top (Sevastopol).

Fig. 2. Red-throated diver *Gavia stellata* in winter plumage, the Kruglaya Bay, 19 January, 2020, photo by V. Giragosov

Conclusion. Observations of the bar-tailed godwit in southern Crimea, where it has not been recorded before (on the Isthmus of Ak-Monay and in the Kruglaya Bay), indicate a significant expansion of the known boundaries of the species distribution area on the peninsula and specify the terms the spring migration begins. January find of the red-throated diver, previously known as an exceedingly rare species on the Crimean Peninsula, is the third reliable record of this species overwintering in Crimea and the first one in Sevastopol area.

Against the backdrop of aquatic and coastal ecosystems' transformation under natural and anthropogenic impact, even single finds of hydrophilic avifauna representatives in atypical spatiotemporal conditions are of interest for the analysis of emerging trends in migration phenology and abundance of both separate species and taxonomic and ecological groups of birds.

Particular attention should be focused on the problem, associated with low ecological culture of the use and disposal of fishing tackles, which have a detrimental effect on hydrophilic birds. Our observations show that fishing hooks have a significant traumatic (often fatal) effect on birds as well. Measures have to be developed to regulate fishing with nets, hooks, and line tackles in the areas of bird mass overwintering and seasonal migrations.

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НОВЫЕ СВЕДЕНИЯ О МАЛОМ ВЕРЕТЕННИКЕ *LIMOSA LAPPONICA* (LINNAEUS, 1758) И КРАСНОЗОБОЙ ГАГАРЕ *GAVIA STELLATA* (PONTOPPIDAN, 1763) НА КРЫМСКОМ ПОЛУОСТРОВЕ (ЧЁРНОЕ МОРЕ)

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Приведены новые данные о распространении малого веретенника *Limosa lapponica* (Linnaeus, 1758) и краснозобой гагары *Gavia stellata* (Pontoppidan, 1763) на Крымском полуострове. На фоне трансформации водных и береговых экосистем под воздействием природных и антропогенных факторов даже единичные находки представителей гидрофильной орнитофауны в нетипичных для них пространственно-временны́х условиях интересны для анализа формирующихся тенденций в фенологии миграций и численности как отдельных видов, так и таксономических и экологических групп птиц. Цель данного исследования — уточнить границы распространения и сроки пребывания на Крымском полуострове двух видов гидрофильных птиц: околоводного вида — малого веретенника — и типичного водоплавающего вида — краснозобой гагары. Наблюдения за птицами проводили в ходе плановых учётов на Акмонайском перешейке (март 2012 г.) и в бухте Круглой (г. Севастополь) (июль 2019 г.). Малый веретенник встречается в Крыму в периоды весенней и осенней миграций. Впервые он обнаружен в северной части Крымского полуострова в 1972 г.; в дальнейшем численность его возрастала и область распространения расширялась. Последние находки особей этого вида в южных районах Крыма значительно расширили известные границы его распространения на полуострове в период миграций. Наблюдение малого веретенника на востоке Крыма 14.03.2012 уточнило дату начала его весеннего пролёта; наблюдение в Севастополе (западное предгорье) 27.07.2019 явилось первым в Горном Крыму. Краснозобая гагара у берегов Крыма — редкая зимующая и пролётная птица. Обнаружение особи краснозобой гагары в бухте Круглой 19.01.2020 стало третьей достоверной регистрацией этого вида на зимовке в Крыму и первой — в Севастопольском регионе. Наблюдение краснозобой гагары на значительном удалении от обычных для этого вида районов зимовки (у юго-западного побережья Чёрного моря) в совокупности с многочисленными фактами расширения гнездовых или зимних ареалов разных видов птиц в северном направлении является подтверждением происходящих климатических и экологических изменений. Отдельно следует заострить внимание на проблеме, которая связана с низкой экологической культурой использования и утилизации рыболовных снастей, оказывающих губительное воздействие на гидрофильных птиц. Необходима разработка мер, регламентирующих рыбную ловлю сетями и крючковой снастью в районах массовой зимовки и сезонных миграций птиц.

Ключевые слова: малый веретенник, краснозобая гагара, Крым, распространение, миграции, сроки пребывания

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BRIEF OVERVIEW ON STUDY OF GENETICALLY MODIFIED ORGANISMS AND ASSESSMENT OF POTENTIAL RISKS OF THEIR USAGE FOR NATURAL SPECIES

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Brief information is summarized on the genetically modified organisms (GMOs), methods of their construction, spheres of usage, potential risks of GMOs propagation, necessity, and ranges of control of their usage, *inter alia* their application to aquatic organisms. The data available allow concluding that the expert community currently has no exact answer concerning the scale of GMOs usage in the country, as well as about the degree of genetic safety of their usage in several fields of industry, especially in semi-closed systems for growing plants and rearing of animals and other living beings in the Russian Federation and other countries. Application of molecular genetic markers and new law regulations will help in monitoring GMOs usage in agriculture and other industries in Russia and responding to requests from the Russian Government and social institutions, as well as many challenges on genetic safety.

Keywords: genetically modified organism, GMO, construction, usage, potential risks, aquaculture, agriculture

A *genetically modified organism* is an organism whose genotype has been artificially altered using genetic engineering techniques (Geneticheski modifitsirovannyi organizm, 2021). Hereinafter, “GMO” and “GM-organism” are used in the meaning defined above. Similarly, the abbreviation “GMO” is used as “genetically modified object” (Ganzha et al., 2011).

This brief overview provides general understanding of GMOs and some of the latest developments in this area. Detailed information can be found in the references cited. To date, GMOs are found in almost all food products offered in stores (Zakharova et al., 2015). The methods for GMOs producing are diverse; they are based on the procedures of transfection and transduction, which add to the normal genome of organisms a set of DNA fragments of other organisms modifying its genotype and phenotype (properties) in the desired way and constructing a new transgenic organism. GMO receives new genetic components through special molecular constructions: vectors. Transfection uses a bacterial plasmid as a vector to transfer new genes (gene), that are specially adapted for this transferring. During transduction, modified retroviruses or their special fragments, capable of enzymatic integration into the DNA of the selected organism, usually are the vector. Both processes have the disadvantage of random insertion into alien DNA. This results in the loss of functionality of the inserted genes themselves or in the activation/deactivation of neighboring genes of the cells in the host organism, loading them with harmful effects, *inter alia* their malignant transformation. In recent years, the technique of genetic material transfer has received significant improvement, which allows more accurate inserting of vectors through the CRISPR/Cas gene editing (Mojica et al., 2005).

GM-organisms can be more efficient and secure in cultivation, breeding, and rearing in closed systems, as well as applicable for the usage in food and medical industries with proper control. As a rule, genetic changes are carried out in accordance with the techniques mentioned above for commercial purposes, but they may be carried out for purely scientific purposes as well. In this overview, only a range of issues related to GMOs usage in human commercial activity is considered. In agriculture and food industry, GMOs mean only organisms modified by the introduction of one or more transgenes into their genome (Kuznetsov, 2005a). The same applies to other living beings, for example, to hydrobionts bred in closed and semi-closed systems; therefore, this is applied to aquaculture farms with full or partial control of reproduction by humans.

The main concerns of GMOs usage relate to their legal, environmental, agrotechnical, and food safety (Kuznetsov, 2005b ; Ganzha & Bannikova, 2010 ; Ganzha et al., 2011 ; Chuyko, 2011), as well as guarantees of the human genome protection under molecular therapy with the CRISPR/Cas gene editing (see https://en.wikipedia.org/wiki/CRISPR), which is already included in the medical practice in several countries.

The Food and Agriculture Organization of the United Nations (FAO) considers the usage of genetic engineering techniques to create transgenic plants or other organisms an integral part of agricultural biotechnology. The transfer of genes responsible for useful traits is a natural consequence of the development of a practice on breeding animals and plants. New techniques expanded the abilities of breeders in terms of controllability and efficacy of the process of creating new strains of broods, in particular being able to transmit useful traits between non-breeding naturally species (FAO, 2004 ; Zhuravleva, 2016). Actual agricultural practice has many examples of the successful usage of transgenic plants, that significantly increase crops. In the early 1980s, the first GMO strains were produced in the United States for commercial use. These strains were extensively tested by government agencies: National Institutes of Health (NIH) and Food and Drug Administration (FDA). After evaluating the evidence for the safety of their usage, these strains of organisms were granted market approval.

In a number of countries, including Russia, construction, production, and usage of GMO products is a subject to government regulation; more than 150 regulatory and legislative acts were passed (Vershkova et al., 2008). In the Russian Federation, several types of transgenic objects have been investigated and approved for usage. Out of them, the most known are: soybean – strains 40-3-2, A 2704-12, and A 5547-127; potato – sorts Russet Burbank Newleaf, Superior Newleaf, Elizabeth 2904/1 kgs, and Lugovskoy 1210 amk; corn – strains GA 21, T-25, NK-603, MON 863, MON 88017, MIR 604, and Bt 11; rice – strain LL 62; and sugar beet – strain H7-1 (Genetically Engineered Mice, 2012 ; Lidder & Sonnino, 2011).

According to the International Service for the Acquisition of Agri-biotech Applications (ISAAA) data, 14 countries were producing crops with biotechnological changes by 2004, and 25 countries – by 2009 (James, 2009). The USA, Brazil, Argentina, India, Canada, and China are leading in terms of GMO producing in crop production. Meanwhile, many countries – Austria, France, Luxembourg, Greece, Switzerland, and New Zealand – have banned the cultivation of GM-plants. Currently, GMOs usage is restricted in a small number of countries (Fig. 1). The distribution of GMOs of plant origin for 25 countries with the specification of breeding objects is presented in a review (Ganzha et al., 2011). Importantly, the introduction of GMOs in growing plants is highly likely to mean their inclusion in the human diet: either directly or through the food chains when used in the form of food and feed additives in animal husbandry. At the same time, there is no control of their effect on the consumer, or this control is insignificant.

Since 01 January, 2011, fish and other products, which are produced from harvested biological resources, including those obtained from the cultivation and rearing of fish and other aquaculture and mariculture objects, are equated to agricultural ones in Russia. By the Order of the Government of Russia dated 30 November, 2010, No. 953 the corresponding amendments were made to the existing norm "On the classification of types of products as agricultural products and primary processing products made from agricultural raw materials of own production".

In the fishery, GMOs are tested both on model organisms and on objects of commercial cultivation (Isaeva & Morozov-Leonov, 2005 ; Mikodina, 2008). Specifically, GMOs are described for Atlantic salmon *Salmo salar*, coho salmon *Oncorhynchus kisutch*, Chinook salmon *O. tshawytscha*, coastal cutthroat trout *O. clarkii clarkii*, Nile tilapia *Oreochromis niloticus*, Mozambique tilapia *O. mossambicus*, Japanese rice fish *Oryzias latipes*, common carp *Cyprinus carpio*, channel catfish *Ictalurus punctatus*, African sharptooth catfish *Clarias gariepinus*, fossil cat *Heteropneustes fossilis*, nigorobuna *Carassius auratus grandoculis*, crucian carp *C. carassius*, yellow pike *Sander vitreus*, northern pike *Esox lucius*, Amur catfish *Parasilurus asotus*, weatherfish *Misgurnus fossilis*, pond loach *M. anguillicaudatus*, gilthead bream *Sparus aurata*, red seabream *Pagrus major*, Wuchang bream *Megalobrama amblycephala*, and zebrafish *Brachydanio rerio* (Ganzha et al., 2011). In the Russian Federation, research on GMOs in aquaculture is carried out on representatives of the most farmed species: rainbow trout, tilapia, carp, *etc.* (Ganzha et al., 2011).

However, there are very contradictory positions regarding the applicability of genomic technologies, *inter alia* widespread GMOs usage, which deserve serious attention of the expert community, specialized departments, and government agencies. Supporters of the applying GMO-technologies argue that only they, with the current world population, can save the planet from the threat of hunger. Opponents of this approach believe that with the actual level of agricultural technology and mechanization of agricultural production, existing plant varieties and animal breeds obtained in the classical way are capable of providing the world population with high-quality food (Obzor pervogo dnya konferentsii, 2019 ; Obzor dokladov za 23 iyunya, 2019) (materials of the Congress of the All-Union Society of Geneticists and Breeders, 2019).

At the International Genetic Congress in 2019 (The VII Congress of the All-Union Society of Geneticists and Breeders), a complex of ethical, social, legal, and scientific norms was considered in the usage of genetic technologies, *inter alia* construction of GMOs and control of their applying (Obzor dokladov za 20 iyunya, 2019). Despite the multitude of documents, both mentioned and new (Ganzha & Bannikova, 2010 ; Chuyko, 2011 ; Agapov & Ganyuhina, 2016), a single set of legislative acts on this issue has not been taken in the Russian Federation. In this regard, the request of the Government of Russia dated 17 March, 2020, No. MN-7/435-AM is extremely timely; its topic is as follows: "Improving the mechanism for controlling the release of genetically engineered organisms and products obtained with the usage of such organisms or obtained from such organisms, as well as monitoring their effect on humans and the environment using modern technologies". The literature sources on these issues are partially annotated; those include 42 articles under the section "Methods for determining GMOs and quality control of organic products" alone (Geneticheski modifitsirovannye organizmy, 2017). In the next section, the author offers his vision of the issues – for their consideration by the expert community, as well as in government departments and other government agencies.

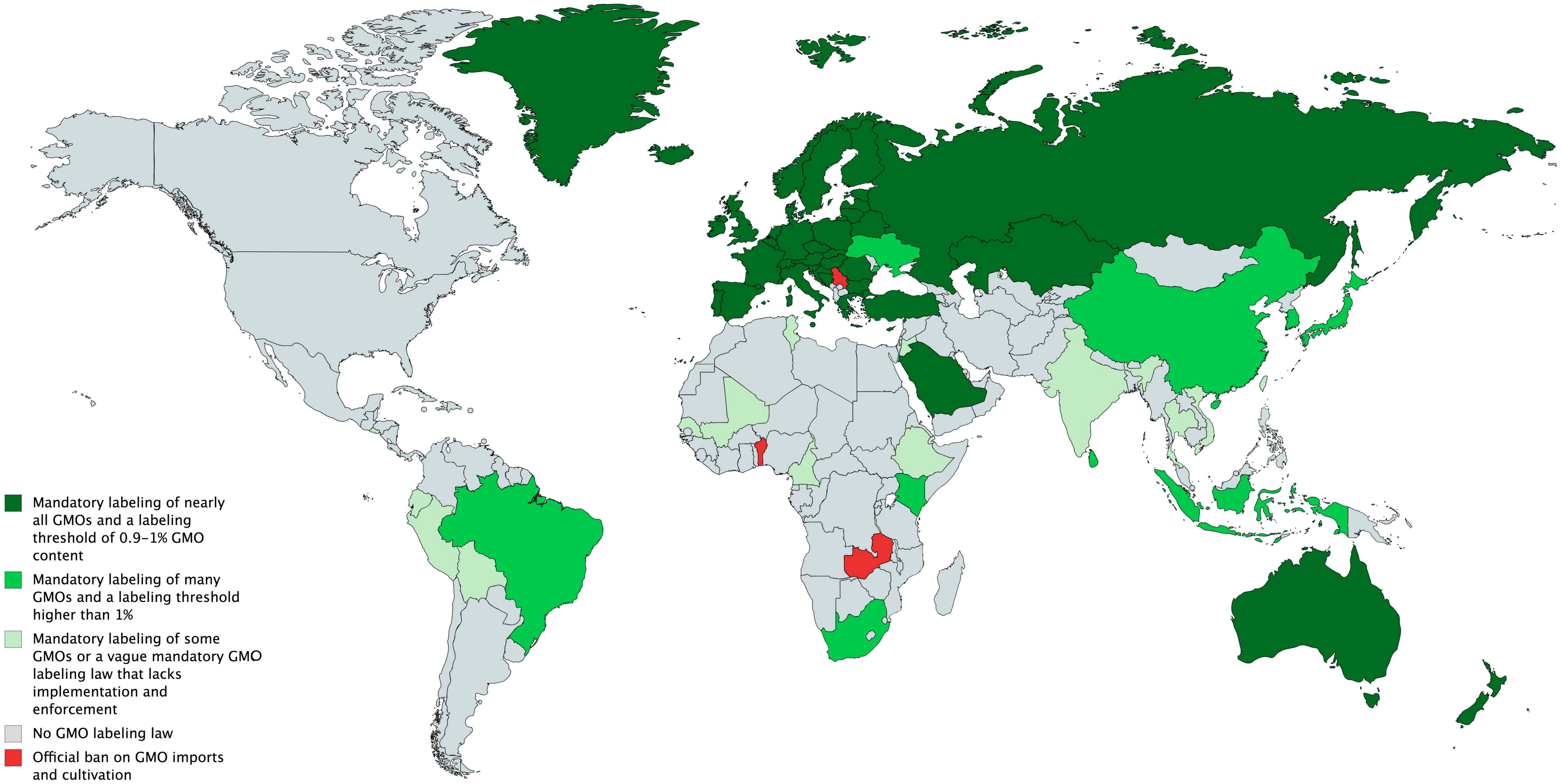

Fig. 1. Prevalence of GMOs in the world. The colors indicate areas of continents with distribution restrictions; the shades of gray indicate areas where GMOs usage is not regulated by law (source: https://www.techpedia.pl)

Identification of GMOs, biodiversity monitoring, and molecular markers. Genetic safety problems. The identification of GMOs is an essential component of controlling the prevalence of their practical application. However, among the most important tasks of biology, there is not only monitoring of GMOs and the safety of the usage of genetic technologies, but also the study of ecological aspects in their contact with natural communities, as it is possible in semi-closed reproduction systems. In this regard, the closest attention should be paid not to full-cycle agricultural and other farms where the reproduction of organisms is fully controlled by humans, but to semi-closed breeding systems, *e. g.* ranching, which is carried out at most salmon marine hatcheries, with partial control of the life cycle by the personnel. During rearing, GMO vectors are capable of entering the natural environment due to horizontal transfer; there, they can convey to species undesirable and even harmful in nature properties of transgenic organisms: instability of localization in the genome and provoking alterations of cellular functions during translocations with unpredictable consequences. The key task is to monitor all the biological diversity and take measures aimed at its preserving in order to maintain a comfortable living environment for current and future generations of Russian citizens. The problems of the society accelerated technologization and food trade globalization entail the need for control the quality of food products, sources for drugs, and their composition as well. All this is impossible without a well-functioning system for identifying organisms based on biological molecular markers.

The origins of the application of the variability of biological macromolecules, *e. g.* in biology, have long roots. In general, biological molecular markers have found application in numerous fields depending on the needs of modern society. Let us define what a biological molecular marker is. A *biological molecular marker* (hereinafter MM) is any macromolecule of a living organism, that can be an identifier of a certain function, results of a biochemical, population, or evolutionary process (Kartavtsev & Redin, 2019). The study and usage of MMs have already become a new branch of biomedical science, as evidenced by the presence of special journals: Biomarkers, Current Biomarker Findings, Biomarker Insights, Barcode Bulletin, *etc.* MMs are used in many fields of biology and medicine.

There are three most significant areas:

1. **DNA barcoding.** Molecular markers are applied in the global program for redescribing biological diversity on a modern molecular and bioinformatics basis: iBOL (see https://ibol.org/). As a standard marker, or DNA barcode, for most invertebrates and vertebrates, the nucleotide sequence of *Co-1* gene encoding cytochrome *c* oxidase subunit I of mitochondrial DNA (mtDNA) is used. For ease of usage, the first half of the gene is used as a barcode, about 650 base pairs (bp). For plants, other MMs, or barcodes, are more suitable, such as *matK* (maturase), *rbcL* (large subunit of the enzyme ribulose-bisphosphate carboxylase), and fragments of internal transcribed spacers of rRNA, ITS1, ITS2, *etc.* (Zhokhova et al., 2018 ; Shneyer & Rodionov, 2018). At this level, the basis for successful identification is low intraspecific variability (weak nucleotide sequence differences between individuals of the same species), but an order of magnitude greater interspecific divergence of samples (between individuals of different species): on average, about 0.5–1.0 % and 10 % divergence for animals, respectively (Kartavtsev, 2011).

One of the applications of MMs is the identification of hybrids and invasive species. Due to globalization and the intensification of international food trade, identification of samples in export-import operations is of great importance. Falsification of brands, *e. g.* trade marks, under which fish fillets, caviar, and other products are sold, can be accurately identified by MMs, and this helps state-owned and private enterprises to avoid significant economic and reputational losses (Nedunoori et al., 2017).

2. **Molecular markers for the identification of stocks, strains, and breeds of animals.** For this level, *Co-1* and other mtDNA molecular markers are not quite suitable, since they are relatively little variable within the species (although there are exceptions). Usually more conservative in animals, nuclear DNA (nDNA) molecular markers are even less applicable at this level. However, microsatellite nDNA loci and single nucleotide polymorphisms are the most effective in identifying differences between animal populations, breeds, and stocks, as well as in their certification in higher organisms.

3. **Molecular markers in the field of medicine** have acquired the greatest importance, especially in the diagnosis of diseases (specifically, breast, prostate, and colon cancer, *etc.*). They are applied in a forensic science as well: aimed at excluding certain individuals from suspects. The scope of MMs also includes monitoring of genetic safety to assess the risks of usage of recombinant DNA and genetically modified products/objects in food and medical industries. There is a special review on the biomedical problems of DNA barcoding (Zhokhova et al., 2018).

In addition to the obvious applied value in medicine and in the biodiversity description, the areas listed above are useful for the paradigms of general biology and evolutionary genetics, as well as for the scientific component of the iBOL program, being of a decisive importance in identifying species by DNA barcoding. As of 07 August, 2020, the iBOL database accumulates the research results as follows: samples of living organisms – 11,429,832; samples with barcodes – 8,466,913; and species identified by barcodes – 314,777 (The Barcode of Life Data System, 2021, see Taxonomy Browser). All this data is accompanied by documentation unified by the iBOL standards and is available to any user *via* the Internet. The contribution of the Russian Federation and RUS-BOL (Shtrikhkodirovanie zhivykh organizmov na osnove DNK, 2021) to research on DNA barcoding is reflected in the Barcode of Life Data System (BOLD) database: these are 42,174 records published, which form 7,972 barcode clusters represented by 263 organizations (laboratories and creative groups). The records made in BOLD refer to 27,320 species names, representing a total of 6,099 species. In terms of activity, Russia is in the middle of the list of program participants, at the level of Brazil and France.

Thus, on the merits of the above-mentioned request of the Government of Russia, it can be stated as follows: **up to date, the expert community does not have an exact answer to the question of the scale of GMOs usage in the country and the degree of genetic safety of their usage in several fields of industry, especially in semi-closed systems for growing plants and rearing of animals and other living beings in the Russian Federation**, as well as in other countries. The usage of MMs and a new regulatory framework will allow carrying out a more accurate monitoring of GMOs applying in agriculture and other industries in the Russian Federation and answering the questions raised by the government, as well as other important public requests regarding genetic safety.

List of genetic safety measures

- Genetic safety of food products. Carrying out large-scale research aimed at development of guidelines for the control of export-import food flows. Development of specialized MMs for monitoring genetic and environmental safety of GMOs usage. Development of recommendations for monitoring the usage of GMO products as food items, food additives, and drugs.
- Monitoring of invasions and hybridization. Ecological and genetic monitoring.
- Monitoring of gene pools of major agricultural crops and commercially important animals and fish. Monitoring of varietal (breed) diversity based on MMs.

- Monitoring of gene pools of human populations based on MMs and omics technologies, *inter alia* the usage of the latest instrumental base for whole genome sequencing, genome editing, transcriptome, proteomic, and other types of analysis to maintain the health of the nation.
- Funding in the amount of 150 million rubles *per* year for 5 years of a targeted federal program for the study and maintenance of biodiversity on the topic "DNA barcoding as the basis of the program for molecular, genetic, and bioinformatic description of biological diversity of living organisms in Russia. Genetic certification of especially valuable populations and species based on new methods for describing biological diversity: biobanking, environmental DNA, transcriptome analysis, and other approaches".
- Prolongation of funding of the relevant topics of the Russian Science Foundation on the assessment of the biodiversity of living organisms and the elaboration of specific research and development projects.
- Organization of a system of monitoring by the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing – of GMO products as food items, food additives, and drugs, as well as food manufacturers and chain stores of the Russian Federation – based on molecular markers.

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КРАТКИЙ ОБЗОР ИЗУЧЕНИЯ ГЕНЕТИЧЕСКИ МОДИФИЦИРОВАННЫХ ОРГАНИЗМОВ И ОЦЕНКА ПОТЕНЦИАЛЬНЫХ РИСКОВ ИХ ИСПОЛЬЗОВАНИЯ ДЛЯ ПРИРОДНЫХ ВИДОВ

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Представлены краткие сведения о генетически модифицированных организмах (ГМО), методах их создания, областях использования, потенциальных рисках применения, а также необходимости и сферах контроля их использования, в том числе применительно к водным организмам. Приведённые материалы позволяют заключить, что экспертное сообщество в настоящее время не имеет точного ответа на вопрос о масштабах использования ГМО в стране, а также о степени генетической безопасности их применения в некоторых сферах производства, особенно в полузамкнутых системах воспроизводства растений, животных и других объектов в Российской Федерации и за рубежом. Использование молекулярных маркеров генов и новая нормативная база позволят осуществить более точный мониторинг применения ГМО в сельском хозяйстве и других отраслях промышленности в РФ, ответить на запросы Правительства России и общества, а также на ряд других важных вызовов относительно генетической безопасности.

Ключевые слова: генетически модифицированный организм, ГМО, создание, применение, потенциальные риски, аквакультура, сельское хозяйство

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SPECIFICATION OF POLYMORPHISM AND CLASSIFICATION OF SHELL COLORATION IN GASTROPODS BY THE EXAMPLE OF *LITTORINA OBTUSATA* (GASTROPODA: LITTORINIDAE)

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Understanding the patterns of microevolutionary processes involves a wide range of population genetic studies on different species. However, the number of genetically studied species is limited due to significant methodological difficulties in testing the genetic conditionality of various traits. Developing population phenetics may become an alternative, which allows considering a large number of new species subject to development of morphologically and genetically-based system to describe the variability and classification of phenotypic traits. Gastropods are a classic object for carrying out population genetic studies based on the analysis of polymorphism of shell coloration. The parametric classification system proposed by S. Sergievsky *et al.* (1995) for periwinkles of the genus *Littorina* may serve as a basis for the developing of a universal system for classifying color traits for that taxonomic group. Since a large amount of new data has been published in recent years, this system requires correction and revision. The study aims to revise the system, taking into account new material on the pigment composition, as well as on the peculiarities of the formation and inheritance of color traits, their joint occurrence, *etc.* A revised and modified classification system for shell coloration traits is presented by the example of the White Sea gastropods *L. obtusata*; this system considers the idea of the formation of a phenotype as a combination of several elementary traits. These are traits associated with the formation of: 1) a shell background color (the ability to include different pigments in the ostracum and the hypostracum color); 2) a pattern of spots (the presence of inclusions of white and/or brown pigment); and 3) wide longitudinal bands (brown, white, and orange). Elementary traits are highlighted taking into account the pigments involved, as well as the mechanisms of their formation and inheritance. When describing the shell coloration, elementary traits are first used to describe relatively simple traits (groups of phenes “Shell background color”, “Hypostracum color”, “Pattern of spots”, and “Wide longitudinal bands”), which are subsequently combined to describe the phenotype as a whole. Our study provides an overview of the available data on the qualitative composition of shell pigments in periwinkles and patterns of formation and inheritance of color traits; their possible combinations are described. The phenes isolated by us are described together with their occurrence, color specification in the RGB system, and the peculiarities of the formation, taking into account the distribution of pigments in the shell. Visible traits, that may be used to assess the distribution of pigments in the shell, are indicated. The ontogenetic changes in traits are described. Despite the fact that the proposed classification system is developed by the example of *L. obtusata*, it can be used for same purposes for other periwinkle species and, with some modifications, for a wide range of gastropod species.

Keywords: polymorphism, shell coloration, classification system, gastropods, *Littorina*

Understanding the patterns of microevolutionary processes involves carrying out a wide range of population genetic studies on different species. At the same time, the number of genetically studied species is limited due to significant difficulties in checking out the genetic conditionality of various traits: problems in keeping and breeding animals, long generational change, *etc.* Therefore, the patterns revealed may be insufficiently characteristic of all living nature. The way out lies in the development of population phenetics, which allows extending population genetic methods to species, whose direct genetic study is difficult or impossible (Yablokov, 1987 ; Yablokov & Larina, 1985). This approach should be based on the formation of reasonable systems for describing variability and classifying phenotypic traits.

Gastropods serve as a classical object for carrying out population genetic studies associated with shell coloration polymorphism (Ito & Konuma, 2020 ; Miura et al., 2007 ; Scheil et al., 2014). The emergence of a sufficiently flexible and well-grounded system for classifying formation and inheritance of shell coloration traits will make it possible to include in consideration a large number of new species. Despite the best knowledge of the genetics of pulmonates (Kozminsky, 2014 ; Backeljau et al., 2001), model objects for the development of such a classification system should be sought among the prosobranchias: more numerous and significantly more varied in color. Molluscs of the genus *Littorina*, which are characterized by high polymorphism (Reid, 1996) in shell coloration, are a promising model object; on its basis, an attempt can be made to create a classification system universal for gastropods.

Molluscs of the genus *Littorina* are a popular subject for numerous studies related to the variability of shell coloration (Estévez et al., 2020 ; Rolán-Alvarez et al., 2015 ; Sokolova & Berger, 2000). So far, several systems have been proposed to describe it. The first one – formulated in the early XX century – was a system for describing variability in *Littorina obtusata*, in which each stable combination of coloration traits (morph) had its own name (Dautzenberg & Fischer, 1915, cited from: Reid, 1996). With some modifications, this approach was used for a long time in population genetic studies of periwinkles (Reimchen, 1979 ; Sacchi, 1974). Its key disadvantage is the problems of studying the variability of elementary traits, that are part of a morph. The attempt to solve those problems by isolating additional morphs leads to the unnecessary bulkiness of the system of variability description (Reid, 1996). For this reason, Pettitt (1973) proposed the system of coloration trait classification in periwinkles, based on the imaginary system of genetic control, with six "loci" for description of elementary coloration traits: background, number and coloration of bands, pattern of spots, *etc.* A similar approach was used to describe variability in *L. obtusata* and formed the basis of the parametric system of coloration trait classification in *L. saxatilis* (Sergievsky et al., 1995). Since there were no data on the mechanisms of inheritance of the shell coloration traits in prosobranchias, Sergievsky *et al.* (1995) used the monogenic polyallelic inheritance pattern characteristic of pulmonates. Unfortunately, later studies (Kozminsky, 2014) showed that this assumption is not true. Among the advantages of this approach, due to which it can serve as a basis for the development of a universal classification system for gastropods, are the description of coloration variants as a combination of independent elementary traits and an attempt to consider the mechanisms of their inheritance. Moreover, the peculiarities of coloration traits formation were partly taken into account (Sergievsky et al., 1995).

Considerable time has passed since the emergence of the parametric system for describing variability in periwinkles, and a lot of new data has appeared. By the example of *L. obtusata*, it was shown that the background coloration in periwinkles is a result of the joint activity of several genetic systems, each of which is responsible for the incorporation of a certain pigment into the shell (Kozminsky, 2014).

The inheritance of bands and a pattern of spots on the periwinkle shell was studied (Kozminsky, 2011, 2016 ; Kozminskii et al., 2010). The concepts of the distribution of pigments in the shell and the formation of elementary coloration traits in periwinkles were substantially supplemented, and the composition of the pigments involved was clarified (Kozminskii & Lezin, 2007). Finally, the efficiency of using shell coloration traits when identifying periwinkle phenotypes was assessed; it was shown that for accurate identification, the peculiarities of the distribution of pigments in the shell thickness have to be considered (Lezin & Kozminskii, 2008).

The aim of this work is to revise the previously proposed classification system for shell coloration traits (Sergievsky et al., 1995) for molluscs of the genus *Littorina*, taking into account new data on the composition of pigments, peculiarities of their distribution in the shell, and inheritance of coloration traits.

RESULTS AND DISCUSSION

The work is based on the data obtained in the study of the White Sea molluscs *Littorina obtusata* (Linnaeus, 1758) and, in some cases, *Littorina saxatilis* (Olivi, 1792). The methods used and results obtained are described in detail in the publications of E. Kozminsky *et al.* (Kozminsky, 2011, 2014, 2016 ; Kozminskii & Lezin, 2006, 2007 ; Kozminsky et al., 2008 ; Kozminskii et al., 2010 ; Lezin & Kozminskii, 2008).

Pigments and coloration of shell areas. The coloration of the gastropod shell consists of a background coloration and a pattern of bands or spots (Sergievsky et al., 1995).

The shell areas of *L. obtusata* can be purple[1], orange, yellow, white, and depigmented. Various pigments are responsible for purple, orange, and yellow coloration. Purple pigment is insoluble in water and organic solvents; it remains in the sediment in the form of brownish flakes when the shell is dissolved by weak acids. Chemical stability and color allow suggesting that purple pigment is melanin. This pigment is widespread in animals (Britton, 1986) and is found in mollusc shells (Lucas, 1974 ; Williams, 2017 ; Williams et al., 2016). Yellow and orange colors are caused by pigments insoluble in water, but extractable with organic solvents: ethanol, chloroform, hexane, and toluene, as well as diethyl and petroleum ethers (Kozminskii & Lezin, 2007 ; Sergievsky et al., 1995). When carrying out paper chromatography, these pigments behave similarly to carotenoids (Zabelensky, Kozminsky, unpublished data). By their visible color, they can belong to both carotenoids and polyenes also found in mollusc shells (Délé-Dubois & Merlin, 1981 ; Hedegaard et al., 2006 ; Williams, 2017). Apparently, guanine is responsible for white coloration, which, strictly speaking, is not a pigment. White color caused by its presence is structural and associated with the reflection of light by ordered guanine microcrystals (Britton, 1986). As known, guanine is responsible for the formation of white coloration in many animals, *inter alia* molluscs (Britton, 1986 ; Lucas, 1974). The incorporation of guanine into the periwinkle shell can explain the formation of a denser than usual microstructure of white areas as well (Kozminskii & Lezin, 2007 ; Sergievsky et al., 1995). Discolored areas of the shell with normal microstructure result from true depigmentation.

Formation of the shell coloration. The major contributor to the formation of the periwinkle shell coloration is the irregular-prismatic layer (ostracum) (Kozminskii & Lezin, 2007). The background shell color can be formed due to the incorporation of one, two, and three pigments into the shell.

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[1] It would be more correct to speak of brown. The latter, with a high pigmentation intensity, looks red-brown and even almost black and was designated by Sergievsky *et al.* (1995) as purple. In this work, the original designation is used.

Elementary traits associated with the formation of a background coloration are the ability to incorporate various pigments into the shell (melanin, two carotenoids) and the ability to incorporate guanine into the shell. Depending on which pigments are incorporated into the shell, different variants of the background color are formed (Fig. 1A–M): monochromatic (pure yellow, orange, and purple), dichromatic (two-layer yellow-purple, yellow-orange, orange-purple, and white-purple), and trichromatic ones (three-layer yellow-orange-purple, yellow-white-purple, and orange-white-purple). In yellow-purple molluscs, the coloration of the shell background depends on the ratio of yellow and purple pigments in the outer ostracum area (Fig. 1H–J). With a higher concentration of yellow pigment, the visible shell color is almost yellow; with its lower concentration, the shell looks brown. With an intermediate concentration of pigment, the shell is olive (greenish). It should be noted that the proper (layer-by-layer) distribution of pigments, which is characteristic of *L. obtusata*, is not necessary. In *L. saxatilis*, in the presence of the same elementary traits, the distribution of pigments in the shell is often chaotic. The pattern of spots is formed on the basis of white or purple pigments (Fig. 1O–R). Separate pattern elements are lenticular incorporations of pigment located in the upper ostracum area. The relative location of the pattern elements varies, as well as the degree of their fusion. The elementary trait highlighted at this stage is the presence of a pattern of white or purple spots or their absence. Considering high variability of the pattern of spots, further studies are required of the formation of this color trait and a highlight of additional elementary traits, *e. g.* the ability to form a zigzag pattern. Bands form as interlayers of brown, white, and orange pigments (Fig. 1S–U). As in the previous case, the elementary trait is the presence of bands of different colors or their absence. The hypostracum of the periwinkle shell can be purple or depigmented (Fig. 1N); elementary trait is colored or depigmented hypostracum.

No one has studied the formation of mollusc shell coloration at histological and biochemical levels. As known, dorsal and ventral glands, as well as the cells of the dorsal epithelium of the mantle margin, are involved in the formation of the periwinkle shell (Bevelander & Nakahara, 1970). The ventral and dorsal glands are responsible for the periostracum formation. The ostracum and hypostracum formation involves the cells of the dorsal epithelium, which are formed in the proliferation zone located at the mantle margin and are gradually displaced in the dorsal direction. It stands to reason that during this displacement, the cells of the dorsal epithelium at certain points in time ensure the synthesis of different pigments and their incorporation into the shell. Moments of switching modes of functional activity of pigment-forming cells are obviously genetically determined. As a result, there are an ordered incorporation of pigments into the shell and a formation of various coloration elements.

When a monochromatic background color is formed, the cells of the dorsal epithelium of the mantle margin synthesize the same pigment. As these cells are displaced from the proliferation zone, their functional activity may decrease. In this case, a pigmentation gradient is formed, with a decrease in intensity from the outer to the inner ostracum area. If this does not occur, the prismatic layer is colored evenly. With the formation of di- and trichromatic variants of the background color, the cells of the dorsal epithelium synthesize one kind of pigment. Then, a synchronous switching of their functional activity occurs from the formation of one pigment to the formation of another. Since the boundaries between areas of different pigmentation are blurred, it can be concluded that functional activity of pigment-forming cells changes smoothly. Apparently, we can talk about a certain pattern in the change in the modes of functional activity of pigment-forming cells: first, yellow and orange pigments and guanine are synthesized; only then, purple pigment is synthesized.

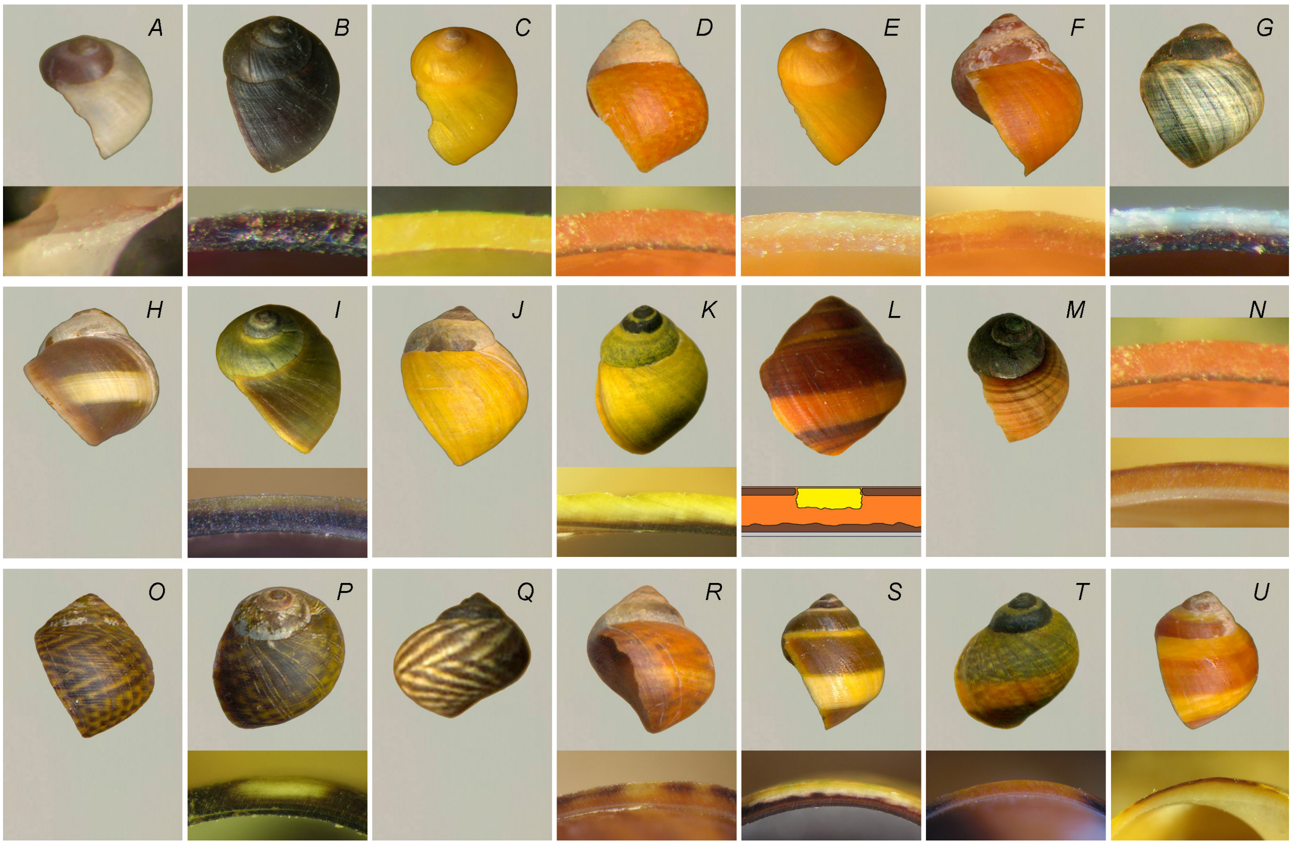

Fig. 1. Variants of the shell background color and the pattern of spots and bands in *Littorina obtusata*. Shell background color: A – depigmented; B – purple; C – yellow; D – orange; E – yellow-orange; F – orange-purple; G – white-purple; H–J – variants of yellow-purple shell background color; K – yellow-white-purple; L – yellow-orange-purple; M – orange-white-purple (by the example of *L. saxatilis*). Hypostracum: N – purple and depigmented. Pattern: of white (O–Q) and purple (R) spots. Wide longitudinal bands: S – white; T – orange; U – brown. The general view of the shells (top panel) and the distribution of pigments in the shell (bottom panel) are presented. This illustrative material is freely available on the website of the Zoological Institute RAS, Saint Petersburg: http://www.zin.ru/projects/litphen/index.html

With the formation of brown (= purple), white, and orange bands, the cells of the dorsal epithelium, located in certain areas of the mantle margin, switch at the appropriate time to the formation of the pigment of the bands. Interestingly, the system sometimes fails: the formation of the background coloration stops, but the synthesis of the pigment of the bands does not occur. In this case, depigmented bands are formed, which we noted in yellow *L. obtusata*; these are homologous to the *hyalozonata* phenotype of other molluscs (Cook, 1967 ; Cook & King, 1966). When a pattern of spots is formed, a change in functional activity of pigment-forming cells in certain areas of the mantle margin occurs periodically. The linear dimensions of such areas are much smaller, and their number is much larger than in the case of bands. As a result, a pattern of pigment spots is formed, the shape and relative position of which vary (Fig. 1O–R).

Switching of the mantle margin cells from the formation of an irregular-prismatic layer to the formation of a lamellar layer is associated, apparently, with a decrease in the number of possible variants of functional activity of pigment-forming cells. In the ostracum formation zone, cells can synthesize purple, orange, and yellow pigments and guanine; in the hypostracum formation zone, purple pigment alone (Fig. 1N).

Possible variants and combinations of color traits. In the series of our research, most of the theoretically possible variants of the background coloration were revealed, which can be formed on the basis of three pigments and guanine. Individuals with depigmented shell were obtained under experimental conditions. *In vivo*, three out of four possible monochromatic color variants (purple, yellow, and orange) were found; all the possible dichromatic color variants involving purple, yellow, and orange pigments and guanine; and three out of four possible trichromatic color variants. At the same time, no molluscs were revealed with monochromatic white, dichromatic yellow- or orange-white, and trichromatic yellow-white-orange background coloration; obviously, white pigment is found only in conjunction with purple one.

White bands were registered in periwinkles with the most varied background colors. Brown bands are characteristic of molluscs, which have a background coloration with orange pigment. Less commonly, they can be found in periwinkles with a background color formed with yellow pigment (yellow and yellow-purple molluscs) or – only under experimental conditions – in depigmented individuals. In *L. saxatilis*, brown bands are found in white-purple individuals alone. Orange bands are recorded mainly in molluscs with yellow-purple background color; occasionally, with pure purple one. Bands of different types can occur simultaneously. Individuals with brown and white bands on the shell are quite common. Periwinkles bearing both white and orange bands were found as well; however, this color variant is very rare. This is probably due to the fact that white and orange bands are formed on the same shell area and “interfere” with each other.

A pattern of white spots blends freely with all the background colors and bands. At the same time, a pattern of purple spots is associated with the presence of orange pigment in the background coloration and often with the presence of brown bands. Both pattern variants can occur at the same time.

Inheritance of shell coloration traits. The information on the inheritance of shell coloration traits in periwinkles is limited. The data of Reimchen (Reimchen, 1974, cited from: Reid, 1996) testify to the hereditary nature of some color variants. In the work (Kozminsky et al., 1995), an attempt was made to explain the inheritance of the background shell coloration in periwinkles based on a single-locus polyallelic inheritance pattern, which is characteristic of pulmonates (Murray, 1975). Unfortunately, in the publication mentioned, little attention was paid to the fact that several different pigments are involved in the formation of the periwinkle shell coloration, and the possibility of multiple paternity was not considered, which doubts the conclusions drawn. In later works (Kozminsky, 2011, 2014, 2016 ; Kozminskii et al., 2010), those shortcomings were eliminated; the results obtained form the basis of the following scheme of inheritance of shell coloration traits in periwinkles (Table 1).

With the formation of the background color in *L. obtusata*, three different groups of genes are responsible for the incorporation of purple, yellow, and orange pigments into the shell (Kozminsky, 2014). The incorporation of pigments into the shell is dominant. In the case of purple and yellow pigments, each group incorporates at least two complementary interacting genes. We have not studied the inheritance of white coloration in *L. obtusata* due to the rarity of the corresponding phenotypes in the White Sea populations[2]. By analogy with pigments, it stands to reason that a separate genetic system is responsible for the incorporation of guanine into the shell as well (it is shown in the table as a separate biallelic gene). In general, the background color of the periwinkle shell is formed as a result of the interaction of several groups of genes, with each being responsible for a certain pigment (synthesis, transport, and incorporation into the shell).

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[2] The corresponding study was carried out by the example of *L. saxatilis*.

Table 1. Inheritance of the shell coloration traits in *Littorina obtusata*

Trait	Gene	Alleles	Genotype / phenotype accordance
Background coloration (see Kozminsky, 2014)			
Purple pigment participates / does not participate in the coloration formation	P_1	P_1 p_1	P_1–P_2– – the pigment is incorporated into the shell (C^P, C^{YP}, C^{WP}, C^{OP}, C^{YOP}, C^{YWP}, C^{OWP})
	P_2	P_2 p_2	$p_1p_1P_2$–; P_1–p_2p_2; $p_1p_1p_2p_2$ – no pigment incorporation into the shell
White pigment participates / does not participate in the coloration formation	W	W w	W– – the pigment is incorporated into the shell (C^{WP}, C^{YWP}, C^{OWP}) ww – no pigment incorporation into the shell
Yellow pigment participates / does not participate in the coloration formation	Y_1	Y_1 y_1	Y_1–Y_2– – the pigment is incorporated into the shell (C^Y, C^{YP}, C^{YO}, C^{YOP}, C^{YWP})
	Y_2	Y_2 y_2	$y_1y_1Y_2$–; Y_1–y_2y_2; $y_1y_1y_2y_2$ – no pigment incorporation into the shell
Orange pigment participates / does not participate in the coloration formation	O	O o	O– – the pigment is incorporated into the shell (C^O, C^{YO}, C^{OP}, C^{YOP}, C^{OWP}) oo – no pigment incorporation into the shell
Bands (see Kozminsky, 2011, 2016 ; Kozminsky, unpublished data)			
Presence/absence of brown bands	B^p	B^p b^p	B^p– – brown bands are formed (B^P) b^pb^p – no brown bands
Presence/absence of white bands	B^w	B^w b^w	B^w– – white bands are formed (B^W) b^wb^w– – no white bands
Presence/absence of orange band	B^o	B^o b^o	B^o – no orange band (B^O) b^ob^o – orange band is formed
Pattern of white spots (see Kozminskii et al., 2010)			
Presence/absence of the pattern	S_1^w	S_1^w s_1^w	S_1^w– S_2^w– – the pattern of spots is formed (S^W)
	S_2^w	S_2^w s_2^w	$s_1^w\,s_1^w\,S_2^w$–; S_1^w– $s_2^w\,s_2^w$; $s_1^w\,s_1^w\,s_2^w\,s_2^w$ – no pattern

Note: designations of phenotypes and further explanations are given in the text; pairs of complementary genes are shown in blue color, and pairs of linked genes, in red color.

One gene with two alleles is responsible for the formation of brown bands in periwinkles (Kozminsky, 2011). The inheritance of white (Kozminsky, 2016) and orange (Kozminsky, unpublished data) bands is also consistent with the hypothesis of a monogenic inheritance pattern. While the presence of brown and white bands is dominant, the presence of orange bands is most likely a recessive trait.

At least two complementary genes are responsible for the presence of a pattern of white pigment spots on the shell (Kozminskii et al., 2010). The high variability of the shape, relative position, and degree of fusion of the pattern elements (Fig. 1O–Q) suggests that the number of genes associated with its formation can be much larger. It is also possible that the manifestation of this trait may depend on environmental factors.

Analysis of the available data indicates a linkage of the loci responsible for the incorporation of orange pigment (O) into the shell and the formation of brown bands (B^{p}). Within the framework of the corresponding hypothesis, the formation of four types of gametes (OB^{p+}, OB^{p-}, oB^{p+}, oB^{p-}) is possible, on the basis of which 10 unique genotypes can be formed. The corresponding phenotypes of individuals can be combined into four groups ("orange banded", "non-orange banded", "orange bandless", and "non-orange bandless"), and each of them should be characterized by its own peculiarities of joint inheritance of background color and bands. In the experiment, in the offspring of orange banded females, banded individuals with orange background color alone split off. In the offspring of yellow banded females, banded individuals had yellow background coloration as well. In the offspring of orange bandless females, only orange bandless individuals were recorded. In the offspring of females of the fourth group, banded individuals (if present) always had orange background color; the corresponding alleles were obtained, apparently, from fathers.

It should be noted as follows: in the cases studied by us, in the offspring of yellow banded females, individuals with bands had yellow background color alone. At first glance, this indicates the emergence of a new linkage pair resulting from a crossing over and the fact that alleles of one gene are responsible for the formation of yellow and orange coloration, which contradicts the data on the inheritance of the background color. However, this is not the case. Let the genes, responsible for the inheritance of orange pigment and bands, and the genes, responsible for the formation of yellow and purple background colors, be in different chromosomes. Then, the crossover gametes oB^{p+} can freely combine with all kinds of gametes, that give different variants of the background color with the participation of yellow and purple pigments (yellow, purple, yellow-purple, and depigmented; we can neglect gametes giving white-purple background color, since this variant is rare in *L. obtusata*). In purple molluscs, the bands will be invisible. We recorded depigmented individuals with brown bands in the experiment (under natural conditions, depigmented individuals most likely do not survive). In reality, only combinations of oB^{p+} gametes with variants of yellow background color (yellow-purple and yellow) will be observed, since they are the most abundant in the population, giving rise to the sensation of apparent linkage of yellow color with brown bands.

There are no data on the inheritance of the pattern of purple pigment spots and hypostracum coloration. A pattern of purple pigment spots is found only in individuals with a background color with the participation of orange pigment, both banded and bandless. It can be both a result of the activity of a separate gene, closely linked to the gene for brown bands, and a by-product of the activity of a "banded" gene. The formation of hypostracum is associated with a change in the mode of functional activity of the mantle margin cells; at the same time, a change in the programs of synthesis and incorporation of pigments into the shell can occur (Fig. 1N). Therefore, it stands to reason that a separate gene (or a group of genes) is responsible for the hypostracum color. It has to be noted that a separate genetic system is probably also responsible for the embryonic coloration of the shell, since the embryonic shell in periwinkles is always purple, even in depigmented individuals.

System for describing variability of shell coloration traits in *Littorina obtusata*

Group of phenes "Shell background coloration" – C.

C^D – "Depigmented" (Fig. 1A). A rare coloration variant: two individuals studied were obtained under laboratory conditions when analyzing the inheritance of shell coloration traits. The shell is depigmented, partially translucent, dull white, and horny in color (RGB[3]: $197{\pm}_{13}$: $180{\pm}_{12}$: $146{\pm}_{14}$). Border and underside are not expressed; shell microstructure is common. The hypostracum is depigmented. The embryonic shell contains a small amount of purple pigment. This phenotype can be confused with very old yellow periwinkles, in which the incorporation of pigment into the shell may have stopped.

C^P – "Purple" (Fig. 1B). One of the most common phenotypes in the White Sea populations (about 40 %). The visible shell color is dark brown, almost black ($77{\pm}_{19}$: $62{\pm}_{15}$: $56{\pm}_{18}$); border and underside are not expressed. The coloration intensity varies slightly. The prismatic layer is colored with purple pigment. In one part of individuals, the ostracum color is even; in other part, a gradient is observed, with a decrease in the pigmentation intensity from the outer to inner shell area (in some cases, the inner ostracum area is practically depigmented). Shell microstructure is common. The hypostracum may be purple or depigmented.

C^Y – "Yellow" (Fig. 1C). Quite a rare phenotype (0.3 %). The visible color is yellow ($195{\pm}_{32}$: $155{\pm}_{31}$: $22{\pm}_{23}$); the intensity varies relatively little. Border and underside are not expressed. The ostracum is colored with yellow pigment; microstructure is common. The coloration of the prismatic layer can be even, or there can be a gradient, with a decrease in the pigmentation intensity from the outer to inner shell area. The hypostracum is usually depigmented, occasionally purple.

C^O – "Orange" (Fig. 1D). Common phenotype (2 %). Visible color is orange, with varying intensity ($179{\pm}_{27}$: $96{\pm}_{12}$: $21{\pm}_{13}$); border and underside are not expressed. The irregular-prismatic layer is colored with orange pigment; microstructure is common. A gradient is possible, with a decrease in the pigmentation intensity from the outer to inner shell area. The hypostracum can be depigmented or colored with purple pigment (the latter variant is common in *L. saxatilis*). The shell may have a pattern of purple spots, which makes the visible coloration darker. On the contrary, a pattern of white spots makes the coloration lighter.

C^{YO} – "Yellow-orange" (Fig. 1E). Quite a rare phenotype (0.3 %). The visible shell color is yellow-orange ($176{\pm}_{31}$: $119{\pm}_{28}$: $9{\pm}_{7}$). There is no expressed border; underside has the same color as other shell areas. Shell microstructure is common. In some individuals, the outer area of the prismatic layer is colored predominantly with yellow pigment, and the inner area is predominantly orange; in most individuals, the pigments are blended. The color of yellow-orange molluscs varies from almost orange to almost yellow, and this can cause problems in identifying the phenotype (Lezin & Kozminskii, 2008). The presence of a white checker lightens the coloration and can also cause identification problems. The hypostracum is usually colorless.

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[3] Color coordinates characterizing the shell coloration elements according to the RGB color model (Kozminskii & Lezin, 2006).

C^{OP} – "Orange-purple" (Fig. 1F). Quite a rare phenotype (0.5 %). The visible shell color is orange ($176\pm_{16}$: $93\pm_{16}$: $38\pm_{18}$); it seems darker due to purple coloration of the inner ostracum area and often incorporation of purple pigment in the form of spots. There is an orange border along the mouth edge. Shell underside is darker and may have a distinct purple tint. The outer area of the prismatic layer is colored with orange pigment, and the inner one is purple. Typically, purple sublayer is very thin. Phenotype identification is difficult, since the incorporation of orange pigment into the shell seems to inhibit the incorporation of purple one, and only a thin, inconspicuous purple sublayer retains at the border with the hypostracum. The latter is colorless or colored with purple pigment.

C^{WP} – "White-purple" (Fig. 1G). A relatively rare coloration variant in the White Sea *L. obtusata* (0.5 %). Unlike the color of depigmented individuals, white color is saturated, shiny ($206\pm_{17}$: $181\pm_{16}$: $111\pm_{24}$). The shell is dense, opaque. Border along the mouth edge is white; shell underside is purple. The outer area of the prismatic layer is colored white, and the inner area is purple. The thickness of a white sublayer varies; accordingly, several variants of white-purple background color, with different intensity, can be distinguished. On thin sections and chips, the shell in the white sublayer area has a denser than usual microstructure with a bluish tint. The hypostracum is always colored with purple pigment.

C^{YP} – "Yellow-purple" (Fig. 1H–J). The most common variant of the background coloration in the White Sea populations (55 %). The visible coloration varies from brown ($116\pm_{8}$: $88\pm_{9}$: $60\pm_{8}$) to deep yellow ($219\pm_{28}$: $181\pm_{17}$: $61\pm_{12}$), but most individuals have olive shell coloration ($123\pm_{25}$: $101\pm_{23}$: $51\pm_{23}$), with yellow border along the mouth edge and purple underside. The outer ostracum area is colored with yellow pigment, the inner one is purple. Shell microstructure is common. Coloration depends on the thickness of yellow pigment sublayer and its pigmentation intensity. In brown individuals, trace amounts of yellow pigment are incorporated into the shell; the outer ostracum area contains traces of purple pigment and appears to be discolored[4]. Olive individuals have a relatively high amount of yellow pigment; when overlaid with traces of purple pigment, a visible greenish coloration is formed in the outer ostracum area. In individuals with deep yellow color, the yellow sublayer is very thick, and the pigmentation intensity is very high; purple pigment is noticeable only in the lower area of the prismatic layer and in the hypostracum. Brown molluscs have a colorless border along the mouth edge; olive and yellow molluscs have a yellow one. The hypostracum is usually purple.

C^{YWP} – "Yellow-white-purple" (Fig. 1K). A very rare variant of the background coloration (0.01 %). The visible color varies from white-purple with a slight admixture of yellow pigment to lemon one ($209\pm_{34}$: $179\pm_{28}$: $33\pm_{14}$). Border along the mouth edge is white or yellowish; underside is purple. The distribution of white and purple pigments corresponds to that of white-purple individuals. White sublayer has a denser microstructure. In the outer ostracum area, a distinct admixture of yellow pigment is noticeable. The hypostracum is purple.

C^{YOP} – "Yellow-orange-purple" (Fig. 1L). Apparently, a rather rare phenotype (0.5 %), but its identification is difficult, since purple sublayer is usually very poorly expressed. The visible color of the shell ($173\pm_{30}$: $116\pm_{29}$: $16\pm_{10}$) corresponds to that of yellow-orange molluscs (if the purple sublayer is weakly expressed) or is slightly darker (if the purple sublayer is expressed enough). In the latter case, a yellow-orange border along the mouth edge and dark shell underside become noticeable. Shell microstructure is common. This variant of the background coloration is characteristic of periwinkles with brown bands.

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[4] Apparently, this is due to the fact that the activity of the gene (or genes) providing the incorporation of yellow pigment into the shell simultaneously inhibits the incorporation of purple pigment into the shell.

Interestingly, in this case, a fairly clear separation of yellow and orange pigments is observed as well: the outer area of the prismatic layer is distinctly yellow outside the brown bands, and the middle area of the prismatic layer is orange; at the border with the hypostracum, the area is purple. The hypostracum can be colorless or colored with purple pigment.

C^{OWP} – "Orange-white-purple" (Fig. 1M). This variant of the background color was found in *L. saxatilis* alone (0.01 %). The detection of similar *L. obtusata* is possible as well, but their occurrence should be very low, since white-purple *L. obtusata* are extremely rare. The visible coloration ($220\pm_{21}$: $148\pm_{25}$: $40\pm_{36}$) varies from white-purple with streaks of orange pigment to reddish-cream. Border is white; shell underside is purple. The distribution of guanine and purple pigment corresponds to that of white-purple individuals; in the outer ostracum area, there is an admixture of orange pigment. The prismatic layer at the location of a white sublayer has a denser than usual microstructure with a bluish tint. The hypostracum is purple.

Group of phenes "Hypostracum coloration" – H.

The hypostracum of the periwinkle shell (Fig. 1N) can be purple (H^P; 66 %) or colorless (H^D; 34 %). The hypostracum discoloration is not associated with the formation of a denser microstructure, which is characteristic of white-colored shell areas. The visible coloration of the hypostracum is affected by the color of the irregular-prismatic layer: for example, in molluscs with yellow and orange ostracum coloration, the colorless hypostracum seems yellowish and orangey. The effect of the hypostracum color on the shell background color is small: in yellow and orange periwinkles, it makes the visible shell coloration darker. No unambiguous relationship was found between the colors of the hypostracum and ostracum. If the color of the prismatic layer is yellow or orange, the hypostracum is usually colorless; if it is purple, the hypostracum is colored purple.

Group of phenes "Pattern of spots" – S.

S^W – "Pattern of white spots" (Fig. 1O–Q). Most periwinkles (55 %) are characterized by the presence of a pattern of white spots on the shell ($198\pm_{31}$: $158\pm_{32}$: $90\pm_{31}$). Separate elements of the spotted pattern are lenticular incorporations of white pigment interconnected by thin layers-anastomoses. The pattern elements are located in the upper area of the prismatic layer. At the location of the pattern elements, the shell has a denser microstructure than in other areas. The shape, relative location, and degree of fusion of the spotted pattern elements are very variable. The traits are found in molluscs with a wide variety of phenotypes. No relationship was found between the presence of a pattern of white spots, bands, and shell background coloration. In periwinkles with yellow and orange shell background color, spots are yellowish. A pattern of white spots is not formed at the location of brown bands, but may be formed at the location of an orange band.

S^P – "Pattern of purple spots" (Fig. 1R). As in the case of a pattern of white spots, separate elements are lenticular incorporations of purple pigment interconnected by thin layers-anastomoses ($125\pm_{43}$: $66\pm_{27}$: $22\pm_{29}$). The pattern elements are located at the top of the ostracum. Microstructure of the irregular-prismatic layer at the location of the pattern elements is common. The trait is typical for periwinkles, which have a background color with the participation of orange pigment and brown bands on the shell. It is found in orange and orange-purple bandless molluscs as well (0.4 %). It appears much later than brown bands and is noticeable only on the last whorls of the shell.

S_o – "No pattern of spots".

Group of phenes "Bands" – B.

B^W – "White bands" (Fig. 1S). A common color variant (2 %). The shell has one or two wide white bands ($232\pm_{20}$: $203\pm_{21}$: $110\pm_{42}$). The main band is located along the whorl periphery; the narrower one, in the suture area of the shell. The bands are layers of additional pigment located in the outer area of the irregular-prismatic layer. At the location of the bands, the shell has a denser microstructure than in other areas. The trait is found in periwinkles with yellow, orange, purple, and yellow-purple background coloration. Since the bands are immersed in the ostracum, their visible color may differ from pure white: in periwinkles with yellow-purple and yellow ostracum, it is yellowish; in orange and orange-purple molluscs, it is orangish. The variability of the trait manifests itself in a change in the pigmentation intensity of the bands (down to complete depigmentation) and in narrowing or complete disappearance of the additional band. In the case of complete depigmentation, in molluscs with a yellow background coloration of the shell, band silhouettes are visible in the lumen in the spots of their usual location.

B^O – "Orange bands" (Fig. 1T). A relatively rare color variant (0.8 %). The shell has one wide orange band ($155\pm_{44}$: $95\pm_{30}$: $29\pm_{24}$) at the whorl periphery. The bands are interlayers of orange pigment with well-defined edges. The location of the bands in the shell thickness is characterized by expressed ontogenetic variability. At the time of their appearance (in the first year of life of a mollusc), those are located in the inner ostracum area; subsequently, they spread to almost the entire ostracum and disappear in the fourth-fifth year shifting to the hypostracum. At the location of the bands, shell microstructure is common. Orange bands are characteristic of molluscs with a yellow-purple shell; sometimes, the trait was observed in individuals with a purple background coloration.

B^P – "Brown (purple) bands" (Fig. 1U). A rare color variant (0.2 %). The shell has two wide bands of different shades of brown, sometimes almost black ($81\pm_{35}$: $50\pm_{23}$: $23\pm_{20}$), located in the upper and lower whorl areas. The bands can either be organized as interlayers of purple pigment in the upper ostracum area, below which a more or less expressed pigment "trail" extends, or occupy the entire shell thickness. At the location of the bands, shell microstructure is common. Brown bands are usually found in *L. obtusata* with a background coloration with orange pigment. Less commonly, this trait is observed in yellow and yellow-purple molluscs; in rare cases, in periwinkles with a depigmented shell[5]. The variability of the trait manifests itself in a change in the coloration intensity of the bands (down to complete depigmentation) and their width, in reduction of separate bands, and in fusion and distribution of bands over the entire shell surface or its part. In yellow-orange periwinkles, depigmentation of the bands results in a color variant when the individuals look like yellow molluscs with two bands. A pattern of spots of white or purple pigment, if present, is always formed outside the area of the bands.

B_o – "No bands".

Differences between two classification systems. The main difference from the previous version of the classification system for coloration traits (Sergievsky et al., 1995) is the correspondence between selected phenotypes and actually existing groups of genes responsible for the formation of various elementary traits. The pre-existing groups of phenes "white color" and "pigment coloration of ostracum" are combined into one group "background shell coloration", since the background color arises as a result of the interaction of a number of substances, some of which (melanin and carotenoids) are pigments, and some (guanine) serve as the basis for the formation of structural color. Quantitative gradations of traits – similar to the previously identified variants W_1–W_3 – are not specially highlighted in the classification system proposed by us. However, they can be useful; if necessary, the corresponding numeric indices can be easily entered into the model.

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[5] In *L. saxatilis*, the trait is always associated with white-purple background coloration.

In the group of phenes "bands", several new phenes were identified, since the results of morphological and genetic studies showed that brown, white, and orange bands are independent structures, which differ in terms of organization, ontogenesis, and inheritance. The "bandless" phenotype is now common to all banded variants, meaning that the genetic systems responsible for the formation of three band types are not active.

The group of phenes associated with the formation of a spotted pattern underwent relatively small changes. However, the data on genetics (Kozminskii et al., 2010) show that the mechanism of inheritance of the trait is more complicated than previously assumed: at least two genes are responsible for the presence/absence of a pattern of white spots. The extremely high variability of the trait allows suggesting that its formation is affected by a much larger number of genes. The emergence of new data on genetics will require clarification or even a significant revision of this group of phenes, with the involvement of new elementary traits. Moreover, this group includes now the previously undescribed "purple-spotted" phenotype. It should be emphasized as follows: although the mechanisms of formation of two variants of the pattern are similar, different genes are most likely responsible for their inheritance.

Conclusion. Despite the widespread use of molecular biological methods, the studies on population and phenetics are still relevant: in particular, when it comes to the investigation of microevolutionary processes directly related to coloration, *e. g.* when the latter performs a protective or thermoregulatory function. A prerequisite for such studies is knowledge of the mechanisms of formation and inheritance of coloration traits. The involvement into the analysis of new species, the genetic study of which is difficult for some reasons, is possible only on the basis of generalization of the available information on the species investigated morphologically and genetically, *i. e.* by methods of population phenetics. In carrying out that kind of research, molecular biological markers rather perform an auxiliary function, since our ideas about the molecular genetic mechanisms underlying the formation and inheritance of complex traits (such as color) are still insufficient to fully replace population-genetic and phenetic methods with molecular biological ones. Moreover, given the high diversity of pigments involved in the formation of shell coloration (Comfort, 1950, 1951 ; Hedegaard et al., 2006 ; Lucas, 1974 ; Williams, 2017), it is hardly possible to speak of the existence of a single "model" of its formation and inheritance. To identify common patterns, it is necessary to use methods of population phenetics and to carry out their joint analysis with molecular biological data as well.

A key prerequisite for the development of the population-phenetic direction of studies is the formation of reasonable systems for description and classification of coloration traits. The system of classification of coloration traits in molluscs of the genus *Littorina* proposed by us can serve as a good basis for the development of such systems.

As noted above, the variety of pigments involved in the formation of shell color in molluscs is great (Comfort, 1950, 1951 ; Hedegaard et al., 2006 ; Lucas, 1974 ; Williams, 2017). Like in periwinkles, in many prosobranchias, the background shell color is formed with the participation of several pigments. It is doubtful whether the incorporation of different pigments into the shell was controlled by a single gene. The implementation of a polygenic inheritance scheme seems to be much more probable (Kozminsky, 2014), with a separate (and, possibly, polyallelic) gene responsible for the incorporation of each pigment into the shell. The monogenic polyallelic inheritance pattern, which is characteristic of pulmonates (Backeljau et al., 2001), is apparently a special case of this more general and universal inheritance pattern. Therefore, any system of classification of coloration traits that claims to be universal should be based on a polygenic scheme for the formation and inheritance of background coloration.

The coloration of the shell of gastropods can be represented as a combination of a number of elementary traits. The elementary traits identified in periwinkles are common in molluscs. Each color trait corresponds to a certain distribution of pigments in the shell, which is a consequence of a regular change in functional activity of pigment-forming cells of the mantle margin (Sergievsky et al., 1995). The peculiarities of the distribution of pigments found in periwinkles with the formation of the background color, bands, and pattern of spots are quite common for molluscs.

Taking into account the variety of shell coloration of gastropods, it is obvious that the elementary traits of coloration in periwinkles and corresponding variants of functional activity of chromatophores do not exhaust all the possible diversity. Specifically, in order to describe a high variety of modes of the pattern of spots on the shell, it is necessary to highlight additional elementary traits and corresponding modes of functional activity of pigment-forming cells, as well as to study the mechanisms of their inheritance. Other possible areas of research are the study of ontogenetic changes in coloration traits and the analysis of the relationship between possible modes of functional activity of pigment-forming cells and formation of different calcium layers of the shell. The introduction of new elementary color traits into consideration will significantly expand the capabilities of the classification system proposed by us.

The above-described classification system for coloration traits has been successfully tested in the study of the inheritance of shell coloration traits in periwinkles (Kozminsky, 2011, 2014, 2016 ; Kozminskii et al., 2010) and the distribution of pigments in the mollusc shell (Kozminskii & Lezin, 2007 ; Lezin & Kozminskii, 2008), as well as in carrying out population biological monitoring in the Kandalaksha State Nature Reserve in 2005–2019 (Kozminsky, 2006, 2020). Due to the use of universal principles of classification of coloration elements (analysis of the diversity of pigments, isolation of elementary traits, and study of the mechanisms of their formation and inheritance), the system developed can be used to describe the shell color not only in periwinkles, but also in the widest range of gastropods.

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ОПИСАНИЕ ПОЛИМОРФИЗМА И КЛАССИФИКАЦИЯ ПРИЗНАКОВ ОКРАСКИ РАКОВИНЫ У БРЮХОНОГИХ МОЛЛЮСКОВ НА ПРИМЕРЕ *LITTORINA OBTUSATA* (GASTROPODA: LITTORINIDAE)

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Понимание закономерностей микроэволюционных процессов предполагает проведение широкого спектра популяционно-генетических исследований на разных видах. Однако количество видов, изученных в генетическом отношении, ограничено вследствие значительных методических трудностей при проверке генетической обусловленности различных признаков. Выход из этого положения заключается в развитии фенетики популяций, которая позволяет включить в рассмотрение большое количество новых видов при условии разработки обоснованных систем описания изменчивости и классификации фенотипических признаков. Классическим объектом для проведения популяционно-генетических исследований, связанных с полиморфизмом по окраске раковины, служат брюхоногие моллюски. В качестве основы для разработки универсальной для этой группы системы описания и классификации признаков окраски может быть использована параметрическая система классификации, предложенная С. О. Сергиевским с соавторами для моллюсков рода *Littorina*. В связи с появлением в последние годы большого количества новых данных, эта система нуждается в коррекции и пересмотре. Целью нашего исследования было провести ревизию этой системы с учётом новых данных о составе пигментов, особенностях формирования и наследования признаков окраски, их совместной встречаемости и т. д. В настоящей работе на примере беломорских моллюсков *L. obtusata* представлена переработанная система классификации признаков окраски раковины, в основу которой положено представление о формировании фенотипа как совокупности ряда элементарных признаков. К их числу отнесены признаки, связанные с формированием: 1) фоновой окраски раковины (способность к включению в остракум разных пигментов и окраска гипостракума); 2) рисунка из пятен (наличие включений белого и/или коричневого пигмента); 3) широких продольных полос (коричневых, белых и оранжевых). Элементарные признаки выделены с учётом задействованных пигментов, механизмов их формирования и наследования. При описании окраски раковины элементарные признаки сначала используются для описания относительно простых признаков (группы фенов «Фоновая окраска раковины», «Окраска гипостракума», «Рисунок из пятен», «Широкие продольные полосы»), которые в последующем комбинируются для описания фенотипа в целом. В работе приведён обзор данных по качественному составу пигментов раковины у литторин и закономерностям формирования и наследования признаков окраски, описаны их возможные варианты и комбинации. Приведены описания выделенных фенов с указанием их встречаемости, количественной характеристики цвета в системе МКО RGB, особенностей их формирования с учётом распределения пигментов в толще раковины. Указаны видимые признаки, которые могут быть использованы при оценке распределения пигментов в раковине; описаны особенности изменения признаков в онтогенезе. Несмотря на то, что предложенная система классификации описана на примере *L. obtusata*, она может быть также использована при описании изменчивости у других видов литторин, а с некоторыми доработками — у самого широкого спектра брюхоногих моллюсков.

Ключевые слова: полиморфизм, окраска раковины, система классификации, брюхоногие моллюски, *Littorina*

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PHENOMENON OF THE COMPLETE SUPPRESSION OF CARDIAC ACTIVITY IN THE BLACK SEA SCORPIONFISH *SCORPAENA PORCUS* (SCORPAENIDAE) DURING AN ALERTNESS REACTION

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Teleosts serve as experimental models for the physiological and pathophysiological processes investigation, in particular those related to the heart work. Methods allowing to analyze the frequency parameters of the heart functioning over a long period of time require taking into account the peculiarities of fish behavioral reactions, that can affect the results of the experiment. The aim of this study was to examine the effect of the simplest test loading (sound stimulus) on the frequency parameters of the heart activity recorded by the fiber-optic method. The objects of the study were adults of *Scorpaena porcus* (12–15 cm long, 80–120 g in weight). In the course of experiments, each scorpionfish was kept in a separate aquarium with seawater (400×400×350 mm), with constant temperature (21 ± 0.5) °C and given oxygen content (5.5–6.7 $mg \cdot L^{-1}$, normoxia). The heart rate (HR) recording was carried out by an invasive fiber-optic method, the essence of which is to transmit the radiation of the infrared semiconductor laser of the photoplethysmograph through a thin fiber-optic cable to the pericardial membrane of the heart and then to fix the signal reflected from the contracting myocardium in the photodetector. During implantation of the photoplethysmograph light guides, the fish were anesthetized by placing them in an anesthetic solution (urethane, 2.4 $g \cdot L^{-1}$ of seawater). In the fornix of the opercular cavity above the area of the conditional heart projection, a minimal dissection of the lining epithelium was performed, through which the underlying tissues were sequentially separated by a blunt method until the pericardial membrane was reached without breaking it. Through the lumen formed in the tissues, two optical light guide sensors were introduced to the surface of the pericardial membrane. Further, free-swimming scorpionfish participated in the experiment after a day of rehabilitation after the surgery. Additionally, we assessed the functional state of the animals by visual fixation of respiratory activity by the quantity of movements of the opercular covers *per* minute. During studying the test loading effect on the correct registration of the scorpionfish HR, the phenomenon of temporary complete suppression of cardiac activity was revealed, which manifested itself upon presentation of sound stimuli (alertness, "freezing" reaction). The duration of cardiac arrest was 31 to 50 seconds; it was accompanied by the cessation of movement of the opercular covers (respiratory arrest, apnea). During the restoration of cardiac activity, two types of physiological reactions were noted. The first type of recovery reaction was characterized by a simultaneous 1.5-fold increase in the HR and a 2-fold enhancement in the photoplethysmograph signal amplitude. The second type of reaction was accompanied by a rise in the HR by 22 % ($p < 0.05$) against the backdrop of a decrement in the signal amplitude of the photoplethysmograph sensors by 28 % ($p < 0.05$); within ~ 120 seconds, the scorpionfish HR returned to baseline. It is assumed that the short-term delay in the scorpionfish cardiac activity is based on the phenomenon of cardiorespiratory coupling and synchronization. The behavioral reaction in the form of suppression for the cardiac and simultaneously respiratory activity generation ensures the complete absence of acoustic and electrical signals, which unmask an ambush predator location, and contributes to the scorpionfish survival.

Keywords: fish, *Scorpaena porcus*, heart, cardiorespiratory coupling, alertness reaction

Teleosts largely retain the similarity of some anatomical and physiological features with mammals, which makes them a convenient and reliable experimental model for studying fundamental / “conserved” physiological and pathophysiological processes occurring in different classes of animals (Gut et al., 2017). The two-chambered fish heart is similar to the human heart in the peculiarities of its development, regeneration, electrical properties of the myocardium, and the presence of several pathological syndromes, in particular long QT syndrome (Sun et al., 2009). There are various approaches to assessing the cardiac activity of teleost fish, which imply the use of variety of instrumental “tools” ensuring the solution of specific experimental problems. In any case, a method allowing to analyze the rate characteristics of the heart rhythm over a long period of time (up to several days) requires taking into account the features of fish behavioral reactions, that can affect the results of the experiment. As an object of research, we chose the Black Sea scorpionfish (sea ruff) *Scorpaena porcus*, which is resistant to a number of stress factors (Kolesnikova & Golovina, 2020 ; Soldatov et al., 2020).

The aim of this study is to examine the effect of the simplest test loading (sound stimulus) on the frequency parameters of the scorpionfish heart activity recorded by the fiber-optic method. The task of the work was to investigate the influence of random environmental factors on the results of recording the scorpionfish heart rate.

MATERIAL AND METHODS

The object of the study was 7 adult specimens of *Scorpaena porcus* Linnaeus, 1758, 12–15 cm long, 80–120 g in weight. The fish were caught in August – September with a fixed seine in Sevastopol water area and delivered to the laboratory in 60-L plastic tanks, with aeration. To relieve stress after transportation, the fish were kept for 7 days in a flow-through aquarium; only actively moving and feeding individuals were used. In the course of experiments, each scorpionfish was kept in a separate aquarium-stand with seawater (400×400×350 mm) under controlled temperature (21 ± 0.5) °C and oxygen content of 5.5–6.7 $\mathrm{mg \cdot L^{-1}}$, normoxia (Soldatov et al., 2020).

The recording of the heart rate (hereinafter HR) was performed by the invasive fiber-optic method for the first time on a representative of teleost fish; previously, a similar method was used on crustaceans (Sladkova et al., 2016). The essence of the fiber-optic method is to transmit the radiation of an infrared semiconductor laser of a photoplethysmograph (LVOF-3, manufactured by Research Innovation Center “EcoContour”, Russia) through a thin fiber-optic cable to the pericardial membrane of the heart (Fig. 1A) and then to fix the signal reflected from the contracting myocardium in the photodetector.

After appropriate amplification, filtration, and digitalization, the cardiac signal was analyzed on a computer and recorded in the form of a photoplethysmogram (Fig. 1B), which allows estimating the dynamics of the HR. The fiber-optic method makes it possible to examine the functional state of fish for a long time without affecting their behavior or causing stress. During implantation of photoplethysmograph light guides, the fish adapted to laboratory conditions were anesthetized by placing them in an anesthetic solution (urethane, 2.4 $\mathrm{g \cdot L^{-1}}$ of seawater) (Soldatov, 2005). Then, in the fornix of the opercular cavity above the area of the heart conditional projection, a minimal dissection of the lining epithelium was performed, through which the underlying tissues were sequentially disunited so as to avoid damaging them, until the pericardial membrane was reached without breaking it. Through the lumen formed in the tissues, two optical sensors of light guides were introduced to the surface of the pericardial membrane.

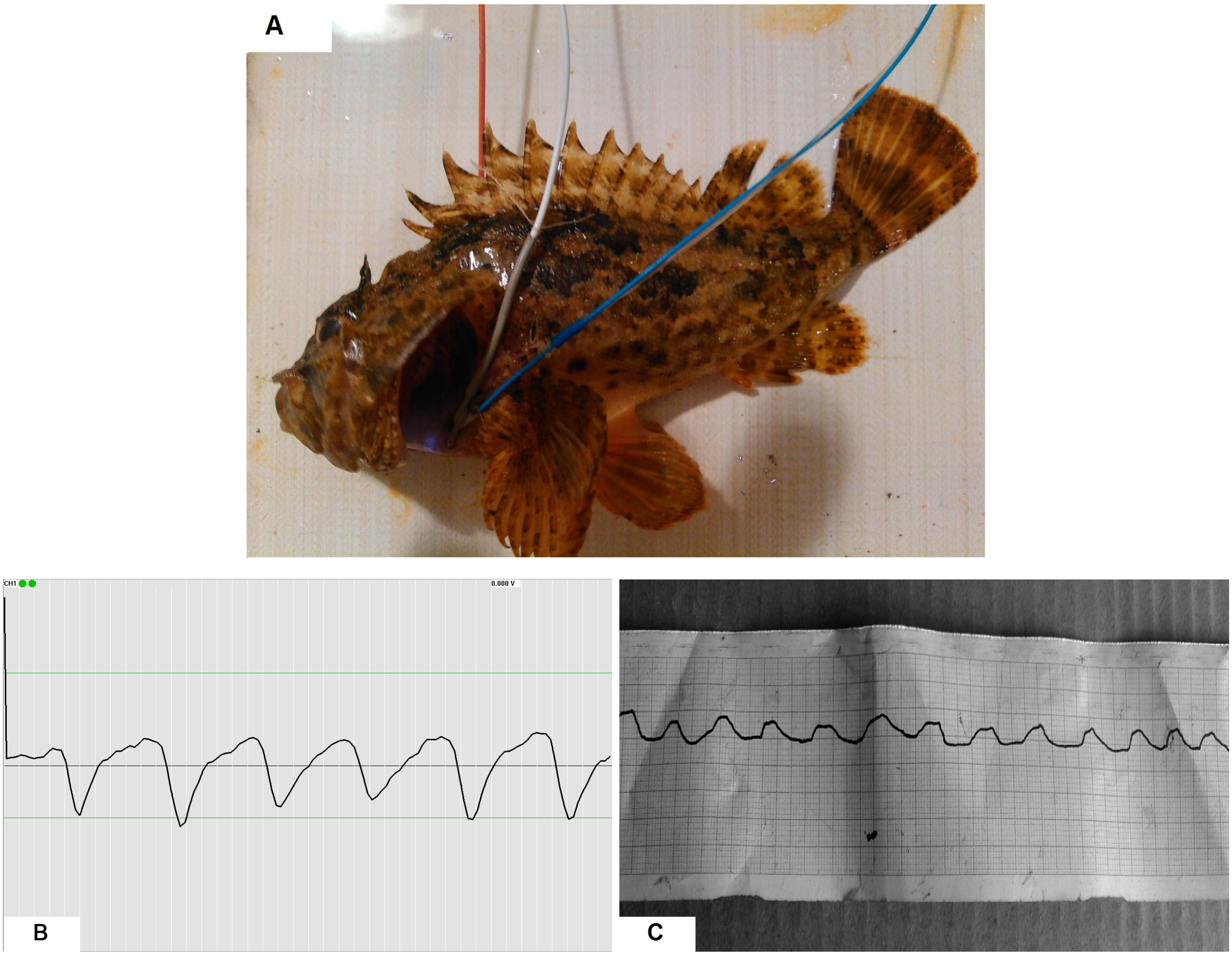

Fig. 1. *Scorpaena porcus* with attached recording sensors (A): 1 and 2 – electrodes of a portable electrocardiograph; 3 and 4 – light guides of photoplethysmograph optical sensors. Steady state cardiac activity of *Scorpaena porcus*: photoplethysmograph registration (B); portable electrocardiograph registration (C)

A stable position of the sensors was ensured by hemming the cables of light guides with silk thread to the skin above the cleithrum and in the dorsal fin area, which made it possible to fix the cables in the vertical position, perpendicular to the longitudinal axis of the scorpionfish body. Later, free-swimming scorpionfish participated in the experiment after a day of rehabilitation after the surgery. Fish reactions were taken into account only to the first two presentations of the stimulus because of partial extinction of subsequent reactions to the stimulus in the form of shortening the time for the manifestation of alertness not canceling the established physiological phenomenon. Photoplethysmograms were compared with ECG (Fig. 1B) obtained using a portable electrocardiograph (EK1T-03M); during ECG recording, modified electrodes were placed at the base of the dorsal and left pectoral fins of the scorpionfish (Fig. 1A). Additionally, the functional state of the animals was assessed by visual fixation of respiratory activity by the number of movements of the opercular covers *per* minute. To study the effect of test loadings on cardiac activity, we chose the simplest sound stimulus: tapping on the aquarium glass, which could affect the physiological reactions of the scorpionfish.

Statistical comparisons were made on the basis of a two-tailed Student's *t*-test; the results are presented as ($M \pm m$).

RESULTS AND DISCUSSION

The obtained photoplethysmograms allowed determining the mean values of the scorpionfish HR at rest, (11.22 ± 1.07) beats·min^{-1}, which corresponded to a similar number of respiratory movements (Fig. 2). When studying the effect of the test loading on the recorded parameters of the myocardium, we revealed the phenomenon of complete suppression of cardiac activity. Thus, a simple tapping on the aquarium glass, along with a hand wave, was accompanied by the emergence of an alertness reaction (“freezing”) in the fish followed by almost complete disappearance of heart contractions (Fig. 2A).

It should be noted that the alertness reaction is related to the cessation of movement of the opercular covers (respiratory arrest, apnea) as well. Several tens of seconds (31 to 50 s; (39.0 ± 7.6) s) after the action of the sound stimulus, the contraction of the scorpionfish heart resumed (Fig. 2B). During the restoration of cardiac activity, two types of physiological reactions were revealed. The first type of recovery reaction ($n = 2$) was characterized by a simultaneous 1.5-fold increase in the HR (tachycardia) and a 2-fold increase in the photoplethysmograph signal amplitude (Fig. 2C). The second type of recovery reaction ($n = 5$) was accompanied by an increase in the HR by 22 % ($p < 0.05$) against the backdrop of a decrease in the signal amplitude of the photoplethysmograph sensors by 28 % ($p < 0.05$) (Fig. 2D). Within approximately 120 s, the scorpionfish HR returned to baseline. Such a reaction of the cardiac muscle of teleost fish has not been previously described in the literature.

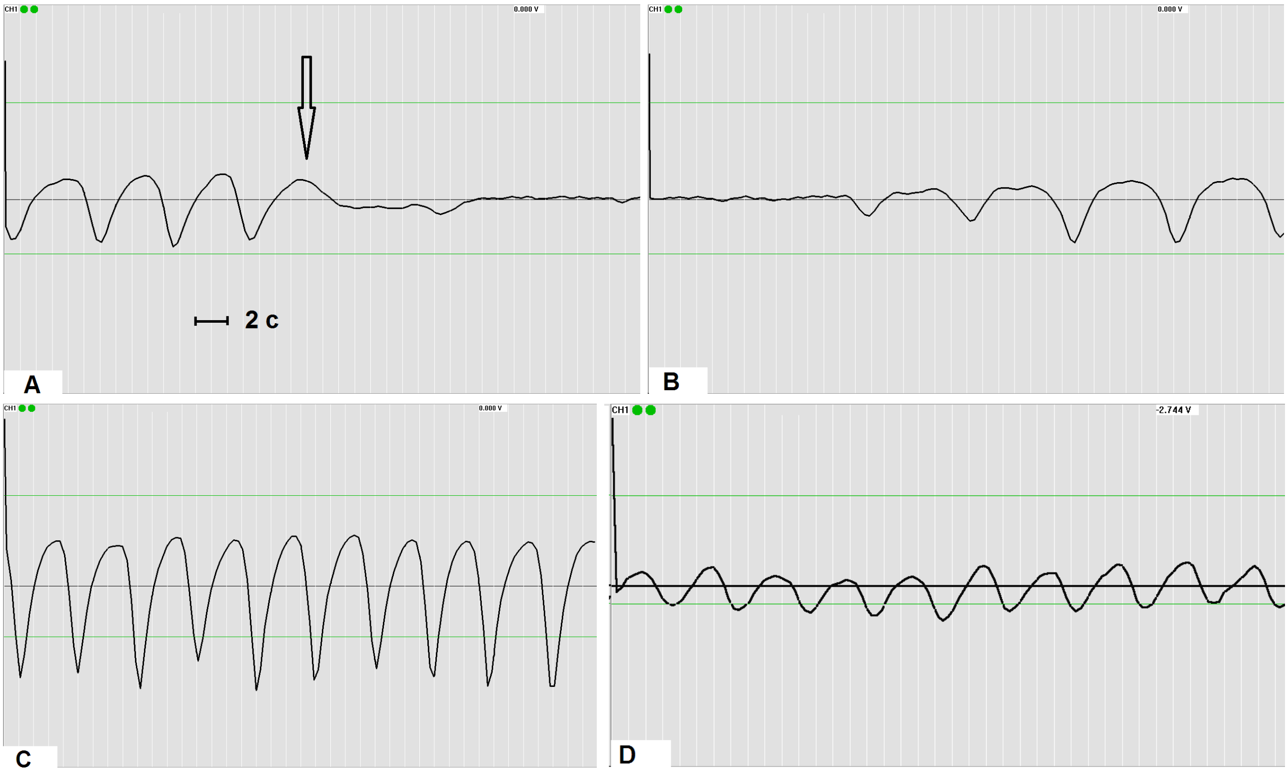

Fig. 2. Photoplethysmograms of the scorpionfish *Scorpaena porcus*: A – delayed cardiac activity of the scorpionfish during the alertness reaction under the tapping on the aquarium wall (the moment of stimulus presentation is marked by an arrow); B – restoration of the scorpionfish cardiac activity after the stimulus and the cancellation of alertness reaction; C – an increase in the heart rate and its amplitude in the period following the cancellation of the alertness reaction (first type of restorative physiological reaction); D – an increase in the heart rate at low amplitude in the period following the cancellation of the alertness reaction (second type of restorative physiological reaction)

As known, the contractions of the heart of vertebrates are initiated by a built-in myogenic generator of heart rhythm; in addition, the steady state cardiac activity values can be regulated by the mechanisms of the central nervous system, which, using the efferent pathways of the autonomic nervous system, have both inhibitory parasympathetic and excitatory sympathetic effects on the heart work (Taylor et al., 2014).

There is a close temporal relationship between cardiac contractions and respiratory activity of fish in the form of cardiorespiratory coupling (hereinafter CRC) and cardiorespiratory synchronization (hereinafter CRS) reaching a ratio of 1:1 (Satchell, 1959 ; Taylor, 1992 ; Taylor et al., 2009); those are determined by the specificity of water flow movement through the branchial apparatus and its perfusion with circulating blood. This close coordination between ventilation and perfusion proportions helps in optimizing gas exchange related to circulatory counterflow in the branchial tissue (Taylor, 1992). CRS appears to depend on a combination of "direct" central and reflex, associated with different receptors, control mechanisms (Taylor, 1992 ; Taylor et al., 1999). The anatomical basis for the GRS manifestation is the close proximity of cardio and respiratory centers in the brainstem, including elements of the motor nuclei of trigeminal (V), facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves (hereinafter CNs); motor nuclei of CNs are connected with each other and with the reticular formation.

Certain CRS elements are also present in mammals in the form of an HR increase during inspiration defined as respiratory sinus arrhythmia (RSA) (Jordan & Spyer, 1987). The cause for RSA is considered to be respiratory-related fluctuations of the inhibitory action of the vagal nerve (*n. vagus*, X CN) on myocardial contractile activity, which are formed under the depressor effect of the inspiratory neurons of the ventral respiratory group localized in the double nucleus (*nucleus ambiguus*) on *n. vagus* preganglionic neurons. In teleost fish, "varying" HR includes "respiratory components" as well. Activity of *n. vagus* can provide the predominant mode of regulation of the fish cardiac function (Taylor et al., 1999), which is eliminated by vagotomy (*n. vagus* transection) (Cambell et al., 2004). As believed, *n. vagus* inhibitory effect is realized through m-cholinergic receptors directly related to the myogenic mechanism of the HR generation (Taylor et al., 1999).

In marine fish, the tonic activity of *n. vagus* cardiac branches is regulated relatively to the influx of afferent impulses from the chemo- (Cambell et al., 2004) and mechanoreceptors of the branchial apparatus (Young et al., 1993). Thus, in an experiment with stimulation of *n. vagus* gill branches, the presence of afferent feedback was established, which probably "originates" from the branchial mechanoreceptors and controls the rhythmic activity of *n. vagus* cardiac branches (Young et al., 1993); moreover, individual *n. vagus* preganglionic neurons show a distinct response to mechanical stimulation of the branchial septa in the form of such a surge in their activity, as a result of which transient bradycardia may develop (Barrett & Taylor, 1985). Pronounced suppression of the HR during stimulation of *n. vagus* is shown in European plaice *Pleuronectes platessa* (Cobb & Santer, 1972) and common carp *Cyprinus carpio* (Saito, 1973). Moreover, it was established that an increase in the HR, that develops after vagotomy, was also accompanied by the cancellation of the so-called approach reflex with the characteristic periods of bradycardia observed during alert in intact rainbow trout *Oncorhynchus mykiss* (Priede, 1974).

As mentioned above, in the scorpionfish, suppression of cardiac muscle contractions was observed simultaneously with the cessation of movements of the opercular covers (respiratory arrest). Fish retain the features of a more primitive metameric organization of the respiratory system compared to mammals (Satchell, 1959), when numerous gill mechanoreceptors are provided with inhibitory afferent fibers in the gill branches of VII, IX CNs, including *n. vagus* (Sutterlin & Saunders, 1969). The afferent impulses input initiated during inspiration from the gill mechanoreceptors is carried by *n. vagus* fibers and is involved in the regulation of gill respiration in fish (Satchell, 1959); probably, the mentioned mechanism in a certain approximation is analogous to the Hering – Breuer reflex of mammals, since in fish, in addition to episodes of bradycardia, stimulation of *n. vagus* is accompanied by breath holding (Satchell, 1959). At the same time, in mammals, breath holding in the form of transient apnea occurs during pharmacological blockade of *n. vagus* (Harris & Milsom, 2001). The question of whether it is possible in fish to situationally eliminate/modify afferent input of *n. vagus* before the development of apnea (Harris & Milsom, 2001) or use the feedback between the reflex cessation of breathing and the activity of *n. vagus*, which mediates cardiac suppression, requires further study.

The hindbrain containing pons and cerebellum is also involved in the formation of respiratory movements in fish (Ballintijn & Roberts, 1982). This conclusion is based on the results of transection of the fish rhomboid brain along the caudal and rostral borders, which was not accompanied by the cancellation of the respiratory activity provided by the somatic muscles of the branchial apparatus. The participation of the coordinating motor center (*inter alia* as a universal regulator of somatic and autonomic functions) in the mechanisms of respiratory activity generation of fish can facilitate the reflex cessation of the branchial apparatus ventilatory movements, which are indirectly associated with the mechanisms of the HR formation.

It can be assumed as follows: the main trigger of the “conservative” CRS mechanism is respiratory modulation of heart contractions (Taylor et al., 2014), which involves *n. vagus* “pathways”. The complete suppression of cardiac activity, that we established during the alertness reaction of the scorpionfish, is a special case of CRS, since the cessation of rhythmic contractions of the heart muscle was noted simultaneously with the termination of movements of the opercular covers providing the respiratory process. Obviously, in the scorpionfish body, CRS degree acquires such a level, at which the reflex cessation of respiratory movements causes a complete suppression of the heart work.

It is known that fish life takes place in a variety of physical fields (light, acoustic, and electric ones) (Barinova & Asylbekova, 2019). At the same time, the visibility range of objects in water is very small and fluctuates within bounded limits (one to two tens of meters). In a dense aquatic environment, mechanical, sound vibrations spread very quickly over considerable distances, which can either contribute to unmasking the animals, or warn in advance about the approach of a potential danger (Protasov, 1965). In addition to acoustic signals (*inter alia* the sounds of working covers of the branchial apparatus, contractions of the heart chambers, crunching of the skeletal joints, and hydrodynamic sounds), very weak electrical discharges resulting from muscle contraction emanate from almost all marine animals (Protasov, 1972), and this attracts predatory fish capable of detecting the electric fields of other living things. Scorpionfish is a recognized ambush predator, for which it is important not to reveal its presence when a potential prey approaches. Apparently, a similar behavioral reaction of the scorpionfish in the form of complete “freezing” should also be manifested in the case when it itself is as a potential prey for larger predators. In both situations, the interruption of generation and the complete absence of acoustic and electrical signals indicating the location of the disguised scorpionfish contribute to its survival as a species. Obviously, the phenomenon we established corresponds to the maxim that physiological transformation presupposes an emphasis on the role of the organism own activity in the evolutionary process, highlighting that “to live means to react, and by no means to be a victim” (Chaikovskii, 1990).

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ФЕНОМЕН ПОЛНОГО ПОДАВЛЕНИЯ СЕРДЕЧНОЙ ДЕЯТЕЛЬНОСТИ ЧЕРНОМОРСКОЙ СКОРПЕНЫ *SCORPAENA PORCUS* (SCORPAENIDAE) ПРИ РЕАКЦИИ НАСТОРОЖЕННОСТИ

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Костистые рыбы известны как экспериментальные модели для изучения физиологических и патофизиологических процессов, в частности связанных с работой сердца. Методы, позволяющие производить анализ частотных характеристик сердечного ритма в течение длительного периода времени, нуждаются в учёте особенностей поведенческих реакций рыб, способных повлиять на результаты эксперимента. Целью работы было изучить воздействие простейшей тестовой нагрузки (звуковой раздражитель) на частотные параметры сердечной деятельности, фиксируемые волоконно-оптическим методом. Объект исследования — взрослые особи *Scorpaena porcus* длиной 12–15 см, массой 80–120 г. В ходе экспериментов каждую скорпену содержали в отдельном аквариуме с морской водой размером 400×400×350 мм с постоянной температурой (21 ± 0,5) °С и регулируемым содержанием кислорода (5,5–6,7 мг·л$^{-1}$, нормоксия). Регистрацию частоты сердечных сокращений (ЧСС) производили инвазивным волоконно-оптическим методом, суть которого состоит в передаче излучения инфракрасного полупроводникового лазера фотоплетизмографа по тонкому волоконно-оптическому кабелю к перикардиальной мембране сердца и в последующей фиксации отражённого от сокращающегося миокарда сигнала в фотоприёмнике. При имплантации световодов фотоплетизмографа рыбу наркотизировали путём помещения в раствор анестетика (уретан, 2,4 г·л$^{-1}$ морской воды). В своде оперкулярной полости над областью условной проекции сердца производили минимальное рассечение выстилающего эпителия, через которое подлежащие ткани последовательно разъединяли тупым методом до достижения перикардиальной мембраны, не прорывая её. Через образовавшийся в тканях просвет к поверхности перикардиальной мембраны вводили два датчика световодов. В дальнейшем свободно плавающие скорпены принимали участие в эксперименте спустя одни сутки после хирургического вмешательства. Дополнительно нами было оценено функциональное состояние животных путём визуальной фиксации дыхательной активности по количеству движений оперкулярных крышек в минуту. При изучении влияния тестовых нагрузок на корректность регистрации ЧСС у скорпены был выявлен феномен кратковременного полного подавления сердечной деятельности, проявлявшийся при предъявлении звуковых стимулов (реакция настороженности, «замирание»). Длительность остановки сердечных сокращений составляла 31–50 с., она сопровождалась прекращением движения оперкулярных крышек (остановка дыхания, апноэ). При восстановлении сердечной деятельности отмечали два типа физиологических реакций. Для восстановительной реакции первого типа характерно одновременное увеличение ЧСС в 1,5 раза и амплитуды сигнала фотоплетизмографа в 2 раза. Второй тип восстановительной реакции сопровождался увеличением ЧСС на 22 % ($p < 0{,}05$) на фоне снижения амплитуды сигнала датчиков фотоплетизмографа на 28 % ($p < 0{,}05$); в пределах ~ 120 с. ЧСС скорпены возвращалась к исходным показателям. Предполагается, что в основе кратковременной задержки сердечной деятельности скорпены лежит явление кардиореспираторного сопряжения и синхронизации. Поведенческая реакция в виде подавления генерации сердечной и одновременно дыхательной активности обеспечивает отсутствие акустических и электрических сигналов, демаскирующих местоположение хищника-засадчика, и способствует выживанию скорпен.

Ключевые слова: рыбы, *Scorpaena porcus*, сердце, кардиореспираторное сопряжение, реакция настороженности

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AUTECOLOGY OF BENTHIC DIATOM *STRIATELLA UNIPUNCTATA* (LYNGBYE) C. A. AGARDH, 1832 – INDICATOR OF ORGANIC WATER POLLUTION (BLACK SEA AND SEA OF JAPAN)

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Analysis of long-term data (1987–2019) was carried out on the morphology and autecology of the benthic colonial large-cell species *Striatella unipunctata* (Lyngbye) C. A. Agardh, 1832 in the microphytobenthos of the Black Sea and Sea of Japan, including water areas of specially protected natural areas of Russia. The species is widely found on natural and artificial substrates in the Black Sea year-round, and in the Sea of Japan, at a water temperature down to −1.5 °C. *St. unipunctata* quantitative data were determined by direct cell counting in the Goryaev camera under light microscopes (LMs) Biolam L-212, Axioskop 40, and Olympus BX41. Species morphology, phytogeography, and ecology are described. The cell size range of populations is presented: for the Black Sea, valves 25–148 µm long, 8–22 µm wide, frustules 36.3–50.4 µm wide, 18–24 fibulae in 10 µm, and 7–8 girdle bands in 10 µm; for the Sea of Japan, valves 85–125 µm long, 12–21 µm wide, 7–8 girdle bands in 10 µm, 20–25 fibulae in 10 µm, frustules 32.0–34.3 µm long, 10–11 µm wide, and 25 fibulae in 10 µm. For the first time, *St. unipunctata* valves and frustules were studied *in vivo* under LMs, and frustule ultrastructure, under a scanning electron microscope (SEM). For the first time, quantitative indicators of the species populations from the Black Sea and Sea of Japan were compared. The morphology of the frustule ultrastructure of *St. unipunctata* was studied under a Hitachi SEM, model SU3500 (Japan), in Leica EM ACE200 gold-palladium-coated samples. In the Kazachya Bay of the Black Sea near the Oceanarium, the absolute maximum abundance was recorded – $41.6\cdot10^3$ cells·cm^{-2} with a biomass of 1.73 mg·cm^{-2} in January (t = +6.9 °C) in the epizoon of the cultured mussel *Mytilus galloprovincialis* Lamarck, 1819 at a depth of 0.5 m at excessive organic pollution of water. The minimum values were of $0.26\cdot10^3$ cells·cm^{-2} and 0.011 mg·cm^{-2}, respectively, in July (t = +23.5 °C) at a depth of 2.5 m. In the Paris Bay (Russky Island) of the Sea of Japan in the water area of the Marine Mammal Research Base of the Primorsky Oceanarium (Vladivostok), the abundance in the asbestos plates periphyton was of $207\cdot10^3$ cells·cm^{-2} in the summer. For the first time, unique micrographs of the species *in vivo* were obtained under a LM, and of purified frustules – under a SEM.

Keywords: benthic diatom *Striatella unipunctata*, morphology, ecology, Black Sea, Sea of Japan

Large-cell diatoms, which have a high biomass, form large accumulations less often than small-cell species, which, under certain environmental conditions, more often reach a high abundance. The first ones include the benthic species *Striatella unipunctata* forming ribbon-like colonies, which are easily recognizable in a light microscope *in vivo* by the chloroplast rosettes resembling a flower. This work is the second communication, after the article on *Cylindrotheca closterium* populations from the Black Sea and Sea of Japan (Ryabushko et al., 2019b), devoted to the generalization of data on some pennate diatom species playing a significant role in coastal ecosystems. Both works are based on the study of morphological, ecological, and phytogeographical characteristics of the species composing microphytobenthos communities and are important in the analysis of the seasonal dynamics of the structure of their natural populations.

In publications on microphytobenthos, generalized data on the species composition, abundance, and biomass of algal communities are usually used. There are practically no data on separate species and their population indicators. Species peculiarities are often studied in cultures. Thus, in laboratory conditions, *St. unipunctata* was found to be dioecious, and reproductive process and auxospore formation were discovered; the latter is typical for diatoms alone (Davidovich & Chepurnov, 1993). So, the combination of the study of the species both from nature and when cultivated in the laboratory expands our understanding of its morphological and ecological peculiarities.

The aim of this study is to summarize long-term data on the morphology, autecology, and phytogeography of the diatom *Striatella unipunctata* (Lyngbye) C. A. Agardh, 1832 inhabiting the microphytobenthos of the Black Sea and Sea of Japan.

MATERIAL AND METHODS

Microphytobenthos was sampled in different ecotopes of the Black Sea and Sea of Japan in 1987–2019 at depths of 0.5–12 m (Fig. 1). The material from great depth was sampled by divers. *St. unipunctata* abundance and cell size were determined in a Goryaev's camera with a volume of 0.9 mm^3 in triplicates in a light microscope (hereinafter LM) Biolam L-212 at magnifications of 10×40×2.5 and 10×90×2.5, as well as in light microscopes C. Zeiss Axioskop 40 (with AxioVision Rel. 4.6 program) and Olympus BX41 UPLanF1 at magnification of 10×40.

The morphology of the ultrastructure of *St. unipunctata* frustules was studied in a Hitachi scanning electron microscope (hereinafter SEM), model SU. Diatom frustules were cleaned from organic matter by the "cold" method: processing with concentrated sulfuric acid followed by washing in distilled water (Ryabushko, 2013 ; Ryabushko & Begun, 2015). Micrographs were taken *in vivo* in the SEM.

Diatom abundance (N, cells·cm^{-2}) and biomass (B, mg·cm^{-2}) were determined by the formulas of V. I. Ryabushko (Ryabushko, 2013):

$$N = n \cdot V / S \cdot V_{\mathrm{h}} ,$$

where n is the number of cells in the Goryaev's camera;

V is the sample volume, mL;

S is the surface area of the substrate, cm^2;

V_{h} is the volume of the Goryaev's camera equal to 0.9 mm^3;

$$B = h \cdot V \cdot b / S \cdot V_{\mathrm{h}} ,$$

where h is algal specific gravity equal to $1.2 \cdot 10^{-9}$ mg·µm^{-3} for benthic diatoms (Oksiyuk & Yurchenko, 1971);

b is the sum of cell biovolumes in the Goryaev's camera.

The surface area of the stones (y, cm^2) was calculated by the formula of R. Calow (1972):

$$y = 2.22 + 0.26(d \cdot n)\,,$$

where d is the maximum length of the stone, cm;

n is the largest perimeter of the stone, cm.

The surface area of mussel shells (S, cm^2) was determined by the formula (Mikhailova et al., 1987):

$$S = 0.956 \cdot L^{2.085}\,,$$

where L is the distance from the shell anterior end to the posterior end, cm.

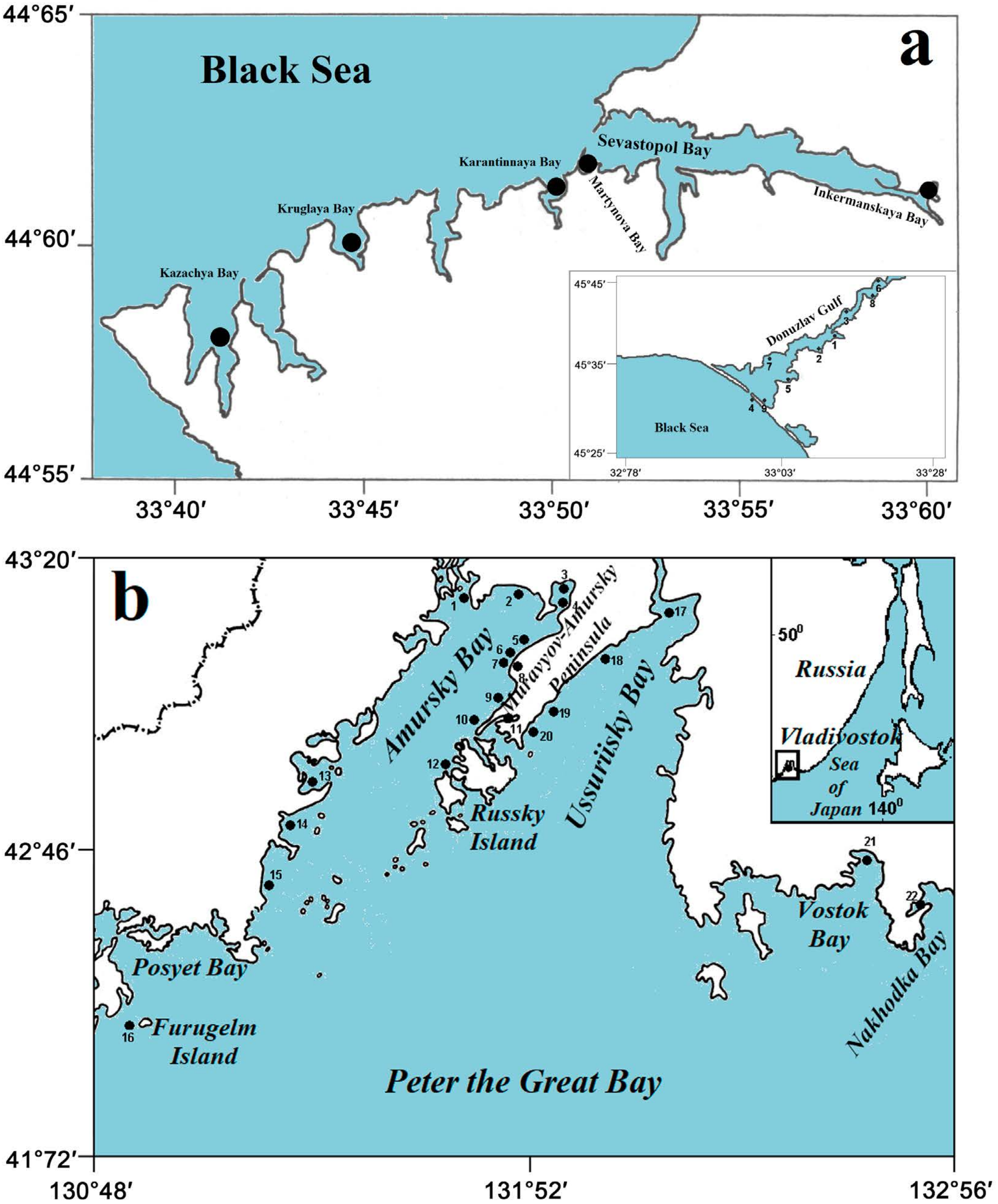

Fig. 1. Stations (●) of microphytobenthos sampling in the bays of the Crimean Peninsula, the Black Sea (a), and in Peter the Great Bay, the Sea of Japan (b)

The surface area of the macrophyte was calculated by the formula for the allometric dependence of the specific surface area of the macrophyte-basiphyte on the diameter of its thallus (Minicheva, 1989):

$$S/W = 3334/d^{0.916}\ ,$$

where S/W is the specific surface area of the macrophyte, $cm^2 \cdot g^{-1}$;

S is the surface area of the macrophyte, cm^2;

W is the wet weight of the macrophyte, g;

d is the diameter of macrophyte thallus, cm.

RESULTS

Morphology, ecology, and phytogeography of the large-celled attached colonial araphid diatom *Striatella unipunctata* (Lyngbye) C. A. Agardh, 1832 were investigated.

Taxonomy. According to the classification (Round et al., 1990), the species belongs to the class Fragilariophyceae, the order Striatellales Round, 1990, the family Striatellaceae Kützing, 1844, and the genus *Striatella* C. A. Agardh, 1832. The species has a broad synonymics, and its taxonomic formation dates back to the XIX century (basionym: *Fragilaria unipunctata* Lyngbye, 1819; synonyms: *Diatoma unipunctata* (Lyngbye) C. A. Agardh, 1824; *Achnanthes unipunctata* (Lyngbye) Carmichael ex Greville, 1827; *Achnanthes unipunctata* Greville, 1828; *Candollella unipunctata* (Lyngbye) Gaillon, 1833; *Tessella pedicellata* Dujardin, 1841; and *Tabellaria unipunctata* (Lyngbye) Schütt, 1896) (Guiry & Guiry, 2020).

Morphology. The description is given according to (Proshkina-Lavrenko, 1955 ; Hendey, 1964) with our additions. *St. unipunctata* cells are quadrangular-tabular (Figs 2 and 3) with cut corners; those are connected in zigzag or linear chains hanging on a long and thick mucilage stalk, with the help of which the species is attached to the substrate (Fig. 2b, c, e). Frustule is rather coarse, with numerous girdle bands, about 8 in 10 µm, with delicate cross fibulae along the edges. The septa are short, flat, about 4 in 10 µm; their free edge is thickened. Valves are linear-lanceolate with widely rounded ends (Fig. 5a); 38–115 µm in length; 8–18 µm wide. The fibulae are very delicate, in sidelong mutually intersecting rows, 18–22 in 10 µm (Fig. 5b). The axial field is linear, with an axial rib (Fig. 5c, d); at each its end, there is one large mucilage pore with rimoportulas [tubes penetrating through the valve and opening inside with the slit; those are indicated by arrows (Fig. 4)]. This allows maintaining the connection of the cell with the environment and attaching to the substrate with the help of a mucilage stalk (rimoportula). Chloroplasts are numerous, radially arranged in the form of a “flower” rosette (Fig. 2).

Micrographs (LM) of *St. unipunctata* cells and colonies found in the Black Sea and Sea of Japan *in vivo* are shown below (Figs 2 and 3). The cells are presented with a mucilage stalk for attaching to the substrate (Fig. 2b, d), which was previously noted by us in the glass plates periphyton in Karantinnaya Bay for another benthic diatom species: *Achnanthes armillaris* (O. F. Müller) Guiry, 2019 (= *Achnanthes longipes* C. Agardh) (Fig. 2e). SEM photographs of the frustule ultrastructure and valves of the Black Sea population are given as well (Figs 4 and 5).

The size range of *Striatella* cells from two seas varies as follows. For the Black Sea, valves are 25–148 µm in length, 8–22 µm wide; frustules are 36.3–50.4 µm wide, 18–24 fibulae in 10 µm (Proshkina-Lavrenko, 1955, 1963); 40–80 µm in length, 10–20 µm wide, 20–24 fibulae in 10 µm (Cleve-Euler, 1953); 60–130 µm in length, 20–36 µm wide (Hendey, 1964); 70–81 µm in length, 18–19 µm wide, 20–22 fibulae in 10 µm (Al-Yamani & Saburova, 2011). For the Sea of Japan, valves are 85–125 μm in length, 12–21 μm wide, 7–8 bands in 10 μm, 20–25 fibulae in 10 μm; frustules are 32–34.3 μm in length, 10–11 μm wide, 25 fibulae in 10 μm (Ryabushko & Begun, 2016). Many details of the thin ultrastructure of *St. unipunctata* frustule are not visible even at high magnification in the LM (Fig. 3a–f); however, these details are very clearly visible in the SEM. The frustules from the Black Sea are 63–66.2 in length (Figs 4 and 5).

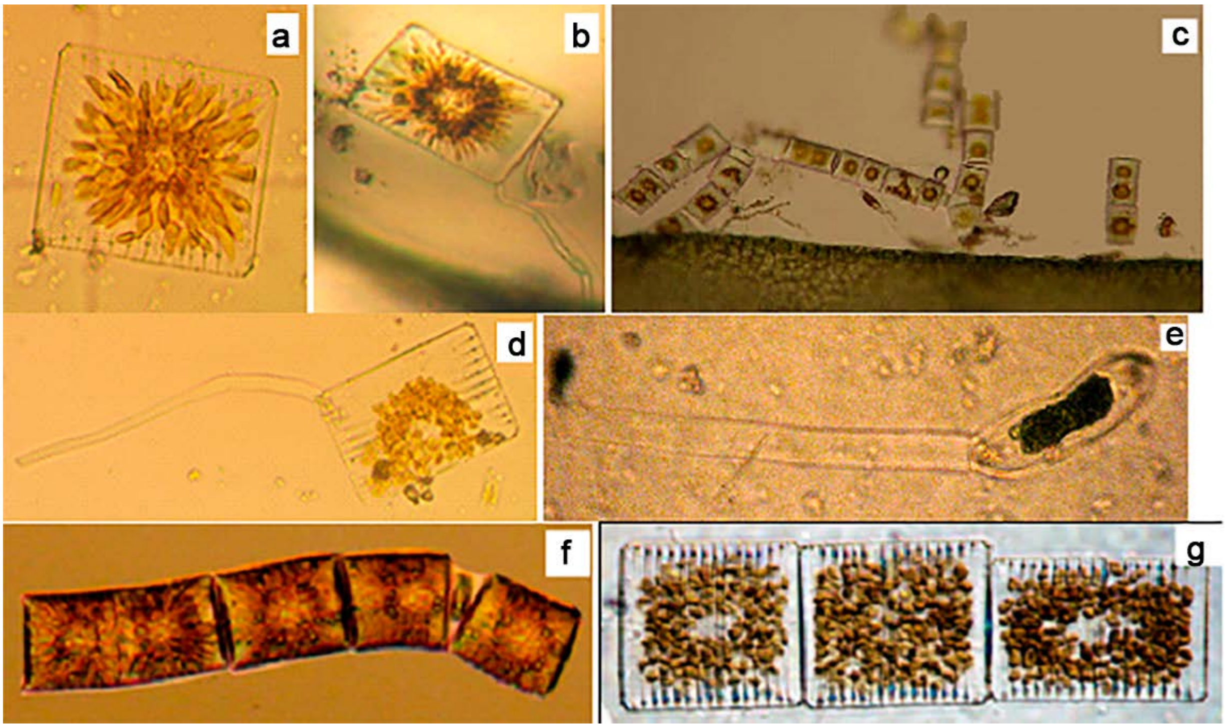

Fig. 2. Frustules of *Striatella unipunctata* with chloroplasts (a–g), cells on a mucilage stalk (b, d), diatom colonies in fouling of macrophyte (c), *Achnanthes armillaris* cell on a mucilage stalk (e), and colonies (f, g). Light microscope. The photos (b, c, e, f) by (Ryabushko, 2013)

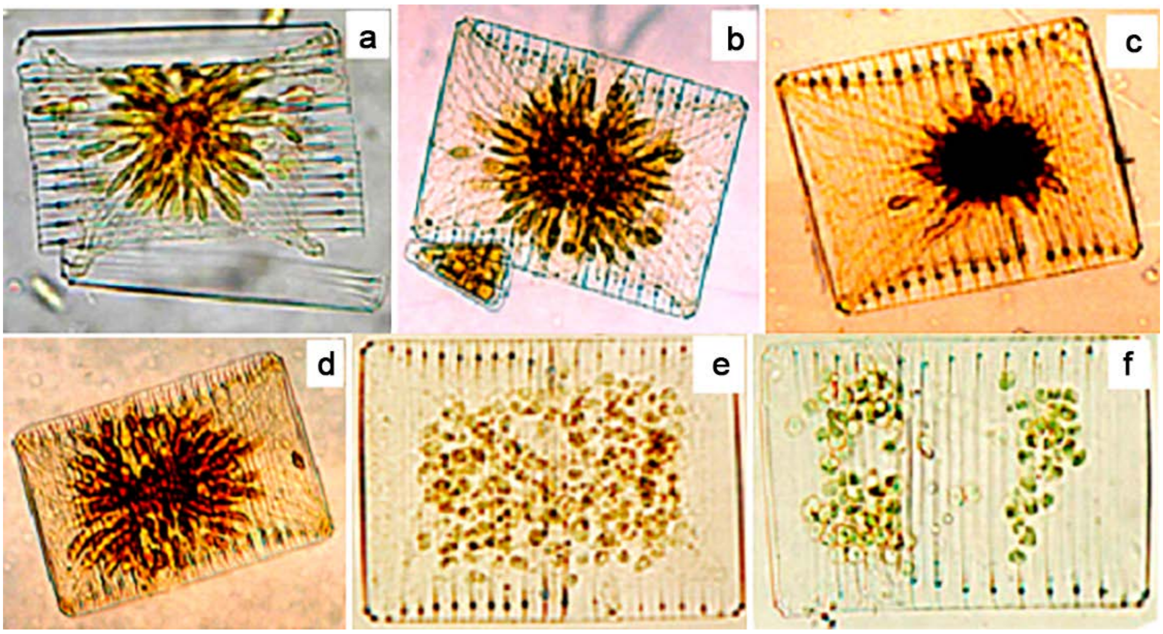

Fig. 3. *Striatella unipunctata* cells *in vivo* (a–d) and in dying state (e, f) with chloroplasts, the Sea of Japan. Light microscope (Ryabushko & Begun, 2016)

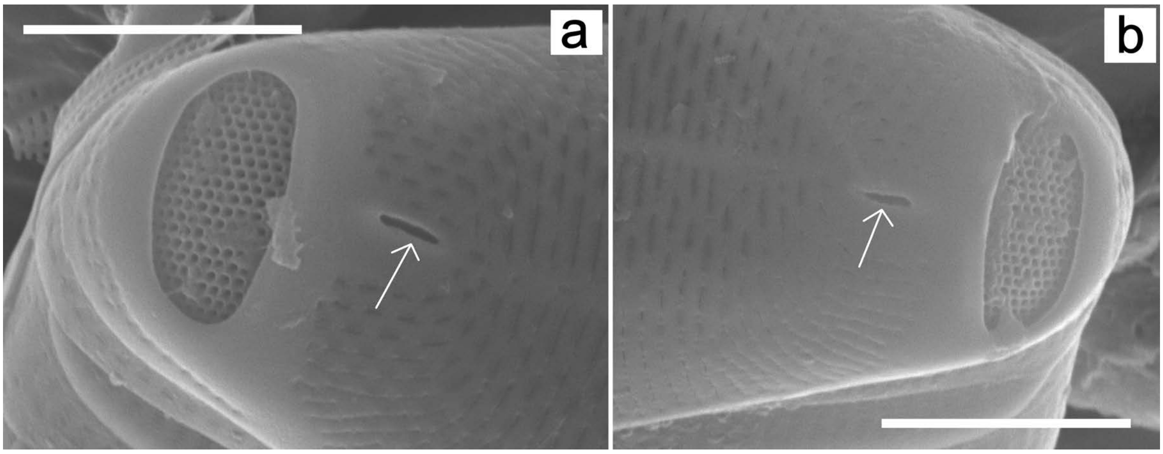

Fig. 4. Fragments of *Striatella unipunctata* external valve view (a, b) with rimoportulas indicated by arrows. SEM. Scale bar: 4 μm

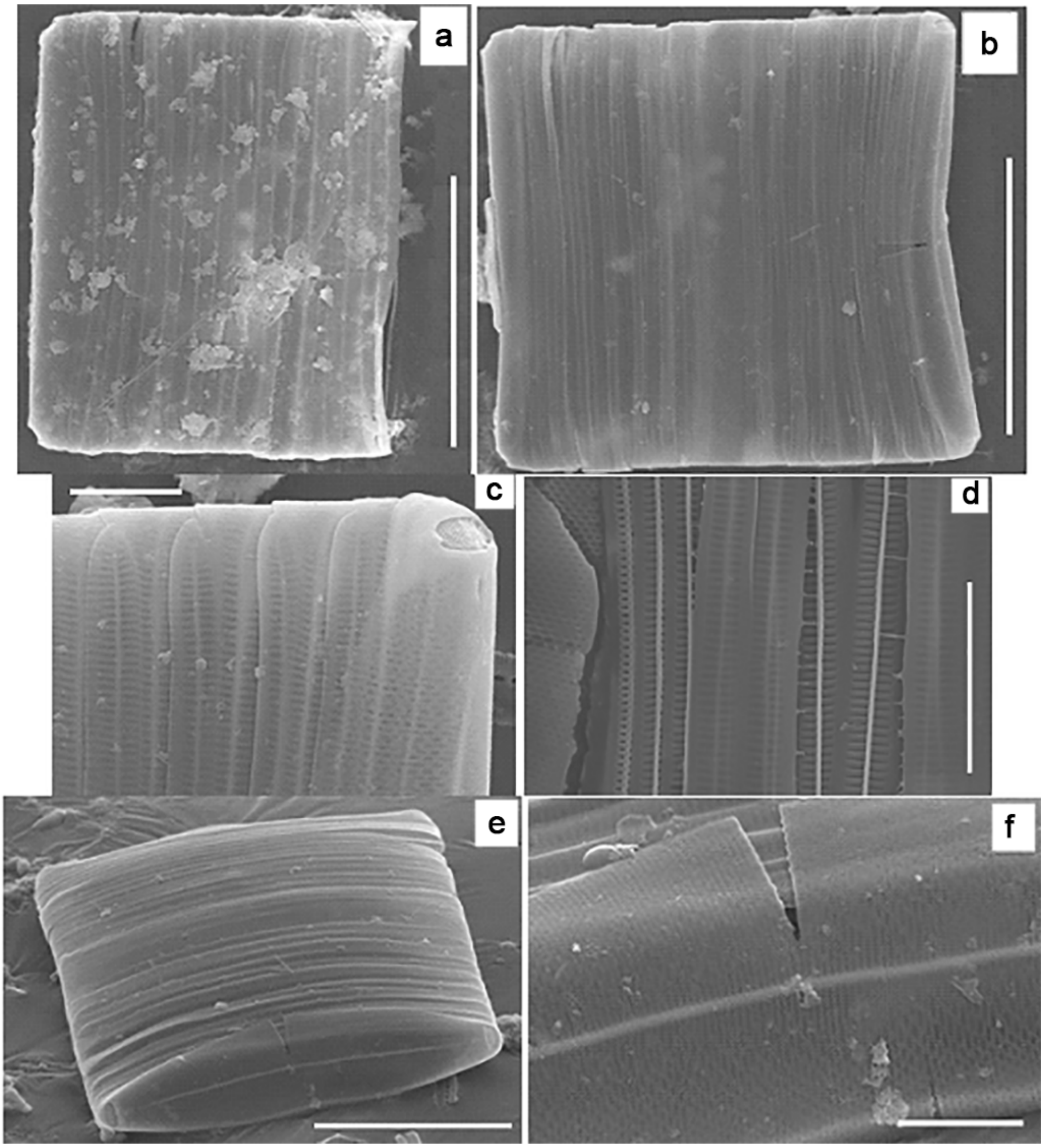

Fig. 5. *Striatella unipunctata* external frustules view with numerous girdle bands with a structure (a–f) and longitudinal axial of rib in the central area (e, f). SEM. Scale bars: 40 μm (a, b), 30 μm (e), 5 μm (c, d, f)

Phytogeography. The species is cosmopolite. It is known in the European seas of both the Northern and Southern hemispheres. The species is found in the Baltic, Barents, and North seas; in the Sea of Japan; in the Caribbean, Mediterranean, Marmara, Aegean, and Black seas; in the Sea of Azov; in Sivash, Bosporus, Great Britain, Ireland, Mexico, Sweden, Finland, the Netherlands, Denmark, Germany, Latvia, France, Kuwait, India, Australia, and New Zealand; and on the Atlantic coast of North America and the Bahamas (Proshkina-Lavrenko, 1955, 1963 ; Ryabushko, 2013 ; Ryabushko & Begun, 2016 ; Al-Yamani & Saburova, 2011 ; Guiry & Guiry, 2020 ; Hendey, 1964).

Ecology. *St. unipunctata* is marine, benthic, eurythermal, euryhaline, and sublittoral species. It is recorded at salinity above 10 ‰ in bays and near the shores of the open sea, rarely in the northwestern Black Sea; in the Crimean coastal area, it is found all year round (Kucherova, 1957 ; Ryabushko, 1994, 2013). In the benthos of Vostok Gulf in the northwestern Sea of Japan, it was registered in the epilithon of stones in winter and summer at a depth of 0.5 m, as well as in the anthropogenic substrate periphyton (Begun, 2012 ; Ryabushko, 1984). In the coastal waters of Rhodes, the Aegean Sea, the species was first found in the epiphyton of the green alga *Bryopsis plumosa* (Hudson) C. Agardh, 1823 (Ryabushko et al., 2019a). It is recorded in plankton, epilithon of stones, and epiphyton of macrophytes of Great Britain (Hendey, 1964), Sweden, and Kuwait (Al-Yamani & Saburova, 2011 ; Kuylenstierna, 1989).

In the microphytobenthos of the coastal waters of the Crimean Black Sea, the study of *St. unipunctata* started in 1987 and was episodic (Ryabushko, 2013). In August 1988, during an emergency discharge of domestic wastewater in the Kalamitsky Bay near the city beach adjacent to Saki Lake, while studying diatoms in the epiphyton of red, brown, and green macrophytic algae at depths of 1.5–12 m at a water temperature of +23 °C, water bloom was registered for the first time, which was caused by the abundant colonies of *St. unipunctata* (Ryabushko, 1997). Other species of pennate diatoms were found as well: from the genera *Amphora* Ehrenberg ex Kützing, 1844, *Cylindrotheca* L. Rabenhorst, 1859, *Licmophora* C. A. Agardh, 1827, *Navicula* Bory, 1822, *Nitzschia* A. H. Hassall, 1845, and *Pleurosigma* W. Smith, 1852. The composition of microalgae at all depths was not very diverse, and those were not abundant. Colonies of *St. unipunctata* were the most abundant out of the colonies of all species, but the greatest abundance was recorded at depths of 2–10 m in the epiphyton of the green alga *Cladophora albida* (Nees) Kützing, 1843 and the brown alga *Feldmannia lebelii* (Areschoug) Hamel, 1939. At a depth of 10 m, *Striatella* frustule was of 44.8–128.8 µm in length and 36.3–50.4 µm wide.

In the relatively clean open coastal waters of the Crimean Black Sea, *St. unipunctata* was less common. In February and November 1990 in Tebenkov Bay (Sevastopol) at a depth of 0.5 m and t = +6 °C, the species was recorded on thalli of *Ericaria crinita* (Duby) Molinari & Guiry, 2020 (= *Cystoseira crinita* Duby, 1830). In April 1998 in Kazachya Bay at 4.5 m and t = +15 °C, the species was registered on thalli of the red alga *Ceramium rubrum* (Hudson) C. Agardh, 1811 (Ryabushko, 2013).

When studying the epiphyton of 15 species of red, brown, and green macrophytic algae in May and August 1990 in the open coastal area of the sea near Omega Cape and in the area of the sanatorium beach at the Kruglaya Bay mouth, not subject to strong anthropogenic impact, no mass development of this alga was noted (Ryabushko, 1996). *St. unipunctata* was found in small abundance only in five out of them. For the first time, quantitative estimates of its abundance were obtained in the epiphyton as follows: of the red algae *Laurencia papillosa* (Forsskål) Greville, 1830 at a depth of 10 m – 200 cells·cm^{-2}, *Phyllophora crispa* (Hudson) P. S. Dixon, 1964 at 20 m – 280 cells·cm^{-2};

of the brown algae *E. crinita* at 5 m – 30 cells·cm^{-2}, *Stilophora rhizodes* (C. Agardh) J. Agardh, 1841 – 110 cells·cm^{-2}; and the green alga *Ulva rigida* C. Agardh, 1823 at a water's edge – 20 cells·cm^{-2}.

Quantitative data on the abundance and biomass of *Striatella* populations in various ecotopes of the coastal waters of the Crimean Black Sea are given below (Tables 1–3).

In the Kazachya Bay April to June 1995 at a depth of 0.5 m, *St. unipunctata* was found on thalli of various macrophytes: *Ulva rigida*, *Enteromorpha prolifera* (O. F. Müller) J. Agardh, 1883, *Chaetomorpha linum* (O. F. Müller) Kützing, 1845, *Sphacelaria cirrosa* (Roth) C. Agardh, 1824, *Ectocarpus siliculosus* (Dillwyn) Lyngbye, 1819, *E. crinita*, *C. rubrum*, and *Laurencia coronopus* J. Agardh, 1852, as well as on the leaves of the eelgrass *Zostera marina* Linnaeus, 1753. Moreover, in 1995–1996 at depths of 0.5–4.5 m in the same bay, annual and seasonal dynamics of the quantitative distribution of *St. unipunctata* populations was studied on the epiphyton of macrophytes and epizoon of shells of the live mussel *Mytilus galloprovincialis* Lamarck, 1819 (Table 1) in the spots with high content of biogenic elements (Ryabushko, 2013). The abundance and biomass of cells ranged 0.26·10^3 to 41.6·10^3 cells·cm^{-2} and 0.011 to 1.73 mg·cm^{-2}. The absolute maximum values were recorded on 29.01.1996 at a water temperature of +6.9 °C with average values of 5.08·10^3 cells·cm^{-2} and 0.21 mg·cm^{-2}. *Striatella* minimum abundance (0.63·10^3 cells·cm^{-2}) and biomass (0.093 mg·cm^{-2}) were registered in the mussel epizoon on 28.11.1995 at a depth of 0.5 m at +12 °C (Table 1).

Table 1. Abundance (N) and biomass (B) of *Striatella unipunctata* populations in different ecotopes of the Kazachya Bay of the Crimean coastal waters of the Black Sea

Sampling date	Ecotope	Depth, m	Temperature, °C	N, ×10^3 cells·cm^{-2}	B, mg·cm^{-2}
17.11.1987	Epiphyton of *Gracilaria verrucosa*	1.0	13.1	often	
17.12.1987	–″–	4.0	10.3	single	
21.01.1988	–″–	4–5	8.2	–″–	
26.01.1988	Epiphyton of *Zostera marina*	1–3	8.2	–″–	
24.02.1988	–″–	–″–	6.0	–″–	
02.03.1988	–″–	–″–	8.0	1.25	0.052
16.03.1988	Epiphyton of *Gr. verrucosa*	–″–	8.0	0.90	0.037
13.04.1988	–″–	5–7	13.0	single	
04.07.1988	–″–	–″–	17.0	–″–	
18.04.1995	Epizoon of mussel	0.5	9.6	–″–	
26.04.1995	–″–	–″–	10.5	9.14	0.38
–″–	Epilithon of stones	–″–	16.0	single	
12.05.1995	Epizoon of mussel	–″–	17.5	1.78	0.074
–″–	Epiphyton of *Ulva rigida*	–″–	–″–	single	
–″–	Epiphyton of *Ceramium rubrum*	–″–	–″–	single	
–″–	Epilithon of stones	–″–	–″–	single	
–″–	Epizoon of mussel	–″–	–″–	1.65	0.070
05.06.1995	–″–	–″–	21.0	3.26	0.14

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Sampling date	Ecotope	Depth, m	Temperature, °C	N, $\times 10^3$ cells·cm^{-2}	B, mg·cm^{-2}
–″–	–″–	–″–	–″–	1.51	0.063
–″–	Epiphyton of *C. rubrum*	–″–	–″–	single	
–″–	Epiphyton of *Chaetomorpha linum*	–″–	–″–	–″–	
–″–	Epiphyton of *Ericaria crinita*	–″–	–″–	–″–	
–″–	Epiphyton of *Ectocarpus confervoides*	–″–	–″–	–″–	
–″–	Epiphyton of *Enteromorpha prolifera*	–″–	–″–	–″–	
–″–	Epiphyton of *U. rigida*	–″–	–″–	–″–	
29.06.1995	Epizoon of mussel	–″–	22.8	0.91	0.038
–″–	Epiphyton of *Ch. linum*	–″–	–″–	single	
–″–	Epiphyton of *E. prolifera*	–″–	–″–	1.50	0.062
27.07.1995	Epizoon of mussel	2.5	23.5	0.96	0.040
–″–	–″–	–″–	–″–	0.33	0.014
–″–	–″–	–″–	–″–	0.26	0.011
01.08.1995	–″–	2.0	24.0	single	
29.08.1995	–″–	0.5	23.5	1.22	0.050
–″–	–″–	–″–	–″–	1.10	0.046
–″–	–″–	–″–	–″–	single	
–″–	Epiphyton of *Ericaria crinita*	–″–	–″–	–″–	
27.09.1995	Epizoon of mussel	–″–	19.0	1.19	0.049
–″–	–″–	2.5	–″–	0.76	0.032
25.10.1995	–″–	0.5	15.0	7.74	0.052
–″–	–″–	2.5	–″–	1.0	0.042
–″–	Epiphyton of *Sphacelaria cirrosa*	0.5; 4.5	–″–	single	
28.11.1995	Epizoon of mussel	0.5	12.0	2.23	0.093
–″–	–″–	–″–	–″–	0.63	0.026
26.12.1995	–″–	–″–	9.2	1.60	0.066
29.01.1996	–″–	–″–	6.9	41.6	1.730
–″–	–″–	–″–	–″–	0.96	0.040
–″–	–″–	2.5	–″–	2.43	0.100
05.03.1996	–″–	0.5	6.8	1.73	0.072
–″–	–″–	–″–	–″–	2.06	0.086
–″–	–″–	2.5	–″–	4.32	0.180
–″–	–″–	–″–	–″–	3.40	0.141
25.03.1996	–″–	0.5	7.7	6.25	0.259
–″–	–″–	–″–	–″–	1.14	0.047
–″–	–″–	2.5	–″–	1.87	0.050
–″–	–″–	–″–	–″–	3.50	0.150
–″–	–″–	–″–	–″–	2.87	0.120
–″–	–″–	4.5	–″–	2.07	0.086

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Sampling date	Ecotope	Depth, m	Temperature, °C	N, $\times 10^3$ cells·cm^{-2}	B, mg·cm^{-2}
–″–	–″–	–″–	–″–	1.39	0.057
–″–	–″–	–″–	–″–	2.73	0.113
25.04.1996	–″–	2.5	10.5	6.64	0.276
–″–	–″–	–″–	–″–	4.51	1.187
–″–	–″–	–″–	–″–	6.64	0.28
27.05.1996	–″–	–″–	18.7	4.64	0.192
–″–	–″–	–″–	–″–	2.73	0.113
–″–	–″–	–″–	–″–	1.22	0.051
–″–	–″–	2.5	–″–	2.89	0.120
–″–	–″–	–″–	–″–	1.56	0.064
–″–	–″–	4.5	–″–	1.98	0.082
–″–	–″–	–″–	–″–	1.67	0.069
26.09.2003	–″–	–″–	20.0	single	
23.01.2004	–″–	–″–	7.1	colonies	

The research was continued in 2018–2019 when studying the epilithon of stones and the epiphyton of macrophytes in Donuzlav Gulf (Western Crimea), as well as in Inkerman and Karantinnaya bays within Sevastopol (Tables 2 and 3). In the epilithon of Donuzlav Gulf during the annual cycle at depths of 0.1–1.0 m and at a water temperature range from +9.0 °C (December) to +26.4 °C (July), *Striatella* maximum abundance was recorded in July in the spots of pollution by domestic wastewater, where high values of BOD$_5$, water oxidizability, nitrates, and phosphates were observed (Ryabushko et al., 2019c). In Inkerman Bay, similar abundance and biomass values were recorded in April and July.

Table 2. Dependence of abundance (N) and biomass (B) of *Striatella unipunctata* in the epilithon of Donuzlav Gulf and Inkerman Bay on environmental factors (Crimea, the Black Sea)

Sampling date	Depth, m	Temperature, °C	Salinity, ‰	N, $\times 10^3$ cells·cm^{-2}	B, mg·cm^{-2}
Donuzlav Gulf					
13.04.2018	0.5	11.0	17.81	2.54	0.01
21.06.2018	1.0	24.4	18.54	1.65	0.011
12.07.2018	0.5	26.4	18.62	5.47	0.065
05.10.2018	0.2	19.8	16.58	2.33	0.02
08.04.2019	–″–	10.6	17.97	2.74	0.02
21.06.2019	0.5	25.3	13.86	1.71	0.046
11.07.2019	–″–	24.6	15.67	0.43	0.001
09.10.2019	1.0	15.9	17.71	2.27	0.016
Inkerman Bay					
24.12.2018	0.3	9.0	16.76	1.34	0.005
25.04.2019	0.1	10.0	16.50	3.58	0.025
25.05.2019	–″–	20.0	13.31	2.05	0.017
08.07.2019	–″–	26.0	12.86	3.88	0.008

For comparison with the epilithon data, the results are presented of studying *Striatella* morphology and quantitative characteristics in epiphyton of 15 species of red, brown, and green macrophytes and eelgrass *Zostera marina* from Donuzlav Gulf and Karantinnaya Bay for 2018–2019 at depths of 0.1–12 m (Table 3).

Table 3. Dependence of abundance (N) and biomass (B) of *Striatella unipunctata* in the epiphyton of Donuzlav Gulf and Karantinnaya Bay on environmental factors (Crimea, the Black Sea)

Sampling date	Macrophyte-basiphyte	Depth, m	Temperature, °C	Salinity, ‰	N, $\times 10^3$ cells·cm^{-2}	B, mg·cm^{-2}
Donuzlav Gulf						
13.04.2018	*Ericaria crinita*	0.5	11.0	17.81	4.06	0.01
19.06.2018	–″–	0.3	23.4	18.66	1.53	0.05
20.06.2018	–″–	0.5	25.0	18.52	2.62	0.005
–″–	*Zostera marina*	–″–	–″–	–″–	single	–
12.07.2018	–″–	4.0	26.0	18.62	single	–
05.10.2018	–″–	0.2	19.8	16.58	1.63	0.06
–″–	*E. crinita*	–″–	–″–	–″–	2.87	0.003
27.05.2018	*Gongolaria barbata*	–″–	20.7	15.56	0.34	0.004
Karantinnaya Bay						
18.05.2018	*Chaetomorpha chlorotica*	0.1	18.0	17.70	2.32	0.01
08.06.2018	*Cladophora liniformis*	–″–	22.6	18.0	0.46	0.014
11.09.2018	*G. barbata*	0.1	22.6	18.0	0.99	0.003
28.02.2019	–″–	0.2	3.4	17.10	0.91	0.001
04.03.2019	*Cl. liniformis*	4.5	10.0	17.24	0.1	0.002
–″–	*Ulva linza*	–″–	–″–	–″–	1.1	0.03
–″–	*Polysiphonia denudata*	–″–	–″–	–″–	0.06	0.001
05.04.2019	*Cl. liniformis*	1.5	10.6	17.70	14.2	0.10
–″–	*Ceramium arborescens*	–″–	–″–	–″–	14.0	0.08
Mussel and oyster farm in Karantinnaya Bay						
20.07 2018	*Nereia filiformis*	12.0	25.0	18.03	3.1	0.014
08.02.2019	*Laurensia coronopus*	4.0	3.4	16.88	4.2	0.10
04.03.2019	*Callithamnion corymbozum*	6.0	10.0	18.0	0.90	0.01
–″–	*Bryopsis plumosa*	–″–	–″–	–″–	17.7	0.10
–″–	*Pyaiella littoralis*	–″–	–″–	–″–	0.6	0.008
04.04.2019	*Ulva clathrata*	3.0	9.8	17.70	1.0	0.006
–″–	*C. arborescens*	–″–	–″–	–″–	1.6	0.03
–″–	*Ulva compressa*	–″–	–″–	–″–	13.3	0.20
–″–	*P. littoralis*	–″–	–″–	–″–	4.0	0.10
14.05.2019	*Feldmannia paradoxa*	2.0	15.2	18.07	7.2	0.27

Striatella reached the highest abundance values of $13.3 \cdot 10^3$ and $14 \cdot 10^3$ cells·cm^{-2} in April in Karantinnaya Bay in the epiphyton of *Ulva compressa*, *Cladophora liniformis*, and *Ceramium arborescens*, with maximum value of $17.7 \cdot 10^3$ cells·cm^{-2} in March on thalli of the green alga *Bryopsis plumosa*.

The data were obtained from the latest sampling of communities of diatoms and cyanobacteria in the epilithon of stones near the beach of Karantinnaya Bay on 12.11.2019 (t = +15 °C; water salinity of 18.5 ‰; depth of 0.3 m) and on a mussel and oyster farm in the same bay on 19.12.2019 (t = +11.2 °C; 17.2 ‰; depth of 0.1 m). The samples showed abundant winter development of *Striatella* colonies along with cyanobacteria. In the quantitative distribution of the species, mosaic structure was observed; the abundance varied 629 to 3383.4 cells·cm^{-2}; the biomass, 0.013 to 0.077 mg·cm^{-2}. At the same time, the species abundance in December was 5.4 times higher than in November.

For comparison with the data for the Black Sea, similar results are presented of *St. unipunctata* study in the microphytobenthos of the northwestern Sea of Japan. The species was registered in different ecotopes: on 10.11.1983 in the epilithon of stones in the Vityaz and Astafyev bays of the Posyet Bay at a depth of 0.2 m (Nikolaev, 1970, 1976); in summer in Vostok Gulf and in winter in Vityaz Bay at a depth of 0.5 m (Ryabushko, 1984, 2014 ; Ryabushko & Begun, 2016); in periphyton, epiphyton of macrophytes, and epizoon of molluscs in Amursky, Ussuriisky, Vostok, and Slavyanka gulfs, in the Golden Horn, Rynda, and Baklan bays, as well as in the epiphyton of the red alga *Mastocarpus stellatus* (Stackhouse) Guiry, 1984 in March 2014 in Troitsa Bay of Posyet Bay at depths of 3–6 m at a water temperature of −1.5 °C. In the periphyton of navigation buoys in the Troitsa Bay (the seaport area contaminated with petroleum products) on 22.11.2011 at depths of 0–8 m, the abundance was of $2.33 \cdot 10^3$ and $31.8 \cdot 10^3$ cells·g^{-1}, respectively (Ryabushko & Begun, 2016).

Moreover, the results were obtained of the species abundance for a 15-day exposure in the periphyton of Rynda Bay on 02.08.2000 on substrates made of plexiglass ($0.6 \cdot 10^3$ cells·cm^{-2}), wood ($20 \cdot 10^3$ cells·cm^{-2}), high-alloy steel ($0.15 \cdot 10^3$ cells·cm^{-2}), and asbestos cement ($0.3 \cdot 10^3$ cells·cm^{-2}), as well as in the epizoon of bay barnacle *Amphibalanus improvisus* (Darwin, 1854), mussel *Mytilus trossulus* Gould, 1850, ascidians *Aplidium tenuicaudum* (Beniaminson, 1974), and *Styela clava* Herdman, 1881 ($0.84 \cdot 10^3$ cells·cm^{-2}) and in the epiphyton of green and brown algae ($1.45 \cdot 10^3$ and $1.01 \cdot 10^3$ cells·g^{-1} of the wet weight of the macrophyte) (Begun, 2012 ; Ryabushko & Begun, 2015, 2016). In the periphyton of asbestos cement plates exposed on 02.07.2013–06.08.2013 in the Paris Bay (Russky Island) in the water area of the Marine Mammal Research Base of the Primorsky Oceanarium of the National Scientific Center of Marine Biology FEB RAS at depths of 0.5–5 m, high values of *Striatella* abundance were obtained: $177.3 \cdot 10^3$ to $207 \cdot 10^3$ cells·cm^{-2}.

DISCUSSION

In the literature sources, *St. unipunctata* is noted in the phytoplankton of the seas (Konovalova, 1984 ; Morozova-Vodyanitskaya, 1948 ; Orlova, 1984 ; Pautova, 1984 ; Pitsyk, 1963 ; Proshkina-Lavrenko, 1955 ; Ryabushko et al., 2004), Eastern Sivash, and Molochnyi Lyman (Ivanov, 1960). This is explained as follows: after being detached from the substrate due to a storm or another sea disturbance or due to death, cells can float into the water column. Therefore, in shallow seawater, when sampling with a bathometer, the species is often registered in the phytoplankton. Z. Kucherova (1957) classified *Striatella* as a year-round benthos species of the Black Sea. Our research confirmed these data.

She registered single cells on the shell surface of the mussel *Mytilus galloprovincialis* in the Sevastopol Bay at a depth of 1 m and in the Ayu-Dag area at 45 m, as well as at a depth of 4 m on the shell of a grass crab inhabiting thickets of macrophytes (Kucherova, 1960).

The first data on *St. unipunctata* abundance in the epiphyton of *Gongolaria barbata* (= *Cystoseira barbata*) in the Kazachya Bay can be found in (Makkaveeva, 1960). In August 1955 at +22.5 °C, the abundance was of $11.9 \cdot 10^3$ cells·cm^{-2}; in September at +19.5 °C, the value was of $1.8 \cdot 10^3$ cells·cm^{-2}; in October at +17.0 °C, the abundance was of $55.4 \cdot 10^3$ cells·cm^{-2}. That information was supplemented by our data on the settlement of various ecotopes in different seasons of the year at depths of 0.1–12 m. *Striatella* size range depends on the life cycle of alga, type of substrate, season of the year, water temperature, and abundance of biogenic elements in habitats. In spring, the species plays a significant role on anthropogenic substrates: glass plates exposed in the sea (Ryabushko, 2013). Also, the species reached high abundance in Karantinnaya Bay in April in the epiphyton of *Ulva compressa*, *Cladophora liniformis*, and *Ceramium arborescens*; the maximum value of $17.7 \cdot 10^3$ cells·cm^{-2} was recorded in March on thalli of the green alga *Bryopsis plumosa*. The minimum values of abundance and biomass in the Black Sea were noted both in winter and summer. In summer on the epiphyton of macrophytes and epizoon of mussel, these indicators decreased, species populations were in a depressed state, and the cells were found singularly.

In the microphytobenthos of the Black Sea and the Sea of Japan, the species is widely registered on natural and artificial substrates (Ryabushko et al., 2018). In the Sea of Japan in the periphyton of various anthropogenic substrates, the highest abundance was recorded on wood (Begun, 2012); that was noted by other authors as well (Bangqin et al., 1989). According to our data, *Striatella* predominantly inhabits the surface of different macrophytes, and its colonies are found in masse in the spots where domestic wastewater is discharged and biogenic pollution increases. Therefore, *St. unipunctata* is classified as an indicator species for the saprobity of organic pollution of the Black Sea water (Ryabushko, 2013, 1997).

Conclusion. A retrospective analysis of long-term own and published data was carried out on various aspects of studying the benthic marine araphid attached pennate diatom *St. unipunctata* inhabiting different ecotopes of the Black Sea and the Sea of Japan. By phytogeographical affiliation, the species is cosmopolite. In the Sea of Japan, the species was recorded at a water temperature down to −1.5 °C. In two seas, the size ranges of cells overlap. In general, regardless of the depth, season, and ecotope inhabited, the species abundance is higher in water with excessive organic pollution, including the spots where molluscs are grown and mammals are kept in oceanariums. *Striatella* predominantly inhabits the surface of benthic vegetation, stones, and mollusc shells; this allows the species to form abundant colonies, which can detach from the substrate and penetrate in the phytoplankton because of the sea waves.

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АУТЭКОЛОГИЯ БЕНТОСНОЙ ДИАТОМОВОЙ ВОДОРОСЛИ *STRIATELLA UNIPUNCTATA* (LYNGBYE) C. A. AGARDH, 1832 — ИНДИКАТОРА ОРГАНИЧЕСКОГО ЗАГРЯЗНЕНИЯ ВОД (ЧЁРНОЕ И ЯПОНСКОЕ МОРЯ)

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Впервые проведён анализ многолетних данных (1987–2019) изучения морфологии и аутэкологии бентосной колониальной диатомовой водоросли *Striatella unipunctata* (Lyngbye) C. A. Agardh, 1832, обитающей в микрофитобентосе Чёрного и Японского морей, включая акватории заповедных и особо охраняемых природных территорий России. Вид широко встречается на природных и искусственных субстратах в Чёрном море круглогодично, в Японском море зарегистрирован при температуре воды до −1,5 °C. Количественные данные *St. unipunctata* определяли прямым подсчётом клеток в камере Горяева, используя световые микроскопы (СМ) типа Биолам Л-212, Axioskop 40 и Olympus BX41. Морфология ультраструктуры панциря *St. unipunctata* изучена в сканирующем электронном микроскопе (СЭМ) Hitachi SU3500 в образцах с золотопалладиевым напылением Leica EM ACE200. Представлен размерный диапазон клеток популяций: для Чёрного моря — створки 25–148 мкм длины, 8–22 мкм ширины, панцири 36,3–50,4 мкм шир., 18–24 штрихов в 10 мкм, 7–8 вставочных ободков в 10 мкм; для Японского моря — створки 85–125 мкм дл., 12–21 мкм шир., 7–8 вставочных ободков в 10 мкм, 20–25 штр. в 10 мкм, панцири 32,0–34,3 мкм дл., 10–11 мкм шир., 25 штр. в 10 мкм. Впервые изучены створки и панцири *St. unipunctata* в прижизненном состоянии в СМ и ультраструктура панцирей в СЭМ. Приведено описание морфологии, фитогеографии и экологии вида. Впервые проведено сравнение количественных показателей черноморской и япономорской популяций вида. В Казачьей бухте Чёрного моря вблизи океанариума зарегистрирована абсолютная максимальная численность клеток — $41{,}6{\cdot}10^3$ кл.$\cdot$см$^{-2}$ при биомассе 1,73 мг$\cdot$см$^{-2}$ в эпизооне культивируемой мидии *Mytilus galloprovincialis* Lamarck, 1819 в январе (t = +6,9 °C) на глубине 0,5 м при избыточном органическом загрязнении вод.

Минимальные значения показателей составляли $0{,}26 \cdot 10^3$ кл.·см$^{-2}$ и 0,011 мг·см$^{-2}$ соответственно в июле (t = +23,5 °C) на глубине 2,5 м. В бухте Парис (остров Русский) Японского моря в акватории Базы исследования морских млекопитающих Приморского океанариума (г. Владивосток) максимальная численность в перифитоне достигала $207 \cdot 10^3$ кл.·см$^{-2}$. Впервые представлены снимки видов в прижизненном состоянии в СМ и очищенные панцири в СЭМ.

Ключевые слова: диатомовая водоросль *Striatella unipunctata*, морфология, экология, Чёрное море, Японское море

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STATUS OF PLANKTON ALGOCENOSIS IN THE WATER AREA OF THE PORT OF TUAPSE AND BEYOND IT IN THE SPRING-SUMMER PERIOD OF 2019

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The article presents the research of qualitative structure and quantitative development of phytoplankton in the water area of the port of Tuapse and beyond it in the spring-summer period of 2019. In April 2019, 43 phytoplankton species of 5 divisions were found. In the port area, the mean values of abundance and biomass were of 568 thousand cells·L^{-1} and 206 mg·m^{-3}, respectively; in the open sea area, the values were 1.5 times higher. In the port area and beyond it, diatoms developed abundantly: 98–99 % of the total phytoplankton abundance and 62–65 % of the biomass. Small-cell species *Pseudo-nitzschia* spp. and *Skeletonema costatum* s. l. formed the basis of the abundance; *Pseudosolenia calcar-avis*, *Dactyliosolen fragilissimus*, and *Chaetoceros affinis* formed the basis of the biomass as well. In June 2019, a decrease was recorded in qualitative (13 species from 4 divisions) and quantitative values of phytoplankton development. In the port area, the mean values of planktonic algae abundance and biomass were of 59 thousand cells·L^{-1} and 81 mg·m^{-3}, respectively; in the open sea area, these values were more than 2 times lower. In the water area of the port, euglenids developed abundantly: 94 % of the phytoplankton abundance and 83 % of the biomass; those were represented by a mesosaprobic species *Eutreptia lanowii*. Beyond the port of Tuapse, euglenids were not found; in terms of abundance, diatoms predominated (95 %): *Skeletonema costatum* s. l. and *Thalassionema nitzschioides*. The following Dinophyta species formed the basis of the phytoplankton biomass (82 %): *Ceratium tripos*, *C. furca*, *Ensiculifera carinata*, *Glenodinium paululum*, *Prorocentrum micans*, and *Protoperidinium crassipes*.

Keywords: phytoplankton, taxonomic composition, abundance, biomass, water area of the port of Tuapse, northeastern Black Sea coast

In recent decades, many researchers have recorded changes in the structure and functioning of marine plankton communities in the waters of Russian Black Sea shelf (Korpakova et al., 2014 ; Safronova et al., 2015 ; Safronova & Naletova, 2017 ; Selifonova & Yasakova, 2012). First of all, zones of environmental stress are the water areas of large seaports, such as the port of Tuapse, with constantly increasing cargo turnover. Phytoplankton studies in the water area of the port of Tuapse and beyond it under progressive pollution of the marine environment were carried out in different seasons of 2009–2011 (Selifonova & Yasakova, 2012 ; Yasakova & Makarevich, 2017). The investigation of qualitative and quantitative characteristics of phytoplankton, the most vulnerable link in marine ecosystems under anthropogenic eutrophication of port waters in the modern period, seems to be urgent. Therefore, the aim of this work is to conduct a study of the species composition and quantity of planktonic algae in the water area of the port of Tuapse and beyond it in spring and summer of 2019 and to compare these indicators with corresponding data for 2009–2011.

MATERIAL AND METHODS

The material for the study was the phytoplankton samples collected at 15 stations in the water area of the port of Tuapse (st. 1–14) and beyond it (st. 15) in April 2019 (Figs 1 and 2). In June 2019, planktonic algae were sampled at 5 stations; st. 1–4 were located in the port water area, and st. 5 – beyond it (Figs 3 and 4). The location of the sampling spots was due to different levels of anthropogenic load on these water areas. During the study period, the water temperature on the sea surface varied +11.1 °C (23.04.2019) to +24 °C (12.06.2019); wind speed was 5–10 $m \cdot s^{-1}$, and sea roughness degree was 1–2. Samples of 1–1.5 L were taken from the sea surface using a 5-L Niskin bottle in the daytime from the research vessel, fixed with formalin up to a final concentration of 1–2 %, and kept in a dark cool place for at least 15 days. Then, the samples were concentrated using a siphon tube with an end bent 2 cm up, which was tightened with a No. 77 sieve. Abundance and volume of phytoplankton cells were counted using 0.05-mL and 0.1-mL cameras under a Mikmed-2 microscope with 10×/0.30 and 40×/0.65 objectives (Kol'tsova et al., 1979 ; Rukovodstvo, 1980 ; Fedorov, 1979). The cells were measured using an eyepiece micrometer; the minimum size of the counted cells was of 3–5 μm. The biomass was calculated by the volumetric counting. The volume of cells was calculated by equating their shape to corresponding geometric figure (Kol'tsova, 1970). The generally accepted species identification guides were used (Kiselev, 1950 ; Konovalova et al., 1989 ; Proshkina-Lavrenko, 1955, 1963 ; Dodge, 1982 ; Identifying Marine Phytoplankton, 1997). Phytoplankton species composition was classified in accordance with S. P. Wasser system (Vodorosli : spravochnik, 1989). The species were considered mass when forming more than 10 % of the total abundance or biomass, common – 1–10 %, and rare – less than 1 %. The arithmetic means of abundance (or biomass) were determined as a number equal to the sum of abundance (or biomass) at each station, divided by the number of stations studied.

RESULTS

Phytoplankton qualitative composition. In April 2019 in the water area of the port of Tuapse and beyond it, 43 phytoplankton species of 5 divisions were found (Bacillariophyta, Dinophyta, Euglenophyta, Cyanophyta, and Cryptophyta), as well as 5 taxonomic units not identified to the species level (Table 1). The maximum species diversity was recorded in diatoms (20 species) and dinophytic algae (20 species). Other divisions were represented by 1 species each. In June 2019, phytoplankton taxonomic composition amounted to 13 species of 4 divisions: Bacillariophyta (2 species), Dinophyta (9 species), Euglenophyta (1 species), and Chlorophyta (1 species).

Table 1. Phytoplankton taxonomic composition in the research area in April and June 2019

Algae divisions and species	23.04.2019				12.06.2019			
	Port of Tuapse		Open sea area		Port of Tuapse		Open sea area	
	N	B	N	B	N	B	N	B
Bacillariophyta								
Amphora sp.	R	R	–	–	–	–	–	–
Bacillaria paradoxa J. F. Gmelin	R	R	–	–	–	–	–	–
Cerataulina pelagica (Cleve) Hendey	R	R	–	–	–	–	–	–
Chaetoceros affinis Lauder	O	O	O	O	–	–	–	–

Continue on the next page…

Algae divisions and species	23.04.2019				12.06.2019			
	Port of Tuapse		Open sea area		Port of Tuapse		Open sea area	
	N	B	N	B	N	B	N	B
Chaetoceros compressus Lauder	R	R	O	R	–	–	–	–
Chaetoceros curvisetus Cleve	R	R	–	–	–	–	–	–
Chaetoceros insignis Proschkina-Lavrenko	R	R	–	–	–	–	–	–
Chaetoceros scabrosus Proschkina-Lavrenko	R	R	R	O	–	–	–	–
Chaetoceros simplex Ostenfeld	R	R	R	R	–	–	–	–
Chaetoceros sp.	R	R	–	–	–	–	–	–
Coscinodiscus sp.	R	O	–	–	–	–	–	–
Dactyliosolen fragilissimus (Bergon) Hasle	R	O	R	O	–	–	–	–
Gyrosigma sp.	R	R	–	–	–	–	–	–
Licmophora gracilis (Ehrenberg) Grunow	R	R	R	R	–	–	–	–
Licmophora flabellata (Greville) C. Agardh	R	R	–	–	–	–	–	–
Melosira moniliformis (O. F. Müller) C. Agardh	R	R	–	–	–	–	–	–
Navicula viridula (Kützing) Ehrenberg	R	R	–	–	–	–	–	–
Nitzschia tenuirostris Mereschkowsky	O	R	R	R	–	–	–	–
Pleurosigma elongatum W. Smith	R	R	–	–	–	–	–	–
Pseudo-nitzschia delicatissima (Cleve) Heiden; *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle (complex)	M	M	M	M	–	–	–	–
Pseudo-nitzschia seriata (Cleve) H. Peragallo	O	O	–	–	–	–	–	–
Pseudosolenia calcar-avis (Schultze) B. G. Sundström	R	M	R	M	–	–	–	–
Skeletonema costatum (Greville) Cleve s. l.	M	O	M	O	–	–	M	O
Synedra sp.	R	R	–	–				
Thalassionema nitzschioides (Grunow) Mereschkowsky	R	O	R	R	O	O	M	M
Dinophyta								
Akashiwo sanguinea (K. Hirasaka) Gert Hansen & Moestrup	–	–	–	–	R	R	R	O
Ceratium furca (Ehrenberg) Claparède et Lachmann	R	O	–	–	R	O	R	M
Ceratium tripos (O. F. Müller) Nitzsch	R	O	–	–	R	O	R	M
Cochlodinium citron Kofoid & Swezy	R	R	R	O	–	–	–	–
Diplopsalis lenticula Bergh	R	R	R	O	–	–	–	–
Dinophysis rotundata Claparède & Lachmann	R	R	–	–	–	–	–	–
Enisculifera carinata Matsuoka, Kobayashi & Gains	R	O	–	–	R	R	R	O
Glenodinium sp.	–	–	–	–	R	R	–	–
Glenodinium paululum Lindernann	–	–	–	–	R	R	O	O
Gymnodinium simplex (Lohmann) Kofoid & Swezy	R	R	–	–	–	–	–	–

Continue on the next page…

Algae divisions and species	23.04.2019				12.06.2019			
	Port of Tuapse		Open sea area		Port of Tuapse		Open sea area	
	N	B	N	B	N	B	N	B
Gymnodinium wulffii J. Schiller	R	R	–	–	–	–	–	–
Gyrodinium spirale (Bergh) Kofoid & Swezy	R	O	–	–	–	–	–	–
Katodinium glaucum (Lebour) Loeblich III	R	R	–	–	–	–	–	–
Lessardia elongata Saldarriaga & F. J. R. Taylor	R	R	–	–	–	–	–	–
Oblea baculifera Balech ex Loeblich Jr. & Loeblich III	R	O	R	O	–	–	–	–
Prorocentrum compressum (Bailey) T. H. Abé ex J. D. Dodge	R	R	R	R	–	–	–	–
Prorocentrum cordatum (Ostenfeld) J. D. Dodge	R	R	–	–	–	–	–	–
Prorocentrum lima (Ehrenberg) F. Stein	R	R	–	–	–	–	–	–
Prorocentrum micans Ehrenberg	R	R	–	–	R	O	R	O
Protoperidinium brevipes (Paulsen) Balech	–	–	–	–	R	O	–	–
Protoperidinium crassipes (Kofoid) Balech	–	–	–	–	R	O	R	M
Protoperidinium pallidum (Ostenfeld) Balech	R	O	–	–	–	–	–	–
Polykrikos kofoidii Chatton	R	M	R	M	–	–	–	–
Pronoctiluca pelagica Fabre-Domergue	R	R	–	–	–	–	–	–
Scrippsiella trochoidea (F. Stein) A. R. Loeblich III	R	R	–	–	R	R	R	O
Dinophyta, cysts	R	R	–	–	–	–	–	–
Cyanophyta								
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová & Cronberg	–	–	R	R	–	–	–	–
Cryptophyta								
Plagioselmis prolonga Butcher ex G. Novarino, I. A. N. Lucas & S. Morrall	R	R	O	R	–	–	–	–
Euglenophyta								
Eutreptia lanowii Steuer	–	–	R	R	M	M	–	–
Chlorophyta								
Pterosperma undulatum Ostenfeld	–	–	–	–	R	R	–	–

Note. Species status in terms of abundance (N) and biomass (B): R – rare species; O – ordinary; M – mass.

Phytoplankton quantitative composition. In April 2019, the mean abundance and biomass in the port water area amounted to 568 thousand cells·L^{-1} and 206 mg·m^{-3}, respectively. Beyond the port, the values were 1.5 times higher: 875 thousand cells·L^{-1} and 334 mg·m^{-3} (Figs 1 and 2). Specifically high values of the abundance in the port were observed at st. 5, 8, 12, and 13 (734–986 thousand cells·L^{-1}), and the minimum ones – at st. 1 and 9 (350–378 thousand cells·L^{-1}). The highest values of the biomass during this period were registered at st. 5 (424 mg·m^{-3}); those were more than 3 times higher than the minimum values noted at st. 1–3 (107–124 mg·m^{-3}).

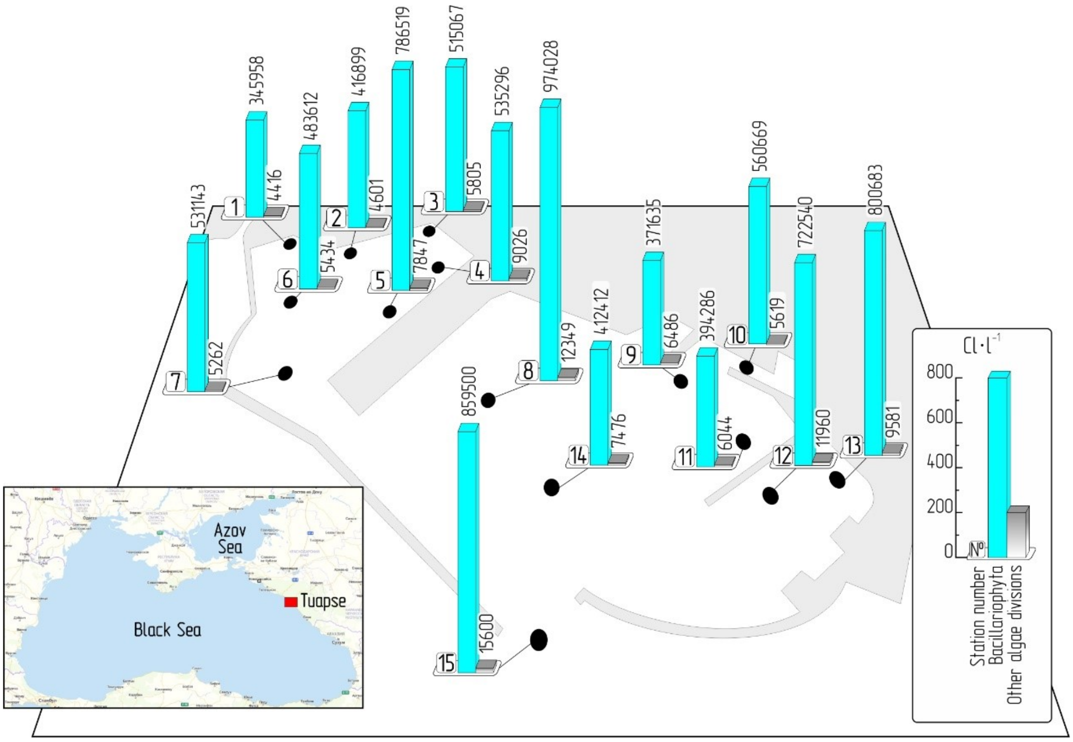

Fig. 1. Distribution of the phytoplankton abundance (cells·L^{-1}) in the water area of the port of Tuapse and beyond it in April 2019

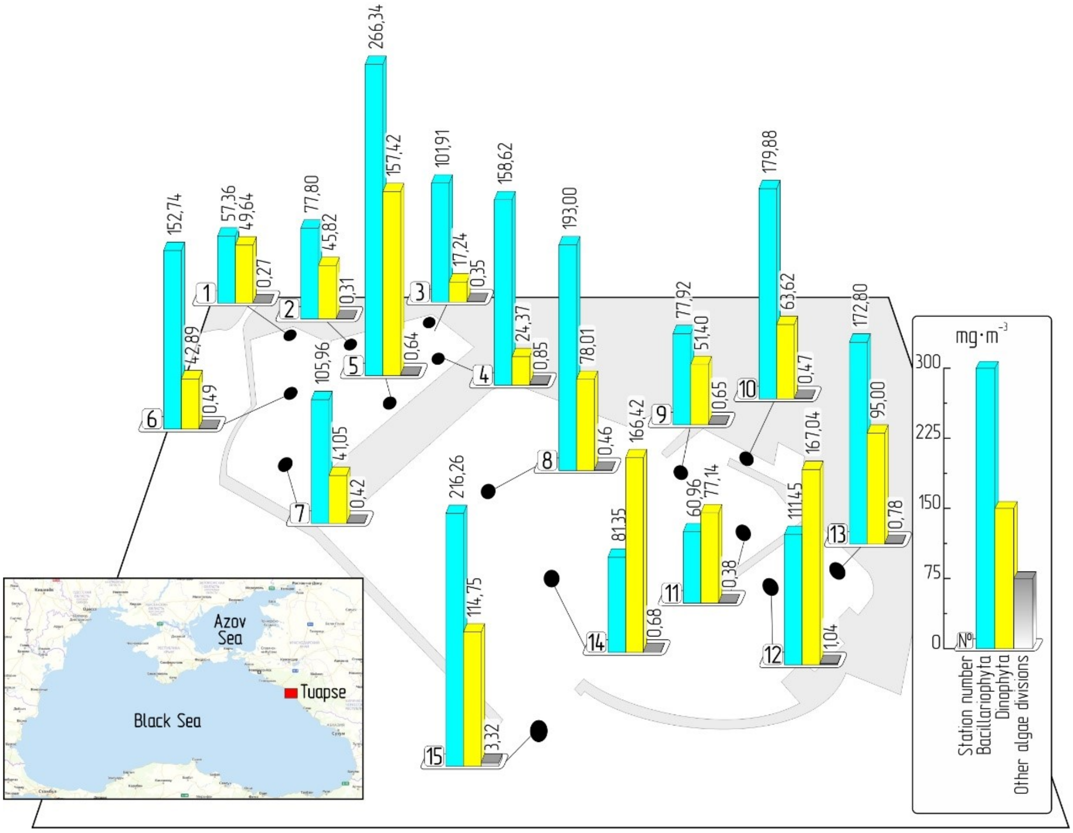

Fig. 2. Distribution of the phytoplankton biomass (mg·m^{-3}) in the water area of the port of Tuapse and beyond it in April 2019

During this period, diatoms were recorded ubiquitously forming 98–99 % of phytoplankton total abundance and 62–65 % of biomass. Out of them, small-cell species *Pseudo-nitzschia* spp. and *Skeletonema costatum* s. l. predominated (72–74 and 21–22 % of the abundance, respectively), whose abundant development is characteristic of the spring season. *Chaetoceros affinis*, *Pseudo-nitzschia seriata*, and *Nitzschia tenuirostris* were found in minor abundance in the port water area (no more than 5 % of the diatom population). In the open sea area, 4 % of the diatom abundance was formed by a complex of species: *Ch. affinis*, *Ch. compressus*, *Ch. scabrosus*, *N. tenuirostris*, and *Thalassionema nitzschioides*.

Pseudo-nitzschia spp., *Pseudosolenia calcar-avis*, *Dactyliosolen fragilissimus*, *Skeletonema costatum* s. l., and *Ch. affinis* formed the basis of diatoms: 86 and 91 % in the port and beyond it, respectively. In the port water area, *P. seriata*, *Th. nitzschioides*, and *Coscinodiscus* sp. amounted to 11 % of the biomass of diatoms; in the open sea area, *Ch. scabrosus* formed more than 5 % of the biomass. For most of common species, a relatively uniform distribution was observed in the port water area. *Ch. affinis* abundance and biomass at st. 5 (100 thousand cells·L^{-1} and 112 mg·m^{-3}, respectively) exceeded these values at other stations by more than an order of magnitude.

In sum, other algae divisions formed no more than 1–2 % of the total phytoplankton abundance. Dinophytes accounted for 0.5 % of the total abundance and 34–37 % of the biomass. In terms of abundance, *Oblea baculifera* predominated: 31–36 % of the abundance and 6 % of the biomass. *Polykrikos kofoidii* was recorded in significant abundance: 14–18 % of the abundance and 55–74 % of the biomass of dinophytic algae. In the port area, a notable proportion of the abundance (37 %) was formed by *Scrippsiella trochoidea*, *Gyrodinium spirale*, *Ensiculifera carinata*, *Gymnodinium simplex*, and *Lessardia elongate*; the large species *Ceratium furca* and *C. tripos* accounted for 22 % of the biomass. In the open sea area, *Diplopsalis lenticula* and *Cochlodinium citron* in sum amounted to 34 % of the abundance and 17 % of the biomass of dinophytes at this time.

Cryptophytic alga *Plagioselmis prolonga* was ubiquitous: from 2 thousand cells·L^{-1} (st. 1) to 10.8 thousand cells·L^{-1} (st. 15); on average, 5 thousand cells·L^{-1}. Whereon, this species formed 1 % of the phytoplankton abundance and 0.3–0.5 % of the biomass. Cyanobacteria and euglenids were recorded in minor abundance (2 thousand cells·L^{-1}) at st. 15 only; that accounted for less than 1 % of the total phytoplankton abundance.

In June 2019, the mean values of the abundance and biomass of planktonic algae in the port amounted to 59 thousand cells·L^{-1} and 81 mg·m^{-3}, respectively (Figs 3 and 4). Beyond the port, the values were more than 2 times lower: 25 thousand cells·L^{-1} and 34 mg·m^{-3}, respectively. During this period, abundant development of euglenids was recorded in the port water area: the mesosaprobic species *Eutreptia lanowii* formed 94 % of the phytoplankton abundance and 83 % of biomass. The diatom *Th. nitzschioides* and dinophytes *C. tripos*, *C. furca*, *Prorocentrum micans*, *Protoperidinium brevipes*, and *P. crassipes* were ubiquitous: 5 % of the abundance and 16 % of the biomass. The representative of green algae *Pterosperma undulatum* was recorded at st. 4 only.

It should be noted that in the water area of the port of Tuapse in the spring-summer period of 2010 and 2011, cyanobacteria and euglenids formed a notable proportion of phytoplankton as well: 35–47 % of the total abundance. The intensive development of these mesosaprobic algae was probably facilitated by the increased concentration of nutrients coming from storm sewers (Yasakova & Makarevich, 2017). Beyond the port, there were no euglenids; diatoms *Skeletonema costatum* s. l. and *Th. nitzschioides* predominated in terms of abundance (95 %). The basis of the phytoplankton biomass (82 %) was formed by dinophytic algae, mainly *Ceratium tripos*, *C. furca*, *Ensiculifera carinata*, *Glenodinium paululum*, *P. micans*, and *Protoperidinium crassipes*.

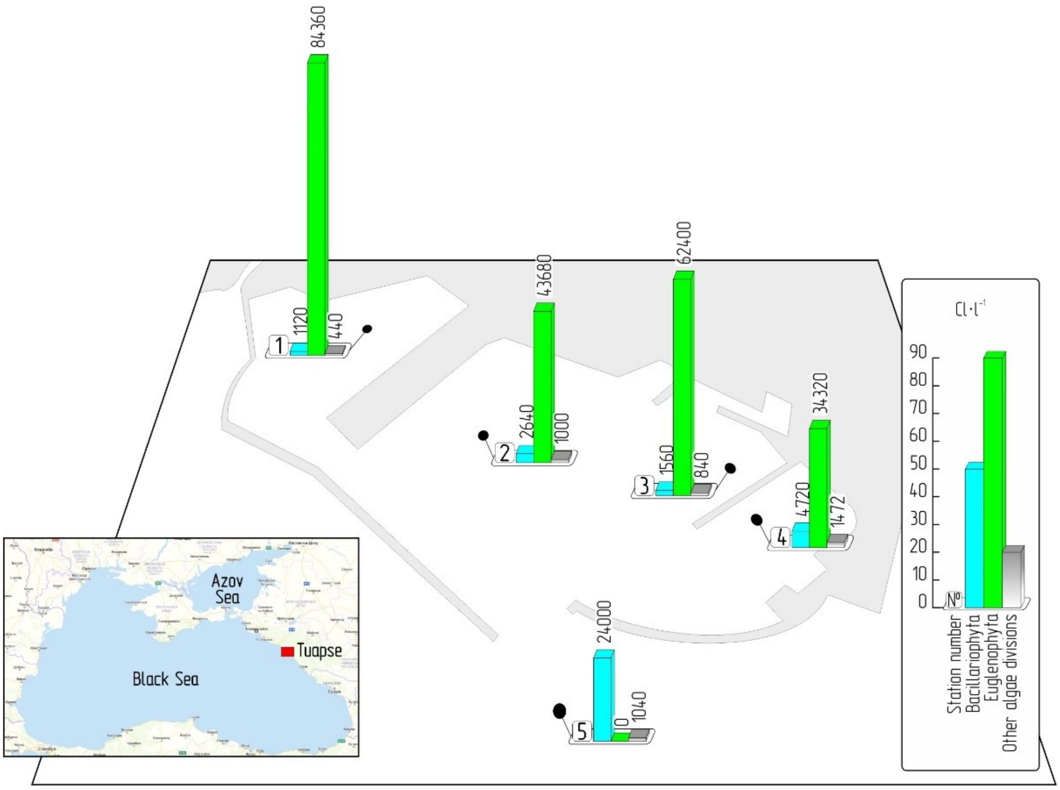

Fig. 3. Distribution of the phytoplankton abundance (cells·L^{-1}) in the water area of the port of Tuapse and beyond it in June 2019

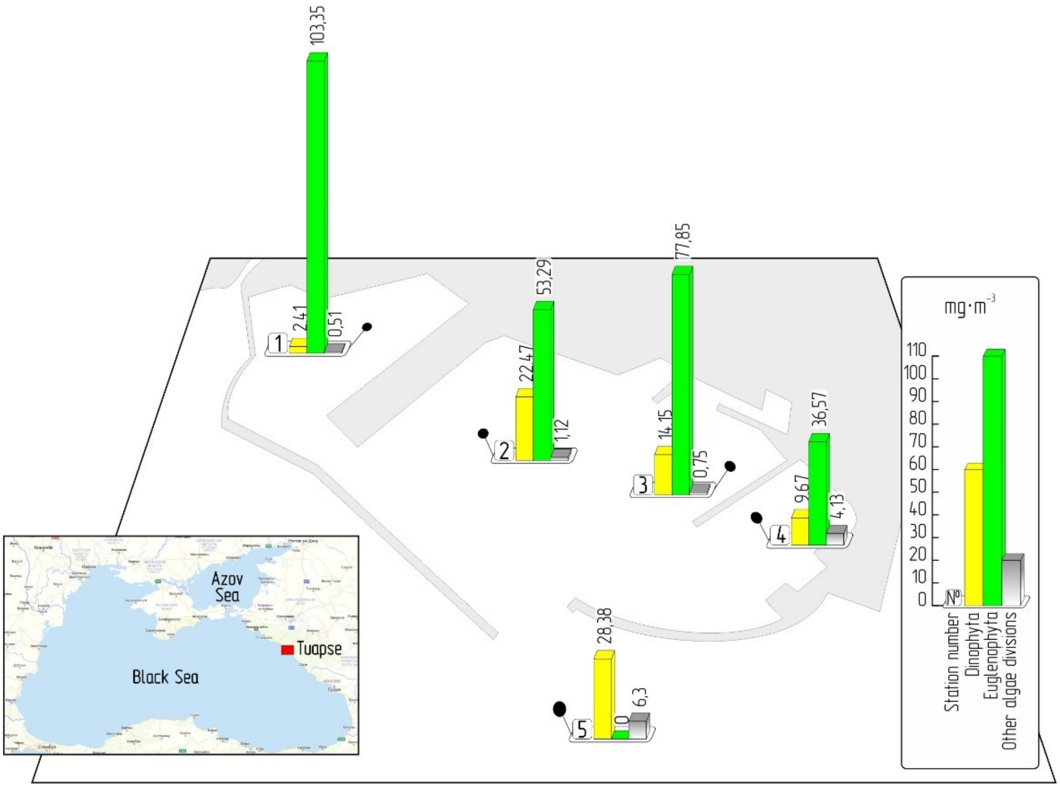

Fig. 4. Distribution of the phytoplankton biomass (mg·m^{-3}) in the water area of the port of Tuapse and beyond it in June 2019

DISCUSSION

In the port, 95 species of planktonic algae were registered earlier (2009–2011); in the open sea area, the species diversity was significantly lower (62 species) (Selifonova & Yasakova, 2012 ; Yasakova & Makarevich, 2017). In the port water area, the mean phytoplankton abundance for the period studied was of 105 thousand cells·L^{-1}, and the biomass was of 0.228 g·m^{-3}. In the open sea area, subject to a lower anthropogenic load, with similar abundance values (120 thousand cells·L^{-1}), the biomass was 2 times higher than in the port water area (0.505 g·m^{-3}), which indicated the presence of large-cell phytoplankton. The peaks of phytoplankton abundance (115–245 thousand cells·L^{-1}) were recorded in March and May 2009 and 2011, as well as in June 2010. Diatoms made a notable contribution to the abundance (35–38 %) and biomass (66–70 %). Moreover, this ratio changed little in the open and port water. The contribution of dinophytes was significant only in the total phytoplankton biomass (26–28 %), wherein they formed 6–7 % of the total abundance. Along with the role of diatoms, the role of primnesian algae, in particular *Emiliania huxleyi*, was great; its abundance was maximum (56 %) in the open sea area, and it was 2 times higher than the values noted in the port (27 %). At the same time, cyanobacteria of the genera *Oscillatoria* and *Lyngbya* and euglenids *Eutreptia lanowii* and *Euglena* sp., presence of which can indicate the unfavorable ecological conditions of coastal water, were a component of phytoplankton in the port water area (11 and 8 % of the total abundance, respectively). In June 2010 and May 2011, their record abundance was registered: in the port water area, they formed up to 35–47 % of the total phytoplankton abundance. Beyond the port, those algae were almost completely absent (no more than 0.02 %).

In 2019, the mean values of the abundance of planktonic algae in the port and beyond it were as follows: 313 thousand cells·L^{-1} in April and 450 thousand cells·L^{-1} in June. Those were almost 2–2.5 times higher than the values for the port water area (145–223 thousand cells·L^{-1}; on average, 184 thousand cells·L^{-1}) and in the open sea (108–207 thousand cells·L^{-1}; on average, 172 thousand cells·L^{-1}) in May and June 2009–2011. Studies carried out shown as follows: in April 2019, the phytoplankton abundance (568 and 875 thousand cells·L^{-1}) was an order of magnitude higher than in June 2019 (59 and 25 thousand cells·L^{-1}). In April 2019, the phytoplankton biomass in the port water area and in the open sea area (on average, 206 and 334 mg·m^{-3}, respectively) was also notably higher (2.5–10 times) than in June 2019 (81 and 34 mg·m^{-3}, respectively). However, those were close to the biomass values observed in the port (80–242 mg·m^{-3}; on average, 175 mg·m^{-3}) and beyond it (165–400 mg·m^{-3}; on average, 293 mg·m^{-3}) in May and June 2009–2011.

The maximum abundance and biomass of planktonic algae were registered in April 2019 beyond the port (875 thousand cells·L^{-1} and 334 mg·m^{-3}, respectively); those were 1.5 times higher than the values for the port water area. This was due to the intensive development of predominantly small-cell diatom species. It should be noted that upwellings, observed in spring along the entire North Caucasus coast, to a significant extent contribute to saturation of coastal water with nutrients and, consequently, to water bloom with small diatoms (Korpakova et al., 2014 ; Proshkina-Lavrenko, 1955). In terms of composition of dominants and the level of abundance, phytoplankton state corresponded to the beginning of the early spring phase of the succession (Makarevich & Oleinik, 2017). The lowest phytoplankton abundance during this period was recorded at the extreme point of the port water area (st. 1). Probably, the hindered water exchange with the open sea resulted there in the formation of unfavorable conditions for the development of planktonic algae.

In June 2019, an increase in the ratio of dinophytes (82 % of the total biomass) was observed in the open sea; that corresponds to the summer phase of the seasonal succession of phytoplankton. In terms of abundance, diatoms predominated again (95 %). Seasonal rearrangement of plant plankton was observed in the port water area as well: there, the predominant plankton component was *E. lanowii*, the mesosaprobic species of euglenids, which formed the basis of the abundance (94 %) and biomass (83 %). In general, the intensive development of euglenids was not typical for the northeastern Black Sea and might manifest the changes in hydrological and hydrochemical environmental conditions: an increase in the eutrophication level, a desalination, and a decrease in the water hydrodynamic activity. The increased level of nutrients in the port water area is also indicated by the twice higher abundance of planktonic algae observed in summer compared to that of the open sea area (st. 5). The minimum phytoplankton abundance in the port water area was again recorded in the tail-end zone (st. 4) characterized by hindered water exchange with the open sea.

In contrast to previous studies, when a significant proportion of the phytoplankton abundance (up to 75 %) at this time of the year was formed by coccolithophorids, the predominant plankton component in 2019 was diatoms and euglenids. Their development may be stimulated by a high concentration of mineral nitrogen and dissolved organic matter in water, while the vegetation of coccolithophorids is limited by mineral phosphorus (Mikaelyan et al., 2013).

Conclusion. The results of the study of phytoplankton, carried out in the spring-summer period of 2019 in the water area of the port of Tuapse and beyond it, notably supplemented the data on the qualitative and quantitative indicators of planktonic microalgae obtained in the previous decade (2009–2011). The state of the plankton community in April 2019 corresponded to the early spring phase of the phytoplankton succession. However, the predominance of the mesosaprobic species of euglenids in plankton in June 2019 may indicate the deterioration of the ecological situation in the port area. Probably, this was facilitated by an increase in the eutrophication level, significant desalination, and stratification of waters, which resulted from the calm weather and an increase in the volume of continental runoff, including wastewater from storm sewers.

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СОСТОЯНИЕ ПЛАНКТОННОГО АЛЬГОЦЕНОЗА В АКВАТОРИИ ПОРТА ТУАПСЕ И ЗА ЕГО ПРЕДЕЛАМИ В ВЕСЕННЕ-ЛЕТНИЙ ПЕРИОД 2019 Г.

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В статье представлены результаты исследования таксономического состава и количества фитопланктона в акватории порта Туапсе и за его пределами в весенне-летний период 2019 г. В апреле 2019 г. обнаружено 43 вида фитопланктона, относящихся к 5 отделам. Средние по акватории порта величи́ны численности и биомассы составили 568 тыс. кл.·л$^{-1}$ и 206 мг·м$^{-3}$ соответственно, что в 1,5 раза ниже, чем в районе открытого моря. Повсеместно в массе (98–99 % общей численности и 62–65 % биомассы фитопланктона) присутствовали диатомовые водоросли. Основу численности составили мелкоклеточные виды *Pseudo-nitzschia* spp.

и *Skeletonema costatum* s. l. Кроме них, основу биомассы формировали *Pseudosolenia calcar-avis*, *Dactyliosolen fragilissimus* и *Chaetoceros affinis*. В июне 2019 г. наблюдали снижение качественных (13 видов из 4 отделов) и количественных величин фитопланктона. Средние значения численности и биомассы планктонных водорослей в порту — 59 тыс. кл.·л$^{-1}$ и 81 мг·м$^{-3}$ соответственно; они в 2 раза превышали величины, отмеченные в открытой части моря. В акватории порта обильно развивались эвгленовые водоросли (94 % численности и 83 % биомассы фитопланктона), представленные мезосапробным видом *Eutreptia lanowii*. За пределами порта Туапсе эвгленовые водоросли отсутствовали, по численности (95 %) доминировали диатомеи — *Skeletonema costatum* s. l. и *Thalassionema nitzschioides*. Основу биомассы (82 %) фитопланктона формировали следующие виды динофитовых водорослей: *Ceratium tripos*, *C. furca*, *Ensiculifera carinata*, *Glenodinium paululum*, *Prorocentrum micans* и *Protoperidinium crassipes*.

Ключевые слова: фитопланктон, таксономический состав, численность, биомасса, акватория порта Туапсе, северо-восточное побережье Чёрного моря

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IN MEMORIAM: YURIY SLYNKO
(09.10.1961 – 14.07.2021)

On July 14, 2021, a talented, extraordinary, and purposeful ichthyologist Yuriy Vladislavovich Slynko passed away.

He was born into a family of genetic scientists in the village of Novovorontsovka, Kherson region. From the age of 5, after the death of his mother, he was raised in the family of his grandfather: the head of the Ukrainian language department at Chernivtsi State University. In 1983, Yu. Slynko graduated from this university with a degree in biology and entered the PhD graduate school in hydrobiology at the Institute of Biology of Inland Waters of the Academy of Sciences of the USSR (Borok, Yaroslavl region). After graduating in 1986, he became a researcher in the laboratory of physiology. Since 1989, he worked as a researcher in the laboratory of ichthyology.

In 2000, Yuriy Slynko successfully defended his PhD thesis “The breeding system of intergeneric hybrids of common roach (*Rutilus rutilus* L.), common bream (*Abramis brama* L.), and blue bream (*Abramis ballerus* L.) (Leuciscinae: Cyprinidae)” in ichthyology at the Zoological Institute of the Russian Academy of Sciences (Saint Petersburg). In two years, he founded a faunogenesis group (later on, the laboratory of evolutionary ecology). Since 2017, he worked at the A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (Sevastopol).

Scientific interests of Yu. Slynko embrace issues of hydrobionts speciation and genetic diversity, distant hybridization, and invasion of alien species into aquatic ecosystems. In search of scientific material, he took part in large-scale expeditions along the Amur, Volga, and Dnieper rivers, as well as along the rivers of Ussuriysk region, Altay, Belarus, Ukraine, Kazakhstan, Mongolia, and Lithuania. He was the manager and participant of 10 grants of the Russian Foundation for Basic Research, 4 programs of the Presidium of the Russian Academy of Sciences, 2 grants of the programs of the Biological Sciences Department of the RAS, and the Federal Science and Technology Program. He participated in international projects with Finland and Mongolia, carried out many economic agreements, and organized scientific symposia “Invasions of alien species in the Holarctic”.

Yuriy Slynko left more than 250 scientific publications and 7 collective monographs. He carried out a large-scale assessment of the genetic diversity of Eurasian freshwater ichthyofauna, described a fish species from the genus *Oreoleuciscus* new to science, formulated a hypothesis of rapid allopatric speciation of the Altai osmans and their origin from minnows at the turn of the Pliocene and Pleistocene, expanded the understanding of evolution mechanisms of endemic species, and determined scale, rate, and direction of fish biological invasions into the largest rivers of the Ponto-Caspian basin. The study of intergeneric hybrids of cyprinids resulted in formulating by him and V. Yakovlev the patterns of inheritance of morphological and genetic features and identifying a new, previously unknown reproductive system in vertebrates: bisexual apomixis. In addition to solving fundamental problems, he was engaged in environmental activities: participated in the work of the commission of the Natural Resources and Ecology Department of Sevastopol on monitoring the state of populations of rare and endangered species of plants and animals.

Under the supervision of Yu. Slynko, four PhD theses were successfully defended. He himself managed to complete the D. Sc. dissertation "Evolutionary and ecological adaptation of alien species of aquatic organisms" and to pass its pre-defense on 10 June, 2021.

The life of Yuriy Slynko was cut short at the peak of his creative achievements: in the autumn of 2021, he would have turned 60. Family, friends, and colleagues will not be able to fill the sudden emptiness in their hearts, but their glorious memory of this bright personality will be the guarantee of the eternal life of Yu. Slynko.

Students, colleagues, and friends

ПАМЯТИ ЮРИЯ ВЛАДИСЛАВОВИЧА СЛЫНЬКО (09.10.1961 – 14.07.2021)

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