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GROWTH OF CULTURES OF MARINE MICROALGAE *PORPHYRIDIUM PURPUREUM* AND *TETRASELMIS VIRIDIS* ON MODIFIED NUTRIENT MEDIA

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Marine species of microalgae are capable of synthesizing a wide range of biologically active substances and are currently considered as the most promising sources of such compounds. Nutrient media for cultivation of microalgae are mostly prepared based on natural or artificial seawater. Modifying the nutrient medium for cultivation of marine microalgae by replacing its natural seawater base with freshwater one seems promising. Unialgal cultures of the marine microalgae *Porphyridium purpureum* and *Tetraselmis viridis* were grown under conditions of replacing sterile seawater with freshwater, with sea salt added up to a concentration of 18 and 28 g·L^{-1} for *T. viridis* and *P. purpureum*, respectively. Based on experimental data obtained, production characteristics of *P. purpureum* and *T. viridis* batch cultures were determined when grown on freshwater-based and seawater-based nutrient media. In general, a change in the density of *P. purpureum* and *T. viridis* cultures during batch cultivation both on freshwater and seawater had a unidirectional character (correlation coefficients in both cases were 0.99), and the water base of the nutrient medium had no significant effect on their growth rate. As shown experimentally, the biomass yield of *P. purpureum* and *T. viridis* using freshwater as a base of the nutrient medium was 3.2–3.4 g of dry weight *per* 1 L of the culture and generally corresponded to the similar parameter of cultures grown using seawater. Despite the fact that the mean growth rate of *T. viridis* cultured in freshwater did not differ significantly from the growth rate of the microalga cultured in seawater, higher mean rates of pigment synthesis and their total accumulation were observed in the culture grown in seawater. In the case of *P. purpureum*, the water base of the nutrient medium had no noticeable effect on B-phycoerythrin synthesis rate and content of this pigment in the culture and biomass of the microalga. The obtained results show that cultures of marine microalgae *P. purpureum* and *T. viridis* can be successfully grown without using natural seawater. It significantly reduces labor costs and biomass production costs; also, it expands geographical perspectives for their mass cultivation.

Keywords: marine microalgae, *Porphyridium purpureum*, *Tetraselmis viridis*, freshwater, productivity, biomass, pigments

Marine species of microalgae are currently considered as the most promising sources of biologically active substances due to their ability to synthesize a wide range of compounds positively affecting organisms of both humans and animals [Minyuk et al., 2008]. Those include polyunsaturated fatty acids, sulfated polysaccharides, photosynthetic pigments (chlorophylls, carotenoids, and phycobiliproteins), vitamins, and other substances with anti-inflammatory, antifungal, immunomodulatory, and antioxidant

properties [Borowitzka, 2013; Chauton et al., 2015; Gaignard et al., 2019; Geada et al., 2021; Li S. et al., 2019]. This allows using marine microalgae biomass and biosynthesis products as dietary supplements and applying them in cosmetology, pharmacology, and food production.

The need for polyunsaturated fatty acids has risen due to the development of aquaculture: for all hydrobionts, microalgae are a key source of valuable long-chain omega-3 fatty acids [Borowitzka, 2013]. Also, marine microalgae are a food supplement for fish. Those contain essential fatty acids, amino acids, polysaccharides, antioxidants, vitamins, and minerals. All these compounds stimulate growth and survival of fish larvae and improve quality of final products [Chauton et al., 2015; Gargouch et al., 2018; Ma, Hu, 2024; Tredici et al., 2009].

The cultivation of marine microalgae in coastal zones is generally less costly due to lower investment, logistics, and operating expenses. However, certain constraints due to competition from recreational, fishing, and fish farming areas and due to urban development force to relocate marine microalgae cultivation complexes to areas far from the shore.

Most nutrient media for microalgae cultivation are prepared on the base of natural or artificial seawater. Various nutrient media for cultivation of microalgae species have been described: F/2, Artificial Seawater Medium, Pm, *etc.* [Fuentes-Grunewald et al., 2015; Gargouch et al., 2018; Kathiresan et al., 2006; Lelekov et al., 2016; Raes et al., 2013; Strizh et al., 2004]. Their composition allows maintaining microalgae cells in a vegetative state. Nevertheless, the culture growth rate and biomass yield may differ significantly depending on starting concentrations of mineral nutrients and cultivation conditions.

Cultivation of marine microalgae becomes unprofitable far from the coastline in case of using nutrient media based on natural seawater. Its mineral composition is unique, and it cannot be properly reproduced under artificial conditions: in addition to mineral salts and trace elements, seawater contains a large number of free ions. One of the ways to reduce the cost of the resulting microalgae biomass is cultivation on nutrient media based on artificial seawater, but its preparation implies additional material and labor expenses. Replacing the base of a nutrient medium with freshwater with natural sea salt added is the way to obtain a base close to natural seawater in its characteristics. Such modification excludes dependence on a natural source of seawater and reduces the cost of biomass; obviously, it is crucial when elevating the efficiency and expanding the area of intensive microalgae cultivation.

A red microalga *Porphyridium purpureum* (Bory) K. M. Drew & R. Ross, 1965 is of interest as a source of sulfated exopolysaccharides, essential fatty acids, and pigments of the phycobiliprotein group. Their biotechnological potential is actively used in producing nutraceuticals, pharmaceuticals, food, and cosmetics; moreover, it is applied in biomedical research and even in clinical diagnostics [Gaignard et al., 2019; Li S. et al., 2019; Manirafasha et al., 2016]. A green microalga *Tetraselmis viridis* (Rouchijajnen) R. E. Norris, Hori & Chihara, 1980 is capable of accumulating significant amounts of polyunsaturated fatty acids. Those play a key role in organisms of humans by participating in metabolic processes. High productivity and nutritional value make the alga promising for production of biologically active substances and food supplements for humans and animals [Borowitzka, 2013; Raes et al., 2013]. Microalgae of the genus *Tetraselmis* are widely used in aquaculture as valuable food enriched with polyunsaturated fatty acids and protein [Borowitzka, 2013; Ma, Hu, 2024; Tredici et al., 2009]. Interestingly, lipids can accumulate in these species in high concentrations (up to 22%) even with relatively high protein levels (31–36%) [Barka, Blecker, 2016].

Accordingly, it seems promising to culture these biotechnologically valuable species of marine microalgae on a nutrient medium modified by replacing its natural seawater base with freshwater one and adding sea salt. Therefore, the aim of this work was to test the cultivation of two marine microalgae, *Tetraselmis viridis* and *Porphyridium purpureum*, on a freshwater-based nutrient medium.

MATERIAL AND METHODS

Objects of the study were unialgal cultures of marine microalgae *P. purpureum* (Rhodophyta) and *T. viridis* (Chlorophyta) from IBSS core facility "Collection of hydrobionts of the World Ocean."

Cultivation was carried out on nutrient media of the following composition [Trenkenshu et al., 1981]:

- For *P. purpureum*: $NaNO_3$, 1.2 g·L^{-1}; $NaH_2PO_4 \times 2H_2O$, 0.45 g·L^{-1}; EDTA-Na_2, 0.037 g·L^{-1}; $FeC_6H_5O_7 \times 3H_2O$, 0.0265 g·L^{-1}; $MnCl_2 \times 4H_2O$, 0.004 g·L^{-1}; $Co(NO_3)_2 \times 6H_2O$, 0.0031 g·L^{-1}; $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 0.0009 g·L^{-1}; and $K_2Cr_2(SO_4)_2 \times 4H_2O$, 0.0017 g·L^{-1}.
- For *T. viridis*: $NaNO_3$, 1.8 g·L^{-1}; $NaH_2PO_4 \times 2H_2O$, 0.3 g·L^{-1}; EDTA-Na_2, 0.037 g·L^{-1}; $FeC_6H_5O_7 \times 3H_2O$, 0.042 g·L^{-1}; $MnCl_2 \times 4H_2O$, 0.008 g·L^{-1}; $Co(NO_3)_2 \times 6H_2O$, 0.00625 g·L^{-1}; $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 0.00183 g·L^{-1}; and $K_2Cr_2(SO_4)_2 \times 4H_2O$, 0.00238 g·L^{-1}.

A nutrient medium for each species was prepared on sterile Black Sea water (for *P. purpureum*, 10 g·L^{-1} of sea salt was added). For modification, seawater was replaced with freshwater to which sea salt (manufactured by Galit production cooperative) was added to concentrations of 18 and 28 g·L^{-1} for *T. viridis* and *P. purpureum*, respectively.

Microalgae were cultured in glass plane-parallel photobioreactors, 25 × 50 cm each. Working thickness was of 2 and 5 cm, and volume was of 1 and 3 L for *T. viridis* and *P. purpureum*, respectively. These volumes were maintained throughout the experiment with daily adding distilled water before sampling to compensate for evaporation. A grid of 18-W fluorescent lamps served as a light source; the mean irradiance intensity at the surface of the photobioreactors was 20 and 40 W·m^{-2} for *P. purpureum* and *T. viridis*, respectively. Irradiance intensity on the surface of the photobioreactors was recorded with a LI-250A light meter with a pyranometer (LI-COR, the USA). Temperature was maintained at +26…+28 °C, and pH of a medium varied from 8 to 10 during cultivation. Cultures were bubbled with air with a Hailea ACO-308 compressor; the rate of air supply was about 0.5 L·L^{-1} culture *per* minute. To increase solubility of atmospheric carbon dioxide, air bubbling was carried out *via* an atomizer: a plastic tube 5 cm long and 5 mm in diameter, with a pore diameter of < 0.1 mm. The experiments lasted for 18 and 10 days for *P. purpureum* and *T. viridis*, respectively.

The optical density of cultures (D_{750}) was measured with a Unico 2100 spectrophotometer in cuvettes with a working length of 5 mm at a wavelength of 750 nm. Dry weight content (DW) was determined by a photometric method [Metody, 1975] by equation $DW = k \times D_{750}$. *Prior* to it, empirical conversion factors from the optical density of the cultures to DW were established (1.4 and 0.8 for *P. purpureum* and *T. viridis* respectively) [Borovkov, Gevorgiz, 2005; Borovkov et al., 2023].

The maximum productivity (P_m) was calculated by approximating empirical data on the linear phase of microalgae cumulative curve using equation (1):

$$B = B_l + P_m \cdot t\,, \tag{1}$$

where B_l is culture density at the beginning of linear growth phase, g·L^{-1};

t is time, days.

Pigment content was recorded spectrophotometrically. Sampling was carried out at different growth phases of a batch culture after thorough mixing. A suspension was centrifuged for 10 min, the supernatant was drained, and the precipitated biomass was used for pigment determination. The spectra of pigment extracts were measured with a SF-2000 spectrophotometer (Russia). For quantitative determination of B-phycoerythrin (hereinafter B-PE), *P. purpureum* biomass was extracted with a phosphate buffer (0.05 M; pH 7–7.5). The optical density of the obtained extracts was registered in the area of characteristic absorption maxima of B-PE (545 nm), R-phycocyanin (615 nm), and allophycocyanin (650 nm), as well as at 750 nm (to account for non-specific absorption of the solution). B-PE concentration in the aqueous extract was calculated according to [Stadnichuk, 1990] using optical density values for corresponding wavelengths:

$$B-PE = 0.1 \times D_{545} - 0.063 \times D_{615} + 0.023 \times D_{650}\,. \quad (2)$$

Chlorophylls and carotenoids were extracted from cells with 100% acetone. Concentrations of chlorophylls and total carotenoids were determined by formulas from [Wellburn, 1994] using optical density values at wavelengths corresponding to absorption maxima of similar pigments:

$$\text{Chl}\,a = 11.75 \times D_{662} - 2.35 \times D_{645}\,;$$

$$\text{Chl}\,b = 18.61 \times D_{645} - 3.96 \times D_{662}\,;$$

$$\text{Carotenoids} = (1,000 \times D_{470} - 2.27 \times \text{Chl}\,a - 81.4 \times \text{Chl}\,b)/227\,.$$

Arithmetic mean ($\bar{x}$), standard deviations (S), standard errors of the mean, and confidence intervals for means ($\Delta\bar{x}$) were established. Calculations were carried out in LibreOffice and SciDAVis (at the significance level, α, of 0.05). In order not to clutter the graphs, statistical indicators characterizing variability of the studied features were omitted without compromising the perception of the results obtained. The tables provide mean values and determined confidence intervals ($\bar{x} \pm \Delta\bar{x}$) for three replications.

RESULTS

Initial culture density for each of two variants (seawater-based and freshwater-based nutrient media) was about 0.2 g·L^{-1} for *P. purpureum* and 0.13 g·L^{-1} for *T. viridis*. Throughout the cultivation, *P. purpureum* biomass increased by more than 15 times compared to the initial one, and that of *T. viridis*, by 25 times (Fig. 1).

During batch cultivation, microalgae culture cells are maintained in a vegetative state due to initial stock of mineral nutrition elements, with the culture density gradually rising and reaching the maximum value which determines the total biomass yield. Throughout the entire period of *P. purpureum* and *T. viridis* cultivation, the character of changes of cumulative curves for seawater- and freshwater-based media was almost the same and had a high correlation (correlation coefficients were of 0.99 for both *P. purpureum* and *T. viridis*). Total biomass growth for analyzed variants also had no significant differences (Fig. 1). Based on data obtained, production characteristics of *P. purpureum* and *T. viridis* batch cultures by biomass on freshwater- and seawater-based nutrient media were determined (Table 1).

The nutrient media used for *P. purpureum* and *T. viridis* cultivation were expected to produce about 4 g of biomass *per* 1 L of culture [Trenkenshu et al., 1981; Upitis et al., 1989]. The calculated biomass growth for the entire cultivation period, taking into account both nitrogen concentration in a medium and dilution, could be about 3–3.5 g·L^{-1} which was consistent with data obtained in the experiment (Table 1).

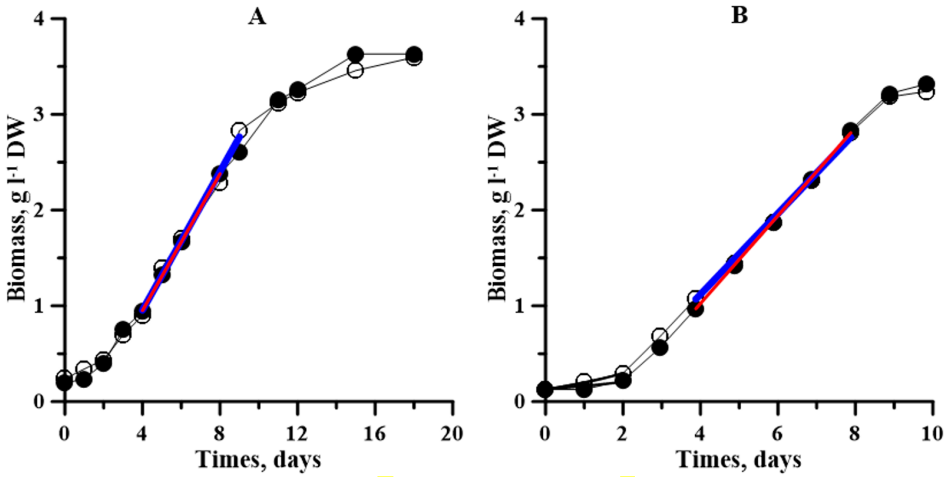

Fig. 1. Dynamics of *Porphyridium purpureum* (A) and *Tetraselmis viridis* (B) batch culture density when grown on a nutrient medium: ●, based on freshwater; ○, based on seawater. Solid lines are an approximation of the linear phase by equation (1) (red, based on freshwater; blue, based on seawater). The values of the coefficients (maximum productivity) are given in Table 1

Biomass productivity of *P. purpureum* and *T. viridis* at the linear growth phase when cultured on freshwater did not differ noticeably from that of corresponding cultures grown on seawater. Both maximum and mean growth rates were slightly higher in *T. viridis* compared to *P. purpureum* which seems to be due to individual characteristics of a culture. However, the specific growth rate of *T. viridis* when cultured on freshwater was lower by almost 2 times compared to the same parameter of the culture grown on seawater (Table 1). *T. viridis* culture was previously grown on an unmodified medium; accordingly, it can be assumed as follows: during the first two days, algal cells adapted to new conditions which is confirmed by some differences in the shape of the accumulation curve (Fig. 1B). In general, a change in *P. purpureum* and *T. viridis* density during batch cultivation on both freshwater and seawater had a unidirectional character, and the productivity of cultures was not much affected by a water base of a nutrient medium.

Table 1. Production characteristics of *Porphyridium purpureum* and *Tetraselmis viridis* batch cultures when grown on freshwater-based and seawater-based nutrient media

Culture	Water base of the nutrient medium	Specific growth rate, day^{-1}	Maximum productivity, $g \cdot L^{-1} \cdot day^{-1}$	Productivity at the linear growth stage, $g \cdot L^{-1} \cdot day^{-1}$	Total biomass growth, $g \cdot L^{-1}$
Porphyridium purpureum	Freshwater	0.45	0.36	0.27 ± 0.02	3.44 ± 0.23
	Seawater	0.35	0.36	0.29 ± 0.01	3.36 ± 0.02
Tetraselmis viridis	Freshwater	0.23	0.46	0.43 ± 0.01	3.19 ± 0.02
	Seawater	0.42	0.46	0.42 ± 0.006	3.11 ± 0.01

During batch cultivation, biochemical composition of resulting microalgae biomass is determined by many parameters, and the key ones are concentration of mineral nutrition elements and cultivation conditions. Therefore, the effect of replacing a water base of a nutrient medium with freshwater on synthesis rate and total pigment accumulation in *P. purpureum* and *T. viridis* was investigated as well. Under these conditions, *P. purpureum* growth was accompanied by a change in B-PE content both in the culture and its cells (Fig. 2).

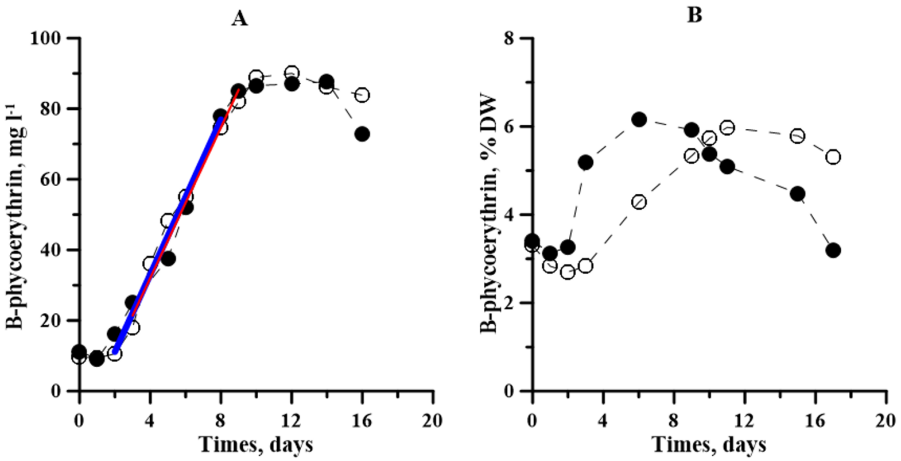

Fig. 2. B-phycoerythrin content in a batch culture (A) and in cells (B) of *Porphyridium purpureum* when grown on a nutrient medium: ●, based on freshwater; ○, based on seawater. Solid lines are an approximation of the linear phase (red, based on freshwater; blue, based on seawater). The values of the coefficients (maximum synthesis rate) are given in Table 2

B-PE content during *P. purpureum* cultivation on both freshwater and seawater gradually increased (9.2–9.5 times) and reached maximum values (88–90 mg·L^{-1}) on the 10th–14th day from the beginning of the experiment (Fig. 2A). A change in B-PE concentration in the culture was unidirectional for both variants of the medium, and the correlation coefficient was of 0.98. An increase in B-PE content in *P. purpureum* cells was less pronounced: it was about 2 times over 7 days and reached 6% of DW on average for each variant (Fig. 2B). We recorded a decrease in B-PE concentration in *P. purpureum* cells in the second half of batch cultivation; such a change has been reported by many researchers and explained by a direct dependence of pigment concentration on nitrogen content in a medium which can drop to minimum values by the final cultivation stage [Fuentes-Grunewald et al., 2015; Li T. et al., 2019]. Data characterizing the rate of B-PE accumulation in the algal culture when grown on freshwater- and seawater-based nutrient media are provided in Table 2.

Table 2. B-phycoerythrin (B-PE) production characteristics of *Porphyridium purpureum* batch culture when grown on freshwater-based and seawater-based nutrient media

Water base of the nutrient medium	Specific synthesis rate (0–3rd days), day^{-1}	Maximum B-PE synthesis rate, mg·L^{-1}·day^{-1}	Mean B-PE synthesis rate (2nd–9th days), mg·L^{-1}·day^{-1}	Total B-PE accumulation, mg·L^{-1}
Freshwater	0.3	12.1	9.8 ± 1.75	77.9 ± 4.7
Seawater	0.2	12.3	10.2 ± 2.3	80.5 ± 5.4

The calculation results showed that the maximum rate of B-PE synthesis in *P. purpureum* culture both on freshwater and seawater was almost the same. The mean rate of B-PE synthesis at the linear stage and the level of pigment accumulation are slightly higher in the culture grown on seawater, but these differences are not significant (Table 2). Despite close values of the culture growth rate (Table 1) and observed rate of B-PE synthesis in two variants, a certain temporal discrepancy was recorded in dynamics of B-PE content in *P. purpureum* biomass (Fig. 2B). This can be explained by a noticeable difference between specific rates of pigment synthesis in two variants in the first three days of the experiment. During this period, the rate of pigment synthesis in a culture grown on freshwater with sea salt added was 1.5 times higher compared to that of a culture grown on seawater. In this case, B-PE could accumulate in *P. purpureum* cells much faster not only in the first day, but also in the next few days.

T. viridis growth on media both with seawater and freshwater was also accompanied by changes in pigment content (Fig. 3). Chlorophyll *a* and chlorophyll *b* concentration during *T. viridis* cultivation (on the 4th–10th days) on both freshwater and seawater gradually rose and reached maximum values by the end of the experiment: an increase by 2.4–3 and 1.7–2.2 times was registered for chlorophyll *a* and chlorophyll *b*, respectively (Fig. 3A, B). A change in content of these pigments in the culture also was unidirectional for two media, and the correlation coefficient was of 0.99. For total carotenoids, a gain in pigment concentration in the culture was recorded as well, but these changes were expressed to a lesser extent than changes in chlorophyll content (Fig. 3C). At comparable density of *T. viridis* culture for two variants, pigment concentration throughout the entire experiment was higher in the culture grown on seawater.

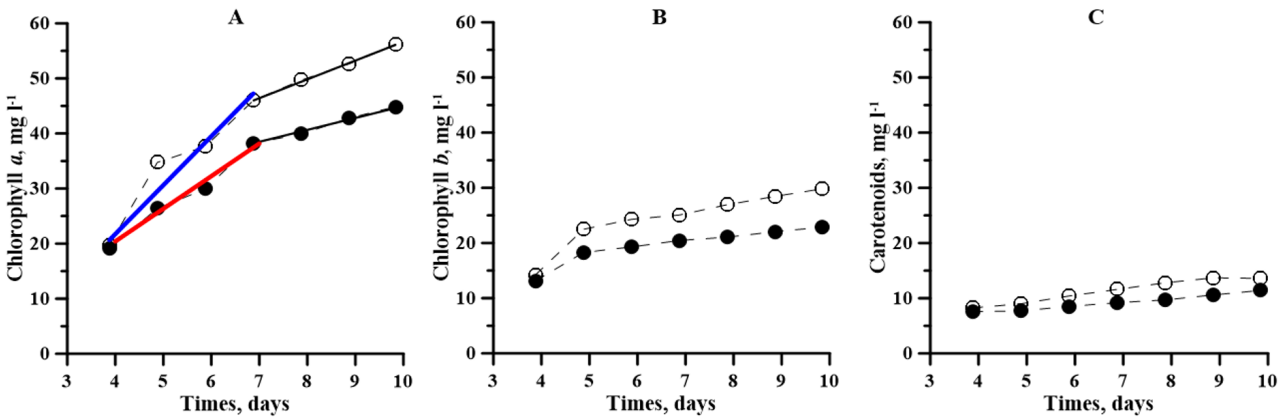

Fig. 3. Content of chlorophyll *a* (A), chlorophyll *b* (B), and carotenoids (C) in *Tetraselmis viridis* batch culture when grown on a nutrient medium: ●, based on freshwater; ○, based on seawater. Solid lines are an approximation of the linear phase (red, based on freshwater; blue, based on seawater). The values of the coefficients (maximum synthesis rate) are given in Table 3

Data characterizing features of pigment accumulation in *T. viridis* batch culture when grown on freshwater and seawater are provided in Table 3.

Table 3. Production characteristics of *Tetraselmis viridis* batch culture for chlorophyll *a*, chlorophyll *b* (chl *a* and chl *b*), and total carotenoids (car) when grown on freshwater-based and seawater-based nutrient media

Water base of the nutrient medium	Maximum pigment synthesis rate, mg·L^{-1}·day^{-1}			Pigment synthesis rate at the linear stage, mg·L^{-1}·day^{-1}			Total pigment accumulation, mg·L^{-1}		
	chl *a*	chl *b*	car	chl *a*	chl *b*	car	chl *a*	chl *b*	car
Freshwater	6.38	–	–	2.16 ± 0.53	0.96 ± 0.11	0.71 ± 0.12	25.77 ± 0.08	9.74 ± 0.62	3.44 ± 0.22
Seawater	8.76	–	–	3.41 ± 0.34	1.47 ± 0.29	1.23 ± 0.21	36.46 ± 1.27	15.60 ± 1.47	5.50 ± 0.04

Maximum and mean synthesis rates of chlorophyll *a* and synthesis rates of chlorophyll *b* and total carotenoids in *T. viridis* batch culture when grown on the seawater-based nutrient medium were more than 1.5 times higher than in case of the freshwater-based one. Such noticeable differences resulted in an increase in pigment accumulation in *T. viridis* culture grown on seawater compared

to that on freshwater by 1.4–1.6 times. Thus, the mean growth rate of *T. viridis* cultured on freshwater was not significantly different from that of the alga cultured on seawater, but higher rates of pigment synthesis and total accumulation were recorded in the culture grown on seawater.

DISCUSSION

Marine microalgae are traditionally cultured on seawater-based nutrient media [Fuentes-Grunewald et al., 2015; Kathiresan et al., 2006; Raes et al., 2013; Strizh et al., 2004]. However, there are data on cultivation of some species of marine microalgae on freshwater-based nutrient media [Gargouch et al., 2018; Lelekov et al., 2016]. Specifically, a marine microalga *Porphyridium marinum* was grown on freshwater-based nutrient medium [Gargouch et al., 2018]. The maximum density of the culture was $(4.6 \pm 0.5) \times 10^6$ cells·mL^{-1}, and B-PE content in biomass was 15.9 mg·g^{-1} in terms of DW. The maximum amount of B-PE in biomass (41 mg·g^{-1}) was registered at the second stage: with an increase in $NaNO_3$ concentration by 2 times, a drop in K_2HPO_4 content to 0 g·L^{-1}, a decrease in light intensity by 2 times, and a rise in volume of micronutrient solution by 1.5 times. However, the density of *P. marinum* batch culture, its productivity, and B-PE content in biomass (at its maximum concentration) are significantly lower than those in the experiment carried out. Moreover, the use of NaCl required adding Mg and Ca naturally contained in seawater, and the application of a two-stage cultivation mode noticeably enlarged the time of cultivation [Gargouch et al., 2018].

In our experiment, when growing *P. purpureum* and *T. viridis* on freshwater-based media, the biomass yield exceeded 3 g of DW *per* 1 L of culture, and the mean growth rate was within 0.3–0.4 g·L^{-1}·day^{-1}. These values are comparable with data obtained earlier, when *P. purpureum* and *Dunaliella salina* (Dunal) Teodoresco, 1905 were cultured on seawater: the mean productivity at the linear growth phase was of 0.37 and 0.2 g·L^{-1}·day^{-1}, respectively [Gudvilovych, Borovkov, 2017; Gudvilovich et al., 2021]. Also, these values are similar to results reported in [Li T. et al., 2019]. Moreover, our data are comparable to production parameters of *T. viridis* when grown on artificial seawater with NaCl concentration of 29 g·L^{-1}: the maximum density of the culture by the 8th day was about 12×10^6 cells·mL^{-1} [Strizh et al., 2004]. Besides, a diatom *Phaeodactylum tricornutum* Bohlin, 1897 was shown to be culturable on a freshwater-based nutrient medium with sea salt added [Lelekov et al., 2016]. In this case, the mean growth rate was of 0.3 g·L^{-1}·day^{-1} which is also comparable to results obtained in our experiment. Notably, *P. purpureum* and *T. viridis* batch cultures were grown without additional carbon: an approved technique of atmospheric air bubbling was applied [Gudvilovych, Borovkov, 2017; Gudvilovich et al., 2021].

Despite the fact that the growth rate of *T. viridis* cultured on freshwater did not differ significantly from the growth rate of this alga on seawater, this variant showed lower rates of pigment synthesis and their total accumulation which may indicate certain non-optimality of cultivation conditions. Apparently, the factor negatively affecting synthesis of *T. viridis* pigments in this case could be an elevated pH level. Throughout the entire experiment, pH of the culture grown on freshwater was higher, by 5% on average, than for the culture grown on seawater. Starting from the 5th day of *T. viridis* culturing on freshwater, when pH values were close to 10, a decrease in the rate of pigment synthesis and production was up to 30–40% in comparison with values for the alga grown on seawater. As known, pH of a medium rises during batch cultivation of microalgae, and its level is critical: it determines solubility and availability of CO_2 and nutrients and noticeably affects microalgae metabolism [Chen, Durbin, 1994; Kumar, Saramma, 2018; López-Elías et al., 2008; Qiu et al., 2017].

Specifically, the effect of pH on growth and biochemical composition of *Dunaliella bardawil* and *Chlorella ellipsoidea* was studied. As shown, a change in pH shifts the direction of biosynthesis in microalgae cells thus affecting biochemical composition of biomass obtained [Khalil et al., 2010]. Both microalgae were capable of growing over a wide pH range (4–9 for *D. bardawil* and 4–10 for *C. ellipsoidea*); however, biomass production in *D. bardawil* reached its maximum at pH of 7.5, and in *C. ellipsoidea*, at pH of 9 [Khalil et al., 2010]. For both species analyzed, the highest values of accumulation of chlorophyll *a*, chlorophyll *b*, and carotenoids were recorded at pH of 7.5. As pH increased (shifted towards alkaline side), content of these three pigments dropped significantly. Importantly, a noticeable decrease in content of all pigments for both *D. bardawil* and *C. ellipsoidea* (more than 2-fold and by 30%, respectively) was observed against the backdrop of pH rise from 9 to 11 which correlates with data obtained in our experiment with *T. viridis*.

Most species used in aquaculture require pH within 6–9. However, various microalgae species have their own optimal pH ranges for biomass production, often strain-specific ones [Khalil et al., 2010; Qiu et al., 2017]. A comparative assessment of production characteristics for *P. purpureum* and *T. viridis* revealed no noticeable differences in the growth rate of the batch cultures when grown on freshwater- and seawater-based nutrient media. Moreover, when using freshwater as a base for *P. purpureum* culturing, we recorded no significant differences in maximum and mean rates of B-PE synthesis and in the level of pigment accumulation in the culture. In general, all these characteristics correspond to similar parameters of B-PE biosynthesis in a culture grown on seawater, both in investigations carried out earlier and in the described experiment [Gudvilovich et al., 2021; Li T. et al., 2019].

Above-mentioned data on the experience of cultivation of *P. purpureum*, *T. viridis*, and *Ph. tricornutum* [Lelekov et al., 2016] representing different systematic groups show the need for further studies on growing other marine microalgae on freshwater-based nutrient media with sea salt added.

Conclusion. As shown, microalgae *Porphyridium purpureum* and *Tetraselmis viridis* can be successfully cultured without the use of natural seawater. Importantly, the water base of a nutrient medium does not significantly affect production parameters of these two species; in the case of *P. purpureum*, it has no effect on the rate of B-phycoerythrin synthesis and its content in both culture and biomass. When culturing *T. viridis*, a possibility of monitoring pH and adjusting it to an optimal level should be considered. The use of freshwater instead of natural seawater, no need for adding mineral salts to prepare artificial seawater, and no need for adding carbon dioxide allow maintaining high growth rate of marine microalgae cultures. It noticeably reduces labor costs and biomass production costs and also expands geographical perspectives for their mass cultivation.

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РОСТ КУЛЬТУР МОРСКИХ МИКРОВОДОРОСЛЕЙ *PORPHYRIDIUM PURPUREUM* И *TETRASELMIS VIRIDIS* НА МОДИФИЦИРОВАННЫХ ПИТАТЕЛЬНЫХ СРЕДАХ

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Морские виды микроводорослей, которые способны синтезировать широкий спектр биологически активных веществ, в настоящее время считают наиболее перспективными источниками таких соединений. Большинство питательных сред для культивирования микроводорослей приготавливают на основе природной или искусственной морской воды. Представляется перспективной модификация питательной среды для выращивания морских микроводорослей путём замены её водной основы с природной морской воды на пресную. Альгологически чистые культуры морских микроводорослей *Porphyridium purpureum* и *Tetraselmis viridis* выращивали, заменяя стерильную морскую воду на пресную, в которую добавляли морскую соль до концентрации 18 и 28 г·л$^{-1}$ для *T. viridis* и *P. purpureum* соответственно. На основании полученных экспериментальных данных определены продукционные характеристики накопительных культур *P. purpureum* и *T. viridis* при их выращивании на пресной и морской водной основе питательной среды. В целом изменение плотности культур *P. purpureum* и *T. viridis* при накопительном культивировании как на пресной, так и на морской воде имело однонаправленный характер (коэффициенты корреляции в обоих случаях 0,99), а водная основа питательной среды не оказывала существенного влияния на скорость их роста. Показано, что выход биомассы *P. purpureum* и *T. viridis* при использовании пресной воды в качестве основы питательной среды составляет 3,2–3,4 г с 1 л культуры (в пересчёте на сухое вещество) и в основном соответствует аналогичному параметру культур, выращенных с применением морской воды. Несмотря на то, что средняя скорость роста *T. viridis* при выращивании на пресной воде

существенно не отличалась от скорости роста культуры на морской воде, отмечены повышенные средние скорости синтеза пигментов и их суммарное накопление у культуры, выращиваемой на морской воде. Для *P. purpureum* водная основа питательной среды не оказывала заметного влияния на такие характеристики, как скорость синтеза B-фикоэритрина и содержание этого пигмента в культуре и в биомассе микроводоросли. Результаты работы показывают, что культуры морских микроводорослей *P. purpureum* и *T. viridis* можно успешно выращивать без использования природной морской воды, что существенно снижает трудозатраты и себестоимость получаемой биомассы, а также расширяет географические перспективы их массового культивирования.

Ключевые слова: морские микроводоросли, *Porphyridium purpureum*, *Tetraselmis viridis*, пресная вода, продуктивность, биомасса, пигменты

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COLOR PATTERNS OF THE THORNBACK SKATE, *RAJA CLAVATA* LINNAEUS, 1758, FROM THE SEA OF MARMARA SUGGESTING POSSIBLE MISIDENTIFICATIONS OF SEVERAL RAJIDS IN THE REGION

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Trawl surveys conducted in shelf waters of the northeastern Sea of Marmara revealed for the first time the occurrence of atypically colored thornback skates, *Raja clavata* Linnaeus, 1758 (Rajiformes: Rajidae), in the region. Since atypical coloring may lead to confusion and misidentification of *R. clavata*, an integrated approach of conventional alpha taxonomy and genetic studies is needed to resolve the taxonomic status of *Raja* species occurring in the Sea of Marmara. Accurate taxonomic resolution is the first step to properly differentiate the populations of the aforementioned species *prior* to performing further study and effective conservation.

Keywords: Rajiformes, polychromatism, aberrations, taxonomy, description

The skates (Chondrichthyes: Rajiformes) currently comprise nearly 304 species representing four families: Arhynchobatidae, or softnose skates; Rajidae, or hardnose skates; Anacanthobatidae, or legskates; and Gurgesiellidae, or pygmy skates [Weigmann, Reinecke, 2023]. Members of the family Rajidae which includes approximately 160 validly described species from 16 genera vary in size from small to very large (total length, TL, ranges 33 to 264 cm as adults) and occur in mainly oceans and seas, from shallow waters down to depth of 4,156 m [Last et al., 2016; Weigmann, Reinecke, 2023]. In the Mediterranean Sea, the family is represented by 4 genera (*Dipturus*, *Leucoraja*, *Raja*, and *Rostroraja*) and 16 species, one of which is the thornback skate, *Raja clavata* Linnaeus, 1758 [Barone et al., 2022]. The thornback skate is one of the first described and best-known members of the family Rajidae which is widespread in the eastern Atlantic and southwestern Indian Ocean, from Iceland to Madagascar, including the Mediterranean Sea, Sea of Marmara, and Black Sea [Last et al., 2016].

The thornback skate is distinguished from other skates in its range by the presence of strong thorny tubercles (bucklers) on both dorsal and ventral surfaces of large specimens and lateral rows of strong hooked thorns along the edge of the tail having dark and light crossbars even in large individuals [Barone et al., 2022; Last et al., 2016]. However, the coloring pattern on the dorsal surface can

be remarkably variable which does not correspond to the generally accepted description of *R. clavata* leading to misidentifications with several other Mediterranean species, such as the spotted skate, *Raja montagui* Fowler, 1910, and the speckled skate, *Raja polystigma* Regan, 1923 [Capapé et al., 2018; Chatzispyrou et al., 2019; Mnasri et al., 2009]. Discrimination of these species which are morphologically very similar but have different life cycles [Ebert, Stehmann, 2013; Last et al., 2016] is due to an unavoidable and unmet need for accurate fisheries management and conservation efforts.

In the present article, the authors report on the occurrence of polychromatic specimens of *R. clavata* in the Sea of Marmara and possible implications of unusual color patterns of the thornback skate on accurate identification.

MATERIAL AND METHODS

During recent scientific bottom trawling surveys in the Sea of Marmara (Fig. 1), several thornback skates with atypical dorsal color patterns (Fig. 2) were caught. On 19 February, 2024, a male specimen (hereinafter referred to as RC1) was captured on a muddy-sandy bottom at depths ranging 85.2 to 87.6 m (trawl positions: start, 40°86.343′N, 29°00.997′E; end, 40°85.515′N, 29°04.568′E). Two more males (hereinafter referred to as RC2 and RC3, respectively) were caught on 22 February, 2024, on a similar bottom type at depths ranging 35 to 50 m (trawl positions: start, 40°95.250′N, 28°98.405′E; end, 40°92.498′N, 28°97.550′E). Morphometric measurements of the captured thornback skates presented as percentages of total length (TL) or disc width (DW) of the mean ± standard deviation (*SD*) (Table 1) were recorded following the procedure outlined by Hubbs and Ishiyama [1968]: either with a measuring type for distances ≥ 10 cm to the nearest 0.5 mm or with a Vernier caliper for distances < 10 cm to the nearest 0.05 mm. TL is the distance from the tip of the snout to the tip of the tail, and DW is the distance between the outermost tips of the pectoral fins [Barone et al., 2022]. The angle of the snout in front of the level of the spiracles was measured according to Ebert and Stehmann [2013]. Maturity stages of the examined specimens were determined in accordance with MEDITS (the international bottom trawl survey in the Mediterranean) maturity scale for oviparous elasmobranchs [Atlas, 2019]. Studied skates are preserved in a deep-freezer (–20 °C) at the Istanbul University, Faculty of Aquatic Sciences, Department of Fisheries Technology and Management laboratories without providing catalogue numbers. Raw data, photographs, and frozen specimens are available upon request for further examination.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The thornback skates were examined within the scope of the ongoing Stock Identification of Demersal Fishes in the Eastern Sea of Marmara project; ethics committee approval granted by the Local Ethics Committee of Istanbul University Animal Experiments covers the present study as well (project ID: 2714; approval granted on 17.04.2015). Before dissection, the thornback skates were kept in well-aerated seawater tanks. Then, each thornback skate was anesthetized by keeping it in seawater with a sufficient dose of MS-222 for at least an hour, and if it did not show signs of life, the specimen was measured and dissected.

RESULTS

The following morphometric and morphological characteristics were registered in the examined specimens. The angle of the snout in front of the level of the spiracles was 114° (RC1), 112° (RC2), and 116° (RC3). Tail length ranged 52.42 to 55.68% of TL (mean was 53.74% of TL). In the specimens

studied, mean interorbital length was 2.34 ± 0.28 times the prenarial length (range of 2.03 to 2.72); mean eye length was 1.25 ± 0.25 times the prenarial length (range of 0.98 to 1.59). Measured morphometric data are provided in Table 1. Buckler thorns were present only on the dorsal surface of RC1 (Fig. 3); however, the dorsal surfaces of all three specimens were entirely prickly and not smooth (Fig. 3). The number of thorns along the midline from nape to the origin of first dorsal fin and interdorsal thorns were counted as 34/2, 32/1, and 32/2, in specimens RC1, RC2, and RC3, respectively. Widely spaced minute lateral buckler thorns were also observed along the tail, but these were not as prominent as those on large juveniles and adults. The dorsal coloring of the examined specimens was yellowish brown with numerous dark spots of various size, denser in the central parts of the disc and widely spaced at the margins; there were dark and light crossbars on the tail (Fig. 2). Dark crossbars were also observed on the dorsal surface of tails of all three specimens (Fig. 3). On the dorsal surface of RC1, two prominent dark colored eye-spots surrounded by cream-colored small spots were present along the line connecting the tips of the disc, and the eye-spots were closer to the midline extending from the snout to the tip of the tail (Fig. 2). Two small but prominent cream-colored spots irregularly surrounded by small dark spots were also present on the dorsal surfaces of specimens RC2 and RC3, which were also in line with the axis connecting the tips of the disc and were closer to the snout-tail tip axis (Fig. 2). The ventral surface was whitish.

The claspers of three specimens were short not reaching the tips of the pelvic fins, not visible from the dorsal view (Fig. 2), and very soft. Therefore, they were considered immature or at stage 1.

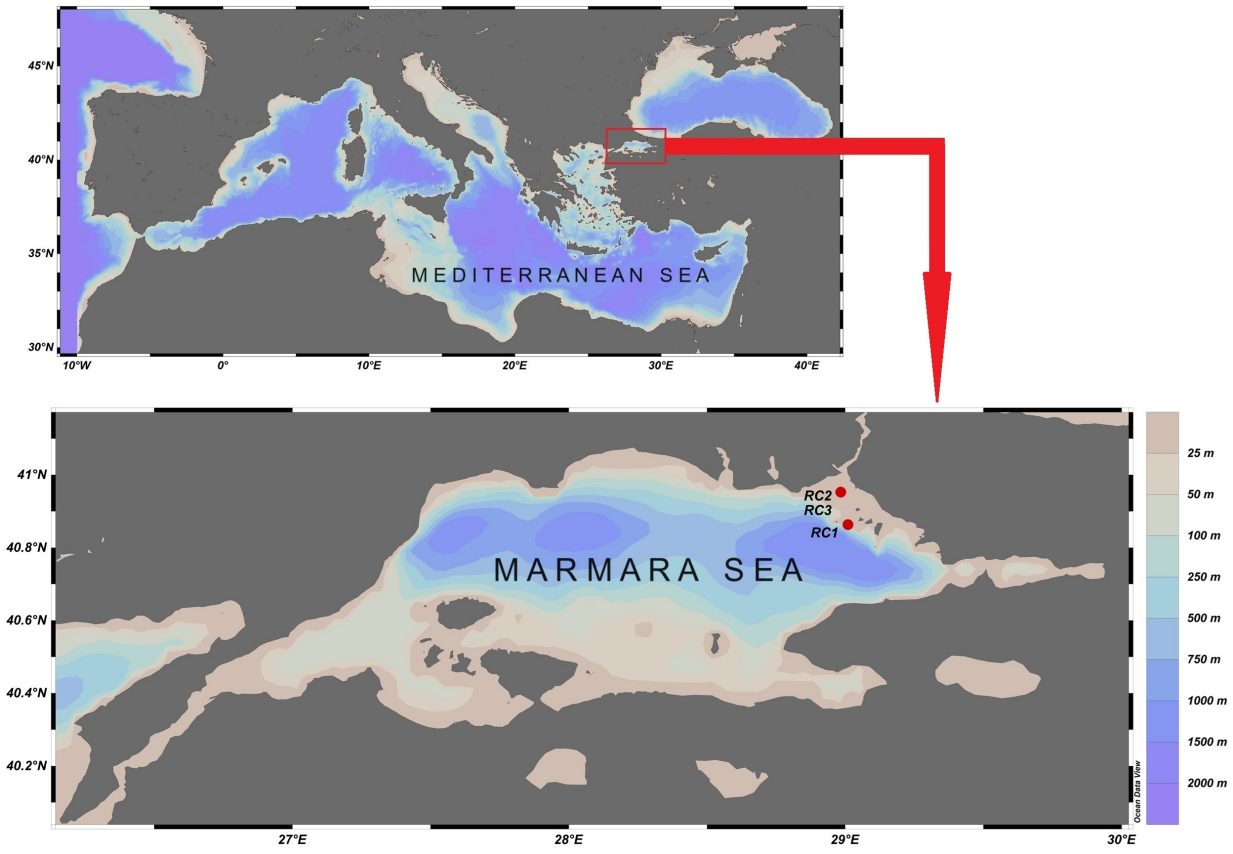

Fig. 1. Small map on the top panel depicts the locality (red rectangle) of the Sea of Marmara in the Mediterranean ecosystem. On the lower panel, approximate localities (red circles) of captures of the examined thornback skates are depicted

Table 1. Morphometric measurements and meristic counts of the examined *Raja clavata* specimens (N/A denotes missing measurement due to dorsal fin and tail tip aberrations)

Measurement, mm	RC1, ♂	RC2, ♂	RC3, ♂	RC1	RC2	RC3	% of TL of the mean	±*SD* of the mean % of TL
	TL 480 mm	TL 308.9 mm	TL 307.1 mm	DW 310 mm	DW 190 mm	DW 210 mm		
	% of TL			% of DW				
Total length	100	100	100	154.84	162.58	146.24	100	0
Disc width	64.58	61.51	68.38	100.00	100.00	100.00	64.82	2.81
Disc length	47.29	48.24	54.05	73.23	78.42	79.05	49.86	2.99
Trunk length	42.08	43.38	46.08	65.16	70.53	67.38	43.85	1.66
Precaudal length	93.75	51.47	N/A	145.16	83.68	N/A	48.41	38.33
Tail length	53.33	55.68	52.43	82.58	90.53	76.67	53.81	1.37
Predorsal-tail length	33.54	3.12	N/A	51.94	5.08	N/A	12.22	15.13
D1 origin to tail tip	19.78	16.75	N/A	30.63	27.24	N/A	12.18	8.7
D1 basal length	5.39	5.55	5.6	8.34	9.03	8.19	5.51	0.09
D1 height	3.31	2.87	3.45	5.13	4.66	5.05	3.21	0.25
D2 basal length	5.88	5.28	3.94	9.1	8.58	5.76	5.03	0.81
D2 height	2.51	2.95	1.79	3.89	4.79	2.62	2.42	0.48
Distance D1/D2	2.46	2.99	2.74	3.81	4.87	4.00	2.73	0.22
Postdorsal length	1.39	3.82	N/A	2.15	6.21	N/A	1.74	1.58
Head length	26.75	28.63	29.18	41.42	46.55	42.67	28.19	1.04
Preocular length	12.41	12.79	14.65	19.21	20.79	21.43	13.28	0.98
Preoral length	10.61	12.35	13.90	16.44	20.08	20.33	12.29	1.34
Prenarial length	9.41	10.84	11.41	14.56	17.63	16.69	10.55	0.84
Internarial length	7.45	6.38	6.51	11.53	10.37	9.52	6.78	0.48
Nasal-curtain length	4.74	4.13	4.33	7.34	6.71	6.33	4.4	0.25
Nasal-curtain width	3.50	3.93	5.06	5.42	6.39	7.40	4.17	0.66
Mouth width	9.56	10.13	10.86	14.81	16.47	15.88	10.18	0.53
Eyeball length	2.91	4.06	4.25	4.5	6.61	6.21	3.74	0.59
Interorbital width	4.63	3.98	5.03	7.16	6.47	7.36	4.55	0.43
Spiracle length	3.43	2.56	2.96	5.31	4.16	4.33	2.98	0.36
Interspiracular width	6.05	6.98	7.57	9.37	11.34	11.07	6.87	0.62
Orbit + spiracle	5.02	5.97	5.88	7.77	9.71	8.6	5.62	0.43
1st gill slit length	1.55	1.96	1.81	2.4	3.18	2.64	1.77	0.17
3rd gill slit length	1.75	1.78	1.99	2.71	2.89	2.90	1.84	0.1
5th gill slit length	1.72	1.89	1.82	2.66	3.08	2.67	1.81	0.07
1st interbranchial width	15.73	14.96	15.65	24.35	24.32	22.88	15.44	0.35
3rd interbranchial width	13.10	12.72	13.04	20.29	20.68	19.07	12.96	0.17
5th interbranchial width	8.41	9.57	9.65	13.02	15.55	14.12	9.21	0.57
Eye-spot length	4.40	1.93	2.31	6.81	3.13	3.38	2.88	1.08
Eye-spot width	4.81	2.12	1.99	7.45	3.45	2.9	2.97	1.3
Between eye-spots	18.43	12.43	14.1	28.53	20.21	20.62	14.99	2.53
Clasper length	5.21	3.56	5.42	8.06	5.79	7.93	4.73	0.83
Clasper width	1.09	0.86	1.12	1.69	1.39	1.64	1.03	0.12
Weight, g	550	139.5	153.9					
Number of thorns in midline	34	32	32					
Number of interdorsal thorns	2	1	2					
Number of orbital thorns	2	2	3					

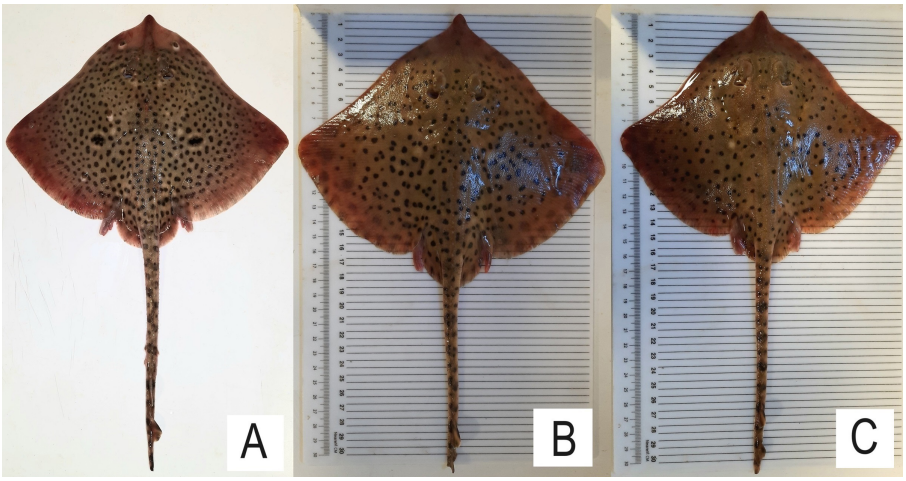

Fig. 2. Examined specimens of *Raja clavata*; A, specimen RC1 (TL 480 mm); B, RC2 (TL 308.9 mm); C, RC3 (TL 307.1 mm)

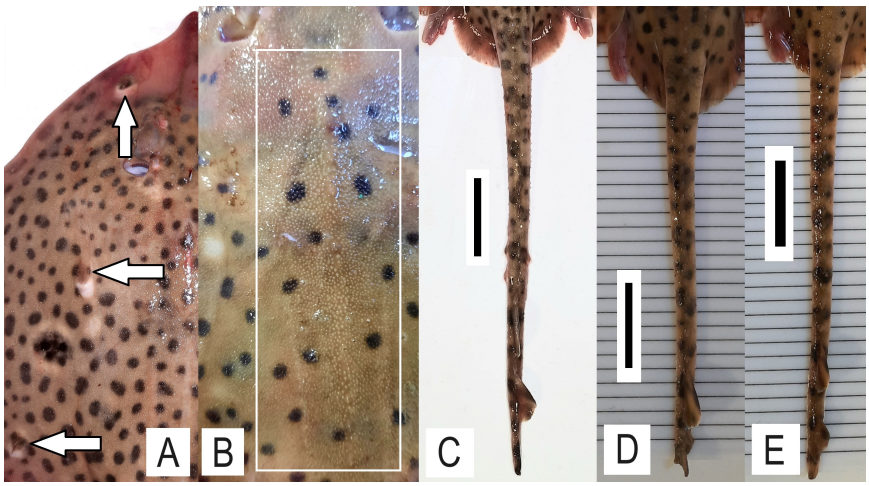

Fig. 3. Squamation and color patterns observed on the examined specimens of *Raja clavata*. A, arrows depict the buckler thorns of specimen RC1; B, rectangle depicts the prickles on dorsal disc of RC2; C, D, and E, dark crossbars on dorsal tails of RC1, RC2, and RC3, respectively. Scale bars are 30 mm

DISCUSSION

Ebert and Stehmann [2013] define *R. clavata* as a "chameleon" among its congeners, mainly due to its dorsal ground color and remarkable variety of patterns. The ground color of this species ranges from brown to grey in light to dark shades, variegated or marbled with dark and light spots or blotches, and it may show a pattern, such as eye-spots; however, single-colored thornback skates have also been recorded [Ebert, Stehmann, 2013; Last et al., 2016]. Based on dorsal coloration and patterns, Mnasri *et al.* [2009] defined 7 types of polychromatism in *R. clavata* specimens caught off the Tunisian coast (the central Mediterranean Sea). Furthermore, 5 out of these 7 types of polychromatism have also been described in thornback skates captured in the eastern Ionian Sea [Chatzispyrou et al., 2019]. Recently, Capapé *et al.* [2018] reported one of the atypical coloring patterns (type 7, vermiculated, *sensu* [Mnasri et al., 2009]) on the dorsal surface of the thornback skate captured off Izmir coast (the eastern Aegean Sea). Although the dilemma of polychromatism or atypical coloring which makes it difficult to distinguish *R. clavata* from *R. montagui*, *R. maderensis*, or *R. polystigma* [Capapé et al., 2018; Chatzispyrou et al., 2019; Mnasri et al., 2009] has been very well documented for the Mediterranean Sea, the present study is the first to report atypically colored thornback skates from the Sea of Marmara.

The types of polychromatism observed in the examined specimens are consistent with type 2 (ocellated) and type 4 (spotted) described by Mnasri *et al.* [2009]. At first sight, the ocellated dorsal pattern of specimen RC1 could be confused with that of *R. polystigma* which has not been recorded in the Sea of Marmara. Furthermore, in contrast to the smooth dorsal surface of *R. polystigma* [Last et al., 2016], the dorsal surface of RC1 was completely prickly, and very few large buckler thorns were also present. On the other hand, the spotted dorsal pattern of specimens RC2 and RC3 resembled the dorsal coloration of *R. montagui*, and this species has been reported from the Sea of Marmara by Bilecenoğlu *et al.* [2014]. Nevertheless, dorsal surfaces of RC2 and RC3 were observed to be completely prickly, and those are completely smooth in young specimens of *R. montagui*. Therefore, one of the main characteristics that allows distinguishing atypically colored *R. clavata* from its congeners *R. montagui* and *R. polystigma* is the presence (*R. clavata*) or absence (*R. montagui* and *R. polystigma*) of prickles.

According to Last *et al.* [2016], the maximum TLs of *R. clavata*, *R. montagui*, and *R. polystigma* are ~130, ~80, and ~71 cm, respectively. Furthermore, males of *R. clavata*, *R. montagui*, and *R. polystigma* reach maturity at 60–77-cm TL, ~40-cm TL, and ~53-cm TL, respectively. As for TL of the examined skates which ranged 307.1 to 480 cm (Table 1), these are clearly juvenile specimens with respect to the above sizes at which males of *R. clavata* and *R. polystigma* reach maturity. However, as males of *R. montagui* reach maturity at around 40-cm TL, the observed TLs of the studied specimens suggest that they are mature or subadult *R. montagui* specimens. According to Last *et al.* [2016], one of the key descriptive characteristics of *R. montagui* and *R. polystigma* is the dorsal disc of these two species which is largely smooth due to the absence of prickles. However, the dorsal discs of the examined specimens were completely prickly and not smooth (Fig. 3). Furthermore, the presence of dark crossbars on the dorsal surface of the tails of studied skates which is another important descriptive characteristic of the thornback skate [Last et al., 2016] also confirms that the examined specimens are *R. clavata*. Although the presence of large buckler thorns is considered as an essential characteristic for positive identification of *R. clavata* [Barone et al., 2022; Last et al., 2016], they are confined to the snout of juvenile and adult males [Last et al., 2016]; those are seen on the snout area of specimen RC1. Moreover, occasional specimens without thorns but with a spinulose dorsal surface have been reported as well [Ebert, Stehmann, 2013]; those are seen on dorsal discs of specimens RC2 and RC3. Therefore, TLs of the studied specimens and the observed squamation patterns were consistent with those reported for *R. clavata* juveniles [Ebert, Stehmann, 2013; Last et al., 2016].

Ratios of interorbital length to prenarial length (IL/PL) and eye length to prenarial length (EL/PL) can also distinguish *R. clavata* (IL/PL ~2 and EL/PL ~1.4) from its congeners *R. montagui* and *R. polystigma* [Last et al., 2016]. Although the mentioned ratios of the examined thornback skates (IL/PL ~2.3 and EL/PL ~1.25) separate them from the ratios of *R. polystigma* (IL/PL ~2 and EL/PL ~2.1 [Last et al., 2016]), they were found to be closer to the ratios of *R. montagui* (IL/PL ~2.5 and EL/PL ~1.2 [Last et al., 2016]) than *R. polystigma*. However, the presence of buckler thorns (RC1) and fully prickly dorsal surfaces (RC2 and RC3) allowed the studied specimens to be positively identified as *R. clavata* rather than *R. montagui* or *R. polystigma*.

Although *R. montagui* has been previously registered in the Sea of Marmara and in the Black Sea (only 1 record; the specimen caught at 41°10′N, 39°36′E) [Bilecenoğlu et al., 2014; Turan, 2008], this species did not occur in the following years in the mentioned marine areas. Therefore, there is a reasonable uncertainty that: 1) *R. montagui* was noted as a result of the vagrant occurrence of the species in the Sea of Marmara and Black Sea and, accordingly, was not found repeatedly in the following years; or 2) atypically colored *R. clavata* individuals could have been misidentified as *R. montagui*.

Although the record of *R. montagui* in the Black Sea is based on genetic material (GenBank accession No. EU476889 [Turan, 2008]), the fact that it has not been registered in the region since 2008 [Karadurmuş, Sarı, 2024] strengthens the possibility of vagrant occurrence or misidentification. According to Bilecenoğlu *et al.* [2014], 13 species of the family Rajidae have been described in Turkish marine waters to date, and 7 out of 13 rajids also occur in the Sea of Marmara. Due to the above-mentioned taxonomic confusion in congeneric *Raja* species, taxonomic issues of the rajids occurring in the Sea of Marmara need to be resolved before conducting future studies on their populations in the region. As highlighted by Pyšek *et al.* [2013], conventional alpha taxonomy integrated with contemporary genetic taxonomic procedures will certainly improve the accuracy of species identification and further refine the taxonomic classification at the population level of rajids occurring in the Sea of Marmara. Combining the observations of Capapé *et al.* [2018] who reported on the occurrence of vermiculated pattern on the thornback skate caught off Izmir coast (the eastern Aegean Sea) with our observations, it is clear that types 2, 4, and 7 of atypically colored *R. clavata* (*sensu* [Mnasri et al., 2009]) occur in Turkish marine waters as well. Atypical coloring in *Raja* species can also be exhibited by several other types [Gajić et al., 2023; Quigley et al., 2018], such as the single unilateral ocellus noted in *R. miraletus* or the xanthochromism observed in *R. montagui* which makes it difficult to correctly identify the species.

Conclusion. Atypical coloring may lead to confusion and misidentification of *Raja clavata*. Therefore, an integrated approach of conventional alpha taxonomy and genetic investigation is needed to resolve the taxonomic status of *Raja* species occurring in the Sea of Marmara. Accurate taxonomic resolution is the first step to properly differentiate populations of the aforementioned species before conducting further studies and effective conservation of them. The Sea of Marmara is considered as an ecological gateway from the Mediterranean to the Black Sea ecosystem; accordingly, accurate identification of the fish fauna of the Sea of Marmara is a clear requirement for a better understanding of the northerly distribution of fish species. Taxonomic issues of the rajids occurring in the Sea of Marmara need to be resolved before conducting studies on their populations in the region.

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ЦВЕТОВОЙ УЗОР МОРСКОЙ ЛИСИЦЫ *RAJA CLAVATA* LINNAEUS, 1758 ИЗ МРАМОРНОГО МОРЯ, ИЗ-ЗА КОТОРОГО ВОЗМОЖНЫ ОШИБКИ ИДЕНТИФИКАЦИИ НЕСКОЛЬКИХ СКАТОВ В РЕГИОНЕ

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Траловые исследования в шельфовых водах северо-восточной части Мраморного моря впервые выявили в этом регионе нетипично окрашенных скатов — морскую лисицу *Raja clavata* Linnaeus, 1758 (Rajiformes: Rajidae). Поскольку нетипичная окраска может привести к путанице и неправильной идентификации *R. clavata*, необходим комплексный подход, сочетающий методы традиционной таксономии и генетических исследований. Точное таксономическое определение скатов рода *Raja* — первый шаг к корректной дифференциации их популяций перед их изучением и разработкой мер эффективной охраны.

Ключевые слова: Rajiformes, полихроматизм, аберрации, таксономия, описание

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THE ELEMENT CONTENTS IN SOFT TISSUES AND SHELLS OF THE BIVALVE *ANADARA KAGOSHIMENSIS* (TOKUNAGA, 1906) FROM THE BLACK SEA AND SEA OF AZOV

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In the ecosystems of the Black Sea and Sea of Azov, the invasive bivalve mollusc *Anadara kagoshimensis* is a poorly studied species. This clam is a valuable object in fishery and mariculture. Currently, there is little information about the element contents in soft tissues and shells of the mollusc living in these two seas. The aim of this work is comparative analysis of the elemental composition of *A. kagoshimensis* from the Black Sea and Sea of Azov. The elemental analysis was carried out using inductively coupled plasma mass spectrometry. The study presents data on the elemental contents in soft tissues and shells of this clam from the two seas. Noticeable differences in contents of elements were found between the sampling areas. These elements include: K, Rb, Cs, Ca, and Ba from the s-element family; the p-elements Al, Ga, Ge, P, As, Bi, and Br; the d-block elements Zn, V, Nb, Ta, Mo, Fe, Ir, and Au; and the f-block elements Pr and Nd. The elemental composition of *A. kagoshimensis* is determined not only by the composition of seawater, which contains mainly s-elements, but also by mollusc adaptation processes in which p- and d-elements are predominantly involved. In soft tissues of the clam from the Black Sea, concentrations of K, Rb, and Cs are significantly higher than in tissues of *A. kagoshimensis* from the Sea of Azov, while the concentration of K is one (the Sea of Azov) to two orders of magnitude (the Black Sea) higher in soft tissues than in shells. In shells of the clam inhabiting the Black Sea, Ca content is significantly higher, and these shells are stronger. Against the high calcium content, relatively low phosphorus content is noted in samples of soft tissues and shells from both seas. In soft tissues of *A. kagoshimensis* from the Black Sea, the contents of P, Al, Ga, Bi, and some heavy metals (Pb and Cd) are significantly higher. The contents of toxic elements in the mollusc from both seas do not exceed the maximum permissible levels. Zn and Mo are accumulated in soft tissues, and Fe is more concentrated in shells. In soft tissues of *A. kagoshimensis* from the Sea of Azov, Zn content is higher than in this clam from the Black Sea. Rare earth elements (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, and Yb) are more concentrated in soft tissues of the mollusc from both seas than in shells, with Pr and Nd contents in specimens from the Sea of Azov being significantly higher than in those from the Black Sea. *Anadara* is capable of concentrating elements depending on their contents in the environment; therefore, the element accumulation in individuals of the same species is primarily a function of the biotope conditions.

Keywords: *Anadara kagoshimensis*, chemical element concentrations, mass spectrometry, Black Sea, Sea of Azov

In the Black Sea and the Sea of Azov, the bivalve *Anadara kagoshimensis* (Tokunaga, 1906) is an invasive and poorly studied species. Due to favorable feeding conditions, high growth rate of anadara is registered [Sahin et al., 2006]. This clam is valuable as a fishing and mariculture target. Specifically, in Thailand, its production reaches 80 million tons *per* year [Suwanjarat et al., 2009].

The content of chemical elements in molluscs is known to be determined by their taxonomic affiliation and genetics [Wala et al., 2016]. The concentration of chemical elements in soft tissues and shells is also dependent on a complex of factors: temperature, salinity, water quality, level of water pollution, *etc.* [Moniruzzaman et al., 2021], with salinity being considered as one of the main environmental parameters altering the functional state of animals [Deaton, 2009]. For example, the concentration of rare earth elements in seawater depends on depth [Elderfield, 1988], and the data on Ce and Eu content indicate the saturation of the marine environment with oxygen and nutrients [Kasper-Zubillaga et al., 2010; Webb, Kamber, 2002]. In soft tissues of mussels sampled in Sevastopol Bay, the concentration of most of the elements considered (54 out of 72) depended on the sampling area [Kapranov et al., 2023].

Levels of chemical elements in molluscs reflect their habitat conditions in different biotopes. The chemical composition of soft tissues characterizes the short-term state of the environment, while the content of chemical elements in shells indicates conditions of the entire life cycle of these hydrobionts [Ravera et al., 2007]. Therefore, when monitoring the metal pollution in the water environment, studying shells of bivalves has methodological advantages compared to the analysis of tissues [Pourang et al., 2014]. At the same time, shells act as accumulators for some metals [Richardson et al., 2001]. Various elements, including heavy metals, can be concentrated in soft tissues and shells of molluscs, and this allows using them as bioindicators of environmental pollution [Hossen et al., 2014]. For example, studies of the content of chemical elements in soft tissues of *Anadara* spp. from the coast of Vietnam showed the following features: As, Sr, Mo, Sn, and Pb contents in clams from the central coastal zone were higher than in clams from other water areas studied, which differences are due to different anthropogenic load [Tu et al., 2011]. Trace element concentrations were within the safe levels for human consumption. Agriculture and fishing are known to result in heavy metals entering the marine environment and affecting the biota [Wijaya et al., 2019]. To date, there is little information on the accumulation of chemical elements in a bivalve *A. kagoshimensis* inhabiting the Black Sea and the Sea of Azov. The aim of this work is to carry out a comparative analysis of concentrations of chemical elements in soft tissues and shells of anadara from these seas.

MATERIAL AND METHODS

The object of research is a bivalve *A. kagoshimensis* from the Black Sea and Sea of Azov (Fig. 1) sampled during the period of its relative sexual maturation resting, when the cellular composition of the gonads does not undergo any changes [Suwanjarat, 1999]. In our work, one hundred mollusc individuals from each sea were used, with the weight (17.6 ± 1.9) g and the shell length (30.5 ± 1.0) mm. In the Black Sea, clams were sampled by divers from the collectors of the marine farm in Karantinnaya Bay, Sevastopol (44°61′83.46″N, 33°50′33.80″E), in October 2022. The sampling depth was 2–3 m, the water temperature was +8 °C, and the salinity was 18‰. In the Sea of Azov, live molluscs were sampled immediately after the storm in Tatarskaya Bay (45°26′51″N, 35°50′46″E) in October 2022. The sea water temperature was +15 °C, and the salinity was 14.83‰. After the mechanical cleaning of clam shells from fouling, they were washed in clean seawater taken from the sampling site. Tissues lining both shells were excised with a plastic scalpel and blotted with filter paper. Soft tissues and shells were dried at +105 °C.

Quantitative elemental analysis was carried out using an inductively coupled plasma mass spectrometer PlasmaQuant MS Elite (Analytik Jena, Germany) with parameters indicated in the paper [Kapranov et al., 2021]. All laboratory vessels were kept for 24 h in a 2% solution of purified nitric acid and rinsed with deionized water. Pre-dried biological samples were mineralized in PTFE tubes by digesting in purified 65% nitric acid and then diluted with deionized water so that the dilution was in the range of 1,000–2,000 mg·L^{-1} (on dry weight basis). Calibration curves were plotted using solutions of a multielement standard IV-ICPMS-71A-D (Inorganic Ventures, the USA, 10 mg·L^{-1}). Samples of the certified reference material (0.1 g) were digested in extra pure nitric acid and diluted with deionized water according to the procedure described above. Coefficients of determination of linear regressions for all calibration plots were no lower than 0.998. The error of quantitative determination in the semi-quantitative analysis of ICP-MS is < 50% [Chen et al., 2008; Krzciuk, 2016]. Two-factor analysis of variance was performed using the PRIMER 6.1.16 and PERMANOVA+ 1.0.6 software.

Fig. 1. Map of the study area with sampling stations

RESULTS AND DISCUSSION

Differences in the content of chemical elements in anadara from the Black Sea and Sea of Azov are statistically significant (Table 1). These elements include: K, Rb, Cs, Ca, and Ba from the s-element family; the p-elements Al, Ga, Ge, P, As, Bi, and Br; the d-elements Zn, V, Nb, Ta, Mo, Fe, Ir, and Au; and the f-elements Pr and Nd. The differences are due not only to the composition of seawater which contains chiefly the s-elements, but also to physiological and biochemical characteristics of the molluscs. The p- and d-elements with atomic numbers 24 to 33 are known to be involved in the functioning of cells of marine organisms as minor constituents of proteins, carbohydrates, lipids, and enzymes [Takarina et al., 2013]. Changes in the chemical composition of clams are likely to result from the effect of the combination of internal and external factors [Osibona et al., 2009]. Sedentary living and filter feeding require relatively small energy costs. Apparently, these molluscs have only two processes related to a significant expenditure of energy: reproduction and linear growth. Therefore, during spawning and growing, the concentration of elements in anadara soft tissues and shells can increase.

Table 1. Elemental concentration in *Anadara kagoshimensis* soft tissues and shells (μg·kg$_{dw}^{-1}$). The differences are significant ($p < 0.05$; $n = 10$): *, between soft tissues and shells of the mollusc from both the Black Sea and Sea of Azov; A or B, between soft tissues and shells of *A. kagoshimensis* from either the Sea of Azov (A), or the Black Sea (B); T, between soft tissues of the clam from the Black Sea and Sea of Azov; S, between shells of *A. kagoshimensis* from the Black Sea and Sea of Azov

Element	Black Sea		Sea of Azov	
	Soft tissues	Shells	Soft tissues	Shells
s-elements				
Li^{AS}	501 ± 379	812 ± 499	320 ± 290	14 ± 9
Be^{*TS}	11 ± 5	6 ± 3	9 ± 8	8 ± 7
Na^{*S}	12,178,992 ± 2,536,968	3,234,141 ± 288,151	13,146,503 ± 2,746,341	1,125,903 ± 273,410
Mg^{*S}	2,875,832 ± 333,062	878,669 ± 668,271	2,503,012 ± 454,165	60,642 ± 15,355
K^{*TS}	3,742,846 ± 474,396	83,002 ± 20,095	2,057,008 ± 227,175	29,845 ± 24,088
Ca^{*TS}	8,311,674 ± 5,209,732	186,445,736 ± 21,517,954	25,067,443 ± 14,688,981	114,796,479 ± 7,733,846
Rb^{*TS}	5,160 ± 1,083	509 ± 191	2,681 ± 322	67 ± 24
Sr^{*S}	56,665 ± 27,128	951,807 ± 113,927	117,496 ± 55,074	560,106 ± 51,275
Cs^{*TS}	44 ± 16	70 ± 32	4.2 ± 3.9	2.6 ± 2.5
Ba^{*TS}	12,768 ± 4,952	17,767 ± 4,834	4,786 ± 2,288	7,375 ± 3,547
p-elements				
B^{*TS}	14,713 ± 2,132	6,626 ± 2,093	11,754 ± 2,206	448 ± 363
Al^{*TS}	173,714 ± 69,956	321,109 ± 113,933	44,931 ± 21,499	24,076 ± 7,625
Si^{*S}	177,381 ± 38,679	298,780 ± 197,663	208,800 ± 54,020	30,776 ± 29,529
P^{*TS}	4,845,357 ± 301,948	245,141 ± 72,786	3,777,277 ± 510,623	55,807 ± 10,943
Ga^{*TS}	179 ± 24	59 ± 25	351 ± 61	14 ± 17
Ge^{*TS}	499 ± 151	1,143 ± 275	807 ± 309	508 ± 261
As^{*TS}	15,756 ± 2,543	1,583 ± 230	8,936 ± 1,505	2,756 ± 130
Se*	5,035 ± 1,729	1,425 ± 954	5,738 ± 1,125	682 ± 488
Br^{*TS}	356,136 ± 126,346	176,248 ± 51,213	218,655 ± 44,940	23,736 ± 7,763
In^{BT}	3 ± 2.9	1.2 ± 0.8	0.7 ± 0.6	0.8 ± 0.5
Sn^{S}	266 ± 185	311 ± 253	139 ± 94	9 ± 5
Sb^{AS}	53 ± 25	57 ± 42	41 ± 10	12 ± 6
Te^{ATS}	9 ± 6	10 ± 6	42 ± 19	23 ± 7
I^{BTS}	31,028 ± 5,276	55,587 ± 17,313	10,552 ± 6,001	7,728 ± 1,904
Tl^{BT}	9 ± 3	2 ± 1	3 ± 2	2 ± 1.8
Pb^{*TS}	1,526 ± 682	776 ± 213	650 ± 408	64 ± 39
Bi^{*TS}	101 ± 80	100 ± 73	5 ± 3	1.3 ± 1.1
d-elements				
Sc^{ATS}	1,056 ± 374	945 ± 396	409 ± 80	66 ± 54
Ti^{AS}	3,843 ± 1,266	4,885 ± 2,442	500 ± 200	2,000 ± 400
V^{*TS}	3,433 ± 1,522	1,634 ± 508	4,227 ± 918	1,530 ± 407
Cr^{TS}	9,906 ± 6,920	7,820 ± 4,605	2,115 ± 261	175 ± 97
Mn*	29,278 ± 10,694	35,069 ± 18,156	19,325 ± 10,248	22,933 ± 17,129
Fe^{*TS}	293,751 ± 67,269	1,564,344 ± 325,172	1,216,741 ± 599,529	5,057,068 ± 2,329,125
Co^{BS}	1,367 ± 976	2,195 ± 147	1,134 ± 283	1,083 ± 137
Ni^{BTS}	4,635 ± 4,164	31,218 ± 4,999	10,943 ± 3,576	8,531 ± 2,079
Cu^{AS}	61,323 ± 20,518	74,990 ± 36,886	47,554 ± 15,261	14,424 ± 5,599
Zn^{*TS}	115,934 ± 59,902	8,268 ± 8,851	181,026 ± 57,602	1,151 ± 649

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Element	Black Sea		Sea of Azov	
	Soft tissues	Shells	Soft tissues	Shells
Y[BT]	1,549 ± 1,212	215 ± 81	608 ± 144	144 ± 48
Zr[TS]	312 ± 140	345 ± 155	107 ± 48	56 ± 25
Nb[*TS]	9 ± 3	25 ± 19	6 ± 3	3 ± 2
Mo[*TS]	2,239 ± 1,645	1,008 ± 704	971 ± 285	55 ± 35
Ru[BS]	1 ± 0.5	8 ± 3	2.2 ± 1.7	3 ± 2
Rh[*S]	28 ± 8	163 ± 42	43 ± 14	134 ± 11
Pd[*T]	191 ± 57	1,193 ± 379	596 ± 107	1,058 ± 100
Ag	780 ± 448	588 ± 252	815 ± 243	569 ± 465
Cd[*T]	15,290 ± 10,450	192 ± 114	6,183 ± 2,524	147 ± 59
La[B]	1,076 ± 1,002	284 ± 113	759 ± 187	231 ± 153
Hf[AT]	7 ± 3	4 ± 2	12 ± 9	2 ± 1.5
Ta[*TS]	1 ± 0.5	0.3 ± 0.2	5 ± 4	0.6 ± 0.4
W[AS]	152 ± 134	73 ± 31	64 ± 17	7 ± 4
Re[AT]	0.6 ± 0.3	0.6 ± 0.3	1.3 ± 0.5	0.3 ± 0.2
Os[AT]	2 ± 1.5	1.6 ± 1.2	20 ± 14	1.9 ± 0.5
Ir[*TS]	0.2 ± 0.1	0.3 ± 0.04	1.2 ± 1.1	0.2 ± 0.1
Pt[A]	3 ± 2	3 ± 2	5.4 ± 5.3	1.1 ± 0.8
Au[*TS]	15 ± 3	21 ± 6	172 ± 86	2 ± 1
Hg[*]	160 ± 90	20 ± 11	193 ± 59	11 ± 6
f-elements				
Ce[*T]	736 ± 490	184 ± 76	1,100 ± 293	473 ± 308
Pr[*TS]	85 ± 66	22 ± 8	158 ± 39	52 ± 34
Nd[*TS]	384 ± 239	89 ± 32	662 ± 142	200 ± 121
Sm[*T]	65 ± 46	19 ± 7	126 ± 31	32 ± 21
Eu[A]	27 ± 15	16 ± 5	32 ± 13	15 ± 9
Gd[*T]	84 ± 63	19 ± 6	160 ± 45	38 ± 24
Tb[*T]	13 ± 9	3 ± 1	21 ± 9	4 ± 3
Dy[*]	61 ± 45	15 ± 5	88 ± 22	15 ± 11
Ho[*]	11 ± 8	3 ± 1	9 ± 3	2 ± 1
Er[*]	33 ± 23	8 ± 3	44 ± 14	7 ± 6
Tm[BT]	5 ± 3	1 ± 0.5	1.2 ± 1.1	0.7 ± 0.6
Yb[*T]	19 ± 12	6 ± 2	29 ± 10	7 ± 5
Lu[BT]	4 ± 3	1 ± 0.4	1 ± 0.9	1 ± 0.7
Th	90 ± 68	84 ± 60	45 ± 35	91 ± 82
U[*T]	51 ± 16	75 ± 25	122 ± 18	72 ± 17

The **s-elements** are distributed differently in *A. kagoshimensis* (Table 1). Noticeable differences in concentrations of K, Ca, Ba, Rb, and Cs were recorded between soft tissues and shells of the clams from both the Black Sea and Sea of Azov. The content of K, Rb, and Cs in tissues of the molluscs from the Black Sea is significantly higher than in individuals from the Sea of Azov, which is mediated by the level of dissolved nutrients in these water areas. In the Black Sea, there is a special type of coastal currents: upwelling. In the areas of upwelling, higher biological productivity is observed due to the remobilization of nutrients from the bottom to surface waters. Most likely, Rb and Cs replace K in its compounds. K plays a key role in the formation of the membrane potential of cells; therefore, K concentration is one (the Sea of Azov) or two (the Black Sea) orders of magnitude higher in anadara

soft tissues than in its shells. In addition, K can affect the thickness of the mollusc shells, and K deficiency leads to a decrease in the thickness of shells [Elegbede et al., 2023]. Thus, shells of the clams from the Black Sea are stronger. This fact should be taken into account when processing soft tissues and shells to produce food supplements, animal feed, mineral fertilizers, *etc.*

A relatively high content of Ca, Na, Mg, and Sr was recorded in *A. kagoshimensis* shells from the Black Sea and Sea of Azov. These are the elements whose compounds make up mollusc shells. Ca content in shells of the clams from the Black Sea is significantly higher than that for the hydrobionts from the Sea of Azov. The higher the Ca content, the stronger the mollusc shell [Dickson, 2013]. Na_2CO_3 and other Na compounds possess binding and moisturizing properties, regulate pH, and provide the shell layers with the ability to stick together and form a compact structure. In our study, against high calcium content in *A. kagoshimensis* soft tissues and shells, relatively low phosphorus concentration was recorded, just as it was established for *Anadara senilis* from Guinea [Elegbede et al., 2023].

The concentration of Li in shells of the anadara from the Sea of Azov is an order of magnitude lower than in shells of the hydrobiont from the Black Sea. Li enters natural springs from sediments; its content in underground waters is consistent with its concentration in sedimentary rocks through which they circulate [von Strandmann, 2020].

Significant differences were revealed between the content of the **p-elements** in *A. kagoshimensis* soft tissues (Table 1). The concentration of P in soft tissues of the Black Sea clams sampled from hanging cages on the mussel-and-oyster farm is higher than that in individuals from the Sea of Azov, and it may evidence for a more intensive metabolism in hydrobionts inhabiting the Black Sea. Anadara grows faster in the water column than in bottom settlements [Acarli et al., 2012]. Al is capable of forming insoluble compounds with P [Haynes, Mokolobate, 2001]; accordingly, Al concentration is significantly higher in soft tissues of the Black Sea clams. Aluminum is considered a toxic element [Toxicological Profile for Lead, 2020].

In soft tissues of *A. kagoshimensis* from the Black Sea, Ga and Bi concentrations are higher than the values for the molluscs from the Sea of Azov. As the occurrence of Ga in water is related to the anthropogenic load, it does not play a noticeable biological role in the life of hydrobionts. The higher content of Bi in soft tissues of the Black Sea clams is likely to result from the higher salinity of the Black Sea water.

Concentrations of P, S, Cl, Pb, Al, Ge, Br, B, Si, Sn, I, Bi, and Sb in shells of anadara from the Black Sea are significantly higher than those for the individuals from the Sea of Azov (Table 1). Probably, shells can concentrate elements in dependence on their content in biotopes, which fact indicates the variability of the elemental composition in both soft tissues and shells of *A. kagoshimensis*.

This species can serve as a bioindicator of environmental pollution with heavy metals. Pb and Cd concentrations significantly differ in soft tissues and shells of clams from the Black Sea and Sea of Azov (Table 1). In general, the content of toxic elements in water from both seas is below the maximum permissible levels established by the requirements of Technical Regulation of the Customs Union 021/2011 [2011]: Pb, 10.0; Cd, 2.0; and Hg, 0.2 $mg \cdot kg^{-1}$. The main sources of Pb in the marine environment are stormwater runoff from inland areas and wastewater inflow from land [El-Sorogy, Youssef, 2015; El-Sorogy et al., 2012; Peters et al., 1997]. High concentrations of heavy metals Pb, Cd, and Hg pose a threat to molluscs [Dabwan, Taufiq, 2016; Isroni, Maulida, 2022].

Concentrations of such **d-elements** as Zn and Mo are higher in *A. kagoshimensis* soft tissues, while Fe content is higher in its shells (Table 1). Iron is important for the metabolism of molluscs [El-Sorogy et al., 2013]. Fe is evenly concentrated in the outer organic layer of the shell, the periosteum,

and is uniformly distributed across the aragonite layers [Duncan et al., 2009] with accumulation in the periostracum. The concentration of Zn is higher in soft tissues of the clams from the Sea of Azov than from the Black Sea. Zinc is required for the activity of 90 enzymes involved in animal metabolism and is an essential trace element for all living organisms [Astuti et al., 2022]. Zn concentration in hydrobionts is higher than in terrestrial organisms. Zn is concentrated in tissues of hydrobionts in the form of insoluble inclusions or bound with macromolecules [Pourang et al., 2014].

Currently, there is little information about the content of rare earth elements in the marine environment, their accumulation in living organisms, and their effect on the biota. Differences in concentrations of rare earth elements (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, and Yb) between the molluscs from the Black Sea and the Sea of Azov are obvious (Table 1). On average, their content is higher in *A. kagoshimensis* soft tissues than in its shells. Only Pr and Nd concentrations in tissues and shells differ significantly, as well as the content in the anadara tissues and shells from the Black Sea and the Sea of Azov. Pr and Nd concentrations in the clams from the Sea of Azov are higher. Like many other rare earth metals, Pr and Nd do not play a key biological role in living organisms. Their occurrence may be related to different anthropogenic load on the water areas studied. Molluscs of the genus *Anadara* are filter feeders; therefore, the concentration of rare earth elements, as a rule, is higher in soft tissues than in shells. These elements can enter the body of clams with bacteria. Bacteria are shown to be able to accumulate metals and, accordingly, affect their transfer in water column [Beveridge, Doyle, 1989].

Conclusion. Concentrations of chemical elements in soft tissues and shells of molluscs depend primarily on environmental conditions. At the same time, the differences in the content are determined not only by the composition of seawater which includes mainly s-elements, but also by physiological processes of mollusc adaptation, since most of the statistically significant differences in this work were revealed among p- and d-elements. It is p- and d-elements that are involved in the functioning of cells of organisms as minor components of proteins, carbohydrates, lipids, and enzymes. Molluscs consume macro elements and trace elements from water and accumulate them in tissues and shells adapting to conditions of their habitat, including salinity. The element contents in *Anadara kagoshimensis* soft tissues and shells are not constant, and the role of certain elements in physiological processes can increase depending on the physiological state of animals. Not all elements accumulated in soft tissues and shells are essential. The process of their accumulation is closely related to the anthropogenic load on the water area.

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СОДЕРЖАНИЕ ХИМИЧЕСКИХ ЭЛЕМЕНТОВ В ТКАНЯХ И РАКОВИНАХ ДВУСТВОРЧАТОГО МОЛЛЮСКА *ANADARA KAGOSHIMENSIS* (TOKUNAGA, 1906) ИЗ ЧЁРНОГО И АЗОВСКОГО МОРЕЙ

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В Чёрном и Азовском морях двустворчатый моллюск *Anadara kagoshimensis* является инвазивным и малоизученным видом. Моллюски — ценный объект промысла и марикультуры. В настоящее время мало сведений об особенностях содержания элементов в тканях и раковинах анадары, обитающей в этих морях. Цель данной работы — провести сравнительный анализ элементного состава тканей и раковин *A. kagoshimensis* Чёрного и Азовского морей. Элементный анализ проводили с помощью масс-спектрометра с индуктивно-связанной плазмой. В работе приведены данные о концентрациях элементов в тканях и раковинах анадары из двух морей. Обнаружены значимые различия концентраций следующих элементов в анадаре из Чёрного и Азовского морей: K, Rb, Cs, Ca и Ba из семейства s-элементов; Al, Ga, Ge, P, As, Bi и Br из числа p-элементов; Zn, V, Nb, Ta, Mo, Fe, Ir и Au из семейства d-элементов; Pr и Nd из числа f-элементов. Содержание элементов в тканях и раковинах *A. kagoshimensis* обусловлено не только составом морской воды, куда входят в основном s-элементы, но и адаптационными процессами в моллюсках, в которых преимущественно участвуют p- и d-элементы. В тканях анадары из Чёрного моря концентрации K, Rb и Cs достоверно выше, чем в тканях особей из Азовского моря, при этом концентрация K на один (Азовское море) или два порядка (Чёрное море) выше в тканях, чем в раковинах. В раковинах *A. kagoshimensis* из Чёрного моря содержание Ca достоверно выше. Раковины анадары из Чёрного моря прочнее. На фоне высокого содержания кальция в образцах тканей и раковин *A. kagoshimensis* из обоих морей зарегистрировано относительно низкое содержание фосфора. В тканях анадары из Чёрного моря концентрация P, Al, Ga и Bi, а также тяжёлых металлов (Pb и Cd) достоверно выше. Содержание токсичных элементов в анадаре из обоих морей не превышает предельно допустимых концентраций. Содержание Zn и Mo выше в тканях, Fe — в раковинах. В тканях *A. kagoshimensis* из Азовского моря концентрация Zn выше, чем в тканях особей из Чёрного моря. Концентрации редкоземельных элементов (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm и Yb) выше в тканях анадары из обоих морей, чем в раковинах. В *A. kagoshimensis* из Азовского моря концентрации Pr и Nd значимо выше, чем в моллюске из Чёрного моря. Анадара способна концентрировать элементы в зависимости от их содержания в среде, поэтому концентрация элементов в моллюсках, принадлежащих к одному виду, в первую очередь зависит от биотопа.

Ключевые слова: двустворчатый моллюск *Anadara kagoshimensis*, концентрация химических элементов, масс-спектрометрия, Чёрное море, Азовское море

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A NEW SPECIES OF ARROW WORMS, *SAGITTA DIMITRYI* SP. NOV. (CHAETOGNATHA, SAGITTOIDEA), FROM THE SEA OF OKHOTSK (NORTHWEST SAKHALIN)

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A new species of chaetognaths, *Sagitta dimitryi* sp. nov., was discovered in the waters of the Sea of Okhotsk, near the northwestern part of Sakhalin. The aim of this article is to describe the new species. A table of identification keys for species of the genus *Sagitta* is given, including *Sagitta dimitryi* sp. nov. The relationship of modern *Sagitta* with ancient Chaetognatha is discussed, including possible reasons for the evolution of the intestinal apparatus.

Keywords: *Sagitta dimitryi* sp. nov., Chaetognatha, Sakhalin, Sea of Okhotsk

The classification of chaetognaths (the phylum Chaetognatha Leucart, 1894) remains a problem since the discovery of this group by M. Slabber [1769]. Only in 1905, the class Sagittoidea Claus et Grobben, 1905 was described. The first researcher attempting to classify chaetognaths was P. Abric [1905]. Despite certain shortcomings, his approach (species classification by the number of paired lateral fins) was not discarded. It was used later: the occurrence of two paired fins was applied as a character of the genus *Sagitta* sensu lato [Ritter-Záhony, 1911]. T. Tokioka [1965] divided the genus proposed by R. Ritter-Záhony into eight genera, with several species being allocated to the new genus *Sagitta* sensu stricto. However, his identification diagnoses did not take into account the occurrence of sac-like gelatinous structures (SGS). With SGS diversity considered in representatives of the family Sagittidae Claus et Grobben, 1905, the subfamilies Flaccisagittinae and Sagittinae were later identified [Kassatkina, 2007].

The difficulties of working with Chaetognatha are partly related to the simplicity of their organization. Those lack not only some organs (permanent oviducts and vas deferens), but also entire organ systems (excretory and respiratory ones). The recently discovered circulatory system is very primitive [Malakhov, Berezinskaya, 2001].

In this paper, we describe a new species to science, *Sagitta dimitryi* Kassatkina & Vasileva sp. nov., from the subfamily Sagittinae.

MATERIAL AND METHODS

Planktonic chaetognaths were sampled during the 82nd cruise of the RV "Professor Gagarinsky" on 03.09.2022 and fixed in 4% formalin. The processing was carried out in such a way as not to damage soft tissues. In a laboratory, the material was examined under an MBS-10 binocular.

Photographs were taken with a Stemi 2000-C stereomicroscope equipped with an AxioCam ICc 3 camera in order to show the differences between healthy specimens and morphologically abnormal animals. The material is kept in the plankton sample storage in the Laboratory for Pollution and Environmental Research of POI FEB RAS (holotype SD N1. 82. 2022 and four paratypes). Immature specimens, about 100 ind., were sampled during the Kuril–Sakhalin expedition (1949), the cruise of the RV "Baidar" (1965), and the 24th cruise of the RV "Akademik Nesmeyanov" (1993). For comparison with characters of mature animals, characters of immature specimens from different plankton samples are given. To establish species affiliation of *Sagitta* at all stages of maturity, animals were stained according to the author's method [Kassatkina, 2008].

RESULTS AND DISCUSSION

Taxonomy. The new species to science belongs to the family Sagittidae, subfamily Sagittinae, genus *Sagitta* sensu stricto Quoy et Gaimard, 1827.

Diagnosis of the species *Sagitta dimitryi* Kassatkina & Vasileva sp. nov. The body is muscular and rigid. The head is the same width as the body; the neck is noticeable. The corona ciliata is short and has a unique shape: paired convexities at the level of the trunk-tail septum (Fig. 1A, m). The shape and location of the corona ciliata relative to the head ganglion are reliable taxonomic features at all stages: both in young, immature animals and in mature ones. The eyes have a dark pigment spot, with its shape being the same in both immature and mature animals (Fig. 1C). The seminal vesicles do not touch either the caudal fin or lateral fins (Fig. 1B, v). Alveolar tissue is absent.

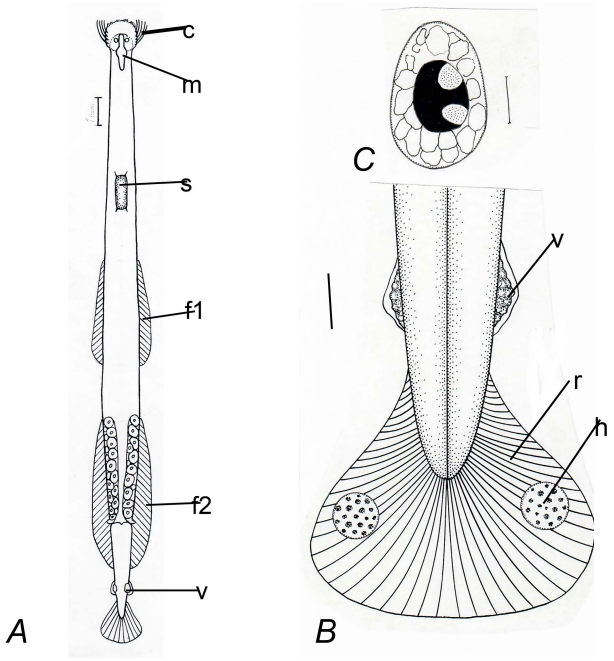

Fig. 1. *Sagitta dimitryi* Kassatkina & Vasileva sp. nov. general view. A: c, hooks; f1, front fin; f2, rear fin; f, tail fin; m, corona ciliata; s, ventral ganglion; v, seminal vesicle. B, rear end of the specimen: h, sensory locomotors organ; r, rays; v, seminal vesicle. C, eye. Scale bar: 1 mm (A); 0.5 mm (B); 0.1 mm (C)

Description of the holotype. Holotype SD N1. 82. 2022 (51.3°N, 144.3°E) is a mature individual at the 4th stage of maturity. Paratype is four mature individuals from one plankton sample taken during the 82nd cruise of the RV "Professor Gagarinsky."

The body length is 20.5 mm. The caudal section is 19.5% of the body length. The upper part of the pharynx is muscular, wider than the midgut (Fig. 2A, d). The midgut wall does not have cells expanded to vacuoles; such cells occur in species of the genera *Parasagitta* and *Aidanosagitta* [Kasatkina, Stolyarova, 2010: Table 3, photos 1 and 3; Table 21, Figs 3–6]. Diverticula are absent, the same as in all *Sagitta* species [Kasatkina, Stolyarova, 2010: Table 29, Fig. 3]. The corona ciliata is short; it has one pair of convexities at the level of the trunk-tail septum. The corona ciliata begins from the brain, and its trunk part is shorter than the part lying on the head (those are almost of the same length). The length of the ventral ganglion is 5.8% of the body length. The anterior margin of the fins of the I pair is located behind the posterior end of the ventral ganglion. The gap between the fins of the I pair and the posterior end of the ventral ganglion is 1.4 times larger than the ganglion and comprises 8.3% of the body length. The fin of the I pair is 17.6% of the body length; it is 1.4 times shorter than the fin of the II pair, and its length is equal to the length of the trunk part of the fins of the II pair. The gap between the lateral fins of the II and I pairs is slightly larger than the gap between the ventral ganglion and the fins of the II pair, with the latter gap being about 9% of the body length. The fin of the II pair is 25% of the body length; its trunk part is 2.3 times longer than the caudal one. The rays in the fins are complete, and there are no rayless zones (Fig. 3B). Alveolar tissue is absent. Sensory-locomotor bodies are few in number; those occur on the caudal fin (Fig. 1B, h). On the head, there are one pair of rows of hooks and two pairs of rows of denticles (Fig. 2B, a, b). There are 7 hooks (Fig. 2B, c), 6 anterior denticles (Fig. 2B, a), and 12 posterior denticles on the left and right sides (Fig. 2B, b). The eyes have a weakly notched central pigment spot (Figs 1C, 3A, g). The seminal vesicles make up 2.6% of the total body length and 17% of the length of the caudal section. Those are located at a great distance from the paired lateral fins (approximately 2 times greater than the distance between the seminal vesicles and the caudal fin). The ovaries constitute 15.6% of the body length; those are located in front of the anterior ends of the fins of the II pair. The diameter of the ovary is equal to the diameter of the egg at its anterior end (0.55 mm) or to the size of the cluster of immature eggs (0.49 mm) at the posterior end. Mature eggs are large; their diameter is 0.55 mm, and it is equal to the length of the seminal vesicles.

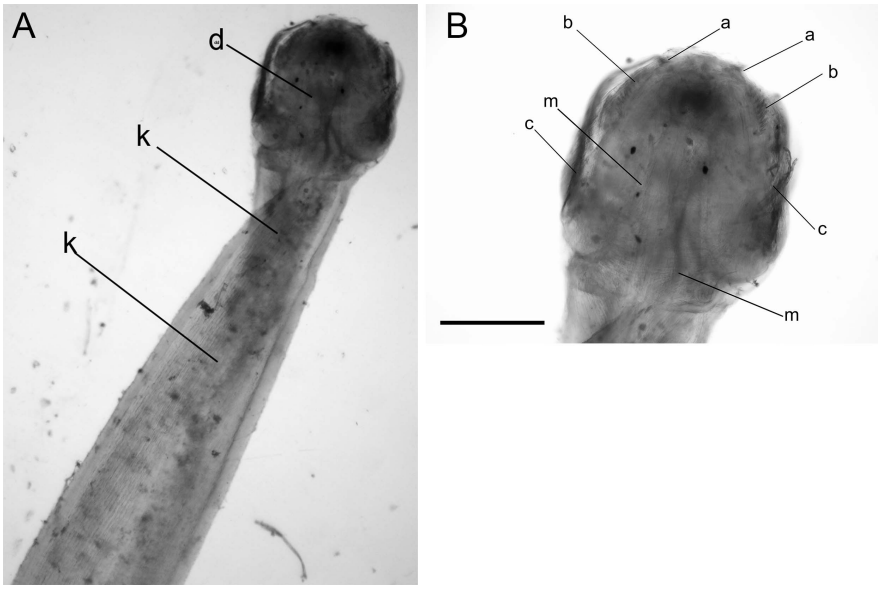

Fig. 2. Holotype. *Sagitta dimitryi* Kassatkina & Vasileva sp. nov. A, dorsal view of anterior part of the body (d, pharynx; k, gut). B, dorsal head (a, anterior teeth; b, posterior teeth; c, hooks; m, corona ciliata). Scale bar: 0.4 mm

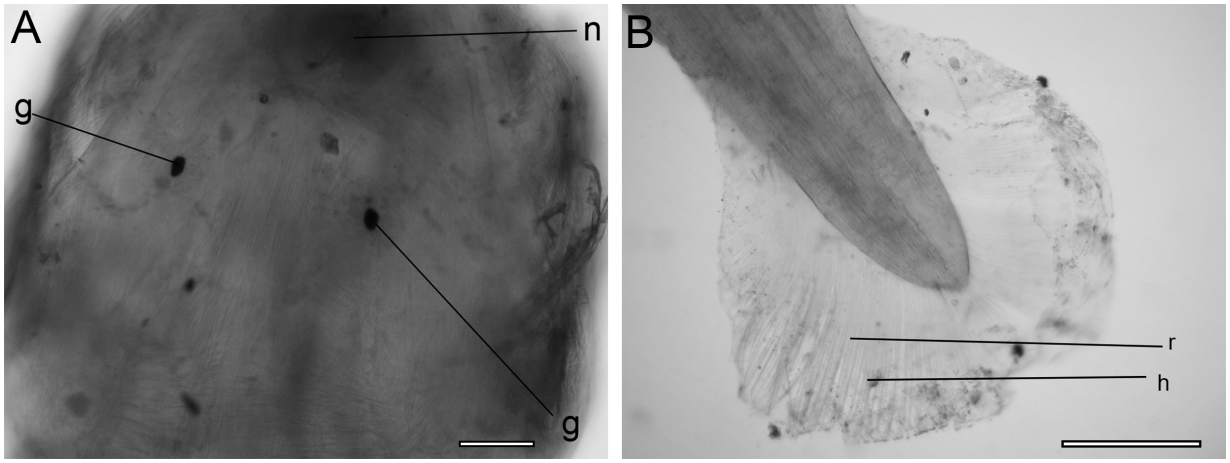

Fig. 3. Holotype. *Sagitta dimitryi* Kassatkina & Vasileva sp. nov. A, dorsal side of the head: g, eyes; n, head ganglion. B, tail fin: r, rays. Scale bar: 0.1 mm (A); 0.5 mm (B)

Morphological features of immature specimens of *Sagitta dimitryi* sp. nov. A striking feature is the shape of the corona ciliata: with paired convexities at the level of the trunk-tail septum. It is small in size and is mainly located on the head. The corona ciliata begins to form already in the embryo of chaetognath, and its shape and location remain constant at all stages of maturity. Therefore, this organ is a reliable species character even for identifying immature animals, while the length of the fins and the distances between the fins change with the growth of an animal.

Description of an immature specimen of *Sagitta dimitryi* sp. nov. from a plankton sample taken on 11.08.1993 from a horizon of 55–23 m during the 24th cruise of the RV "Akademik Nesmeyanov," station 2393 (Fig. 4).

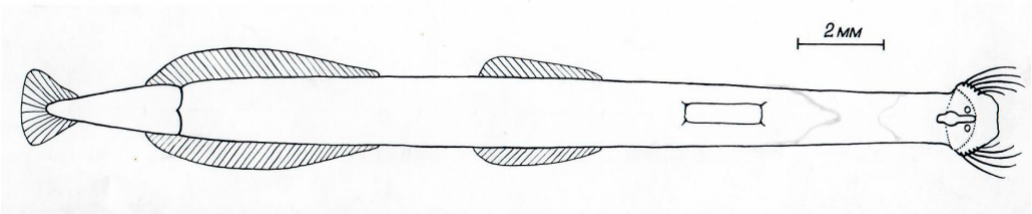

Fig. 4. *Sagitta dimitryi* sp. nov. general view. Scale bar: 2 mm

The total body length is 11.1 mm. The muscles are rigid. The caudal section makes up about 13% of the body length. The upper part of the pharynx is muscular, wider than the midgut (Fig. 5, p). The trunk-caudal septum is wide; it clearly separates the pharynx from the midgut (Fig. 5, s). From the very beginning, from the trunk-caudal septum, the midgut is filled with large food which by its shape may be mistaken for outgrowths of the midgut, *i. e.*, diverticula (Fig. 5, f). However, diverticula are a paired organ (with a clearly defined epithelium). *Sagitta dimitryi* sp. nov. has no diverticula, the same as all *Sagitta* species. The corona ciliata is short; it has one pair of convexities at the level of the trunk-tail septum. The corona ciliata begins from the brain, and its trunk part is shorter than the part lying on the head (those are almost of the same length). The length of the ventral ganglion is about 8% of the body length. The anterior edge of the fins of the I pair is located behind the posterior end of the ventral ganglion. The gap between the fins of the I pair and the posterior end of the ventral

ganglion is slightly larger than the ganglion and is about 9% of the body length. The fin of the I pair constitutes 13% of the body length; it is 1.8 times shorter than the fin of the II pair and shorter than the trunk part of the fins of the II pair. The gap between the lateral fins of the II and I pairs is slightly larger than the gap between the ventral ganglion and the fins of the I pair, with the latter gap being about 9% of the body length. The fin of the II pair is about 20% of the body length; its trunk part is 5.7 times longer than the tail one. The rays in the fins are complete, and there are no rayless zones (Fig. 3B). Alveolar tissue is absent. On the head, there are one pair of rows of hooks and two pairs of rows of denticles. There are 5 hooks, 3 anterior denticles, and 7 posterior denticles.

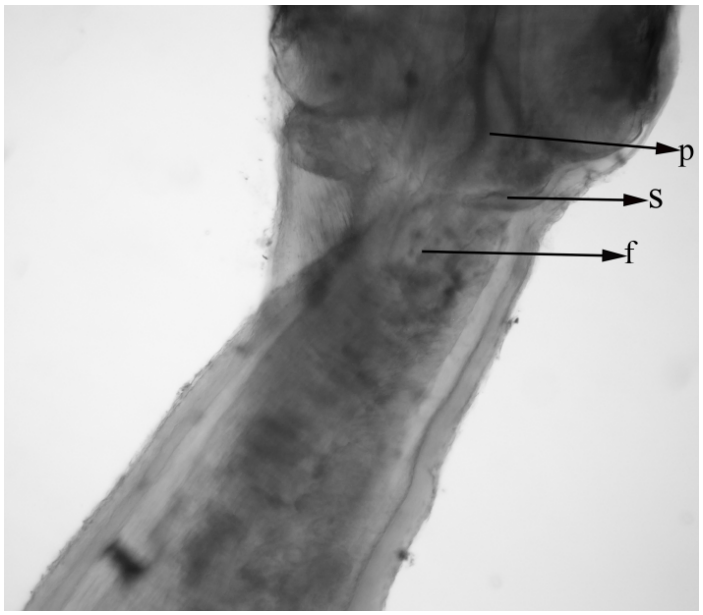

Fig. 5. Dorsal view of anterior part of *Sagitta dimitryi* sp. nov. body: p, pharynx; s, trunk-tail septum; f, lump of food

Description of an immature specimen of *Sagitta dimitryi* sp. nov. from a plankton sample taken on 08.08.1949 from a horizon of 200–0 m during the expedition of the Zoological Institute of RAS, station 75. The length is 9.5 mm. The length of the ventral ganglion is about 8% of the body length (coincides with the length of the ganglion in the 11.1-mm individual). Other parameters do not coincide (the caudal section is 1.7 mm, 18%).

Description of an immature specimen of *Sagitta dimitryi* sp. nov. from a plankton sample taken on 20.08.1965 from a horizon of 75–0 m during the cruise of the RV "Baidar." The length is 10.5 mm. The length of the ventral ganglion is about 7.9% of the body length (almost the same as the length of the ganglion in the 11.1-mm individual). Other parameters do not coincide (the caudal section is 1.5 mm, 13%).

Differential diagnosis. The new species differs from *Sagitta nagae*, *S. bedoti*, and *S. pulchra* in the position of the fins of the I pair relative to the ventral ganglion. Our specimens differ from *S. bruuni*, *S. izuensis*, and *S. abyssicola* not only in the position of the anterior pair of fins, but also in the location of the seminal vesicles relative to the fins (caudal one and lateral ones). It differs from *S. euneritica* and *S. modesta* in the length of the gap between the posterior end of the ventral ganglion and the fins of the I pair (in those species, this gap is smaller than the ganglion). Moreover, the new species differs from *S. modesta* in the absence of a rayless zone in the caudal fin.

The species closest to the new one is *Sagitta nutana* (by the location of the seminal vesicles and the length of the gap between the ganglion and the front fins). However, *S. nutana* has extensive alveolar tissue on the dorsal side which is absent in specimens of the new species; also, the corona ciliata has no convexities in *S. nutana*. The new species differs from *S. glacialis* in the shape and length of the corona ciliata and the absence of alveolar tissue: in *S. glacialis*, the corona ciliata has no convexities, and its part on the trunk is 2.5 times larger than the part located on the head. The new species differs from *S. setosa* in the location of the seminal vesicles: in *S. setosa*, those are tightly adjacent to the lateral fins. The new species differs from *S. sublica* in the occurrence of complete rays in the fins and long fins of the I pair, as well as in the smaller gap of the body between the ventral ganglion and the fins of the I pair.

Distribution. Mature individuals of the new species were found in the coastal, neritic zone on the Sea of Okhotsk side of Sakhalin. Immature animals were caught both in the neritic zone and in the central Sea of Okhotsk. For several years, we encountered hundreds of immature individuals of the species in the open area of the Sea of Okhotsk; we were unable to describe it, for new species can only be described based on mature animals. Apparently, mature individuals come to the coastal zone to spawn. As noted, mature animals go to the depths after spawning, and part of the population stays in this zone and reproduces again [Alvariño, 1968; Kasatkina, Stolyarova, 2010; Russel, 1932]. There is an assumption that adult animals leave the spawning ground after spawning as a part of a conservation strategy for the species [Russel, 1932].

In order to show the differences between the new species and other ones of this genus, we provide a key for identification of mature animals of all known *Sagitta* species in the world fauna (keys do not include animals at the early stages of maturity).

Key for identification of mature individuals of species and subspecies of *Sagitta* s. str. of the world fauna

1 (2) Anterior end of the fins of the II pair and the posterior end of the fins of the I pair appear fused from the ventral and dorsal sides. However, it is visible from the lateral side that the fins do not touch, but are located parallel to each other ..... *S. sceptrum*
2 (1) Front and rear fins are distant from each other 3
3 (4) Fins of the I pair begin in front of the anterior end of the ventral ganglion ..... *S. nagae*
4 (3) Fins of the I pair begin behind the anterior end of the ventral ganglion 5
5 (10) Fins of the I pair begin almost in the middle of the ventral ganglion 6
6 (15) Fins of the I pair begin in front of the posterior end of the ventral ganglion 7
7 (10) Front fins are longer than rear fins 8
8 (9) Seminal vesicles are located close to both the lateral fins and the caudal fin ..... *S. bedoti*
9 (8) Seminal vesicles are distant from the lateral fins and touch the caudal fin ..... *S. pulchra*
10 (5) Fins of the I pair begin at the level of the posterior end of the ventral ganglion 11
11 (12) Seminal vesicles touch both the lateral fins and the caudal fin ..... *S. bruuni*
12 (11) Seminal vesicles are distant from either the lateral fins or the caudal fin 13
13 (14) Seminal vesicles are distant from the lateral fins and touch the caudal fin ..... *S. izuensis*
14 (13) Seminal vesicles touch the lateral fins and are distant from the caudal fin ..... *S. abyssicola*
15 (6) Fins of the I pair begin behind the posterior end of the ventral ganglion 16
16 (17) A gap between the posterior end of the ventral ganglion and the fins of the I pair is less than ½ the length of the ganglion ..... *S. bipunctata*

17 (16) A gap between the posterior end of the ventral ganglion and the fins of the I pair is equal to the length of the ganglion or is more than ½ the length of the ganglion 18
18 (21) A gap between the posterior end of the ventral ganglion and the fins of the I pair is more than ½ the length of the ganglion, but less than the length of the ganglion 19
19 (20) The seminal vesicles touch the lateral fins and are located at a short distance from the caudal fin. The caudal fin has complete rays; there are no rayless zones ..... *S. euneritica*
20 (19) Seminal vesicles are located at a considerable distance from the lateral fins and the caudal fin. The caudal fin has an internal rayless zone ..... *S. modesta*
21 (18) A gap between the posterior end of the ventral ganglion and the fins of the I pair is more than ½ the length of the ganglion and larger than the ganglion 22
22 (25) A gap between the posterior end of the ventral ganglion and the fins of the I pair exceeds the length of the ganglion, but no more than 1.5 times 23
23 (24) Corona ciliata is smooth and has no convexities; the corona ciliata is mainly located on the trunk part, not on the head ..... *S. nutana*
24 (23) Corona ciliata has paired convexities; the corona ciliata is mainly located on the head part, not on the trunk part ..... ***S. dimitryi* sp. nov.**
25 (22) A gap between the posterior end of the ventral ganglion and the fins of the I pair exceeds the length of the ganglion by more than 1.5 times 26
26 (29) There are no rayless zones in any of the fins ..... *S. glacialis*
27 (28) Relative length of the caudal section exceeds 17%; the length of the fins of the I pair exceeds 13%; and the length of the fins of the II pair exceeds 19% of the body length ..... *S. glacialis glacialis*
28 (27) Relative length of the caudal section does not exceed 17%; the length of the fins of the I pair does not exceed 13%; and the length of the fins of the II pair does not exceed 19% of the body length ..... *S. glacialis baltica*
29 (26) There may be rayless zones in the fins 30
30 (35) Seminal sacs either touch the lateral fins or are located at a distance that is significantly shorter than the sacs 31
31 (32) Seminal sacs touch the lateral fins and are located at a distance from the caudal fin ..... *S. setosa*
32 (31) Seminal sacs are located at a distance from the lateral fins and do not touch the caudal fin 33
33 (34) Corona ciliata has no convexities ..... *S. euxina*
34 (33) Corona ciliata has paired convexities ..... *S. sublica*
35 (30) Seminal sacs are located at a distance from the lateral fins and almost touch the caudal fin; this gap between the sacs and the caudal fin is visible only under high magnification ..... *S. kussakini*

The genus *Sagitta* is morphologically closer than *Parasagitta* to the ancient Chaetognatha called protoconodonts. It is *Parmia anastassiae* [Gnilovskaya, 1998] from the Proterozoic layers of the northeast of the Russian Platform (the age of 1 billion years) [Gnilovskaya et al., 2000]. Their evolution was directed towards increasing the complexity of the organization (the appearance of grasping hooks-setae, eyes, and corona ciliata); it was a response to the oxygenation of the environment; and it was aimed at protecting metabolic pathways [Fedonkin, 2003: p. 10].

Assumably, with such a tool, hooks, ancient chaetognaths scraped off unicellular organisms from microbial mats which they fed on, the same as protoarticulates did [Ivanzov, 2011].

The size of food corresponded to the width of the intestine; therefore, the ancient Chaetognatha (*P. anastassiae*) had no intestinal diverticula. Their absence was also noted in *Protosagitta spinosa* [Hu, 2005] from the Lower Cambrian (540–520 million years). Apparently, there was no larger

prey in the Vendian and Lower Cambrian [Fedonkin, 2003; Hu, 2005]. This ancient morphological character (absence of diverticula and narrow intestine) has been preserved in some modern Chaetognatha. The evolution of chaetognaths (a rise in the level of organization or degradation of the general structure) depends entirely on external environmental factors [Gasmi et al., 2014; Kasatkina, 2022]. An increase in the width of the intestine during evolution (the appearance of diverticula) was noted in *Paucijaculum samamithion* Schram, 1973 (the Paleozoic Era, Carboniferous Period, from the Pennsylvanian Subperiod layer). Presumably, it was associated with the ability to feed on larger prey.

For chaetognaths with intestinal diverticula, T. Tokioka [1965] formed the genus *Parasagitta* which is close to *Sagitta* in other morphological features. In 1847, J. Müller described the species *Sagitta setosa* [Müller, 1847] and provided drawings clearly showing the midgut without diverticula. However, some researchers, *e. g.*, [Müler et al., 2019], mistakenly attributed *S. setosa* to *Parasagitta* whose representatives have diverticula. Genetic studies have shown that the branches with *Parasagitta* species are definitely different from the branch with *Sagitta* species [Gasmi et al., 2014]. We also believe that the occurrence of diverticula on the trunk (midgut) is a feature of the genus in the taxonomy of chaetognaths. The species *S. setosa* cannot be classified as a *Parasagitta* one. We have included *S. setosa* in the "Key for identification of mature individuals of species and subspecies of *Sagitta* s. str. of the world fauna."

Chaetognaths are a sensitive and convenient indicator of the state of the marine environment. Its anthropogenic pollution of any origin (in particular, radioactive) causes changes in the shape of the body and organs of these animals [Kassatkina et al., 2017]. The pattern of alterations in tissues of chaetognaths and the percentage of abnormal individuals in samples can be used to predict a forthcoming of such a natural disaster as the earthquake: when it is coming, intestinal walls (Fig. 2A, k) are destroyed, the symmetry of the eyes (Fig. 3A, g) is disrupted, and the central pigment spot disappears. Elevated levels of radioactivity in seawater affect the fins of chaetognaths. Specifically, the rays on the fins (Fig. 3B, r) stick out the fin plate [Kasatkina, 1995; Kassatkina, Stolyarova, 2016].

Conclusion. The finding of a new Chaetognatha species adds to our knowledge of marine biodiversity. Comparative analysis of the morphological features of the discovered species showed as follows: the absence of intestinal diverticula brings *Sagitta dimitryi* sp. nov. closer to fossil and some modern species. The new organ (diverticula) is thought to have evolved as a result of animals gaining the ability to feed on larger prey. In terms of general morphology, the genus *Sagitta* s. str. is closest to the genus *Parasagitta*. However, genetic studies have shown the isolation of these two genera. By the location of the fins, the occurrence of the gap between the ganglion and the front fins, the location of the seminal vesicles, the occurrence of the gap between the rear and the front fins, the absence of alveolar tissue, and the occurrence of convexities of the corona ciliata at the level of the trunk-tail septum, *S. dimitryi* sp. nov. is an independent species new to science. It is clearly distinguishable from *Sagitta* species which is reflected in the "Key for identification of mature individuals of species and subspecies of *Sagitta* s. str. of the world fauna."

Etymology. The species *Sagitta dimitryi* Kassatkina & Vasileva sp. nov. is named in honor of a historian and famous writer Dmitry Kasatkin, may his memory be blessed.

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НОВЫЙ ВИД ЩЕТИНКОЧЕЛЮСТНЫХ *SAGITTA DIMITRYI* SP. NOV. (CHAETOGNATHA, SAGITTOIDEA) ИЗ ОХОТСКОГО МОРЯ (СЕВЕРО-ЗАПАДНЫЙ САХАЛИН)

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В статье описан новый вид щетинкочелюстных *Sagitta dimitryi* sp. nov., обнаруженный в водах Охотского моря у северо-западной части Сахалина. Дана таблица определительных ключей для видов рода *Sagitta* с включением *Sagitta dimitryi* sp. nov. Обсуждается родство современных *Sagitta* с древними Chaetognatha, в том числе возможные причины эволюции кишечного аппарата.

Ключевые слова: *Sagitta dimitryi* sp. nov., Chaetognatha, Сахалин, Охотское море

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BIOLUMINESCENT BACTERIA OF THE BLACK SEA AND SEA OF AZOV

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The aim of the present study was to isolate bioluminescent strains from the northern Black Sea and Sea of Azov, analyze their morphological and biochemical characteristics, and identify them based on 16S rRNA, *recA*, and *gyrB* gene sequences. Nine isolates were isolated from hydrobionts, and twelve, from seawater. Results of biochemical and molecular genetic identification revealed that isolated luminous strains represent the genera *Vibrio*, *Aliivibrio*, and *Photobacterium*. All five cultivated luminescent strains isolated from water and hydrobionts of the Sea of Azov belong to the species *Photobacterium leiognathi*. Cultivated luminous bacteria of the Black Sea are assigned to the genera *Aliivibrio* and *Vibrio*. The genus *Aliivibrio* is represented by two *Aliivibrio fischeri* strains related to various hydrobionts. Fourteen strains of the genus *Vibrio* belong to the species *Vibrio campbellii*, *V. jasicida*, *V. harveyi*, *V. owensii*, and *V. aquamarinus* sp. nov. Thus, it was shown that taxonomic composition of the cultivated luminescent bacteria differs greatly in the Black Sea and Sea of Azov.

Keywords: luminous bacteria, identification, taxonomic composition, biodiversity, Black Sea, Sea of Azov

Currently, bacteria are among the most common model biological objects in basic and applied research. Out of them, bioluminescent bacteria are a special natural phenomenon. Those are intensively studied all over the world and used in solving various problems of biology, genetics, and biotechnology.

Bioluminescence of bacteria formed the basis of many analysis methods widely applied in practice. These are bioluminescent testing of humoral and cellular bactericidal blood systems *in vitro* and study of the characteristics of the infectious process on models *in vivo* [Deryabin, 2009]. In the practical application of bioluminescent bacteria, a significant role is played by the analysis of the integral toxicity of various natural environments. Natural and recombinant luminescent microorganisms have become a recognized tool for environmental monitoring [Baumstark-Khan et al., 2007; Chugunova et al., 2016; Ivask et al., 2007; Niu et al., 2008; Sazykin et al., 2015, 2016; Sönmez et al., 2016; Tsybulskii, Sazykina, 2010] and analysis of new substances and materials [Kovalenko et al., 2013; Kuryanov et al., 2011; Zheng et al., 2010].

Luminescent bacteria studies served as the basis for the discovery of quorum sensing – a phenomenon of general biological significance [Taga, Bassler, 2003]. First, this genetic mechanism was noted in marine luminous bacteria *Aliivibrio fischeri* and *Vibrio harveyi*; later, it was found in many other species of bacteria as a regulator of manifestations of numerous properties, including pathogenic ones. Thus, bacterial bioluminescence opens up exceptional methodological opportunities in a variety of applications

in biology, ecology, and medicine. Despite noticeable progress in the investigation of physiology, biochemistry, and genetics of luminous bacteria, many issues of their species composition and distribution in ecosystems remain unclear.

Bioluminescent bacteria are widespread in nature [Ast et al., 2009; Baumann, et al., 1984; Dunlap, Urbanczyk, 2013; Thompson et al., 2004; Urbanczyk et al., 2011]. The list of luminescent bacteria is being expanded by both discovering new species [Ast et al., 2007; Cano-Gómez et al., 2010; Gomez-Gil et al., 2003; Lucena et al., 2012; Wang et al., 2010; Yoshizawa et al., 2009a, b, 2010a, b, 2012] and reclassification of long-known ones [Labella et al., 2017; Thompson et al., 2003; Urbanczyk et al., 2007]. However, due to anthropogenic load, the composition of biological species in ecosystems often changes. In many cases, the only ways to preserve natural biodiversity, *inter alia* that of microorganisms, are to carry out a detailed analysis of natural communities of luminescent bacteria by species and environmental criteria and to provide preservation of biological genotypes in collections.

Bioluminescent bacteria of the Black Sea and Sea of Azov remain poorly studied [Katsev, 2002; Katsev, Makemson, 2006; Maligina, Katsev, 2003; Tsybulskii, Sazykina, 2010]. Species composition of luminescent bacteria and their spatial distribution are of great interest due to features of these water areas. One of the key peculiarities of the Black Sea and Sea of Azov is the fact that many rivers flow into them; this results in lower salinity compared to values for other seas and oceans (16–18‰ for the Black Sea and 10–13‰ for the Sea of Azov).

A distinguishing feature of these water areas is noticeable seasonal variation in temperature which is more typical for the shallow Sea of Azov. This sea is also characterized by higher values of water temperature in summer, which, along with low salinity, leads to higher biological activity and more significant biodiversity compared to those for the Black Sea. Therefore, the aim of this work was to study species composition, biochemical properties, and characteristics of the distribution of luminous bacteria in coastal waters of the northern Black Sea and Sea of Azov.

MATERIAL AND METHODS

Sampling. To isolate bacteria in the northern coastal zone of the Black Sea and Sea of Azov, seawater and various hydrobionts were sampled May to October 2016–2018 (Fig. 1). The samples were placed in sterile containers and transported to a laboratory for further processing, which was carried out no later than 24 h after sampling. Details of the sampling location and their characteristics are provided in Table 1.

Fig. 1. Sampling sites for the isolation of luminescent bacteria

Table 1. Description of sampling sites

Site No.	Sample (water/hydrobiont)	Site	Location	
The Black Sea				
1	Water	Kerch city	N45.254692°	E36.430439°
2	Water	Kerch city	N45.230556°	E36.414444°
3	Water	Sudak city	N44.840932°	E34.964564°
4	Water	Malyi Mayak village	N44.603173°	E34.372549°
5	Water	Partenit village	N44.559875°	E34.346913°
6	Water	Partenit village	N44.572081°	E34.346930°
7	Water	Sudak city	N44.831895°	E34.987922°
8	Water	Solnechnaya Dolina village	N44.863852°	E35.138874°
9	Water	Alushta city	N44.647252°	E34.401669°
10	Water	Partenit village	N44.561823°	E34.347663°
11	The Mediterranean mussel *Mytilus galloprovincialis*	Sudak city	N44.816742°	E35.049346°
12		Alushta city	N44.633788°	E34.392279°
13		Sevastopol city	N44.441391°	E33.640501°
14		Partenit village	N44.549120°	E34.347217°
15	The horse mackerel *Trachurus trachurus*	Sudak city	N44.797715°	E35.070433°
16	The brown shrimp *Crangon crangon*	Alushta city	N44.696810°	E34.444220°
The Sea of Azov				
17	Water	Shchelkino town	N45.426850°	E35.809528°
18	Water	Shchelkino town	N45.445819°	E35.846986°
19	Gobiidae gen. sp. (Pisces)	Shchelkino town	N45.451294°	E35.820341°
20	The Mediterranean green crab *Carcinus aestuarii*	Shchelkino town	N45.452265°	E35.852640°
21	Amphipoda fam. gen. sp.	Shchelkino town	N45.416109°	E35.791470°

Isolation of luminescent bacteria. After transportation to the laboratory, water samples were concentrated by filtration through a 0.45-μm membrane filter (Sartorius AG, Germany). The volume of the filtration sample varied within 10–50 mL depending on seawater temperature. After sample concentration, the filter was placed on the surface of solid media (HiMedia, India) containing 3% of sodium chloride. In summer (July and August), plating of water on a solid medium was carried out without *prior* concentration. In total, 200–500 μL of samples were applied to the surface of the nutrient agar in a Petri dish. The samples were incubated at +15…+25 °C, with periodical visual analysis of the results in a dark room. Upon detection of luminescent spots on the surface of the nutrient medium, pure bacterial culture was isolated by standard microbiological techniques.

Also, experimentally designed selective media based on water salinity in the sampling site were used for isolation of luminescent bacteria [Patent 2358009 RU, 2009; Patent 2368658 RU, 2009]. Bioluminescent bacteria were isolated, and the media were prepared as described in [Tsybulskii, Sazykina, 2010]. To isolate luminescent bacteria from fish samples, pieces of biomaterial less than 1–2 cm in size were placed in a sterile container and ⅔ covered with 3% solution of sodium chloride. Following incubation, bioluminescence analysis and bacteria isolation were carried out as described above. When using other hydrobionts as sources for bacteria isolation, those were removed from their shells (mussels),

or chitinous exoskeleton (crabs and shrimps) was broken; then, biological material (samples 1–2 cm in size) was processed as described above using 3% solution of sodium chloride. The isolated pure cultures of luminescent bacteria were stored as museum cultures in semi-liquid agar under a vaseline oil layer.

Identification of luminescent bacteria. Bacteria were identified by standard microbiological techniques. Morphological properties of bacteria, as well as their growth and bioluminescence characteristics, were evaluated at different temperatures (+10, +20, +25, +30, +35, +37, and +44 °C) and NaCl contents (0.5, 1, 2, 3, 4, 5, 6, 7, and 8%). Also, their enzymatic properties and ability to ferment various sugars (maltose, D-mannitol, and sucrose) were examined.

Bioluminescence kinetics. To study the kinetics of the luciferase reaction, enzyme preparations isolated from biomass of luminescent bacteria were used. Bacteria were cultivated, and the biomass was accumulated on a liquid nutrient medium under constant stirring at the optimal for each isolate temperature for 24 h. Bacterial cells were separated from the medium by centrifugation at 5,000 rpm for 30 min. The obtained biomass was washed with a 3% sodium chloride solution; then, it was suspended in a 0.01 M phosphate buffer in the ratio of 1 / 10 (biomass / buffer solution), pH = 7.0, at +4 °C. Cell destruction was carried out by 3-fold freezing–thawing, additionally using ultrasonic treatment and avoiding temperature increase of the samples to values above +15 °C. Leftover cellular debris was separated by centrifugation at 5,000 rpm for 30 min. The enzyme preparation containing luciferase was isolated from the resulting supernatant by ammonium sulphate precipitation, 25–80% of saturation.

To evaluate the kinetic characteristics of luciferase reaction, the protein precipitate obtained at the previous stage was dissolved in 0.1 M phosphate buffer, pH of 7.0. Then, 500 μL of 0.1 M phosphate buffer, pH of 7.0, 20–50 μL of the enzyme preparation (working dilutions were selected experimentally for each strain separately), and 20 μL of the 0.001% aqueous suspension of dodecanal (Sigma-Aldrich) were mixed in a chemiluminometer cuvette. The suspension of aldehyde was prepared by solvent exchange method. Dodecanal solution in ethanol was mixed with water in ratio 1 : 100. Luciferase reaction was initiated by adding 400 μL of photoreduced $FMNH_2$ (Sigma-Aldrich) at the concentration of 5×10^{-5} M containing 1×10^{-3} M of Trilon B. Bioluminescent signal was registered for 5 min after adding $FMNH_2$ till complete luminescence decay. The obtained graphical dependence of bioluminescence intensity on time was used for calculating the constant of the first-order bioluminescence decay rate (k, s^{-1}):

$$k = (\ln I_0/I)/t \,,$$

where I_0 and I are intensity of bioluminescence at the initial moment and after the time period t, respectively.

Also, according to the diagrams, half-decay time of bioluminescence ($t_{½}$, s) was determined. Bioluminescence intensity dependence on time was recorded with a chemiluminometer Lum 100 (DISoft Ltd, Russia).

Molecular characteristics. Isolation of genomic DNA from microbial isolates. For the purpose of molecular genetic identification, total genomic DNA was isolated from the isolates. To isolate genomic DNA, the overnight culture of microorganisms was grown in 50-mL Erlenmeyer flasks on a liquid LB medium with addition of 3% NaCl. Twenty mL of medium was introduced into a flask and cultivated for 18 h in an incubator shaker at +25 °C and 170 rpm. Bacterial cells were precipitated by centrifugation in 2-mL screw-cap microtubes at 6,000 g for 2 min; ~ 75 mg of glass beads, 0.25 mm in diameter, was added to the obtained precipitate.

Then, 350 μL of guanidine solution (guanidine HCl 240 mM), 350 μL of detergent solution (Tris-HCl 500 mM, pH 8.0; SDS 2%, laurylsarcosinate 4%), and 400 μL of phenol-chloroform mixture were introduced into the tubes. Cells were destroyed by shaking on a laboratory vibrating mill Mixer Mill MM 400 (Retsch, Germany) for 2 min with a shaking frequency of 30 Hz. After that, the tubes were centrifuged for 7 min at 14,000 g; supernatant was taken; and 400 μL of chloroform was added to it and thoroughly mixed with a vortex. Then, it was centrifuged as in the previous step. Subsequently, the aqueous phase was separated, and DNA was precipitated with an equal volume of isopropanol. The precipitate was washed twice with 70% ethanol, dried, and dissolved in 100 μL of deionized water. Discrete PCR amplicon bands were resolved on agarose gel. The PCR amplicon was purified to remove contaminants by the Cleanup Standard kit (Evrogen, Russia).

16S rRNA, *recA*, and *gyrB* gene amplification. To identify the isolated strains, amplification of 16S rRNA gene, as well as housekeeping genes encoding recombinase A (*recA*) and DNA gyrase B subunit (*gyrB*), was carried out. It was followed by determination of their nucleotide sequence. Sequencing of 16S rRNA gene and *gyrB* and *recA* housekeeping genes was performed by Sanger method [Sanger et al., 1977]. The structure of the primers used to obtain the target amplicons was taken from [Ast et al., 2009] and is shown in Table 2. Comparison of the sequence data and their differentiation was carried out using BLAST (https://blast.ncbi.nlm.nih.gov/). Phylogenetic relationships among submitted species were examined by means of MEGA X [Kumar et al., 2018]. Phylogenetic tree was constructed by neighbor-joining. A bootstrap analysis to investigate the stability of the tree was performed in 1,000 replicates. Also, the sequences of the following reference strains (https://www.ncbi.nlm.nih.gov) were added to the phylogenetic tree for comparison: *Vibrio harveyi* SB1 (NZ_CP125875.1), *Vibrio campbellii* BoB-53 (NZ_CP026321.1), *Photobacterium leiognathi* subsp. *mandapamensis* Lk8.2 (NZ_CP131594.1), *Aliivibrio wodanis* Vw11 (LR813705.1), and *Aliivibrio logei* 6Go0121 (MZ005969.1).

Table 2. The structure of the primers used to obtain the target amplicons

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Amplicon size (bp)
16S rRNA	AGAGTTTGATCCTGGCTCAG	TACGGYTACCTTGTTACGACTT	~ 1,500
recA	TCAAATTGAAAAACAATTTGGTAAAGG	ATCTTATCACCATTGTAGCTGTACC	~ 900
gyrB	GAAGTTATCATGACGGTACTTC	AGCGTACGAATGTGAGAACC	~ 1,200

To amplify the target genes, the following PCR modes were used. For 16S RNA gene, +95 °C – 2 min, 35 cycles (+95 °C – 20 s; +48 °C – 15 s; and +72 °C – 1 min); +72 °C – 5 min. For *recA* gene, +95 °C – 2 min, 35 cycles (+94 °C – 20 s; +45 °C – 15 s; and +68 °C – 1 min); +72 °C – 7 min. For *gyrB* gene, +95 °C – 2 min, 35 cycles (+95 °C – 20 s; +49 °C – 15 s; and +72 °C – 1 min); +72 °C – 5 min. Amplicons were purified by cutting an agarose gel (1% agarose, TBE buffer, 6 V·cm^{-1}) strip stained with SYBR Green (DNK-Sintez, Russia). The target product was isolated from the agarose gel using the Cleanup Standard kit (Evrogen, Russia) according to the manufacturer's instructions. The amplified fragments of 16S rRNA, *recA*, and *gyrB* genes were sequenced at Evrogen company (Moscow).

RESULTS AND DISCUSSION

Field studies conducted May to October 2016–2018 in various coastal areas of the Black Sea and Sea of Azov (Fig. 1) allowed us to identify 21 bacterial isolates with visible bioluminescence. In the spring–summer season (May to June), when the Black Sea water had not yet fully warmed up,

and its temperature averaged +16…+20 °C, the abundance of luminescent bacteria was low. Accordingly, the bacteria were isolated with preliminary concentration on filters. In summer (July to August), seawater temperature in the Black Sea reached +25 °C, and luminescent forms of bacteria were plated from water without additional concentration of samples. Isolation and research on luminescent bacteria of the Sea of Azov were carried out in August, when seawater temperature reached +30 °C. Luminescent forms occurred everywhere and were isolated both from seawater and various marine hydrobionts.

Table 3 provides biochemical characteristics of cultured luminescent bacteria strains isolated from water and hydrobionts of the Black Sea and Sea of Azov. For isolated strains, sequence data on 16S rRNA gene and *gyrB* and *recA* housekeeping genes were obtained (GenBank accession numbers were MK692515–MK692535). The results of phylogenetic analysis based on the comparison of 16S rRNA genes sequences are presented in Fig. 2 (compared to some reference bacteria strains). The data on molecular genetic identification are also provided in Table 3. The analysis revealed the presence of three well-supported clades. All the isolated luminous strains tested here resolved unambiguously either to the *Vibrio* clade, or to the *Aliivibrio* clade, or to the *Photobacterium* clade. Cells of all isolated strains are gram-negative. They are capable of growing at +15…+35 °C, with the optimum of +20…+30 °C, at 0.5–5.0% NaCl (weight/volume, w/v), with the optimum of 1.5–3.0 %, and at pH 6.0–8.0, with the optimum of 7.0–8.0. All of them are able to ferment glucose and mannose with acids formation.

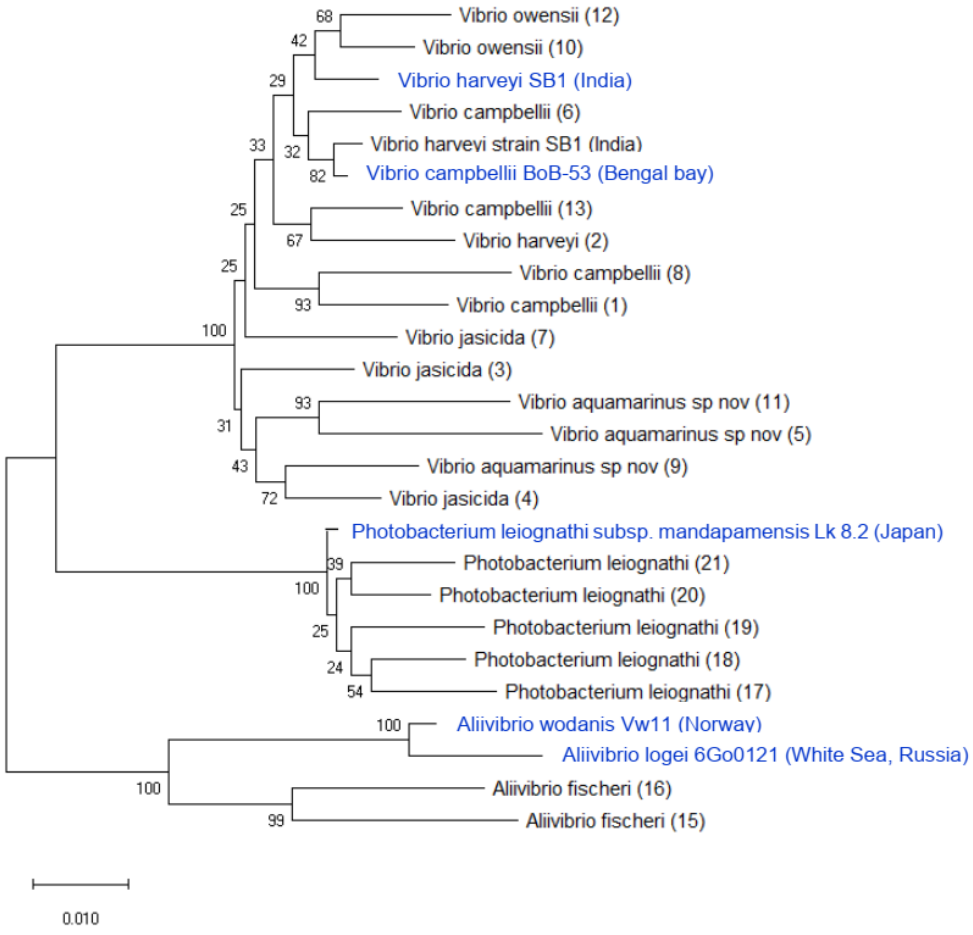

Fig. 2. Phylogenetic tree based on the analysis of 16S rRNA sequences, constructed by neighbor-joining [Saitou, Nei, 1987]. Bootstrap percentages from 1,000 replicates appear next to respective branches. The scale bar indicates the number of inferred nucleotide changes. The strain numbers (1–21) correspond to the numbers given in Table 3

Table 3. Results of identification of cultivated strains of bioluminescent bacteria isolated from the Black Sea and Sea of Azov

No.	Result of molecular genetic identification	Kinetics of luciferase reaction			Fermentation of sugars		
		k, s^{-1}	$t_{½}$, s	type	maltose	D-mannitol	sucrose
The Black Sea							
The genus *Vibrio*							
1	*Vibrio campbellii*	0.038	18.2	S	+	+	–
2	*Vibrio harveyi*	0.040	17.3	S	+	+	+
3	*Vibrio jasicida*	0.036	19.3	S	+	+	–
4	*Vibrio jasicida*	0.037	18.7	S	+	+	–
5	*Vibrio aquamarinus* sp. nov.	0.038	18.2	S	+	+	–
6	*Vibrio campbellii*	0.036	19.3	S	+	+	–
7	*Vibrio jasicida*	0.043	16.1	S	+	+	–
8	*Vibrio campbellii*	0.050	13.9	S	+	+	–
9	*Vibrio aquamarinus* sp. nov.	0.045	15.4	S	+	+	–
10	*Vibrio owensii*	0.059	11.7	S	+	+	+
11	*Vibrio aquamarinus* sp. nov.	0.040	17.3	S	+	+	+
12	*Vibrio owensii*	0.041	16.9	S	+	+	+
13	*Vibrio campbellii*	0.059	11.7	S	+	+	+
14	*Vibrio owensii*	0.056	12.4	S	+	+	+
The genus *Aliivibrio*							
15	*Aliivibrio fischeri*	0.38	1.8	F	+	–	–
16	*Aliivibrio fischeri*	0.43	1.6	F	+	–	–
The Sea of Azov							
The genus *Photobacterium*							
17	*Photobacterium leiognathi*	0.45	1.5	F	–	–	–
18	*Photobacterium leiognathi*	0.48	1.4	F	–	–	–
19	*Photobacterium leiognathi*	0.37	1.9	F	–	–	–
20	*Photobacterium leiognathi*	0.42	1.7	F	–	–	–
21	*Photobacterium leiognathi*	0.36	1.9	F	–	–	–

Note: S, slow-type luciferase kinetics; F, fast-type luciferase kinetics.

The following criteria and recommendations were used for primary identification of isolated bacteria. The presence of yellow pigment and the fast-type luciferase kinetics, as well as the ability of maltose fermentation, determined the bacteria species *A. fischeri* [Farmer III, Michael Janda, 2015; Farmer III et al., 2015]. These strains had average range of substrate specificity (3 out of 5 studied carbohydrates). Other isolates capable of fermenting maltose and D-mannitol, containing no yellow pigment, and characterized by the slow-type luciferase kinetics were assigned to the genus *Vibrio* [Farmer III, Michael Janda, 2015; Farmer III et al., 2015]. Bacteria not capable of fermenting D-mannitol and sucrose, having no yellow pigment, and characterized by the fast-type luciferase kinetics were classified as *Photobacterium*. Isolated strains of this genus capable of growing at +30 °C and non-fermenting maltose were assigned to *Photobacterium leiognathi* [Moi et al., 2017; Thyssen, Ollevier, 2015]. Later, molecular genetic identification of the studied isolates confirmed their belonging to indicated genera.

The obtained data revealed the following phenotypic features of the strains. All the Black Sea *Vibrio* strains isolated from mussels (isolates 11–14) had the slow-type luciferase kinetics and the ability to ferment D-mannitol and sucrose. On the other hand, vibrions obtained from seawater in various

coastal zones of the Black Sea (isolates 1–10) were characterized by the slow-type luciferase kinetics and the ability to ferment D-mannitol, but only 2 out of 10 strains utilized sucrose: *V. owensii* and *V. harveyi*. None of *V. jasicida* isolates fermented sucrose; among the isolated *V. campbellii* and *V. aquamarinus* strains, there were ones capable and incapable of utilizing sucrose. Thus, all *Vibrio* isolates had the slow-type luciferase kinetics and an extended range of utilized sugars (4–5 out of 5 studied). This coincides with the *Bergey's Manual* data: as indicated there, only 83% of strains of this genus have the ability to utilize sucrose [Baumann, et al., 1984; Farmer III, Michael Janda, 2015; Farmer III et al., 2015].

Cultural, biochemical, and genetic identification of luminescent bacteria isolated from water and hydrobionts of the Sea of Azov showed that all of them belong to *P. leiognathi*. This species has bright bioluminescence, the fast-type luciferase kinetics, and a narrow range of utilized substrates (2 out of 5 studied sugars). It was practically not encountered during the study of samples of the Black Sea water and hydrobionts. Apparently, high temperature of seawater, its low salinity, and, consequently, high biological activity of the sea cause the predominance of this species in the Sea of Azov water and also lead to the colonization of hydrobionts inhabiting it.

Fig. 3 provides the results of occurrence of different types of cultivated luminescent bacteria isolated in the studied water areas of the Black Sea and Sea of Azov. The ratio of isolated strains was as follows: *V. harveyi*, 4.76%; *A. fischeri*, 9.52%; *V. jasicida*, 14.29%; *V. aquamarinus* sp. nov., 14.29%; *V. owensii*, 14.29%; *V. campbellii*, 19.04%; and *P. leiognathi*, 23.81%.

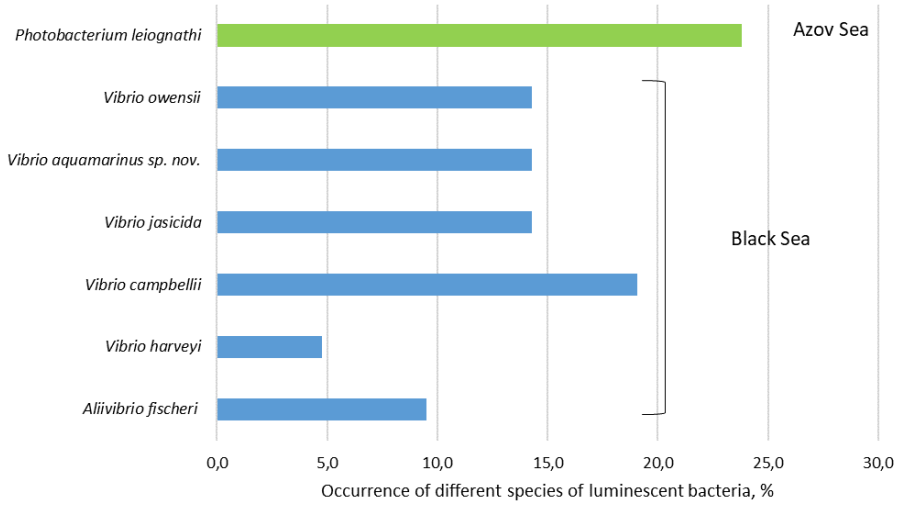

Fig. 3. Occurrence (%) of different species of cultivated luminescent bacteria isolated from water and hydrobionts of the Black Sea and Sea of Azov

Concerning the object of isolation, it should be noted as follow: 9 isolates were isolated from hydrobionts, and 12, from seawater. Strains isolated from seawater belong to the species *V. harveyi* and *V. jasicida*. *Aliivibrio fischeri* strains were isolated from hydrobionts alone, while *V. campbellii*, *V. owensii*, and *V. aquamarinus* sp. nov. strains were isolated from both water and hydrobionts. Interestingly, all the strains of the genus *Vibrio* isolated from hydrobionts had the ability to utilize sucrose, while *V. campbellii* and *V. aquamarinus* sp. nov. isolated from seawater did not ferment sucrose. Apparently, in these species, this ability is associated with symbiosis and depends on the ecological niche occupied by a certain strain.

Notably, the new isolates confirmed the occurrence of a new species of the genus *Vibrio*, *V. aquamarinus*, in the Black Sea. Isolates are deposited in the Russian National Collection of Industrial Microorganisms (*V. aquamarinus* VKPM B-11245) and German Collection of Microorganism and Cell Culture (*V. aquamarinus* DSM 26054).

Conclusion. Studies have shown that a significant difference in environmental conditions between the Black Sea and Sea of Azov in summer leads to prevalence of different taxa of luminescent bacteria. The genus *Photobacterium* represented by the species *P. leiognathi* dominates the Sea of Azov characterized by low salinity and high water temperatures. The genus *Vibrio* represented by the species *V. campbellii*, *V. jasicida*, *V. harveyi*, *V. owensii*, and *V. aquamarinus* sp. nov. can be considered the prevailing genus of luminous bacteria that inhabit the Black Sea water and live in its mussels. The obtained results showed variability of *V. campbellii* and *V. aquamarinus* sp. nov. strains by the ability to ferment sucrose depending on the isolate source (water or hydrobionts). Investigations on cultivated luminescent bacteria of the northern Black Sea have also revealed the occurrence of *Aliivibrio fischeri* associated with various hydrobionts (pelagic fish and shrimps).

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БИОЛЮМИНЕСЦЕНТНЫЕ БАКТЕРИИ ЧЁРНОГО И АЗОВСКОГО МОРЕЙ

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Целью настоящего исследования было выделить биолюминесцентные бактерии из прибрежных акваторий Чёрного и Азовского морей, изучить их морфологические и биохимические характеристики и идентифицировать их на основе последовательностей генов 16S рРНК, *recA* и *gyrB*. Из морских гидробионтов выделены 9 изолятов, из морской воды — 12. Результаты биохимических и молекулярно-генетических исследований показали, что выделенные светящиеся бактерии относятся к родам *Vibrio*, *Aliivibrio* и *Photobacterium*. Установлено, что все 5 люминесцентных штаммов, выделенных из воды и гидробионтов Азовского моря, принадлежат виду *Photobacterium leiognathi*. Бактерии, выделенные из Чёрного моря, отнесены к родам *Aliivibrio* и *Vibrio*. Род *Aliivibrio* представлен 2 штаммами *Aliivibrio fischeri*, ассоциированными с различными гидробионтами; 14 штаммов рода *Vibrio* отнесены к видам *Vibrio campbellii*, *V. jasicida*, *V. harveyi*, *V. owensii* и *V. aquamarinus* sp. nov. Таким образом, таксономический состав культивируемых люминесцентных бактерий в Азовском и Чёрном морях существенно различается.

Ключевые слова: люминесцентные бактерии, идентификация, таксономический состав, биоразнообразие, Чёрное море, Азовское море

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INDICES IN THE EVALUATION OF THE FUNCTIONAL ACTIVITY OF BLOOD CELLS OF THE BOTTLENOSE DOLPHIN *TURSIOPS TRUNCATUS* (MONTAGU, 1821)

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The content of cationic protein in granulocytes of the bottlenose dolphin *Tursiops truncatus* (Montagu, 1821) was established by calculating the average cytochemical coefficient. Its shortcomings were substantiated in visually determining the intensity of staining of the product of a cytochemical reaction on blood products and distributing cells into groups according to the amount of protein they contain. To assess the activity of a substance in a cell, computer programs were applied, and a light microscope was used which allows to minimize errors in morphometric measurements of objects. Individual parameters were calculated for the degree of filling and intensity of staining of cationic protein in granulocytes in bottlenose dolphins with and without taking into account the protein content in the entire blood volume. Such indicators allow carrying out comparative age-related, intraspecific, and interspecific studies in animals. As established, the content of cationic protein in granulocytes can vary greatly in different individuals of bottlenose dolphins, and its amount changes slightly with age.

Keywords: morphometry, average cytochemical coefficient, integral cytochemical index, cationic protein, granulocytes, bottlenose dolphin

In Russia, marine mammals are kept in oceanariums and dolphinariums mainly for commercial purposes. Only few of such organizations carry out research to assess health of pinnipeds and cetaceans and their immune status [Andreeva et al., 2013; Derko et al., 2018; Duvanova, Denisenko, 2018; Kaganova, 2018; Lauderdale et al., 2021; Romanov et al., 2023; Semenov et al., 2020; Vasileva, 2019; Zakharenko, 2019]. Clinical, biochemical, and cytomorphological blood tests are widely used to determine the functional health of marine mammals. To date, a large number of such studies have been carried out applying hematological and biochemical analyzers, flow cytometers, *etc.* [CRC Handbook, 2018; Keogh et al., 2011; Lauderdale et al., 2021; Nouri-Shirazi et al., 2017; Tryland et al., 2006]. Sampling of blood from animals in their natural habitat and its delivery are often limited by the distance from laboratories and acceptable storage periods for biomaterial. Blood smears from animals, both in captivity and in the wild, can be stored for a long time and allow describing and assessing the health of mammals later, with modern equipment used.

Precise mathematical measurements of cellular structures using computer morphometry allow carrying out comparative age, intraspecific, and interspecies studies of animals. Quantitative and qualitative techniques are effective in establishing the functional activity of mammalian blood cells. Quantitative analysis is aimed at identifying individual cell groups and determining their ratio (*e. g.*, white blood cell count), estimating the number of granules and nuclear elements in individual cells, and measuring areas of a substance stained by various cytochemical reactions. Qualitative analysis allows registering the intensity of staining of the cytochemical reaction product. Each of these techniques involves the use of dimensional (length, width, radius, area, and so on) and quantitative (number of granules and segments) characteristics of the objects investigated.

Qualitative analysis requires determining not only dimensional characteristics, but also color ones (*e. g.*, optical density) of the reaction product. Such an assessment of the functional activity of a cell involves the use of special equipment. At the same time, accurate dimensional and color characteristics do not depend on subjectivity of a researcher and their experience. When determining morphometric indices of cells on blood smears, absolute and relative parameters are used. Absolute ones are cell area, its diameter, and its shape, as well as number of granules and cellular elements in the cell. To establish these parameters, in each smear, cells are measured which are freely located in the visible field without overlapping and deformation from nearby cells: this allows to exclude their compression depending on the smear density. The use of relative parameters helps in recording characteristics of cellular structures regardless of the smear density.

Quantitative characteristics are simple and convenient for determining the functional activity of cells, but those provide a subjective and only general assessment of the intensity of cellular processes. A semiquantitative method is widely used: the calculation of the average cytochemical coefficient [Letsky, 1973] which allows estimating the average cytochemical activity of a living organism based on the distribution pattern of the stained substance in a cell. White blood cell count is effectively applied; based on it, leukocyte indices are determined [Davis et al., 2008; Garkavi et al., 1990; Kal'f-Kalif, 1941; Mustafina et al., 1999; Ostrovsky et al., 2006, 2007; Speransky et al., 2009]. The comprehensive use of hematological indices provides a large amount of information and helps in assessing development, severity, and course of an inflammatory process and endogenous intoxication and in analyzing the general immunological reactivity of an organism. Application of these coefficients and indices is informative both separately and together. At the same time, some quantitative indicators are not perfect.

The aim of this work is to study the possibility of using additional coefficients and indices to assess the functional activity of proteins and enzymes in the bottlenose dolphin blood cells on the example of cationic protein in granulocytes.

MATERIAL AND METHODS

The object of the study are dolphins *Tursiops truncatus* (Montagu, 1821) aged 1 to 16 years. Material from 14 individuals was obtained in the Sevastopol oceanarium. Blood was sampled from caudal veins of bottlenose dolphins. Blood smears were prepared in accordance with a generally accepted technique and fixed in methanol for 5 min before staining. The preparations were stained with fast green after M. Alfert and I. Geschwind [Butenko et al., 1974] and investigated using oil immersion under an Axio Imager M1 microscope equipped with an AxioCam digital video camera and AxioVision software for analyzing images of microobjects (manufactured by Zeiss). To register the content of cationic

protein (hereinafter CP), the average cytochemical coefficient was calculated [Letsky, 1973], the cell area was determined, and area and optical density of the cytochemical reaction product were recorded. Two cytochemical characteristics were established: cell filling index (hereinafter CFI) and integral cytochemical index (hereinafter ICI) [Slavinsky, 2000].

RESULTS AND DISCUSSION

To calculate the average cytochemical coefficient (hereinafter ACC), the degree of reaction intensity is determined visually by the amount of the stained substance in the cell cytoplasm (Fig. 1). Granulocytes are divided into groups: 0 (no staining or granules in the cytoplasm); A, low-active cells (the occurrence of single granules or staining); B, moderately active (the studied substance fills almost the entire cell in leukocytes, but unstained areas of the cytoplasm may remain); and C, highly active (intensively stained granules [substance] fill the entire cytoplasm). ACC is calculated by the formula ACC = (3C + 2B + A) / 200. In each smear, 200 granulocytes were taken into account.

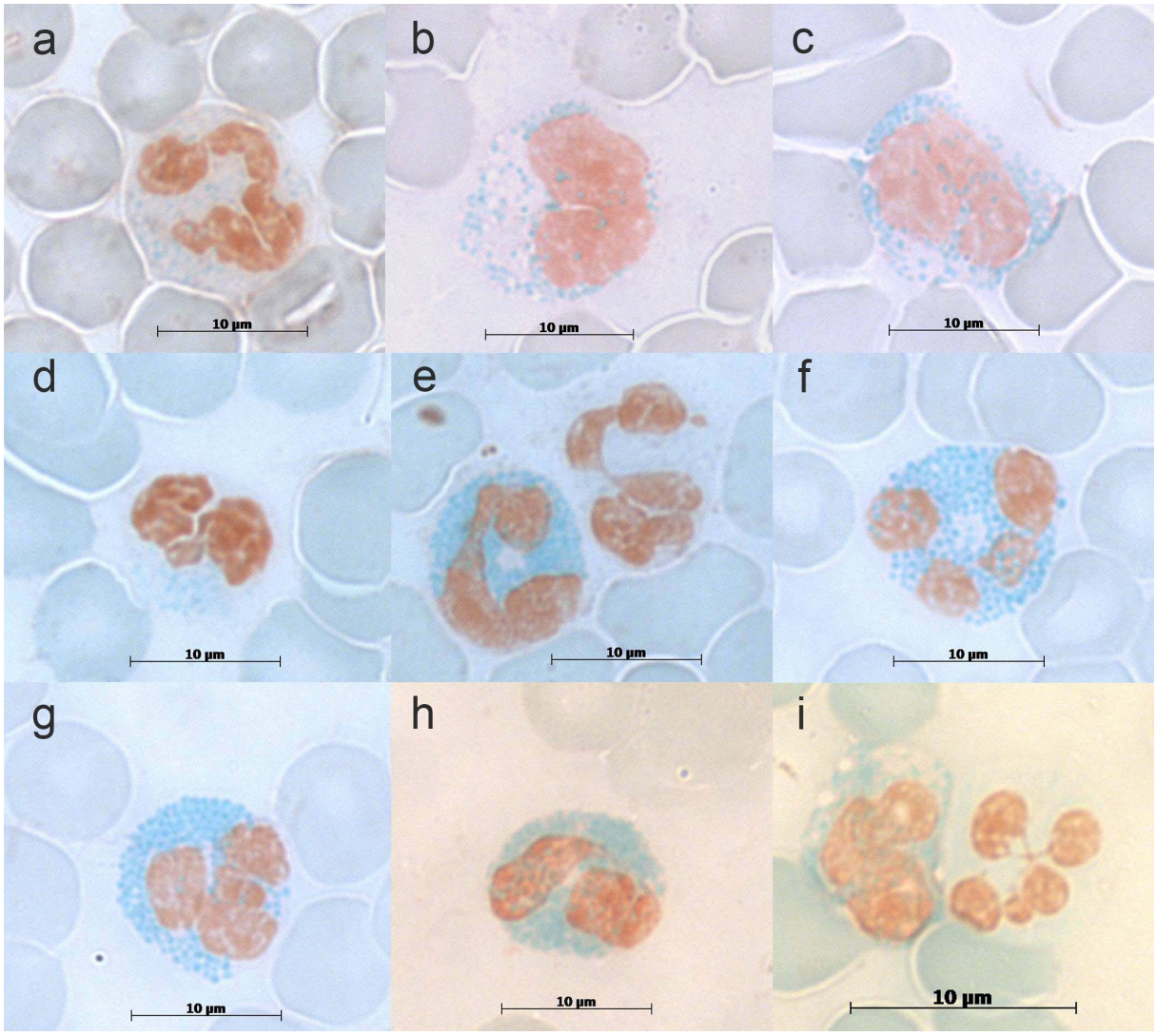

Fig. 1. Bottlenose dolphin granulocytes. Staining for cationic protein after M. Alfert and I. Geschwind [Butenko et al., 1974] (see text for explanation)

Visually, it is hard to distribute cells into the above-described groups, especially to assign them to categories A and B. Low-active cells with single granules (A) can include both a cell with one granule and a cell with a small area of the stained substance filling a quarter of a cell or less (Fig. 1a, b, d). Moderately active granulocytes (B) are also represented by a wide range of patterns: from a third of the stained substance in a cell (Fig. 1c, i) to almost complete filling with granules or the active substance (Fig. 1e, f, g, h). When distributing cells into groups, highly active granulocytes (C) can be the ones where the active substance occupies almost the entire cell, with only small areas being free of granules or the stained substance (Fig. 1g, h); however, according to the classification, this type of staining belongs to group B.

To reduce the share of stained cells mistakenly assigned to a particular group, we used additional parameters for assessing the activity of the substance. We determined cell area, optical density, and area of the cytochemical reaction product (Fig. 2). CFI and ICI were calculated [Slavinsky, 2000]. CFI is the share of the total area of the structures measured (stained CP granules) in the cell area (Fig. 2a, b). ICI is the product of the total area of the cytochemical reaction product in the cell and its optical density corresponding to the amount of stained CP (Fig. 2b).

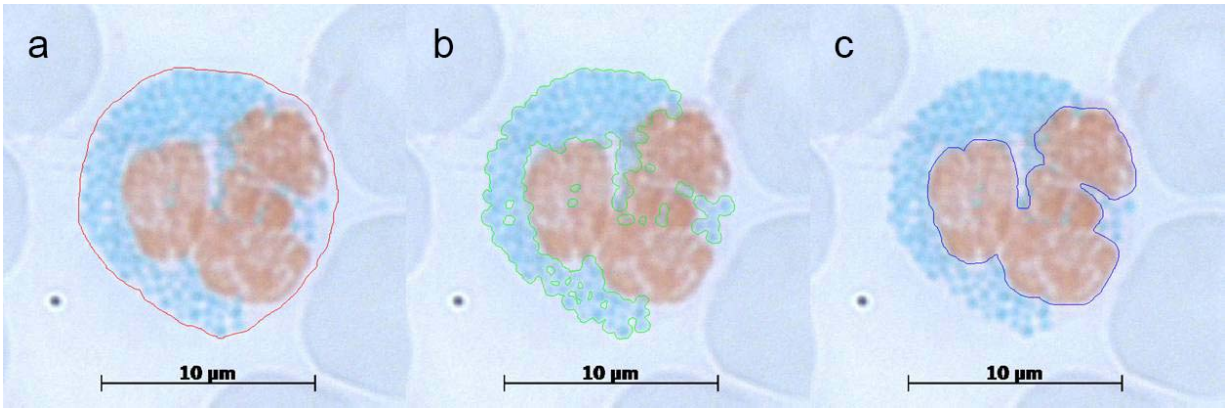

Fig. 2. Bottlenose dolphin granulocytes: a, the structures of the entire cell are highlighted; b, the area of the cytochemical reaction product is highlighted; c, the area of the nucleus is highlighted. Staining for cationic protein after M. Alfert and I. Geschwind [Butenko et al., 1974]

CFI is a convenient morphometric parameter: it is relative and allows measurements to be taken regardless of the smear density. At the same time, this index is not informative enough and requires an addition to the calculation formula. Apparently, it will be more accurate if the area of the nucleus (Fig. 2c) is subtracted from the cell area (Fig. 2a), and this difference is divided by the total area of the structures measured (stained CP granules) (Fig. 2b). In this case, it becomes possible to estimate the intensity of filling with the active substance directly in the volume of the cell cytoplasm, not in the entire cell volume. ICI shortcoming is that it requires the analysis of cells freely located in the visible field, so that the optical density of the stained substance does not change when cells overlap or are compressed.

The results of determining ACC, ICI, and CFI in granulocytes of the bottlenose dolphin are provided in Figs 3 and 4. The amount of CP-containing leukocytes (CP + leukocytes), intensity of staining, and degree of cell filling (CFI) with the cytochemical reaction product (ICI) change in these mammals with age. ACC values of bottlenose dolphins are higher than those of gray and harp seals, but lower than those of humans (Fig. 3).

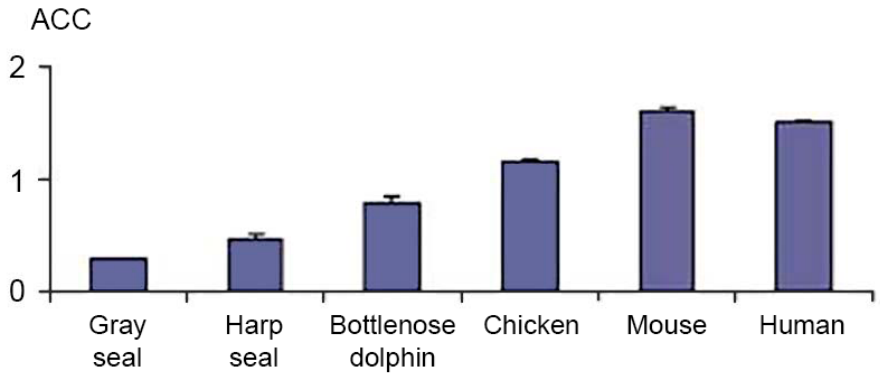

Fig. 3. The content of cationic protein in granulocytes of adult animals of different species (according to: [Budyka et al., 2009; Kletikova, 2010; Stoiko, Ermakov, 2004])

We introduced two more coefficients. Thus, mean values of CFI and ICI for samples of 200 granulocytes were expressed as the cell filling index coefficient (cCFI) and integral cytochemical index coefficient (cICI). These two parameters were used to compare groups and individual species of animals that differ significantly in protein activity in cells (ACC is applied for the same purpose). Specifically, in female bottlenose dolphins aged 4 to 5 years (circled in Fig. 4), against the backdrop of a high CFI value (*i. e.*, at the highest intensity of filling the cytoplasm of CP + leukocytes with stained granules) and a medium ICI value, the lowest ACC, cCFI, and cICI are observed which is due to a low percentage of CP + leukocytes in a mammal. Such individual fluctuations in the distribution of the active substance in leukocytes (by CFI) in bottlenose dolphins indicate an age-related decrease according to the trend line on the graph. Averaging the data obtained, both on the intensity of cell filling with granules of the active substance and on the degree of protein staining in cells, shows as follows: the amount of CP in granulocytes of an animal changes slightly with age. In seals, with medium ACC values, the content of active cells is high, but the intensity of filling with the active substance is low or medium.

The above-described indices and coefficients have their advantages and shortcomings. Application of ICI requires strict adherence to a staining technique in terms of the ratio of staining components and time of the procedure, as well as the use of non-overlapping cells. The core is that smear compaction leads to the stain thickening and, accordingly, to misinterpretation of the results obtained. CFI is convenient to use on any smears regardless of their density. Determination of all morphometric indicators is time-consuming, but computer technologies and automatic measurement programs allow to reduce time costs. Importantly, the use of ICI and CFI separately provides few data. It is way more reasonable to apply them combined with each other and with several other qualitative and quantitative parameters to assess the functional activity of the substance in studied cells. Consequently, comparative studies of the functional state of an animal organism based on various color-and-brightness characteristics of cells require calculations of additional indices and coefficients, as shown on the example of determining the content of CP in the bottlenose dolphin granulocytes. These indicators can be effectively used not only in assessing the content of CP in blood cells, but also in establishing the activity of other enzymes (alkaline phosphatase, succinate dehydrogenase, myeloperoxidase, *etc.*).

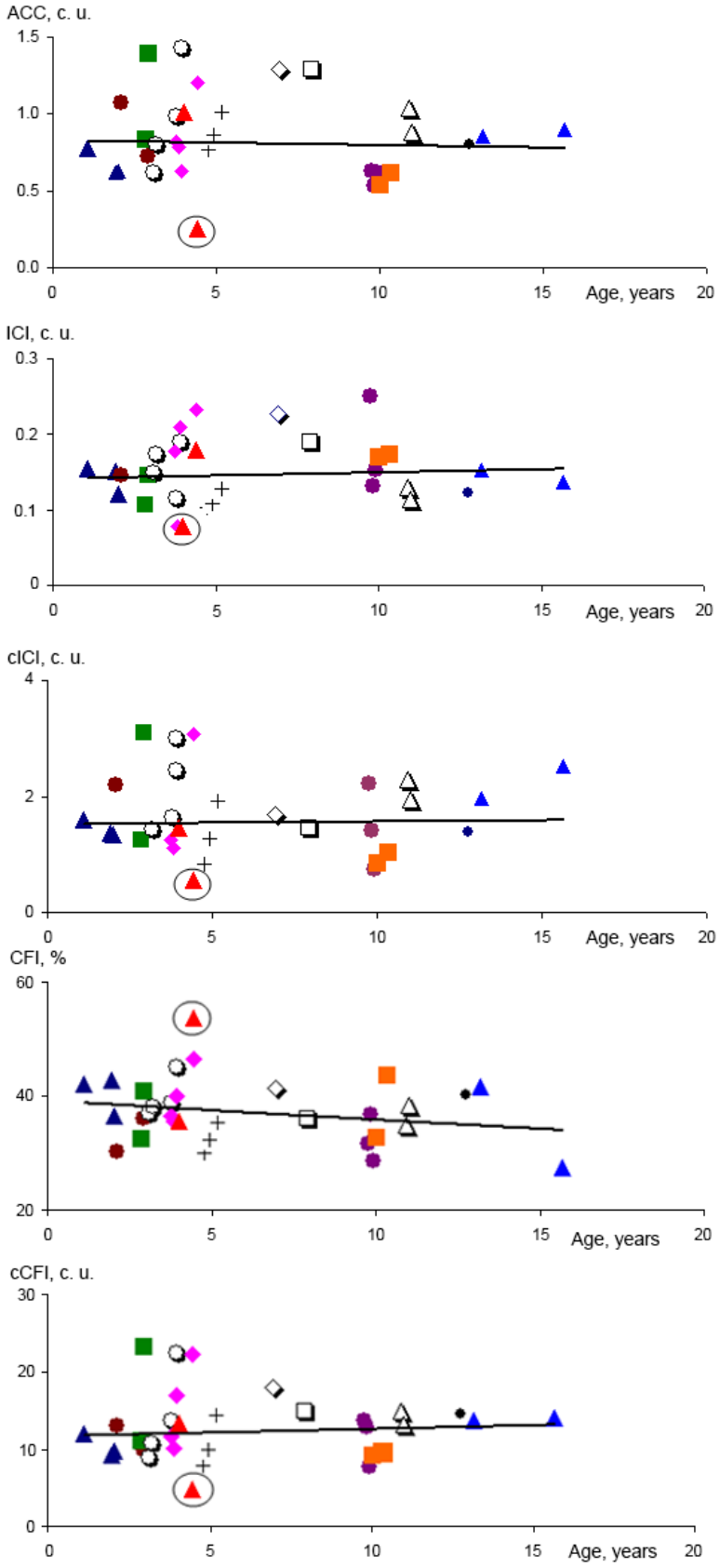

Fig. 4. Age-related changes in cytochemical parameters of the content of cationic protein in granulocytes of bottlenose dolphins (each individual animal is marked with its own symbol)

Morphometric measurement of a cell as a separate structure allows determining individual parameters for each organism. Despite the labor intensity of additional measurements and calculations when applying computer morphometry, indices provide accurate quantitative data on the content of substances in cells. The results of analysis of morphometric cellular parameters of marine mammals can be an important source of additional information for assessing the immunological status of animals. This is especially relevant for pinnipeds and cetaceans during their adaptation and long-term maintenance in oceanariums and dolphinariums. Biomaterial obtained from marine mammals in their natural habitat is often very disparate in age, sex, time, weight, and species characteristics of animals or even spoiled or insufficient in volume (only blood smears are preserved). Precision and maximum information content of microscopy of blood of animals allow carrying out comparative age, intraspecific, and interspecies studies of marine mammals.

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ИНДЕКСЫ В ОЦЕНКЕ ФУНКЦИОНАЛЬНОЙ АКТИВНОСТИ КЛЕТОК КРОВИ ДЕЛЬФИНА-АФАЛИНЫ *TURSIOPS TRUNCATUS* (MONTAGU, 1821)

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Содержание катионного белка в гранулоцитах дельфина-афалины *Tursiops truncatus* (Montagu, 1821) определяли методом расчёта среднего цитохимического коэффициента. Обоснованы его недостатки при визуальном установлении интенсивности окрашивания продукта цитохимической реакции на препаратах крови и при распределении клеток на группы по количеству в них белка. Применены современные методы оценки активности вещества в клетке с использованием компьютерных программ и светового микроскопа, что позволяет минимизировать погрешности морфометрических измерений объектов. Рассчитаны индивидуальные параметры по степени заполнения и интенсивности окрашивания катионного

белка в гранулоцитах у афалин с учётом и без учёта содержания белка во всём объёме крови. Такие показатели позволяют проводить сравнительные возрастные, внутри- и межвидовые исследования животных. Установлено, что содержание катионного белка в гранулоцитах может сильно различаться у разных особей афалин, а с возрастом его количество меняется незначительно.

Ключевые слова: морфометрия, средний цитохимический коэффициент, интегральный цитохимический показатель, катионный белок, гранулоциты, афалина

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CURRENT STATE OF THE POPULATION AND FEATURES OF THE DISTRIBUTION OF THE SOFT-SHELL CLAMS *MYA ARENARIA* LINNAEUS, 1758 IN THE KOLA BAY OF THE BARENTS SEA

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The soft-shell clam *Mya arenaria* Linnaeus, 1758 is a boreal bivalve. The range of this species covers coastal waters of the Atlantic Ocean, the northeastern Pacific Ocean, and seas of the Arctic Ocean (the Barents and White seas). *M. arenaria* settlements can occupy vast areas along the coasts, where the molluscs form large aggregations and prevail in biomass among representatives of littoral macrozoobenthos. This species can withstand fluctuations in environmental factors and affect detritus formation and sedimentation. The mollusc juveniles inhabiting upper layers of the sediment are an important food object for seabirds and commercial fish species. High tolerance allows considering *M. arenaria* as an indicator of the effect of climate change on the Arctic ecosystem. Obtaining new data on peculiarities of the species biology is necessary to identify general patterns of development of benthic organisms under varying conditions of the marine environment, to understand adaptive characteristics of certain long-lived high-tolerant molluscs, and to assess the effect of environmental factors on them. The investigation of *M. arenaria* biology may be of practical significance as well: this species may become one of promising objects of mariculture in the Arctic region. The paper provides the results of a study of the current state of the soft-shell clam population and features of its distribution in the Kola Bay of the Barents Sea. Material was sampled during MMBI RAS coastal expedition in 2021. Quantitative characteristics and size and age structure of the mollusc settlements were analyzed. *M. arenaria* aggregations were recorded in the intertidal zone of the western and eastern shores of the middle and southern bay areas. The mollusc settlements in the intertidal zone off the Elovyi Cape (the Tuloma River mouth) were found for the first time during the entire period of research in the Kola Bay (1921–2021). The highest abundance was registered in the Khlebnaya Bay (67.1 ind.·m^{-2}), and the lowest one was noted in the Belokamennaya Bay (5.0 ind.·m^{-2}). There were no abundant aggregations in the intertidal zone off the cape Abram-mys and in the Vayenga Bay. Settlements in the Kola Bay are represented by the soft-shell clams aged 4 to 14 years, with the size varying 17.5 to 91.2 mm. Apparently, *M. arenaria* distribution and quantitative and morphometric characters of its settlements are related to hydrological features of the bay (the intensity of movement of water masses in small bights and cyclonic movement of water masses in the southern bay area). An increase in the mollusc abundance and an expansion of its range may be interpreted as a response to climate change in the Arctic region and an indicator of reduction of anthropogenic load on coastal communities throughout the Kola Bay.

Keywords: *Mya arenaria*, distribution, state of the population, density, biomass, size and age structure, intertidal zone, Kola Bay

Mya arenaria Linnaeus, 1758 is a bivalve of boreal origin that digs into surrounding sediment to a depth of 40 cm [Pfitzenmeyer, Drobeck, 1963, 1967; Sveshnikov, 1963]. Its range covers coastal temperate waters of the northern Atlantic Ocean and northeastern Pacific Ocean, as well as the Barents and White seas of the Arctic Ocean. The species is reported for the Atlantic and Pacific coasts of North America and for the Baltic, Black, and Mediterranean seas of the Eastern Atlantic [Carlton, 1992; Fedyakov, 1986; Golikov et al., 1985; Strasser, 1999; Wheaton et al., 2008; Zhang et al., 2018]. *M. arenaria* settlements can occupy vast areas along the coasts, where the molluscs form large aggregations and prevail in biomass among representatives of littoral macrozoobenthos. The soft-shell clams are able to withstand fluctuations in environmental factors in wide ranges [Fedyakov, 1986]. This species is resistant to salinity within 1–30‰ [Berger, 1986; Khlebovich, Stankyavichyus, 1979], and its favorable temperature is within +4…+16 °C [GISD, 2023]. *M. arenaria* are pretty resistant to hydrogen sulfide content in water and oxygen deficiency [Sveshnikov, 1963; Thumdrup, 1935]. High tolerance allows considering the soft-shell clams as indicators of the effect of long-term climate change on the Arctic ecosystem.

M. arenaria is a key component of coastal communities. Its aggregations affect the processes of detritus formation and sedimentation, and this determines the role of the mollusc as an ecosystem engineer. The soft-shell clam juveniles inhabiting upper layers of silty sediment are an important food object for seabirds and commercial fish species [Marshall, Elliott, 1997; Piersma et al., 1998; Sutherland et al., 2000]. *M. arenaria* filter suspended organic matter of the water column thus improving water quality [Forster, Zettler, 2004]. Like other filter-feeding bivalves, these molluscs can serve as indicators of the aquatic environment conditions. By filtering water, the soft-shell clams are capable of accumulating various toxins, oxidizing organic matter, and even regulating the trophic state of water bodies to some extent [Loo, Rosenberg, 1996].

Obtaining new data on *M. arenaria* biology is necessary to identify general patterns of development of benthic organisms under dynamic conditions of the marine environment, to understand adaptive characteristics of certain long-lived high-tolerant molluscs, and to assess the effect of environmental factors on them. Studying the biology of soft-shell clams may have practical significance. In North America, it is an important commercial species [Beal, 2002; Brousseau, 1979; Connell et al., 2007; Newcombe, 1935, 1936]. Interestingly, in Europe, there is practically no commercial exploitation. Nevertheless, there are publications investigating *M. arenaria* growth characteristics. Thus, a Danish researcher considered the possibility of using it as a commercial object [Munch-Petersen, 1973]. The mollusc may become one of promising objects of mariculture in the Arctic region.

In Russia, studies on this species are concentrated mainly in the White and Baltic seas [Cardoso et al., 2009; Gerasimova et al., 2016; Maksimovich, 1978]. Also, there are investigations of *M. arenaria* from the Sea of Azov–Black Sea Basin [Savchuk, 1970; Savikin, 2020; Zolotnitskiy, Sytnik, 2020] – the area for which this mollusc is an invader. In the Far Eastern seas, comprehensive taxonomic analysis of *Mya japonica* Jay, 1857 is carried out – a species closely related to *M. arenaria* [Zhang et al., 2018]. The research is focused on clarifying the taxonomic status and geographic range of the soft-shell clams. It shows that *M. arenaria* range covers the northeastern Pacific Ocean, while the closely related *M. japonica* is distributed in its northern part.

Information on the biology and distribution patterns of the soft-shell clams from the Kola Bay is patchy. For the first time, the molluscs of the Kola Bay shallows were described by K. Deryugin [1915]. In 1921–1925, the staff of the Murmansk Biological Station studied several coastal areas;

according to the results obtained, *M. arenaria* was recorded from the Olenya Bay in the northern bay area to the Lavna River mouth in the southern one [Gur'yanova et al., 1929]. In the following 40 years, there were no regular investigations of the bay bottom communities. The monitoring was resumed only in the 1970s. In 1984, the staff of the Polar Research Institute of Marine Fisheries and Oceanography studied the central bay. In 1989, the staff of MMBI repeated this survey. These investigations were carried out from a vessel with a Van Veen grab and covered bottom communities of the deep-water bay area. In 1991–1993, there was a benthic survey of the littoral zone of the Tuloma River mouth, and the molluscs were not noted [Gudimov, Frolov, 1997]. In 2005–2007, during coastal expeditions of MMBI, *M. arenaria* was registered in the upper sublittoral of the Kola Bay from the village of Retinskoye to cape Abram-mys at depths of 4 to 12 m [Frolov, 2009]; in the littoral zone, the soft-shell clams were not found [Lyubina et al., 2009].

The Kola Bay is the largest bay in the Russian part of the Barents Sea. It is a typical fjord of tectonic, erosional, and glacial origin [Berega, 1991]. The depth of the bay gradually decreases from the entrance in the northern area to the head in the southern one. There are many bights jutting into the bay coast, and the Tuloma and Kola rivers flow into the bay head. The length along the alignment lines is 58.7 km; the straight line distance from the entrance to the head is 51 km. The total area of the bay is about 180 km^2. Its hydrological regime is affected by the warm Murmansk Coastal Current. In the apex, meteorological conditions of the adjacent land and freshwater runoff have the most significant effect.

Since the second half of the XX century, the mean global air temperature rises [Zhilina, 2021]. The Arctic is one of the most vulnerable regions of the Earth in terms of climate change: there, warming occurs faster than on the planet as a whole [Ponomarev et al., 2005; Zhilina, 2021]. To date, the Arctic seas are characterized by a tense environmental situation, with the main problems being pollution, effect of consequences of ongoing global climate change, decrease in biodiversity, and reduction of marine biological resources [Nersesov, Rimsky-Korsakov, 2021; Stishov et al., 2013; Yakimenko, Ivanenko, 2021]. Over the past 30 years, temperature has risen in all areas of the northern polar region. In general for 30 years, a linear increase in the mean annual temperature is about 2.43 °C (http://www.aari.ru). For the Arctic seas in 1936–2019, the trend sign is positive everywhere. During the last 30-year period, the Barents Sea water has warmed by about 2.2 °C. Since the 1990s, the consequences of climate change have been repeatedly recorded in the water area and coastal zone of the Kola Bay [Antsiferova, Davydov, 2009; Davydov, 2001; Dzhenyuk et al., 1997]. Climate change affects the state of ecosystems, and boreal species are especially sensitive to warming; those respond to rising temperature by expanding their range and increasing abundance [Matishov et al., 2014]. Investigations on the biology and life conditions of hydrobionts are extremely important for monitoring changes in the coastal zone.

The aim of this work is to study the distribution of the bivalve *Mya arenaria* in the Kola Bay of the Barents Sea, to assess the current state of its population, to describe and analyze morphometric indicators, and to identify factors of the key effect on the mollusc distribution in the bay.

MATERIAL AND METHODS

Data on *M. arenaria* distribution in the Kola Bay were obtained during a comprehensive coastal expedition of MMBI RAS in 2021. The survey was carried out in the littoral zone of the middle and southern bay areas, from the Retinskaya Bay to Cape Usov in the Tuloma River mouth on the western shore of the bay and from the Vayenga Bay to Fadeev Stream on the eastern shore. In the southern bay area (from the Cape Usov to cape Abram-mys), sampling was carried out in June – after the complete

melting of the ice cover and fast ice that form there in winter. In spots of the littoral zone of the middle bay area (the Belokamennaya, Retinskaya, Khlebnaya, and Vayenga bays) which are accessible all year round, sampling was carried out in March. A total of 234 specimens were sampled during the research period.

The quantitative recording of the soft-shell clams was carried out in the littoral zone at low tide with a 0.1-m^2 frame. The sediment was removed to a 30–40-cm depth and washed through a sieve with a 0.5-mm mesh. In each area, 10 samples were taken. Water salinity and temperature were measured simultaneously with the sampling using a portable refractometer and thermometer with an accuracy of 1‰ and 1 °C, respectively. During the morphometric analysis, the shell length (L, mm), height (H, mm), and convexity (D, mm) were determined for each mollusc with a caliper with an accuracy of 0.01 mm (Fig. 1). The total weight of the soft-shell clam (W, g), the weight of the shell (W_r, g), and the weight of soft tissues (W_m, g) were measured. The weight of mantle water (W_w, g) was calculated as the difference between the total weight of *M. arenaria* and the weight of the shell and soft tissues. For this purpose, before weighing, the molluscs were kept in containers with seawater. Weighing was carried out on electronic scales with an accuracy of 0.01 g. The weight of soft tissues was determined after their drying on filter paper. The proportions of the shell weight and soft tissue weight in the total weight of the soft-shell clams were estimated (W_r/W and W_m/W, respectively). When describing size and weight characteristics, for each study area, mean value (*M*, mm) and standard deviation (±*SD*) were calculated. *M. arenaria* age was determined by counting the annual rings on the shell; those are formed during the winter growth cessation and are thickened growth lines [Haskin, 1954; Metody, 1990].

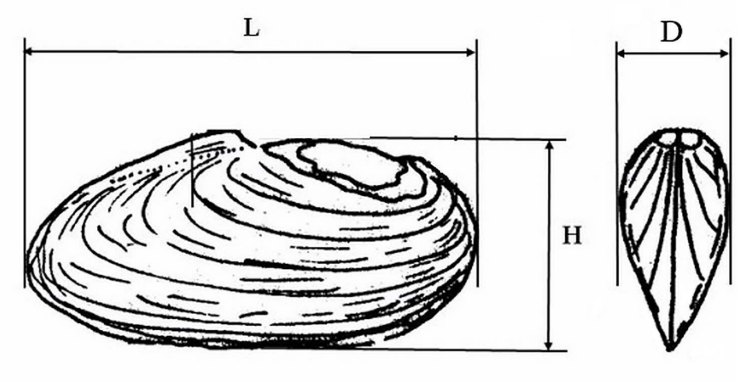

Fig. 1. Scheme of measurements of a bivalve shell [Naumov, 2006]: L, length; H, height; D, convexity

The distribution pattern of quantitative and dimensional characters of the soft-shell clams was assessed by the Kolmogorov–Smirnov test. The significance of differences was determined by the Wilcoxon–Mann–Whitney test. Differences were considered insignificant at $p \geq 0.05$. Mathematical calculations and data classification were performed using the STATISTICA 10.0 software package and MS Office Excel 2010. The relationships within the entire set of morphometric characters of the molluscs were estimated by cluster analysis. Multivariate analysis was carried out using the values of morphometric characters (body size and weight). As the main procedures, two methods were selected – hierarchical analysis and *k*-means clustering. Distances between groups of characters were assessed by the Ward's method with Euclidean distances indicated. The significance of differences between means in groups was tested using variance analysis at a significance level of $p \geq 0.05$.

RESULTS

In the Kola Bay, *M. arenaria* settlements were registered in the littoral zone in the Retinskaya, Belokamennaya, and Khlebnaya bays and in the littoral zone off the Elovyi Cape (Fig. 2). In the areas indicated, the bivalve forms aggregations chiefly in the middle horizon of the littoral zone. The occurrence in samples was of 70–100%.

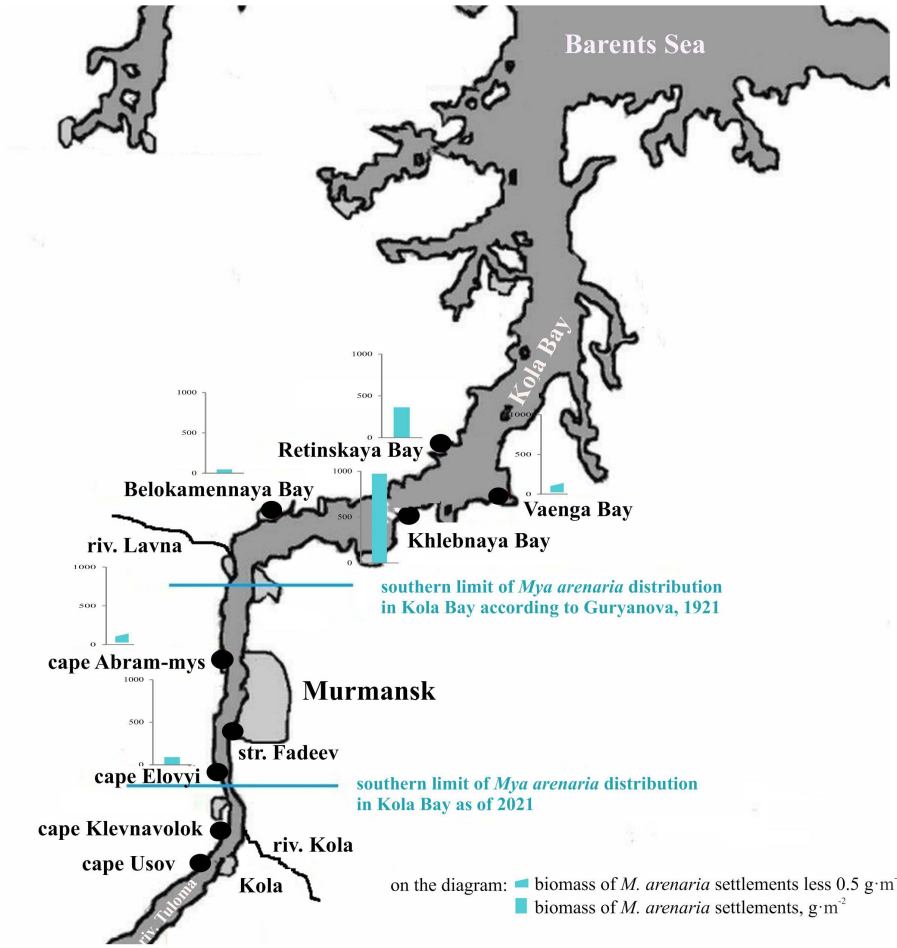

Fig. 2. Distribution and quantitative characteristics of the bivalve *Mya arenaria* settlements in the Kola Bay of the Barents Sea

M. arenaria was also recorded in the littoral zone off the cape Abram-mys and in the Vayenga Bay, but the molluscs do not form dense aggregations in these biotopes. The occurrence of the soft-shell clams there was 10–30%. The bivalves were not noted southward of the Elovyi Cape (the Klevnavolok and Usov capes). The northernmost spot covered by the research was the Retinskaya Bay. In the studied areas, *M. arenaria* mean size varied from (32.8 ± 5.11) mm in the littoral zone of the Vayenga Bay to (55.6 ± 13.4) mm in the Retinskaya Bay, with the mean body weight of (7.0 ± 1.5) g and (18.2 ± 9.6) g, respectively. The molluscs sampled in the Khlebnaya and Retinskaya bays and off the Elovyi Cape were characterized by the highest values of these characters, while the hydrobionts from the Vayenga Bay and the littoral zone off the cape Abram-mys were characterized by the lowest ones. The maximum sizes were 91.2 × 51.5 × 36.8 mm, and the body weight was 36.8 g (this mollusc was found in the littoral zone of the Khlebnaya Bay).

Individual settlements differ significantly in the size and age structure. The largest aggregation is confined to the eastern shore of the middle bay area, to the biotope of silted fine sand of the Khlebnaya Bay. This settlement occupies the southeastern bay area near the stream and is represented by the molluscs aged 4 to 14 years, with a shell length of 30.0 to 91.2 mm. The soft-shell clams inhabit mostly the middle and lower horizons of the littoral zone. The density of the settlement is 67.1 ind.·m^{-2}, and the biomass is 974 g·m^{-2}. In the settlement, the molluscs aged 7–8 years prevail (40%), with a shell length of 50.0–59.9 mm (Fig. 3). The littoral zone of the Khlebnaya Bay is flat and is represented by different sediment types. Water salinity in the bay near the waterline is 16–31‰, and water temperature varies from +1 °C in March to +16 °C in August.

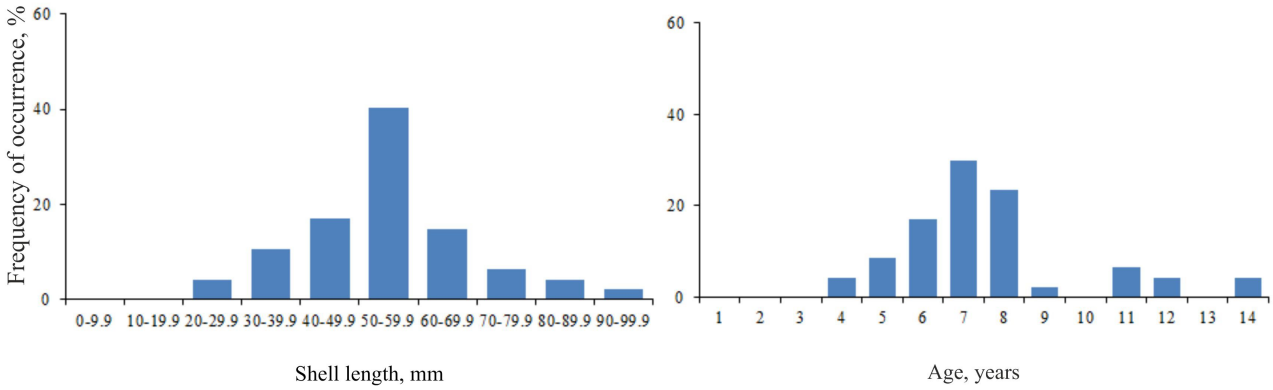

Fig. 3. Frequency distribution of the size and age structure of the molluscs *Mya arenaria* in the Khlebnaya Bay

The southern boundary of *M. arenaria* distribution in the Kola Bay is a small littoral zone of the southern area off the Elovyi Cape (the Tuloma River mouth). The density of the mollusc settlement there is 8.2 ind.·m^{-2}, and the biomass is 92.3 g·m^{-2} (Fig. 2). The sizes vary 23.4 to 68.2 mm. The age structure covers individuals 4 to 11 years. The soft-shell clams aged 6–7 years prevail (56%); their shell length ranges 40.0 to 70.0 mm (Fig. 4). The proportion of hydrobionts of older age groups (9–10 years) in the settlement is 17.4%. The bivalves of 4 and 11 years are encountered singly. Based on the sampling data, it can be concluded as follows: 2015 was the most favorable year for replenishment of this settlement with juveniles. The biotope of the littoral zone of the Tuloma River mouth is distinguished by the occurrence of numerous littoral baths. Those are separated from each other by ridges of stones and sand. The largest littoral baths have drains. The littoral zone off the Elovyi Cape is 150 m long and is represented by silty-sandy sediment with boulder fractions with fucoids. The biotope is characterized by a high degree of desalination. The salinity at the water's edge at low tide can be only 7‰; water temperature in the littoral puddles in June is +7 °C.

In the Retinskaya Bay, *M. arenaria* settlement is located at the estuary of the inflowing stream. It is represented by the soft-shell clams aged 5 to 10 years, with a shell length of 30.5 to 80.0 mm. The settlement density is 35.0 ind.·m^{-2}, and the biomass is 364.0 g·m^{-2}. The molluscs aged 6, 7, and 8 years prevail (> 60%), with a shell length of 50 mm or more. Water salinity in the bay throughout the year is 10–25‰, with the value dropping to 3‰ in May at the estuary of the stream. The width of the littoral zone is 30–50 m. The sediment is chiefly silty sand.

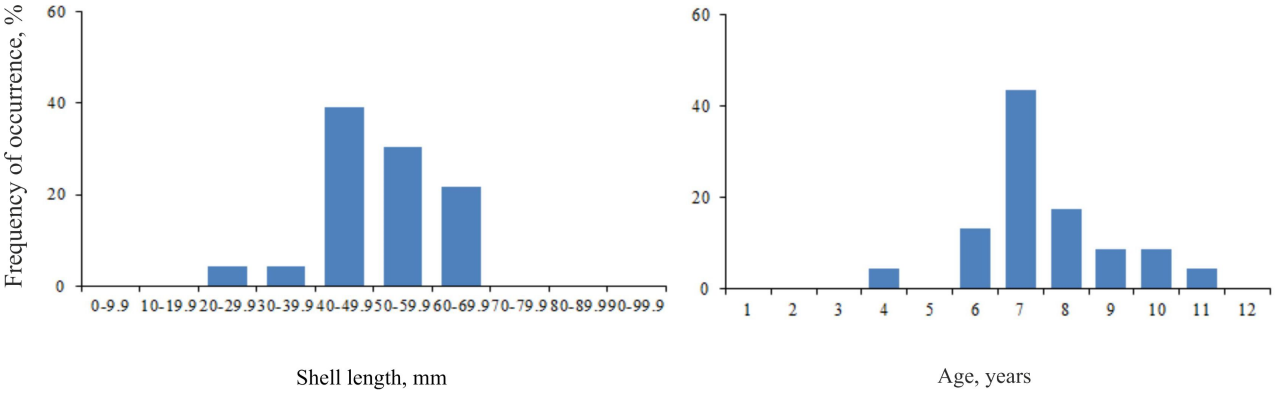

Fig. 4. Frequency distribution of the size and age structure of the molluscs *Mya arenaria* off the Elovyi Cape

In the Belokamennaya Bay, the aggregation is situated near the stream flowing out into the littoral zone. *M. arenaria* settlement in the littoral zone of this bay is in a depressed state. There, the lowest values of density and biomass are recorded: 5 ind.·m^{-2} and 48 g·m^{-2}, respectively (Fig. 2). The size distribution is characterized by the occurrence of two groups of the soft-shell clams – 3 to 5 years, with a shell length of 10–30 mm (39%), and 6 to 8 years, with a shell length of more than 50 mm (47.7%). The mean size of the hydrobionts is (43.4 ± 15.2) mm, and the body weight is (10.0 ± 8.9) g. The Belokamennaya Bay is not sharply separated from the Kola Bay; it is a small bight on its western shore located to the north of the Belokamenny Cape. The littoral zone is wide (the length from the shore to the water's edge at low tide is 130 m), with numerous boulder fractions and a belt of brown algae in the lower horizon. Water salinity at low tide near the water's edge varies 14 to 22‰.

In the Vayenga Bay, *M. arenaria* are found singly. The density does not exceed (0.3 ± 0.04) ind.·m^{-2}, and the biomass is 0.13 g·m^{-2} (Fig. 2). This bight is situated on the eastern shore of the Kola Bay and forms a wide open bay. The Vayenga River, a large one, flows into the bay apex desalinating the studied area to 16‰. The sediment in the Vayenga Bay is represented mainly by rocky placers only occasionally replaced by small spots of silt. Their fauna is extremely poor [Gur'yanova et al., 1929]. In the littoral zone off the cape Abram-mys, the soft-shell clams are encountered singly. The distribution density does not exceed (0.1 ± 0.03) ind.·m^{-2}, and the biomass is 0.47 g·m^{-2}. The biotope is characterized by intensive water movement and significant fluctuations in salinity due to the runoff of large rivers – Kola and Tuloma. At high tide, salinity is 34‰, while at low tide, the value is 10‰ [Malavenda, Malavenda, 2012]. The width of the intertidal zone is about 100 m, and the slope of the littoral zone does not exceed 5° [Kol'skii zaliv, 2009]. The size and weight indicators of *M. arenaria* found in the littoral zone of the Vayenga Bay and cape Abram-mys are provided in Table 1.

Table 1. Size and weight indicators of the molluscs *Mya arenaria* inhabiting the intertidal zone of the Vayenga Bay and cape Abram-mys

Studied area	L, mm	H, mm	D, mm	W, g	W_r, g	W_m, g	W_w, g
Vayenga Bay (N = 6)	32.8 ± 5.1	24.9 ± 4.5	15.0 ± 4.2	7.0 ± 1.5	2.7 ± 1.0	3.9 ± 0.9	1.5 ± 0.03
Cape Abram-mys (N = 3)	34.3 ± 10.5	16.3 ± 1.8	8.4 ± 1.3	12.1 ± 0.2	4.6 ± 0.1	3.8 ± 0.2	1.2 ± 0.03

Note: N, the number of molluscs. The abbreviations used are explained in "Material and Methods" section.

In the studied areas, the relative shell weight varies 38 to 61% of the total body weight of the hydrobionts (Fig. 5). The highest values were revealed for the Khlebnaya Bay, and the lowest ones, for the Belokamennaya Bay. The proportion of *M. arenaria* shell weight (W_r/W) in the Retinskaya Bay and off the cape Abram-mys was 55%; in the littoral zone of the Elovyi Cape, 48%; and in the Vayenga Bay, 39%. The relative weight of soft tissues is the highest for the molluscs of the Vayenga Bay (56%) and the lowest for the soft-shell clams of the Belokamennaya Bay (33%). The mean amount of mantle water was 6.5%, with fluctuations within 2% (the Khlebnaya Bay) to 29% (the Belokamennaya Bay). The ratio of the shell weight and soft tissue weight in *M. arenaria* from the littoral zone off the cape Abram-mys and Elovyi Cape was almost 1 : 1, and the proportion of mantle water did not exceed 0.5%.

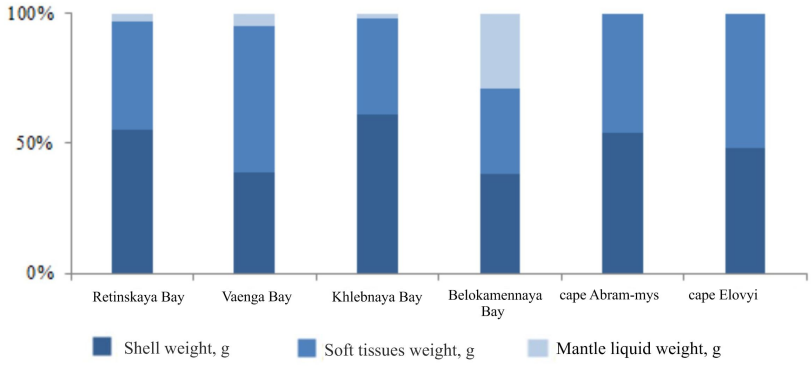

Fig. 5. The ratio between weight parameters of the molluscs *Mya arenaria* in the studied areas of the Kola Bay

No changes were revealed in the body size and weight depending on the latitudinal location of the studied areas. Therefore, to determine relationships within the entire set of morphological characters for *M. arenaria* sampled in the littoral zone of the Kola Bay, cluster analysis was applied (Fig. 6).

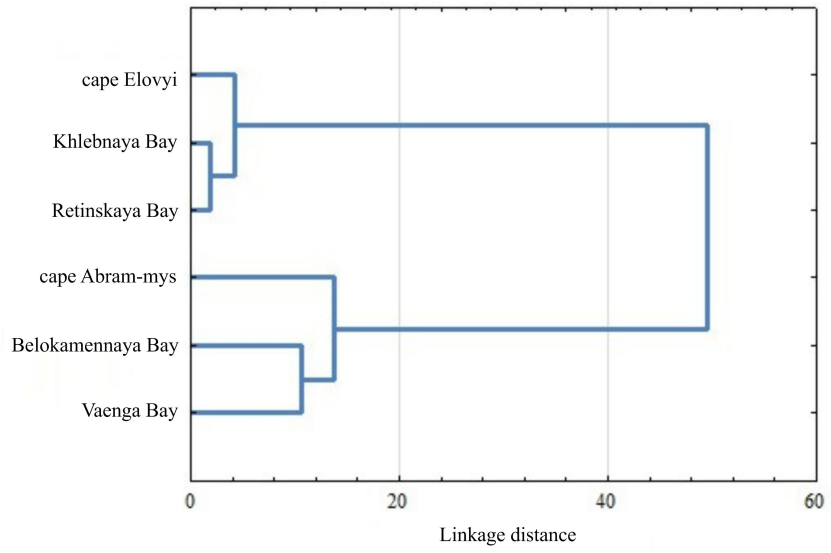

Fig. 6. Dendrogram of similarity of morphometric characters for settlements of the molluscs *Mya arenaria* in the intertidal zone in the studied areas of the Kola Bay

The analyzed set was divided into two clusters. Cluster 1 consisted of the molluscs inhabiting the biotopes of the Retinskaya and Khlebnaya bays and the Elovyi Cape. These settlements had the highest density and biomass, and the molluscs were characterized by the maximum mean values of the shell length, height, and convexity. Cluster 2 consisted of the soft-shell clams from the Vayenga and Belokamennaya bays and the cape Abram-mys vicinity. In these biotopes, the settlements had the lowest density and biomass, and the hydrobionts were characterized by lower values of morphometric characters compared to those of cluster 1 (Table 2).

Table 2. Classification of morphometric characters of the molluscs *Mya arenaria* (*k*-means clustering) and assessment of the significance of differences (*F*-test)

Character	Cluster 1, $M \pm SD$	Cluster 2, $M \pm SD$	Analysis of variance	
			F	significance level (*p*)
Shell length (L, mm)	54.0 ± 1.83	36.8 ± 5.74	24.37953	0.007830
Shell height (H, mm)	32.6 ± 0.87	22.3 ± 5.18	11.61329	0.027081
Shell convexity (D, mm)	20.3 ± 1.03	13.1 ± 4.13	8.43078	0.043960

Note: *M*, the mean value; *SD*, the standard deviation.

The data obtained were supplemented by the results of *k*-means clustering. The dispersion analysis showed noticeable differences between two clusters ($p < 0.05$). In cluster 1, the highest similarity of size characteristics was recorded for the molluscs of the Khlebnaya and Retinskaya bays (Euclidean distance 0.82), and in cluster 2, it was registered for the soft-shell clams of the Vayenga and Belokamennaya bays (Euclidean distance 1.58).

DISCUSSION

M. arenaria distribution and quantitative and morphometric characters of settlements in different areas of the Kola Bay are very diverse. The highest settlement density was determined for the Khlebnaya Bay. Relatively high abundance values were obtained for the Retinskaya Bay. The lowest density was noted in the littoral zone of the Belokamennaya Bay. In shallow areas off the cape Abram-mys and in the Vayenga Bay, the molluscs do not form dense aggregations. The abundance values of the hydrobionts are comparable with quantitative characteristics of the individuals from other areas. Similar abundance values were determined for the White Sea [Smolkova, 2021] and for the Kerch Strait of the Black Sea [Ivanov, Sinegub, 2007]. The quantitative characters of the soft-shell clam settlement of the Khlebnaya Bay which belongs to moderately polluted areas of the Kola Bay [Informatsionnyi byulleten', 2012] are close to abundance values of *M. arenaria* in the southeastern Onega Bay of the White Sea [Smolkova, 2021] and exceed the indicators of settlements in shallow and cleaner areas of the Eastern Murmansk [Smolkova, Meshcheryakov, 2023]. The size and age composition of the studied settlements of the Kola Bay covers individuals of 30.0 to 91.2 mm and 4 to 14 years, with a prevalence of middle-aged hydrobionts, 6 to 8 years (frequency of occurrence is 47 to 69%). In the samples, there were no molluscs aged 1 and 2 years and no yearling; it is quite natural for settlements of this species and was described earlier [Maksimovich, 2004]. The heterogeneity of the size and age characteristics of the settlements arises due to differences in the patterns of replenishment with juveniles, intraspecific and interspecific competition, and *M. arenaria* death at early stages of its development.

Apparently, the key factors affecting the soft-shell clam distribution in the bay and quantitative characters of settlements are hydrological processes (water dynamics, currents, and water exchange). The total water transport in the bay consists of tidal, runoff, and wind currents. The most significant role is played by tidal currents caused by the Barents Sea tidal wave. There, tidal currents are regular semi-diurnal; those vary depending on the cross-sectional area of the bay. The current velocity decreases from the surface horizon to the bottom; at the horizon of less than 10 m, it does not exceed 5–10 $cm \cdot s^{-1}$ in the middle bay area and 5 $cm \cdot s^{-1}$ in the northern one. The highest current velocity is characteristic of the southern bay area: there, the values reach 100 $cm \cdot s^{-1}$ [Kol'skii zaliv, 1997]. Tidal currents in the Kola Bay cover almost the entire water mass in bays and bights and serve as the main source of organic matter and oxygen necessary for the vital activity of the molluscs. However, with intensive movement of water masses, young individuals that just settled may be subject to postlarval passive transfer in the water column [Roegner et al., 1995].

As shown for the southeastern North Sea, with intensive movement of water masses together with bottom sediments, the transfer of *M. arenaria* juveniles in large numbers, with a shell length up to 15 mm, is possible [Emerson, Grant, 1991]. In the Baltic Sea, passive transfer in the water column was recorded for individuals with a shell length up to 25 mm [Kube, 1996]. Coastal areas off the Elovyi and Abram-mys capes are characterized by the highest dynamics of water mass movement; therefore, juveniles inhabiting surface layers of the sediment can be transferred by a current into more closed water areas of the bay, with a less dynamic hydrological regime. The apex spots of the Retinskaya and Khlebnaya bays are closed from the main bay area; those have a flat littoral and are characterized by weak tidal currents. The streams flowing into the apex spots of these bays have a moderate desalinating effect and provide the removal of organic matter suitable for mollusc feeding. There, the conditions are quite favorable for *M. arenaria*, and it is reflected in relatively high density and biomass of its settlements.

In addition to hydrological conditions, the distribution of the soft-shell clams in the Kola Bay can be affected by climate change and associated processes of primary production and suspended matter transfer. Because of a rise in the mean annual water and air temperature, coastal waters of the bay became more susceptible to desalination due to intensive snow melting in spring. A gain in freshwater runoff in the bay apex determines an increase in the intensity of water movement [Kravets, 2012]. In the zone of seawater and freshwater mixing, the content of suspended matter is high [Mityaev, Gerasimova, 2009]. There, intensive formation of primary production occurs due to the production activity of phytoplankton [Makarevich et al., 2004]. The distribution of suspended matter is associated with the cyclonic movement of water masses in the bay. From the south, desalinated water masses spread along the eastern shore of the southern bay area. In the zone of conjugation of the southern and middle bay areas, those collide with seawater moving from the north and, turning around, return along the western shore of the southern bay area. The eastern shore of the southern bay area is a zone of transit of suspended matter, and the western one is a zone of its concentration and, apparently, accumulation [Mityaev, Gerasimova, 2009].

Suspended matter is very important in the vital activity of inhabitants of the littoral zone. *M. arenaria* are sedentary seston feeders and filter feeders [Beskupskaya, 1963; Metody, 1990]. The soft-shell clams need a constant influx of suspended matter for their feeding: these hydrobionts filter the bottom water and pick up nutrient particles from it. The main food components are suspended detritus, diatoms of the genus *Coscinodiscus*, and particles of a macrophyte *Ascophyllum nodosum* [Beskupskaya, 1963]. Moreover, a sedentary lifestyle reduces the ability of these molluscs to compete for favorable

areas of the littoral zone with more mobile invertebrates inhabiting spots with similar conditions (other Bivalvia species, Polychaeta representatives, *etc.*) [Sveshnikov, 1963]. Accordingly, *M. arenaria* have adapted to inhabit biotopes inaccessible to many other species (highly desalinated apex areas of bays and gulfs, with salinity of 10‰); there, food resources are sufficient for the soft-shell clams, and the costs of competition are reduced [Smolkova, 2012].

In the early XX century, the southern boundary of *M. arenaria* distribution in the Kola Bay was the pre-estuarine area of the Lavna River on the western shore [Gur'yanova et al., 1929]. When moving southward along the Tuloma River mouth to the Nemetsky Island, the molluscs were not encountered. Starting from the Nemetsky Island, researchers registered a gradual transition from marine fauna to freshwater one. According to our survey in 2021, the southern boundary of *M. arenaria* distribution in the bay is the littoral zone off the Elovyi Cape which is 15 km south of the Lavna River. The littoral zone off the Elovyi Cape where we have recorded *M. arenaria* (for the first time during the entire period of investigation in the littoral zone of the Kola Bay) is located on the western shore of the southern bay area, in the spot of suspended matter concentration and accumulation.

Differences in the quantitative characters of the mollusc settlements in the bay may also be associated with the effect of anthropogenic factors on the habitat. Even with a high threshold of resistance to fluctuations in environmental factors, *M. arenaria* is in a depressed state in silted eutrophic areas of the littoral zone – in biotopes subject to significant anthropogenic load. Severe siltation negatively affects the vital activity of the soft-shell clams [Winther, Gray, 1985]. Due to the occurrence of floating layers of the sediment, non-floating burrows cannot be formed, and this leads to the death of the molluscs [Sveshnikov, 1963]. Earlier, when describing *M. arenaria* aggregations in the littoral zone of the Khlebnaya Bay, a strong inverse relationship was established between the content of small silt and pelitic fractions in the sediment and the settlement density [Smolkova, Meshcheryakov, 2023]. In areas with waters subject to higher anthropogenic load and a more intensive eutrophication (the Vayenga and Belokamennaya bays and the cape Abram-mys), the settlements are in a depressed state, and the values of quantitative characters are low. In areas with lower anthropogenic load (the Retinskaya and Khlebnaya bays), aggregations have higher density and biomass. At the same time, the occurrence of the molluscs in the southern Kola Bay may be associated not only with the effect of climate change, but also with improved water quality and a decrease in anthropogenic load.

The Kola Bay is classified as a moderately polluted area of marine waters in the Murmansk coastal zone of the Barents Sea [Informatsionnyi byulleten', 2012]. The current level of background pollution of waters and especially bottom sediments of the bay is still quite high; however, a gradual decrease recorded since the 1980s undoubtedly results in an improvement in the state of the bottom fauna [Pavlova et al., 2019]. According to the annual monitoring of the State Oceanographic Institute [2021], the water quality in the water post area in the Murmansk Commercial Seaport has improved significantly in 2021. The water pollution index (WPI = 0.70) dropped to class II ("clean"). In 2018–2020, WPI was of 0.93–1.13 and was attributed to class III ("moderately polluted"). The content of phosphates decreased in 2021 by an average of 1.5 times compared to that for the previous year. The priority pollutants are still petroleum hydrocarbons, copper, and iron. In 2021, the concentration of petroleum hydrocarbons was below the maximum permissible concentration (MPC) for the first time and amounted to 0.034 mg·dm^{-3}. The highest value, 1.3 MPC, was recorded in May. The mean concentration of copper became lower and slightly exceeded the standard (5.2 mg·dm^{-3}). The mean annual content of iron was also lower compared to that for the last year; the value amounted to 0.46 MPC.

The concentration of oxygen in the surface water layer in the water post area (a water sampling station closest to the southern border of the mollusc distribution in the bay) dropped: the mean annual concentration was 9.3 mg $O_2 \cdot dm^{-3}$ *vs.* 11.8 mg $O_2 \cdot dm^{-3}$ in 2020.

Conclusion. New data were obtained on the distribution of the bivalve *Mya arenaria* in the littoral zone of the Kola Bay of the Barents Sea. For the first time during the research period there (1921–2021), the molluscs were registered in its mouth, in the littoral zone off the Elovyi Cape. Apparently, it is a response to climate change occurring in the Arctic region, and it indicates the formation of favorable habitat conditions in areas previously unsuitable for this. The largest settlement of the soft-shell clams is located on the southeastern shore of the middle bay area in the biotope of silted fine sand of the Khlebnaya Bay. The littoral area of the western shore off the Elovyi Cape (the Tuloma River mouth) is defined as the southern boundary of the species distribution in the Kola Bay. *M. arenaria* distribution is most likely determined by the hydrological features of the Kola Bay (the intensity of movement of water masses in bays and bights, the cyclonic movement of water masses in the southern bay area, and the degree of siltation and eutrophication of the studied areas). The obtained data on the biology and distribution of this bivalve in the Kola Bay of the Barents Sea will serve as a basis for monitoring possible changes caused by anthropogenic load or climate fluctuations.

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СОВРЕМЕННОЕ СОСТОЯНИЕ ПОПУЛЯЦИИ И ОСОБЕННОСТИ РАСПРОСТРАНЕНИЯ ДВУСТВОРЧАТЫХ МОЛЛЮСКОВ *MYA ARENARIA* LINNAEUS, 1758 В КОЛЬСКОМ ЗАЛИВЕ БАРЕНЦЕВА МОРЯ

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Mya arenaria Linnaeus, 1758 (мия) — двустворчатый моллюск бореального происхождения. Ареал вида охватывает прибрежные умеренные воды Атлантического океана и северо-восточную часть Тихого океана, а также моря Северного Ледовитого океана — Баренцево и Белое. Поселения мии могут занимать обширные пространства в прибрежной полосе, где моллюски образуют большие скопления и являются доминирующим по биомассе видом среди представителей литорального макрозообентоса. *M. arenaria* способны выдерживать флуктуации факторов среды, а также оказывать влияние на процессы детритообразования и осадконакопления. Молодь моллюсков, заселяющая верхние слои грунта, представляет собой важный кормовой объект для морских птиц и промысловых видов рыб. Высокая эврибионтность позволяет рассматривать мию в качестве индикатора для оценки влияния климатических изменений на природную среду Арктики. Получение новых данных об особенностях биологии *M. arenaria* необходимо как для выявления общих закономерностей развития бентосных организмов в динамичных условиях морской среды, так и для понимания адаптивных особенностей отдельных видов долгоживущих эврибионтных моллюсков и оценки влияния на них различных экологических факторов. Изучение биологии *M. arenaria* может иметь практическое значение: не исключено, что мия станет перспективным объектом марикультуры в Арктическом регионе. В работе представлены результаты исследования современного состояния популяции и особенностей распространения моллюсков в Кольском заливе Баренцева моря. Материал собран в ходе экспедиции ММБИ РАН в 2021 г. Изучены количественные характеристики и размерно-возрастной состав поселений мии. Скопления *M. arenaria* зафиксированы на литорали западного и восточного берегов среднего и южного колен залива. Впервые за весь период исследований Кольского залива (1921–2021 гг.) моллюски обнаружены в эстуарной его части — на литорали

у мыса Еловый (устье реки Тулома). Наибольшие показатели обилия отмечены на восточном берегу среднего колена залива — в губе Хлебная (67,1 экз.·м$^{-2}$), наименьшие зарегистрированы на западном берегу — в губе Белокаменная (5,0 экз.·м$^{-2}$). На мелководных участках в районе Абрам-мыса и в губе Ваенга мия плотных скоплений не образует. Исследованные поселения залива представлены особями в возрасте от 4 до 14 лет с вариацией размеров от 17,5 до 91,2 мм. Распространение *M. arenaria*, а также количественные и морфометрические характеристики её поселений связаны, вероятно, с гидрологическими особенностями залива (с интенсивностью движения водных масс в губах и бухтах, а также с циклоническим перемещением водных масс в южном колене). Отмеченные увеличение численности моллюсков и расширение ареала можно, по-видимому, интерпретировать как отклик вида на климатические изменения, происходящие в Арктическом регионе, и показатель снижения антропогенной нагрузки на прибрежные сообщества залива в целом.

Ключевые слова: двустворчатый моллюск *Mya arenaria*, распространение, состояние популяции, плотность, биомасса, размерно-возрастная структура, литораль, Кольский залив

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SPECIES COMPOSITION, ABUNDANCE, AND BIOMASS OF PHYTOPLANKTON IN THE KERCH STRAIT IN 2009–2019

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The results of studies of planktonic algae developing in the Kerch Strait in various seasons of 2009–2019 are presented. Phytoplankton included 114 species and several taxa identified down to the genus level covering 11 classes of algae, *inter alia* 64 Dinophyceae species and 32 Bacillariophyceae species. Mean values of abundance and biomass were 140 thousand cells·L^{-1} and 0.386 g·m^{-3}, respectively. Cyanophyceae prevailed accounting for 44% of the total phytoplankton abundance. Bacillariophyceae and Dinophyceae formed a significant part of the total phytoplankton abundance (19 and 18%) and biomass (62 and 35%). Cryptophyceae, Coccolithophyceae, and Chlorophyceae amounted to 18% of the total phytoplankton abundance. In spring, small-cell diatoms *Skeletonema costatum* and *Cyclotella caspia* dominated. In summer, large- and small-cell species of Bacillariophyceae and Dinophyceae prevailed, along with a Coccolithophyceae representative *Emiliania huxleyi*. In autumn, species of Cyanophyceae (*Planktolyngbya limnetica*), Cryptophyceae (*Plagioselmis*), and Chlorophyceae (*Binuclearia* and *Nannochloris*) were the most abundant ones. Bacillariophyceae (*Pseudosolenia calcar-avis*) and Dinophyceae (*Prorocentrum*, *Protoperidinium*, and *Ceratium* species) formed the major part of the phytoplankton biomass.

Keywords: phytoplankton, species composition, abundance, biomass, Kerch Strait

The Kerch Strait connects the Sea of Azov and the Black Sea. Its inhabitants are exposed to effect of both natural and anthropogenic factors: heavy shipping traffic, construction of hydraulic structures, operation of port and roadstead transshipment complexes, exploitation of coastal oil terminals in Taman and the port of Kavkaz, and transshipment of oil and bulk cargo. Also, the problem of the input of biogenic elements with domestic wastewater is becoming more and more urgent [Zhugailo et al., 2011]. Against the backdrop of climate change, increase in the shipping intensity, development of tourism, and industrialization of water recreation, constant monitoring of the environment in the Sea of Azov–Black Sea Basin is required [Matishov, Ivanov, 2012; Matishov et al., 2013]. In this regard, the study of current state of a plankton phytocenosis of the Kerch Strait is of significant interest, for it is one of the most sensitive components of marine ecosystems. Despite the fact that plankton communities of the Kerch Strait are being investigated, data on the seasonal dynamics of phytoplankton composition, abundance, and biomass in this area are insufficient. The aim of our research is to analyze spatial and temporal variability of phytoplankton species richness and abundance under the effect of environmental factors for 2009–2019.

MATERIAL AND METHODS

The provided results were obtained during expeditions covering the Kerch Strait water area (Fig. 1) on the RV "Deneb" of SSC RAS in April 2009, July 2010, June and September 2011, July 2012, and May and July 2013, as well as on the RV "Peleng" of the Sevastopol Branch of the N. N. Zubov's State Oceanographic Institute in August 2016 and 2019, under the projects EMBLAS-II (Improving Environmental Monitoring in the Black Sea – Phase II, ENPI/2013/313-169) and EMBLAS-Plus (Selected Measures, ENI/2017/389-859).

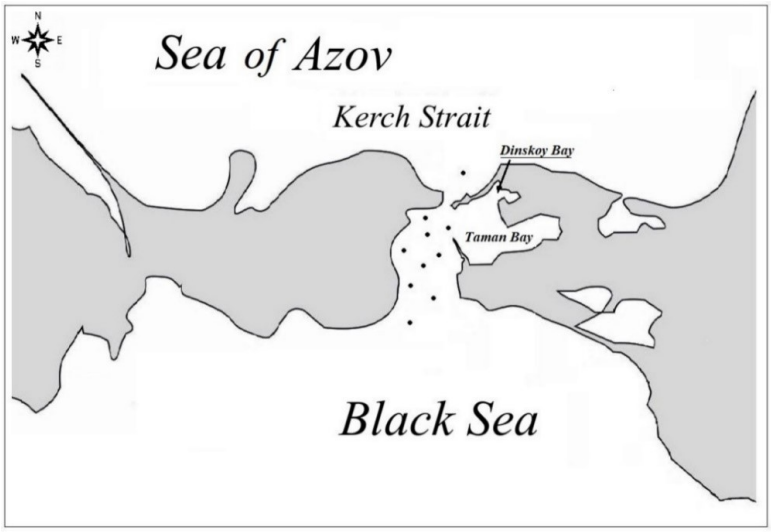

Fig. 1. A scheme of phytoplankton sampling sites in the Kerch Strait waters in 2009–2019

A total of 51 samples were taken and processed. In September 2011, May and July 2013, and August 2016, sampling was carried out in the upper layer of the sea, 0–1 m. In April 2009, June 2011, and July 2010, the vertical structure of phytoplankton was analyzed at two horizons, on the surface and at the bottom. In July 2012 and August 2019, it was studied at horizons of 0–1 m, 5 m, and 10 m, as well as at the bottom. Water was sampled during the daytime from aboard the RV with bathometers, thickened by sedimentation, and fixed with 5% Lugol's solution, neutral formalin, or Watermael acidic solution to a final concentration of 1–2% [Makarevich, Druzhkov, 1989]. Phytoplankton was recorded quantitively under a Mikmed-2 microscope with a magnification of ×100, ×200, and ×400 in a 0.05-mL Nageotte counting chamber. The minimum size of cells to be counted was 3–5 μm. For trichome cyanoprokaryotes, a colony 50–100 μm long was taken as one conventional unit for counting. Species were identified according to guidelines [Dedusenko-Shchegoleva et al., 1959; Dodge, 1982; Gollerbakh et al., 1953; Identifying Marine Phytoplankton, 1997; Kiselev, 1950; Kosinskaya, 1948; Proshkina-Lavrenko, 1955, 1963]. Phytoplankton biomass was determined based on size and shape of cells in accordance with their similarity to the closest stereometric figures, and specific weight of algae was considered equal to one [Bryantseva et al., 2005]. In our work, algal classification ranking follows AlgaeBase [2023]. The Sørensen–Czekanowski coefficient [Clarke et al., 2014] was applied to assess the similarity of taxonomic composition for microalgal communities in the Kerch Strait in different study periods.

RESULTS

1. Taxonomic composition of phytoplankton. In the Kerch Strait, we registered 114 species and several algae not identified down to the species level (Table 1). Those represent 11 classes. Dinophyceae (64 species) and Bacillariophyceae (32 species) were distinguished by high species diversity.

In June 2011 and August 2019, species composition of phytoplankton (Table 1) was the richest (58 and 53 species, respectively). In July 2013, the lowest diversity was observed (16 species). At other times, the total number of algal species varied within 34–45. In June 2011 and August 2019, the maximum diversity of dinoflagellates was noted (37 and 41 species, respectively); in other study periods, the number of species was 6 to 27. Diatoms were most widely represented in April 2009 (19 species); values in other periods were 7 to 14. The number of species from other classes varied depending on season and year in a range from 3 (July 2012 and July 2013) to 10 (April 2009 and July 2010).

Table 1. Species composition of phytoplankton in the Kerch Strait in 2009–2019

Year, month / Algal class and species	2009	2010	2011		2012	2013		2016	2019
	IV	VII	VI	IX	VII	V	VII	VIII	VIII
BACILLARIOPHYCEAE									
Chaetoceros affinis Lauder	–	+	++	–	+	–	++	–	+
Chaetoceros curvisetus Cleve	++	–	+	–	–	–	–	–	–
Chaetoceros peruvianus Brightwell	–	–	–	–	–	–	–	+	–
Chaetoceros scabrosus Proshkina-Lavrenko	–	–	–	–	–	–	–	+	–
Chaetoceros simplex Ostenfeld	+	–	–	–	–	–	–	+	–
Chaetoceros subtilis Cleve	+	–	–	–	–	–	–	–	–
Chaetoceros spp.	+	+	–	–	–	–	–	–	–
Cerataulina pelagica (Cleve) Hendey	+	++	+	–	–	–	–	+	–
Climaconeis inflexa (Brébisson ex Kützing) E. J. Cox [= *Amphora inflexa* (Brébisson ex Kützing) Cleve]	+	–	+	–	–	–	–	–	+
Cocconeis scutellum Ehrenberg	+	–	–	–	–	–	–	+	–
Coscinodiscus granii L. F. Gough	++	+	+	++	–	++	–	+	–
Coscinodiscus janischii A. W. F. Schmidt	–	–	+	+	–	++	++	–	–
Coscinodiscus subtilis Ehrenberg	–	–	–	+	–	–	–	–	–
Coscinodiscus sp.	++	+	+	+	–	+	–	+	–
Cyclotella caspia Grunow	+++	+++	–	++	–	–	–	–	–
Cyclotella sp.	–	–	–	+	–	+	–	+	–
Cylindrotheca closterium (Ehrenberg) Reimann & J. C. Lewin	–	–	–	–	–	–	–	+	–
Dactyliosolen fragilissimus (Bergon) Hasle	+	–	+	–	–	–	–	–	++
Ditylum brightwellii (T. West) Grunow	–	+++	–	++	–	–	–	–	–
Gyrosigma sp.	++	–	–	+	–	–	–	+	–
Halamphora hyaline (Kützing) Rimet & R. Jahn (= *Amphora hyaline* Kützing)	+	–	–	–	–	–	–	–	+
Hemiaulus hauckii Grunow ex Van Heurck	–	–	–	+	–	–	–	–	–

Continued on the next page…

Year, month / Algal class and species	2009	2010	2011		2012	2013		2016	2019
	IV	VII	VI	IX	VII	V	VII	VIII	VIII
Leptocylindrus danicus Cleve	–	–	–	–	–	–	–	++	–
Licmophora ehrenbergii (Kützing) Grunow	+	–	–	–	–	–	++	–	–
Licmophora flabellata (Greville) C. Agardh	+	–	–	–	–	+	–	–	–
Melosira moniliformis (Link) C. Agardh	–	–	–	–	–	+	–	–	–
Nitzschia tenuirostris Manguin	++	+++	+	+	+	–	–	++	–
Pleurosigma elongatum W. Smith	+	+	+	–	+	–	+	+	–
Pleurosigma sp.	+	+	–	–	–	–	–	–	+
Proboscia alata (Brightwell) Sundström	–	–	–	–	+	–	–	–	++
Pseudosolenia calcar-avis (Schultze) B. G. Sundström	–	+++	++	++	+++	+++	+++	+++	+++
Pseudo-nitzschia pseudodelicatissima (Hasle) Hasle (complex)	+	++	+	++	++	–	+	+	+++
Pseudo-nitzschia sp.	+	–	+	–	–	–	–	+	–
Skeletonema costatum (Greville) Cleve	+++	++	++	–	–	–	–	+	–
Striatella delicatula (Kützing) Grunow ex Van Heurck	+	–	–	–	–	–	–	–	–
Striatella unipunctata (Lyngbye) C. Agardh	+	–	–	–	–	–	–	–	–
Surirella gemma (Ehrenberg) Kützing	–	–	–	–	–	+	–	–	–
Thalassionema nitzschioides (Grunow) Mereschkowsky	+	+++	+++	++	++	+	+	+	++
Thalassiosira sp.	+	+	–	++	–	–	–	+	–
DINOPHYCEAE									
Akashiwo sanguinea (K. Hirasaka) G. Hansenet Moestrup (= *Gymnodinium sanguineum* K. Hirasaka)	++	–	+	+	+	–	–	+	+
Alexandrium tamarense (Lebour) Balech	–	–	–	–	–	–	–	–	+
Amphidinium sp.	–	–	–	–	–	–	–	+	–
Amphidinium crassum Lohmann	–	+	–	–	–	–	–	–	–
Amphidinium longum Lohmann	–	–	+	–	–	++	–	–	–
Amphidinium flagellans J. Schiller	–	–	–	–	–	–	–	–	+
Amphidinium fusiforme G. W. Martin	–	+	–	–	–	–	–	–	–
Blixaea quinquecornis (T. H. Abé) Gottschling (= *Peridinium quinquecorne* Abé)	–	–	–	–	–	–	–	+	–
Dinophysis acuminata Claparède & Lachmann	+	–	+	–	–	+	–	–	–
Dinophysis acuta Ehrenberg	–	–	+	+	–	–	–	–	+
Dinophysis caudata Kent	–	+	++	–	+	–	–	+	+
Dinophysis fortii Pavillard	–	+	+	–	–	–	–	–	–
Dinophysis sacculus F. Stein	+	–	+	–	+	+	–	–	–
Dinophysis sp.	–	–	+	–	–	–	–	–	–
Diplopsalis lenticula Bergh	+	+	–	+	++	+	–	+	+
Diplopsalis sp.	–	–	–	+	–	–	–	–	–

Continued on the next page…

Year, month / Algal class and species	2009	2010	2011		2012	2013		2016	2019
	IV	VII	VI	IX	VII	V	VII	VIII	VIII
Glenodinium pilula (Ostenfeld) J. Schiller	–	–	+	+	–	+	–	–	+
Glenodinium sp.	+	+	+	–	–	+	–	–	–
Gymnodinium agiliforme J. Schiller	–	–	–	–	–	+	–	–	+
Gymnodinium blax T. M. Harris	–	–	+	–	++	–	++	–	++
Gymnodinium elongatum B. Hope	–	–	–	–	–	++	–	–	–
Gymnodinium simplex (Lohmann) Kofoid & Swezy	+	–	+++	–	++	–	–	+	++
Gymnodinium wulffii J. Schiller	+	+	++	–	+	+	+	+	+
Gymnodinium spp.	++	+++	+++	+	++	+++	++	++	+
Gyrodinium lacryma (Meunier) Kofoid & Swezy	–	–	–	–	–	–	–	–	+
Gyrodinium fusiforme Kofoid & Swezy	+	++	+++	++	++	++	–	+	+
Gyrodinium spirale (Bergh) Kofoid & Swezy	–	–	+	+	+	–	–	–	++
Gyrodinium sp.	+	+	+	++	++	++	++	+	++
Gonyaulax digitale (Pouchet) Kofoid	–	+	–	+	+	–	–	–	–
Gonyaulax spinifera (Claparède & Lachmann) Diesing	–	+	–	+	+	–	–	–	–
Gonyaulax polygramma F. Stein	–	–	–	–	–	–	–	–	+
Gonyaulax sp.	–	–	–	–	–	–	–	+	+
Katodinium glaucum (Lebour) A. R. Loeblich III	–	+	++	+	+	–	–	+	++
Lingulodinium polyedra (F. Stein) J. D. Dodge	–	–	+	–	–	–	–	–	–
Margalefidinium citron (Kofoid & Swezy) F. Gómez, Richlen & D. M. Anderson (= *Cochlodinium citron* Kofoid & Swezy)	–	–	+	–	–	–	–	–	+
Mesoporos perforatus (Gran) Lillick	–	–	–	–	–	–	–	–	+
Heterocapsa rotundata (Lohmann) G. Hansen [= *Katodinium rotundatum* (Lohmann) Loeblich III]	–	–	+	–	–	–	–	–	–
Heterocapsa triquetra (Ehrenberg) F. Stein	–	–	+	+	+	–	–	–	–
Heterocapsa sp.	–	–	–	–	–	–	–	+	+
Ensiculifera carinata Matsuoka, Kobayashi & Gains	–	–	+	++	+	–	–	+	–
Oblea baculifera Balech	+	–	–	–	–	–	–	–	–
Oblea rotunda (Lebour) Balech	+	+	–	+	+	–	–	+	++
Oxyrrhis marina Dujardin	–	+++	+	+	–	–	++	–	–
Oxytoxum caudatum J. Schiller	–	+	–	–	–	–	–	–	–
Phalacroma rotundatum (Claparéde & Lachmann) Kofoid & J. R. Michener [= *Dinophysis rotundata* (Claparède & Lachmann) Balech]	–	–	+	+	+	+	–	+	+
Polykrikos kofoidii Chatton	–	+	+	+++	+++	+	–	++	+
Polykrikos schwartzii Bütschli	–	+	+	–	–	–	–	–	–

Continued on the next page…

Year, month / Algal class and species	2009	2010	2011		2012	2013		2016	2019
	IV	VII	VI	IX	VII	V	VII	VIII	VIII
Pronoctiluca pelagica Fabre-Domergue	–	–	+	–	–	–	–	–	++
Prorocentrum compressum (Bailey) T. H. Abé ex J. D. Dodge	+	+	++	+	+	+	–	+	+
Prorocentrum cordatum (Ostenfeld) J. D. Dodge [= *P. minimum* (Pavillard) J. Schiller]	+	++	++	+	++	++	+	+	++
Prorocentrum micans Ehrenberg	+	++	++	+++	++	+++	++	+++	++
Prorocentrum sp.	–	–	–	–	–	–	–	+	–
Protoceratium reticulatum (Claparède & Lachmann) Bütschli	–	+	–	–	++	–	–	–	++
Protoperidinium bipes (Paulsen) Balech	+	–	+	–	–	–	–	–	+
Protoperidinium brevipes (Paulsen) Balech	–	–	+	–	–	–	–	+	+
Protoperidinium conicum (Gran) Balech	–	–	–	–	–	+	–	–	+
Protoperidinium crassipes (Kofoid) Balech	–	–	–	–	–	–	–	–	+
Protoperidinium depressum (Bailey) Balech	–	–	–	–	–	+	–	+	++
Protoperidinium divergens (Ehrenberg) Balech	–	+	–	++	++	++	–	–	++
Protoperidinium excentricum (Paulsen) Balech	–	–	–	–	–	–	–	+	–
Protoperidinium globulus (F. Stein) Balech	–	+	–	–	–	–	–	–	–
Protoperidinium granii (Ostenfeld) Balech	+	–	+	–	–	–	–	–	+
Protoperidinium knipowitschii (Usachev) Balech	–	–	–	+	–	–	–	–	–
Protoperidinium pallidum (Ostenfeld) Balech	–	–	+	–	–	–	–	–	+
Protoperidinium pellucidum Bergh	–	–	+	–	–	–	–	–	–
Protoperidinium steinii (Jørgensen) Balech	+	–	–	+	+	+	–	–	+
Protoperidinium spp.	++	+	–	++	+	–	–	+	–
Scrippsiella acuminata (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S. Soehner, Kirsch, Kusber & Gottschling [= *Scrippsiella trochoidea* (F. Stein) A. R. Loeblich III]	++	+	–	+	+	+	+	–	++
Speroidium fungiforme (Anisimova) Moestrup & Calado [= *Katodinium fungiforme* (Anisimova) A. R. Loeblich III]	–	–	+	–	–	–	–	–	–
Torodinium robustum Kofoid & Swezy	–	+	+	–	+	–	–	–	++
Tripos furca (Ehrenberg) F. Gómez [= *Ceratium furca* (Ehrenberg) Claparède & Lachmann]	–	–	+	++	++	++	–	+	++
Tripos fusus (Ehrenberg) F. Gómez [= *Ceratium fusus* (Ehrenberg) Dujardin]	–	–	+	+	+	+	–	–	++
Tripos muelleri Bory [= *Ceratium tripos* (O. F. Müller) Nitzsch]	–	–	++	–	–	++	–	–	++
Warnowia aff. *maculate* (Kofoid & Swezy) Lindemann	–	–	–	–	–	–	–	–	+

Continued on the next page…

Year, month / Algal class and species	2009	2010	2011		2012	2013		2016	2019
	IV	VII	VI	IX	VII	V	VII	VIII	VIII
COCCOLITHOPHYCEAE									
Emiliania huxleyi (Lohmann) W. W. Hay & H. P. Mohler	+	+	+++	–	+++	+++	–	–	++
CRYPTOPHYCEAE									
Plagioselmis spp.	+	+++	++	++	++	++	+++	++	++
EUGLENOPHYCEAE									
Eutreptia lanowii Steuer	+	+	+	–	+	–	++	+	–
Euglena viridis (O. F. Müller) Ehrenberg	–	–	–	–	–	–	–	–	+
Euglena sp.	–	+	+	++	–	–	–	+	–
ULVOPHYCEAE									
Binuclearia lauterbornii (Schmidle) Proshkina-Lavrenko	+	+++	+	++	–	+	–	+	–
CHLOROPHYCEAE									
Ankistrodesmus convolutus Corda	+	–	+	+	–	–	–	–	–
Golenkinia radiata Chodat	+	–	–	–	–	–	–	–	–
Monoraphidium contortum (Thuret) Komàrková-Legnerová	+	++	+	–	–	++	–	+	–
Scenedesmus bicaudatus Dedusenko	–	+	–	–	–	–	–	–	–
Scenedesmus falcatus Chodat	+	–	–	–	–	–	–	–	–
Scenedesmus obliquus (Turpin) Kützing	–	+	–	–	–	–	–	–	–
Scenedesmus quadricauda Chodat	+	+	+	+	–	–	–	–	–
Tetraselmis sp.	–	–	–	+	–	–	–	–	–
TREBOUXIOPHYCEAE									
Oocystis sp.	+	–	–	–	–	–	–	–	–
aff. *Nannochloris* sp.	–	+	+	++	–	–	–	–	–
PYRAMIMONADOPHYCEAE									
Pterosperma undulatum Ostenfeld	–	+	+	–	+	+	–	–	+
Pterosperma sp.	+	+	–	–	–	–	–	+	–
DICTYOCHOPHYCEAE									
Octactis octonaria (Ehrenberg) Hovasse	–	–	–	–	–	–	–	–	+
CYANOPHYCEAE									
Anabaena flos-aquae Brébisson ex Bornet & Flauhault f. *major* Elenkin	–	–	–	–	–	–	++	+	–
Anabaena spiroides Klebahn	–	–	–	–	–	–	–	++	–
Anabaena sp.	–	+	–	+	–	–	++	++	–
Aphanizomenon sp.	–	+	–	+	–	+	–	–	–
Merismopedia punctata Meyen	+	+	–	–	–	–	–	–	–
Microcystis aeruginosa (Kützing) Kützing	–	–	–	++	–	–	–	–	–
Oscillatoria spp.	+	++	+	–	–	–	–	+	–
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová & Cronberg	+	+	+	+++	–	+	++	+++	–
Spirulina sp.	+	–	–	–	–	–	–	–	–

Note: +, rare species; ++, common; +++, abundant; and –, absent. Abundant species formed more than 10% of total phytoplankton abundance or biomass; common ones, 1 to 10%; and rare ones, up to 1%.

The highest similarity rates for taxonomic composition of microalgal communities in the Kerch Strait [Sørensen–Czekanowski (Dice) coefficient ≥ 60%] were recorded during the warm season (Table 2), at maximum water temperatures (+21…+28 °C). In this period, high species diversity of microalgae was registered (Table 3).

Table 2. Indicators of similarity [Sørensen–Czekanowski (Dice) coefficient] of taxonomic composition for microalgal communities in the Kerch Strait during different study periods

Year, month		2009	2010	2011		2012	2013		2016
		IV	VII	VI	IX	VII	V	VII	VIII
2010	VII	52							
2011	VI	52	55						
2011	IX	45	55	54					
2012	VII	44	62	59	63				
2013	V	43	44	50	52	51			
2013	VII	33	40	35	33	42	32		
2016	VIII	52	53	54	49	55	44	36	
2019	VIII	37	42	62	46	67	51	26	41

Note: cells highlighted in pale pink depict the coefficient values within 26–37; light pink, 40–49; pink, 50–59; and dark pink, 62–67.

Table 3. The basic hydrological and phytoplankton characteristics for the Kerch Strait in 2009–2019

Month	Year	Water temperature, °C / depth, m	Wind, m·s^{-1} / swell, points	Phytoplankton		
				number of species	abundance, thousand cells·L^{-1}	biomass, mg·m^{-3}
April	2009	+9.8…+9.9 / 7–11	no data	45	130	57
May	2013	+19…+19.8 / 10–12	E-ES 5–9 / 1–2	34	58	145
June	2011	+22.6…+24 / 6–11	0 / 0	58	62	82
July	2010	+26.8…+27 / 3–15	E-ES 2–3 / 1	44	101	418
	2012	+22…+23 / 4–14	NW 5.3–5.7 / 1–2	37	72	217
	2013	+24…+24.4 / 10	S 2–3 / 0–1	16	19	242
August	2016	+26.2…+28 / 4–16	ES 2–3 / 0–1	39	719	1,922
	2019	+25.8…+26 / 24–28	S 2–2.5 / 0–1	53	22	302
September	2011	+21.3…+21.5 / 3–12	SW 6–8 / 1–2	39	77	93
				114 in total	140 on average	386 on average

In phytoplankton of the Kerch Strait, the most abundant species are those common to the Black Sea and Sea of Azov: diatoms (*Cerataulina pelagica*, *Chaetoceros affinis*, *Chaetoceros curvisetus*, *Chaetoceros subtilis*, *Coscinodiscus granii*, *Cyclotella caspia*, *Ditylum brightwellii*, *Nitzschia tenuirostris*, *Pseudo-nitzschia pseudodelicatissima*, *Pseudosolenia calcar-avis*, *Skeletonema costatum*, and *Thalassionema nitzschioides*), dinoflagellates (*Akashiwo sanguinea*, *Diplopsalis lenticula*, *Gymnodinium simplex*, *Gyrodinium fusiforme*, *Prorocentrum cordatum*, *Prorocentrum micans*, *Protoperidinium granii*,

Protoperidinium divergens, *Protoperidinium steinii*, and *Scrippsiella acuminata*), and a coccolithophore (*Emiliania huxleyi*) [Studenikina et al., 1999]. Against the backdrop of noticeable abundance of diatoms and dinoflagellates, brackish-water species of the classes Cryptophyceae, Euglenophyceae, Chlorophyceae, and Cyanophyceae developed significantly; these species are widely distributed in the Sea of Azov plankton. Low depths and high hydrodynamic activity of the Kerch Strait contribute to phytoplankton enrichment with periphytic diatoms (*Climaconeis inflexa*, *Cocconeis scutellum*, *Halamphora hyalina*, *Licmophora ehrenbergii*, *Licmophora flabellata*, *Melosira moniliformis*, and *Pleurosigma elongatum*) and representatives of the genera *Gyrosigma* and *Striatella*.

Species richness of phytoplankton we observed in the Kerch Strait in 2009–2019 (114 species) is higher than that described earlier (42–90 species) [Bryantseva et al., 2010; Chernikova, 2004; Zaremba, 2013]. This is probably due to longer period of our research and the coverage of three seasons (spring, summer, and autumn). However, taxonomic composition of planktonic algae was significantly lower than the number of species recorded for a larger area of the Kerch Strait including Dinskoy Bay and Taman Bay in 1997–2000 (154 species) [Kovaleva, 2008].

2. Seasonal dynamics of phytoplankton abundance and biomass. In the Kerch Strait, values of phytoplankton abundance and biomass during the study period (April 2009 to August 2019) varied widely: 19 to 719 thousand cells·L^{-1} and 0.06 to 1.92 g·m^{-3}, respectively. Those averaged (140 ± 220) thousand cells·L^{-1} and (0.386 ± 0.587) g·m^{-3} (Table 3, Fig. 2).

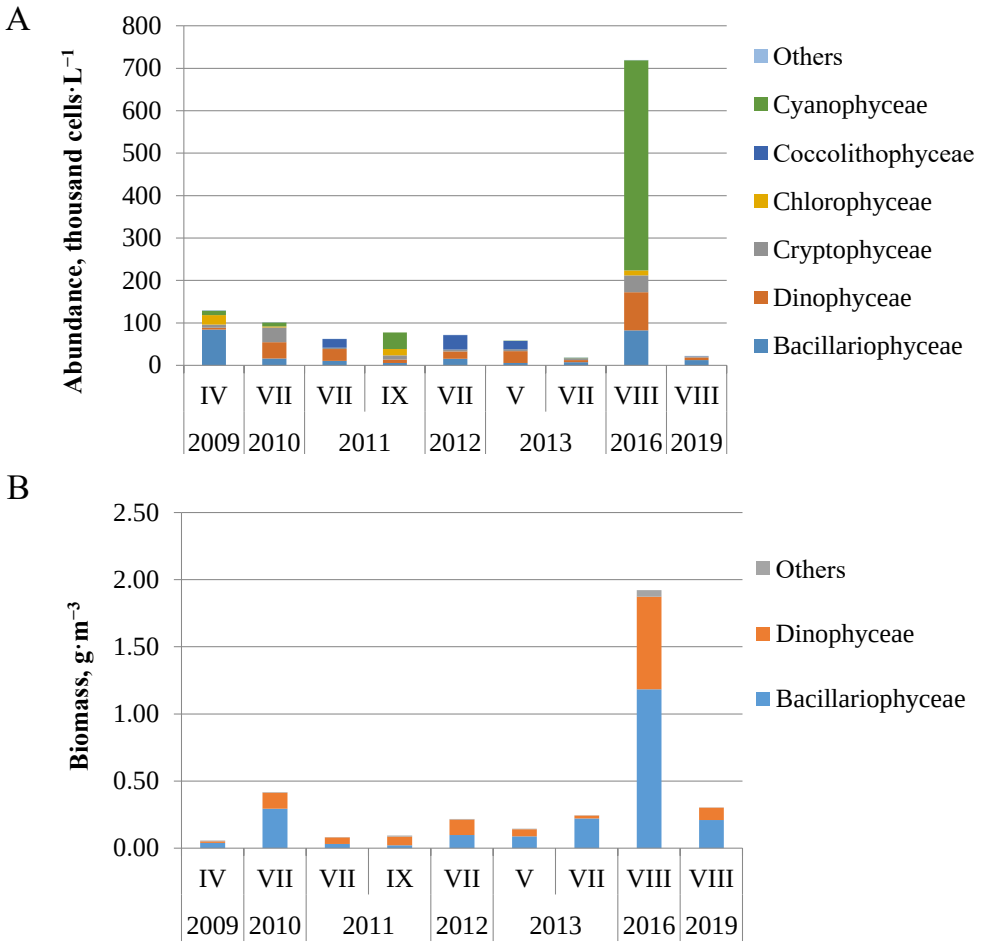

Fig. 2. Abundance (A) and biomass (B) of planktonic algae in the Kerch Strait area in 2009–2019

Cyanoprokaryotes dominated by abundance (44% on average over the study period). Diatoms and dinoflagellates constituted a noticeable part of the total abundance (19 and 18% on average, respectively) and biomass (62 and 35%). Cryptophyceae, Coccolithophyceae, and Chlorophyceae formed 18% of abundance and 3% of biomass of the plankton phytocenosis. Representatives of other classes accounted for < 1% of abundance and biomass in total. The maximum development of planktonic algae was noted in August 2016: 719 thousand cells·L^{-1} and 1.922 g·m^{-3}. Accordingly, mean values of phytoplankton abundance and biomass in summer (166 thousand cells·L^{-1} and 0.531 g·m^{-3}, respectively) were 2–5 times higher than values registered in spring and autumn (94 and 77 thousand cells·L^{-1} and 0.101 and 0.093 g·m^{-3}).

In spring and summer 2009–2019, vertical distribution of phytoplankton abundance and biomass was uneven and depended on the hydrodynamic activity of waters contributing to a more uniform distribution of planktonic algae in a water column. Specifically, in July 2012, because of intensive wind activity, uniform distribution of coccolithophores was noted (those made up 43–50% of the total abundance) in the water column (0–14 m). In June 2011, in calm weather, coccolithophores were concentrated (42% of the abundance) in the upper layer of the sea (0–1 m) and were absent at the bottom (6–11 m). Due to mass development of large species (up to 200 μm) of benthic–planktonic diatoms in the bottom layer of the pelagic zone, their biomass at the bottom was 1.4–2 times higher than in the upper layers of the strait (0–5 m) in April 2009, June 2011, and August 2019.

Spring. In April 2009, due to intensive development of mostly medium- and large-sized dinoflagellate *A. sanguinea* (40–200 μm) and periphytic diatoms of the genera *Gyrosigma* and *Striatella*, phytoplankton abundance in bottom layers of the strait was 1.3 times higher than on the surface, and biomass was 1.9 times higher. Diatoms averaged 65% of abundance and 69% of biomass of pelagic phytoplankton; those formed 61% of abundance and 49% of biomass on the surface of the strait and 68 and 79%, respectively, at the bottom. Out of them, *S. costatum* and *C. caspia* developed most intensively, while *N. tenuirostris* and *C. curvisetus* were subdominant species. *Coscinodiscus*, *Thalassiosira*, and *Gyrosigma* representatives formed the basis of biomass of diatoms, along with *S. costatum*, *C. caspia*, and *C. curvisetus* prevailing by abundance. A key role in formation of the total biomass of communities (21% on average) was played by dinoflagellates: 33% on the surface of the strait and 14% at the bottom. Interestingly, their share in the total abundance did not exceed 2%. Out of them, the main ones were *Oblea baculifera*, *S. acuminata*, *A. sanguinea*, and *P. micans*, as well as *Gymnodinium* and *Protoperidinium* representatives. Also, high values of abundance were registered for several species of green algae (*Binuclearia lauterbornii* and *Monoraphidium contortum*), cyanoprokaryotes (*Oscillatoria* and *Planktolyngbya* representatives), and cryptophytes (*Plagioselmis* spp.). Representatives of these classes formed 17, 8, and 6% of phytoplankton abundance, respectively, and about 9% of its biomass in total. Small flagellate algae with a mean abundance of 2 thousand cells·L^{-1} were occasionally encountered in the studied water area.

In May 2013, diatoms accounted for 9% of phytoplankton abundance and 60% of its biomass. Out of them, a large-cell species *P. calcar-avis* developed most intensively (89% of abundance and 88% of biomass of diatoms). *T. nitzschioides* and *Coscinodiscus* representatives were registered as subdominants. The main species of coccolithophores, *E. huxleyi*, accounted for 35% of the total abundance and, due to small size of its cells, only 2% of biomass. Dinoflagellates formed the basis of phytoplankton abundance (48%) and a noticeable part of its biomass (37%). Out of them, the most abundant

species were *Gymnodinium* and *Prorocentrum* representatives. The core of biomass of dinoflagellates included *P. micans* and large species of the genera *Protoperidinium* and *Tripos*. Representatives of other classes of algae accounted for no more than 6% of abundance and 1% of biomass.

Summer. In June 2011, on the surface, *E. huxleyi* contribution was noticeable: this coccolithophore formed 42% of phytoplankton abundance. At the bottom, it did not develop which seems to result from darkening of the photic layer because of churning. In the upper layer, abundance of algae (98 thousand cells·L^{-1}) was almost 4 times higher, while their biomass (0.067 g·m^{-3}) was 1.4 times lower than at the bottom (26 thousand cells·L^{-1} and 0.096 g·m^{-3}, respectively). Dinoflagellates prevailed accounting for 44 and 54% of abundance and 67 and 56% of biomass on the surface and at the bottom, respectively. Out of them, the main species in terms of abundance were *Gymnodinium* representatives and *G. fusiforme*. *P. minimum* and *Gyrodinium spirale* were found in smaller abundance. Biomass of dinoflagellates chiefly included *Tripos furca*, *Tripos muelleri*, and *Gyrodinium* representatives. Diatoms formed 17% of phytoplankton abundance on average (46% at the bottom and 8% on the surface) and 37% of its biomass (27 and 44%, respectively). *T. nitzschioides* was the most abundant species; *P. pseudodelicatissima*, *C. affinis*, and *C. granii* were less abundant ones. Biomass was mainly formed by large cells of *P. calcar-avis*, *C. granii*, and an abundant species *T. nitzschioides*. Cryptophytes and green algae accounted for about 5% of the total phytoplankton abundance.

In July 2010, a decrease in abundance was recorded with a change in depth: from 116 thousand cells·L^{-1} on the surface to 86 thousand cells·L^{-1} at the bottom. Biomass was almost equal at all horizons: 0.41–0.43 g·m^{-3}. On the surface, dinoflagellates formed 40% of phytoplankton abundance and 31% of its biomass; at the bottom, 36 and 27%, respectively. Out of them, *Oxyrrhis marina*, *P. micans*, and representatives of *Amphidinium* and *Gymnodinium* were highly abundant on the surface, while *P. cordatum*, *G. fusiforme*, and *Katodinium glaucum* were abundant at the bottom. The biomass was mainly formed by large *Dinophysis fortii*, *P. divergens*, *D. lenticula*, and *Polykrikos kofoidii*, as well as by abundant *P. micans*, *O. marina*, and *K. glaucum*. Diatoms constituted 15–16% of abundance and 67–71% of biomass on the surface and at the bottom; *C. caspia*, *D. brightwellii*, *N. tenuirostris*, *T. nitzschioides*, and *P. calcar-avis* prevailed in abundance. The basis of biomass was formed by *C. granii*, *P. calcar-avis*, *D. brightwellii*, and *Coscinodiscus* sp. At the bottom, the role of *P. calcar-avis* rose (26% of the total biomass), and on the surface, the role of *D. brightwellii* increased (46% of biomass). High abundance of cryptophytes *Plagioselmis* spp. and cyanoprokaryotes of the genera *Oscillatoria*, *Planktolyngbya*, and *Aphanizomenon* was revealed: 30–38 and 9–10% of the total abundance, respectively. A brackish-water species *B. lauterbornii* and a marine one *Pterosperma undulatum* developed predominantly at the upper horizon forming 4% of the total abundance.

In July 2012, a relatively uniform vertical distribution of phytoplankton abundance and biomass was established (67–80 thousand cells·L^{-1} and 0.203–0.228 g·m^{-3}, respectively). The same as during the summer 2011, a coccolithophore *E. huxleyi* prevailed in abundance (47%). Its maximum (34–38 thousand cells·L^{-1}) was observed in the upper layer of the strait (0–5 m); the value decreased with depth (29 thousand cells·L^{-1}). Due to small size of its cells, *E. huxleyi* formed < 2% of the total biomass. Diatoms constituted 22% of abundance on average (from 15% at the bottom to 21–28% in the layer of 0–5 m) and 45% of biomass (from 33% on the surface and at the bottom to 67% at the horizon of 5 m). In terms of abundance, *P. pseudodelicatissima*, *T. nitzschioides*, and *P. calcar-avis* dominated, with the latter one forming about 42% of the total biomass. Intensive development of this thermophilic species is associated with high temperature on the surface of the strait (+23 °C).

C. affinis, *Proboscia alata*, *N. tenuirostris*, and *P. elongatum* were subdominants. Maximum values for the latter two species were recorded at the bottom. Dinoflagellates formed 24% of phytoplankton abundance and 53% of its biomass. *Gymnodinium* and *Gyrodinium* species developed in abundance; out of them, *Gymnodinium blax*, *G. simplex*, and *G. fusiforme* predominated. *P. cordatum*, *P. micans*, and *P. kofoidii* were abundant as well. Biomass of dinoflagellates was chiefly formed by large species *T. furca*, *P. kofoidii*, and *P. divergens*. Interestingly, the role of the first one increased at the bottom, while the second and third ones prevailed in the upper layer of the strait (0–5 m). *Dinophysis caudata*, *Phalacroma rotundatum*, *D. lenticula*, *P. micans*, and *Protoceratium reticulatum* contributed to formation of biomass as well. In the water layer from 5 m down to the bottom, cryptophytes predominated (5% of the total abundance); on the surface, a mesosaprobic species *Eutreptia lanowii*, a euglenid, vegetated, with abundance up to 2 thousand cells·L^{-1}. Out of green algae, a marine species *P. undulatum* was found.

In July 2013, diatoms *C. affinis* and *P. pseudodelicatissima* dominated (39% of abundance and 91% of the total biomass of phytoplankton). The species *C. affinis*, *P. pseudodelicatissima*, and *P. calcar-avis* formed 58% of the abundance and 96% of the biomass of diatoms. Against the backdrop of their intensive development, dinoflagellates formed no more than 27% of phytoplankton abundance and 8% of its biomass. Out of them, the key ones were small- and medium-sized species: *Gymnodinium nana*, *Gymnodinium* sp., *O. marina*, and *P. micans*. Biomass was chiefly formed by *O. marina*, *P. micans*, *S. acuminata*, and *Gyrodinium* sp. (96% of biomass of dinoflagellates). The share of cryptophytes and cyanoprokaryotes of the genera *Planktolyngbya* and *Anabaena* accounted for 30% of the total abundance of phytoplankton. Euglenids were characterized by low abundance (3%).

In August 2016, the highest values of abundance and biomass of planktonic algae (3,046 thousand cells·L^{-1} and 4.589 g·m^{-3}, respectively, *i. e.*, at the level characteristic of water bloom) were observed at individual stations in areas further from the coast; there, we recorded high values for a mesosaprobic dinoflagellate *P. micans* (272 thousand cells·L^{-1} and 2.960 g·m^{-3}) and for cyanoprokaryotes (2,411 thousand cells·L^{-1} and 0.197 g·m^{-3}). The lowest values (34–44 thousand cells·L^{-1} and 0.109–0.664 g·m^{-3}) were revealed at coastal stations. In general, cyanoprokaryotes, *Planktolyngbya limnetica* and *Anabaena* species, dominated in the studied area of the strait: 49 and 20% of the total abundance, respectively. Diatoms and dinoflagellates formed a significant part of the total abundance of phytoplankton (11 and 12%, respectively) and were the main component of its biomass (62 and 36%). Out of diatoms, *P. calcar-avis* developed in mass (5% of the total abundance of communities and 60% of their biomass). Also, we registered vegetation of *Leptocylindrus danicus*, *N. tenuirostris*, *T. nitzschioides*, and species of the genus *Pseudo-nitzschia* which formed 56% of the total abundance of diatoms. Out of dinoflagellates, *Gymnodinium* and *Heterocapsa* representatives prevailed in abundance, along with *P. micans* and *P. cordatum* (80% of dinoflagellates). A significant part of phytoplankton biomass was formed by *P. micans* cells (34%).

In August 2019, diatoms and dinoflagellates dominated accounting for an average of 57 and 28% of the total abundance of phytoplankton and 69 and 30% of its biomass. A coccolithophore *E. huxleyi* and cryptophytes formed no more than 13% of the total abundance. Other classes of planktonic algae provided less than 2% of abundance and 1% of biomass. The highest values (24 thousand cells·L^{-1} and 0.361 g·m^{-3}) were revealed in the water layer of 12–30 m; the indicators were 1.3–2 times lower on the surface (18 thousand cells·L^{-1} and 0.185 g·m^{-3}). At lower horizons (30 m), the maximum shares of abundance (71%) and biomass (92%) were formed by diatoms; at the horizons of 0–25 m, values decreased (52–56% of abundance and 64–78% of biomass). In the middle water layer (12–25 m),

shares of dinoflagellates in abundance and biomass were of 32 and 35%, respectively. In the lower layer (30 m), these algae formed 17% of abundance and 8% of biomass; on the surface, 26 and 21%. Values for cryptophytes in the entire studied water column were of 9–12% of the total abundance. On the sea surface, coccolithophores constituted more than 8% of the total phytoplankton abundance. Out of diatoms, *P. pseudodelicatissima* and *P. calcar-avis* were the most abundant species in the studied area. Subdominants *P. alata* and *T. nitzschioides* formed 6% of abundance of communities. Out of dinoflagellates, the key species were *P. micans*, *P. cordatum*, *S. acuminata*, *Pronoctiluca pelagica*, *K. glaucum*, and *Torodinium robustum*, as well as *Tripos*, *Gymnodinium*, and *Gyrodinium* representatives. The basis of phytoplankton biomass was formed by a large-cell diatom *P. calcar-avis* and dinoflagellates: *P. kofoidii*, *P. divergens*, *P. reticulatum*, *Tripos* species, and representatives of the genera *Prorocentrum*, *Gymnodinium*, and *Gyrodinium* prevailing in terms of abundance.

Autumn. In September 2011, cyanoprokaryotes, cryptophytes, and green algae dominated by abundance (50, 13, and 19% of the total abundance, respectively). However, those accounted for < 7% of phytoplankton biomass. In the Sea of Azov, the most abundant cyanoprokaryote was *P. limnetica*; less abundant ones were *Microcystis aeruginosa*, *Merismopedia punctata*, and representatives of *Aphanizomenon* and *Anabaena*. Out of green algae, *B. lauterbornii* and *Nannochloris* species prevailed; *Scenedesmus quadricauda*, *M. contortum*, and *Ankistrodesmus convolutus* developed at the level of subdominants. Diatoms and dinoflagellates formed no more than 7 and 10% of phytoplankton abundance, respectively, and the core of its biomass: 22 and 69%. Out of dinoflagellates, the prevailing species were *P. micans*, *P. cordatum*, *G. fusiforme*, *Ensiculifera carinata*, *K. glaucum*, *A. sanguinea*, *P. kofoidii*, *S. acuminata*, and *Gymnodinium* representatives. The basis of biomass was formed by *P. micans*, *E. carinata*, rare large-cell species of the genus *Protoperidinium*, and *P. kofoidii*. Out of diatoms, the main species were *C. caspia*, *P. pseudodelicatissima*, and *T. nitzschioides*, while subdominants included *P. calcar-avis* and *Thalassiosira* sp. In terms of biomass, a diatom *P. calcar-avis* and abundant *Thalassiosira* sp. were of greatest importance (58%). About 40% of biomass was formed by *C. caspia*, *D. brightwellii*, *T. nitzschioides*, and representatives of *Gyrosigma* and *Coscinodiscus*.

DISCUSSION

The hydrological regime of the Kerch Strait is governed by its shallowness, active water exchange between the Black Sea and Sea of Azov, and meteorological conditions [Sytnik et al., 2017]. Water masses heat up and cool down quickly throughout the water column. The annual course of water temperature is characterized by significant amplitude with a pronounced minimum in February–March (down to −1 °C) and a maximum in July–August (up to +30 °C); mean long-term values are +2.0 and +24.2 °C, respectively. The mean annual water temperature in the strait is about +13 °C. In the deeper central area of the strait, the wave height can be of 2–3 m, while off the coast, especially with the wind blowing from the shore, the wave height does not exceed 1.5 m. Water salinity usually fluctuates 12.0 to 18.0‰ and depends on the prevailing type of currents. Wind conditions in the strait area are varied which is determined by the diversity of synoptic situations and the nature of the relief. The frequency of calms is 1 to 2%. The mean annual wind speed is 5.3–6.9 $m \cdot s^{-1}$, and the maximum one can be of 40 $m \cdot s^{-1}$. Water masses in the strait are easily identified by salinity and content of biogenic elements and also visually: by water color and transparency. The total frequency of currents for the year, with direction of mixed flows taken into account, averages 62% for the Sea of Azov flow and 38% for the Black Sea one. With southern winds, the Black Sea current becomes dominant.

A total of 154 algal species were registered in phytoplankton of the Kerch Strait, Dinskoy Bay, and Taman Bay (Table 4). Those are diatoms, dinoflagellates, green algae, ochrophytes, cryptophytes, cyanoprokaryotes, prasinophytes, haptophytes, and euglenophytes. Phytoplankton is represented by marine, freshwater, and brackish-water species. Such diversity was facilitated by wide ranges of water salinity and temperature. High values of phytoplankton biomass were confined to the warm season: October–December 2007, August 2008 and 2009, and July 2010. Medium ones were noted in May 2003, October 2005, June and September 2008, August 2011, and March 2020. Low values were observed at other times. In August 2009, abundant development of planktonic algae (at the level characteristic of water bloom) was recorded. At other times, abundance of phytoplankton cells was noticeably lower. The results of studies carried out in the western and central Kerch Strait in August 2009 showed as follows: within this relatively small water area, values of phytoplankton abundance and biomass can vary by several orders of magnitude [Bryantseva et al., 2010].

In the Kerch Strait area, significant seasonal and interannual fluctuations in species richness, abundance, and biomass of planktonic algae were noted. In spring, small diatoms *S. costatum* and *C. caspia* dominated which is typical for this time of year [Makarevich, 2022]. In summer, both small diatoms (*P. pseudodelicatissima*, *C. caspia*, and *T. nitzschioides*) and large ones (*P. calcar-avis* and *D. brightwellii*) prevailed, as well as relatively small dinoflagellates of the genera *Gymnodinium*, *Gyrodinium*, *Oxyrrhis*, and *Prorocentrum* and large ones representing *Dinophysis*, *Tripos*, *Polykrikos*, and *Protoperidinium*. The role of large species of diatoms and dinoflagellates usually increases in the Black Sea and Sea of Azov during late summer and autumn [Makarevich, 2022; Yasakova, Makarevich, 2017]. In autumn 2011, the most abundant algae were a cyanoprokaryote (*P. limnetica*), cryptophytes of the genus *Plagioselmis*, and green algae representing *Binuclearia* and *Nannochloris*. The probable reason for the dominance of brackish-water forms of planktonic flora was a drop in salinity values in September 2011 due to a strong northeastern wind preceding the investigations: it formed the Sea-of-Azov type of waters in the Kerch Strait [Ivanov et al., 2014]. Throughout the study period, the basis of phytoplankton biomass were large species of diatoms (*P. calcar-avis*) and dinoflagellates (*Protoperidinium* and *Tripos* representatives). Interestingly, *P. micans* often causes red tides in the shallow northwestern Black Sea, and the bloom of cyanoprokaryotes during formation of a blocking layer can result in hypoxia in the coastal zone of the Sea of Azov [Matishov, Fushtei, 2003; Nesterova, 2001].

The development of a coccolithophore *E. huxleyi* in the Kerch Strait area was observed in June 2011, July 2012, and May 2013, and this species accounted for 34–47% of phytoplankton abundance. In the Black Sea, abundance of this alga tends now to reach the level characteristic of water bloom annually; moreover, there is a trend towards its intensifying development which is confirmed by satellite observations [Mikaelyan et al., 2006, 2011; Silkin et al., 2009]. An abnormally intensive and long-lasting (May to July) *E. huxleyi* bloom in the Black Sea was registered in 2012 after a cold winter and active wind mixing [Yasakova, Stanichny, 2012]. Due to the ability of this species to regulate CO_2 level in the atmosphere and, accordingly, to affect the temperature regime and climatic conditions of our planet, investigations on the dynamics of *E. huxleyi* development in the modern period are of particular importance [Yasakova et al., 2017].

In August 2019, phytoplankton abundance and biomass in the Kerch Strait were noticeably lower than in 2016: by 32 and 6 times, respectively. The likely reason for such a drop in abundance could have been the construction of hydraulic structures of the Crimean Bridge in 2017–2019. A significant content

of heavy metals (1–2.5 MPC) and petroleum products in bottom sediments could negatively affect phytoplankton abundance and photosynthetic activity of algae sensitive to the darkening of the photic layer and to occurrence of various pollutants in water (Table 5).

Table 4. Main hydrological characteristics, number of taxa, and mean abundance and biomass of phytoplankton in the Kerch Strait and adjacent waters in 1989–2020

Study period	The Kerch Strait area	Number of species	Water temperature, °C / salinity, ‰	Abundance, thousand cells·L^{-1}	Biomass, mg·m^{-3}	Reference
May–September 1989	The northern area	90 taxa	+17…+25 / 11–16	–	–	Chernikova, 2004
June 1997 and 2000; December 1998 and 1999	The Kerch Strait, Dinskoy Bay, and Taman Bay	154	0…+26 / 5–27	–	–	Kovaleva, 2008
November 2003	The southern area	44	–	43.5	81.9	Zaremba, 2011
October 2005	–″–	48	–	69.1	355.1	–″–
October 2007	–″–	46	–	275.6	1,514.7	–″–
September 2008	–″–	46	–	79.5	378.6	–″–
June 2009	The southern area	47	–	27.8	111.3	Zaremba, 2013
June 2010	–″–	46	–	26,9	263,3	–″–
June 2011	–″–	42	–	90,0	202,3	–″–
June 2012	–″–	54	–	65.9	209.2	–″–
October 2007	The central area	–	–	220	4,500	Matishov et al., 2013
November 2007	–″–	42	+7.2…+11 / –	365	5,800	–″–
December 2007	–″–	–	–	250	1,500	–″–
April 2008	–″–	–	–	405	200	–″–
June 2008	–″–	–	–	145	400	–″–
August 2008	–″–	–	–	205	1,200	–″–
August 2009	The central and western areas	27	– / 11–14	2,298 ± 1,945 (96.12–9,754.4)	4,128 ± 2,023 (162.2–9,887.55)	Bryantseva et al., 2010
August 2011	The central area	72	+27.6 / 12.22–16.59	45.6	412.95	Trotsenko et al., 2012
May–December 2000–2011	The southern area	–	+5…+27 / 15.11–17.78	–	436 (80–1,400)	Zhugailo et al., 2011
September 2018	The southern area	84	+20…+21 / –	105.7 ± 22	227 ± 32	Remizova, Teyubova, 2021
March 2020	The central area	33	–	389.2 (356.8–421.6)	426.4 (346.0–506.7)	Zagorskaya et al., 2021
June 2020	–″–		–	582.9 (553.3–612.4)	66.1 (53.1–79.0)	–″–
August 2020	–″–		–	37.9 (35.1–40.6)	28.4 (26.2–30,6)	–″–
November 2020	–″–		–	24.6 (20.6–28.5)	35.8 (34.1–37.4)	–″–

Note: a dash (–) denotes no data; in parentheses, the range of values are given.

Table 5. The content of pollutants in bottom sediments (from the surface layer of the bottom, 0.0–0.2 m) of the Crimean Bridge construction area [Sytnik et al., 2017]

Chemical element / pollutant	MPC, mg·kg^{-1}	Content in sediment samples, mg·kg^{-1}
Mercury	2.1	3.6–5.5
Petroleum products	no data	725.9–1,147.4
Lead	32	14.3–27.4
Arsenic	2	0.9–1.8
Cadmium	0.5	0.6–1.32
Nickel	20	1.1–3.2
3.4-benz(a)pyrene	0.01	≤ 0.01

Conclusions:

1. Taxonomic composition of phytoplankton in the Kerch Strait in 2009–2019 included 114 species of microalgae. The highest species diversity of diatoms was recorded in spring (April 2009), and that of dinoflagellates, in summer (June 2011 and August 2019). Species richness of representatives of other phytoplankton classes varied slightly depending on the season. Waters of the strait were mainly characterized by occurrence of diatoms, dinoflagellates, and coccolithophores common to the Black Sea and Sea of Azov, as well as brackish-water species and periphytic diatoms.
2. The highest similarity rates for taxonomic composition of microalgal communities of the Kerch Strait [Sørensen–Czekanowski (Dice) coefficient ≥ 60%] were registered during the warm season (June to September).
3. In spring, small-cell diatoms dominated; in summer, coccolithophores prevailed, as well as small and large diatoms and dinoflagellates; and in late summer and autumn, the role of cyanoprokaryotes and large diatoms and dinoflagellates increased.
4. Phytoplankton abundance in the Kerch Strait area in 2009–2019 varied from 19 to 719 thousand cells·L^{-1}, and its biomass, from 0.057 to 1.92 g·m^{-3}. The highest values were observed in August 2016 when cyanoprokaryotes and dinoflagellates were the most abundant. At this time, biomass was chiefly formed by a large diatom *Pseudosolenia calcar-avis* and abundant dinoflagellate *Prorocentrum micans*. High biomass values for the summer of 2010, 2012, and 2013 were determined by the occurrence of a large-cell species *P. calcar-avis* in plankton. This alga developed intensively due to the onset of warm weather.
5. Vertical distribution of phytoplankton abundance and biomass was uneven and depended on the hydrodynamic activity of waters.
6. The construction of hydraulic structures of the Crimean Bridge in 2017–2019 seemed to cause a noticeable decrease in phytoplankton abundance and biomass in the Kerch Strait during the late summer 2019: the values dropped by 32 and 6 times, respectively, compared to those for 2016.

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ВИДОВОЙ СОСТАВ, ЧИСЛЕННОСТЬ И БИОМАССА ФИТОПЛАНКТОНА В КЕРЧЕНСКОМ ПРОЛИВЕ В 2009–2019 ГГ.

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В работе представлены результаты исследований планктонных водорослей Керченского пролива в весенне-осенний период 2009–2019 гг. В составе фитопланктона обнаружено 114 видов и несколько таксонов, определённых до рода, из 11 классов водорослей, в том числе 64 вида динофитовых и 32 вида диатомовых. Средние значения численности и биомассы — 140 тыс. кл.·л$^{-1}$ и 0,386 г·м$^{-3}$ соответственно. Цианопрокариоты доминировали по численности (44 % общего числа клеток). Диатомовые и динофитовые составили основу (62 и 35 %) биомассы и значительную часть численности фитопланктона (19 и 18 %). Представители криптофитовых, кокколитофорид и зелёных водорослей в сумме формировали 18 % общего обилия фитопланктона. Весной доминировали мелкие диатомовые *Skeletonema costatum* и *Cyclotella*

caspia. В летний период преобладали мелкие и крупные виды диатомовых и динофитовых, а также кокколитофорида *Emiliania huxleyi*. Осенью наиболее многочисленными были цианопрокариоты (*Planktolyngbya limnetica*), криптофитовые (из рода *Plagioselmis*) и зелёные водоросли (из родов *Binuclearia* и *Nannochloris*). Диатомовые (*Pseudosolenia calcar-avis*) и динофитовые из родов *Prorocentrum*, *Protoperidinium* и *Ceratium* формировали основу биомассы фитопланктона.

Ключевые слова: фитопланктон, таксономический состав, численность, биомасса, Керченский пролив

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THE KUBAN RIVER BASIN, A NEW PAGE IN THE EXPANSION OF THE ASIAN CLAM *CORBICULA FLUMINEA* (O. F. MÜLLER, 1774) (BIVALVIA: CYRENIDAE)

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The invasive bivalve *Corbicula fluminea* (O. F. Müller, 1774) was found in the Kuban River basin. Three live Asian clams were recorded in the Protoka River near the settlement of Grivenskaya (Krasnodar Krai) in the autumn of 2022. Assumably, high invasive potential of this species and its ability to withstand salinity up to 5‰ will allow the clam to inhabit not only freshwater bodies, but also estuarine zones of rivers and Azov limans. *C. fluminea* is a food item for fish, and its naturalization can increase the resource potential of water bodies in the south of Russia.

Keywords: invaders, biological invasion, European Russia, freshwater and estuarine ecosystems

The freshwater gold clam *Corbicula fluminea* (O. F. Müller, 1774) is a bivalve with a typical habitat in Guangzhou, China [Araujo et al., 1993]. In the early XX century, this mollusc penetrated into North and South America [Araujo et al., 1993; Counts, 1981]; since the 1980s, it has been actively settling in freshwater and estuarine water bodies of Europe [Allen, 2019]. In 2015, this bivalve was first found in European Russia – in the basin of the Northern Dvina River [Bespalaya et al., 2016] and in the Gorky Reservoir of the Volga River [Pryanichnikova et al., 2019]. In 2017, *C. fluminea* was recorded in the Don River basin [Zhivoglyadova, Revkov, 2018]. The listed findings were confined to water bodies heated by discharge warm water from the Arkhangelsk, Kostroma, and Novocherkassk power plants. Later, the Asian clam was registered in the Don basin outside the anomalous temperature zone – in the main river bed, below the Manych River mouth [Zhivoglyadova et al., 2018].

In 2013–2018, findings of a closely related species, *Corbicula fluminalis* (O. F. Müller, 1774), were reported for water bodies of the Caspian Sea coast [Khlopkova et al., 2019; Nabozhenko, Nabozhenko, 2016]. In 2019 and 2021, *C. fluminea* settlements were noted in the Dagestan sector of the Caspian Sea, including those in sympatry with *C. fluminalis* [Khlopkova et al., 2023].

This communication provides data on *C. fluminea* record in the basin of the Kuban River, a major waterway in the south of Russia. Three live freshwater gold clams were found during ichthyological survey in November 2022 in the Protoka River (right branch of the Kuban River) near the settlement of Grivenskaya (Kalininsky District, Krasnodar Krai). Coordinates of the spot are N45.656877°, E38.129956°. Bivalves were noted in shallows along the water edge. The substrate of the sampling area is represented by silty sand and pebbles. Shell length (maximum distance between its anterior and posterior ends) was 14.8–16.3 mm; shell height (maximum dorsoventral size measured from the top) was 14.3–15.7 mm; and shell convexity (maximum distance with closed valves) was 10.7–11.8 mm. Weight of specimens after mantle fluid was removed amounted to 1.4–2.1 g.

The species was identified based on conchological characters [Hubenov et al., 2013; Kamburska et al., 2013; Korniushin, 2007; Son, 2007; Zhadin, 1952]. Shells of all the clams are oval-triangular, with a broad top in the center (Fig. 1). Shell radial ribs are well pronounced. Each specimen has 10 ribs *per* 1 cm of its height. The inner surface of shells is light-colored, with violet darkening in the area of muscle scars.

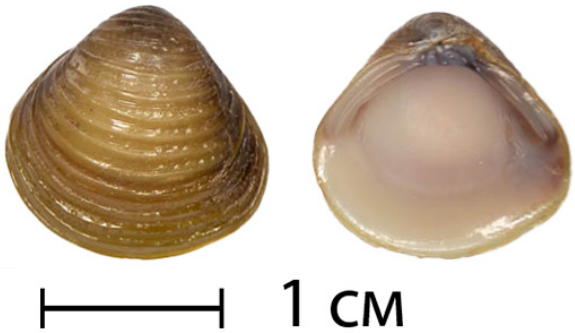

Fig. 1. Shell of *Corbicula fluminea* from the Protoka River (right branch of the Kuban River)

The success of global expansion of *Corbicula* representatives is likely to be related to their effective reproductive strategy and ecological plasticity [Allen, 2019]. These clams can reproduce both sexually and by producing clones [Pigneur et al., 2011]. Also, those are known for their early maturity (starting from 3 months of age) and high fecundity (up to 570 pediveligers *per* day) [McMahon, 2000]. These bivalves can inhabit various substrates [Sousa et al., 2008], are not demanding to the type of water body, and successfully survive in different ecological environments – from drainage channels to large rivers, ponds, lakes, and estuaries [Karatayev et al., 2007; McMahon, 2000; Sousa et al., 2008]. These molluscs are relatively resistant to hypoxia: with oxygen concentration 1–3 mg·L^{-1}, their growth slows down, but they remain viable [Karatayev et al., 2007]. Moreover, *C. fluminea* is more tolerant to salinity compared to freshwater mussels, such as unionids and *Dreissena bugensis* (Andrusov, 1897). Under natural conditions, the Asian clam tends to inhabit only upper estuarine zones with salinity up to 5‰; however, features of osmoregulation allow this species to withstand salinity up to 14–17‰ [Karatayev et al., 2007; McMahon, 2000].

The distribution of *C. fluminea* in the Kuban River is still unclear. However, given its high ecological plasticity and tolerance to salinity, it can be assumed as follows: the distribution of the freshwater gold clam and its naturalization are possible in freshwater bodies of the lower river basin, including reservoirs and irrigation canals, and in estuarine zones of water bodies in the south of Russia.

Potential recipient ecosystems include the Azov limans as well, with water salinity being up to 5‰. Since small clams (up to 5 mm in size) are a food object for benthos-feeding fish [Robinson, Wellborn, 1988], the new invasive species may become an additional food resource and increase the receiving capacity of water bodies.

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БАССЕЙН РЕКИ КУБАНЬ — НОВАЯ СТРАНИЦА В ЭКСПАНСИИ АЗИАТСКОГО МОЛЛЮСКА *CORBICULA FLUMINEA* (O. F. MÜLLER, 1774) (BIVALVIA: CYRENIDAE)

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В бассейне реки Кубань обнаружен двустворчатый инвазивный моллюск *Corbicula fluminea* (O. F. Müller, 1774). Три живые особи найдены в реке Протока вблизи станицы Гривенская Краснодарского края осенью 2022 г. Предполагается, что высокий инвазионный потенциал вселенца и способность осваивать среды с солёностью до 5 ‰ позволят этому виду заселить не только пресноводные водоёмы, но и эстуарные зоны рек и азовские лиманы. Моллюск является кормовым объектом рыб, его натурализация может увеличить ресурсный потенциал водных объектов юга России.

Ключевые слова: вселенцы, биологическая инвазия, европейская часть России, пресноводные и эстуарные экосистемы

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THE FIRST FINDINGS OF NEW SPECIES OF AMPHIPODS IN THE SEA OF AZOV

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In 2022–2023, 8 species and 4 genera of Amphipoda new to the Sea of Azov were found near the Cape Kazantip (the Crimea; Golubniki, Russkaya, and Shirokaya bays). All specimens are stored in IBSS Collection of Hydrobionts of the World Ocean. The following species were recorded: *Ampelisca sevastopoliensis* Grintsov, 2011 (the family Ampeliscidae); *Apohyale crassipes* (Heller, 1866) (Hyalidae); *Microprotopus* cf. *maculatus* (Microprotopidae); *Monocorophium insidiosum* (Crawford, 1937) (Corophiidae); *Nototropis massiliensis* (Bellan-Santini, 1975) (Atylidae); *Orchestia mediterranea* A. Costa, 1853 (Talitridae); *Orchestia montagui* Audouin, 1856 (Talitridae); and *Pleonexes helleri* (Karaman, 1975) (Ampithoidae). New genera were registered: *Apohyale* Bousfield & Hendrycks, 2002; *Monocorophium* Bousfield & Hoover, 1997; *Nototropis* A. Costa, 1853; and *Pleonexes* Spence Bate, 1857. Seven species were represented by adult males and females, as well as juveniles. Two *Orchestia* species were identified by adult males. Individuals of species new to the Sea of Azov were found in the coastal zone in the following biotopes: supralittoral, macrophytes on the beach (*O. mediterranea* and *O. montagui*); detached macrophytes off the coast (*A. crassipes*); sand on the bottom at a depth of 0.2–1.5 m (*A. sevastopoliensis* and *N. massiliensis*); seagrass beds (*M. insidiosum* and *Microprotopus* cf. *maculatus*); and attached macrophytes on the bottom at a depth of 0.2–1.0 m (*P. helleri*). The occurrence of these species in the Sea of Azov may be associated with an increase in the salinity of its waters.

Keywords: Amphipoda, the first findings, Sea of Azov

New crustacean species periodically invade the Sea of Azov [Timofeev, Bondarenko, 2022; Timofeev et al., 2019], and the order Amphipoda is no exception. In recent years, we have recorded 9 species new for the Sea of Azov and 5 new genera. The material was sampled from various biotopes: pebble-sandy and rocky beaches (splash), sand on the bottom, algal associations, periphyton, and rocks on the bottom (Fig. 1). Qualitative samples were taken by hand or with a scraper (0.5-mm net mesh) or a frame with a net (0.5-mm net mesh). The samples were fixed with 96% ethanol. In the laboratory, amphipods were selected from the samples under an MBS-9 microscope at ×16 or ×32 magnification.

In the subcollection of amphipods of IBSS Collection of Hydrobionts of the World Ocean, the following species are stored:

- *Ampelisca sevastopoliensis* Grintsov, 2011 (No. IBSS.bent.567Amph.as. V10);
- *Apohyale crassipes* (Heller, 1866) (No. IBSS.bent.568Amph.ac. V200);
- *Microprotopus* cf. *maculatus* (No. IBSS.bent.569Amph.mm. V20);

- *Monocorophium insidiosum* (Crawford, 1937) (No. IBSS.bent.569Amph.mm. V20);
- *Nototropis massiliensis* (Bellan-Santini, 1975) (No. IBSS.bent.571Amph.nm. V1);
- *Orchestia mediterranea* A. Costa, 1853 (No. IBSS.bent.572Amph.omd. V1);
- *Orchestia montagui* Audouin, 1856 (No. IBSS.bent.573Amph.omt. V3);
- *Pleonexes helleri* (Karaman, 1975) (No. IBSS.bent.575Amph.ph. V5).

Fig. 1. Map of the sampling

The following species were recorded: *Ampelisca sevastopoliensis* Grintsov, 2011 (the family Ampeliscidae) (Fig. 2A); *Apohyale crassipes* (Heller, 1866) (Hyalidae) (Fig. 2B); *Microprotopus* cf. *maculatus* (Microprotopidae) (Fig. 2C); *Monocorophium insidiosum* (Crawford, 1937) (Corophiidae) (Fig. 2D); *Nototropis massiliensis* (Bellan-Santini, 1975) (Atylidae) (Fig. 2E); *Orchestia mediterranea* A. Costa, 1853 (Talitridae) (Fig. 2F); *Orchestia montagui* Audouin, 1856 (Talitridae) (Fig. 2G); and *Pleonexes helleri* (Karaman, 1975) (Ampithoidae) (Fig. 2H).

The following new genera were registered: *Apohyale* Bousfield & Hendrycks, 2002; *Monocorophium* Bousfield & Hoover, 1997; *Nototropis* A. Costa, 1853; and *Pleonexes* Spence Bate, 1857. Seven Amphipoda species (with the exception of two *Orchestia* species) were represented by both adults and juveniles. Two *Orchestia* representatives were identified by adult males (*Orchestia* females and juveniles are morphologically similar). All taxa were previously reported for the Black Sea [Grintsov, 2022, 2023; Grintsov, Sezgin, 2011]. The appearance of these species in the Sea of Azov seems to be related to the recent increase in the salinity level of its waters [Kosenko et al., 2017].

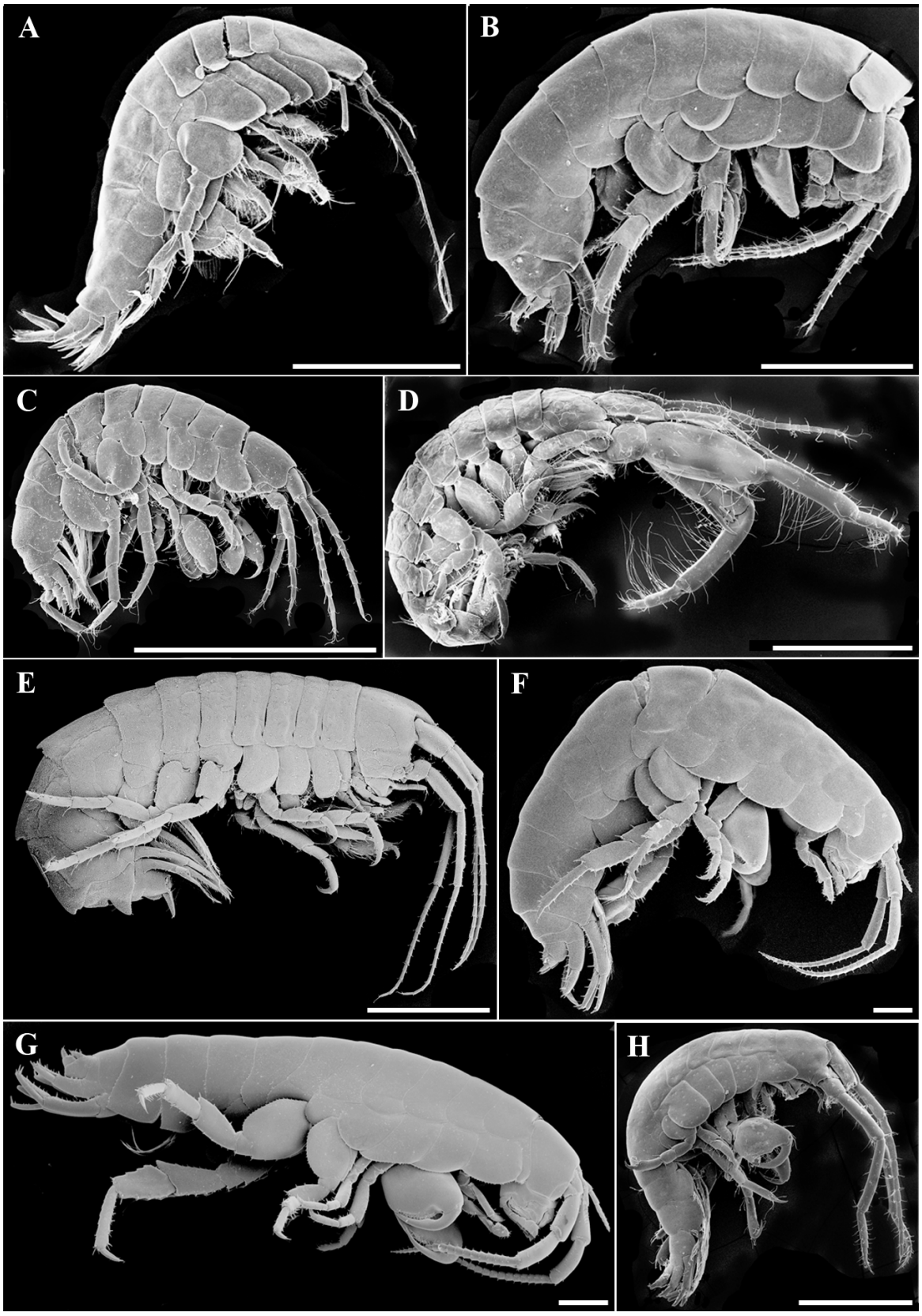

Fig. 2. Habitus of adult Amphipoda specimens not registered in the Sea of Azov earlier: A, *Ampelisca sevastopoliensis*, female; B, *Apohyale crassipes*, male; C, *Microprotopus* cf. *maculatus*, male; D, *Monocorophium insidiosum*, male; E, *Nototropis massiliensis*, male; F, *Orchestia mediterranea*, male; G, *Orchestia montagui*, male; H, *Pleonexes helleri*, male. Scale bars are 1 mm

All Amphipoda species were found in the coastal area of the Sea of Azov near the Cape Kazantip at depths of 0–1.5 m. *Orchestia* species were noted in supralittoral in algae discharge and under stones (the Shirokaya Bay, the Kazantip Nature Reserve). Few *A. sevastopoliensis* individuals were recorded on sand on the bottom at a depth of 0.2–1.5 m in the Russkaya Bay which borders the Kazantip Nature Reserve. *A. crassipes* was registered in mass in the Shirokaya Bay in aggregations of detached macrophytes. Numerous *Microprotopus* cf. *maculatus* individuals were found on seagrasses off the coast in the Russkaya Bay area which borders the Golubniki Bay belonging to the Cape Kazantip. *M. insidiosum* was noted there in mass as well. Few *N. massiliensis* individuals were recorded on sand on the bottom, in aggregations of detached macrophytes, and in bivalve aggregations in the Russkaya Bay at a depth of 0.5–1.5 m. *P. helleri* was registered in all the studied bays on macrophytes off the coast.

This work was carried out within the framework of IBSS state research assignment "Comprehensive study of the functioning mechanisms of marine biotechnological complexes with the aim of obtaining bioactive substances from hydrobionts" (No. 124022400152-1).

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ПЕРВЫЕ НАХОДКИ НОВЫХ ВИДОВ АМФИПОД В АЗОВСКОМ МОРЕ

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В 2022–2023 гг. у мыса Казантип (полуостров Крым, бухты Русская, Голубники и Широкая) обнаружены 8 видов и 4 рода амфипод, новых для Азовского моря. Все экземпляры хранятся в подколлекции амфипод Коллекции гидробионтов Мирового океана ФИЦ ИнБЮМ.

Зарегистрированы следующие виды: *Ampelisca sevastopoliensis* Grintsov, 2011 (семейство Ampeliscidae); *Apohyale crassipes* (Heller, 1866) (Hyalidae); *Microprotopus* cf. *maculatus* (Microprotopidae); *Monocorophium insidiosum* (Crawford, 1937) (Corophiidae); *Nototropis massiliensis* (Bellan-Santini, 1975) (Atylidae); *Orchestia mediterranea* A. Costa, 1853 (Talitridae); *Orchestia montagui* Audouin, 1856 (Talitridae) и *Pleonexes helleri* (Karaman, 1975) (Ampithoidae). Отмечены новые для Азовского моря роды: *Apohyale* Bousfield & Hendrycks, 2002; *Monocorophium* Bousfield & Hoover, 1997; *Nototropis* A. Costa, 1853 и *Pleonexes* Spence Bate, 1857. Семь видов представлены взрослыми самцами и самками, а также молодью. Два вида из рода *Orchestia* идентифицированы по взрослым самцам. Особи новых для Азовского моря видов обнаружены в прибрежной зоне в следующих биотопах: супралитораль, макрофиты на пляже (*O. mediterranea* и *O. montagui*); неприкреплённые макрофиты у берега (*A. crassipes*); песок на дне на глубине от 0,2 до 1,5 м (*A. sevastopoliensis* и *N. massiliensis*); заросли морской травы (*M. insidiosum* и *Microprotopus* cf. *maculatus*) и прикреплённые макрофиты на дне на глубине от 0,2 до 1,0 м (*P. helleri*). Появление этих видов в Азовском море может быть связано с повышением в нём солёности вод.

Ключевые слова: Amphipoda, первые находки, Азовское море

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ABOUT FINDING *POLYDORA WEBSTERI* HARTMAN IN LOOSANOFF & ENGLE, 1943 (ANNELIDA: SPIONIDAE) IN THE SEA OF AZOV

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The research was carried out in 2023–2024 near the Kazantip Peninsula (the Sea of Azov). In this area, blisters were found in the shells of the mussel *Mytilus galloprovincialis* for the first time. The blisters occupied ⅕ to ⅓ of the shells area. The blisters contained boring polychaetes. Polychaetes were identified as *Polydora websteri* Hartman in Loosanoff & Engle, 1943 (Annelida: Spionidae). The results obtained must be taken into account when planning and organizing mussel farms in this area.

Keywords: Polychaeta, invasive species, *Mytilus galloprovincialis*, mariculture

The Sea of Azov is a promising area for aquaculture development. Already in the middle of the XX century, there were recommendations to farm the bivalve *Mytilus galloprovincialis* Lamarck, 1819 in the northern Sea of Azov [Spichak, 1979]. Another potential commercial species is the bivalve *Anadara kagoshimensis* (Tokunaga, 1906), the mollusc that invaded the Sea of Azov and formed dense settlements there [Syomin et al., 2021]. When selecting areas for the organization of mariculture farms, data are required on biology and ecology of molluscs under natural conditions. Studying morphophysiological characteristics of the above-mentioned species, we found cavities filled with black detritus, *i. e.*, blisters containing polychaetes, in *M. galloprovincialis* shells. The aim of this work is to identify polychaetes registered in the bivalve shells from the Sea of Azov.

The research was carried out June 2023 to January 2024 in the Kazantip Peninsula vicinity (the Sea of Azov) (Fig. 1). Bivalves were sampled in the Russkaya Bay (45°26′58″N, 35°49′29″E; 0.1-m depth; sandy-silty sediments), the Golubniki Bay (45°27′14″N, 35°49′6″E; 1.5-m depth; coastal rocks), and the Shirokaya Bay (45°28′19″N, 35°51′8″E; 0.5-m depth; rocky-sandy sediment).

To determine salinity, an OHAUS Starter ST20S meter was used. The value in the bays was of 12.9‰. Monthly, 30 specimens of each species were sampled in the bays investigated. The hydrobionts were measured with a caliper with 0.1-mm accuracy and opened with a scalpel. The contents of the blisters were viewed under an MBS-10 binocular. The polychaetes found in the blisters were removed and placed in Petri dishes with seawater for further identification. Photographs were taken with a Sony Cyber-shot camera.

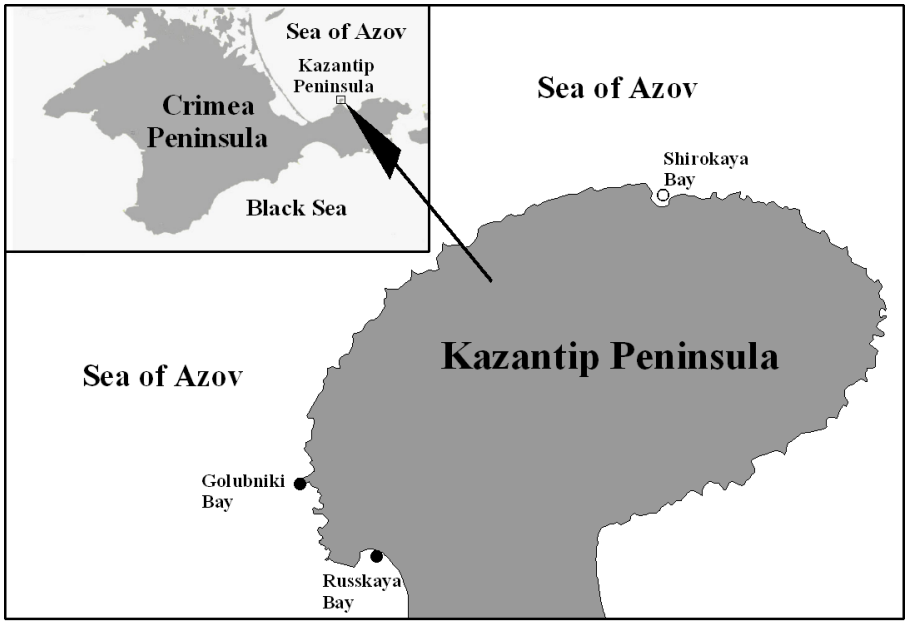

Fig. 1. Map of the study area

Blisters on the inside of the shells were registered in mussels sampled in September and December 2023 in the Russkaya Bay. Out of 30 hydrobionts examined, 2 specimens were affected which amounted to 7% of the sample. In the Golubniki Bay in November, the blisters were noted in 8 specimens which accounted for 27% of the sample. Sizes of the affected mussels were as follows: shell length, (57.7 ± 2.9) mm; height, (30.1 ± 1.6) mm; and width, (22.0 ± 1.4) mm. The blisters occupied ⅕ to ⅓ of the shell surface (Fig. 2A). In the Shirokaya Bay, affected mussels were not recorded. In *A. kagoshimensis* shells, there were no blisters.

One live polychaete was found inside each blister in the shells of mussels from the Russkaya Bay. The blisters of specimens from the Golubniki Bay were filled with black detritus with the smell of hydrogen sulfide; extracted worm fragments were macerated, and only 2 live polychaetes were extracted from one blister. A total of 6 worms were extracted. The live polychaetes were yellow, with translucent red blood vessels (Fig. 2B). The body length was 20–25 mm, and the number of chaetigers was 80–90.

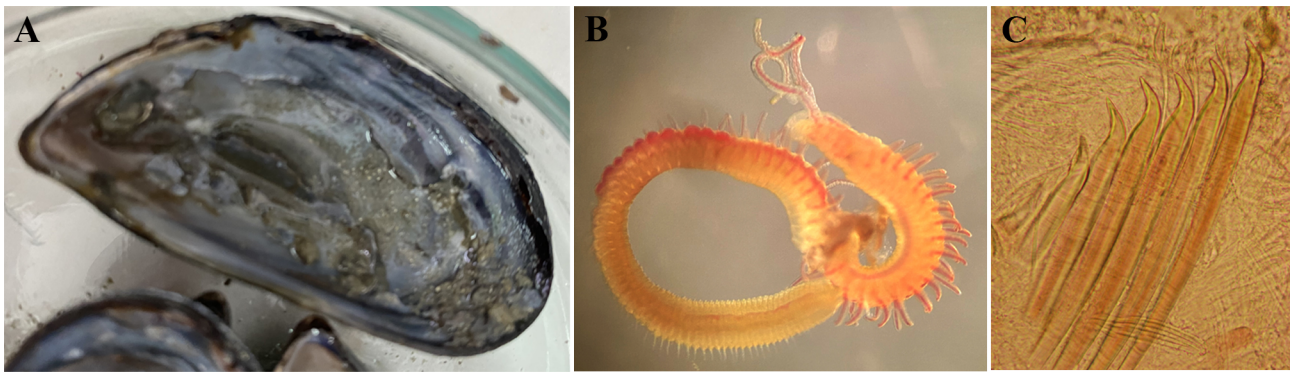

Fig. 2. *Polydora websteri*: A, a mussel shell with a blister; B, *Polydora* from a blister; C, chaetae of chaetiger V

The maximum-size worm had 97 chaetigers and reached 28 mm in length and 0.6 mm in width. Prostomium with small notch in front. Caruncle to middle of chaetiger III; no occipital papilla. Eyes 2 pairs, black in color. Palps long (to about 10–12 chaetigers), transparent, with 2 black longitudinal pigmented stripes along the groove. Notochaetae absent on chaetiger I, with 3–4 simple chaetae in neuropodia. From chaetiger II to chaetiger IV, only chaetae in dorsal and ventral branches of parapodia. Chaetiger V with large specialized dorsal chaetae (6–7), without lateral denticle; in some specimens, a ridge is clearly visible, and accompanying chaetae are lanceolate (Fig. 2C). Abdominal chaetae capillary. In neuropodia from chaetiger VII, hooded bidentate hooked chaetae appear (5–9), while in notopodia, only capillary chaetae appear. Branchiae begin from chaetiger VII; those are absent on the last 24–32 chaetigers. Pygidium small, in the form of a rounded anal papilla, with a notch on the dorsal side. By morphological characters, the polychaetes extracted from the blisters corresponded to the descriptions of *Polydora websteri* Hartman in Loosanoff & Engle, 1943 [Radashevsky, 1999; Read, 2010; Surugiu et al., 2012] and also were similar to *Polydora* representatives found earlier in the Black Sea and in the Kerch Strait [Lisitskaya et al., 2010; Syomin et al., 2021]. The obtained material is stored in IBSS Collection of Hydrobionts of the World Ocean (IBSS.bent.: 1Ann.aa.v1; 2Ann.aa.v2; 3Ann.aa.v3).

P. websteri is a widespread species in the World Ocean. This *Polydora* representative perforates calcareous substrates, as well as shells of gastropods and bivalves; it is one of the main pests of molluscs grown on marine farms [Read, 2010]. In the Black Sea, *P. websteri* was registered for the first time in 2005 among rocks off the Romanian coast [Surugiu, 2005]. In the following years, the polychaete widely distributed in the northern Black Sea. This worm was reported from shells of cultured oyster *Crassostrea gigas* (Thunberg, 1793), shells of the invasive mollusc *Rapana venosa* (Valenciennes, 1846), and limestones off the Crimean coast [Boltachova et al., 2021; Bondarev, Boltachova, 2021; Lisitskaya et al., 2010; Surugiu et al., 2012]. In the Black Sea, *P. websteri* was not found on mussel shells. In the Sea of Azov, this species was not registered earlier [Kiseleva, 2004]. In 2020, this boring polychaete was noted in the Kerch Strait on the invasive bivalve *A. kagoshimensis* [Syomin et al., 2021].

The expansion of ranges of both native and invasive species is likely to result from an increase in the Sea of Azov salinity up to 14.83‰ [Berdnikov et al., 2022]. As believed, the mass development of *Anadara* in the Sea of Azov after the salinization contributed to the distribution of the boring polychaete [Syomin et al., 2021]. *Polydora* larvae are known to settle on calcareous substrate. In the Sea of Azov, rocky sediments are rare, and native mollusc species have thin-walled shells. Apparently, due to the lack of substrate required, *P. websteri* larvae can settle not only on denser *Anadara* shells, but also on mussel shells.

Thus, our results show that the polychaete *Polydora websteri* has distributed in the Sea of Azov. This fact should be taken into account when planning and organizing mussel farms in this area.

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О НАХОЖДЕНИИ *POLYDORA WEBSTERI* HARTMAN IN LOOSANOFF & ENGLE, 1943 (ANNELIDA: SPIONIDAE) В АЗОВСКОМ МОРЕ

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Исследование проводили в 2023–2024 гг. в районе полуострова Казантип, Азовское море. Впервые в раковинах мидии *Mytilus galloprovincialis* обнаружены заполненные детритом блистеры, которые занимали от ⅕ до ⅓ площади створок. В блистерах находились полихеты-перфораторы. Полихеты идентифицированы как *Polydora websteri* Hartman in Loosanoff & Engle, 1943 (Annelida: Spionidae). Полученные данные необходимо учитывать при планировании и организации мидийных ферм в этом регионе.

Ключевые слова: Polychaeta, инвазивные виды, *Mytilus galloprovincialis*, марикультура

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REVIEWS

CYSTOSEIRA PHYTOCENOSIS AS A BIOLOGICAL BARRIER FOR HEAVY METALS AND ORGANOCHLORINE COMPOUNDS IN THE SPNA CAPE MARTYAN MARINE AREA (THE BLACK SEA): REVIEW OF THE ARTICLE

The article "*Cystoseira* phytocenosis as a biological barrier for heavy metals and organochlorine compounds in the SPNA Cape Martyan marine area (the Black Sea)" published by V. Egorov and co-authors in the prestigious journal "Regional Studies in Marine Science" is focused on the genus *Cystoseira* and one of its numerous ecological communities [Egorov et al., 2021]. *Cystoseira*, brown algae of the order Fucales, comprise fucoids; those contain antherozoids without stigmata, few antheridial branches, trichothallic hairs in conceptacles, large ovoid oospheres, and eggs remaining attached to the surface of receptacles *via* mucilaginous stalks until after fertilization [*Cystoseira*, 2024].

Cystoseira species are currently thought to occur in the Mediterranean and Black seas and Northeast Atlantic. Species from the Indian and Pacific oceans are now classified within different genera. *Cystoseira* representatives are important habitat-forming species in coastal waters of the Mediterranean Sea and Northeast Atlantic. They require good water quality and can be used as bioindicators of pollution levels.

In addition to *Cystoseira barbata*[*] and *Cystoseira boshorica*[*] reported for the Black Sea, 19 more species are listed in [*Cystoseira*, 2024]. Many of them are poorly studied and require validation. *C. barbata* and *C. bosphorica* form significant populations along the Romanian Black Sea coast in the southern sector between Constanța and Vama Veche – with its hard substrate and rocky bottom – and dominate in the supra-, mid-, and infralittoral zones [Black Sea Biological Diversity, 1997; Exotic Species, 2001]. Their biomass measurably (up to 80%) decreased after severe frosts in the winter of 1970–1971 [Bologa et al., 1996]. Now, they seem to be recovering.

The study [Egorov et al., 2021] was aimed at assessing the role of a *Cystoseira* phytocenosis as a biological barrier in seawater purification in the specially protected natural area (hereinafter SPNA) Cape Martyan from pollution by heavy metals (V, Fe, Co, Ni, Cu, Zn, Hg, Mo, Ag, Cd, Sb, and Pb) and organochlorine compounds (DDT, DDE, DDD, ∑DDT, PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180).

The "Introduction" section provides a description of Cape Martyan. The "Material and Methods" section presents data of hydrobotanical observations, methods for analyzing pollutants, and the concept of flows. The results of hydrobotanical studies are given, as well as information on flows of pollutants: heavy metals and organochlorine compounds (hereinafter HMs and OCs, respectively).

[*]Note from the Scientific Editor:
1. *Cystoseira barbata* is unaccepted; the synonym is *Gongolaria barbata* (Stackhouse) Kuntze, 1891.
2. *Cystoseira bosphorica* is unaccepted; the synonym is *Ericaria bosphorica* (Sauvageau) D. Serio & G. Furnari, 2021.

Based on the results of the research, the following key conclusions were drawn:

1. The parameters of a *Cystoseira* biotope in the SPNA Cape Martyan marine area were determined: area of 309,000 m^2, algae reserve of 1,425.6 tons, and production of 3,136.3 tons *per* year.
2. For most of the trace elements studied (Co, Cu, Zn, Pb, Sb, Mo, Cd, Ag, Ni, V, and Hg), concentrations in the SPNA Cape Martyan marine area did not reach international reference values established for both acute and chronic effects. In general, this evidenced for a favorable environmental status of the water area in terms of its pollution by HMs.
3. In the *Cystoseira* biotope, the deposition of 9÷99% HMs and 55÷96% OCs is governed by its high specific biomass (4.6 $kg \cdot m^{-2}$) and the ability to concentrate pollutants characterized by accumulation factors within the ranges from $n \times 10^2$ to $n \times 10^5$ for HMs and from $n \times 10^2$ to $n \times 10^3$ for OCs.
4. Localization of *Cystoseira* phytocenoses in recreational zones is the key biological factor in seawater self-purification from HMs and OCs. As a result of production processes in the *Cystoseira* phytocenosis in the SPNA Cape Martyan marine area, the extraction of HMs is 0.1÷967.6 $kg \cdot year^{-1}$, and the extraction of OCs is 0.044÷8.360 $g \cdot year^{-1}$. In the biotope, the turnover of HMs and OCs varies mainly from daily one to seasonal.
5. An equation is proposed for assessing the maximum capacity of the *Cystoseira* phytocenosis to purify water from HMs and OCs. Preservation of *Cystoseira* phytocenoses in coastal areas and measures to increase their reserve and productivity are among optimal mechanisms for high-quality management of recreational zones, with the factor of pollution of the marine environment taken into account.

The work concludes with 54 references, 19 of which are related to the Black Sea. Some other literature sources could be added, for example, the following paper: Celan M. Notes sur la flore algologique du littoral roumain de la mer Noire. I. Sur les *Cystoseira*. *Bulletin de la Section Scientifique de l'Académie Roumaine*, 1935, vol. 17, pp. 81–94.

Thus, the publication by D. Sc. V. Egorov and his colleagues is a valuable scientific contribution to the study of the Black Sea macrophytobenthos based on an original approach, new concept and methodology. This once again confirms the ecological significance of a *Cystoseira* community in the marine benthic ecosystem.

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ОБЗОР СТАТЬИ *CYSTOSEIRA* PHYTOCENOSIS AS A BIOLOGICAL BARRIER FOR HEAVY METALS AND ORGANOCHLORINE COMPOUNDS IN THE SPNA CAPE MARTYAN MARINE AREA (THE BLACK SEA)

Проанализирована статья академика РАН В. Н. Егорова и соавторов, посвящённая исследованию роли видов *Cystoseira* как биологического барьера для потоков загрязняющих веществ — тяжёлых металлов и хлорорганических соединений — в акватории особо охраняемой природной территории «Мыс Мартьян».

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