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WORKING COLLECTION OF CAROTENOGENIC MICROALGAE LIVING CULTURES OF A. O. KOVALEVSKY INSTITUTE OF BIOLOGY OF THE SOUTHERN SEAS

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The article contains information on the specialized working collection of carotenogenic microalgae maintained by the staff of the animal physiology and biochemistry department of A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (IBSS). The collection was established within the framework of IBSS scientific and applied research to study the mechanisms of stress tolerance in eurybiontic and extremophilic single-celled phototrophs and to identify commercially significant sources of highly valuable ketocarotenoids of astaxanthin group used for medicine and food production. The collection contains 44 microalgal strains of various taxonomic and ecological specialization with a pronounced ability to hypersynthesize secondary carotenoids and lipids under extreme conditions (drying, nutrient starvation, high-intensity illumination, extreme temperature and salinity, effect of toxicants, *etc.*). The main ways to replenish the fund are direct exchange of carotenogenic species with leading Russian and foreign collections of microalgae and own field sampling in the Black Sea areas of Crimea and Caucasus. The majority of strains in the collection represent two orders of the class Chlorophyceae: Chlamydomonadales (25 strains) and Sphaeropleales (15 strains), since the phenomenon of secondary carotenogenesis is widespread in these orders. Out of them, inhabitants of ephemeral freshwater ponds predominate, as well as aerophilic and soil microalgae. All strains are maintained under controlled conditions on agarized mineral media as pure cultures. Description of the collection accession includes the following data: a) current taxonomic status of the species verified according to updated information from corresponding collections and algological databases, namely AlgaeBase and NCBI Taxonomy Browser; b) species basionym and known synonyms; c) date and source of the strain deposition; d) author's surname, geographic location, and biotope, from which the strain was isolated; e) accession number of sequences associated with the strain in NCBI (if any); and f) nutrient medium, on which the strain is maintained in the IBSS collection. The significance of the collection for morphological, biological, physiological, and biochemical studies of growth, secondary carotenogenesis, and biotechnological potential in green microalgae is discussed.

Keywords: carotenogenic microalgae, collection storage, Chlorophyta, carotenoids, astaxanthin

The concept of carotenogenic microalgae implies a group of eukaryotic algae, taxonomically and ecologically heterogeneous ones, with a characteristic stress response – massive accumulation of specific secondary (extraplastid) carotenoids, which are structurally and functionally unrelated to photosynthesis and perform a protective function. In terms of chemical structure, such carotenoids in most microalgae are products of the enzymatic oxidation of β-carotene to astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) occurring on the surface of cytoplasmic lipid globules (oleosomes). The main functional significance of this stress reaction – secondary carotenogenesis – is to reduce the intensity of oxidative stress, which inevitably develops under acute negative effects, to a level adequate to possibility of formation of resting phases by vegetative cells, ensuring preservation of their viability under extreme conditions for a long time (Shah et al., 2016 ; Solovchenko, 2015).

The discovery of extremely high antioxidant and regulatory activity in astaxanthin and its closest precursors (Capelli et al., 2019 ; Han et al., 2013) caused a high interest in the late 1990s in the problem of secondary carotenogenesis in microalgae and a boom in growth and metabolic research of the most prominent astaxanthin producer – the planktonic green microalga *Haematococcus pluvialis* Flotow (Chlorophyceae, Chlamydomonadales) – which became a classic model object in corresponding investigations and the first species cultivated commercially as an algal astaxanthin source. Over the last 20 years, numerous studies on various aspects of *H. pluvialis* viability and mass cultivation were carried out in different countries; their main results are summarized in several widely known reviews serving as a reference point in the continuously growing information flow on this problem (Lemoine & Schoefs, 2010 ; Li et al., 2011 ; Shah et al., 2016 ; Solovchenko, 2015 ; Zhang et al., 2020).

The patterns of secondary carotenogenesis revealed on the example of *H. pluvialis* are extrapolated by many authors to all groups of carotenogenic microalgae without considering their origin and features of biology; this can be true only partially due to lack of reliable factual data on other species. In reviews, the lists of carotenogenic microalgae usually contain no more than 10–15 names of single representatives of 5–7 genera of the class Chlorophyceae (*Chlorella*, *Coelastrella*, *Scenedesmus*, *Ankistrodesmus*, *Chlorococcum*, *etc.*). Moreover, the experimental data on the specificity of secondary carotenogenesis in such species and their potential as astaxanthin sources are still few and episodic (references can be found in the reviews listed above).

In fact, the phenomenon of secondary carotenogenesis in microalgae is much more widespread in nature and is characteristic of representatives of two algae phyla (Chlorophyta and Euglenophyta) and five classes (Chlorophyceae, Trebouxiophyceae, Eustigmatophyceae, Ulvophyceae, and Euglenophyceae). In the overwhelming majority of cases, these are aerophilic and soil species, phycobionts of epilithic lichens, planktonic inhabitants of drying eutrophic ponds, cryophilic inhabitants of snow and ice, *etc.*, which repeatedly experience during their annual life cycles acute-extreme conditions: starvation, dehydration, high UV exposure and temperature, and so on (Minyuk, 2020). In most of such species, growth characteristics in culture and peculiarities of physiological, biochemical, and molecular genetic mechanisms of induction and regulation of astaxanthin biosynthesis are still poorly studied, although, considering high ecological plasticity of terrestrial species, among them there may be highly productive and unpretentious in mass production sources of astaxanthin and ketocarotenoids similar in structure and biological activity.

A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS (hereinafter IBSS) is one of the pioneers of research on physiology and metabolism of astaxanthin producers in the post-Soviet space. The basic condition and tool for the development of this direction was the establishment of the own experimental fund of carotenogenic microalgae living cultures (the working collection). The main principles are as follows: algae ought to represent the most abundant taxa and ecological groups of astaxanthin producers, have a reliably established taxonomic status, and be stored under identical, strictly controlled conditions. The collection began to form in 2002: from the Komarov Botanical Institute of RAS, the first strain of *Haematococcus pluvialis* Flotow (LABIK 927-1) was obtained (from V. M. Andreeva) and cultivated in the laboratory. To date, the collection includes 44 strains of carotenogenic Chlorophyceae, is a structural part of the IBSS general collection of microalgae living cultures (acronym is IBSS; registration number in the World Data Center for Microorganisms is 1201) (World Data Centre for Microorganisms, 2021), and has an internal identifier IBSSca. Since 2017, the IBSS collection is included in the national depository bank of living systems "Noah's Ark" – Lomonosov Moscow State University project aimed at creating a multifunctional network storage of biological material. The collection ID in the "Noah's Ark" database is IBSS-ALGAE (Mikroorganizmy i griby, 2020).

The main ways to replenish the IBSSca fund are direct exchange of carotenogenic species with leading Russian and foreign collections (their list is given in Material and Methods section) and own field sampling in the Black Sea areas of Crimea and Caucasus (Dantsyuk et al., 2015 ; Chelebieva et al., 2018). Most of the strains represent two orders of the class Chlorophyceae [Chlamydomonadales (25 strains) and Sphaeropleales (15 strains)], in which the phenomenon of secondary carotenogenesis is most widely represented. Out of them, inhabitants of ephemeral freshwater ponds prevail, as well as aerophilic and soil species. The collection includes 4 strains of the halophilic microalga *Dunaliella salina* (Dunal) Teodoresco, 1905 isolated from Crimean hypersaline lakes by IBSS staff. The species is distinguished by a unique form of secondary carotenogenesis: its final product is β-carotene (Ben-Amotz & Avron, 1990).

The features of some strains obtained from Russian and Ukrainian collections are as follows: their European and American origin, long-term collection storage (up to 100 years in some cases), and a multistage path of transfer from depositing collections to the storages in the Commonwealth of Independent States. Some strains were isolated in China and obtained directly from the Institute of Hydrobiology of the Chinese Academy of Sciences; those are absent in other Russian collections. Several strains were isolated by us from field samples.

Every strain has an electronic passport including the following information: a) current taxonomic status of the species verified according to updated data of the depositing collections and algological databases – AlgaeBase (https://www.algaebase.org/) and NCBI Taxonomy Browser (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/); b) basionym and known synonyms of the species; c) date and source of the strain deposition into the IBSS collection; d) author's surname, as well as date, geographic location, and biotope, from which the natural sample was isolated; e) identifiers (ID) of nucleotide sequences in the NCBI database (if any); and f) nutrient medium, on which the strain is maintained in the IBSS collection.

It should be noted that in carotenogenic microalgae, astaxanthin biosynthesis is always associated with massive accumulation of neutral lipids suitable for obtaining high-quality biofuel; therefore, the species can serve as sources of two products highly demanded by the market (Minyuk et al., 2017, 2020 ; Solovchenko, 2015). This fact significantly expands the borders of research and practical use of varieties of the IBSSca collection.

The aim of this work was to distribute information about IBSS freshwater and terrestrial strains of microalgae, which are active producers of astaxanthin and lipids, among specialists of the corresponding profile to expand scientific contacts and cooperation in the sphere of fundamental and applied problems of algology.

MATERIAL AND METHODS

Data on the Collections, That Provided IBSS With Strains of Carotenogenic Microalgae. Acronym of every collection, its affiliation, registration number in the World Data Centre for Microorganisms (WDCM), and link to a website or catalogue are given in Table 1.

Table 1. Data on the collections of living cultures, that provided strains of carotenogenic microalgae

Collection acronym	Collection name, scientific organization, and country	No. in WDCM	Website or collection catalogue	Number of strains transferred to IBSS
ACKU	The Algae Culture Collection at the Kyiv University, Ukraine	994	https://biology.univ.kiev.ua/ (Kostikov et al., 2009)	14
CALU	The Collection of the Living Cultures of Cyanobacteria, Algae, and Algal Parasites at the Saint-Petersburg State University, Russia	461	https://researchpark.spbu.ru/collection-ccem-rus/1628-ccem-kollekciya-calu-rus	5
IPPAS	The Collection of Microalgae and Cyanobacteria at the Timiryazev Institute of Plant Physiology of RAS, Russia	596	http://cellreg.org/catalog/	4
FACHB	The Freshwater Algae Culture Collection at the Institute of Hydrobiology, Wuhan, China	873	http://algae.ihb.ac.cn/english/Cultrues.aspx	4
UTEX	The Culture Collection of Algae at the University of Texas, Austin, the USA	606	http://www.utex.org	2
PLY	The Marine Biological Association Culture Collection, Plymouth, the UK	128	https://www.mba.ac.uk/facilities/culture-collection	1

Field Sampling Areas. Own microalgae strains were isolated from field samples collected in three local climatic zones of the Black Sea area (humid subtropics, marine temperate continental climate, and highlands of the Central Caucasus), as well as in the Arctic climate zone (Svalbard archipelago). Detailed information on biotopes and geographic locations of the spots, where the strains were isolated from the natural environment, is given in Strain Systematic List section.

Obtaining Pure Cultures and Identification of Microalgae. Most of our own strains were isolated from dry orange-red or yellow-brown deposits on stones or from the walls of various water tanks and were mature resting stages of microalgae (aplanospores or cysts). This circumstance made it much easier to obtain microalgae pure cultures since dry samples exposed to open solar irradiation practically did not contain associated species. A particle of the sample was placed in a well of a glass slide and moistened with a drop of distilled water; then, under an MBS-10 microscope or XS-6320 binocular (Ningbo Shengheng, China), single cells were isolated with a dissecting needle and a micropipette into a glass tube with 0.5–1.0 mL of 2–3-times diluted sterile BBM nutrient medium. The tube was closed with a cotton-gauze plug and left for several days at room temperature and twilight light for spore germination and vegetative cell accumulation. Further sample purification was carried out by standard streaking technique on agarized media (1.5–2.0 %) in Petri dishes (Temraleeva et al., 2014 ; Brand et al., 2013).

Algae taxonomic identification was carried out by morphometric and biological features using the species guides (Andreeva, 1998 ; Anisimova & Gololobova, 2006 ; Dedusenko-Shchegoleva et al., 1959), as well as the results of molecular genetic analysis of fragments of nucleotide sequences of the nuclear 18S rRNA gene and internal transcribed spacer ITS2. Genetic analysis included procedures for the isolation of total DNA; amplification of target fragments; their electrophoretic detection and purification followed by sequencing; preliminary analysis and search for homologues by the BLAST algorithm in the GenBank database (NCBI); construction of multiple alignments; selection of an evolutionary model; and building of a phylogenetic tree (Chelebieva et al., 2013, 2018 ; Minyuk et al., 2017).

Storage Conditions for Microalgae Cultures. Pure microalgae strains are maintained in the collection in an active vegetative state by subculture method on slant agarized (1.5–2.0 %) nutrient media (Gaisina et al., 2008 ; Temraleeva et al., 2014 ; Brand et al., 2013 ; Lourenço, 2020), depending on the biological peculiarities of the species (Ben-Amotz & Avron, 1990 ; Bischoff & Bold, 1963 ; Fučíková & Lewis, 2012) (Table 2).

Table 2. Nutrient media composition for carotenogenic microalgae collection storage

Component	Nutrient medium		
	OHM (Fábregas et al., 2000)	BBM (Bischoff & Bold, 1963)	Ben-Amotz (Ben-Amotz & Avron, 1990)
KNO_3	410 $mg \cdot mL^{-3}$		505 $mg \cdot mL^{-3}$
$NaNO_3$		250 $mg \cdot mL^{-3}$	
$CaCl_2 \cdot 2H_2O$	110.9 $mg \cdot mL^{-3}$	25 $mg \cdot mL^{-3}$	
$CaCl_2$			22.2 $mg \cdot mL^{-3}$
$FeC_6H_5O_7 \cdot 5H_2O$	2.62 $mg \cdot mL^{-3}$		
$FeCl_3$			0.325 $mg \cdot mL^{-3}$
$FeSO_4 \cdot 7H_2O$		4.98 $mg \cdot mL^{-3}$	
$MgSO_4 \cdot 7H_2O$	246.5 $mg \cdot mL^{-3}$	75 $mg \cdot mL^{-3}$	
$MgSO_4$			600 $mg \cdot mL^{-3}$
Na_2EDTA			1.86 $mg \cdot mL^{-3}$
Na_2HPO_4	30 $mg \cdot mL^{-3}$		
$NaHCO_3$			4.2 $mg \cdot mL^{-3}$
K_2HPO_4		75 $mg \cdot mL^{-3}$	
KH_2PO_4		175 $mg \cdot mL^{-3}$	27.2 $mg \cdot mL^{-3}$
$MnSO_4 \cdot H_2O$	0.85 $mg \cdot mL^{-3}$		
$MnCl_2$			0.882 $mg \cdot mL^{-3}$
$ZnSO_4 \cdot 7H_2O$		8.82 $mg \cdot mL^{-3}$	
$ZnCl_2$			0.136 $mg \cdot mL^{-3}$
$CuCl_2$			0.945 $mg \cdot mL^{-3}$
$CuSO_4 \cdot 5H_2O$	0.012 $mg \cdot mL^{-3}$	1.57 $mg \cdot mL^{-3}$	
$Na_2MoO_4 \cdot 2H_2O$	0.12 $mg \cdot mL^{-3}$		
$(NH_4)_6 \cdot Mo_7O_{24}$			1.164 $mg \cdot mL^{-3}$
MoO_3		0.71 $mg \cdot mL^{-3}$	
$CoCl_2 \cdot 6H_2O$	0.011 $mg \cdot mL^{-3}$		
$CoCl_2$			0.130 $mg \cdot mL^{-3}$
$Co(NO_3)_2 \cdot 6H_2O$		0.49 $mg \cdot mL^{-3}$	
Cr_2O_3	0.076 $mg \cdot mL^{-3}$		
SeO_2	0.005 $mg \cdot mL^{-3}$		
H_3BO_3		11.42 $mg \cdot mL^{-3}$	
KOH		31 $mg \cdot mL^{-3}$	
NaCl		25 $mg \cdot mL^{-3}$	58.5 $mg \cdot mL^{-3}$
Biotin	25 $\mu g \cdot mL^{-3}$		
Vitamin B_1	17.5 $\mu g \cdot mL^{-3}$		
Vitamin B_{12}	15 $\mu g \cdot mL^{-3}$		

Agar stocks (two replicates for each strain) are stored under controlled conditions in a Snaige modified refrigerated display case, which is equipped with Feron DL 20W T4 6400K fluorescent lamps (Russia) and two additional thermostats (F/2000 type, FTWOF PRODIGY manufacturer), at a temperature of +12…+14 °C and continuous illumination of 2000 Lx.

Culture reseedings are performed according to the schedule, every 2–3 months (depending on the algae growth rate), observing all necessary aseptic techniques (Temraleeva et al., 2014 ; Brand et al., 2013). Those include two steps: 1) transferring reddened palmelloid cells from agar stock into liquid culture medium to obtain actively dividing vegetative cells; 2) sowing young vegetative cells on agar stock. The best algae growth on solid media was observed when using agar-agar for microbiological purposes (manufactured by Laboratorios Conda, Spain).

Current control of functional state of liquid cultures at the subculture stage (purity, cell division rate, cell shape and size, chlorophyll content, ratio of viable cells in culture, *etc.*) is carried out by the same methods as in our experimental studies using a Goryaev chamber (MiniMed, Russia), light microscope Leica DM1000 (Germany), digital camera Leica Microsystem AG (Germany), computer program ImageJ, and flow cytometer Cytomics FC 500 Beckman Coulter (USA) (Chelebieva et al., 2013, 2018 ; Chubchikova et al., 2011 ; Minyuk et al., 2014, 2016, 2017).

RESULTS AND DISCUSSION

Strain Systematic List and Description of the IBSS Working Collection of Carotenogenic Microalgae.

Class Chlorophyceae.

1. ***Deasonia granata*** (Starr) Ettl & Komárek, 1982. **Strain IBSS-11.** Chlamydomonadales, Actinochloridaceae. Obtained from the Saint-Petersburg State University collection in 2006 as *Chlorococcum granatum* CALU-859 = CCAP-213-1a. Isolated by E. G. Pringsheim in 1928 from soil in the vicinity of Prague (Czech Republic). Basionym: *Chlorococcum humicolum*. Subcultures: SAG 213-1a; UTEX 116 (as *Neospongiococcum granatum* Deason, 1971). GenBank nucleotide sequence identifiers (GenBank IDs): KM020105; MK541716. Medium: BBM + agar.
2. ***Deasonia granata*** (Starr) Ettl & Komárek, 1982. **Strain IBSS-94.** Chlamydomonadales, Actinochloridaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 566-06. Isolated by E. G. Pringsheim from soil in the vicinity of Prague, presumably before 1928. Heterotypic synonyms: *Chlorococcum multinucleatum* Starr, 1955; *Neospongiococcum granatum* Deason, 1971. Deposited into SAG (213-1a). Subcultures: CCAP 213/1A; UTEX 116 (as *Neospongiococcum granatum* Deason, 1971). GenBank ID: KM020105. Medium: BBM + agar.
3. ***Tetracystis* sp. / (*Macrochloris* sp.**?). **Strain IBSS-95.** Chlamydomonadales, Chlorococcaceae/Actinochloridaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 170-02. Isolated by I. Yu. Kostikov from soil of a coniferous forest in the Ardennes, Wiltz, near the city of Kanndorf, Geisht (Luxembourg). Medium: BBM + agar.
4. ***Bracteacoccus giganteus*** Bischoff et Bold, 1963. **Strain IBSS-96.** Sphaeropleales, Bracteacoccaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 461-06. Isolated by I. Yu. Kostikov (B-145) in 1996 from acid brown soil in Belgium (High Ardennes, Waroneu experimental polygon, spruce forest). Deposited by I. Yu. Kostikov. Medium: BBM + agar.
5. ***Bracteacoccus minor*** (Chodat) Petrová, 1931. **Strain IBSS-97.** Sphaeropleales, Bracteacoccaceae. Obtained from the Kyiv University collection in 2009 as strain ACKU 506-06. Deposited

into SAG (221-1). Isolated by R. Chodat in 1913 from soil. Subcultures: CCAP 221/1; UTEX 66. Basionym: *Botrydiopsis anglica* Fritsch et John, 1942; *Botrydiopsis minor* Schmidle ex Chodat, 1913; *Dictyococcus minor* (Schmidle) Pascher, 1937. GenBank IDs: KF673367; KT199253.1. Medium: BBM + agar.

6. ***Bracteacoccus* sp. Strain IBSS-104.** Sphaeropleales, Bracteacoccaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 65-02. Isolated by I. Yu. Kostikov in 1996 from soil of an oak forest in Belgium (High Ardennes, Waroneu experimental polygon, sampling spot QL-120, sample B-25). Deposited by I. Yu. Kostikov. Medium: BBM + agar.
7. ***Chromochloris zofingiensis*** (Dönz) Fučíková & L. A. Lewis, 2012. **Strain IBSS-20.** Sphaeropleales, Chromochloridaceae. Obtained from the Saint-Petersburg State University collection in 2006 as *Chlorella zofingiensis* Dönz, 1933, strain CALU-190. Isolated from soil in the vicinity of Zofingen (Switzerland). Taxonomic status was changed based on molecular genetic analysis (Fučíková & Lewis, 2012). Subcultures: CCAP-211/14 = CAUP H 1905 = UTEX 32 = SAG 211-14 = ATCC 30412. Homotypic synonyms: *Chlorella zofingiensis* Dönz, 1934; *Muriella zofingiensis* (Dönz) Hindák, 1982; *Mychonastes zofingiensis* (Dönz) Kalina & Puncochárová, 1987. GenBank IDs: GU827478.1; HQ902940; KR904902; KP645230; HQ902932; HQ902929. Medium: BBM + agar.
8. ***Chlamydomonas* cf. *debaryana*** Goroschankin, 1981. **Strain IBSS-105.** Chlamydomonadales, Chlamydomonadaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 45-02. Isolated by E. N. Demchenko in the Cherkasy Region (Pekari village, Kaniv District, Ukraine) from a puddle on a dirt road. Deposited by E. N. Demchenko. In AlgaeBase and NCBI Taxonomy Browser, *Chlamydomonas debaryana* Goroschankin, 1891 is currently regarded as *Edaphochlamys debaryana* (Goroschankin) Pröschold & Darienko, 2018 (Pröschold et al., 2018). Medium: BBM + agar.
9. ***Chlamydomonas montana*** Romanenko, 1999. **Strain IBSS-106.** Chlamydomonadales, Chlamydomonadaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 167-03. Isolated by E. N. Demchenko in 2002 from cracks of granite outcrops in the regional landscape park Granite-steppe lands of Buh, Gard tract (Yuzhnoukrainsk, Mykolaiv Region, Ukraine). Deposited by E. N. Demchenko. Medium: BBM + agar.
10. ***Chlamydomonas* sp. Strain IBSS-88.** Chlamydomonadales, Chlamydomonadaceae. Isolated by I. N. Chubchikova and N. V. Dantsyuk in 2006 from a freshwater spring pond in the vicinity of Sevastopol (Sakharnaya Golovka village). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: BBM + agar.
11. ***Ettlia carotinosa*** Komárek, 1989. **Strain IBSS-98.** Chlamydomonadales, Chlamydomonadales incertae sedis. Obtained from the Kyiv University collection in 2009 as strain ACKU 573-06. Isolated by F. Mainx, presumably before 1954, from soil in the Czech Republic. Deposited into SAG (213-4) by E. G. Pringsheim in 1954. Heterotypic synonyms: *Chlorococcum wimmeri* Rabenhorst; *Neochloris wimmeri* (Hilse) P. A. Archibald & Bold; *Protococcus wimmeri* Hilse. Subcultures: CCAP 213/4; UTEX 113. GenBank IDs: KR181935; KR181934; GU292342. Medium: BBM + agar.
12. ***Neospongiococcum gelatinosum*** (Archibald & Bold) Ettl & Gärtner, 1987. **Strain IBSS-99.** Chlamydomonadales, Chlorococcaceae. Obtained from the Kyiv University collection in May 2009 as strain ACKU 631-06 (15 K1A). Isolated by P. A. Archibald, presumably before 1970, from soil of a peat bog in the Elkhart County (Indiana, the USA). Deposited into SAG (64.80) by P. A. Archibald, presumably before 1980. According to molecular data of SAG and (Kawasaki et al., 2015),

it is identified as *Chlorococcum oleofaciens* Trainor & Bold, 1954. Basionym: *Chlorococcum gelatinosum* Archibald et Bold, 1970. Subculture: UTEX 1773. GenBank IDs: KX147356; KM020103; JN968584; KX782323; AB983631; AB983613. Medium: BBM + agar.

13. ***Pseudospongiococcum protococcoides*** Gromov & Mamkaeva, 1974. **Strain IBSS-10.** Chlamydomonadales, Chlorococcaceae. Obtained from the Saint-Petersburg State University collection in March 2006 as strain CALU-221. Isolated by B. V. Gromov in 1962 from surface soil layer in the vicinity of Sevastopol. GenBank ID: KU057947. Medium: BBM + agar.
14. ***Spongiochloris spongiosa*** (Vischer) Starr, 1955. **Strain IBSS-100.** Chlamydomonadales, Chlorococcaceae. Basionym: *Asterococcus spongiosus* Vischer, 1945. Obtained from the Kyiv University collection in 2009 as strain ACKU 649-06, Vischer 318. Isolated by W. Vischer in 1942 from soil in Unterengadin (Switzerland). Deposited into SAG (280-2b) by E. G. Pringsheim in 1954. Subcultures: CCAP 3/1; UTEX 1. GenBank IDs: KR607497; MK541715; AF395511; U34776.1. Medium: BBM + agar.
15. ***Chlorosarcinopsis* sf *dissociata*** Herndon, 1958. **Strain IBSS-107.** Chlamydomonadales, Chlorosarcinaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 309-04. There is no information on the strain origin in the ACKU catalogues. Medium: BBM + agar.
16. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-79.** Chlamydomonadales, Dunaliellaceae. Isolated by N. V. Shadrin in 2006 from a salt lake at Khersones Cape near Sevastopol. Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
17. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-92.** Chlamydomonadales, Dunaliellaceae. Isolated by A. B. Borovkov in 2008 from the Donuzlav Salt Lake (Crimea, Russia). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
18. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-86.** Chlamydomonadales, Dunaliellaceae. Isolated by N. V. Shadrin in 2008 from the Sivash Salt Lake (western shore) (Krasnoperekopsky District, Crimea). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
19. ***Dunaliella salina*** (Dunal) Teodoresco, 1905. **Strain IBSS-78.** Chlamydomonadales, Dunaliellaceae. Isolated by T. A. Kukhareva in May 2017 from the Koyashskoye Salt Lake (southern Kerch Peninsula, Crimea). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: Ben-Amotz + agar.
20. ***Dunaliella tertiolecta*** Butcher, 1959. **Strain IBSS-87.** Chlamydomonadales, Dunaliellaceae. Obtained in January 2003 from the collection of algae of the Turkish Institute of Marine Sciences (Erdemli) as PLY-83. The authentic strain was deposited in the Marine Biological Association Culture Collection (Plymouth, the UK). Isolated by B. Føyn in 1928 in the Oslofjord inlet (Atlantic Ocean) off the coast of southeastern Norway. Subcultures: CCAP19/6B; UTEX LB999; CCMP364. GenBank IDs: AY572957; HM243579; HQ828109; JF260981; KJ094612; KJ756820. Medium: Ben-Amotz + agar.
21. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-16.** Chlamydomonadales, Haematococcaceae. Obtained from the collection of green algae cultures of the algology laboratory of the Komarov Botanical Institute of RAS (from V. M. Andreeva) in 2002 as strain LABIK 92-1 (Mainx). Isolated in the Czech Republic. The exact date and spot of isolation are unknown. Is identical to the strain CALU-79 *Chlorococcum wimmeri* Rabenhorst by Mainx = *Haematococcus pluvialis* Flotow emend. Wille; Coll. Pringsheim, Praha, A-93. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875 (Nakada & Ota, 2016). Medium: OHM + agar.

22. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-18.** Chlamydomonadales, Haematococcaceae. Isolated by G. S. Minyuk in 2003 in the vicinity of Adler from a reddish-brown deposit on the walls of an empty pool for *Arthrospira platensis* cultivation at the AgroViktoriya enterprise (the Imereti Lowlands, Veseloe-Psou village, Adler Region, Krasnodar Territory). Spot geographic coordinates are 43°25′07″N, 40°00′09″E; height above sea level is 7 m. Isolated into pure culture by O. A. Galatonova. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank ID: KU193764.1. Medium: OHM + agar.
23. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-17.** Chlamydomonadales, Haematococcaceae. Obtained from the Timiryazev Institute of Plant Physiology collection in 2004 as strain IPPAS H-239. It was transferred to the IPPAS collection from the Czechoslovak Academy of Sciences collection in 1958 as strain A-63, Prague, Prát. Isolated by W. Vischer in 1923 in Switzerland (Botanical Garden of the University of Basel). Deposited into SAG (3-1d) by E. G. Pringsheim in 1954. Subcultures: CCAP 34/1D = CALU-333 = JSBG BS-2. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank IDs: KC153467; MG022681. Medium: OHM + agar.
24. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-73.** Chlamydomonadales, Haematococcaceae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-712. Isolated in 2007 from the Donghu Lake (Wuhan, Hubei Province, China). Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. Medium: OHM + agar.
25. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-74.** Chlamydomonadales, Haematococcaceae. Isolated by N. V. Shadrin in 2008 in the Central Caucasus area from a puddle of melted snow on the left glacial slope of the Bezengi Gorge (Misses-Kosh, Kabardino-Balkaria). Spot geographic coordinates are 43°03′25″N, 43°05′49″E; height above sea level is 2200 m. Isolated into pure culture by N. V. Dantsyuk. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank ID: KU193763.1. Medium: OHM + agar.
26. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-75.** Chlamydomonadales, Haematococcaceae. Isolated by I. N. Drobetskaya in 2008 in Sevastopol from red-brown sediment at the bottom of a water tank (balcony of a multi-storey residential building). Spot geographic coordinates are 44°36′00″N, 33°32′00″E; height above sea level is 232 m. Isolated into pure culture by N. V. Dantsyuk. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. GenBank ID: KU193762.1. Medium: OHM + agar.
27. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-108.** Chlamydomonadales, Haematococcaceae. Isolated by D. A. Davydov in 2011 on Nordaustlandet (Svalbard archipelago, Norway) from a pond with red deposit on pebbles (0.1-m depth). The material was obtained in 2015. Isolated into pure culture by N. V. Dantsyuk in 2017. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. Medium: OHM + agar.
28. ***Haematococcus pluvialis*** Flotow, 1844. **Strain IBSS-111.** Chlamydomonadales, Haematococcaceae. Isolated by I. N. Chubchikova in 2018 in Sevastopol (Maksimova dacha) from a red-brown deposit on the walls of a plastic water tank. Isolated into pure culture by N. V. Dantsyuk. Synonym: *Haematococcus lacustris* (Girod-Chantrans) Rostafinski, 1875. Medium: OHM + agar.
29. ***Neochloris oleoabundans*** S. Chantanachat & H. C. Bold, 1962. **Strain IBSS-101.** Sphaeropleales, Neochloridaceae. Obtained from Ikhlyas-Agro-Energiya enterprise (Saki, Crimea) in 2009 as a strain from the University of Texas collection; the number is unknown. Isolated

by S. Chantanachat in 1958–1962 in Saudi Arabia in sand dunes of the Rub' al Khali desert. Deposited into UTEX by H. C. Bold in 1962. Synonym: *Ettlia oleoabundans* (S. Chantanachat & H. C. Bold) J. Komárek, 1989 (Chlorophyceae; Chlamydomonadales; Chlamydomonadales incertae sedis) (Komárek, 1989). GenBank IDs: KX350066; JX978410. Genome and transcriptome assembly data in GenBank: PRJNA412701; PRJNA354501; PRJNA305197; PRJNA297494; PRJNA79207. Medium: BBM + agar.

30. ***Coelastrella rubescens*** Kaufnerová & Eliás, 2013. **Strain IBSS-12.** Sphaeropleales, Scenedesmaceae, Coelastroideae. Obtained from the Timiryazev Institute of Plant Physiology collection in 2006 as *Scotiellopsis rubescens* Vinatzer, 1975, strain IPPAS H-350. It was transferred to the IPPAS collection from the Institute of Botany at the University of Innsbruck (from J. Lukavský) in 1988 as strain Vinatzer/Insbruck V195 (CCALA 475). Isolated by G. Vinatzer in 1988 from soil in the Dolomites (South Tyrol, Italy). GenBank ID: KT962984.1. Medium: BBM + agar.
31. ***Coelastrella* sp. Strain IBSS-112.** Sphaeropleales, Scenedesmaceae, Coelastroideae. Obtained from the University of Tehran branch in 2020 as strain KNUA037. Isolated on the Caspian Sea coast (Nur-Sultan, Kazakhstan). GenBank ID: KT883911. Medium: BBM + agar.
32. **sf *Scotiellopsis* sp. Strain IBSS-109.** Sphaeropleales, Scenedesmaceae, Coelastroideae. Obtained from the Kyiv University collection in 2011 as strain ACKU 14-02. There is no information on the strain origin in the ACKU catalogues. Medium: BBM + agar.
33. ***Acutodesmus dimorphus*** (Turpin) P. M. Tsarenko, 2001. **Strain IBSS-89.** Sphaeropleales, Scenedesmaceae. Isolated by N. V. Dantsyuk and I. N. Chubchikova in 2006 from a freshwater pond in the vicinity of Sevastopol (Sakharnaya Golovka village). Isolated into pure culture by N. V. Dantsyuk. Identified by P. M. Tsarenko in 2008. Medium: BBM + agar.
34. ***Desmodesmus communis*** (E. Hegewald) E. Hegewald, 2000. **Strain IBSS-82.** Sphaeropleales, Scenedesmaceae, Desmodesmoideae. Obtained from the Timiryazev Institute of Plant Physiology collection in May 2007 as *Scenedesmus quadricauda* (Turpin) Brébisson, 1835, strain IPPAS S-313, Greifswald/15. Isolated in the vicinity of Greifswald (Germany). Subcultures: CAUP H-522 = CCALA-463. GenBank ID: MN178487. Medium: BBM + agar.
35. ***Scenedesmus obliquus*** (Turpin) Kützing, 1833. **Strain IBSS-9.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Obtained from the Saint-Petersburg State University collection in March 2006 as strain CALU-13. It was transferred to the CALU collection from the Czechoslovak Academy of Sciences collection in 1960 as strain Pringsheim, Praha, A-125. Subcultures: CCALA 45; IPPAS S-305. Synonyms: *Tetradesmus obliquus* (Turpin) M. J. Wynne, 2016; *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata, 2000 (Wynne & Hallan, 2015). Medium: BBM + agar.
36. ***Scenedesmus obliquus*** (Turpin) Kützing, 1833. **Strain IBSS-81.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-12. Isolated in Hebei Province (China) in 1960. Medium: BBM + agar.
37. ***Scenedesmus rubescens*** (Dangeard) Kessler et al., 1997. **Strain IBSS-91.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Synonym: *Halochlorella rubescens* P. J. L. Dangeard, 1966. Obtained from the Timiryazev Institute of Plant Physiology collection in 2007 as strain IPPAS D-292. It was transferred to the IPPAS collection from the Institute of Botany of the Academy of Sciences of the Uzbek SSR in 1989. Isolated on the Kamchatka Peninsula from a deposit

on a lake shore. Subculture: CALU-449. Last identification: E. S. Chelebieva and S. V. Skrebovskaya in 2013 (Chelebieva et al., 2013). Currently regarded as *Halochlorella rubescens* (Wynne & Furnari, 2014). GenBank ID: KU057946. Medium: BBM + agar.

38. ***Scenedesmus rubescens*** (Dangeard) Kessler et al., 1997. **Strain IBSS-102.** Sphaeropleales, Scenedesmaceae, Scenedesmoideae. Obtained from the Kyiv University collection in 2009 as strain ACKU 64-06. Isolated by F. Dangeard in 1965 near Bordeaux (France) from a culture of brown algae. Deposited into SAG by E. Kessler. Subcultures: SAG 595 = CCAP 232/1. Synonyms: *Halochlorella rubescens* P. J. L. Dangeard, 1966; *Chlorella fusca* var. *rubescens* Kessler et al., 1968. GenBank IDs: X74002; MK975491; HG514422; HG514373; HG514402. Medium: BBM + agar.
39. ***Ankistrodesmus* sp.** Corda, 1838. **Strain IBSS-85.** Sphaeropleales, Selenastraceae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-49. Isolated by K. Lin in 1979 in Wuhan (China). Identified by L. Li. Medium: BBM + agar.
40. ***Ankistrodesmus* sp.** Corda, 1838. **Strain IBSS-93.** Sphaeropleales, Selenastraceae. Isolated by N. V. Dantsyuk in 2008 from a freshwater spring pond in the vicinity of Sevastopol (Sakharnaya Golovka village). Isolated into pure culture and identified by N. V. Dantsyuk. Medium: BBM + agar.

Class Trebouxiophyceae.

41. ***Chlorella fusca*** Shihira et Krauss, 1965. **Strain IBSS-110.** Chlorellales, Chlorellaceae. Obtained from the Kyiv University collection in 2011 as strain ACKU 38-04. Isolated by E. N. Demchenko in 2009 in the regional landscape park Granite-steppe lands of Buh (Ukraine). Currently regarded as *Desmodesmus abundans* (Kirchner) E. H. Hegewald, 2000 (AlgaeBase, 2021). Medium: BBM + agar.
42. ***Chlorella* sp. Strain IBSS-103.** Chlorellales, Chlorellaceae. Obtained from Ikhlyas-Agro-Energiya enterprise (Saki, Crimea) in 2009 as a strain from the University of Texas collection. There is no detailed information on the strain. Medium: BBM + agar.
43. ***Botryococcus braunii*** Kützing, 1849. **Strain IBSS-76.** Trebouxiales, Botryococcaceae. Obtained from the collection of the Institute of Hydrobiology of the Chinese Academy of Sciences in 2007 as strain FACHB-759. Isolated by C.-H. Xu from a lake in Kunming (Yunnan Province, China). Isolated into pure culture by Q. Lin; identified by R. Li. Medium: BBM + agar.

Class Eustigmatophyceae.

44. ***Chlorobotrys neglectus*** Pascher & Geitler, 1925. **Strain IBSS-90.** Eustigmatales, Eustigmataceae. Isolated by G. S. Minyuk in 2006 from a deposit on the walls of an empty freshwater tank (balcony of a multi-storey building) in Sevastopol. Identified by P. M. Tsarenko. Synonym: *Chloridella neglecta* (Pascher & Geitler) Pascher. Medium: BBM + agar.

Conclusion. The specialized working collection of microalgae IBSSca contains 44 strains of eurybiontic and extremophilic Chlorophyceae species with a pronounced ability to hypersynthesize secondary carotenoids and lipids under extreme external effects. The establishment and replenishment of the collection is the basic condition for carrying out comparative research on the peculiarities of growth and secondary carotenogenesis in microalgae of various taxonomic and ecological specialization, aimed at identifying physiological and biochemical mechanisms of stress tolerance in extremophilic species and searching for new commercially promising astaxanthin producers and technically valuable lipids. Using the material of research carried out on the basis of the collection in 2005–2020, more than 30 articles were published in national and foreign scientific journals indexed in the RSCI, Scopus, and WoS (the key works are cited above), 23 reports were made at international and regional conferences, and 3 patents were registered for the invention of methods for cultivating three microalgae species to obtain carotenoids and lipids (Patent 2541455, 2015 ; Patent 2661086, 2018 ; Patent 2715039, 2020).

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РАБОЧАЯ КОЛЛЕКЦИЯ ЖИВЫХ КУЛЬТУР КАРОТИНОГЕННЫХ МИКРОВОДОРОСЛЕЙ ИНСТИТУТА БИОЛОГИИ ЮЖНЫХ МОРЕЙ ИМЕНИ А. О. КОВАЛЕВСКОГО

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В статье приведены сведения о специализированной рабочей коллекции каротиногенных микроводорослей отдела физиологии животных и биохимии Федерального исследовательского центра «Институт биологии южных морей имени А. О. Ковалевского РАН» (ФИЦ ИнБЮМ), созданной в рамках научной и прикладной тематик института для исследования механизмов стресс-толерантности у эврибионтных и экстремофильных одноклеточных фототрофов, а также для выявления коммерчески значимых источников высокоценных в медицинском и пищевом отношении кетокаротиноидов группы астаксантина. Коллекция насчитывает 44 штамма микроводорослей различной таксономической и экологической специализации с выраженной способностью к гиперсинтезу вторичных каротиноидов и липидов при экстремальных внешних воздействиях (высыхание, острое голодание, высокая освещённость, температура и солёность, действие токсикантов и др.). Основными способами пополнения фонда являются направленный обмен каротиногенными видами с ведущими российскими и зарубежными коллекциями микроводорослей и собственные полевые сборы в причерноморских зонах Крыма и Кавказа. Большинство штаммов в коллекции — представители двух порядков класса Chlorophyceae [Chlamydomonadales (25 штаммов) и Sphaeropleales (15 штаммов)], так как именно в этих порядках явление вторичного каротиногенеза распространено наиболее широко. Среди них преобладают обитатели эфемерных пресноводных водоёмов, аэрофильные и почвенные микроводоросли. Все штаммы поддерживаются в состоянии альгологически чистых культур при контролируемых условиях на агаризованных минеральных средах. Описания вариететов коллекции включают следующие сведения: а) современный таксономический статус вида, верифицированный с учётом обновлённых данных депонирующих коллекций и альгологических баз AlgaeBase и NCBI Taxonomy Browser; б) базионим и известные синонимы вида; в) время и источник поступления штамма в коллекцию; г) фамилию автора, географическое место и биотоп, из которого штамм был изолирован; д) номер штамма в NCBI (если есть); е) питательную среду, на которой штамм поддерживается в коллекции ФИЦ ИнБЮМ. Проанализировано значение коллекции для проведения морфобиологических и физиолого-биохимических исследований особенностей роста, вторичного каротиногенеза и биотехнологического потенциала зелёных микроводорослей.

Ключевые слова: каротиногенные микроводоросли, коллекционное хранение, Chlorophyta, каротиноиды, астаксантин

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CONTENT OF NUTRIENTS AND LIMITATION OF PHYTOPLANKTON PRIMARY PRODUCTION IN THE SPECIALLY PROTECTED NATURAL AREA "CAPE MARTYAN" (BLACK SEA)

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The results are presented of studying the content of nitrogen compounds and mineral phosphorus, as well as phytoplankton primary production (PPP). The research was carried out in 2017–2019 in the marine area of the specially protected natural area "Cape Martyan" located on the southern coast of Crimea (Black Sea). As found, during summer in the surface seawater layer, PPP can be limited by both nitrogen and phosphorus. The dependence of PPP variation on the concentration of total nitrogen in water is not significant, while the dependence on the concentration of phosphorus is significant. It is shown that during the entire annual cycle, concentrations of nitrites, nitrates, ammonium, and mineral phosphorus vary but remain within the limits that do not lead to water hypereutrophication. A high ecological significance of precipitation was revealed: the related increase in PO_4 concentration caused a transition in PPP limitation mode from phosphorus to nitrogen one. Using theoretical concepts, it is substantiated that, under oligotrophic conditions, an increase in the concentration of the substrate limiting PPP in water results in an increase in the rate of its uptake from the environment in accordance with the negative feedback of natural regulation of ecosystem homeostasis. Under conditions of eutrophication, the effect of production processes on water conditioning by the factor of nutrients decreases.

Keywords: Black Sea, Cape Martyan, nitrogen compounds, phosphates, precipitation, plankton, primary production, limitation

The Crimean Black Sea coast is a zone of intensive natural resource management (The Current State of the Crimean Coastal Zone, 2015). The complex anthropogenic load on it is determined by the influx of a wide range of pollutants with wastewater and slope runoff, as well as urbanization and functioning of objects of transport infrastructure (*inter alia* intensive shipping), industrial centers, and recreational and tourist centers. According to classification of the marine environment quality, the Crimean coast is a critical zone (Zaitsev & Polikarpov, 2002), in which the content of pollutants can exceed both natural levels and maximum permissible concentrations established based on sanitary and hygienic requirements (Egorov et al., 2013). To date, one of the most urgent environmental issues is water hypereutrophication (Vinogradov et al., 1992). It is caused by the influx of excessive amounts of nutrients into the marine environment (Yunev et al., 2019) resulting in an increased phytoplankton primary productivity. This leads to changes in structural and functional organization of marine ecosystems (Zaitsev, 1998) and to deterioration in seawater quality, that is both a habitat for hydrobionts and a recreational resource.

In 1973, in Crimea, on the basis of the Nikitsky Botanical Gardens, a specially protected natural area (hereinafter SPNA) was established: the "Cape Martyan" Nature Reserve (the Decree of the Council of Ministers of the Ukrainian SSR dated 20.02.1973, No. 84). Currently, it has the status of a natural park of regional significance (Ob utverzhdenii polozheniya, 2018). It is both a terrestrial and aquatic object since half of its total area of 2.4 km^2 falls on the Black Sea coastal waters. Comprehensive study of this object allows revealing ecological and biogeochemical peculiarities of the coastal water area, which is an integral part of the whole nature protection and recreational complex functioning in the coast – sea system.

The aim of this study was to assess the annual trends in the variation in primary production processes and the content of nitrogen mineral forms (nitrites, nitrates, and ammonium) and mineral phosphorus in the surface water layer of the SPNA "Cape Martyan" coast considering precipitation intensity and peculiarities of limitation of phytoplankton production by nutrients, as well as to give theoretical interpretation of the survey data.

MATERIAL AND METHODS

Sampling was carried out in 2017–2019 in the surface water layer at a distance of 60–70 m from the shoreline from a pier located within the boundaries of the economic zone of the SPNA "Cape Martyan" water area (the sampling point coordinates are 44°30′19.1″N, 34°14′19.5″E) (Fig. 1).

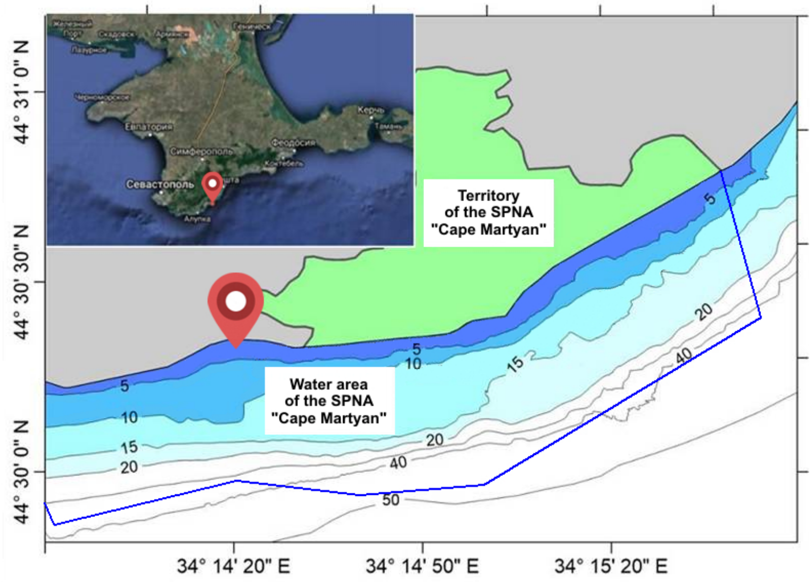

Fig. 1. Studied area localization and schematic map; 📍 denotes the sampling point

Tendencies in annual variation in the surface water temperature in the studied area are given according to the material from the website (Temperature, 2020); the amount of precipitation, according to the data provided by Nikitsky Botanical Gardens agrometeorological station. Hydrochemical indicators of the water samples were determined in a certified hydrochemical laboratory of the IBSS aquaculture and marine pharmacology department applying generally accepted methods (Rukovodstvo, 1977). The results of determining the concentrations of nutrients in water had ranges and mean relative errors as follows: nitrate ions, 5 to 500 μg·L^{-1}, with an error of 2.7–7.39 %; nitrite ions, 0.5 to 1000 μg·L^{-1}, with an error of 1.53–18.02 %; ammonium nitrogen, 15 to 1500 μg·L^{-1}, with an error of 1.69–11.4 %; and phosphate ions, 5 to 100 μg·L^{-1}, with an error of 4.6 %. The data on phytoplankton primary production (hereinafter PPP) obtained by the radiocarbon method (Egorov et al., 2018b) were used, as well as the data on the concentration of nutrients in the SPNA "Cape Martyan" seawater in 2017–2018 (Egorov et al., 2018a).

To determine the limiting nutrient factor, the Redfield stoichiometric ratio (R_{at}) (Redfield, 1958) was applied. With the dimension of included parameters in μg·L^{-1}, the ratio had the following form:

$$R_{at}(N/P) = 1.53\ (1.35NO_2 + NO_3 + 3.44NH_4)/PO_4\ , \tag{1}$$

where NO_2, NO_3, NH_4, and PO_4 are the concentrations (μg·L^{-1}) of nitrogen (in nitrites, nitrates, and ammonium) and mineral phosphorus PO_4 in the surface water, respectively.

According to the methodology of applying the Redfield ratio, at $R_{at} > 16$, PPP was limited by phosphorus, and at $R_{at} < 16$, by nitrogen (Zilov, 2009).

RESULTS

The results of determining the concentration of nutrients in seawater, measuring of PPP, and calculating the Redfield ratio are given in Table 1.

Table 1. Results of measuring the concentration of nutrients and phytoplankton primary production and assessment of the Redfield ratio in the SPNA "Cape Martyan" water area

Date	Concentration					PPP, mg C·m^{-3}·day^{-1}*	Redfield ratio (R_{at})
	NH_4 ± SD, μg·L^{-1}	NO_2 ± SD, μg·L^{-1}	NO_3 ± SD, g·L^{-1}	ΣN, μg·L^{-1}	PO_4 ± SD, μg·L^{-1}		
2017							
19.04.2017	15.00 ± 0.70	0.50 ± 0.10	17.00 ± 0.50	32.5	0.50 ± 0.10	3.4	211.98
30.06.2017	30.00 ± 1.00	0.80 ± 0.10	7.60 ± 0.20	38.4	14.40 ± 0.20	112.8	11.28
14.12.2017	21.00 ± 1.00	0.40 ± 0.10	6.50 ± 0.20	26.9	3.00 ± 0.20	23.3	40.43
2018							
09.01.2018	5.00 ± 2.50	1.40 ± 0.10	11.80 ± 0.30	18.2	3.00 ± 1.10	10.2	15.75
20.04.2018	18.00 ± 0.86	0.40 ± 0.01	29.20 ± 0.87	47.6	1.40 ± 0.10	22.7	100.17
29.07.2018	30.00 ± 1.40	1.60 ± 0.02	20.00 ± 0.60	51.6	8.50 ± 0.10	n. d.	22.56
29.10.2018	15.00 ± 0.72	0.60 ± 0.01	12.20 ± 0.36	27.8	2.90 ± 0.04	n. d.	34.08
26.11.2018	50.00 ± 2.40	1.40 ± 0.02	14.00 ± 0.42	65.4	30.30 ± 0.45	n. d.	9.48
18.12.2018	40.00 ± 1.92	2.20 ± 0.03	10.20 ± 0.31	52.4	6.80 ± 1.00	n. d.	33.92
2019							
30.01.2019	28.50 ± 0.23	4.80 ± 0.07	12.00 ± 0.36	45.3	6.50 ± 1.00	n. d.	27.43
04.04.2019	8.00 ± 0.40	0.80 ± 0.01	34.00 ± 1.00	42.8	1.50 ± 0.02	n. d.	63.85
29.04.2019	10.00 ± 0.48	1.00 ± 0.02	34.80 ± 1.44	45.2	1.50 ± 0.02	n. d.	71.96
26.06.2019	12.00 ± 0.58	1.00 ± 0.02	7.90 ± 0.24	20.9	10.00 ± 0.15	n. d.	7.73
10.10.2019	5.00 ± 0.24	1.60 ± 0.02	7.00 ± 0.21	13.6	3.40 ± 0.05	n. d.	11.86
28.11.2019	4.00 ± 0.20	2.00 ± 0.03	10.20 ± 0.30	16.2	10.20 ± 0.20	n. d.	4.00
23.12.2019	2.50 ± 0.12	1.80 ± 0.03	28.00 ± 3.00	32.3	3.40 ± 0.05	n. d.	17.56

Note: SD denotes standard deviation; * denotes data given according to (Egorov et al., 2018a); n. d. denotes no data available.

The results obtained show as follows: during the survey period, the concentration of nitrogen in water in the form of ammonium varied 2.5 to 50.0 $\mu g \cdot L^{-1}$; in the form of nitrites, 0.4 to 4.8 $\mu g \cdot L^{-1}$; in the form of nitrates, 6.5 to 34.8 $\mu g \cdot L^{-1}$. The total concentration of mineral nitrogen compounds varied 13.6 to 65.4 $\mu g \cdot L^{-1}$; the concentration of phosphates was 0.5 to 30.3 $\mu g \cdot L^{-1}$. In different seasons of the year, PPP varied within 3.4–112.8 mg $C \cdot m^{-3} \cdot day^{-1}$. The value of the Redfield ratio varied 7.73 to 211.98, and this indicated PPP limitation by both nitrogen compounds and mineral phosphorus.

DISCUSSION

From 19.04.2017 to 20.04.2018, in the SPNA "Cape Martyan" water area, minimum PPP was recorded during the winter season, when the daylight duration was less than 10 hours, and the surface water temperature did not exceed +10 °C (Fig. 2a–2d). At the same time, $R_{at} \approx 16$, which indicated the absence of primary production processes limitation by nutrients (Fig. 2e).

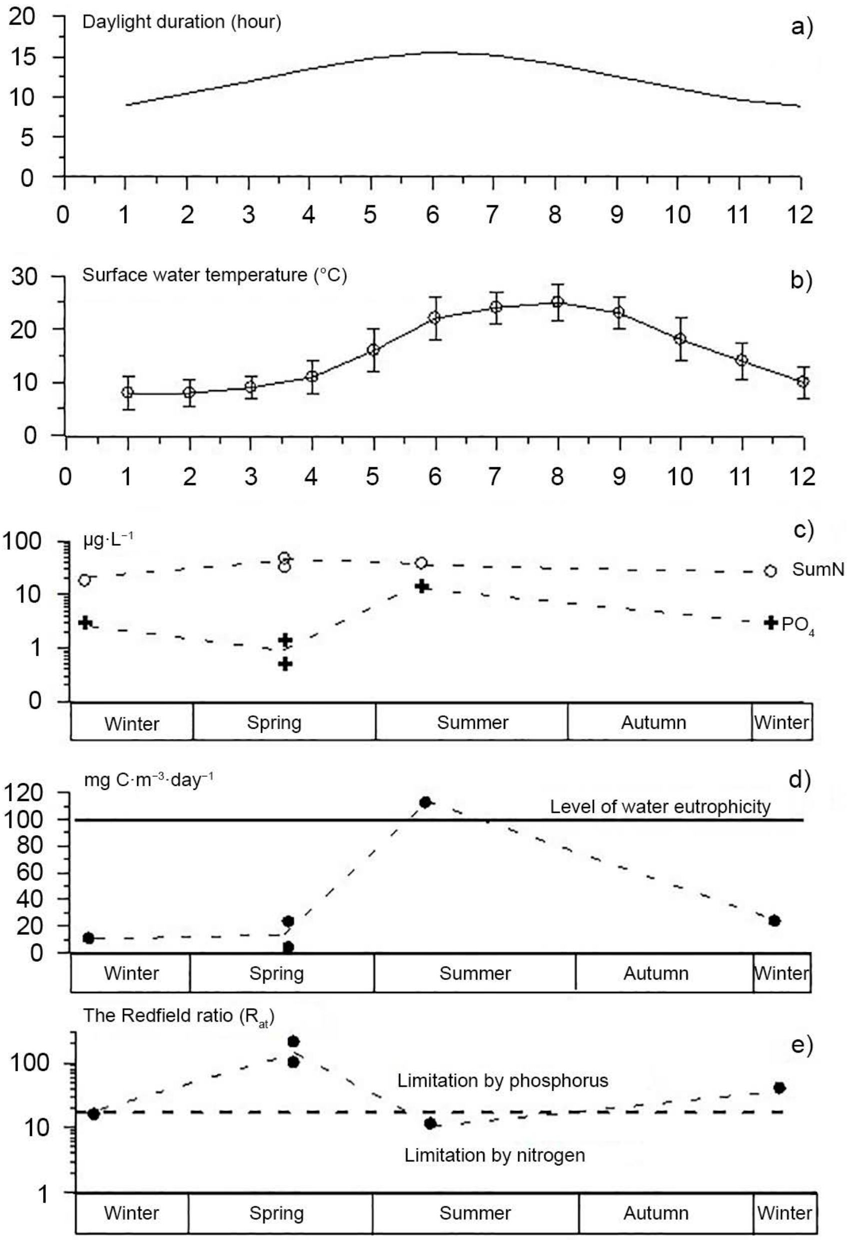

Fig. 2. Annual dynamics of environmental indicators in the SPNA "Cape Martyan" and phytoplankton production indicators in its marine area according to 2017–2018 data: a) daylight duration; b) mean temperature of the surface seawater layer; c) concentration of the sum of nitrogen and mineral phosphorus compounds in the surface seawater layer; d) phytoplankton primary production; e) the Redfield ratio

Spring began under conditions of production processes limitation by phosphorus; PPP maximum level (112.8 mg C·m^{-3}·day^{-1}) exceeding the lower limit of water eutrophicity (100 mg C·m^{-3}·day^{-1}) was reached in summer under conditions of microalgae growth limitation by nitrogen. In general, in the water area studied, PPP variations were characterized by a statistically significant dependence (R^2 = 0.960) on phosphorus concentration (Fig. 3a) and a dependence with a lower significance (R^2 = 0.134) on nitrogen concentration (Fig. 3b). The recorded effects can be explained by the following fact: in 80 % of cases, during the survey period, PPP was limited by phosphorus concentration (Fig. 3c) at $R_{at} \geq 16$.

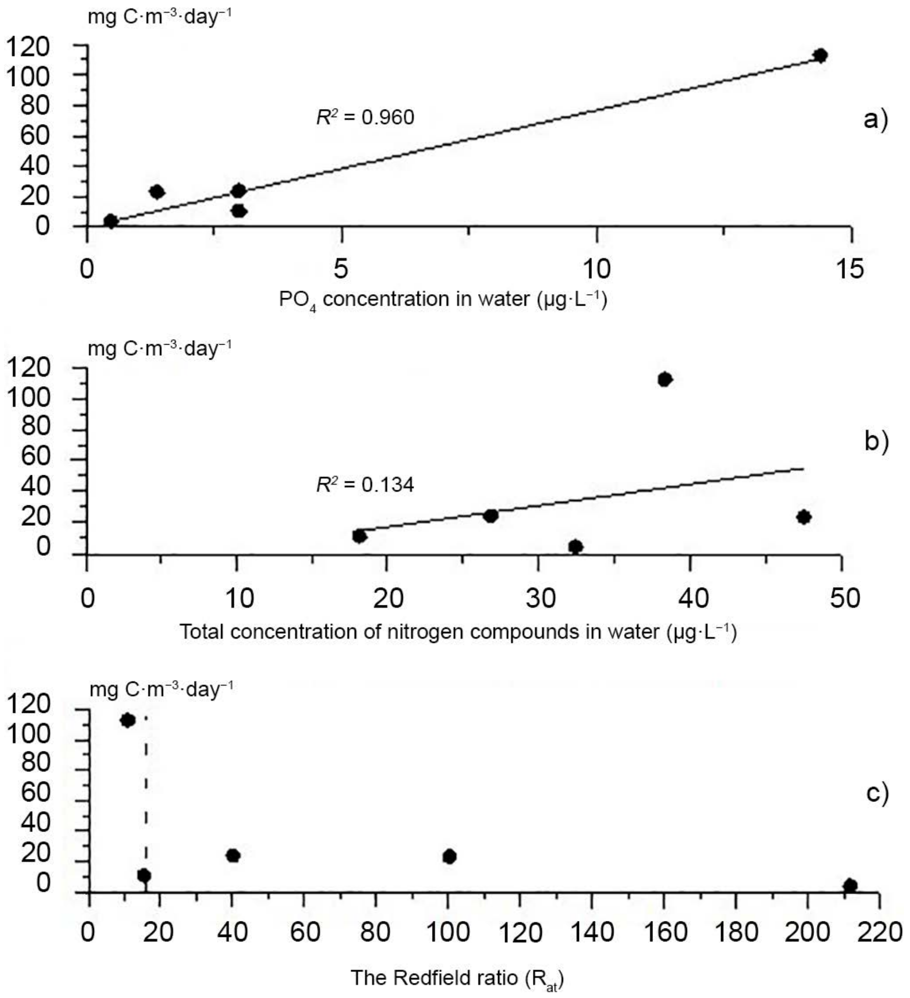

Fig. 3. Dependence of phytoplankton primary production in the surface seawater layer of the SPNA "Cape Martyan" marine area on the concentration of mineral phosphorus compounds (a) and the sum of nitrogen compounds (b), as well as on the value of the Redfield ratio (c)

The survey results showed as follows: in the spring of 2018 in the SPNA "Cape Martyan" water area, multidirectional simultaneous processes were observed: an increase in the concentration of nitrogen in the form of ammonium and nitrates and a decrease in the content of nitrites and mineral phosphorus (Fig. 4a, b).

During that period, the dilution of NO_2 and PO_4 concentration was likely to be due to intensive precipitation (Fig. 4b). In late spring and in summer, the concentration of the sum of nitrogen mineral forms (SumN) in water stabilized (Fig. 4a); this was probably caused by a relatively high consumption of nitrates by phytoplankton under conditions of production processes limitation by phosphorus (Fig. 4c) against the backdrop of an increase in nitrites and ammonium content. No significant effect of precipitation on hydrochemical indicators of the surveyed water area (Fig. 4b) was revealed.

In the autumn of 2018, an increase in precipitation intensity was recorded (see Fig. 4b). It resulted in a slight rise in the concentration of all forms of mineral nitrogen and a significant increase in the content of mineral phosphorus in seawater, causing a transition in PPP limitation mode from phosphorus to nitrogen one (Fig. 4c).

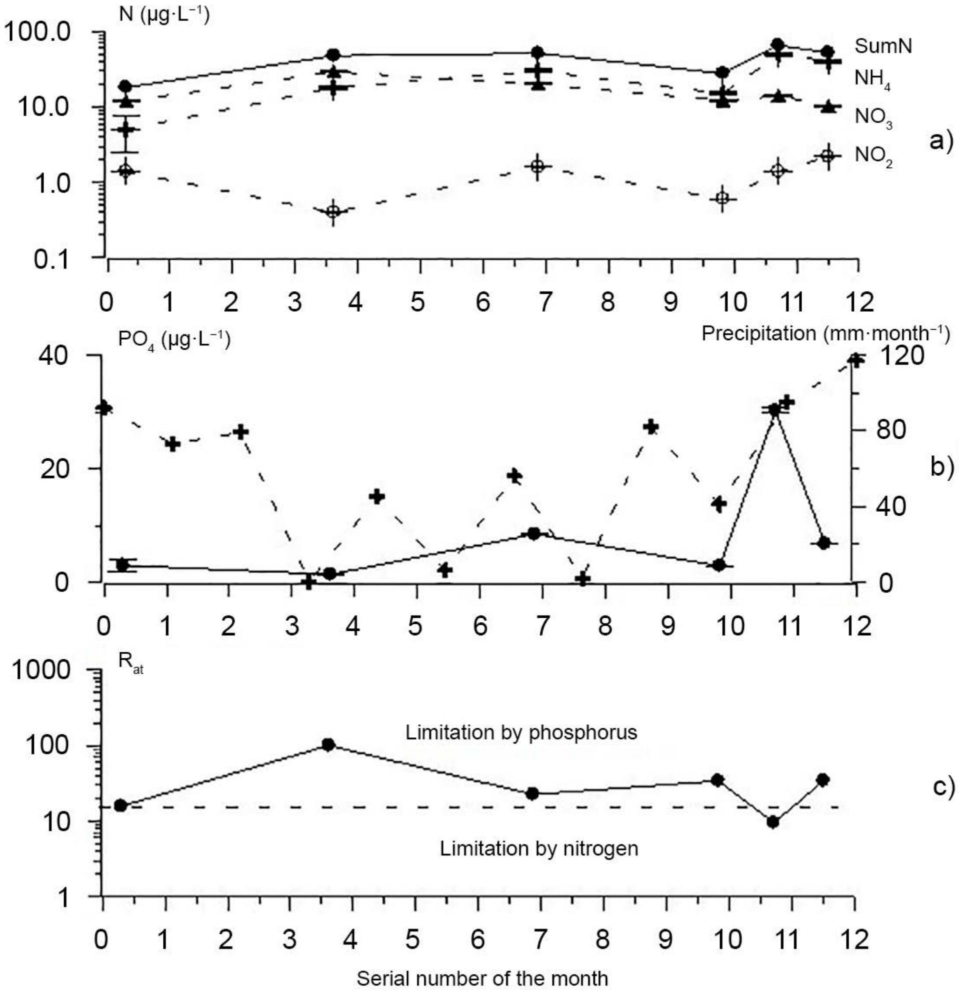

Fig. 4. Dynamics of environmental indicators in the SPNA "Cape Martyan" and content of nutrients in its marine area in 2018: a) concentration of nitrogen compounds in the surface water layer; b) concentration of mineral phosphorus compounds (●) in the surface water layer and precipitation volume (+) in the studied area; c) the Redfield ratio

In the spring of 2019, against the backdrop of a decrease in precipitation intensity in the SPNA "Cape Martyan" water area, an increase in ammonium concentration was recorded (Fig. 5b), with a simultaneous decrease in the content of nitrates, nitrites, and mineral phosphorus (Fig. 5a, b) under conditions of PPP limitation by phosphorus (Fig. 5c).

An increase in precipitation intensity [it peaked in late spring and early summer (see Fig. 5b)], almost did not change the content of nitrates and ammonium (see Fig. 5a) but resulted in a significant rise in the concentration of mineral phosphorus in the surveyed water area (see Fig. 5b). This caused transition in phytoplankton mineral nutrition mechanism from limitation by phosphorus to limitation by nitrogen (Fig. 5c), accompanied by intense absorption of nitrates (see Fig. 5a) from aquatic environment. The autumn peak of precipitation (see Fig. 5b) coincided with a slight increase in the ammonium concentration and a significant rise in PO_4 concentration in seawater, which resulted in an increase in the level of PPP limitation by nitrogen.

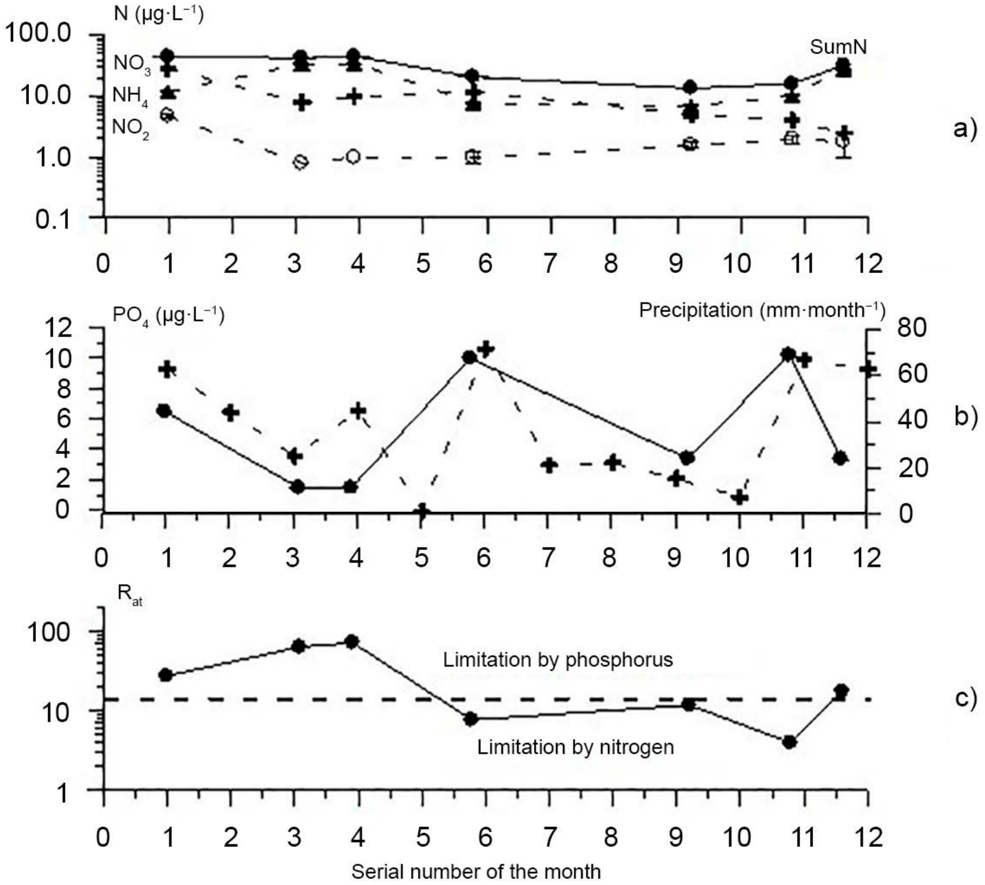

Fig. 5. Dynamics of environmental indicators in the SPNA "Cape Martyan" and content of nutrients in its marine area in 2019: a) concentration of nitrogen compounds in the surface seawater layer; b) concentration of mineral phosphorus compounds (●) in the surface seawater layer and precipitation volume (+) in the studied area; c) the Redfield ratio

In general, the survey carried out in 2017–2019 in the SPNA "Cape Martyan" coastal and marine area showed as follows: in summer, PPP can be limited by both nitrogen compounds and mineral phosphorus compounds. The observed phenomena set the task of theoretical interpretation and assessment of practical significance of the biochemical processes of PPP limiting factors' transition.

For the first time, the dependence of the specific growth rate indicator of a microorganism culture (μ, with the dimension inverse to time: 1 *per* time unit) on a weight concentration of the limiting substrate in the medium (C_v, concentration units *per* medium volume unit) was described by the Monod function (Monod, 1942):

$$\mu = \frac{\mu_{max} C_v}{K_m + C_v} , \qquad (2)$$

where μ_{max} is an indicator of the maximum physiologically possible specific growth rate of a cell culture (1 *per* time unit);

K_m is an indicator (concentration units *per* medium volume unit), that characterizes the intensity of catalytic processes; it is numerically equal to C_v value, at which $\mu = 0.5 \cdot \mu_{max}$, and is usually called the Michaelis–Menten constant.

When using the Monod function to assess the level of PPP limitation, it seems reasonable to consider two biochemical situations with extreme values: $C_v << K_m$ and $C_v >> K_m$. In the first case ($C_v << K_m$), C_v value in denominator of the expression (2) can be neglected. Therefore, the equation (2) turns into a linear function $\mu = (\mu_{max} / K_m) \cdot C_v$, in which $\mu_{max} / K_m = \text{const}$. That is, at $C_v << K_m$, which corresponds to oligotrophic conditions, the specific productivity of microalgae rises with increasing concentration of the limiting substrate in the environment. In the second case ($C_v >> K_m$), K_m value in the expression (2) can be neglected. Then, $\mu \approx \mu_{max}$. So, at $C_v >> K_m$, which corresponds to eutrophic conditions, PPP is maximum and does not depend on the concentration of the limiting substrate in the environment.

Importantly, the results of many *ex situ* and *in situ* observations have shown that all living matter, including phytoplankton, can absorb different specific amounts of substrates depending on their content in the environment. For each substrate (C_f) potentially limiting PPP, there is a minimum intracellular concentration q_{min}, which ensures microalgae viability. During the growing season, phytoplankton can accumulate limiting substrates up to C_f levels significantly exceeding q_{min}. As the stored substrates become depleted in the environment, they can be used by microalgae to continue cell division, which is accompanied by an increase in the level of limitation of the growth rate until their intracellular concentration (C_f) decreases down to $C_f = q_{min}$ level. For these conditions, the dependence of the indicator of the specific growth rate of a microorganism culture (μ) is described by the Droop equation (Droop, 1974):

$$\mu = \mu_{max}(1 - q_{min}/C_f)\ . \tag{3}$$

In the formula (3), q_{min}/C_f ratio refers to the substrate limiting PPP at present. When shifting limiting factors (see Figs 4 and 5), the expression (3) should take into account q_{min}/C_f ratio for the factor controlling the production process. In the equation (3), it is permissible to use not q_{min}/C_f, but an indicator limiting cell division by the effect of ecotoxicological factors. Therefore, to solve the problems of PPP processes limitation, it is necessary to assess the dependence of C_f on C_v considering modern concepts of sorption and metabolic interactions of phytoplankton with chemical elements, *inter alia* nutrients, of the marine environment (Polikarpov & Egorov, 1986).

In experimental studies with a ^{32}P radioactive label, a differential equation was obtained for the kinetics of phosphorus metabolism in unicellular algae in time (t), which had the following form (Egorov et al., 1982):

$$\frac{dC_f}{dt} = \frac{V_{max}C_v}{K_m + C_v} - [r + \mu_{max}(1 - q_{min}/C_f)]C_f\ , \tag{4}$$

where r is an indicator of the rate of phosphorus metabolism in unicellular algae (1/t);

V_{max} is the maximum physiologically possible specific rate of the substrate intracellular absorption (concentration units *per* microalgae mass unit *per* time unit).

In (4), the first term on the right-hand side is the ratio proposed by Dugdale (1967) to determine the rate of nutrient absorption by algae in accordance with the Michaelis–Menten equation. The term r considers the metabolic peculiarities of intracellular phosphorus metabolism, and the second term in parentheses on the right reflects production processes limitation by a substrate C_f in accordance with the Droop equation (3).

Application of the equation (3) for oligotrophic stationary conditions, when $C_v << K_m$, showed that the dependence of C_f on the change in C_v value has the following form:

$$C_f = \frac{V_{max}}{K_m(r + \mu_{max})} C_v - \frac{q_{min}\mu_{max}}{r + \mu_{max}} \,. \tag{5}$$

It can be seen in the formula (5) that its parameters V_{max}, K_m, r, μ_{max}, and q_{min} are constant within the limits of the theoretical concepts used and at $C_v << K_m$. Hence, under oligotrophic conditions, the relationship between C_v and C_f is linear. Therefore, substitution into the equation (3) of C_v values from the expression (5) instead of C_f will not change the hyperbolic increase in the rate of microalgae cell division (μ) with an increase in the concentration of the primary production-limiting substrate (C_f) in the environment.

At $C_v >> K_m$, which corresponds to the conditions of water eutrophication, in the first term of the right-hand side of the equation (4), K_m value can be neglected. Considering this, under stationary conditions, the dependence of C_v on the indicators of (4) will have the following form:

$$C_f = \frac{\mu_{max} q_{min}}{r + \mu_{max}} \,. \tag{6}$$

When substituting C_f value from the formula (6) into the equation (3), μ value will not depend on the change in the concentration of the limiting substrate in the environment (C_v). This indicates as follows: under conditions of water eutrophication, the specific growth rate of microalgae (μ) reaches its maximum values, but with an increase in the concentration of the limiting substrate in the environment (C_v), the relative rate of its extraction from water decreases.

As shown by the analysis, various theoretical approaches used to assess the limiting role of nutrients in PPP processes reveal the same physiological regularities. Both the Monod function (Monod, 1942) and Droop equation (Droop, 1974) show as follows: under oligotrophic conditions, a rise in the concentration of the limiting substrate in water results in an increase in the rate of its extraction from the environment. Thus, natural regulation of ecosystem homeostasis is carried out in accordance with the principle of negative feedback (Egorov, 2019). Under conditions of eutrophication, with an increase in C_v, the effect of production processes on water conditioning by the factor of nutrients decreases.

Conclusion. Research carried out in 2017–2019 in the SPNA "Cape Martyan" showed that in summer, phytoplankton primary production in its coastal and marine area can be limited by both nitrogen compounds and mineral phosphorus. As found, in the surface seawater, the dependence of PPP change on nitrogen concentration in water is less significant than the dependence on phosphorus concentration, which indicates the linear nature of the functional relationship. At the same time, during the entire annual cycle, the concentrations of nitrites, nitrates, ammonium, and phosphates vary but remain within the limits that do not lead to water hypereutrophication related to PPP. It was revealed that the variation in the concentration of nutrients in seawater correlates with precipitation volume. The influx of nutrients can occur directly with precipitation or with an increased slope runoff during precipitation. Survey shows that it is the slope runoff that has the greatest effect on the water composition of the studied water area in terms of nutrients. As found, summer and autumn peaks of precipitation to a greater extent increase the influx of mineral phosphorus into the water area than nitrogen compounds. The resulting increase in the concentration of mineral phosphorus in seawater leads to a transition in PPP limitation mode from phosphorus to nitrogen one. It was shown that, under oligotrophic conditions, an increase in the concentration of the substrate limiting PPP in water results in a rise in the rate of its uptake from the environment in accordance with the principle of negative feedback regulating the natural homeostasis of the ecosystem. Under conditions of eutrophication, the effect of the production processes of microalgae on water conditioning by the factor of nutrients decreases.

This work was carried out within the framework of IBSS state research assignment "Molismological and biogeochemical fundamentals of marine ecosystems homeostasis" (No. 121031500515-8) and NBG – NSC state research assignment "Monitoring research and determination of the current biota state of the State Nature Reserve 'Cape Martyan'" (No. AAAA-A20-120110690010-4).

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СОДЕРЖАНИЕ БИОГЕННЫХ ЭЛЕМЕНТОВ И ЛИМИТИРОВАНИЕ ПЕРВИЧНОЙ ПРОДУКЦИИ ФИТОПЛАНКТОНА В АКВАТОРИИ ООПТ «МЫС МАРТЬЯН» (ЧЁРНОЕ МОРЕ)

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Представлены результаты изучения содержания соединений азота и минерального фосфора, а также первичной продукции фитопланктона (ППФ) в 2017–2019 гг. в морской акватории ООПТ «Мыс Мартьян», расположенной на Южном берегу Крыма (Чёрное море). Установлено, что в летний период в поверхностном слое морской воды ППФ может лимитироваться как по азоту, так и по фосфору. Зависимость изменения ППФ от концентрации общего азота в воде обладает малой значимостью, а зависимость от концентрации фосфора — высокой. Показано, что в течение всего годового цикла концентрации нитритов, нитратов и аммония, а также минерального фосфора изменяются, но остаются в пределах, не приводящих к гиперэвтрофикации вод. Выявлена высокая экологическая значимость атмосферных осадков: связанное с ними повышение концентрации PO_4 обусловливало изменение режима лимитирования ППФ с фосфорного на азотный. С использованием теоретических представлений обосновано, что в олиготрофных условиях увеличение концентрации лимитирующего ППФ субстрата в воде приводит к возрастанию скорости его извлечения из среды в соответствии с отрицательной обратной связью природного регулирования гомеостаза экосистем. В условиях эвтрофирования влияние продукционных процессов на кондиционирование вод по фактору биогенных элементов снижается.

Ключевые слова: Чёрное море, мыс Мартьян, соединения азота, фосфаты, атмосферные осадки, планктон, первичная продукция, лимитирование

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INTENSIVE CULTURE OF *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN ET LEWIN ON THE NUTRIENT MEDIUM WITH SODIUM BICARBONATE

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The possibility is shown experimentally of using sodium bicarbonate in a nutrient medium to provide *C. closterium* culture with carbon under conditions of intensive cultivation without supplying CO_2 to the suspension. After *C. closterium* adaptation to a nutrient medium with sodium bicarbonate with a concentration of 1.2 $g{\cdot}L^{-1}$, active growth is observed, with a maximum productivity of 0.6–0.7 $g{\cdot}(L{\cdot}day)^{-1}$ of dry weight. Carbon penetrates into diatom cells both in the form of carbon dioxide and bicarbonate ions. However, all nutrient media for artificial cultivation of diatoms still require using CO_2 from the atmosphere or from a gas cylinder. The aim of this work is to assess the possibility of using sodium bicarbonate to provide *C. closterium* with carbon under conditions of intensive cultivation without supplying CO_2 to the suspension. The culture was grown in the mode of accumulative cultivation in a 1-L flask on the RS nutrient medium prepared with sterile Black Sea water; its composition was as follows ($g{\cdot}L^{-1}$): $NaNO_3$ – 0.775; $NaH_2PO_4{\cdot}2H_2O$ – 0.0641; $Na_2SiO_3{\cdot}9H_2O$ – 0.386; Na_2EDTA – 0.0872; $FeSO_4{\cdot}7H_2O$ – 0.045; $CuSO_4{\cdot}5H_2O$ – $0.2{\cdot}10^{-3}$; $ZnSO_4{\cdot}7H_2O$ – $0.44{\cdot}10^{-3}$; $CoCl_2{\cdot}6H_2O$ – $0.2{\cdot}10^{-3}$; $MnCl_2{\cdot}4H_2O$ – $0.36{\cdot}10^{-3}$; and $NaMoO_4{\cdot}H_2O$ – $0.12{\cdot}10^{-3}$. Previously, 1.2 $g{\cdot}L^{-1}$ of sodium bicarbonate was dissolved there. Cell suspension was stirred with a magnetic stirrer (250 rpm). On the 4th day of the experiment, 1 g of $NaHCO_3$ and 2 mL of 0.1 N hydrochloric acid were added to the culture in order to lower the medium pH down to 8.6. From the 2nd day of the experiment, active growth was observed, with a maximum productivity of 0.6 $g{\cdot}(L{\cdot}day)^{-1}$. After adding 1 $g{\cdot}L^{-1}$ of sodium bicarbonate to the actively growing culture and lowering pH down to 8.6, the growth rate approached almost zero, but considering the increase rate of the medium pH during adaptation, the culture actively absorbed bicarbonate ions. The possibility of cultivating the benthic diatom *C. closterium* on a nutrient medium with a high sodium bicarbonate content is experimentally shown. As found, on the RS nutrient medium with 1.2 $g{\cdot}L^{-1}$ of sodium bicarbonate added under conditions of intensive cultivation, *C. closterium* maximum productivity reaches 0.7 $g{\cdot}(L{\cdot}day)^{-1}$, with a significant increase in the medium pH. According to our data, optimal medium pH for *C. closterium* growth is in the range of 8.4–9.4. At higher values ($pH > 9.4$), the growth of diatoms slows down; at $pH = 9.9$, the culture enters the dying phase.

Keywords: nutrient medium, cultivation, diatoms, sodium bicarbonate

Diatoms have a rather efficient carbon-concentrating mechanism (Lebeau & Robert, 2003 ; Matsuda et al., 2017 ; Matsuda & Kroth, 2014). In terms of quantitative and qualitative composition of carbonic anhydrases, diatoms are superior to other algae species; therefore, they inhabit various water bodies with different CO_2 and HCO_3^- concentrations (Lebeau & Robert, 2003 ; Roberts et al., 2007).

Being representatives of secondary endosymbiosis (Keeling, 2010), diatoms have inherited the ability to synthesize ten unique carbonic anhydrases of α-, β-, γ-, and θ-class; those are located along the entire pathway of inorganic carbon transport from the environment to the chloroplast (Berges et al., 2002 ; Jensen et al., 2019 ; Matsuda & Kroth, 2014). This structure, combined with the urea cycle and the ability of diatoms to C_4 carbon fixation, significantly reduces CO_2 loss by a cell and allows diatoms to survive under unfavorable conditions (Horne, 1972 ; Obata et al., 2013 ; Reinfelder et al., 2004).

Inorganic carbon is known to penetrate into cells mainly in the form of CO_2, by free diffusion, as well as by active HCO_3^- transport due to ATP energy (Lebeau & Robert, 2003 ; Matsuda et al., 2017). Despite the fact that the ability of diatoms to use hydrocarbons has been known for a long time (Matsuda & Kroth, 2014 ; Matsumoto et al., 2017 ; Obata et al., 2013), all nutrient media for artificial cultivation of diatoms still require using CO_2 from the atmosphere or from a gas cylinder, *inter alia* for industrial growth of high-density cultures (Lebeau & Robert, 2003 ; Matsumoto et al., 2017 ; Reinfelder et al., 2004). In the literature, there is no information on the adaptive ability of diatoms to a medium with high concentration of hydrocarbons and high pH values; there is also no data on the use of nutrient media with hydrocarbons for intensive cultivation of high-density cultures.

Out of variety of marine diatoms, *C. closterium* is one of the most convenient study objects. Moreover, *C. closterium* is a promising object for industrial cultivation. Therefore, the aim of this work was to assess the possibility of using sodium bicarbonate to provide *C. closterium* with carbon under conditions of intensive cultivation without supplying CO_2 to the suspension.

MATERIAL AND METHODS

C. closterium from IBSS culture collection was adapted to the conditions of intensive cultivation on a luminostat for two weeks. The culture was grown in the storage cultivation mode in a 1-L flask, on the RS nutrient medium prepared with sterile Black Sea water. The composition was as follows ($g \cdot L^{-1}$): $NaNO_3$ – 0.775; $NaH_2PO_4 \cdot 2H_2O$ – 0.0641; $Na_2SiO_3 \cdot 9H_2O$ – 0.386; Na_2EDTA – 0.0872; $FeSO_4 \cdot 7H_2O$ – 0.045; $CuSO_4 \cdot 5H_2O$ – $0.2 \cdot 10^{-3}$; $ZnSO_4 \cdot 7H_2O$ – $0.44 \cdot 10^{-3}$; $CoCl_2 \cdot 6H_2O$ – $0.2 \cdot 10^{-3}$; $MnCl_2 \cdot 4H_2O$ – $0.36 \cdot 10^{-3}$; and $NaMoO_4 \cdot H_2O$ – $0.12 \cdot 10^{-3}$ (Zheleznova et al., 2015). The algae were grown at a constant temperature of (20 ± 1) °C and 24-hour illumination with LB 40 lamps with a mean illumination of the working surface of 27 $W \cdot m^{-2}$ (12 klx). During adaptation, the culture was bubbled with air by a compressor unit (0.5 L of air *per* 1 L of culture *per* minute).

The first stage of the experiment. Upon reaching a culture density of 1 $g \cdot L^{-1}$ of dry weight, the part of the culture volume was centrifuged (3 minutes at 1450 g). After removing supernatant, fresh RS nutrient medium was added to a raw biomass, where 1.2 $g \cdot L^{-1}$ of sodium bicarbonate was previously dissolved. The resulting suspension with a volume of 1 L and a density of 1.2 $g \cdot L^{-1}$ was placed in a flask mounted on a magnetic stirrer. The suspension surface area (phase separation) was of 50 cm^2. Throughout the experiment, the culture was grown in the storage cultivation mode at a constant stirring speed of 250 rpm. The experimental setup is shown in Fig. 1.

The culture density was determined daily by the method of iodate oxidation (Gevorgiz et al., 2015), and pH value was measured with an accuracy of 0.01 by the Aqua Medic pH Controller equipped with a combined electrode.

The second stage of the experiment. On the 4th day of the experiment, 1 g of $NaHCO_3$ and 2 mL of 0.1 N hydrochloric acid were added to the culture to lower pH down to 8.6.

Fig. 1. *C. closterium* cultivation on a nutrient medium with sodium bicarbonate as the sole carbon source

RESULTS AND DISCUSSION

The dynamics of the culture density and the nutrient medium pH are shown in Fig. 2. At the first stage of the experiment, the culture was adapted to the nutrient medium with sodium bicarbonate within a day; some cells died, as evidenced by decreases in the culture density down to 0.9 g·L^{-1} and in the medium pH down to 8.77. From the 2nd day of the experiment, an active culture growth was observed, with a maximum productivity of 0.6 g·(L·day)$^{-1}$. This growth was accompanied by a significant increase in the medium pH: cells actively assimilated carbon in the form of HCO_3^-. After adding 1 g·L^{-1} of sodium bicarbonate to actively growing culture and lowering pH down to 8.6 at the second stage of the experiment, the culture growth rate decreased to almost zero, but considering the medium pH increase during adaptation period, the culture actively absorbed bicarbonate ions (Fig. 2). After adaptation, there was an active culture growth, with a maximum productivity of 0.7 g·(L·day)$^{-1}$, which was accompanied by a high rate of the medium alkalization as well. When the medium pH reached 9.4, the culture growth slowed down; at pH = 9.9, the growth stopped completely. A day later, the culture entered the dying phase.

Based on the results obtained and considering the fact that dissolved carbon dioxide is practically absent in the nutrient medium with pH > 8.4 (Kratkaya khimicheskaya entsiklopediya, 1961 ; Sonnenfeld, 1988 ; Horne, 1972), it can be argued as follows: *C. closterium* culture grew actively, absorbing HCO_3^- ions from the nutrient medium (Kupriyanova & Samylina, 2015). Thus, in nutrient media for intensive cultivation of marine diatoms, it is quite possible to use $NaHCO_3$ (1 g·L^{-1} or more) as the sole carbon source.

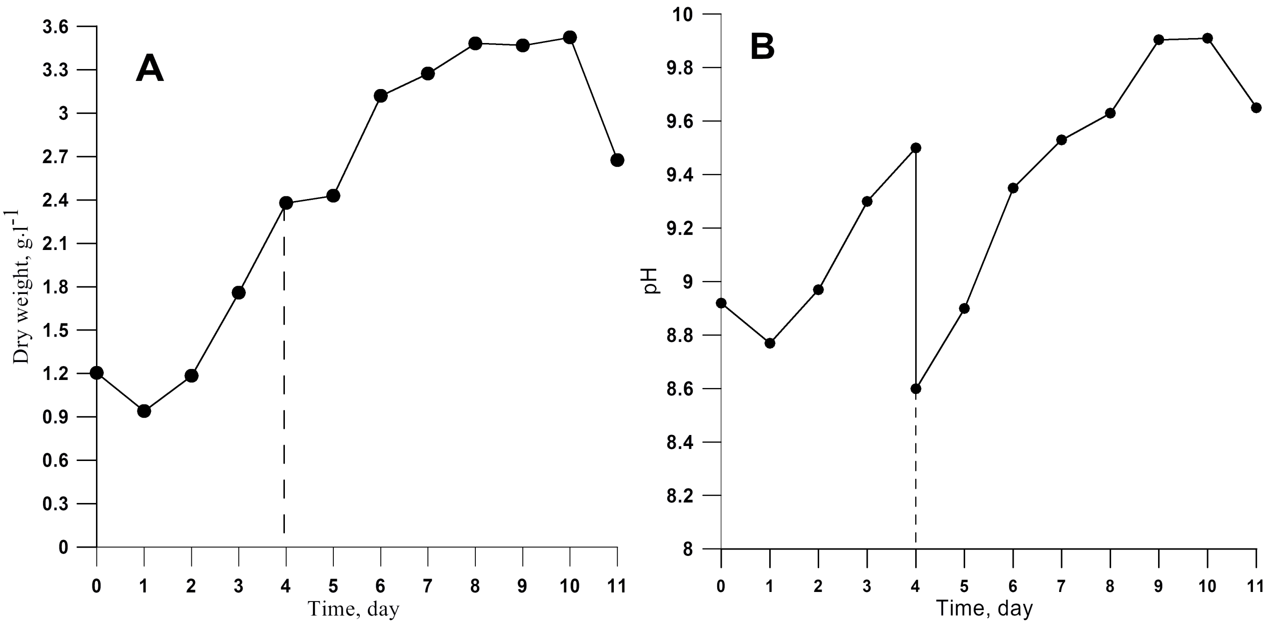

Fig. 2. Dynamics of the culture density when using sodium bicarbonate as the sole source of carbon (A) and pH dynamics during cultivation (B). The dotted line indicates the moment of adding 1 g of $NaHCO_3$ to the culture and lowering pH down to 8.6

Let us estimate limit values for an algal yield obtained on the nutrient medium with sodium bicarbonate. In general, when a nutrient from a dissolved inorganic salt is completely transformed into organic mass (without losses), and there are no losses related to the synthesis of extracellular metabolites, the maximum possible yield (B_{MAX}) is:

$$B_{MAX} = \frac{M(S)}{Y_S \cdot M(SX)} \cdot m(SX)\,, \tag{1}$$

where Y_S is nutrient content in the biomass;

M(S) and M(SX) are molar masses of a nutrient and salt containing a nutrient, respectively, $g{\cdot}mol^{-1}$;

m(SX) is the mass of salt dissolved in the nutrient medium, $g{\cdot}L^{-1}$.

Carbon content in biomass of many microalgae species is about 50 % (Horne, 1972 ; Allen et al., 2011). However, due to high ash residue content in biomass of benthic diatoms, this value varies considerably (Anderson, 1995 ; Brown & Jeffrey, 1995). According to the literature, in the active growth phase, *Cylindrotheca* sp. microalgae biomass includes as follows: total proteins – 41 % of dry weight (Brown & Jeffrey, 1995 ; Brown et al., 1997); carbohydrates – 25 % (Gügi et al., 2015 ; Nesara & Bedi, 2019); and lipids – 1 % (Ying & Kangsen, 2005). Considering that carbon content in proteins averages 52 %; in carbohydrates, 40 %; and in lipids, 75 % (Kratkaya khimicheskaya entsiklopediya, 1961), we can assume that carbon content in the organic part of *C. closterium* biomass is 48 %.

Considering ash residue content in microalgae, the expression (1) takes the form:

$$B_{MAX} = \frac{M(C)}{(1-z) \cdot Y_C^{\mathrm{ORG}} \cdot M(NaHCO_3)} \cdot m(NaHCO_3)\,, \tag{2}$$

where z is ash residue content in the biomass;

Y_C^{ORG} is carbon content in the organic part of the biomass;

M(C) and M($NaHCO_3$) are molar masses of carbon and sodium bicarbonate, respectively, $g \cdot mol^{-1}$;
m($NaHCO_3$) is the mass of sodium bicarbonate dissolved in the nutrient medium, $g \cdot L^{-1}$.

According to our data, the ash residue content in *C. closterium* biomass is 33 % (Gevorgiz et al., 2015); in the experiment, the weighed portion of $NaHCO_3$ dissolved in the nutrient medium is 1.2 $g \cdot L^{-1}$. Therefore, substituting these values into (2), the maximum biomass increase (yield, B_{MAX}) is 0.53 $g \cdot L^{-1}$. Considering carbon (total carbon in the form of HCO_3^- and CO_3^2) in Black Sea water, the concentration of which reaches 0.007 $g \cdot L^{-1}$ (Kratkaya khimicheskaya entsiklopediya, 1961), B_{MAX} is 0.575 $g \cdot L^{-1}$.

For 4 days in the experiment, the yield was 1.2 $g \cdot L^{-1}$ (see Fig. 2A), which is more than double the limiting estimate. On the other hand, 1.2 g of $NaHCO_3$ was added to the nutrient medium. In the experiment, the increase was 1.2 $g \cdot L^{-1}$ of algal dry mass; then, taking into account carbon in Black Sea water, at least 2.66 g of $NaHCO_3$ should be consumed. This results from the fact as follows: in 1.2 g of biomass, the organic part is $1.2 \times (1 - 0.33) = 0.8$ g; carbon content in the organic part is $0.8 \times 0.48 = 0.384$ g; and carbon content in $NaHCO_3$ is 14.3 %. It has to be considered that in a nutrient medium at pH > 8.4, due to the hydrolysis of bicarbonate ions (Skopintsev, 1975 ; Sonnenfeld, 1988 ; Horne, 1972), the equilibrium in

$$HCO_3^- + HO^- + H^+ \rightleftarrows H_3O^+ + CO_3^{2-} \tag{3}$$

is shifted to the right, towards CO_3^{2-} formation; so, in the nutrient medium at high pH values, some carbon is in the form inaccessible to photosynthesis (Kupriyanova & Samylina, 2015 ; Jansson & Northen, 2010). Consequently, obviously, not all carbon from $NaHCO_3$ salt, which is dissolved in the nutrient medium, is absorbed by cells for photosynthesis; some carbon in the carbonate form remains in the nutrient medium.

Let us estimate the amount of $NaHCO_3$ to be used to increase biomass by 1.2 g at pH > 8.4. Phototrophic cells are known to release 1 mole of OH^- hydroxide ions into the nutrient medium (Jansson & Northen, 2010) when absorbing 1 mole of HCO_3^- hydrogen carbonate ions for photosynthesis. As a result, the equilibrium in (3) is shifted to the right, and 1 mole of CO_3^{2-} carbonate ions is formed. Thus, a decrease in HCO_3^- in the nutrient medium is associated both with the removal of bicarbonate ions by cells for photosynthesis and with the formation of carbonate ions in the nutrient medium. Therefore, in order to obtain 1.2 g of biomass, it is necessary to use at least $1.2 \times (1 - 0.33) \times 0.48 / 0.143 \times 2 = 5.4$ g of $NaHCO_3$, which is more than 4 times a weighed portion of sodium bicarbonate dissolved in the nutrient medium in the experiment.

Such an obvious discrepancy is likely to be due to the fact that atmospheric CO_2 was actively dissolved in the culture medium. Even though the interface-specific phase in the experiment was small, the dissolution rate of carbon dioxide in the nutrient medium was sufficiently high for *C. closterium* intensive growth. The 2nd to the 4th day of the experiment, the biomass increase was estimated at 1.2 $g \cdot L^{-1}$ (see Fig. 2A); 1.2 g of *C. closterium* dry biomass contained 0.387 g of organic carbon. Thus, 0.171 $g \cdot L^{-1}$ of inorganic carbon was added to the nutrient medium. Within two days of the experiment, at least $0.387 - 0.171 = 0.216$ g of carbon was dissolved in the nutrient medium, or 0.4 $g \cdot (L \cdot day)^{-1}$ of CO_2. Importantly, the estimate is approximate; to calculate the absorption rate of atmospheric CO_2 by *C. closterium* culture, further special research is required.

Conclusion. The possibility of cultivating the marine benthic diatom *C. closterium* on a nutrient medium with high sodium bicarbonate content has been experimentally shown. As found, on the RS nutrient medium with 1.2 $g \cdot L^{-1}$ of sodium bicarbonate added under conditions of intensive cultivation, *C. closterium* productivity reaches 0.7 g·(L·day)$^{-1}$, with a significant increase in the medium pH. According to our data, optimal medium pH for *C. closterium* growth is in the range of 8.4–9.4. At higher values (pH > 9.4), the growth of diatoms slows down; at pH = 9.9, the culture enters the dying phase.

The development of nutrient media with sodium bicarbonate for intensive diatom cultivation is a promising task, since it greatly facilitates the supplying of culture with carbon, especially on an industrial scale. Adding bicarbonates to a culture medium increases the system buffer capacity and excludes sharp changes in pH, as well as the loss of carbon in the form of CO_2. Moreover, the use of culture media with hydrocarbons does not exclude the processes of CO_2 absorption from the atmosphere, even with a small interface area. According to the experimental data, during active growth, the culture received at least 50 % of atmospheric carbon.

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ИНТЕНСИВНАЯ КУЛЬТУРА *CYLINDROTHECA CLOSTERIUM* (EHRENBERG) REIMANN ET LEWIN НА ПИТАТЕЛЬНОЙ СРЕДЕ С ГИДРОКАРБОНАТОМ НАТРИЯ

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Экспериментально показана возможность использования гидрокарбоната натрия в питательной среде для обеспечения культуры *C. closterium* углеродом в условиях интенсивного культивирования без подачи CO_2 в суспензию. После адаптации *C. closterium* к питательной среде с гидрокарбонатом натрия с концентрацией 1,2 г·л$^{-1}$ наблюдался активный рост с максимальной продуктивностью 0,6–0,7 г·(л·сут)$^{-1}$ сухой массы. В клетки диатомовых водорослей углерод проникает как в форме углекислого газа, так и в форме гидрокарбонат-ионов. Однако все питательные среды для искусственного культивирования диатомей по-прежнему предполагают применение CO_2 из атмосферы или баллона. Цель работы — оценить возможность использования гидрокарбоната натрия для обеспечения *C. closterium* углеродом в условиях интенсивного культивирования без подачи CO_2 в суспензию. Культуру выращивали в режиме накопительного культивирования в колбе объёмом 1 л на питательной среде RS, приготовленной на стерильной черноморской воде, следующего состава (г·л$^{-1}$): $NaNO_3$ — 0,775; $NaH_2PO_4\cdot 2H_2O$ — 0,0641; $Na_2SiO_3\cdot 9H_2O$ — 0,386; Na_2EDTA — 0,0872; $FeSO_4\cdot 7H_2O$ — 0,045; $CuSO_4\cdot 5H_2O$ — $0{,}2\cdot 10^{-3}$; $ZnSO_4\cdot 7H_2O$ — $0{,}44\cdot 10^{-3}$; $CoCl_2\cdot 6H_2O$ — $0{,}2\cdot 10^{-3}$; $MnCl_2\cdot 4H_2O$ — $0{,}36\cdot 10^{-3}$; $NaMoO_4\cdot H_2O$ — $0{,}12\cdot 10^{-3}$. Предварительно в ней растворили 1,2 г·л$^{-1}$ гидрокарбоната натрия. Суспензию клеток перемешивали посредством магнитной мешалки (250 оборотов в минуту). На 4-й день эксперимента в культуру добавили 1 г $NaHCO_3$ и 2 мл 0,1 н соляной кислоты, чтобы снизить pH до 8,6. Со 2-го дня эксперимента зарегистрирован активный рост с максимальной продуктивностью 0,6 г·(л·сут)$^{-1}$. После добавления в активно растущую культуру 1 г·л$^{-1}$ гидрокарбоната натрия и снижения pH до 8,6 наблюдали снижение скорости роста практически до нуля, однако, судя по скорости повышения pH среды́ за время адаптации, культура активно поглощала гидрокарбонат-ионы. Экспериментально показана возможность культивирования бентосной диатомовой водоросли *C. closterium* на питательной среде с высоким содержанием гидрокарбоната натрия. Установлено, что на питательной среде RS с добавлением 1,2 г·л$^{-1}$ гидрокарбоната натрия в условиях интенсивного культивирования максимальная продуктивность *C. closterium* достигает 0,7 г·(л·сут)$^{-1}$, при этом отмечено существенное повышение pH среды́. По нашим данным, оптимальное значение pH среды́ для роста *C. closterium* находится в диапазоне 8,4–9,4. При pH > 9,4 рост диатомовых водорослей замедляется, а при достижении в питательной среде значения pH 9,9 культура переходит в фазу отмирания.

Ключевые слова: питательная среда, культивирование, диатомовые водоросли, гидрокарбонат натрия

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STEROID HORMONES, SELENIUM, AND ZINC IN THE GONADS – GAMETES – LARVAE BIOLOGICAL SYSTEM OF THE MUSSEL *MYTILUS GALLOPROVINCIALIS* LAM.

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Assessment of the interaction of marine farms with the environment in the industrial cultivation of the mussel *Mytilus galloprovincialis* is very important. In the mussel farm – environment system, biotic fluxes of chemical compounds through gonads, gametes (sperm and eggs), and larvae make a considerable contribution to this interaction. Since gonads play a key role in the mussel reproduction, it is interesting to study the budget of materials, that are directly involved in this process. Out of these materials, testosterone, estradiol, fatty acids, and some trace minerals, such as Se and Zn, are known to affect spawning, growth, and development. The molluscs absorb these materials from food and water. These materials are partly metabolically assimilated by mussels and partly excreted into the environment with gametes. The aim of this study was to estimate the components of the budget of steroid hormones, fatty acids, and two essential trace elements (Zn and Se) in mussel gonads, gametes, and larvae. The total testosterone and estradiol in gonads and gametes were quantified by enzyme-linked immunosorbent assay. The contents of the trace elements were found using inductively coupled plasma mass spectrometry. The fatty acid composition was determined by means of gas chromatography–mass spectrometry. The contents of Se and Zn in mussel gonads and gametes were found to depend on the stage of the reproductive cycle. In female gonads, Se and Zn concentrations were higher than in male ones. The highest concentration of Se was recorded in eggs: (14.7 ± 2.9) $\mu g \cdot g^{-1}$ dry weight (d. w.). In sperm, it was (14.4 ± 1.8) $\mu g \cdot g^{-1}$ d. w. Zn content in gonads before spawning was higher than in gametes. In male gonads and in sperm, its values were (27.5 ± 3.7) and (19.3 ± 6.4) $\mu g \cdot g^{-1}$ d. w., respectively. In female gonads and eggs, the contents of zinc were (53.6 ± 10.9) and (49.3 ± 8.2) $\mu g \cdot g^{-1}$ d. w., respectively. In spring, the mean values of Se and Zn assimilation degree (q) in gonads of the mussel were within 0.1–0.6. The limit values of the alimentary accumulation coefficient (K_{lim}) of Se and Zn ranged 0.6 to 1.4. While spawning, mussels excrete polyunsaturated fatty acids (PUFA), which are probably used by other marine organisms. Up to 56.2 % of PUFA are excreted with sperm, and 48.1 %, with eggs, whereas in larvae this fraction does not exceed 10.2 %. The data obtained indicate that the molluscs assimilate sex hormones, fatty acids, selenium, and zinc to maintain vital processes: prostaglandins are synthesized from PUFA in the body, and testosterone esters are formed from testosterone. Se and Zn, when coupled with proteins, play a key role in the reproduction and formation of larval shells.

Keywords: mussel *Mytilus galloprovincialis*, gametes, larvae, selenium, zinc, testosterone, estradiol, fatty acids, Black Sea

Testosterone, estradiol, polyunsaturated fatty acids (hereinafter PUFA), and trace elements possess high biological activity (Kapranova et al., 2019 ; Nikonova et al., 2017). Selenium protects an organism, as well as sperm vitality, against reactive oxygen species. Its absence during spermatogenesis affects the sperm quality and fertility of animals (Ahsan et al., 2014). Zinc is known to be used to maintain the functions of the reproductive system, as well as to activate enzymes, DNA synthesis, and proteins in the body. Therefore, the studies that allow assessing the content of steroids, fatty acids (hereinafter FA), Se, and Zn in the reproductive system of hydrobionts are of particular relevance.

Marine mussel farms are an important component of coastal marine ecosystems. In the mussel farm – environment interaction, a key role is played by biotic fluxes of chemical compounds and energy in the matter → gonads → gametes (sperm and eggs) → larvae system. The balance approach allows estimating the fluxes of the compounds through these components. The energy balance of the Black Sea mussel settlements in natural populations has been studied in most detail (Finenko et al., 1990). For marine farms, a similar approach was implemented in the investigation of the flux of carotenoids in the environment – mussel (*Mytilus galloprovincialis*) – mussel biodeposition system determining the qualitative and quantitative composition of carotenoids in various organs of *M. galloprovincialis*, depending on the season and assessment of the pigment assimilation by the mollusc (Pospelova & Nekhoroshev, 2003). As a result of the study of individual carotenoids' transformation in the process of metabolism and quantitative assessment of their assimilation, the number of carotenoids assimilated and excreted by molluscs was determined.

However, the data on the element balance of other biologically active substances involved in the metabolism, such as steroid hormones, FA, and biogenic trace elements, in mollusc gonads and gametes are very limited. It determined the aim of this work.

MATERIAL AND METHODS

The material for the research was the bivalve *Mytilus galloprovincialis* Lamarck, 1819, grown in the mollusc farms of Sevastopol: in Laspi Bay (44°24.56′N, 33°42.19′E) and Karantinnaya Bay (44.61°N, 33.49°E). Mussels were collected by diving from a depth of 2–3 m in April and May 2020. The water temperature range was +7…+21 °C. A total of about 600 uniform-size mussels with a shell length of 50–60 mm was processed. Before the experiment, the molluscs were kept in filtered seawater for 3–6 hours to clear the digestive tracts. Mussel sex and sexual maturation stage were determined on gonadal smears under a microscope, basing on the analysis of histological preparations of the gonads (Pirkova et al., 2019). Mussel sperm and eggs were obtained by the method previously described by Nikonova *et al.* (2017). The dry weight (hereinafter d. w.) of gametes was determined after drying 1 mL of the suspension of homogenized sperm and eggs at +105 °C. At the same temperature, the dry weight of gonads was determined.

The total concentration of testosterone and estradiol in gonads and gametes was quantified by enzyme-linked immunosorbent assay (Nikonova et al., 2017). The trace element concentration was determined by inductively coupled plasma mass spectrometry using multielement standard IV-ICPMS-71A (Inorganic Ventures, the USA). The relative composition of FA was determined according to the method developed by Kapranova *et al.* (2019). The larvae were obtained and their FA composition was determined using the method described in (Kapranova et al., 2020). Measurements of trace elements and FA composition were carried out at the "Spectrometry and Chromatography" core facility at IBSS.

RESULTS AND DISCUSSION

The results of the analysis of steroid hormones, Se, and Zn concentration in gonads, eggs, and sperm of the mussel *M. galloprovincialis* at different ripening stages are given in Table 1.

Table 1. Concentration of steroid hormones, selenium, and zinc in gonads, eggs, and sperm of the mussel *M. galloprovincialis*

Gonadal ripening stages (gametes)	Concentration of steroid hormones, × 10^{-6} µg·g^{-1} d. w.				Concentrations of trace elements, µg·g^{-1} d. w.			
	Total testosterone		Estradiol		Selenium		Zinc	
	♂	♀	♂	♀	♂	♀	♂	♀
1	7757.8 ± 2315.2	2154.5 ± 643.1	90.1 ± 28.6	512.5 ± 33.1	n. d.	n. d.	n. d.	n. d.
2	2453.1 ± 1409.8	592.1 ± 112.8	120.9 ± 27.8	623.0 ± 40.8	12.0 ± 1.5	n. d.	33.3 ± 6.6	n. d.
3	781.1 ± 60.1	210.3 ± 30.0	104.7 ± 30.1	747.0 ± 30.0	8.7 ± 0.7	10.8 ± 1.7	24.6 ± 3.6	35.6 ± 11.8
4	979.0 ± 83.9	352.0 ± 192.0	119.5 ± 26.3	636.7 ± 22.0	9.3 ± 1.9	9.0 ± 2.5	16.3 ± 1.9	45.5 ± 31.2
5A	975.1 ± 464.3	859.0 ± 116.1	132.2 ± 34.3	529.0 ± 26.1	10.7 ± 2.8	9.7 ± 3.3	22.9 ± 5.7	56.3 ± 17.9
5B	692.2 ± 115.4	144.2 ± 14.4	110.6 ± 20.4	501.8 ± 34.4	8.8 ± 1.4	13.7 ± 1.9	27.5 ± 3.7	53.6 ± 10.9
Eggs	n. f.	10.1 ± 4.8	n. f.	539.5 ± 122.8	n. f.	14.7 ± 2.9	n. f.	49.3 ± 8.2
Sperm	14 284.8 ± 259.2	n. f.	194.4 ± 59.2	n. f.	14.4 ± 1.8	n. f.	19.3 ± 6.4	n. f.

Note: n. f. denotes not found; n. d., no data; 5A and 5B, gonads before and after spawning.

Selenium concentrations in gonads and gametes of female and male mussels (Table 1, Fig. 1) change similarly to the concentrations of steroid hormones (Nikonova et al., 2017) and depend on the mollusc reproductive cycle (Goede et al., 1993). Se concentration in male gonads is positively correlated with testosterone concentration. The Pearson correlation coefficient is 0.89 ($p = 0.045$).

The maximum concentration of selenium is determined in the gametes: in eggs, (14.7 ± 2.9) µg·g^{-1} d. w., and in sperm, (14.4 ± 1.8) µg·g^{-1} d. w. The decrease in Se content in mussel gonads after spawning is due to the beginning of the post-spawning restructuring phase.

Selenium is an essential element for mussels. The percentages of its entry into the body are 90 % with food and 10 % with water (Ahsan et al., 2014). All this Se is in a bivalent organic form, with selenocysteine (SeCys) predominating in animal products and selenomethionine (SeMet) predominating in plant products. Selenium enters the animal body mainly in the form of selenomethionine. Se is transported and deposited by selenoproteins containing selenocysteine. Selenoproteins are selenocysteine-containing redox proteins involved in the antioxidant reaction. Se is an integral part of selenoproteins, which protect sperm during maturation against oxidative damage and also serve as structural components of mature sperm. Thus, selenium and selenoproteins provide sperm viability, as well as protection against reactive oxygen species. Genetic studies of selenoproteins have shown that their absence during spermatogenesis results in abnormal sperm development, which in turn affects sperm quality, fertility, and libido (Ahsan et al., 2014).

In the study of selenoprotein from the pearl mussel *Cristaria plicata*, it was possible to express messenger ribonucleic acid (mRNA) from tissues of the mantle, gills, hemocytes, muscles, and hepatopancreas. The highest expression occurred from hepatopancreas tissues (Hu et al., 2014 ; Kopp et al., 2018).

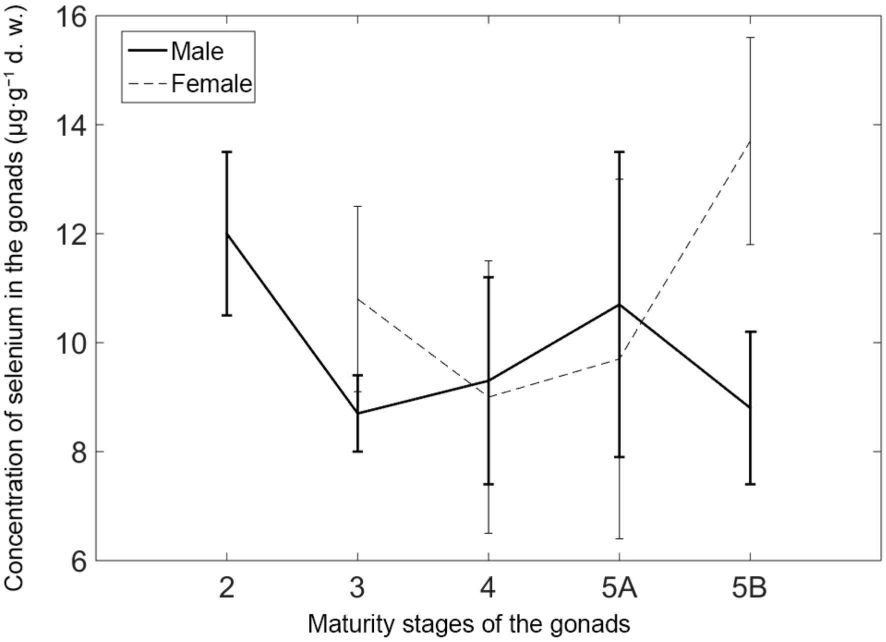

Fig. 1. Concentration of selenium in gonads of the mussel *M. galloprovincialis*: 5A and 5B denote gonads before and after spawning

Zinc is used by the animal body to activate enzymes, synthesize DNA and proteins, and maintain the functions of the reproductive system. It also acts as antioxidant inhibiting the ability of free radicals to damage cell tissue and genetic material. Zn excretion from the soft tissues of mussels seems to occur through eggs during spawning (Lowe & Moore, 1979). In *M. galloprovincialis* collected in several seaports of Western Algeria, zinc concentration varied within 87.1–731.5 µg·g^{-1} d. w. (Hadj et al., 2012). In the Black Sea mussel sperm, Zn concentration was (30.4 ± 6.4) µg·g^{-1} d. w.; in eggs, it was (115.4 ± 24.2) µg·g^{-1} d. w. (Karavantseva et al., 2012). In the present work, the higher Zn content was recorded in gonads before spawning than in gametes, which indicates a partial excretion of zinc with sperm and eggs. In male gonads before spawning, Zn content was (27.5 ± 3.7) µg·g^{-1} d. w., and in sperm, it was (19.3 ± 6.4) µg·g^{-1} d. w. In female gonads, the concentration was (53.6 ± 10.9) µg·g^{-1} d. w., and in eggs, it was (49.3 ± 8.2) µg·g^{-1} d. w.

Zinc assimilated with food is related mainly to the mussel soft tissues, while Zn from seawater is deposited mostly in shells (Fisher et al., 1996). Changes in the mass of soft tissues and shells in *M. galloprovincialis* are likely to result from different levels of Zn accumulation. Specifically, the weight of zinc-contaminated mussel shells increased after a 51-day cleaning period (Soto et al., 2000).

In our studies, a negative correlation between zinc content and estradiol concentration in female gonads at different ripening stages was revealed. The Pearson correlation coefficient was −0.98 ($p = 0.024$). Estradiol is recognized as an important regulator of both food intake and energy consumption (Mauvais-Jarvis et al., 2013). Estradiol regulates body weight, contributing to its increase (Mauvais-Jarvis et al., 2013).

As shown in (Soto et al., 2000), Zn was accumulated in the soft tissues proportionally to its concentration in seawater, while its content in hemolymph was slightly higher than in the environment. Zinc assimilation was through intestines, mantle, and gills. Zn was transported to the kidneys from gills and intestines ($t_{½} \approx 8$ days) through the hemolymph either as a high-molecular complex or as granular amoebocytes. Most of zinc was in granular amoebocytes, which were found either in all body tissues or in intestines and kidneys. Thus, in the mussel *Mytilus edulis*, the kidney is an organ for the storage of many trace elements; it contains 30 % (about 1000 $\mu g \cdot g^{-1}$ d. w.) of total zinc concentration in the body. In this case, Zn is localized in the form of insoluble granules in some intracellular organelles, which occupy about 20 % of the cell volume. Zinc is excreted by defecation, exocytosis of renal granules to the urine, and diapedesis of amoebocytes (George & Pirie, 1980).

To assess the assimilation degree of Se and Zn with food by hydrobionts, a mathematical model was applied based on the equation of G. G. Polikarpov and V. N. Egorov (1986):

$$\frac{dC_{\text{mussels}}}{dt} = R(C_f q - C_{\text{mussels}} q_f) - C_{\text{mussels}}\, p \,, \tag{1}$$

where C_{mussels} and C_f are concentrations of a chemical element in the hydrobiont and its food, $\mu g \cdot g^{-1}$;

R is the relative food intake rate, day^{-1};

q is the degree of an element assimilation from food;

q_f is the degree of food assimilation for growth (= K_2);

p is the element exchange rate of the hydrobiont, day^{-1}.

The equation proposed by G. G. Polikarpov and V. N. Egorov (1986) for describing the kinetics of trace element exchange in hydrobionts during element assimilation with food can be applied for mussels if the concentration of trace elements in their gonads and gametes is measured given the known coefficient K_2. The degree of trace element assimilation from food is estimated from the coefficient q, which may be an important characteristic determining the need of marine organisms for the trace elements in question.

By transforming this equation (Pospelova et al., 2018), a formula was obtained for estimating the assimilation degree of selenium (zinc) q from the measurement data of element concentrations in mussel tissues and gametes:

$$q = \frac{C_{\text{gon.}} q_f}{C_{\text{gon.}} q_f + C_{\text{gametes}}(1 - q_f)} \,, \tag{2}$$

where q is the degree of element assimilation from food;

$C_{\text{gon.}}$ is the concentration of a chemical element in gonads, $\mu g \cdot g^{-1}$;

C_{gametes} is its concentration in mussel sperm or eggs, $\mu g \cdot g^{-1}$;

q_f is the degree of food assimilation for growth (= K_2).

It should be noted that this approach did not take into account Se and Zn fractions that could be excreted through biodeposition.

Previously, a study of the kinetics of trace element content in *M. galloprovincialis* showed as follows: the concentration and exchange of a trace element in mussels could be considered as an integral process in ontogenesis (Polikarpov & Egorov, 1986). In the first approximation, the mean annual value of the food assimilation for growth – q_f (K_2) (Finenko et al., 1990) – can be used to assess the element assimilation by molluscs. For q_f limits (0.14 and 0.42), q values were determined for Se (Zn) in spring (Table 2).

In mussel gonads in spring, the mean annual q values for selenium fluctuate in the range 0.1 to 0.4, which is lower than the degree of food assimilation for growth (K_2). It is known that if the efficiency of trace element assimilation from food is lower than the degree of food assimilation for growth, the transfer of matter along the trophic chain proceeds with a decrease in the rate of contamination in the subsequent link (Polikarpov & Egorov, 1986). Mean annual q values for zinc are 0.1–0.6.

Table 2. Assimilation of selenium and zinc with food (q) by the mussel *Mytilus galloprovincialis*

Gonadal ripening stages	q Se ($q_f = 0.14$)		q Zn ($q_f = 0.14$)	
	♂	♀	♂	♀
1	n. d.	n. d.	n. d.	n. d.
2	0.12 ± 0.03	n. d.	0.22 ± 0.08	n. d.
3	0.09 ± 0.03	0.11 ± 0.07	0.17 ± 0.01	0.11 ± 0.01
4	0.10 ± 0.04	0.09 ± 0.07	0.12 ± 0.05	0.13 ± 0.02
5A	0.11 ± 0.06	0.10 ± 0.08	0.16 ± 0.01	0.16 ± 0.01
5B	0.09 ± 0.04	0.13 ± 0.08	0.19 ± 0.01	0.15 ± 0.01
Gonadal ripening stages	q Se ($q_f = 0.42$)		q Zn ($q_f = 0.42$)	
	♂	♀	♂	♀
1	n. d.	n. d.	n. d.	n. d.
2	0.38 ± 0.09	n. d.	0.55 ± 0.07	n. d.
3	0.30 ± 0.06	0.35 ± 0.02	0.48 ± 0.01	0.34 ± 0.03
4	0.32 ± 0.01	0.31 ± 0.01	0.38 ± 0.08	0.40 ± 0.06
5A	0.35 ± 0.02	0.32 ± 0.02	0.46 ± 0.02	0.45 ± 0.03
5B	0.31 ± 0.11	0.40 ± 0.20	0.51 ± 0.07	0.44 ± 0.20

Note: n. d. denotes no data; 5A and 5B, gonads before and after spawning.

With known values of the degree of an element assimilation from food (q) and the degree of food assimilation for growth (q_f), it is possible to determine the limiting coefficient of food accumulation of a trace element according to the equation $K_{lim} = Rq / (Rq_f + p)$ (Polikarpov & Egorov, 1986). From this equation (at $p = 0$, where p is the element exchange rate in the hydrobiont, day^{-1}), $K_{lim} = q / q_f$. That is, K_{lim} is equal to the ratio of the degree of Se or Zn assimilation from food to the degree of food assimilation for growth (Table 3).

Table 3. Maximum coefficient of food accumulation of trace element (K_{lim}) by the mussel *M. galloprovincialis*

Gonadal ripening stages	K_{lim} Se ($q_f = 0.14$)		K_{lim} Zn ($q_f = 0.14$)	
	♂	♀	♂	♀
1	n. d.	n. d.	n. d.	n. d.
2	0.86 ± 0.21	n. d.	1.57 ± 0.59	n. d.
3	0.64 ± 0.23	0.79 ± 0.52	1.21 ± 0.07	0.79 ± 0.07
4	0.71 ± 0.31	0.64 ± 0.53	0.86 ± 0.35	0.93 ± 0.12
5A	0.79 ± 0.42	0.71 ± 0.62	1.14 ± 0.07	1.14 ± 0.07
5B	0.64 ± 0.74	0.93 ± 0.61	1.36 ± 0.07	1.07 ± 0.07
Gonadal ripening stages	K_{lim} Se ($q_f = 0.42$)		K_{lim} Zn ($q_f = 0.42$)	
	♂	♀	♂	♀
1	n. d.	n. d.	n. d.	n. d.
2	0.90 ± 0.20	n. d.	1.31 ± 0.2	n. d.
3	0.71 ± 0.12	0.83 ± 0.05	1.14 ± 0.02	0.81 ± 0.07
4	0.76 ± 0.02	0.74 ± 0.02	0.90 ± 0.23	0.95 ± 0.14
5A	0.83 ± 0.05	0.76 ± 0.05	1.10 ± 0.05	1.07 ± 0.07
5B	0.74 ± 0.23	0.95 ± 0.54	1.21 ± 0.22	1.05 ± 0.54

Note: n. d. denotes no data; 5A and 5B, gonads before and after spawning.

K_{lim} values for selenium and zinc in mussel gonads in spring are 0.64 to 1.36, and those are higher than the degree of assimilation and involvement in biochemical processes (q) of the considered trace elements. This fact shows that Se and Zn are excreted with gametes into the environment.

Since we used mussels grown on marine farms, we calculated the concentration of steroid hormones, selenium, and zinc in gametes of one ton of molluscs – 71,124 specimens with 51–60 mm in size (Kholodov et al., 2017) (Table 4).

Table 4. Concentration of testosterone, estradiol, selenium, and zinc *per* one ton of the mussel *M. galloprovincialis*

Stages	Content of steroid hormones, $\times 10^{-6}$ g·t^{-1} d. w.				Content of trace elements, g·t^{-1} d. w.			
	Total testosterone		Estradiol		Selenium		Zinc	
	♂	♀	♂	♀	♂	♀	♂	♀
5A	975.1 ± 464.3	859.0 ± 116.1	132.2 ± 34.3	529.0 ± 26.1	10.7 ± 2.8	9.7 ± 3.3	22.9 ± 5.7	56.3 ± 17.9
5B	692.2 ± 115.4	144.2 ± 14.4	110.6 ± 20.4	501.8 ± 34.4	8.8 ± 1.4	13.7 ± 1.9	27.5 ± 3.7	53.6 ± 10.9
Eggs	n. f.	10.1 ± 4.8	n. f.	539.5 ± 122.8	n. f.	14.7 ± 2.9	n. f.	49.3 ± 8.2
Sperm	14 284.8 ± 259.2	n. f.	194.4 ± 59.2	n. f.	14.4 ± 1.8	n. f.	19.3 ± 6.4	n. f.

Note: n. f. denotes not found; 5A and 5B, gonads before and after spawning.

According to Table 4 and literature data (Kapranova, 2020 ; Scott, 2018), steroids and trace elements are excreted by molluscs into the aquatic environment with gametes. In spring, during mass spawning, testosterone, estradiol, and selenium are largely transferred to gametes. Zinc is only partly excreted, which is most likely due to the portioned mussel spawning. Mass mussel spawning in the Black Sea is repeated twice a year: in spring and autumn. The maximum number of spawning mussels is recorded in mid-April. Mass spawning in autumn begins in September – October and continues in November – December (Kholodov et al., 2017). The peak of mussel spawning in the southeastern Crimean coast of the Black Sea is recorded in December – January, and the lesser one is registered in May – June. Thus, the seasonal duration of the mass spawning averages 4 months *per* year.

The work of A. V. Pirkova *et al.* (2019) shows the dynamics of gonad maturation and sex ratio of mussels depending on the season. The maximum number of males spawn in July, and the ratio of females to males in the sample (%) is 25.0 : 75.0.

The elements of FA balance in the mussel *M. galloprovincialis* can be represented in the *gonads – gametes – trochophores* conditional system as shown in Table 5.

Changes in FA profile in mussel gonads and gametes depending on the maturation stage have already been discussed in (Kapranova et al., 2019). The content of SFA in mussel trochophores is approximately equal to the total content of SFA in eggs and sperm (Kapranova et al., 2020) (Table 5). This dependence can be explained by the fact that before the formation of organs and tissues, mussel trochophores are passively fed; SFA seem to perform mainly a protective function, forming the shells of cell membranes (Fokina et al., 2010). Moreover, at the initial maturation stages, FA are probably involved in the esterification of steroid hormones since this process involves predominantly C16 and C18 SFA (Scott, 2018).

Table 5. Fatty acid content (% of total lipids) in gonads, reproductive products, and trochophores of the mussel *M. galloprovincialis*

Gonadal ripening stages (gametes)	Saturated fatty acids (SFA)			Monounsaturated fatty acids (MUFA)			Polyunsaturated fatty acids (PUFA)		
	♂	♀	Larvae	♂	♀	Larvae	♂	♀	Larvae
1	100	100	58.2	n. f.	n. f.	31.6	n. f.	n. f.	10.2
2	48.2	100		15.8	n. f.		36.0	n. f.	
3	35.1	100		11.3	n. f.		53.6	n. f.	
4	42.5	81.1		35.9	3.7		21.6	15.2	
5	100	44.4		n. f.	22.1		n. f.	33.5	
Eggs	n. f.	46.7		n. f.	5.2		n. f.	48.1	
Sperm	34.0	n. f.		9.8	n. f.		56.2	n. f.	

Note: n. f. denotes not found.

MUFA content in both female and male gametes of mussels is almost two times lower than in gonads. This fact shows that both saturated and unsaturated FA enter the mussel body with food and water and then are used in processes maintaining their vital activity (Pospelova et al., 2018 ; Orban et al., 2002). Similar to MUFA content, PUFA content in larvae is an order of magnitude lower than the total PUFA content in female and male gametes since most of FA enter the mussel tissues from microalgae and are accumulated throughout the life cycle. PUFA are necessary for adaptation to environmental conditions (temperature, salinity, *etc.*) (Fokina et al., 2010 ; Orban et al., 2002). In addition, in molluscs, PUFA are probably predecessors of prostaglandins (Rowley et al., 2005). Total prostaglandin content in molluscs is low (Tadasi & Hiroshi, 1976); nevertheless, prostaglandins and related eicosanoids, being oxygen-containing metabolites of C20 PUFA, have a physiological effect on bivalve spawning (Stanley-Samuelson, 1994). Excessive amounts of PUFA are possibly released into the aquatic environment and then absorbed by other hydrobionts.

Conclusion. The data obtained indicate that the mussels are most likely unable to synthesize testosterone, estradiol, and fatty acids. Molluscs assimilate selenium, zinc, sex steroids, and fatty acids with food and water to maintain vital functions. In the process of biochemical transformations, prostaglandins are synthesized from PUFA in the body, and testosterone esters are formed from testosterone. Se and Zn play a key role in mollusc reproduction. Selenium content in male gonads is positively correlated with testosterone content. Zinc affects the weight of mussel gonads. Zn concentration in female gonads is negatively correlated with estradiol content. By the example of selenium and zinc in spring, the values of the assimilation degree of these elements by mussel gonads from food (q) were quantified along with the limiting coefficient of food accumulation of these trace elements (K_{lim}). For the considered trace elements, q values are lower than the degree of food assimilation for growth (K_2), which indicates an active interaction of mussels with the environment. Excessive amounts of free forms of testosterone, estradiol, fatty acids, Se, and Zn are excreted with gametes in order to maintain a balance between free and bound forms of these substances. Together with gametes, the necessary amounts of steroids and trace elements are transferred to the nascent larvae, which are endogenously fed during the first few days. During spawning, one ton of mussels is capable of excreting into the environment with sperm up to $14.28\cdot10^{-3}$ mg of testosterone, $0.19\cdot10^{-3}$ mg of estradiol, 14.4 g of zinc, and 19.3 g of selenium. With eggs of one ton of mussels, $0.54\cdot10^{-3}$ mg of estradiol, 14.7 g of Se, and 49.3 g of Zn are excreted. Mussels serve as a source of PUFA, which are probably used by other hydrobionts. With sperm, up to 56.2 % and with eggs, up to 48.1 % of PUFA is excreted; in larvae, this value does not exceed 10.2 %.

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СТЕРОИДНЫЕ ГОРМОНЫ, СЕЛЕН И ЦИНК В БИОЛОГИЧЕСКОЙ СИСТЕМЕ ГОНАДЫ — ПОЛОВЫЕ ПРОДУКТЫ — ЛИЧИНКИ МИДИИ *MYTILUS GALLOPROVINCIALIS* LAM.

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Оценка взаимодействия морских хозяйств с окружающей средой при промышленном выращивании мидии *Mytilus galloprovincialis* весьма актуальна. В системе мидийная ферма — среда важную роль играют биотические потоки веществ через гонады, половые продукты (сперма и яйцеклетки) и личинки. Поскольку гонады выполняют ключевую роль в размножении мидий, представляется интересным рассмотреть элементы баланса веществ, принимающих непосредственное участие в этом процессе. Тестостерон, эстрадиол, жирные кислоты, а также селен и цинк, потребляемые моллюсками вместе с пищей и водой, прежде всего необходимы им для осуществления нереста, а также для роста и развития. Часть потребляемых веществ задействуется организмом мидий в процессе метаболизма, а часть экскретируется в водную среду вместе с половыми продуктами. Цель данной статьи — провести количественное определение элементов баланса стероидных гормонов, жирных кислот и биогенных микроэлементов в гонадах, половых продуктах и личинках моллюсков, играющих важную роль в метаболизме их организма. Концентрацию общего тестостерона и эстрадиола в гонадах и половых продуктах определяли методом твёрдофазного иммуноферментного анализа. Содержание микроэлементов измеряли методом масс-спектрометрии с индуктивно-связанной плазмой. Относительный состав жирных кислот гонад, половых продуктов и личинок мидий определяли методом хромато-масс-спектрометрии. Концентрации селена и цинка в гонадах и половых продуктах мидий зависят от стадии репродуктивного цикла. В женских гонадах содержание селена и цинка выше, чем в мужских. Наибольшая концентрация селена обнаружена в яйцеклетках — (14,7 ± 2,9) $мкг·г^{-1}_{сух}$. Концентрация в сперматозоидах — (14,4 ± 1,8) $мкг·г^{-1}_{сух}$.

Содержание цинка в гонадах до нереста выше, чем в половых продуктах. В гонадах самцов до нереста концентрация цинка составляет (27,5 ± 3,7) $мкг·г^{-1}_{сух}$, в сперматозоидах — (19,3 ± 6,4) $мкг·г^{-1}_{сух}$. В гонадах самок — (53,6 ± 10,9) $мкг·г^{-1}_{сух}$, в яйцеклетках — (49,3 ± 8,2) $мкг·г^{-1}_{сух}$. В весенний период значения степени усвоения селена и цинка из пищи (q) в гонадах мидий колеблются в диапазоне от 0,1 до 0,6. Значения предельного коэффициента пищевого накопления селена и цинка ($К_п$) составляют от 0,6 до 1,4. Мидии служат источником полиненасыщенных жирных кислот (ПНЖК), которые, вероятно, используются другими гидробионтами. Со спермой выделяется до 56,2 % ПНЖК, с яйцеклетками — 48,1 %, тогда как в личинках этот показатель не превышает 10,2 %. Полученные данные свидетельствуют о том, что моллюски потребляют гормоны, жирные кислоты, селен и цинк из пищи и воды для поддержания жизненных процессов: из ПНЖК в организме образуются простагландины, из тестостерона — сложные эфиры тестостерона. Селен и цинк, соединяясь с белками, играют ключевую роль в размножении и формировании оболочек личинок.

Ключевые слова: мидия *Mytilus galloprovincialis*, половые продукты, личинки, селен, цинк, тестостерон, эстрадиол, жирные кислоты, Чёрное море

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ASSESSMENT OF CONTAMINATION OF THE ESTUARINE AREA OF THE NORTHERN DVINA RIVER BY CALCULATING BACKGROUND CONCENTRATIONS (Fe, Mn, Zn, Cu, Cd, Pb, and Ni)

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This publication is a continuation of research on the quantitation of the heavy metal contamination level (Fe, Mn, Zn, Cu, Cd, Pb, and Ni) in the ecosystem components of the estuarine area of the Northern Dvina River. To assess the contamination level with these metals in the ecosystem of the Northern Dvina estuarine area in the spot of the highest anthropogenic load (the area of Novodvinsk – Arkhangelsk – Severodvinsk urban agglomeration), we use the method of comparative analysis of the study object with the background plot – the river section above urban development. Using spatial mapping of the enrichment factor values for the aquatic ecosystem components, areas with abnormal excess of metal content in mollusc tissues, bottom sediments, and bottom water layer were determined. As established, the area adjacent to the city center and to an industrial zone on the left bank is subject to the highest anthropogenic load in the water area of the river delta top. The most likely sources of contamination with the considered heavy metals are the inflow of surface wastewater (meltwater and rain storm runoff), untreated municipal wastewater, and direct short-range atmospheric transport. The analysis of mollusc tissues proved to be a more informative and indicative approach to the assessment of the heavy metal contamination in water areas with complex hydrological and hydrochemical environmental gradients than the analysis of bottom water layer and bottom sediments.

Keywords: estuarine area of the Northern Dvina River, background concentrations, heavy metals, enrichment factor, bivalves

Rivers draining huge basins are the main source of inflow from land to sea of dissolved and suspended matter, *inter alia* organic matter, trace elements, and biogenic substances. The bulk of these substances, inputted from drainage basins, are deposited in estuarine areas of rivers – in the zone of freshwater and saltwater mixing; hence, the information on the state of the estuarine areas of rivers can help in characterizing both the marine environment and drainage basin area (Kotova et al., 2020). Moreover, coastal cities and manufacturing enterprises are mostly concentrated in the estuarine areas of rivers since there is a need for a constant source of freshwater. The works carried out to assess the heavy metal content in the ecosystem components of the estuarine area of the Northern Dvina River (Neverova et al., 2014, 2016) using threshold limit values (hereinafter TLVs) revealed the need to apply an approach with the calculation of conditional background concentrations typical for the research area. Mosaicity and high variability of the metal content in abiotic and biotic components of the aquatic ecosystem of the estuarine area of the Northern Dvina were recorded as well. The highest values of the heavy metal concentrations in water, bottom sediments, and hydrobiont tissues were observed (with no regularities) in local areas and for certain metals.

At the first stage of the work (Neverova et al., 2014), to quantify the heavy metal content in bottom water, bottom sediments, and tissues of macrobenthic species in the Northern Dvina delta, we compared the results obtained with the generally accepted state standards – TLVs. Importantly, concentrations of all metals in the entire studied section exceeded the standard. The results of observations carried out by the Northern Directorate for Hydrometeorology and Environmental Monitoring (Obzory zagryazneniya okruzhayushchei sredy, 2021) also annually show increased concentrations (up to 2–6 TLV) of Mn, Fe, Zn, and Cu. In various documents issued by environmental organizations, such excess is associated with the consequences of economic activities. However, in the estuarine area of the Northern Dvina River, there are no enterprises with high heavy metal content in technological processes. This fact demonstrates as follows: the existing state standards (TLVs) cannot be unified for all the types of aquatic ecosystems and for different climatic, geographic, and geochemical conditions. To date, most researchers dealing with this problem (Venitsianov et al., 2015 ; Wozniak & Lepikhin, 2018 ; Khorudzhaya, 2002) agree that it is unacceptable to apply uniform TLV norms to all regions; technogenic loads on ecosystems have to be standardized based on regional geochemical peculiarities. Moreover, the TLVs themselves have not been established for the vast majority of the aquatic ecosystem components, in particular for bottom sediments and tissues of benthic species.

To quantify the contamination level of the ecosystem components when having no TLVs, several models are usually applied to compare heavy metal concentrations obtained with metal natural content in the ecosystem (background values). The values of the indicator, which were established for the area not under direct anthropogenic load, can be used as the background ones. However, almost all the types of aquatic ecosystems are now affected by human activities (for example, resulting from long-range atmospheric transport or agricultural work in the depths of the drainage basins), and we can only talk about conditional background values. In this work, we chose as a background plot the river section above the spot of the highest anthropogenic load (above the area of Novodvinsk – Arkhangelsk – Severodvinsk urban agglomeration). To assess the background values of the heavy metal concentrations in water, bottom sediments, and mollusc tissues, we use the median and mean absolute deviation (*MAD*) obtained for a set of sampling points in the background plot. This method is recognized as the most sensitive one for calculating metal background levels in the analysis of contamination of components of the environment (Lukashev, 2007 ; Reimann et al., 2005).

The aim of this work is to quantify the level of heavy metal contamination in the Northern Dvina River in the spot of the highest anthropogenic load, applying the method of comparative analysis with conditional background values and calculating the mean metal concentrations in the tissues of hydrobionts and in their habitat by nonparametric statistics methods; based on the spatial distribution of the enrichment factor values, to draw a conclusion about the main sources of contamination in the studied river section (household wastewater of Arkhangelsk and Novodvinsk, industrial enterprises in these cities, *etc.*).

MATERIAL AND METHODS

The Northern Dvina River and its tributaries in the northern Russian Plain belong to the White Sea basin. In the Northern Dvina basin, the main part of the region's industrial potential is concentrated. The estuarine area of the river can be attributed to the most developed territories from the standpoint of industry and transport in the north of the Russian Federation; the area is under significant anthropogenic load. The estuarine area of the Northern Dvina includes a vast section of the lower river reach from the Pinega confluence to the delta sea edge and the southeastern Dvina Bay of the White Sea (Fig. 1). "The upper boundary of the estuarine area is at the Pinega River estuary, where tidal level fluctuations are no longer observed. The sea boundary runs along the slope of depths in the Dvina Bay, approximately along the 8-meter isobath. The river section in the estuarine area has a length of 135 km along the navigable fairway" (Gidrologiya ust'evoi oblasti Severnoi Dviny, 1965).

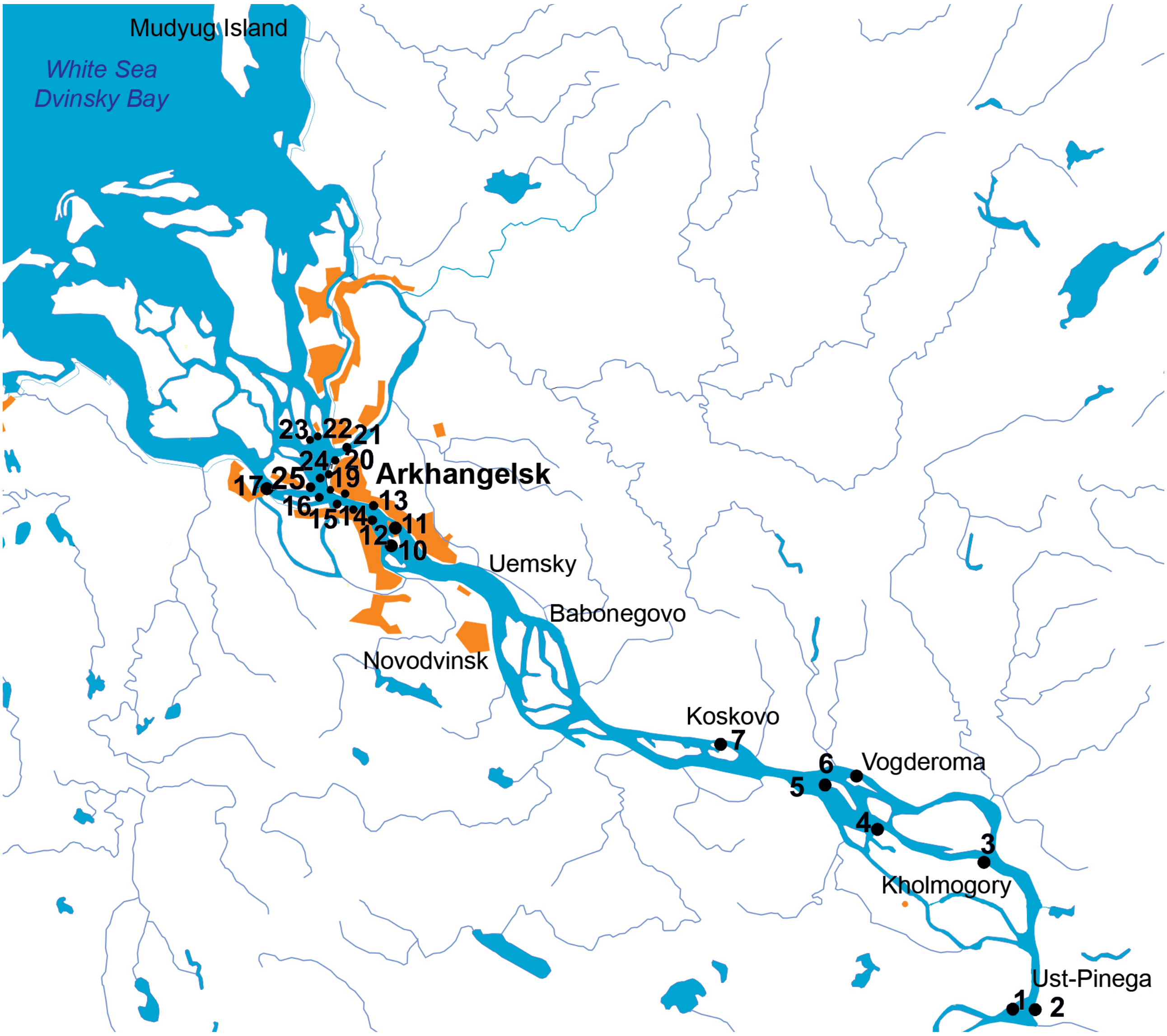

Fig. 1. Schematic map of the estuarine area of the Northern Dvina River with sampling points of bottom water layer, bottom sediments, and bivalves (points 1–7, the background plot; points 10–25, Arkhangelsk)

On the Northern Dvina, about 50 km from its confluence into the White Sea, Arkhangelsk is located; city quarters stretch for about 40 km along the right river bank. On the left bank, within the boundaries of Arkhangelsk, there are numerous industrial sites and low-rise residential areas, wooden buildings mostly, which stretch all the way to Novodvinsk.

To compare the heavy metal content, we chose the river section from Koskovo village to the upper boundary of the estuarine area (Ust-Pinega village) as the background plot (see Fig. 1, sampling points 1–7). Bivalve molluscs were sampled from their habitats simultaneously with bottom sediments and water in the estuarine area of the Northern Dvina at late July – August in 2009–2018. Molluscs were sampled by standard methods (Rukovodstvo po metodam, 1983) manually with a hydrological landing net or, at depths exceeding 1.5 m, with diving equipment. For the analysis, molluscs of the same age and size were selected from the sample and placed in vessels with water to cleanse their intestines; after this procedure, soft tissues were separated from shells and subjected to mineralization with concentrated nitric acid when heated. The preparation of biological samples for analysis is described in detail in the State Standard 26929-94 (2010).

The dominant benthic species in the studied river section, depending on biotope, are as follows: for solid substrate, bivalves *Dreissena polymorpha* (Pallas, 1771); for silted sand, *Anodonta anatina* (Linnaeus, 1758), *Unio pictorum* (Linnaeus, 1758), and *Pisidium* sp.; and for silt, Oligochaeta and Ghironomidae. To identify bivalve species, molecular genetic analysis was carried out according to the method (Bolotov et al., 2015). It showed that we have selected the most typical representatives of large bivalves for our area as objects for studying the heavy metal accumulation in tissues: *Anodonta anatina* (hereinafter duck mussel) and *Unio pictorum* (hereinafter painter's mussel). For *Dreissena*, no genetic analysis was performed: according to (Makhnovich, 2016), the mussels of this genus in the Northern Dvina belong to the species *Dreissena polymorpha* (Pallas, 1771). The analysis carried out earlier (Neverova et al., 2014, 2016) revealed that oligochaetes accumulate the maximum amount of metals; at the same time, due to technical difficulties in their sampling (primarily because of their small size), large bivalves are a more convenient object for studying the heavy metal accumulation.

Bottom water layer was sampled in accordance with the State Standard R 51592-2000 (2008), fixed with nitric acid, and, when transferred to a laboratory, subjected to mineralization when heated for analysis according to the Federal Environmental Regulatory Document 14.1:2.214-06 (2006). After preliminary drying to an air-dry state, bottom sediment samples were subjected to water bath–assisted extraction with 5 M nitric acid solution according to the Guidance Document 52.18.191-89 (Metodika vypolneniya izmerenii, 1990). All the solutions obtained (extracted tissues and bottom sediments; water) were analyzed by atomic absorption spectrometry on a novAA 315 spectrometer (Analytik Jena AG); in them, total content of Fe, Mn, Zn, Cu, Cd, Pb, and Ni was determined. The analysis was carried out at "Critical technologies of the Russian Federation in the field of environmental safety in the Arctic" core facility at the Federal Center for Integrated Arctic Research (Arkhangelsk).

All the empirical data obtained were preliminary assessed for compliance with the law of normal distribution using the Shapiro–Wilk test (Subbotina & Grzhibovskii, 2014). It showed that the distribution of most of the values obtained on the metal content in mollusc tissues, bottom water, and bottom sediments differs from the normal one. Therefore, to estimate the mean heavy metal content in mollusc tissues and in mollusc habitat at the background plot, we used the criteria of nonparametric statistics:

the median and *MAD*, defined as the median of absolute deviations from the median of all data (Lukashev, 2007 ; Reimann et al., 2005 ; Tukey, 2008). In this case, the background values were calculated by the formula:

$$C_f = Me_f \pm 2MAD\,, \tag{1}$$

where C_f is the background value of the studied indicator;

Me_f is the median value of a set of measured values (X_f) for the background plot;

MAD is the median of a set of absolute values of the differences between the measured X_f value and the median of a set of measured values for the background plot ($|X_f - Me_f|$).

To quantify the contamination level in certain areas of the riverbed, the metal content at a separate point was compared with the value of the upper limit of the background concentration using their ratio – the enrichment factor:

$$X_i/(Me_f + 2MAD)\,, \tag{2}$$

where X_i is the measured concentration value at the *i*-th sampling point.

If the ratio of the obtained metal concentration at a sampling point to the value of the upper limit of the background concentration is ≤ 1, the metal concentration at this point will be considered within the background fluctuation; if >1, the concentration will be considered increased.

Within the framework of the statistical analysis, to check the homogeneity of the sets of the heavy metal concentrations obtained for the background plot and for the area under direct anthropogenic load, an analysis was performed using the nonparametric Mann–Whitney *U* test.

To assess the spatial distribution of the enrichment factor values in heavy metals of the ecosystem components according to the data obtained at the sampling points, we used isoline schematic maps. Those were built in the Surfer software package; a data grid was created by the radial basis function interpolation. Since it is not an accurate interpolation method, the limits of maximum and minimum values were set manually.

RESULTS AND DISCUSSION

Bottom water layer. After processing the data obtained with the criteria of nonparametric statistics (the median and *MAD*), the following heavy metal concentrations were obtained in the bottom water layer for the background plot (points 1–7): Ni – (0.005 ± 0.003) mg·dm^{-3}; Cd – (0.0038 ± 0.0026) mg·dm^{-3}; Zn – (0.015 ± 0.011) mg·dm^{-3}; Cu – (0.006 ± 0.001) mg·dm^{-3}; Mn – (0.087 ± 0.023) mg·dm^{-3}; and Fe – (0.420 ± 0.060) mg·dm^{-3}. Pb content was below the detection limit. When testing the hypothesis that two compared sets (for the background plot and the river area within the city limit) belong to the same general population using the Mann–Whitney *U* test, we obtained statistically significant differences at $p \leq 0.05$ for Ni concentration alone.

Bottom sediments. Background concentrations for upper layer of bottom sediments had the values as follows (points 1–7): Ni – (3.64 ± 1.10) mg·kg^{-1}; Pb – (1.02 ± 0.68) mg·kg^{-1}; Cd – (0.14 ± 0.05) mg·kg^{-1}; Zn – (9.91 ± 6.44) mg·kg^{-1}; Cu – (4.65 ± 6.94) mg·kg^{-1}; Mn – (86.85 ± 43.30) mg·kg^{-1}; and Fe – (422.53 ± 298.60) mg·kg^{-1}. When testing the hypothesis that two compared sets belong to the same general population, we obtained statistically significant differences at $p \leq 0.05$ for Cd and Pb content in bottom sediments for areas above and below the city limit. At the same time, the median of Cd concentration in the section of points 1–7 (see Fig. 1) was higher than in the river section adjacent to the city quarters. We associate this result with the noted increased Cd content in sediments in the drainage basin of the Northern Dvina in the background plot (Zimovets & Fedorov, 2013) and Cd behavior at the chemical boundary of the Pinega and Northern Dvina rivers mixing, when Cd can be displaced from compounds with organic ligands and deposit in bottom sediments in the form of a poorly soluble hydroxide (Dinu & Shkinev, 2020).

Bivalve molluscs. To assess the differences in the heavy metal accumulation in the tissues of two bivalve species, we used the Mann–Whitney U test for the entire set of samples of the estuarine area of the river. This test revealed no differences in the processes of the heavy metal accumulation in the tissues of painter's and duck mussels at $p \leq 0.05$ for all metals studied. In addition to analysis of the main pair of species, *Anodonta* and *Unio*, a pairwise comparison of the sets with another bivalve, *Dreissena polymorpha*, was carried out. For this species, the difference was shown in accumulation of all metals. However, in the present work, we do not use this species, though it is promising for ecotoxicological research, since we did not obtain sufficient field material in the studied section of the Northern Dvina. Due to the unfavorable effect of abiotic factors on molluscs of this species (lack of substrates for attachment, high reach velocities, rather low summer water temperatures, *etc.*) (Makhnovich, 2016), the density of *Dreissena* settlements does not provide enough material for monitoring. Perhaps, we will carry out a similar work based on *Dreissena polymorpha* analysis later.

For painter's mussel samples for areas above and below the city limit, the test did not reveal significant differences at $p \leq 0.05$ for all metals studied. For duck mussel, statistically significant differences at $p \leq 0.05$ were recorded for Zn, Cu, and Ni. For painter's mussel, background concentrations calculated by the formula (1) had the values as follows: Ni – (0.13 ± 0.04) $\text{mg}\cdot\text{kg}^{-1}$; Pb – (0.10 ± 0.09) $\text{mg}\cdot\text{kg}^{-1}$; Cd – (0.09 ± 0.03) $\text{mg}\cdot\text{kg}^{-1}$; Zn – (18.46 ± 8.93) $\text{mg}\cdot\text{kg}^{-1}$; Cu – (1.01 ± 0.33) $\text{mg}\cdot\text{kg}^{-1}$; Mn – (308.91 ± 128.21) $\text{mg}\cdot\text{kg}^{-1}$; and Fe – (91.38 ± 93.93) $\text{mg}\cdot\text{kg}^{-1}$. For duck mussel, the values were the following: Ni – (0.06 ± 0.08) $\text{mg}\cdot\text{kg}^{-1}$; Pb – (0.12 ± 0.01) $\text{mg}\cdot\text{kg}^{-1}$; Cd – (0.06 ± 0.03) $\text{mg}\cdot\text{kg}^{-1}$; Zn – (11.61 ± 5.37) $\text{mg}\cdot\text{kg}^{-1}$; Cu – (0.59 ± 0.11) $\text{mg}\cdot\text{kg}^{-1}$; Mn – (252.33 ± 182.83) $\text{mg}\cdot\text{kg}^{-1}$; and Fe – (86.06 ± 86.06) $\text{mg}\cdot\text{kg}^{-1}$. We published the data on the background heavy metal content in the bivalves in the estuarine area of the Northern Dvina River earlier in (Neverova & Chupakov, 2018).

Thus, a comparative analysis of the sets of metal concentrations for the background plot (B) and the city area (C) using the Mann–Whitney U test revealed several differences between them. The following significant relationships were established: for bottom water layer, B(Ni) < C(Ni); for bottom sediments, B(Pb) < C(Pb) and B(Cd) > C(Cd); and for *Anodonta*, B(Ni) < C(Ni), B(Zn) < C(Zn), and B(Cu) < C(Cu). These results characterize the effect of household activity on Ni, Pb, Zn, and Cu content in the ecosystem components of the estuarine area of the river. However, analyzing the data obtained, we note a high mosaicity in the metal concentration in the river water area (Neverova et al., 2014, 2016), which indicates a key role of local peculiarities of the sampling point (presence of a local source of contamination and spots with different hydrodynamic characteristics of waters and other granulometric composition of bottom sediments, as well as many other local factors – up to the predominant macrophytic species). Therefore, along with the "classical" scheme for the analysis of the results obtained (comparison of the sets), in order to quantify the differences in the metal content in mollusc tissues between the background plot and the river area within the boundaries of Arkhangelsk, we compared metal content at separate points with the value of the upper limit of the background concentration (formula (2), Table 1).

Table 1. Values of $X_i / (Me_f + 2MAD)$ enrichment factor in the study objects

Sampling point No.	Object	Ni	Pb	Cd	Zn	Cu	Mn	Fe
10	Bottom water layer	0.56	–	0.25	**1.00**	**1.07**	0.27	0.41
	Bottom sediments	0.81	0.65	0.47	0.46	0.20	0.39	**1.37**
	Anodonta	–	–	–	–	–	–	–
	Unio	–	–	–	–	–	–	–
11	Bottom water layer	0.78	–	0.26	0.67	**1.21**	0.36	0.45
	Bottom sediments	**1.24**	**1.05**	0.37	0.79	0.61	**1.13**	**1.90**
	Anodonta	0.95	**1.12**	0.77	0.96	0.97	0.88	0.4
	Unio	–	–	–	–	–	–	–
12	Bottom water layer	**1.05**	–	0.65	0.38	0.52	0.21	0.16
	Bottom sediments	0.74	0.05	0.03	0.29	0.31	0.35	0.33
	Anodonta	0.8	0.41	**1.08**	0.87	**1.21**	0.16	0.19
	Unio	–	–	–	–	–	–	–
13	Bottom water layer	**3.44**	–	0.45	**2.00**	0.52	0.23	0.23
	Bottom sediments	0.60	0.40	0.07	0.32	0.29	0.41	0.33
	Anodonta	–	–	–	–	–	–	–
	Unio	–	–	–	–	–	–	–
14	Bottom water layer	**1.00**	–	–	0.39	0.50	0.37	0.42
	Bottom sediments	**1.27**	0.47	0.08	0.51	0.28	0.76	0.31
	Anodonta	0.6	–	**1.58**	**1.37**	**1.63**	0.44	0.71
	Unio	–	–	–	–	–	–	–
15	Bottom water layer	**2.13**	–	–	0.95	0.52	0.29	0.33
	Bottom sediments	**1.45**	0.54	0.14	0.63	0.31	0.93	0.36
	Anodonta	–	–	–	–	–	–	–
	Unio	0.22	0.07	0.96	0.34	0.8	0.44	**1.18**
16	Bottom water layer	–	–	0.43	0.50	0.36	0.42	0.67
	Bottom sediments	**3.12**	**2.03**	0.63	**1.48**	**3.44**	**2.01**	**1.71**
	Anodonta	**1.46**	**1.06**	0.95	**1.38**	**1.56**	0.92	**1.79**
	Unio	–	–	**1.84**	**1.53**	0.08	0.13	0.53
17	Bottom water layer	0.56	–	0.32	**1.23**	0.36	0.17	0.24
	Bottom sediments	**1.16**	0.14	0.56	0.52	0.36	0.48	0.38
	Anodonta	–	–	–	–	–	–	–
	Unio	–	–	–	–	–	–	–
18	Bottom water layer	0.56	–	0.02	0.67	0.71	0.49	0.62
	Bottom sediments	0.94	0.90	0.14	0.84	0.26	0.28	0.32
	Anodonta	**2.07**	–	**54.15**	**1.05**	**1.44**	0.42	**1.31**
	Unio	0.31	0.16	0.73	0.59	0.55	0.22	0.54
19	Bottom water layer	**1.34**	–	0.04	**1.70**	0.50	0.47	0.22
	Bottom sediments	0.83	**6.42**	0.04	0.39	0.27	0.49	0.33
	Anodonta	0.91	0.58	0.69	**1.72**	**1.08**	0.92	0.83
	Unio	–	–	–	–	–	–	–

Continue on the next page…

Sampling point No.	Object	Ni	Pb	Cd	Zn	Cu	Mn	Fe
20	Bottom water layer	**1.89**	–	0.08	0.90	0.50	0.25	0.13
	Bottom sediments	0.83	0.28	0.10	0.31	0.25	0.75	0.34
	Anodonta	0.8	0.12	0.83	**1.15**	0.99	0.4	0.61
	Unio	–	–	–	–	–	–	–
21	Bottom water layer	0.11	–	0.11	1.00	0.50	0.31	0.54
	Bottom sediments	0.54	0.38	0.33	0.26	0.31	0.36	0.96
	Anodonta	**1.09**	**1.55**	0.85	**1.15**	**1.27**	0.44	0.96
	Unio	–	–	–	–	–	–	–
22	Bottom water layer	0.44	–	0.16	0.10	0.71	0.09	0.23
	Bottom sediments	**2.77**	**1.25**	0.63	**1.45**	**1.08**	**1.47**	0.66
	Anodonta	0.56	0.42	0.68	0.48	0.71	0.45	0.14
	Unio	–	–	–	–	–	–	–
23	Bottom water layer	0.67	–	–	0.47	0.52	0.33	0.50
	Bottom sediments	0.83	0.13	0.05	0.31	0.21	0.28	0.36
	Anodonta	**2.02**	–	**1.19**	**1.07**	**1.41**	0.15	0.52
	Unio	–	–	–	–	–	–	–
24	Bottom water layer	**1.91**	–	–	0.41	0.51	0.15	0.13
	Bottom sediments	0.58	0.17	0.09	0.20	0.20	0.44	0.30
	Anodonta	0.53	–	**2.29**	**1.62**	0.67	0.45	0.35
	Unio	0.4	**1.09**	0.61	0.26	0.20	0.16	0.17
25	Bottom water layer	–	–	0.18	1.00	0.71	**1.34**	**1.41**
	Bottom sediments	0.95	0.38	0.12	0.68	0.33	0.44	**2.37**
	Anodonta	–	–	–	–	–	–	–
	Unio	0.60	0.62	0.69	0.77	0.66	0.58	0.98

Note: a dash (–) denotes either no molluscs found or metal concentrations being below the detection limit; in bold, the values corresponding to excess of the calculated background level are highlighted.

Visualizing the results obtained (see Table 1), we built schematic maps of the contamination in the river water area with heavy metals (Figs 2–5). The Mann–Whitney U test did not show differences in the accumulation processes between two mollusc species; therefore, when building the maps and when having data for duck mussel and painter's mussel, we used the arithmetic mean of the coefficient values in their tissues. As mentioned above, we did not apply an accurate interpolation method. For calculating the data grid for isoline maps, we used radial basis function interpolation: the location of sampling points is heterogeneous, and the use of accurate interpolation methods, *e. g.* triangular linear interpolation, is difficult. On the schematic maps 2–5, colored fields of values of enrichment factors > 1 may slightly deviate from sampling points, overlap land areas, *etc.*; these are common errors in the building of isoline maps. However, the general trends in the data distribution undoubtedly persist, and the clarity of the information presentation covers minor inaccuracies.

At all sampling points located in the area, where the river flows through the city center, there is (see Table 1) an excess of the calculated background concentrations in terms of Ni content in bottom water layer (Fig. 2A) and in bottom sediments (Fig. 2C), as well as in Zn content in mollusc soft tissues (Fig. 2F). At local sampling points in the city center, concentrations higher than the calculated background ones were recorded for two heavy metals: for Zn, in upper layer of bottom sediments (Fig. 2D); for Ni, in bivalve tissues (Fig. 2E).

Fe and Mn content in bottom water layer mostly does not exceed the background values calculated by us throughout the entire area (Fig. 3A, B). In upper layer of bottom sediments, Mn concentrations were higher than the background ones in the entire area of the Northern Dvina River adjacent to the city center; Fe concentrations (Fig. 3C), in local areas upstream and downstream of the city center. It needs to be highlighted that it is Fe and Mn compounds in water that are considered the main contaminants throughout the Northern Dvina watercourse – from the headwaters to the estuarine area (Gosudarstvennyi doklad, 2013 ; Kharakteristika zagryazneniya, 2021). In mollusc tissues, Fe concentrations higher than the calculated background ones were recorded at all points in the city center; Mn concentrations were lower than the background ones at all sampling points (Fig. 3E).

Cd content in the studied river section did not exceed the calculated background concentrations in bottom water layer and bottom sediments (Fig. 4A, C). As mentioned above, the median of Cd content was higher in the background plot than in the area of the river adjacent to the city quarters. In mollusc tissues, Cd concentration was higher than calculated for the entire water area in the city center (Fig. 4E). In 2014, in this area, the maximum absolute value of Cd concentration (5.12 $mg \cdot kg^{-1}$ wet weight) in duck mussel tissues was recorded; it exceeded the calculated background concentrations by more than 50 times (Table 1), wherein mollusc tissues were dark in color. This indicator was excluded from the set when building the maps due to its extreme value.

Cu content in bottom water layer above the calculated background concentration was registered in local areas of the watercourse along the right bank adjacent to the center of Arkhangelsk (Fig. 4B, D). When comparing this indicator with the TLV for the fishery industry, we saw as follows: in these areas, Cu content was ten times higher than the standardized one (Neverova et al., 2016). Cu concentration in bottom sediments above the calculated background one was recorded at points downstream of the central city quarters, in spots, where reaches slow down due to morphological features of the riverbed (Fig. 4D).

Pb content in bottom water layer throughout the studied section was below the detection limit of the method; in upper layer of bottom sediments, it exceeded the background values calculated by us at all sampling points in the area, where the river flows through the central city quarters (Fig. 5A). In mollusc tissues, Pb concentrations exceeded the background ones in sampling points above and below the city center (Fig. 5B).

As for bottom sediments, two areas are distinguished, where background concentrations are exceeded for all metals. These are sampling points 16 (the area at the Nikolsky branch mouth) and 22 (near Molodezhny Island) (see Fig. 1). Point 16 is located downstream of numerous industrial areas on the left bank of the Northern Dvina, in spots, where reaches slow down, which is associated with morphological features of the riverbed. Point 22 is located in the zone of macrophyte thickets, which is also the spot, where the reach slows down. As a result of these peculiarities, in these areas, bottom sediments deposit and accumulate, which are carried by reaches with metals sorbed on them.

In contrast to concentrations in bottom water layer and bottom sediments, in mollusc tissues, there is an excess of content of most of the metals studied: values above the calculated upper background ones are recorded at all sampling points along the riverbed in the city center – both on the right and left banks (Figs 2E, F; 3E; 4E, F; 5E, F).

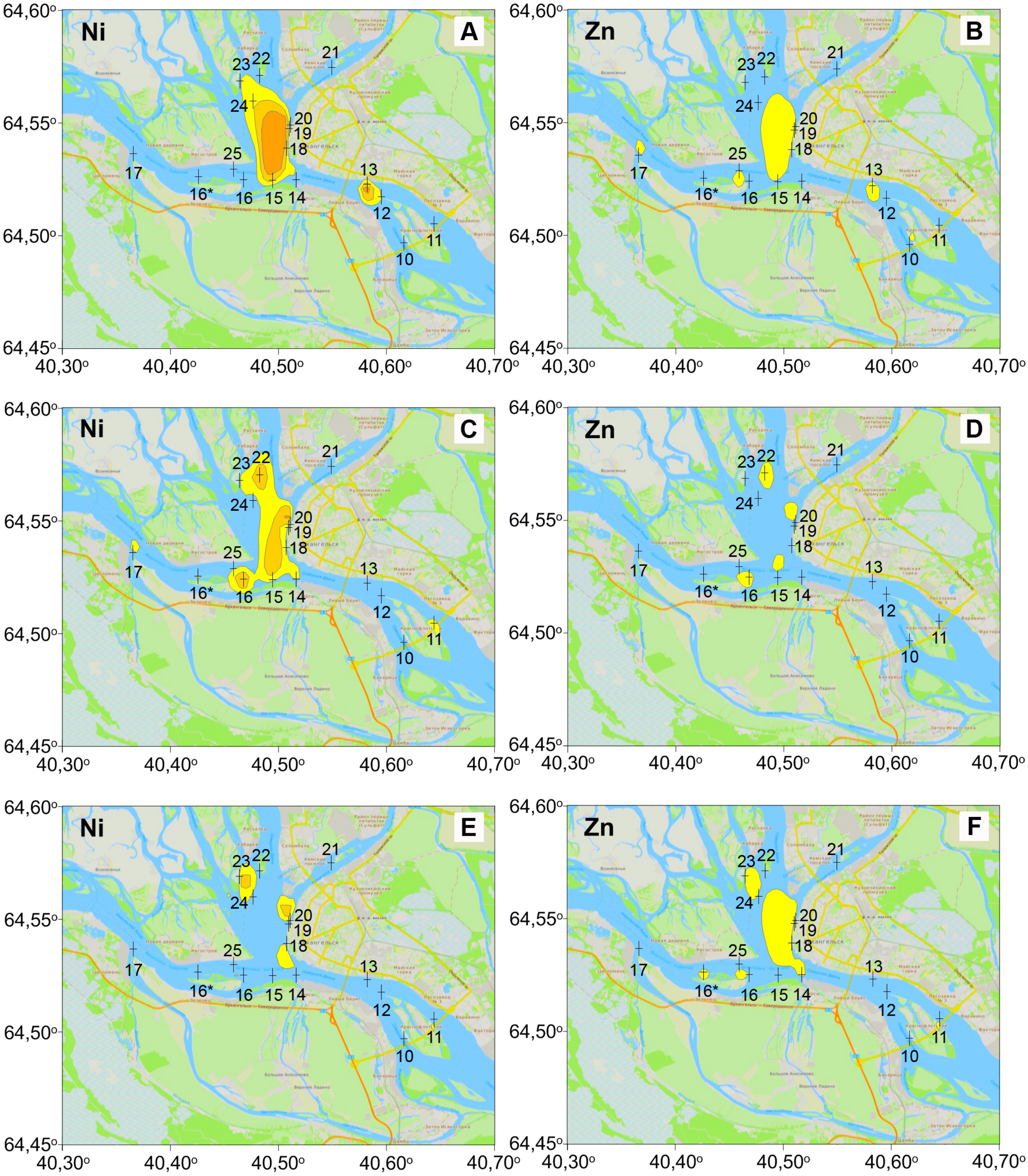

Fig. 2. Excess of the background level of Ni content (A, in bottom water layer; C, in bottom sediments; E, in mollusc tissues) and Zn content (B, in bottom water layer; D, in bottom sediments; F, in mollusc tissues). The gradation scale is shown in Fig. 5

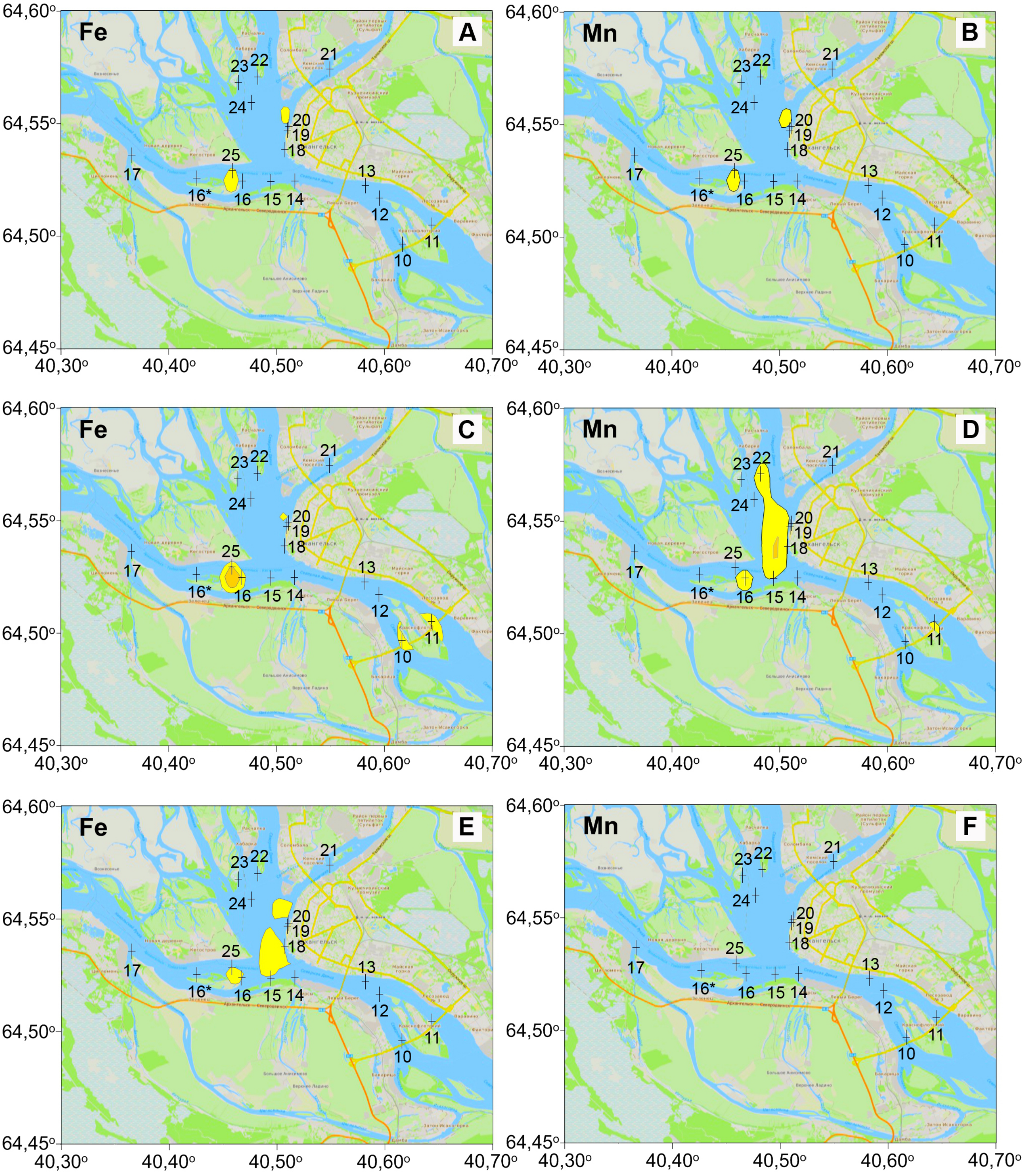

Fig. 3. Excess of the background level of Fe content (A, in bottom water layer; C, in bottom sediments; E, in mollusc tissues) and Mn content (B, in bottom water layer; D, in bottom sediments; F, in mollusc tissues). The gradation scale is shown in Fig. 5

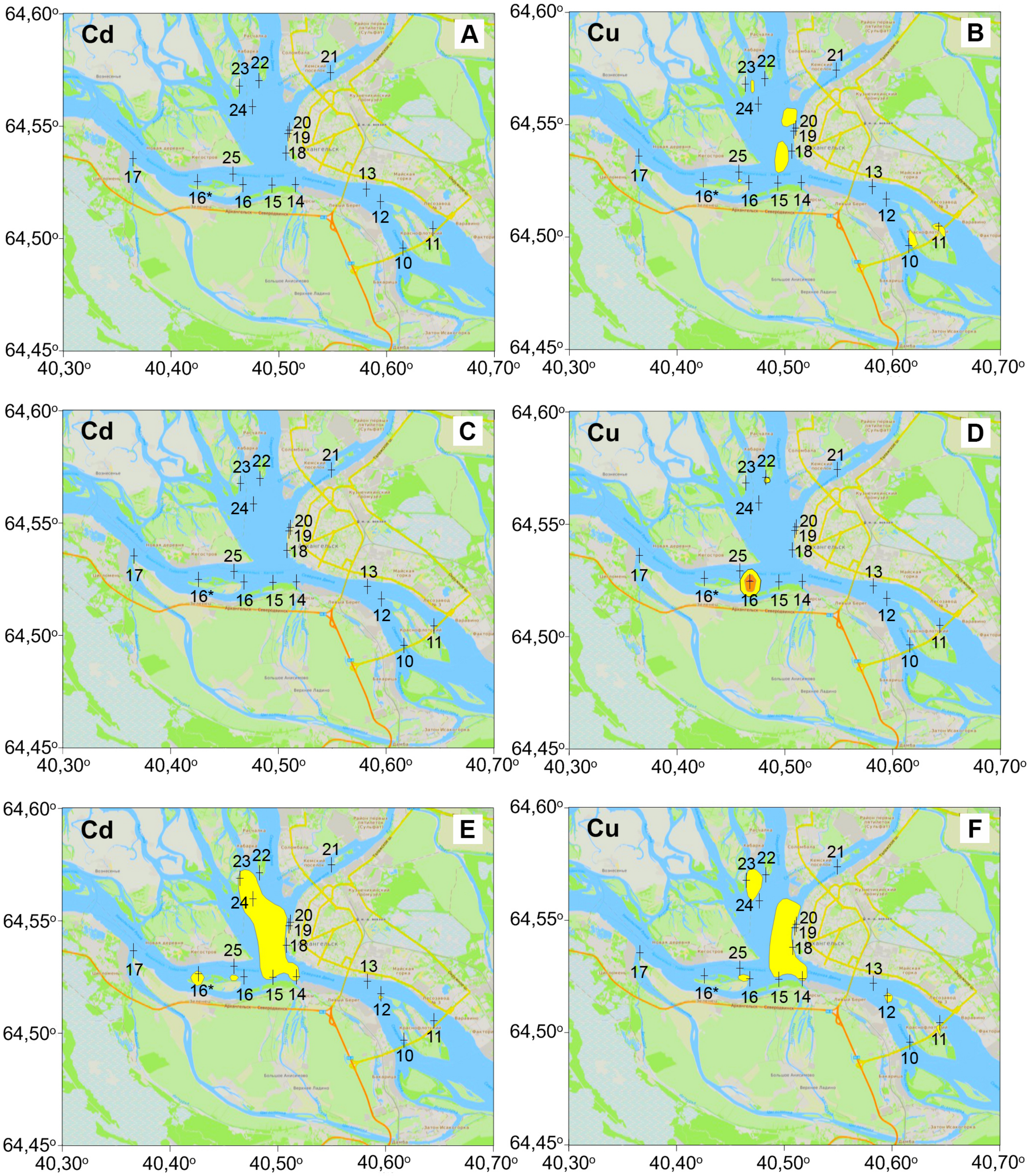

Fig. 4. Excess of the background level of Cd content (A, in bottom water layer; C, in bottom sediments; E, in mollusc tissues) and Cu content (B, in bottom water layer; D, in bottom sediments; F, in mollusc tissues). The gradation scale is shown in Fig. 5

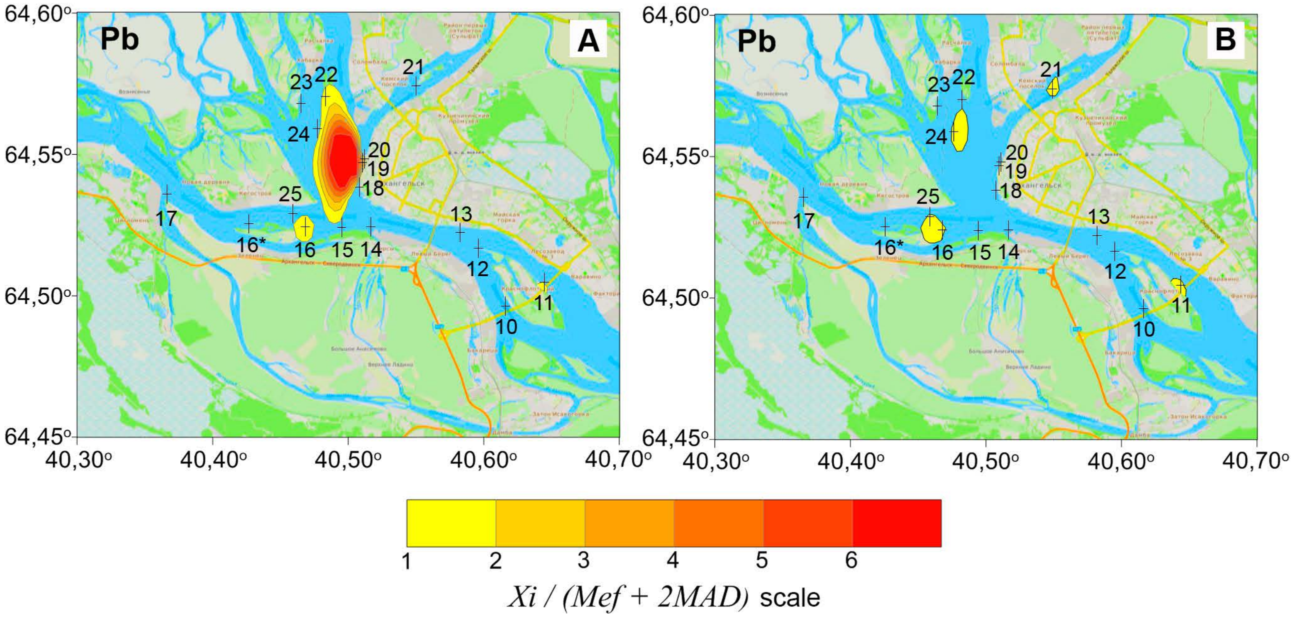

Fig. 5. Excess of the background level of Pb content (A, in bottom sediments; B, in mollusc tissues)

Analyzing the data obtained, it can be concluded as follows: Ni and Zn are inputted into bottom water layer from a single source (there is a significant positive Ni/Zn correlation; $R = 0.55$; $p \leq 0.05$; $N = 16$), with drains of drainage and storm sewers into the river water area on the right bank. There are local inflows of metals from an industrial hub located along the left bank of the Northern Dvina, where, along the river, insufficiently treated drains of domestic and drainage and storm sewers from the southern Arkhangelsk are inputted. The inflow of untreated domestic wastewater into the area of the city beach (points 18–20, Fig. 1) of the Northern Dvina is mentioned already in the publication of the 1920s (Lopato, 1927). Bottom sediments of the river section studied are enriched in Pb [> 6 background concentrations (b. c.)] and Ni (> 3 b. c.) throughout the entire area, where the river flows through the city center, both on the right and left banks. Local areas of the river in the city center and above and below central quarters are enriched in Fe (> 1 b. c.), Mn (> 1 b. c.), Cu (> 3 b. c.), and Zn (> 1 b. c.). In mollusc tissues, the calculated background values were exceeded for all metals, except for Mn.

Conclusions:

1. The excess of the heavy metal content over conditional background concentrations in the section of the Northern Dvina River adjacent to the central city quarters was revealed, which is associated with the inflow of rainwater and meltwater into the water area with insufficiently treated drains of domestic and drainage and storm sewers. For Cd and Pb, short-range atmospheric transport can be another major source of input. As shown in the study of the White Sea drainage basin contamination with the heavy metals from the atmosphere (Shevchenko et al., 2020), the proximity to a motorway significantly affects the enrichment of the snow cover with these two elements.
2. The analysis of the heavy metal content in mollusc tissues is more informative and indicative than the analysis of bottom water layer and bottom sediments. Sedentary benthic species, in particular bivalves, can serve as indicators of this contamination in the aquatic ecosystems: being filter feeders,

they accumulate relatively higher heavy metal concentrations in their tissues during feeding and respiration than in the habitat. Moreover, being less mobile than bottom sediments and water, molluscs can concentrate contaminants in a spatial aspect.

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ОЦЕНКА ЗАГРЯЗНЕНИЯ УСТЬЕВОЙ ОБЛАСТИ РЕКИ СЕВЕРНОЙ ДВИНЫ МЕТОДОМ РАСЧЁТА ФОНОВЫХ КОНЦЕНТРАЦИЙ
(Fe, Mn, Zn, Cu, Cd, Pb, Ni)

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Данная публикация является продолжением работ по количественной оценке уровня загрязнения тяжёлыми металлами (Fe, Mn, Zn, Cu, Cd, Pb, Ni) компонентов экосистемы устьевой области реки Северной Двины. Для оценки уровней загрязнения указанными металлами экосистемы устьевой области Северной Двины в районе наибольшего антропогенного воздействия (район городской агломерации Новодвинск — Архангельск — Северодвинск) мы используем метод сравнительного анализа объекта исследования с условно-фоновым участком, приняв за него участок реки, расположенный выше городской застройки. С применением пространственного картирования значений коэффициентов обогащения для компонентов водной экосистемы были выделены районы с аномальным превышением содержания металлов в тканях моллюсков, донных отложениях и придонном слое воды. Установлено, что наибольшей антропогенной нагрузке в акватории вершины дельты реки подвержен район, примыкающий к центральной части города и к промышленной зоне на левом берегу. Наиболее вероятными источниками загрязнения рассмотренными тяжёлыми металлами можно назвать попадание поверхностных сточных вод (талые и дождевые ливневые стоки), неочищенные коммунально-бытовые стоки и прямой ближний атмосферный перенос. Анализ тканей моллюсков проявил себя как более информативный и показательный подход к оценке загрязнения тяжёлыми металлами акватории со сложными гидрологическими и гидрохимическими градиентами среды, чем анализ придонного слоя воды и донных отложений.

Ключевые слова: устьевая область реки Северной Двины, фоновые содержания, тяжёлые металлы, коэффициент обогащения, двустворчатые моллюски

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REDOX CONDITIONS OF BOTTOM SEDIMENTS AND MACROZOOBENTHOS CHARACTERISTICS IN THE KRUGLAYA AND KAZACHYA BAYS (SEVASTOPOL)

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A lack of systemic and environmentally efficient approach to exploitation of the Kruglaya and Kazachya bays (Sevastopol) resulted in their severe pollution. The conjunction of natural and anthropogenic factors led to deterioration of habitat conditions of benthic communities. The aim of this work was to carry out complex research of Kruglaya and Kazachya bays' ecosystems to study peculiarities of formation of redox conditions in bottom sediments and bottom water layer, as well as their effect on macrozoobenthos characteristics. Bottom sediments were sampled by diver in plexiglass tubes hermetically sealed at the top and bottom; it helped in preserving a fine structure of bottom sediments and bottom water layer. To study benthic communities, samples were taken in the same spot with a manual sampler. To obtain chemical composition of pore waters with high vertical resolution, the voltammetry analysis was carried out. Calculation of an oxygen flux at the boundary and in the upper layer of bottom sediments was performed according to the vertical profile of oxygen concentration in pore waters and geochemical analysis with applying the equation for the Fick's first law and considering concentration gradient and molecular diffusion of oxygen in pore waters. Standard hydrobiological methods were applied for the analysis of benthic material. When calculating the values of the Shannon diversity index (*H'*), the binary logarithm was used. The data analysis showed as follows: a high level of anthropogenic load and restricted water dynamics resulted in siltation of bottom sediments in the studied water areas, which obstructed oxygen penetration; the accumulation of organic carbon contributed to its active consumption. Stratification of a water column due to limited water exchange, high temperature of bottom water, accompanied by a decrease in oxygen solubility, and finely dispersed nature of bottom sediments contributed to the fact that the rate of oxygen input was lower than the rate of oxygen consumption for organic matter oxidation. This was followed by the development of oxygen deficiency zones and emergence of reduced compounds, in particular hydrogen sulfide. Importantly, suboxic conditions prevailed in the upper sediment layer, and anaerobic conditions prevailed below. Due to this, the main forms of macrozoobenthos were species tolerant to oxygen deficiency and organic pollution. Specifically, in some spots of the Kazachya Bay, polychaetes alone were recorded. At the same time, in the Kruglaya Bay mouth area, intense water dynamics and morphological peculiarities of bottom sediments contribute to saturation of the upper sediment layer with oxygen. Based on oxygen concentration data for the surface (0–5 mm) sediment layer (pore waters) and on geochemical peculiarities of bottom sediments (moisture and porosity), the oxygen flux at st. 4 (the Solenaya Bay) was calculated; the value was 0.73 $\mathrm{M \cdot m^{-2} \cdot year^{-1}}$. Considering oxygen concentration in bottom water layer (259 μM), the time for complete depletion of oxygen or its renewal is about 5 months if taking into account biogeochemical processes alone. Hence, it can be assumed that the ecosystems of the Kruglaya and Kazachya bays are in the stage of degradation. Their further exploitation without a developed systemic and rational approach will result in a critical deterioration of the ecosystems – the emergence and spread of environmental risk zones. It will reduce the recreational and socioeconomic attractiveness of these areas.

Keywords: sediments, pore water, oxygen, hydrogen sulfide, organic carbon, macrozoobenthos, coastal waters, Black Sea

Marine coastal ecosystems play an important role in human life (Harley et al., 2006). Those are involved in all spheres of economic activity and are largely subject to anthropogenic load, which affects their physicochemical and biological characteristics, as well as ecological state of the ecosystem in general (Cabral et al., 2019 ; Ducrotoy, 2021 ; Rabalais et al., 2009). Any external effects have a fairly quick response. Under favorable conditions, ecosystems are capable of recovering; however, under prolonged anthropogenic load and exposure to certain natural physical and physicochemical factors, natural balance is disrupted, and the system degrades (Rabalais et al., 2009). Out of coastal marine ecosystems, those with limited water exchange are the most susceptible to destruction. There, under certain conditions, water stratification occurs, sediments are silted, and pollutants and organic carbon accumulate in bottom sediments, which affects the state of benthic communities and results in formation of zones of ecological risk (Ovsyanyi & Orekhova, 2019 ; Orekhova et al., 2019a).

The bays of the Sevastopol region belong to the type of ecosystems, in which the accumulation of organic matter in bottom sediments prevails over destruction (Ignat'yeva et al., 2008 ; Orekhova & Konovalov, 2018b ; Orekhova et al., 2019b ; Osadchaya et al., 2003). Depletion of oxygen for the oxidation of organic matter and other reduced compounds leads to a shift in the processes, which occur due to anaerobic oxidation of organic matter, closer to the surface of bottom sediments (Orekhova & Konovalov, 2009, 2018a, 2018b). Thus, in the upper sediment layer, reduced forms of nitrogen, metals, and sulfur predominate, and anoxic zones with anaerobic conditions are formed. An increase in the content of reduced compounds, in particular the concentration of sulfides, results in a rise in their flux into bottom water layer (Orekhova & Konovalov, 2018b). In this case, anaerobic conditions are formed not only in bottom sediments, but also in water column (Berlinsky et al., 2003 ; Meysman et al., 2003). Such changes in physicochemical characteristics of ecosystems are reflected in their ecological status and, as a result, in the socioeconomic attractiveness of the region.

To study the ecological state of Sevastopol coastal waters, the Kazachya and Kruglaya bays were chosen, which have high social and recreational significance for the region.

Earlier studies of these bays (Zaika et al., 2011 ; Mironov et al., 2002, 2003 ; Mironova & Pankeeva, 2019 ; Sanitary and Biological Studies, 2018 ; Soloveva et al., 2019) showed as follows: over the last 10–15 years, their ecosystems have undergone certain changes due to an increase in anthropogenic load. However, there was no comprehensive study of the accumulation of organic matter in bottom sediments, as well as of redox conditions of the environment (including oxygen deficiency) in them and their effect on a benthic community. These investigations are relevant for the region due to an increase in the number of sources and flux of organic matter inputted into the water area of the bays.

MATERIAL AND METHODS

We used the data on the analysis of bottom water layer, pore waters, and bottom sediments sampled in two bays in September 2019.

The Kazachya Bay is located in the southwestern Heracles Peninsula (Fig. 1) (Mironov et al., 2002 ; Ovsyanyi & Orekhova, 2019). This water area is characterized by free water exchange with the deep sea and is one of the objects of the nature reserve fund of the Sevastopol region (Mironov et al., 2003). Until recently, it was considered one of the cleanest Sevastopol bays (Mironov et al., 2003). To date, its coast is actively used as a recreational area: sandy and pebble beaches are located in the top of the bay. However, active construction on the bay coast and development of a new residential zone with the appropriate infrastructure, with year-to-year rising area and population, led to a significant increase in anthropogenic load on its ecosystem (Soloveva et al., 2019).

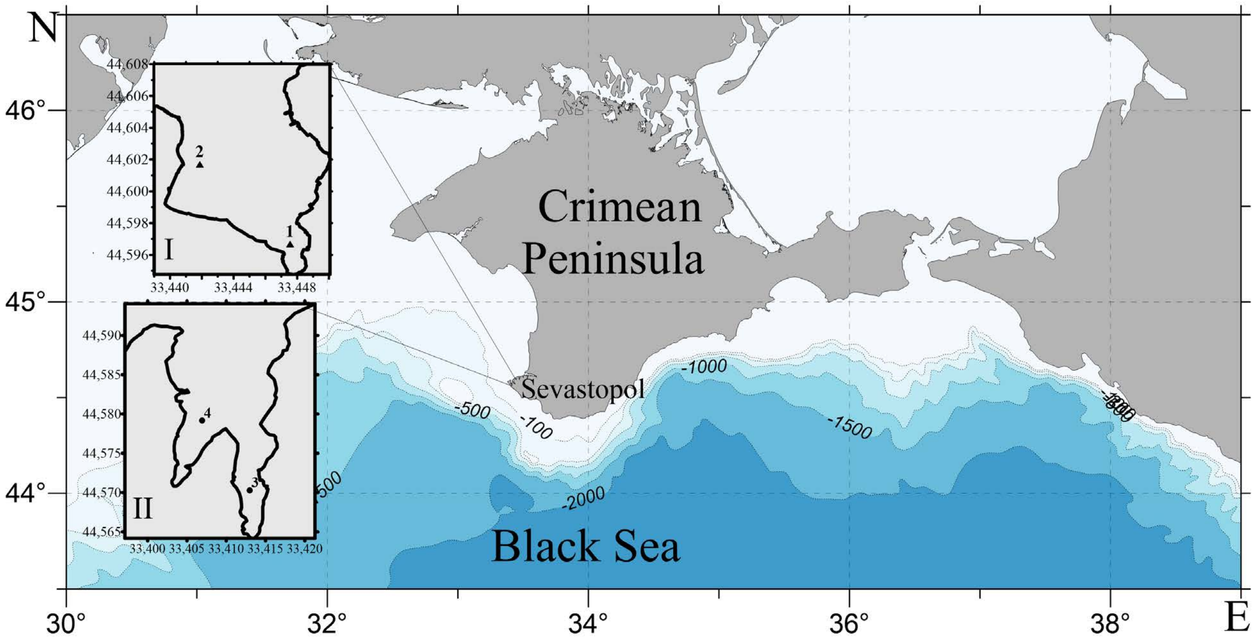

Fig. 1. Scheme of sampling stations in the bays of the Sevastopol region: I, the Kruglaya Bay; II, the Kazachya Bay

The Kruglaya Bay, the shallow one, is located in the northern Heracles Peninsula between Dvoinaya and Streletskaya bays (Fig. 1); it is of the semi-enclosed type (Mironova & Pankeeva, 2019), with limited water exchange (Zaika et al., 2011). During cold season, the bay waters are well aerated due to their dynamics and vertical convective mixing. During warm period, because of a seasonal thermocline determining the two-layer structure of waters, vertical water stratification and weak ventilation of bottom layer at a relatively high temperature are characteristic (Zaika et al., 2011). An increase in temperature promotes intensive oxygen consumption when it is involved in various biological and chemical processes (Zaika et al., 2011). The Kruglaya Bay is a recreational area as well, with resting places, city beach, and entertainment centers. At the same time, there is an emergency runoff of sewage water; therefore, during summer, sanitary and epidemiological indicators often do not meet the standards (Sanitary and Biological Studies, 2018). Intensive construction of the microdistrict, arrangement of the beach area, and a rise in the number of small boats on the eastern coast resulted in an increase in anthropogenic load on the bay ecosystem (Sanitary and Biological Studies, 2018). All this negatively affects its ecological state. There can be an additional source of organic matter and pollutant input into the bay due to water exchange with the adjacent area: contiguous semi-enclosed bays with numerous permanent, local, emergency, and unauthorized wastewater discharges (Kuftarkova et al., 1999). The maximum load on the bay waters falls on summer.

The samples were taken by diver in plexiglass tubes hermetically sealed at the top and bottom. Depth of the tube immersion was limited by the nature of the sediments. The columns sampled were transferred in a strictly vertical position to preserve a fine structure of bottom sediments and bottom water layer. At each point, bottom sediment columns were sampled in one replication. To study benthic communities, samples were taken in the same spot in duplicate, with a manual sampler with an area of 0.025 m^2. Water temperature in the samples was of +22…+24 °C.

In the Kruglaya Bay, bottom sediment columns were sampled in the apex (st. 1; N44.597°, E33.448°; Fig. 1) and in the mouth area (st. 2; N44.602°, E33.442°; Fig. 1). The sampling points were chosen considering different sedimentation conditions and sources of organic matter input. The shallow (sampling depth of 0.5 m) bay apex is most exposed to anthropogenic load, and due to the remoteness from the bay mouth, water stagnation often occurs there. Moreover, in the bay apex, there are sources of pollution: runoff from storm sewers and permanent outlets of untreated domestic wastewater (Kuftarkova et al., 2008). This contributes to organic matter accumulation and oxygen consumption in bottom sediments and bottom water layer. In the bay mouth area (st. 2), the depth is of 10 m; an underwater coastal abrasion slope is located there (Mironova & Pankeeva, 2019). In this area, active hydrodynamic effect, peculiarities of bottom topography, and absence of significant and permanent sources of organic matter contribute to the saturation of bottom water layer and sediment surface with oxygen.

In the Kazachya Bay, samples were taken in the central areas of the eastern branch (st. 3; N44.570°, E33.413°; Fig. 1) and of the western one – in the Solenaya Bay (st. 4; N44.579°, E33.407°; Fig. 1). Sampling depths were of 3.5 and 13.6 m for st. 3 and st. 4, respectively. The water exchange is not limited (Mironov et al., 2003), but these areas are under significant anthropogenic load due to dense settlement of the shores and location of infrastructure facilities (Ovsyanyi & Orekhova, 2019).

The voltammetry analysis with the use of a glass Au/Hg microelectrode was carried out to obtain a chemical profile of pore waters with high vertical resolution (1–5 mm) (Brendel & Luther, 1995 ; Orekhova & Konovalov, 2009). This method allows to quantify electrochemically active compounds in pore waters, primarily oxygen, sulfides and other reduced forms of sulfur, and reduced forms of iron and manganese; the method error does not exceed 10 % (Orekhova & Konovalov, 2018b).

Organic carbon content (C_{org}; % dry weight) in bottom sediments was determined by coulometry on an AN-7529 express analyzer according to a technique adapted for marine sediments (Ljutsarev, 1986). The method error does not exceed 10 % (about ± 0.2 % dry weight).

The calculation of an oxygen flux at the boundary and in the upper layer of bottom sediments was carried out according to the data of voltammetry and geochemical analyses with applying the equation for the Fick's first law considering concentration gradient and molecular diffusion of oxygen in pore waters (Hyacinthe et al., 2001):

$$J = -\varphi D_s dC/dZ \; , \tag{1}$$

where J is the flux, mol·($year^{-1}$·m^{-2});

φ is porosity;

D_s is the coefficient of molecular oxygen diffusion taking into account the viscosity, m^2·$year^{-1}$;

dC/dZ is the concentration gradient, mol·m^{-4}.

Molecular oxygen diffusion coefficients in pore waters were taken from (Meysman et al., 2003). When calculating these coefficients, temperature of bottom water layer, which was measured immediately at the time of sampling, was taken into account.

Considering the high rate of sedimentation processes in the coastal zone, short time for diagenetic processes of bottom sediments compaction, high intensity of intra-annual dynamics of bottom sediments, and high rate of redox biogeochemical processes, the effect of advective currents was not taken into account.

Benthic samples were washed through a sieve with a 1-mm mesh and fixed in ethanol. Further processing of the fixed material was carried out under laboratory conditions. Species were identified according to the guides (Opredelitel', 1968, 1969, 1972). Bivalves were weighted after their dissection and removing the fixative solution from the mantle cavity. At each station, biomass (g·m^{-2}) and abundance (ind.·m^{-2}) of a separate species were calculated from the total benthic sample. The values of the Shannon diversity index (*H'*) (the binary logarithm was used) and Pielou evenness index were calculated in the Diverse application of the PRIMER-5 package.

RESULTS

The height of the column sampled in the Kruglaya Bay apex (st. 1) was of 25 cm. Sediment is represented by gray and dark gray silted sand. The sediment surface in the sampling area was covered with a layer of vegetation. Based on the works of Mironova and Pankeeva *et al.* (Mironova & Pankeeva, 2019 ; Pankeeva et al., 2019), it can be assumed that this is a community of seagrasses and green algae, mainly Cladophora species on silty sediments.

The finely dispersed nature of sediment and the organic matter content of about 1.97 % contributed to active oxygen consumption. Thus, in bottom water layer, oxygen saturation of 56 % was observed; directly above the surface of seagrasses and on the sediment surface, it dropped down to 19 %, which corresponds to hypoxia (Zaika et al., 2011). In the layer down to 7 mm, no voltammetrically active compounds were detected (Brendel & Luther, 1995 ; Orekhova & Konovalov, 2009), which may indicate the occurrence of processes with oxidized forms of nitrogen in this depth interval (Orekhova & Konovalov, 2009). Below, hydrogen sulfide appears, with non-uniform distribution (Fig. 2). In the depth interval of 8–30 mm, hydrogen sulfide concentration varies within 9–31 μM. This is followed by the interval of 32–40 mm, in which hydrogen sulfide concentration is below the detection limit (3 μM) or there is no hydrogen sulfide. In the layer

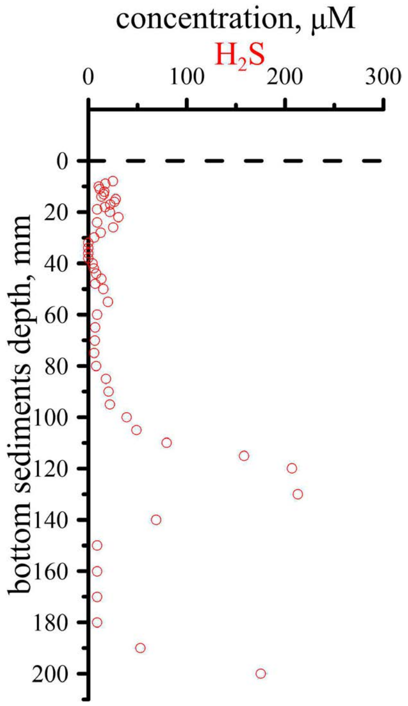

Fig. 2. Vertical profile of hydrogen sulfide in pore waters of bottom sediments sampled in the Kruglaya Bay (st. 1)

of 42–140 mm, hydrogen sulfide concentration increases with depth, reaching a maximum of 207 μM at 120 mm, and then decreases. In the depth interval of 150–180 mm, hydrogen sulfide concentration is constant and amounts to 9 μM; it sharply increases from 190 mm, reaching 175 μM at 200 mm. Further profiling was not performed.

At st. 2 (see Fig. 1), sediment is represented by sand; the height of the column sampled is of 9 cm. Free water exchange, bottom peculiarities (Mironova & Pankeeva, 2019), and absence of organic matter sources contribute to oxygenation of bottom water layer. In bottom water layer and in upper sediment layer, oxygen saturation of 100 % was recorded.

In the central area of the eastern Kazachya Bay (st. 3, Fig. 1), a significant predominance of silty material contributed to organic carbon accumulation (to date, its content reaches ≈ 7 %). High content of organic matter leads to oxygen consumption in bottom water layer and on the sediment surface for its oxidation (Orekhova & Konovalov, 2009). Oxygen concentration in bottom water layer (10 cm above the sediment surface) corresponded to saturation of 65 %. Such oxygen concentration at a sampling depth of 3.5 m indicates the development of oxygen deficiency.

On the sediment surface, oxygen concentration decreases down to 100 μM (saturation of 40 %); below, all oxygen is depleted (Fig. 3a). No voltammetrically active compounds were found in the depth interval of 1–20 mm (Orekhova & Konovalov, 2009, 2018b), which allows to suggest the occurrence of biogeochemical processes of organic matter mineralization mainly with nitrates/nitrites involved. Mosaic signals corresponding to reduced iron and manganese with concentrations of about 2 and 3 μM, respectively (Fig. 3a), may indicate the occurrence of processes with these compounds involved. Below, hydrogen sulfide appeared (Fig. 3a). With depth, concentration of sulfides varied within 36–346 μM; other components were not detected.

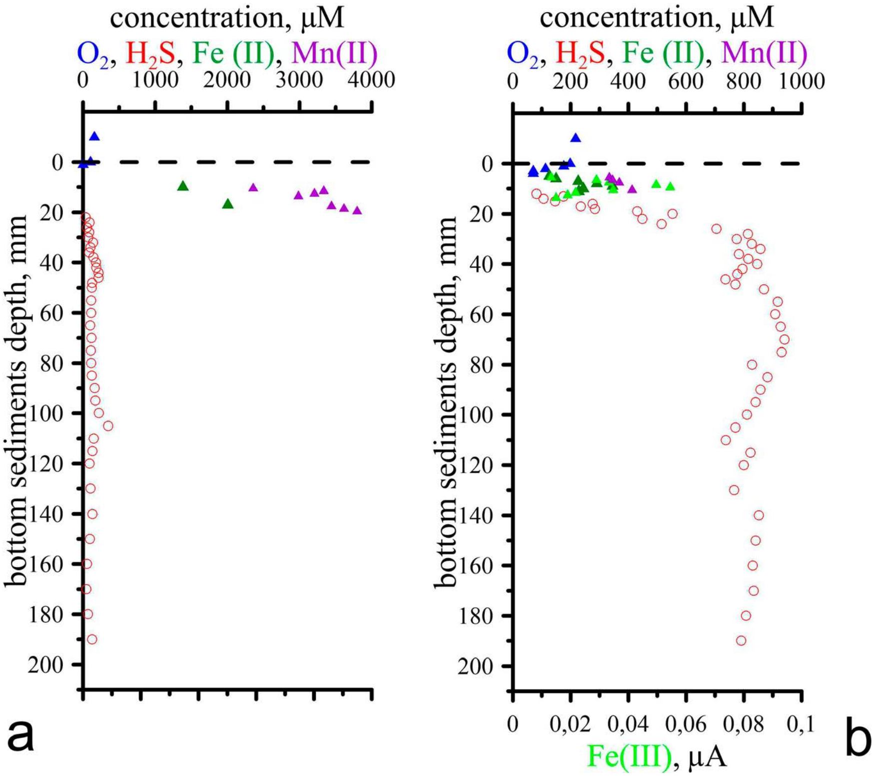

Fig. 3. Vertical profile of the chemical composition of pore waters of bottom sediments sampled at stations 3 (a) and 4 (b) in the Kazachya Bay

A sampling depth in the Solenaya Bay (st. 4, Fig. 1) located in the western Kazachya Bay, was of 13.6 m. The column height was of 34 cm; sediment was mainly represented by a finely dispersed fraction. However, the appearance of sampled sediment differed from the previous ones: in the upper 2-mm layer, a fluff layer was recorded; in the depth interval of 2-20 mm, gray-brown sand was found; and below, homogeneous dark gray silted sand was registered.

In bottom water layer, oxygen concentration corresponded to saturation of 97 %. On the sediment surface, oxygen concentration decreases down to 200 μM (saturation of 80 %). It penetrated into the sediment down to 4 mm (Fig. 3b). From 5 mm, Fe(III) appears; its intensity [due to the formation of colloidal solutions, Fe(III) concentration cannot be determined analytically (Brendel & Luther, 1995)] increases with depth, reaching a maximum at 9 mm, and then decreases; from 14 mm, its signal disappears. In the layer of 5–11 mm, signals are recorded corresponding to reduced forms of iron and manganese. Iron and manganese concentrations averaged 230 and 365 μM, respectively (Fig. 3b). From 11 mm, hydrogen sulfide appears; its concentration is 82 μM; it increases up to 942 μM with depth. In hydrogen sulfide distribution, two “depth intervals” can be distinguished: 12–110 mm (there, concentration of sulfides first increases, reaching a maximum value (942 μM) at 70 mm, and then decreases) and 120–200 mm (it is a layer of insignificant increase and decrease in sulfide concentration, but the value varies within 767–852 μM).

Based on the data on oxygen concentration in the surface (0–5 mm) sediment layer (pore waters), as well as on material on geochemical composition of bottom sediments, the oxygen flux at st. 4 was calculated: 0.73 $\mathrm{M{\cdot}m^{-2}{\cdot}year^{-1}}$. Considering oxygen concentration in bottom water layer (259 μM), the time for complete depletion of oxygen is about 5 months if taking into account biogeochemical processes alone.

Table 1 shows the data on oxygen concentration on the sediment surface, depth of emergence of hydrogen sulfide and its concentration, and quantitative indicators of the benthic community in the water areas studied. Table 2 contains the data on macrozoobenthos characteristics (abundance, biomass, Shannon index, and Pielou evenness index) of the Kruglaya and Kazachya bays.

Table 1. Geochemical characteristics of bottom sediments and macrozoobenthos abundance

Station	Oxygen concentration on the sediment surface, μM	Depth of hydrogen sulfide emergence, mm	Mean hydrogen sulfide concentration in bottom sediment column ± *SD*, μM	Hydrogen sulfide concentration range, μM	Macrozoobenthos abundance, ind.·m^{-2}
St. 1 (the Kruglaya Bay)	< 20	8	31 ± 30	< DL…213	2,480
St. 2 (the Kruglaya Bay)	238	–	–	–	360
St. 3 (the Kazachya Bay)	< 20	20	126 ± 42	< DL…346	40
St. 4 (the Kazachya Bay)	177	11	696 ± 196	< DL…941	80

Note: *SD* denotes standard deviation; < DL is below the detection limit (3 μM).

Table 2. Macrozoobenthos characteristics (abundance, biomass, Shannon index, and Pielou evenness index) of the water area studied

Class	Species	St. 1		St. 2		St. 3		St. 4	
		N	M	N	M	N	M	N	M
Bivalvia	*Chamelea gallina* (Linnaeus, 1758)	–	–	40	7.52	–	–	–	–
	Mytilaster lineatus (Gmelin, 1791)	80	0.012	120	0.04	–	–	–	–
	Abra segmentum (Récluz, 1843)	1,640	220.8	–	–	–	–	–	–
	Cerastoderma glaucum (Bruguière, 1789)	80	63.6	–	–	–	–	–	–
	Lucinella divaricata (Linnaeus, 1758)	–	–	–	–	–	–	40	0.012
Gastropoda	*Tritia neritea* (Linnaeus, 1758)	40	6.68	–	–	–	–	–	–
	Tritia pellucida (Risso, 1826)	80	33.44	–	–	–	–	–	–
Crustacea	*Ampelisca diadema* (Costa, 1853)	–	–	40	0.32	–	–	–	–
Insecta	*Chironomus* sp.	40	0.016	–	–	–	–	–	–
Harpacticoida		40	0.004	–	–	–	–	–	–
Polychaeta	*Capitella capitata* (Fabricius, 1780)	40	0.008	–	–	–	–	–	–
	Heteromastus filiformis (Claparède, 1864)	40	0.004	–	–	40	0.008	–	–
	Notomastus lineatus (Claparède, 1863)	40	0.004	–	–	–	–	–	–
	Nephtys hombergii Savigny in Lamarck, 1818	–	–	–	–	–	–	40	3.12
	Cirriformia tentaculata (Montagu, 1808)	–	–	160	3.04	–	–	–	–
	Glycera alba (O. F. Müller, 1776)	40	1.56	–	–	–	–	–	–
Ascidiacea		320	17.64	–	–	–	–	–	–
In total		2,480	343.77	360	10.88	40	0.008	80	3.132
Shannon index ($\log_2$ based)		1.93	1.56	1.75	1.06	–	–	1.0	0.04
Pielou evenness index		0.46	0.39	0.88	0.53	–	–	1.0	0.04

Note: N denotes abundance, ind.·m^{-2}; M, biomass, g·m^{-2}.

Macrozoobenthos maximum abundance (2,480 ind.$\cdot$m^{-2}) was recorded in the Kruglaya Bay apex; in its upper layer of bottom sediments, hypoxic conditions were noted. In the bay mouth area, with the maximum oxygen concentration (238 µM) on the sediment surface and with no hydrogen sulfide, macrozoobenthos abundance was an order of magnitude lower (360 ind.$\cdot$m^{-2}) due to a decrease in bivalve abundance and absence of gastropods, ascidians, insects, and harpacticides (Table 2). In the Kazachya Bay, abundance of hydrobionts was significantly lower; the minimum value (40 ind.$\cdot$m^{-2}) was registered at the minimum oxygen concentrations on the sediment surface (Table 1).

In the Kruglaya Bay, bivalve molluscs predominated in terms of abundance and biomass. *Mytilaster lineatus* was recorded at both stations; at st. 2, it was the most abundant bivalve species (Table 2). At this station, *Chamelea gallina* predominated in terms of biomass, wherein at st. 1, the species was not found. Gastropods were not recorded. In the bay apex (st. 1), the predominant species in terms of abundance and biomass was the detritus feeder *Abra segmentum*. The same values of abundance were observed for the filter feeders *Mytilaster lineatus* and *Cerastoderma glaucum*. Gastropods were represented by two species: *Tritia neritea* and *Tritia pellucida*.

In the bay apex, ascidians and four polychaetes with the same abundance values were recorded as well: *Capitella capitata*, *Heteromastus filiformis*, *Notomastus lineatus*, and *Glycera alba* (biomass-dominant species). In the bay mouth area, ascidians were absent, and polychaetes were represented by one species: *Cirriformia tentaculata*.

The results of our research show low values of species diversity and abundance for molluscs in the Kazachya Bay. At st. 4, only the bivalve *Lucinella divaricata* was found; gastropods were not recorded. High biomass values in the Kazachya Bay were noted for polychaetes (Table 2) represented by two species of the same abundance: *Heteromastus filiformis* at st. 3 and *Nephtys hombergii* at st. 4. At st. 3, no other representatives of benthic fauna were registered.

DISCUSSION

The results of geochemical analysis provide a basis for understanding the ongoing biogeochemical processes (Volkov, 1973 ; Mitropol'skii et al., 1982 ; Rozanov & Volkov, 2009 ; Khimiya okeana, 1979 ; Orekhova & Konovalov, 2009). Pore waters are an integral part of bottom sediments (Rozanov & Volkov, 2009). The study of their characteristics allows to assess redox and acid-base properties of bottom sediments, content of mobile forms of metals, and possibility of their binding and transition into a solid form of sediments or into the water column (Kuftarkova et al., 1999 ; Mironov et al., 2003).

According to our material and the data of Soloveva *et al.* (2019), mean organic carbon content in bottom sediments of the Kruglaya Bay is 1.67–1.97 %, which is more than 2 times higher than mean organic carbon content in the New Black Sea sediments (0.71 %) and bottom sediments of the Black Sea shelf (Mitropol'skii et al., 1982). Such organic carbon content in bottom sediments contributes to active oxygen consumption and development of its deficiency.

A significant rise in anthropogenic load on the Kazachya Bay water area and its coast resulted in an increase in organic carbon content. According to (Ovsyanyi & Orekhova, 2019), C_{org} content in 2015 averaged 2.74 % (35 % higher than in 2002). To date, C_{org} concentration is about 5.8 %

(the range is 4.32–7.86 %), which is comparable to organic carbon content in bottom sediments of the Sevastopol Bay (Inkerman area, the Yuzhnaya Bay) – the most anthropogenically stressed water area of the Sevastopol region (Orekhova et al., 2019b).

According to the data obtained, in bottom sediments of the Kruglaya Bay, the main processes of organic matter mineralization [their sequence is determined by the thermodynamic characteristics of the system and is described in detail in (Orekhova & Konovalov, 2009)] were carried out with oxidized forms of nitrogen involved (equation 2) in the upper 7-mm layer and sulfates involved (equation 3) in a layer of 8–200 mm in the bay apex (st. 1):

$$C_{106}H_{175}O_{42}N_{16}P + 104HNO_3 \leftrightarrow 106CO_2 + 60N_2 + H_3PO_4 + 138H_2O\,, \tag{2}$$

$$C_{106}H_{175}O_{42}N_{16}P + 59H_2SO_4 \leftrightarrow 106CO_2 + 16NH_3 + H_3PO_4 + 59H_2S + 62H_2O\,. \tag{3}$$

This was facilitated by the presence of a permanent source of organic matter, shallow depth, high temperature of bottom water layer (+24 °C), and limited water exchange.

Hydrogen sulfide distribution obtained may indicate a non-uniform organic matter accumulation due to a shift in the load level in different periods, redeposition resulting from turbidity, *etc.*

In the bay mouth area (st. 2), the absence of a permanent source of organic matter and water dynamics (Zaika et al., 2011 ; Kuftarkova et al., 2008) contributed to the fact as follows: during the period studied, there was enough oxygen to oxidize all organic matter. In the upper layer of bottom sediments (0–20 mm), aerobic conditions were recorded, and the main processes of organic matter oxidation were carried out with the oxygen involved (equation 4) (Orekhova & Konovalov, 2009):

$$C_{106}H_{175}O_{42}N_{16}P + 150O_2 \leftrightarrow 106CO_2 + 16HNO_3 + H_3PO_4 + 78H_2O\,. \tag{4}$$

Chemical composition of pore waters and geochemical characteristics of bottom sediments, *inter alia* organic carbon accumulation, as well as water circulation affect characteristics of benthic communities. As known, benthic diversity largely depends on redox conditions of the environment, presence of oxygen / hydrogen sulfide (Zaika et al., 2011), and organic matter content.

Despite the state corresponding to hypoxia in bottom water layer and an insignificant oxygen content (< 20 μM) on the sediment surface, the Kruglaya Bay apex was characterized by the highest macrozoobenthic diversity. In addition to bivalves, polychaetes and ascidians were noted. At the same time, two out of five recorded mollusc species in the Kruglaya Bay, *Abra* and *Cerastoderma*, are resistant to organic pollution (Tikhonova, 2010 ; Tikhonova & Rubtsova, 2012). This fact also indicates a fairly high level of organic pollution. The decrease in macrozoobenthos abundance at st. 2 is determined by geological and geomorphological structure of the underwater relief [it is an underwater coastal abrasion slope composed of psephite deposits with outcrops of bedrock (Mironova & Pankeeva, 2019)] and rather intensive water circulation in this area (Zaika et al., 2011). However, polychaetes are found there as well; a decrease in abundance and biomass (by almost an order of magnitude) of bivalves, absence of gastropods, and presence of crustaceans are registered. The polychaete *Cirriformia tentaculata* recorded in the bay mouth area is less resistant to organic pollution than species previously noted in other bay areas. They are found in spots at some distance from the source of organic matter, in so-called intermediate zones (Dean, 2008). Absence of ascidians in the Kruglaya Bay is characteristic of this water area: 2000 to 2009, they were not found as well (Mironov et al., 2003 ; Sanitary-Biological Investigations, 2009).

Thus, the greatest macrozoobenthic diversity in the Kruglaya Bay is confined to the apex station with silty and sandy bottom sediments (st. 1). In terms of its composition, this area has always significantly differed from other coastal areas (Mironov et al., 2003). On sandy and pebble sediments (st. 2), the diversity and quantitative parameters of macrobenthos are lower than in the mouth area, which also coincides with the data of earlier studies (Mironov et al., 2003). Species composition at the stations corresponds to that in earlier periods.

The Kruglaya Bay water area is distinguished by fluctuations in abundance and biomass, as well as a variability in the predominant species. Specifically, at st. 1 in 1999 and 2002–2004, the mollusc *C. glaucum* prevailed; in 2000 and 2004, *H. acuta*. In the recent period, a variety of crustaceans and polychaetes has been recorded in macrozoobenthos (Mironov et al., 2003). In 2001, a sharp decrease in species abundance was revealed in this area, with polychaetes predominating among four species registered (Mironov et al., 2003). During that period, significant abundance of *M. lineatus* juveniles was observed there (Mironov et al., 2003), as in the present study. It was noted earlier that at st. 1, the level of pollution of marine sediments with petroleum products is still not high enough to exert a depressing effect on species sensitive to this pollutant (Mironov et al., 2003). Besides pollutants, a wave effect has a significant impact on the formation of the community; however, in the points studied at st. 2, it is less notable, and at st. 1, it is practically absent. The latter favorably affects the development of malacofauna (including filter feeders); the accumulation of silt particles in bottom sediments contributes to an increase in the diversity and abundance of detritus feeders (Mironov et al., 2003).

In the Kazachya Bay, despite the better water exchange (Mironov et al., 2002) than in the Kruglaya Bay, the presence of permanent sources of organic matter led to its significant accumulation. Due to high organic carbon concentrations (4.32–7.86 %), oxygen is not the main component during its oxidation, and anaerobic conditions prevail in bottom sediments. There, hydrogen sulfide concentration in bottom sediments is significantly higher than in other bays of the Sevastopol region (Orekhova et al., 2019a ; Orekhova & Konovalov, 2018b). However, different conditions of sedimentation and sources of organic carbon determine as follows: at st. 3 in the upper sediment layer, suboxic conditions are observed, and the main biogeochemical processes are carried out with nitrates/nitrites involved (equation 2) in the 20-mm sediment layer. Below, sulfate reduction occurs, and one of its products is hydrogen sulfide (equation 3). In pore waters of bottom sediments at st. 4, the maximum hydrogen sulfide concentrations (about 900 μM) were noted; however, aerobic conditions were recorded in the upper 4-mm layer, which is probably due to the influx of "fresh" oxygen-enriched waters because of ventilation. Below, anaerobic conditions prevail, with their development resulting from sulfate reduction (equation 3). Moreover, for the Kazachya Bay area, the presence of reduced forms of iron and manganese was registered (Fig. 3) in the upper layer (0–20 mm), which may be associated with their local input *prior* to sampling.

In the bottom sediments of the Kazachya Bay, oxygen deficiency in the upper sediment layer and high hydrogen sulfide concentrations in bottom sediment column led to the fact that the benthic community was not characterized by high abundance and diversity, and polychaetes were the predominant class (Table 2). According to (Mironov et al., 2002 ; Belan & Moshchenko, 2009), *Capitella capitata* is a recognized positive indicator species of organic pollution, resistant to oxygen deficiency and reaching its maximum abundance under severe environmental pollution; *Heteromastus filiformis* is an indicator species of eutrophic waters (Losovskaya, 2011).

In recent years, changes have been observed in the macrozoobenthic community of loose sediments in the Kazachya Bay. According to AZTI Marine Biotic Index (AMBI), in 2003 and 2009, at most of the stations studied in the bay, the "undisturbed" state of benthic communities was recorded, while in 2006, "slightly disturbed" state was registered (Tikhonova & Alyomov, 2012). On sandy sediments, 13 Gastropoda taxa and 11 Bivalvia taxa were found. The ratio of gastropods was 55 and 75 % in 2011–2012 and 2018–2019, respectively; the ratio of bivalves was 45 and 25 %. In 2018–2019, there was a slight decrease in Mollusca abundance: Gastropoda, two times; Bivalvia, four times (Makarov, 2020). In terms of abundance, gastropods *Hydrobia acuta* (Draprnaud, 1805) prevailed: 55 and 70 % in 2011–2012 and 2018–2019, respectively; in terms of biomass, bivalves *Cerastoderma glaucum* (Bruguière, 1789) prevailed: 54 %. The mean abundance of molluscs decreased on average by three times compared to the values of 2011–2012, but the species composition was typical for loose sandy sediments (Makarov, 2020). These data differ from the values obtained in our study. The disparities revealed can be associated with the nature of bottom sediments (loose sandy sediments and silty sediments), sampling depth (coastal samples from a depth of 0.1 m and samples from a depth of more than 13 m), mosaicity of the benthic community distribution, and different oxygen regimes. Similar rearrangements in the benthic community were registered not only on loose sediments of the Kazachya Bay, but also in the epiphyton of *Zostera* seagrass. The number of species in 1970–1971 and 2006–2007 was the same – 13. Species composition for the indicated period practically did not differ. In 2006–2007, instead of *Steromphala divaricata* (Linnaeus, 1758), the species of the same genus was recorded – *Steromphala adriatica* (Philippi, 1844); instead of *Mytilus galloprovincialis* Lamarck, 1819 juveniles, a closely related Mytilidae species was noted – *M. lineatus*. However, mollusc mean abundance and mean biomass decreased significantly. Specifically, in 1970–1971, the mean abundance of Mollusca was 6,182 ind.$\cdot$kg^{-1}, while in 2006–2007, only 257 ind.$\cdot$kg^{-1} (it decreased by almost 25 times). The mean biomass in the early 1970s was 44.2 g$\cdot$kg^{-1}, while in the early 2000s, 8 g$\cdot$kg^{-1} (it decreased by more than 5 times). Moreover, there was a change in the predominant species. In the early 2000s, in terms of abundance and biomass, *Tricolia pullus* (Linnaeus, 1758) prevailed. In the early 1970s, *Rissoa membranacea* (J. Adams, 1800) definitely prevailed, and *Tricolia* was a non-abundant species (Makarov, 2018).

It can be assumed that the changes occurring in the macrozoobenthic community in the Kazachya Bay are caused not only by the anthropogenic factor (hypoxia development due to a recent increase in anthropogenic load on the water area because of the massive construction on the bay coast), but also by long-term fluctuations in abundance of the species themselves, characteristic of this region (Makarov, 2018), and active water dynamics in winter.

Moreover, there is a gradation of a particular species belonging to a certain group in terms of sensitivity to pollution: sensitive, indifferent, and stable ones (Mironov et al., 2003). Sensitive species are those found only in bottom sediments with the I–III pollution levels [these levels were determined by the content of chloroform extractable substances in them (Mironov et al., 2003)] or those characterized by a pronounced decrease in the frequency of occurrence with an increase in the pollution level of bottom sediments. Macrozoobenthic species characterized by the opposite tendency (an increase in the frequency of occurrence with an increase in the pollution level) are attributed to the ecological group of stable. According to this scale, the species recorded in the Kruglaya Bay water area are classified as stable ones (*A. segmentum*, *C. glaucum*, *T. neritea*, *T. pellucida*, and *C. capitata*), sensitive ones (*C. gallina* and *A. diadema*), and indifferent ones (*M. lineatus* and *H. filiformis*). For the Kazachya Bay, one sensitive species was recorded (*L. divaricata*), as well as two indifferent ones (*H. filiformis* and *N. hombergii*).

For the Kruglaya Bay, the Shannon indices calculated for abundance were 1.93 and 1.75 (Table 2). For the Kazachya Bay, the values were much lower: 0 and 1.0. Comparison of values obtained with those of previous studies shows that they are commensurate in Kruglaya Bay: earlier, the index varied 1.14 to 2.14 (Mironov et al., 2003). In the Kazachya Bay, the index is currently significantly lower. Earlier, it averaged 2.04–2.41 and reached 3.47 at some stations (Mironov et al., 2003). The results obtained can be associated with the mosaicity of the benthic community distribution over the bay water area (in the bay apex, the index values are lower), with a small number of samples analyzed, and with changes in habitat conditions. According to the classification of the state of the macrozoobenthic community for loose sediments considering the Shannon index (Water Framework Directive, 2009), it can be assessed as bad for the Kazachya Bay and moderate for the Kruglaya Bay. The Shannon indices calculated for biomass in the bays were low: the values did not exceed 1.56, and at some stations those were close to 0 (Table 2). The Pielou evenness index was rather low in the Kruglaya Bay, which indicates the predominance of particular species in the benthic community (Table 2). One species was recorded in the Kazachya Bay (st. 3), which makes it impossible to calculate the index. At st. 4, the Pielou evenness index is 1.

Analysis of species diversity in the water areas studied shows as follows: the formation of the benthic community, along with concentration of oxygen, hydrogen sulfide, and organic matter in bay bottom sediments, is influenced by habitat conditions of hydrobionts as well: presence of a food base, speed of currents, intensity of organic matter input, granulometric composition of bottom sediments, and wave processes (Neyman & Karpinsky, 2013).

The values of the oxygen flux calculated (0.73 $\text{M}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) at water – bottom sediments boundary and time of hypoxia development in bottom water layer in the Solenaya Bay area (st. 4) indicate the following: while maintaining the level of anthropogenic load with no water dynamics there, anoxia conditions in bottom water layer can develop in 5 months. This will undoubtedly result in the appearance of reduced forms of components (metals, nutrients, and sulfur) in the upper sediment layer and worsen habitat conditions of benthic communities.

Conclusion. Stratification of a water column due to limited water exchange, high temperature of bottom water layer, accompanied by a decrease in oxygen solubility, finely dispersed nature of bottom sediments, and high content of organic carbon (up to 7 % in bottom sediments of the Kazachya Bay and up to 2 % in bottom sediments of the Kruglaya Bay) contribute to the fact that the rate of oxygen consumption for organic matter oxidation exceeds the rate of its input. This is accompanied by the development of zones of oxygen deficiency on the surface of bottom sediments and reduced forms of compounds, in particular hydrogen sulfide, in their column.

In the upper sediment layer of the areas studied, suboxic conditions prevail; below, anaerobic ones. This led to the fact that the main forms of macrozoobenthos are species tolerant to oxygen deficiency and organic pollution. Specifically, in the investigated spots of the Kazachya Bay, polychaetes alone were present. The maximum hydrogen sulfide concentrations (up to 900 μM) in bottom sediments of the Solenaya Bay (the Kazachya Bay) are probably due to the presence of an additional source of organic matter there. The calculated time for complete depletion of oxygen in the upper sediment layer (where it was recorded), taking into account biogeochemical processes alone at this station, is about 5 months.

Species diversity of the benthic community in bottom sediments of the studied bays indicates that the determining factors in its formation are not only concentrations of oxygen, hydrogen sulfide, and organic matter in bottom sediments, but also presence of a food base, speed of currents, intensity of organic matter input, granulometric composition of bottom sediments, wave processes, *etc.*

Hence, it can be assumed that a lack of systemic and environmentally efficient approach to exploitation of the Kruglaya and Kazachya bays resulted in their severe pollution, which contributed to the deterioration of habitat conditions of benthic communities. The development of hypoxic conditions on the sediment surface and hydrogen sulfide emergence in the upper sediment layer may indicate that the ecosystems of the Kruglaya and Kazachya bays are in the stage of degradation.

Their further exploitation without reducing anthropogenic load will result in the emergence and spread of environmental risk zones. This will worsen the recreational and socioeconomic attractiveness of these areas.

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ОКИСЛИТЕЛЬНО-ВОССТАНОВИТЕЛЬНЫЕ УСЛОВИЯ ДОННЫХ ОТЛОЖЕНИЙ И ХАРАКТЕРИСТИКИ МАКРОЗООБЕНТОСА БУХТ КРУГЛОЙ И КАЗАЧЬЕЙ (Г. СЕВАСТОПОЛЬ)

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Отсутствие системного и экологически рационального подхода при эксплуатации бухт Севастопольского региона (бухт Круглой и Казачьей) привело к значительному их загрязнению. Комбинация ряда естественных и антропогенных факторов обусловила ухудшение условий существования бентосных сообществ. Целью данной работы было провести комплексные исследования экосистем бухт Круглой и Казачьей для изучения особенностей формирования окислительно-восстановительных условий в донных отложениях и придонном слое вод, а также их влияния на характеристики макрозообентоса. Пробы донных отложений отбирал водолаз трубками из оргстекла, герметично закрываемыми сверху и снизу, что позволило сохранить тонкую структуру донных отложений и придонного слоя вод. Для изучения бентосных сообществ в этом же месте отбирали пробы с помощью ручного пробоотборника. Для получения химического состава поровых вод с высоким вертикальным разрешением использовали полярографический метод анализа. Расчёт потока кислорода на границе и в верхней части донных отложений выполняли по данным вертикального профиля концентрации кислорода в поровых водах и геохимического анализа, используя уравнение для первого закона Фика с учётом градиента концентраций и молекулярной диффузии кислорода в поровых водах. Для анализа бентосного материала применяли стандартные гидробиологические методы. При расчёте значений индекса разнообразия Шеннона (H') использовали логарифм по основанию 2. Анализ полученных данных показал, что высокий уровень антропогенного воздействия и ограниченная динамика вод привели к заиливанию донных отложений исследуемых акваторий, что затрудняло поступление кислорода в них, а накопление в осадках органического углерода обусловили активное его расходование. Стратификация водной толщи за счёт ограниченного водообмена, высокая температура придонного слоя вод, сопровождающаяся снижением растворимости кислорода, и мелкодисперсный характер донных отложений способствовали тому, что скорость поступления кислорода была меньше скорости его потребления на окисление органического вещества; это сопровождалось развитием зон дефицита кислорода и появлением восстановленных соединений, в частности сероводорода. Отмечено, что в верхнем слое донных отложений преобладали субкислородные условия, ниже — анаэробные. Это привело к тому, что основными формами макрозообентоса являлись виды, толерантные к дефициту кислорода и к загрязнению органическими веществами. Так, на некоторых участках бухты Казачьей присутствовали только полихеты. При этом отмечено, что в районе выхода из бухты Круглой интенсивная динамика вод и морфологические особенности дна способствуют насыщению верхнего слоя отложений кислородом. На основании данных о концентрации кислорода в поверхностном (0–5 мм) слое осадка (в поровых водах), а также данных о геохимических характеристиках (влажность, пористость) донных отложений рассчитан поток кислорода на ст. 4 (бухта Солёная); его величина составила 0,73 $\text{М}\cdot\text{м}^{-2}\cdot\text{год}^{-1}$. Принимая в расчёт концентрацию кислорода в придонном слое вод (259 мкМ), можно сказать, что время развития аноксии с учётом только биогеохимических процессов составляет около 5 месяцев. Таким образом, можно предположить, что экосистемы бухт Круглой и Казачьей находятся в стадии деградации. Дальнейшая их эксплуатация без выработанного комплексного и рационального подхода приведёт к критическому ухудшению их экосистем — к появлению и распространению зон экологического риска, что снизит рекреационную и социально-экономическую привлекательность данных районов.

Ключевые слова: донные отложения, поровые воды, кислород, сероводород, органический углерод, макрозообентос, прибрежные акватории, Чёрное море

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ON THE PRESENCE OF THE SPINNER SHARK *CARCHARHINUS BREVIPINNA* (MÜLLER & HENLE, 1839) (CHONDRICHTHYES: CARCHARHINIDAE) IN MALDIVIAN WATERS

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We present the first confirmed report on the presence of spinner shark, *Carcharhinus brevipinna*, in Maldivian waters. The species was observed in front of Kooddoo's harbour, in Villingili pass, Northern Huvadhoo Atoll, Southern Maldives in March 2020.

Keywords: shark, spinner shark, *Carcharhinus brevipinna*, Huvadhoo Atoll, ecotourism

To date, a total of 36 species of sharks have been recorded in Maldivian waters, including 13 requiem sharks (family Carcharhinidae) (De Maddalena & Galli, 2017). Detailed lists of the shark species recorded in Maldivian waters have been published by various authors (Adam et al., 1998 ; Ali & Sinan, 2015 ; Anderson & Ahmed, 1993 ; Anderson et al., 1998 ; De Maddalena & Galli, 2017); however, the spinner shark *Carcharhinus brevipinna* (Müller & Henle, 1839) was not listed in any of them. In this article, we report for the first time in scientific literature the presence of *C. brevipinna* in Maldivian waters.

The spinner shark belongs to the order Carcharhiniformes and to the family Carcharhinidae. It is a pelagic species found on continental and insular shelves, at depths ranging from the surface down to at least 100 m, in the Atlantic waters, Mediterranean Sea, and Pacific and Indian oceans (De Maddalena et al., 2015). The spinner shark is active and fast, observed both solitary and in large groups. Its diet includes bony fish, rays, cephalopods, and crustaceans. It feeds on small schooling fishes by swimming fast through the school with an open mouth, spinning along the body axis, and leaping out of the water. It is a placental, viviparous species. The male attains sexual maturity at 170 cm; the female, at 180 cm. After a 12–15-month gestation, the female gives birth to 2–15 young ones ranging 46–80 cm in total length. It can attain a maximum size of 278 cm and can live up to 27 years (Castro, 1983 ; Compagno, 1984 ; De Maddalena et al., 2015 ; Last & Stevens, 1994 ; Randall, 1986).

In the past, the Maldives severely depleted shark species due to overfishing for dried meat exports, oil, and fins. Following concerns by the tourism sector of shark fishing at major dive sites, the Government of the Maldives announced in 1995 the establishment of 15 Protected Marine Areas and the total protection of the whale shark *Rhincodon typus*. In 1999, further nine Protected Marine Areas were identified. In 1998, the government implemented a 10-year moratorium on all types of shark fishing inside and within the rim of seven major tourist atolls in the Maldives. A failure to enforce the moratorium and effectively ban fishing at Protected Marine Areas led to a total ban on all fishing, capture, killing, or extraction of sharks from Maldivian waters since 15 March, 2010 (Ali & Sinan, 2015 ; Anderson & Ahmed, 1993). To date, the Republic of Maldives is among few countries that have declared a complete ban on shark fishing. The driving force of this fundamental change has been the blossoming of the ecotourism industry, which allows the general public to observe sharks in their natural environment. Apart from *C. brevipinna*, shark species regularly encountered by divers in the waters of Kooddoo include grey reef shark *Carcharhinus amblyrhynchos*, blacktip reef shark *C. melanopterus*, silvertip shark *C. albimarginatus*, and whitetip reef shark *Triaenodon obesus*.

MATERIAL AND METHODS

The observation took place on two different days, on 2 and 9 March, 2020, while the first author (F. R.) was scuba diving, in the waters in front of Kooddoo's harbour, in Villingili pass, Northern Huvadhoo Atoll, or Gaafu Alifu, Southern Maldives, Central Indian Ocean (Fig. 1). Both encounters took place 400–500 m distance from the harbour entrance and 350 m from the beach, in 20-m deep waters, with the sharks swimming at depths ranging from the surface down to 15 m. Fish wastes were used as chum and bait by local fishermen to attract the sharks to the site and keep them interested around the divers for viewing purposes.

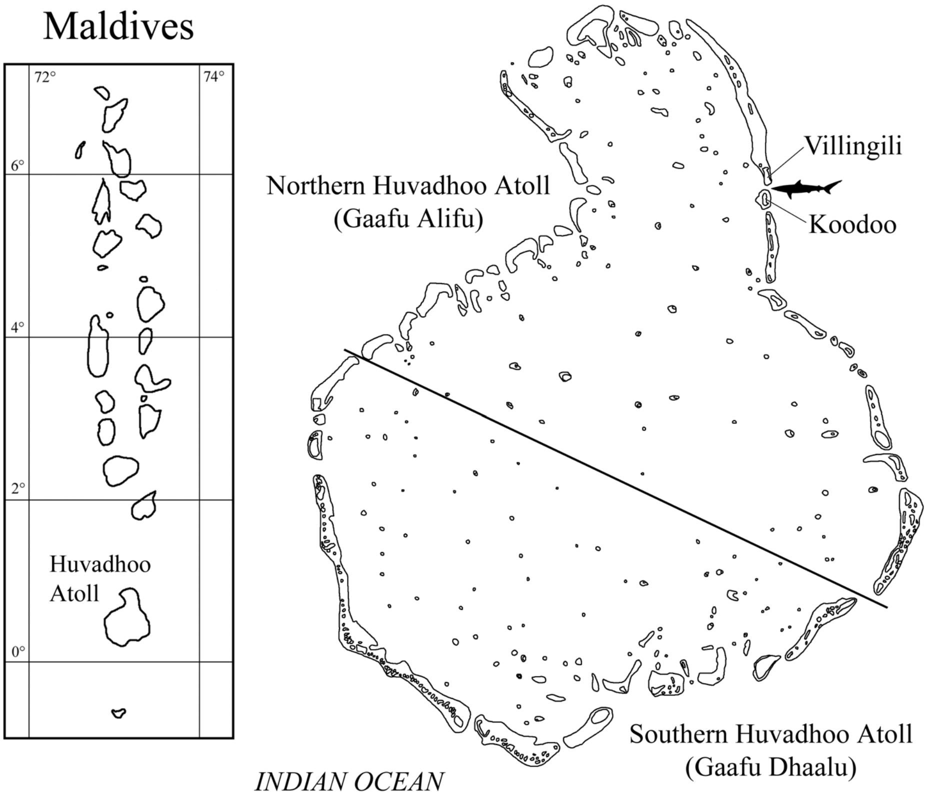

Fig. 1. Map showing the location where the spinner sharks *Carcharhinus brevipinna* were observed off the Northern Huvadhoo Atoll, Southern Maldives

On 2 March, 2020, approximately 20 spinner sharks were observed for 45 minutes, 14:40 to 15:25. It was a sunny day, with +28.8 °C mean temperature (maximum: +30.8 °C; minimum: +28.8 °C), calm seas, and no current. Underwater visibility was sufficient at the beginning of the dive (between 10 and 5 m) but became very poor near the end (less than 5 m), due to chumming. The size of the spinner shark was estimated from 1.5–1.6 m in total length for the smaller individuals up to 1.9–2.0 m in total length for the larger ones. Most individuals were female but a few males were observed as well (the males have two copulatory organs called claspers, which are located at the base of the pelvic fins). The divers remained suspended half-way in the water column, close to each other, and in vertical position. There was no interaction between the sharks, and they showed no interest in the divers. The sharks appeared interested only in the fish wastes thrown overboard by the fishermen. In order to attract the sharks and keep them around the divers, the fishermen were using the delayed surface marker buoy as the target to throw the fish wastes into the water.

On 9 March, 2020, approximately 20 spinner sharks were observed for 46 minutes, 14:37 to 15:23. It was a sunny day, with a +30.3 °C mean temperature (maximum: +31.2 °C; minimum: +30.0 °C), calm seas, and no current. Underwater visibility was good at the beginning of the dive (between 15 and 10 m) and remained sufficient until the end (between 10 and 5 m). The size of the spinner sharks was the same as observed on the previous encounter. Similarly, the large majority of individuals were female; only one male was later spotted in the filmed footage. The behavior of the sharks was similar to that during the previous dive. The sharks hit the delayed surface marker buoy several times and finally broke it.

Two spinner sharks had a fishing line wrapped around their head, and one of them was spotted on both 2 and 9 March.

On both dives, other fishes were attracted to the site by the chumming, including species of the families Acanthuridae and Caesionidae, at least a humphead wrasse *Cheilinus undulatus*, several great barracudas *Sphyraena barracuda*, and a green sea turtle *Chelonia mydas*. The sharks were accompanied by some live sharksuckers *Echeneis naucrates*.

Videos of the sharks were taken with an action cam full HD for subsequent analysis (Fig. 2).

The species identification was based mainly on the morphological descriptions given in Castro (1983), Compagno (1984), and De Maddalena *et al.* (2015).

Fig. 2. Spinner shark *Carcharhinus brevipinna* accompanied by live sharksuckers *Echeneis naucrates*, observed in the waters in front of Kooddoo's harbour, in Villingili pass, Northern Huvadhoo Atoll, Southern Maldives. Photo by Fabio Russo

RESULTS AND DISCUSSION

There could be multiple reasons to explain why the spinner shark was not previously recorded from the area. This may be due to the lack of specific surveys in the waters of the Northern Huvadhoo Atoll. Moreover, spinner sharks are sometimes mistaken for other requiem shark species because of their similar morphology. Species, with which the spinner shark may be confused, that have been recorded in Maldivian waters, include the silky shark *C. falciformis* and the blacktip shark *C. limbatus*. Further observations of *C. brevipinna* are needed to reveal whether this species is an occasional visitor in the area or there is a stable population that reproduces in Maldivian waters.

Kooddoo Fisheries Complex started its operations in 1996; it is the main cold storage facility of Maldives Industrial Fisheries Company Ltd. This facility has an alongside berth which can accommodate vessels up to 2500 deadweight tonnage. The fish processing station grew bigger in 2006, with a consequent increase in the processing wastes that are thrown at sea just out of the harbour. People noticed that fish wastes had started to attract a good amount of spinner sharks in the area, and diving companies began promoting shark diving on the site. The concentration of spinner sharks may reach 40 individuals encountered on a single dive. The fishermen are now getting paid by diving cruise boats companies to throw the waste when the divers are in the water.

Such collaboration between diving companies and fishermen is a good element in this particular scenario, since the various parts draw an economic profit from the conservation of that population of spinner sharks, and then there can hopefully be successful conditions to conserve this newly discovered local natural resource. It is also positive that the sharks are being attracted by using fish wastes rather than fishes specifically killed to feed the sharks.

On the other hand, the fact that on diving days the spinner sharks can be fed for several hours in a row, may alter the normal predatory behavior of this species in the area. Therefore, feeding fish wastes to sharks should be kept to a minimum.

Conclusion. Two major economic activities in the Maldives are fishing and tourism. In 1992, the number of dives *per* year at 35 shark observation dive sites was estimated at over 70,000, and the amount of money spent on those dives was estimated at US$2.3 million. Based on these numbers, it was estimated that a single reef shark was worth about US$33,500 *per* year, at the most popular dive site, and an average of US$3,300, when taking into account all the shark observation dive sites (Anderson & Ahmed, 1993). Since grey reef sharks can live more than 25 years, a single grey reef shark may be worth over US$800,000 during its whole lifetime (De Maddalena & Galli, 2017). In contrast, a dead grey reef shark was calculated to have a one-time value of about US$32, to a local fisherman (Anderson & Ahmed, 1993). Therefore, a grey reef shark is worth up to 25,000 times more alive than dead.

Ecotourism is an extremely powerful and effective tool for animal protection, when properly managed. By setting solid guidelines, monitoring diving activities, and enforcing the rules, the operators and enforcement personnel will make sure that shark diving is a useful activity and not just an alternative way to simply make a profit from the exploitation of wild animals, which often ends up harming them, alters their natural behavior, and damages their habitat (De Maddalena & Galli, 2017).

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О НАЛИЧИИ КОРОТКОПЁРОЙ СЕРОЙ АКУЛЫ *CARCHARHINUS BREVIPINNA* (MÜLLER & HENLE, 1839) (CHONDRICHTHYES: CARCHARHINIDAE) В АКВАТОРИИ МАЛЬДИВ

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Это первое подтверждённое сообщение о наличии короткопёрой серой акулы *Carcharhinus brevipinna* в акватории Мальдив. Особь была замечена перед гаванью Кудду на перевале Виллингили (атолл Северный Хувадху, Южные Мальдивы) в марте 2020 г.

Ключевые слова: акула, короткопёрая серая акула, *Carcharhinus brevipinna*, атолл Хувадху, экотуризм

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COMPARATIVE CHARACTERISTICS OF THE TRACE ELEMENTAL COMPOSITION OF CHUM SALMON *ONCORHYNCHUS KETA* WALBAUM, 1792 FROM THE SEA OF JAPAN AND THE SEA OF OKHOTSK

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Content of trace elements (iron, zinc, copper, nickel, lead, and cadmium) was determined in the most popular species of Pacific salmon on the consumer market – chum salmon *Oncorhynchus keta*, which is the second largest catch in Russian waters (after pink salmon *O. gorbuscha*). Metal content in fish organs and tissues (muscle tissue, liver, and gonads) was determined by the atomic absorption method using a Shimadzu AA-6800 spectrophotometer in flame and flameless atomizers from sample extracts; the latter ones were obtained by decomposition of weighed portions of concentrated HNO_3 (high purity) in a MARS 6 complex using standard samples with known concentrations. The data was statistically processed in SPSS Statistics 21. Sexually mature individuals of chum salmon (five specimens of males and females on each spot) were sampled in autumn 2018 in storage cages of salmon hatcheries on the Firsovka River (southeastern Sakhalin, Gulf of Patience) and Reidovaya River (Iturup Island, Kuril Islands), as well as in October 2019 in breeder holding cages on the Poima River (southwestern Primorye, the Sea of Japan). Muscle tissue, liver, and gonads were sampled from every individual. The elements determined were clearly divided according to content values into two groups depending on the sampling spot: Cd and Pb prevailed in organs and tissues of chum salmon from the Sakhalin-Kuril Region of the Sea of Okhotsk, whereas Fe, Zn, Cu, and Ni prevailed in the Sea of Japan fish. The reason for heavy metal contrasting distribution in fish organs and tissues is obviously environmental geochemical conditions formed in salmon feeding areas and on migration routes. In the Sea of Japan, practically enclosed, poorly connected with the Pacific Ocean by several shallow straits, the aquatic environment is under significant anthropogenic, technogenic, and terrigenous load (household and industrial drains, surface washing from coastal areas, and combustion of hydrocarbon fuel by ships). In the Sakhalin-Kuril basin, it is affected by natural phenomena: surface and underwater volcanism and post-volcanism of the Kuril Islands, as well as upwellings, which carry from the depths of the Kuril–Kamchatka Trench into the surface layer practically the entire set of chemical elements of the Mendeleev's periodic table. At the same time, Pb, with its unusually high sorbability, is retained on any suspended particles, both living and non-living, and enters fish organisms with food. The distribution of trace elements over chum salmon organs and tissues differs significantly. Specifically, in fish muscle tissues, the lowest metal content is observed, regardless of the catch spot. The liver is characterized by increased levels of all metals, except for Ni, with the highest Fe, Zn, and Cu content recorded in the Sea of Japan fish liver. The distribution of metals in fish gonads is characterized by its own specificity, with very clear manifestation in the Primorye salmon. Thus, in female gonads, Fe, Zn, and especially Cu predominated: Cu content in female gonads from the Poima River was the same as in liver. Female gonads of the Sea of Japan chum salmon had the highest Ni concentration, although male gonads were characterized by a high, albeit very variable, content of this element as well. As for Pb and Cd, the most toxic elements monitored by sanitary services (for technical reasons, we determined their amount in the Sea of Okhotsk fish only), a well-known pattern was observed in their distribution in organs and tissues: maximum content was found in fish liver. Pb content exceeded the sanitary standard only in some individuals that came to spawn in the Firsovka River in the southeastern Sakhalin. Cd concentration in Sakhalin fish liver was 2.5–4.0 times higher than threshold limit value, and in Kuril fish liver, 2.1–5.0 times higher.

Keywords: Pacific salmon, chum salmon, Sakhalin-Kuril Region, Sea of Okhotsk, Primorsky Krai, Sea of Japan, trace elements

Chum salmon is one of six Pacific salmon species of the genus *Oncorhynchus*. It inhabits both coasts of the Pacific Ocean and is one of the most abundant salmon species. In the present work, mature chum salmon individuals were studied, that came to spawn and were caught in the rivers of the southeastern Sakhalin, Iturup Island, and southern Primorye. In chum salmon soft organs and tissues, the content of six chemical elements was investigated: iron, zinc, copper, nickel, lead, and cadmium.

Out of six trace elements analyzed, three – Cu, Zn, and Fe – are essential (necessary), or true ones, and other three – Ni, Cd, and Pb – are non-essential, but almost constantly present in organs and tissues. In addition to their biological significance, these trace elements differ in geoecological peculiarities. Cu and Zn, if they do not enter the environment from copper-zinc industries or spots where these metals, their alloys, and compounds are used in technology (mining, enrichment, smelting, work with electroplated and anti-corrosion coatings, *etc.*), are the tracers of technogenic load on the environment and are present in all household drains; Ni contamination occurs mainly during the combustion of hydrocarbon fuel, wood, and waste, as well as because of nickel production and use (Khristoforova et al., 2018b ; Kobzar & Khristoforova, 2015).

Iron enters the environment due to widespread use of a variety of surface and underwater steel structures and their rusting, as well as due to river, terrigenous, and surface runoff, stirring up of loose bottom sediments in coastal waters, and decomposition of dead hydrobionts, in which Fe is an obligatory component (macroelement) (Khristoforova, 1989).

In the 1960s and 1970s, Pb and Cd were, along with Hg, at the top of all the blacklists of heavy metals in organisms due to their toxic effect. Now, they are considered as the tracers of technogenic load on the environment (Khristoforova, 1989).

Chum salmon *Oncorhynchus keta* (Walbaum, 1792) is the species in demand and the most numerous species of Pacific salmon in the food markets of Russia and other countries. Due to its high commercial quality and a distinct homing, chum salmon currently accounts for the overwhelming part of all artificially reproduced salmon along both the Asian and American coast of the North Pacific. In Russia, in particular in Sakhalin, high chum salmon catches are mainly due to the work of the hatcheries. In our country, artificial reproduction of chum salmon is carried out in Primorsky Krai, Kamchatka, Khabarovsk, Magadan, and Sakhalin regions.

The Sakhalin Region has exceptionally favorable environmental conditions for a large-scale development of aquaculture. In 2019–2020, over 60 fish hatcheries of different forms of ownership were engaged in artificial reproduction of Pacific salmon in the Sakhalin-Kuril Region. Due to their stable and systematic work, salmon hatcheries release more than 800 million ind. of juveniles every year, ensuring the sustainable development of the fishing industry in the region. In 2019, more than 1 billion ind. of juveniles were released there, *inter alia* 263.2 million ind. of pink salmon and 863.6 million ind. of chum salmon (Ofitsial'naya informatsiya o rybovodnykh predpriyatiyakh, 2020b).

The main species of Pacific salmon reproduced in the Sakhalin Region are autumn chum salmon and pink salmon. Most chum salmons from the Sakhalin-Kuril basin are of artificial origin. Due to the operation of fish hatcheries, more than 20 thousand tons of salmon enter the coastal area of the Iturup Island alone annually, while before the start of the fish farming program in the Kuril Islands, the comebacks of wild fish were, at best, 500–700 tons (Ofitsial'naya informatsiya o rybovodnykh predpriyatiyakh, 2020b).

The large-scale work of the salmon hatcheries, as well as natural reproduction, annually ensure stable approaches of salmon to the rivers of Sakhalin and the Kuril Islands. In 2018, in the Sakhalin-Kuril Region, according to official data of the Sakhalin Government, 126 thousand tons of Pacific salmon were caught by commercial fishing; out of them, there were 85 thousand tons of pink salmon and more than 40 thousand tons of chum salmon. In 2019 – the less productive year – salmon caught in the Sakhalin-Kuril waters amounted to 75.5 thousand tons; out of them, there were more than 28 thousand tons of pink salmon and 45.5 thousand tons of chum salmon.

The Primorye chum salmon is characterized by a southern distribution within the species range. The very fact of fish entering local rivers, although in small abundance, indicates the passage of chum salmon through the southern Sea of Japan (Goryainov et al., 2007 ; Shuntov et al., 2004).

In the Primorsky Krai, there are several fish hatcheries of different forms of ownership engaged in the Pacific salmon reproduction. Out of them, there are two state enterprises: Ryazanovka Experimental Commercial Fish Hatchery and Barabashevka Fish Hatchery. Both are located in the Khasansky District, have been operating since the mid-1980s, and are structural subdivisions of the Federal State Budgetary Institution "Glavrybvod". For the hatcheries, the main farming object is autumn chum salmon. Its basic watercourses are the Ryazanovka and Barabashevka rivers. Eggs for laying are mostly obtained on the Ryazanovka River. Eggs are collected on the Poima River as well, which flows into the Baklan Bay of the Peter the Great Gulf of the Sea of Japan (the Khasansky District, near the Slavyanka urban-type settlement).

In the Khasansky District, hatchery chum salmon schools are of mixed origin since in different years, fertilized eggs were delivered to the Primorye hatcheries not only from Primorsky Krai (Olginsky District), but also from the southwestern Sakhalin.

Over the past 20 years, stable approaches of chum salmon have been observed in the areas where the hatcheries operate. The annual release is 25–30 million ind. of juveniles. During the 2019 season, more than 39 million chum salmon eggs were collected and incubated at the Primorsky Krai hatcheries (Ofitsial'naya informatsiya o rybovodnykh predpriyatiyakh, 2020a). According to V. G. Markovtsev (2006), it is the operation of these two fish hatcheries that ensures salmon fishing in the Primorye.

The aim of the study is to compare the content of trace elements in chum salmon that came to spawn in the rivers of the Sakhalin-Kuril Region and Primorsky Krai, which was collected from the storage cages of three salmon hatcheries (on the Iturup Island, in Sakhalin, and in the southwestern Primorye).

MATERIAL AND METHODS

The material for research was sampled during the period of spawner accumulating and egg laying for incubation in the spots of egg collecting – on the basic watercourses of Sakhalin fish hatcheries: in October 2018, on the Firsovka River (Firsovka Salmon Hatchery, Gulf of Patience, southeastern Sakhalin) and Reidovaya River (Reidovaya Salmon Hatchery, Iturup Island, Greater Kuril Chain); in October 2019, on the Poima River of the Khasansky Dictrict, Primorsky Krai (the basic watercourse of the Ryazanovka Experimental Commercial Fish Hatchery) (Fig. 1).

Fig. 1. Chum salmon sampling areas

In total, five chum salmon males and five chum salmon females were sampled (randomly) at each hatchery from breeder holding cages. Muscle tissue, liver, and gonads were sampled from every individual.

The time required for chum salmon spawners to mature directly depends on the feeding efficiency in the marine life period, especially at its early stage, and can range for this species 2 to 5 years. According to official information from the Sakhalin branch of VNIRO, chum salmon from the Reidovaya River in most cases (54.5 %) comes back to spawn at the age of 3+. Based on our data, 80 % of the studied spawners of the Primorye chum salmon are individuals aged 5+, *i. e.* feeding areas for the Sea of Japan chum salmon are likely to be less forage (according to information of the Primorsky branch of "Glavrybvod", 53 % of mature individuals come back at the age of 4+; 44 %, at the age of 3+). We determined the age of chum salmon from the Firsovka River as 3+.

All spawners were at the 4th–5th stage of maturity. In Table 1, fish length and mean weight values are given.

As can be seen, the Poima River fish is of maximum weight, whereas the Firsovka River individuals are of minimum one. At the same time, salmon of average weight from the Reidovaya River are the longest ones, especially males.

All the elements were determined from sample extracts according to the State Standard 26929-94 (2010) using a Shimadzu AA-6800 spectrophotometer (Cd and Pb, by graphite furnace atomic absorption; the other trace elements, by flame atomization). The work was carried out at the "Center for Landscape Diagnostics and GIS Technologies" core facility at the Pacific Geographical Institute FEB RAS. Sample extracts were obtained by decomposition of weighed portions of fish organs and tissues with concentrated HNO_3 (high purity) by microwave mineralization in a MARS 6 complex.

Table 1. Morphometric characteristics of *Oncorhynchus keta* from the Firsovka, Reidovaya, and Poima rivers (2018–2019)

Sampling date	Sampling spot, sex of fish	Mean body weight of fish, g (min–max)	Mean length of fish, cm (min–max)	
			SL	SCL
October 2018	Reidovaya River, ♀	2423 (2232–2774)	67.0 (65–69)	63.0 (61–65)
	Reidovaya River, ♂	3115 (4834–2154)	71.0 (66–82)	67.0 (62–78)
October 2018	Firsovka River, ♀	2096 (1626–2402)	56.6 (53–59)	53.4 (50–56)
	Firsovka River, ♂	3008 (2086–3008)	60.8 (53–60)	56.8 (53–60)
October 2019	Poima River, ♀	3220 (2350–4250)	66.6 (60–71)	61.6 (55–66)
	Poima River, ♂	3330 (3000–3800)	68.6 (67–70)	64.6 (63–67)

Note: SL denotes length according to Smith (up to middle rays of caudal fin); SCL, length up to end of scale cover.

The accuracy of the element determination and possible contamination of the samples during the analysis were controlled by comparison with calibration solutions, *inter alia* with a "blank" (zero) one (concentrated HNO_3, 10 mL after heating in a microwave mineralizer, evaporated and diluted in the same ratio with 0.1 M solution).

The accuracy and precision of the method used were confirmed by regular analysis of the standard reference material (SRM) 1566a (NIST, oyster tissue) for each element determined in the samples (Table 2). The mean value, standard deviation (*SD*), and significance of the compared differences (applying the Mann–Whitney *U* test) were calculated in SPSS Statistics 21 for macOS. The detection limits were quantified as 3×*SD* of 10 fish samples mixed with a known minimum amount of the elements analyzed. For analytes that could not be determined in mixed samples, the detection limits were found as the amount of substance in the sample in accordance with the minimum concentration of the calibration standard. The minimum amount of the calibration standard was determined based on the State Standard 30178-96 (2010).

Total concentrations (mean ± *SD*) for Fe, Zn, Cu, Ni, Cd, and Pb were quantified in $\mu g \cdot g^{-1}$ wet weight.

Table 2. Comparison of the results of trace metal determination ($\mu g \cdot g^{-1}$ dry weight) in certified material [SRM 1566a (oyster tissue)] with passport data; limit of detection (LOD)

	Fe	Zn	Cu	Pb	Ni	Cd
Certified value	539 ± 15	830 ± 57	66.30 ± 4.3	0.371 ± 0.014	2.25 ± 0.44	4.15 ± 0.38
Present study	554 ± 16	821 ± 51	61.09 ± 3.2	0.358 ± 0.012	2.52 ± 0.48	4.19 ± 0.37
Limit of detection	0.2	0.01	0.02	0.01	0.05	0.01

As seen, the results of the control determination of the trace elements in the certified sample are comparable with the passport data of the reference material.

RESULTS

The trace element concentration in organs and tissues of chum salmon that came back to spawn in the rivers of the southeastern Sakhalin, Iturup Island, and southern Primorye differs significantly (Table 3).

Table 3. Trace elements content in *Oncorhynchus keta* organs and tissues from the Firsovka, Reidovaya, and Poima rivers ($\mu g \cdot g^{-1}$ wet weight)

Organs and tissues	Fe	Zn	Cu	Pb	Ni	Cd
Sakhalin Island, Firsovka River (October 2018)						
Muscle	7.6 ± 2.5	1.66 ± 0.23	0.52 ± 0.15	0.40 ± 0.15	0.29 ± 0.10	0.06 ± 0.02
Liver	*60.2 ± 34.6*	3.48 ± 1.24	0.54 ± 0.22	**1.18 ± 0.36**	0.23 ± 0.10	**0.66 ± 0.15**
Male gonads	13.0 ± 6.2	1.74 ± 0.23	0.39 ± 0.12	0.48 ± 0.17	0.19 ± 0.05	0.12 ± 0.03
Female gonads (eggs)	15.6 ± 2.4	1.92 ± 0.09	0.33 ± 0.05	0.45 ± 0.14	0.26 ± 0.04	0.04 ± 0.01
Iturup Island, Reidovaya River (October 2018)						
Muscle	11.4 ± 3.9	1.74 ± 0.29	0.49 ± 0.12	0.45 ± 0.07	0.26 ± 0.11	0.05 ± 0.02
Liver	*48.1 ± 19.1*	3.35 ± 1.22	0.55 ± 0.315	0.87 ± 0.19	0.31 ± 0.07	**0.72 ± 0.3**
Male gonads	10.4 ± 4.0	2.18 ± 0.23	0.38 ± 0.14	0.64 ± 0.09	0.23 ± 0.05	0.09 ± 0.03
Female gonads (eggs)	16.2 ± 4.3	2.60 ± 0.41	0.46 ± 0.01	0.57 ± 0.05	0.20 ± 0.06	0.07 ± 0.00
Southern Primorye, Poima River (October 2019)						
Muscle	10.5 ± 3.0	*5.20 ± 0.65*	0.76 ± 0.28	N. d.	*0.48 ± 0.14*	N. d.
Liver	121.9 ± 95.2	33.463 ± 14.83	5.49 ± 3.36	N. d.	*0.51 ± 0.21*	N. d.
Male gonads	*20.0 ± 10.2*	*7.17 ± 1.18*	0.76 ± 0.28	N. d.	0.97 ± 0.93	N. d.
Female gonads (eggs)	*23.4 ± 5.5*	20.67 ± 0.82	5.68 ± 0.40	N. d.	0.75 ± 0.38	N. d.

Note. N. d. denotes "were not detected"; in italic, increased concentrations for each of trace elements are highlighted; in bold, their highest values. According to sanitary rules and regulations, threshold limit values ($\mu g \cdot g^{-1}$ wet weight) for seafood are as follows: Pb, 1.0; Cd, 0.2 (SanPiN 2.3.2.1078-01, 2002).

Specifically, the trace elements are ranked according to their content as follows: Fe opens the row; Zn follows it; Cu and Pb share the 3rd and 4th places; Ni is noticeably behind them; and Cd closes the row.

The Sea of Japan chum salmon was characterized by higher concentrations of all elements determined (p = 0.012…0.048). Particularly noteworthy is Fe content in liver of the Poima River fish: it was 2 times higher than in the Firsovka River chum salmon and almost 3 times higher than in the Reidovaya River fish. At the same time, in muscles of chum salmon from any river, approximately equal or similar values were recorded. Zn clearly predominated in the Primorye chum salmon, with its high content not only in liver ($p = 0.03$), but also in female gonads (eggs) ($p = 0.032$). Cu concentrations both in liver and eggs of the Sea of Japan chum salmon females were an order of magnitude higher than those in the Kuril ($p = 0.02$) and Sakhalin fish ($p = 0.029$). Ni content in all organs and tissues of the Sea of Japan chum salmon was higher as well (p = 0.018…0.045) than in the Sakhalin and Kuril fish; however, male and female gonads were characterized by both higher Ni concentration and its higher variability.

While Ni was almost evenly distributed between organs and tissues of the Sea of Okhotsk chum salmon, Cd clearly (by an order of magnitude or more) predominated in liver. Primorye salmons were characterized by both higher Ni content in general and its different distribution. Specifically, the difference in Ni concentrations in the Sea of Japan chum salmon in muscles and liver reached

2 times ($p = 0.019$), whereas in both female and male gonads the content was 3–4 times higher ($p = 0.012$) than in muscles. The highest Ni concentration was observed in male gonads (its content in male gonads was 1.3 times higher than in female gonads, although it was very variable).

DISCUSSION

Increased Fe concentration in male and female gonads of the Sea of Japan chum salmon and the highest Fe content in fish liver clearly indicate a higher terrigenous load on the water area of the almost enclosed Sea of Japan and on its inhabitants, as well as the detoxification of the trace element excess by liver.

Besides Fe, other trace elements enter the coastal waters of the Sea of Japan with terrigenous and surface runoff as well (Khristoforova & Chernova, 2005). The presence of Zn (the second highest concentration trace element in fish organs and tissues) confirms a much higher anthropogenic load on the water area of the Sea of Japan than on the water area of the Sea of Okhotsk. Another trace element indicating anthropogenic load on the environment and organisms is Cu. Its concentration neither in liver nor in male and female gonads of the Sea of Okhotsk salmon did not differ significantly from the trace element content in fish muscles. However, in chum salmon that came to spawn in the Poima River, Cu concentration in liver was in sharp contrast to its value in muscles; this indicates the detoxification of its excess by liver. Moreover, there was a strong difference between Cu concentration in female gonads and male gonads in the Sea of Japan chum salmon, which was not observed in the Sea of Okhotsk fish. Since copper is both biophilic and biocidic trace element, much higher Cu content in female gonads is likely to indicate a stronger physiological control over the trace element concentration in tissues of females and excretion of its excess from the organism both with gonads and because of liver detoxification. When studying in detail the behavior of metals in commercial molluscs in the Peter the Great Bay (Khristoforova et al., 1994) and paying attention to species peculiarities, seasonal variability in the distribution of the trace elements in organs and tissues, and age differences in their content, we repeatedly observed sex differences in toxic metal concentrations (Cu, Pb, and Cd), registered the ability of molluscs to limit copper bioaccumulation, and noted, as well as other authors, that this ability is higher in females under conditions of increased Cu content in the environment. Excess of Zn (really biophilic trace element, unlike Cu) is also excreted from the organism and in the same ways, if taking into account its significant predominance in liver and female gonads.

Nickel predominance in fish liver was not revealed in the Kuril, Sakhalin, or even Primorye individuals. However, there was a clear excess of its concentration in gonads of the Sea of Japan fish, especially in females; this seems to result from gonad excretion into the environment (Khristoforova et al., 1994).

On the example of the Sea of Japan fish, it can be seen as follows: only a small amount of the trace element entering the organism is assimilated and redistributed in the organs and tissues; most of it is transported to the excretion system; as well as other authors, we have previously observed it when studying bivalves (Khristoforova et al., 1994).

As known, within the same species, older or larger individuals, weighing much more than younger ones, have higher trace element concentrations (Kelly et al., 2008 ; Khristoforova et al., 2019b). It seems likely that older age (5+) and, consequently, longer feeding period and larger mass of the Sea of Japan chum salmon than of the Sakhalin and Iturup fish result in higher trace element concentrations in the Sea of Japan chum salmon.

After a release, juveniles from the salmon hatcheries in the southeastern Sakhalin and Iturup Island of the Kuril Chain enter the coastal area of the southwestern Sea of Okhotsk; juveniles from the Primorye hatcheries enter the Peter the Great Bay of the Sea of Japan.

Quite recently, the Sea of Japan was not even considered as a feeding and wintering area for chum salmon. However, not all juveniles from the Sea of Japan basin go to the Sea of Okhotsk and the Pacific Ocean in the first year of life (Shuntov & Temnykh, 2011 ; Salo, 1991). A. I. Smirnov (1975) pointed out that the Primorye chum salmon and part of the Amur, Sakhalin, and Hokkaidō chum salmons winter and feed in the Sea of Japan, to the east and southeast of the Korean Peninsula. It was proved in the late 1990s that some chum salmons winter in the Sea of Japan (Semenchenko et al., 1997). Several authors believe that all underyearlings of the Primorye chum salmon winter in the Sea of Japan and only the next year partially leave it (Goryainov et al., 2006).

The role of the Sea of Japan as a feeding area for chum salmon, especially in the first – the marine one – year of life, is not known in detail. Unlike pink salmon and masu salmon, chum salmon have not been caught in winter and winter to spring periods within the Russian economic zone in the Sea of Japan (Atlas rasprostraneniya, 2002 ; Shuntov et al., 2004). It has not also been mentioned in the works on drifter salmon cruises to the central and southern Sea of Japan. There is no actual data on chum salmon specific wintering areas and conditions in the Sea of Japan. However, there is the point of view that some chum salmons, living side by side with pink salmons, winter in the Sea of Japan (Goryainov et al., 2007). It is evidenced by their joint occurrence in catches in wintering areas and on the routes of anadromous migrations confirmed by long-term studies. In winter, in the Sea of Japan, aggregations of pink salmon, and, consequently, chum salmon, are distributed from the Korea Bay to the Noto Peninsula, in two wintering areas. The first one is in the latitudinal direction from the Korea Bay to the island of Honshu; the second one is to the north – along the shores of the island of Hokkaidō. The second wintering area is characterized by lower temperatures; in terms of wintering conditions, it is like an ocean one. Analyzing the migration of the Primorye pink salmon in the Sea of Japan in winter, I. B. Birman (1986) noted as follows: during winter, “pink salmon, that came from the north to the waters of Korea, migrates eastward to the Noto Peninsula area” – at the confluence of the southern and northern branches of the Tsushima current. Undoubtedly, the currents supply fish with food. In April, fish migrates back to the west, and when reaching the Korea Bay, it comes to the north along the coast of Primorye. This seems to be associated with a water warming and increase in plankton abundance.

During the northern summer salmon migrations, chum salmon can be found in small quantities in the open waters of Primorye. It was established in a large series of TINRO summer trawl surveys (Atlas rasprostraneniya, 2002 ; Shuntov et al., 2004), as well as during drifter cruises in May and June in the 1990s and early 2000s (Dudarev et al., 2004 ; Semenchenko et al., 1997). These facts allow suggesting as follows: a small part of chum salmon at the age of 3+ and 4+ does not go to the ocean for feeding at all (Shuntov & Temnykh, 2011). Migrations of chum salmon of all ages, breeding in the western Sea of Japan, *inter alia* the Korean Peninsula, have not actually been traced (Shuntov & Temnykh, 2008).

The main feeding areas for chum salmon in Asian waters are the western Bering Sea, Sea of Okhotsk, and Kamchatka and Kuril waters of the northwestern Pacific Ocean (Shuntov & Temnykh, 2011). In early winter, juveniles of the Sakhalin and Kuril pink salmon and chum salmon, along with other juvenile schools, go to feed in a wide front through the northern Kuril straits to the open areas of the Pacific Ocean (N40° to N45°). For the first time in its life, salmon crosses the geochemically impact and at the same time high-feeding zone: the Kuril Chain and Kuril–Kamchatka Trench. For the second time, the pink salmon crosses the impact zone the next year, during anadromous migration, when going in the opposite direction, to the spawning areas (Khristoforova et al., 2019b). As for chum salmon, according to data of Shuntov and Temnykh (2011), during its marine life period (usually, 3–4 years), it can go several times along the Kuril Islands, with the trace element concentration in tissues increasing. Possessing a powerful growth potential, which is characteristic of all Pacific salmon, chum salmons significantly increase in length and body weight during the months spent in the sea.

Making extensive migrations, chum salmon goes after wintering to the Commander–Aleutian Trench, enters the deep-water western Bering Sea, and, after feeding in this area, goes along the Kamchatka, gradually moving to the polar front for wintering. Having completed at least three cycles of passage to the Bering Sea and back, it finally descends the feeding route along the Kamchatka, Kuril Islands, and Kuril–Kamchatka Trench; through the southern Kuril straits, the fish goes to the rivers on the Asian coast of the mainland, Sakhalin, and Kuril Islands (Shuntov & Temnykh, 2008, 2011).

On its way to feeding and back, to native rivers, the Primorye chum salmon goes through the Sea of Japan. The fish passes this sea twice in its life, following migratory routes to feeding areas and back – to spawn in the Primorye rivers. The Peter the Great Bay of the Sea of Japan and its bays, *inter alia* Amur and Posyet ones, where chum salmon juveniles migrate primarily, are characterized by high anthropogenic and terrigenous load on the environment and biota (Khristoforova et al., 1994 ; Kobzar & Khristoforova, 2015). Thus, different feeding conditions and migration routes of chum salmon of "Primorye" and "Sakhalin" or "Kuril" origin result in different trace element composition of its organs and tissues. Estimating heavy metal content in pink salmon and masu salmon in our previous studies (Khristoforova et al., 2015, 2018a, 2018b, 2019a, 2019b), we also noted that pink salmon from the Sea of Okhotsk and Sea of Japan had significant differences in the trace element concentration in organs and tissues. Specifically, the Sea of Japan pink salmon was characterized by Zn predominance; salmon from the Sakhalin-Kuril Region, which went to feed in the Pacific Ocean and twice crossed the geochemically impact and at the same time high-feeding zone (the Kuril Islands and Kuril–Kamchatka Trench), was characterized by Pb and Cd predominance.

Conclusions:

1. Kuril and East Sakhalin chum salmon, which winters in the ocean and feeds along the high-feeding zone, accumulates increased amounts of Pb and Cd in its organs and tissues. This results from volcanism and upwellings, which carry nutrients from the depths into the surface layer and cause rapid plankton development, as well as from geochemical impact of the region related to a high sorbability of these trace elements on nutrient suspension particles.
2. Chum salmon wintering and feeding in the enclosed Sea of Japan, which is poorly connected with the ocean and subject to terrigenous, anthropogenic, and technogenic load, are accompanied by Fe, Zn, Cu, and Ni accumulation in fish organs and tissues. The elements are tracers of this load and indicate intense shipping, household and industrial drains, and almost complete surrounding by land.
3. A predominant metal accumulation in liver, typical for fish of the areas studied (except for Ni, which is almost evenly distributed between organs and tissues of Kuril and East Sakhalin fish), and an increase in the element concentrations in female gonads, especially noticeable in the Sea of Japan chum salmon, where Ni clearly predominates, indicate the regulatory capabilities of salmon organisms and the entry (redistribution) of element excess into the excretion system.

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СРАВНЕНИЕ МИКРОЭЛЕМЕНТНОГО СОСТАВА КЕТЫ *ONCORHYNCHUS KETA* WALBAUM, 1792 ИЗ ЯПОНСКОГО И ОХОТСКОГО МОРЕЙ

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Определено содержание микроэлементов (железа, цинка, меди, никеля, свинца и кадмия) в наиболее востребованном на потребительском рынке виде тихоокеанских лососей — кете *Oncorhynchus keta*, являющейся в российских водах вторым по объёму вылова объектом (после горбуши *O. gorbuscha*). Концентрации металлов в органах и тканях рыб (мышечная ткань, печень, гонады) определены атомно-абсорбционным методом на спектрофотометре Shimadzu AA-6800 на пламенном и беспламенном атомизаторах из минерализатов проб, полученных при разложении навесок концентрированной HNO_3 марки ОСЧ в комплексе MARS 6, с использованием стандартных образцов с известными концентрациями. Статистическая обработка данных выполнена в программе SPSS Statistics 21. Половозрелые особи кеты (по 5 экз. самцов и самок в каждом месте сбора) отобраны осенью 2018 г. в садках-накопителях лососевых рыбоводных заводов на реках Фирсовка (юго-восток Сахалина, зал. Терпения) и Рейдовая (о-в Итуруп, Курильские острова), а также в октябре 2019 г. в садках для выдерживания производителей на р. Пойма (юго-запад Приморья, Японское море). От всех особей отдельно взяты пробы мышечной ткани, печени и гонад. Определяемые элементы чётко разделились по величинам концентраций на две группы в зависимости от места сбора проб: Cd и Pb преобладали в органах и тканях кеты из Сахалино-Курильского региона Охотского моря, а Fe, Zn, Cu и Ni — в япономорских рыбах. Причиной контрастного распределения тяжёлых металлов в органах и тканях рыб являются, очевидно, геохимические условия среды, формируемые в акваториях нагула и миграционных путей лососей. Если в практически замкнутом Японском море, слабо связанном с Тихим океаном малочисленными мелководными проливами, водная среда находится под сильным воздействием антропогенных, техногенных и терригенных факторов (хозяйственно-бытовых и промышленных стоков, поверхностных смывов с прибрежных территорий, сжигания судами углеводородного топлива), то в Сахалино-Курильском бассейне она испытывает влияние природных явлений — надводного и подводного вулканизма и поствулканизма Курильских островов, а также апвеллингов, выносящих из глубин Курило-Камчатской впадины в поверхностный слой практически весь набор химических элементов периодической системы Д. И. Менделеева. При этом Pb, обладающий высокой способностью к сорбции, задерживается на любых взвешенных частицах (как живых, так и неживых) и поступает в организмы рыб с пищей. Распределение микроэлементов по органам и тканям кеты существенно различается: в мышечной ткани зарегистрированы наименьшие концентрации металлов независимо от мéста вылова, а печень характеризуется повышенными уровнями всех, кроме Ni, металлов, причём в печени япономорских рыб определены самые высокие концентрации Fe, Zn и Cu. Для распределения металлов в гонадах рыб характерна своя специфика, особенно ярко проявившаяся в приморских лососях. Так, в гонадах самок преобладали как Fe, так и Zn, но особенно Cu: концентрация меди в гонадах самок кеты из р. Пойма была такой же, как в печени.

Гонады самок япономорской кеты имели наибольшие концентрации Ni, хотя гонады самцов тоже отличались высоким, пусть и очень изменчивым, содержанием этого элемента. Что касается Pb и Cd, наиболее токсичных и контролируемых санитарными службами элементов (их количество по техническим причинам нам удалось определить только в охотоморских рыбах), то в их распределении по органам и тканям отмечена хорошо известная картина: максимальная концентрация выявлена в печени рыб. При этом содержание Pb превосходило санитарную норму лишь в отдельных особях, пришедших на нерест в р. Фирсовка на юго-востоке Сахалина; по Cd превышение ПДК в печени всех сахалинских рыб составляло 2,5–4,0 раза, а для всех курильских рыб — 2,1–5,0 раз.

Ключевые слова: тихоокеанские лососи, кета, Сахалино-Курильский регион, Охотское море, Приморский край, Японское море, микроэлементы

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NOTES

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CARBOHYDRATES AS AN ORGANIC SUBSTRATE FOR MICROALGAE *TISOCHRYSIS LUTEA* (HAPTOPHYTA) UNDER CONDITIONS OF LABORATORY CULTURE

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The possibility was studied of using carbohydrates in order to optimize the cultivation process of microalgae *Tisochrysis lutea* (Haptophyta). The effect of D-galactose, glucose, and sucrose at concentrations of 100 and 200 mg·L^{-1} on the dynamics of *T. lutea* abundance was analyzed. As found, adding of all the studied carbohydrates stimulated microalgae growth, with sucrose at a concentration of 200 mg·L^{-1} having the most pronounced effect.

Keywords: *Tisochrysis lutea*, cell abundance, carbohydrates, aquaculture

Microalgae are a food source for various groups of hydrobionts. Among marine microalgae, *Tisochrysis lutea* is of great interest as a food object for invertebrates: mussels, oysters, scallops, and sea cucumbers. Moreover, it is used to feed larvae of salmon, flounder, and representatives of other fish families. Controlling the growth parameters of algae cultures (enriching media composition, introducing nutrient substrates, *etc.*) results in growth acceleration (Bigagli et al., 2018). Currently, one of the objectives of cultivation is to obtain high abundance of microalgae in a short time.

The aim of this work is to assess the effect of carbohydrates on the growth of marine microalgae *Tisochrysis lutea*.

MATERIAL AND METHODS

The study object was the culture of unicellular algae *T. lutea* (Haptophyta) – MBRU_Tiso-08 strain from the Marine Biobank resource collection at NSCMB FEB RAS (Efimova et al., 2016). *T. lutea* was cultivated on the nutrient medium *f*, with D-galactose, glucose, and sucrose added at concentrations of 100 and 200 mg·L^{-1}, at a temperature of +20 °C, a light intensity of 50 µmol·m^{-2}·s^{-1}, in a visible light area, and a light/dark cycle 12 h : 12 h. Microalgae were grown in 250-mL Erlenmeyer flasks with 100 mL of nutrient medium, under conditions of a periodic cultivation mode. As a control, an alga grown on the medium *f* with no carbohydrates added was used. The experiments lasted 21 days. *T. lutea* abundance was counted under a microscope in a Goryaev chamber. Algae biomass (wet weight) was calculated by the formula from (Levich et al., 1997). The experiments were carried out in three biological replicates. Mean values and standard deviations were calculated. The data was statistically processed in Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Up to the 18th day, D-galactose adding at both concentrations stimulated *T. lutea* growth and biomass increase. On the 21st day, these values were lower than the control ones at 100 mg·L^{-1} of D-galactose (Table 1).

Table 1. Effect of carbohydrates on *Tisochrysis lutea* abundance and biomass

Day	Control	D-galactose		Glucose		Sucrose	
		100 mg·L^{-1}	200 mg·L^{-1}	100 mg·L^{-1}	200 mg·L^{-1}	100 mg·L^{-1}	200 mg·L^{-1}
Cell abundance, × 10^4 *per* mL							
0	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2	21 ± 0.2
2	24.5 ± 0.1	29 ± 0.3	23.8 ± 0.1	21.2 ± 0.2	21.7 ± 0.4	23.7 ± 0.4	32.3 ± 0.9
4	26.5 ± 0.3	34.5 ± 0.5	29.8 ± 0.4	31.7 ± 0.5	25.5 ± 0.5	35.0 ± 0.7	31.2 ± 1.2
7	33.8 ± 0.3	52.8 ± 1.0	51.0 ± 1.5	36.7 ± 0.7	59.2 ± 0.8	49.4 ± 0.5	47.0 ± 0.9
9	64.5 ± 0.6	84.9 ± 0.8	93.2 ± 0.9	74.2 ± 0.9	74.7 ± 1.0	70.4 ± 0.7	69.9 ± 1.2
11	102.8 ± 0.7	121.0 ± 1.2	121.0 ± 1.3	96.8 ± 0.3	97.5 ± 0.9	246.6 ± 1.0	294.6 ± 1.0
14	122.0 ± 0.4	134.6 ± 1.6	149.8 ± 1.4	142.0 ± 1.3	168.5 ± 1.2	157.2 ± 0.9	165.9 ± 0.9
16	167.0 ± 0.9	176.2 ± 0.9	178.1 ± 0.9	191.2 ± 0.8	194.5 ± 0.8	219.7 ± 1.5	219.0 ± 0.7
18	479.8 ± 1.2	634.3 ± 1.3	585.4 ± 1.7	424.8 ± 1.4	434.2 ± 1.3	745.3 ± 1.8	792.8 ± 1.6
21	587.2 ± 1.4	509.0 ± 1.7	581.7 ± 1.0	607.8 ± 1.0	574.5 ± 1.5	750.3 ± 0.9	833.6 ± 0.5
Biomass, mg·L^{-1}							
0	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.3
2	0.7 ± 0.2	0.9 ± 0.4	0.7 ± 0.2	0.6 ± 0.5	0.7 ± 0.2	0.7 ± 0.2	0.9 ± 0.4
4	0.8 ± 0.4	1.0 ± 0.6	0.9 ± 0.5	1.0 ± 0.6	0.8 ± 0.4	1.0 ± 0.4	1.0 ± 0.6
7	1.0 ± 0.9	1.6 ± 0.4	1.6 ± 0.7	1.1 ± 0.5	1.8 ± 0.8	1.5 ± 0.5	1.4 ± 0.8
9	1.9 ± 0.3	1.5 ± 1.2	1.6 ± 0.9	2.2 ± 0.8	2.2 ± 0.4	2.1 ± 1.2	2.1 ± 0.8
11	3.1 ± 1.1	3.7 ± 0.7	3.7 ± 1.1	2.9 ± 1.1	3.0 ± 1.2	7.5 ± 1.1	8.9 ± 1.3
14	3.7 ± 1.1	4.0 ± 1.2	4.4 ± 1.5	4.3 ± 0.9	5.1 ± 1.0	4.8 ± 0.8	5.0 ± 1.5
16	5.0 ± 0.9	5.4 ± 0.9	5.4 ± 0.9	5.8 ± 1.3	5.9 ± 0.7	6.7 ± 0.9	6.7 ± 1.2
18	14.5 ± 1.8	19.2 ± 1.3	17.8 ± 1.9	12.9 ± 1.7	13.1 ± 1.6	22.7 ± 1.9	24.1 ± 2.0
21	17.8 ± 1.6	15.5 ± 1.8	17.7 ± 1.3	18.5 ± 1.8	17.4 ± 1.3	22.8 ± 2.2	25.3 ± 1.9

Note: mean values and standard deviations are given.

With glucose adding in both concentrations, an increase in *T. lutea* abundance and biomass was registered on the 7th–16th days of the experiment (see Table 1). Sucrose stimulated algae growth, especially from the 11th day (see Table 1). The most pronounced effect was recorded at 200 mg·L^{-1}. As shown in the literature, microalgae growth intensifies with organic substrates adding (*inter alia* carbohydrates): in *Chlamydomonas reinhardtii* and *Haematococcus pluvialis* (Jeon et al., 2006), in *Chlorella sorokiniana* and *Chlorella* sp. (Heredia-Arroyo et al., 2010), and in *Nannochloropsis* sp. (Hu & Gao, 2003).

Conclusion. D-galactose, glucose, and especially sucrose adding results in an increase in *T. lutea* abundance and biomass. The effect of carbohydrates on microalgae growth is dose-dependent. Hence, carbohydrates adding is a promising method of *T. lutea* cultivation.

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УГЛЕВОДЫ КАК ОРГАНИЧЕСКИЙ СУБСТРАТ ДЛЯ МИКРОВОДОРОСЛИ *TISOCHRYSIS LUTEA* (НАРТОРНУТА) В УСЛОВИЯХ ЛАБОРАТОРНОЙ КУЛЬТУРЫ

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Исследована возможность применения углеводов для оптимизации процесса культивирования микроводоросли *Tisochrysis lutea* (Haptophyta). Проанализировано влияние D-галактозы, глюкозы и сахарозы в концентрациях 100 и 200 $мг·л^{-1}$ на динамику численности популяции *T. lutea*. Установлено, что добавление всех изученных углеводов стимулировало рост микроводоросли, однако наибольший эффект оказала сахароза в концентрации 200 $мг·л^{-1}$.

Ключевые слова: *Tisochrysis lutea*, численность клеток, углеводы, аквакультура

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CHRONICLE AND INFORMATION

“STUDY OF AQUATIC AND TERRESTRIAL ECOSYSTEMS: HISTORY AND CONTEMPORARY STATE”. INTERNATIONAL SCIENTIFIC CONFERENCE DEDICATED TO THE 150th ANNIVERSARY OF THE SEVASTOPOL BIOLOGICAL STATION – A. O. KOVALEVSKY INSTITUTE OF BIOLOGY OF THE SOUTHERN SEAS AND TO THE 45th ANNIVERSARY OF THE RESEARCH VESSEL “PROFESSOR VODYANITSKY”

The international scientific conference was held 13 to 18 September, 2021, on the basis of the Federal Research Center “A. O. Kovalevsky Institute of Biology of Southern Seas of RAS” (IBSS). The conference “Study of Aquatic and Terrestrial Ecosystems: History and Contemporary State” was dedicated to the 150th anniversary of the Sevastopol Biological Station – IBSS and the 45th anniversary of the RV “Professor Vodyanitsky”. It was organized by IBSS and supported by the Ministry of Science and Higher Education of the Russian Federation, the Government of Sevastopol, the Head Production and Technical Enterprise “GRANIT”, the Russian Hydrobiological Society of RAS, the Sevastopol City Branch of the Russian Geographical Society, the Parasitological Society of RAS, and the Sevastopol State University.

More than 370 researchers took part in the conference representing 95 scientific institutions, educational institutions, and departments of Russia, Belarus, Abkhazia, Romania, Spain, Japan, Vietnam, USA, Mexico, Bulgaria, and Switzerland.

At the opening plenary session, eight reports were presented:

1. “Evolution of research of IBSS radiation and chemical biology department on the problem of the interaction of biotic and abiotic matter with radioactive and chemical components of the marine environment” (V. N. Egorov, D. Sc., Academician of RAS, IBSS).
2. “Genetic signatures of historic translocation in contemporary white-tailed deer populations” (Michael Douglas, D. Sc., Fayetteville, USA).
3. “Prospects for the mariculture developing in the Sea of Azov – Black Sea basin” (E. A. Kozhurin, the head of the Sea of Azov – Black Sea branch of the VNIRO).

4. “Aquatic ecotoxicology in solving the problems of assessing the water quality and “health” of ecosystems” (T. I. Moiseenko, D. Sc., Corresponding Member of RAS, V. I. Vernadsky Institute of Geochemistry and Analytical Chemistry of RAS).
5. “Paradoxes of anamniotes’ bioenergetics” (A. A. Soldatov, D. Sc., Prof., IBSS).
6. “Marine aspects of the carbon polygon in the Kaliningrad region” (V. V. Sivkov, PhD, M. O. Ulyanova, PhD, Immanuel Kant Baltic Federal University).
7. “The history of the development of the scientific direction “marine biotechnologies” in IBSS” (V. I. Ryabushko, D. Sc., V. E. Erokhin, PhD, IBSS).
8. “The research vessel “Professor Vodyanitsky”: 45 years of worthy service (anniversary retrospective at the turn of the century)” (O. A. Rylkova, PhD, N. A. Gavrilova, PhD, S. M. Ignatyev, IBSS).

The work of the conference was continued in the following sections:

- “Biodiversity of aquatic and terrestrial ecosystems and its conservation”.
- “Structure, functioning, and dynamics of aquatic and terrestrial ecosystems”.
- “Ecology, physiology, behavior, and biochemistry of hydrobionts”.
- “Molecular biology and genetics of hydrobionts”.
- “Problems of aquatic ecosystem pollution and marine radiochemoecology”.
- “Biological resources, biotechnology, and aquaculture”.
- “Sustainable development of coastal areas”.
- “Methane, its genesis, and participation in biological and geological processes”.

The results were widely presented of fundamental and applied scientific research in biodiversity and functioning of aquatic and terrestrial ecosystems. The prospects were discussed for the practical application of these results for the development of innovative technologies. The problems were highlighted of environmental protection and rational nature management. The aspects were emphasized of the reproduction of biological resources and aquaculture. In total, 220 oral, 70 virtual, and 70 poster reports were presented.

The work of the conference was accompanied by an exhibition of IBSS scientific literature and innovative developments.

Three roundtable discussions were held within the framework of the conference.

At the roundtable discussion “Ways of coordinating the activities of libraries of Russian marine research centers” (moderated by O. A. Akimova, IBSS), issues were highlighted on the establishment of the Association of Scientific Libraries and Information Centers in the field of marine and aquatic sciences in Russia and its further activities.

At the roundtable discussion “Floating universities: opportunities and prospects in the Russian Federation” (moderated by N. E. Ryazanova, PhD, MGIMO University), the best practices were emphasized for the implementation of domestic and international projects, as well as issues of grant and public-private support, regulatory aspects, and problems and prospects for interdepartmental cooperation in planning and carrying out relevant projects.

At the roundtable discussion “Towards Blue Growth. Prospects for the development of the Blue Economy, research, and innovations in the Black Sea region” (moderated by T. I. Andreenko, PhD, IBSS, A. D. Lappo, MIREA – Russian Technological University), the prospects were discussed for international cooperation in the field of the Blue Economy and for the Blue Growth development in the Black Sea region.

Two seminars were held as well: “Management of the state of aquatic ecosystems: theory and practice” (the speaker was A. G. Degermendzhi, D. Sc., Academician of RAS, Institute of Biophysics of SB RAS) and “General ecological problems in the context of ecological biophysics” (the speaker was S. I. Bartsev, D. Sc., Institute of Biophysics of SB RAS).

High level of the works, interesting oral and poster presentations, and lively discussion of the key scientific issues contributed to creating favorable atmosphere, exchanging views and information, broadening horizons and scientific contacts, and deepening understanding of problems and prospects for studying aquatic and terrestrial ecosystems.

The research results presented at the conference are published in the book of abstracts. It is uploaded in the IBSS Open Access Repository (https://doi.org/10.21072/978-5-6044865-5-9) and in the national bibliographic database Russian Science Citation Index (https://elibrary.ru/item.asp?id=46714524).

«ИЗУЧЕНИЕ ВОДНЫХ И НАЗЕМНЫХ ЭКОСИСТЕМ: ИСТОРИЯ И СОВРЕМЕННОСТЬ». МЕЖДУНАРОДНАЯ НАУЧНАЯ КОНФЕРЕНЦИЯ, ПОСВЯЩЁННАЯ 150-ЛЕТИЮ СЕВАСТОПОЛЬСКОЙ БИОЛОГИЧЕСКОЙ СТАНЦИИ — ИНСТИТУТА БИОЛОГИИ ЮЖНЫХ МОРЕЙ ИМЕНИ А. О. КОВАЛЕВСКОГО И 45-ЛЕТИЮ НИС «ПРОФЕССОР ВОДЯНИЦКИЙ»

Международная научная конференция, посвящённая 150-летию Севастопольской биологической станции — Института биологии южных морей имени А. О. Ковалевского и 45-летию НИС «Профессор Водяницкий», прошла с 13 по 18 сентября 2021 г. В работе конференции приняли участие более 370 исследователей, представлявших 95 научных, образовательных и ведомственных учреждений. Представлено 220 очных, 70 дистанционных и 70 постерных докладов.

Participants of the conference “Study of Aquatic and Terrestrial Ecosystems: History and Contemporary State” (2021)

1871
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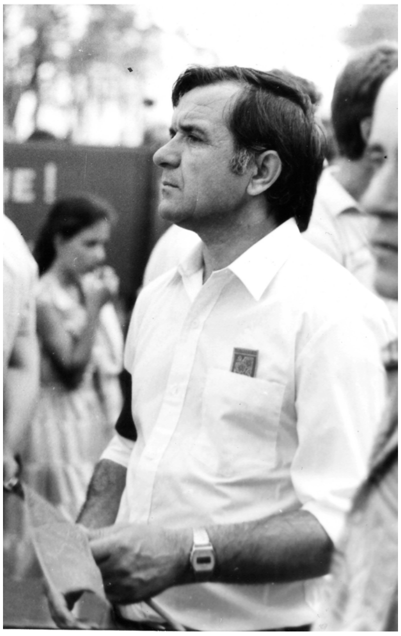
IN MEMORIAM: ZOSIM FINENKO
(17.11.1938 – 15.11.2021)

On 15 November, 2021, after a serious and prolonged illness, Zosim Finenko passed away, a well-known hydrobiologist, D. Sc., Professor, laureate of the State Prize of Ukraine, and chief researcher at A. O. Kovalevsky Institute of Biology of the Southern Seas.

Z. Finenko was born in Berdyansk, Zaporizhzhya region, on 17 November, 1938. In 1961, he successfully graduated from the Belarusian State University specializing in biology and zoology; later, he entered PhD graduate school there and studied under the guidance of G. Vinberg, the classic of Russian hydrobiology. After graduating from the university (1961), Zosim Finenko began his career at IBSS. He became one of the first Russian hydrobiologists, who applied the radiocarbon method for measuring primary production in the Black Sea. During these years, he participated in many expeditions to the Black Sea and Sea of Azov. Together with L. Sushchenya, under the EQUALANT international program, he took part in the research cruise of the RV "Mikhail Lomonosov" to the Tropical Atlantic. Based on the results of those studies, he defended his PhD thesis in hydrobiology "Primary production of the Black Sea, Sea of Azov, and the Tropical Atlantic" (1966). The subsequent years were very productive. Specifically, participation in expeditions of the Institute of Oceanology to the Pacific Ocean broadened his horizons and brought new ideas and impressions. Moreover, he met interesting people, and many of them remained his friends forever. During this period, in addition to field research, he was involved in experimental work. Its material formed the basis for his D. Sc. dissertation in hydrobiology "Ecological and physiological foundations of primary production at the sea" (1977). In 1978, he was awarded the academic title of senior researcher; in 2000, the title of professor.

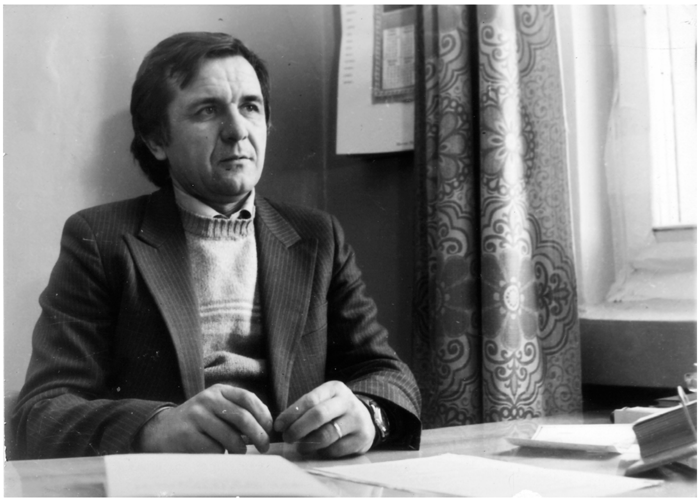
In 1980, on the initiative of Z. Finenko, algae ecological physiology department was created at IBSS of the Academy of Sciences of the Ukrainian SSR. Until 2020, he headed this department. In 1980–1988, being deputy director for scientific work, he contributed much in development of IBSS activity programs and in planning numerous scientific expeditions to various areas of the World Ocean. With his direct leadership and participation, several research cruises were carried out to the Black and Mediterranean seas, as well as to the Atlantic, Pacific, and Indian oceans. Many of our colleagues shared with him all the joys and difficulties of sea expeditions, in which he was always a key member of the team – knowledgeable, aimed at discovering something new, interested in scientific results of other employees, and a cheerful companion with a subtle sense of humor. In different years, Zosim Finenko worked at leading research institutes in France, Italy, and the UK on EU and NASA grants.

For many years, he was a member of the IBSS scientific council, a member of the specialized council for the defense of PhD thesis and D. Sc. dissertations in hydrobiology, and a member of the editorial boards of Marine Ecological Journal and Marine Biological Journal. In recent years, he headed the subsection of the Presidium of the Russian Academy of Sciences project “Combined effect of environmental factors on the phytoplankton community development”. In 2016–2018, he headed the Russian Foundation for Basic Research project “Alternative approach to assessing biomass and growth rate of phytoplankton in the Black Sea using satellite data”.



Z. Finenko was a well-known specialist in the field of microalgae physiology, production processes in seas and oceans, primary production modeling, and theory of the functioning of phytoplankton community and aquatic ecosystems. He carried out fundamental research on algae physiology and phytoplankton productivity, as well as on modeling production processes and photosynthesis production with satellite data applying. He theoretically substantiated and experimentally confirmed the quantitative regularities of the relationship between biosynthesis rate and organic matter destruction in algae and their size and weight. Due to in-depth analysis of these data, theoretical concepts were significantly developed concerning both the physiological mechanisms of algae adaptation to environmental conditions and relationship between microalgae growth rate and intensity of photosynthesis and respiration, as well as intracellular content of organic carbon and pigments. He developed a semi-analytical model describing the combined effect of light, temperature, and nutrients on variability in growth rate and pigment content in algae of various taxonomic groups. General quantitative regularities of chlorophyll *a* vertical distribution in the southern seas were substantiated and confirmed on extensive material. For the first time, convincing evidence was presented that the rate of nutrient flow from deeper layers to the surface and adaptation of phytoplankton community to light are the main factors regulating the shape of chlorophyll vertical profile and its relative content in phytoplankton. The results of Zosim Finenko’s fundamental research served as the basis for the development and substantiation of modern methods for determining primary production using satellite measurements of chlorophyll *a* concentration and solar radiation. This allowed to evaluate production in real time, on scales from several tens to thousand kilometers. On this basis, new data were obtained on global phytoplankton productivity in the vast water areas of the Black Sea and Atlantic Ocean, its dynamics, and relation with climatic changes. Those could not be obtained earlier, applying other methods.

Until the last days of his life, Z. Finenko worked with enthusiasm on the problem of the spatiotemporal variability of phytoplankton biomass and primary production in the Black Sea. He endeavored to find out the main factors and processes determining the trends in the variability of primary production indicators at the current stage of the Black Sea ecosystem development. His extensive scientific heritage is an invaluable contribution to the Russian science. His activity is reflected in more than 150 articles and 5 collective monographs.



Zosim Finenko was a talented teacher and a man of encyclopedic knowledge. He shared his skills and knowledge with numerous students and created his own scientific school. Among his students, there is 1 D. Sc. and 13 PhDs.

Z. Finenko left a noticeable mark in the history of Russian hydrobiology and in the hearts of everyone he worked with and was friends with. The bright memory of him will always live in our hearts.

Students, colleagues, and friends

ПАМЯТИ ЗОСИМА ЗОСИМОВИЧА ФИНЕНКО (17.11.1938 – 15.11.2021)

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