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ZOOPLANKTON PRODUCTIVITY IN THE COASTAL AREA OF THE SOUTHERN BARENTS SEA IN SPRING

© 2020 **V. G. Dvoretsky and A. G. Dvoretsky**

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The results of the analysis of zooplankton assemblage state of the southern Barents Sea are presented. Zooplankton samples were collected during the cruise of the RV “Dalnie Zelentsy” in May 2016. Hydrological conditions were typical for Murmansk coastal water this season. A total of 47 zooplankton taxa were identified. Taxa number varied between stations, ranging 18–29, with copepods being a dominant group in zooplankton. The most frequent ones were *Calanus finmarchicus*, *Metridia longa*, *Metridia lucens*, *Microcalanus* spp., *Oithona atlantica*, *Oithona similis*, *Pseudocalanus* spp., copepod nauplii and ova, as well as cladoceran *Evadne nordmanni*, larvae of Echinodermata and Polychaeta, chaetognath *Parasagitta elegans*, and early stages of the euphausiids of the genus *Thysanoessa*. In populations of common copepod species *Pseudocalanus* spp. and *Oithona similis*, early age stages dominated, which indicated their continued reproduction. Total zooplankton abundance ranged from 748 to 6576 ind.·m^{-3}, averaging 3012. Total zooplankton biomass varied from 17 to 157 mg of dry mass per m^3, with a mean value of 83. The data obtained were comparable to those registered in Murmansk coastal water in July 2008 and were higher than those in August 2007. The authors suggest that it might be related to the differences in sampling seasons and hydrological conditions. Daily zooplankton production was estimated to be 0.49–4.04 mg of dry mass per m^3, averaging (2.17 ± 0.17). These estimates were about twice as high as mean values, registered in Murmansk coastal water during summer period. This seems to be due to higher phytoplankton concentrations in spring. Total zooplankton stock for water area studied (25.8 thousand km^2) was estimated to be 425,000 thousand tons of dry mass. Cluster analysis revealed four groups of stations that differ in relative abundance of *Calanus finmarchicus*, Copepoda nauplii, *Oithona similis*, larvae of Echinodermata, and appendicularian *Fritillaria borealis*. Spatial variation of zooplankton abundance was closely related to station location (latitude, longitude, and sampling depth), as well as bottom layer temperature and mean salinity at the station.

Keywords: zooplankton assemblage, copepods, pelagic ecosystem

Zooplankton is a key component of pelagic ecosystems, providing energy transfer from primary producers to higher trophic levels (Kiselev, 1980). It is an important part of commercial fish diet in the northern seas. The southern Barents Sea is characterized by high biological productivity (Zelikman & Kamshilov, 1960 ; Zenkevich, 1963). In its coastal zone, spawning and feeding areas of many fish species are located (Dalpadado & Mowbray, 2013 ; Orlova et al., 2011). Data on zooplankton composition and quantitative characteristics make it possible to assess the availability of food resources for commercial hydrobionts (Dvoretsky & Dvoretsky, 2015 ; Orlova et al., 2004 ; Raymont, 1983).

The most important phase of plankton succession cycle in the Arctic seas is spring (Timofeev, 2000 ; Orlova et al., 2011): in this period, primary production reaches its maximum values. Its significant part is utilized by zooplankton and subsequently used on its growth and development (Orlova et al., 2004 ; Plankton morei…, 1997). Phytoplankton abundance in spring largely determines zooplankton stock and its production and, ultimately, total amount of food available for fish (Kiselev, 1980 ; Orlova et al., 2004 ; Raymont, 1983).

The aim of this work is to study zooplankton assemblage structure, abundance, biomass, and production characteristics in the coastal area of the southern Barents Sea in spring.

MATERIAL AND METHODS

The study was carried out in May 2016 during a cruise of the RV “Dalnie Zelentsy” (Fig. 1, Table 1). Murmansk coastal water area was considered a coastal zone. Data on temperature and salinity were obtained using a SBE 19plus V2 SeaCAT profiler. Information on chlorophyll *a* concentration was taken from (Chislenko, 1968).

During the study period, a total of 25 zooplankton samples were collected with a Juday net (mouth diameter of 37 cm; mesh size of 180 μm). The samples were fixed with 4 % formalin. The scheme of sampling stations is shown in Fig. 1. Material processing was carried out in a coastal laboratory by standard methods (Instruktsiya po sboru…, 1971 ; ICES Zooplankton…, 2000). Zooplankton representatives were identified up to species level, if possible. From each sample, three subsamples with a volume of 5–10 mL were taken; the results of their quantitative analysis were averaged and calculated in $\text{ind.}\cdot\text{m}^{-3}$ and $\text{ind.}\cdot\text{m}^{-2}$. Zooplankton biomass was calculated using nomograms, tables of individual standard weight of marine hydrobionts, and length-weight equations (Chislenko, 1968 ; Richter, 1994). All values were converted to dry mass in accordance with transition coefficients for main systematic groups (Dvoretsky & Dvoretsky, 2015 ; ICES Zooplankton…, 2000). Daily zooplankton production was calculated by the method (Dvoretsky, 2012 ; Dvoretsky & Dvoretsky, 2015, 2018), based on mean water temperature, biomass, and potential growth rate of hydrobionts. For hydrobionts, individual dry/carbon mass was calculated, as well as egg production rate / growth rate. Their values were taken from sources published [complete list is given in (Dvoretsky, 2012 ; Dvoretsky & Dvoretsky, 2015, 2018)].

The data obtained were processed by the methods of variation statistics, with mean values and standard errors being determined. The analysis of zooplankton assemblage structure was based on the calculation of Bray – Curtis indices. To establish the relationship between zooplankton abundance and environmental factors, BIO-ENV routine of Primer 5.0 software package was used. Calculations of zooplankton stock and integrated daily production for water area studied were performed using a GIS CardMaster (VNIRO, Moscow) (Bizikov et al., 2007).

RESULTS AND DISCUSSION

Mean water temperature in the sampling layer ranged from +4.1 to +5.4 °C, and salinity – from 34.15 to 34.66 ‰. The estimates obtained were typical for Murmansk coastal water (Ozhigin & Ivshin, 1999). According to (Vodopyanova et al., 2017), chlorophyll *a* concentration varied in a photic layer from 0.11 to 0.91 $\text{mg}\cdot\text{m}^{-3}$; at a depth of 75 m (station 4), a local maximum (1.26 $\text{mg}\cdot\text{m}^{-3}$) was recorded; in bottom layers, pigment content was 0.15–0.30 $\text{mg}\cdot\text{m}^{-3}$ (Vodopyanova et al., 2017). Similar mosaic distribution of phytopigments was observed in previous years (Plankton morei…, 1997), being typical for Kola Peninsula coastal water (Plankton morei…, 1997).

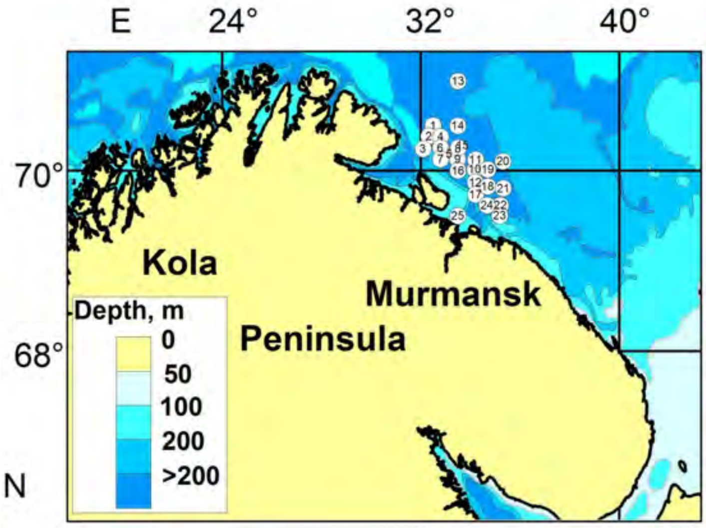

Fig. 1. Scheme of zooplankton sampling stations in the southern Barents Sea in May 2016

Table 1. Characteristics of zooplankton sampling stations in the southern Barents Sea

Station	Date	Sampling time (UTC+3:00)	Depth, m	Sampling layer, m	Coordinates	
					N	E
1	22.05.2016	23:58	284	270–0	70°30′	32°31′
2	23.05.2016	20:35	181	170–0	70°23′	32°20′
3	24.05.2016	12:37	300	280–0	70°15′	32°06′
4	24.05.2016	17:30	261	250–0	70°23′	32°49′
5	24.05.2016	23:43	192	180–0	70°11′	33°09′
6	25.05.2016	03:52	169	160–0	70°15′	32°48′
7	25.05.2016	10:05	137	130–0	70°08′	32°48′
8	25.05.2016	19:00	247	240–0	70°15′	33°30′
9	26.05.2016	01:07	238	230–0	70°08′	33°30′
10	26.05.2016	04:52	214	210–0	70°01′	34°11′
11	26.05.2016	09:25	249	240–0	70°07′	34°13′
12	27.05.2016	00:10	208	200–0	69°52′	34°12′
13	27.05.2016	12:16	219	210–0	70°60′	33°31′
14	27.05.2016	16:35	247	240–0	70°30′	33°30′
15	27.05.2016	19:25	246	240–0	70°15′	33°31′
16	27.05.2016	21:54	147	140–0	70°00′	33°31′
17	28.05.2016	00:51	180	170–0	69°44′	34°13′
18	28.05.2016	04:04	213	200–0	69°50′	34°42′
19	28.05.2016	06:45	244	240–0	70°01′	34°42′
20	28.05.2016	09:37	215	200–0	70°06′	35°19′
21	28.05.2016	20:05	233	220–0	69°48′	35°20′
22	28.05.2016	23:00	187	180–0	69°37′	35°13′
23	29.05.2016	01:43	176	160–0	69°30′	35°11′
24	29.05.2016	05:35	167	160–0	69°37′	34°41′
25	29.05.2016	14:35	263	250–0	69°30′	33°30′

A total of 47 zooplankton taxa were found in samples (Table 2). The most common (> 80 % of the samples studied) were *Calanus finmarchicus*, copepod nauplii and ova, *Metridia longa*, *Metridia lucens*, *Microcalanus* spp., *Oithona atlantica*, *Oithona similis*, *Pseudocalanus* spp., *Evadne nordmanni*, larvae of Echinodermata and Polychaeta, *Parasagitta elegans*, and early stages of *Thysanoessa* spp. Taxa number at the stations varied from 18 to 29. The maximum was recorded at st. 13, and the minimum – at st. 20. The southern Barents Sea is the richest area in plankton species composition: more than 280 animal taxa have been found here (Dvoretsky & Dvoretsky, 2015, 2010). In spring, species number increases due to emerge of meroplankton, which consists mainly of larvae of benthic invertebrates (Plankton morei…, 1997 ; Timofeev, 2000 ; Orlova et al., 2011); that was confirmed by our study.

Table 2. List of zooplankton taxa, their frequency in samples, and mean abundance in the southern Barents Sea in May 2016

Taxon	Frequency in samples, %	Mean abundance, ind.·m^{-3}
Acartia longiremis	64	1
Anomalocera patersoni	4	< 1
Calanus finmarchicus	100	1743
Calanus hyperboreus	36	< 1
Centropages hamatus	32	< 1
Copepoda ova	100	12
Copepoda nauplii	100	400
Metridia longa	96	36
Metridia lucens	100	16
Microcalanus pusillus	100	8
Microcalanus pygmaeus	100	98
Oithona atlantica	100	10
Oithona similis	100	308
Triconia borealis	16	< 1
Paraeuchaeta spp. I–IV	20	< 1
Paraeuhaeta norvegica V–VI	12	< 1
Pseudocalanus spp. I–IV	100	26
Pseudocalanus minutus V–VI	100	10
Pseudocalanus acuspes V–VI	96	< 1
Temora longicornis	40	< 1
Evadne nordmanni	100	6
Podon leuckartii	32	< 1
Aglantha digitale	48	< 1
Rathkea octopunctata	8	< 1
Bivalvia juv.	56	7

Continue on the next page…

Taxon	Frequency in samples, %	Mean abundance, ind.·m^{-3}
Cirripedia nauplii	8	< 1
Echinoidea (echinopluteus larvae)	100	126
Gastropoda larvae	72	3
Ophiuroidea (ophiopluteus larvae)	100	36
Polychaeta larvae	88	11
Chionoecetes opilio zoea + megalopa	12	< 1
Hyas spp. zoea	4	< 1
Paralithodes camtschaticus zoea	4	< 1
Pandalus borealis larvae	8	< 1
Boroecia borealis	68	< 1
Pisces larvae	64	< 1
Limacina helicina larvae	4	< 1
Parasagitta elegans	88	< 1
Meganyctyphanes norvegica	8	< 1
Thysanoessa inermis	4	< 1
Thysanoessa raschii	4	< 1
Thysanoessa spp. calyptopis	100	49
Thysanoessa spp. nauplii	96	23
Thysanoessa spp. furcilii	100	24
Fritillaria borealis	52	43
Oikopleura labradoriensis	40	13
Nemertini pilidium larvae	4	< 1

Total zooplankton abundance varied over a wide range 748–6576 ind.·m^{-3}, averaging (3012 ± 255). Zooplankton abundance in coastal water depends largely on external factors: circulation system, presence of frontal zones, and freshwater runoff (Dvoretsky & Dvoretsky, 2015 ; Timofeev, 2000). The highest variability in zooplankton abundance is characteristic of the southeastern area of the sea and some bays (Zelikman, 1977 ; Zenkevich, 1963 ; Plankton morei…, 1997), having favorable conditions for forming plankton aggregations.

Total zooplankton biomass varied from 17 to 157 mg of dry mass per m^3; mean value was (83 ± 7). The data were comparable to those, obtained in the southern Barents Sea in July 2008 (Dvoretsky & Dvoretsky, 2015), and exceeded estimates, recorded in Kola Bay area in August 2007 (Dvoretsky & Dvoretsky, 2012), which, in turn, determine the phase of seasonal development of zooplankton assemblage (Zelikman, 1977 ; Orlova et al., 2011 ; Raymont, 1983).

Zooplankton production varied from 0.49 to 4.04 mg of dry mass per m^3 per day, with a mean value of (2.17 ± 0.17). The values obtained were approximately twice as high as those recorded for Murmansk coastal water (2008–2013) during summer period: 0.64–1.25 mg of dry mass per m^3 per day (Dvoretsky & Dvoretsky, 2015, 2016, 2018). This is probably due to food abundance for zooplankton in May. It is known that an increase in phytoplankton abundance is observed in spring and usually followed by a peak in zooplankton biomass (Zenkevich, 1963 ; Kiselev, 1980 ; Timofeev, 2000). In summer, microalgae concentration is significantly lower; so, the level of zooplankton production decreases (Plankton morei…, 1997). The waters at the junction of frontal zones are considered to be the most productive ones. In August 2010, zooplankton production within the frontal zones in the central Barents Sea was 0.18–4.02 mg of dry mass per m^3 per day (Dvoretsky & Dvoretsky, 2017). In May 2016, the estimates were quite similar, confirming high zooplankton productivity in the coastal area. In other seasons, daily zooplankton production can be much higher than in spring. In the Pechora Sea in June 2001, maximum copepod production reached 14.6 mg of dry mass per m^3 per day; in Dvorovaya Bay in July 2008, maximum zooplankton production exceeded 28.3 mg of dry mass per m^3 per day (Dvoretsky & Dvoretsky, 2015). As a rule, zones of increased productivity are registered in bays with waters, enriched with nutrients and suspended matter (Dvoretsky & Dvoretsky, 2015, 2016 ; Timofeev, 2000). Daily P/B ratio (production/biomass ratio) of zooplankton was 0.02–0.03, which is comparable to the estimates, obtained previously in Barents Sea coastal water (Dvoretsky & Dvoretsky, 2015, 2016, 2012).

Copepods were a dominant zooplankton group during the period of our study: on average, they accounted for 90 % of zooplankton abundance, 96 % of biomass, and 94 % of total production. This result is quite expected, since copepods are the major constituent of zooplankton in the Arctic seas throughout the year (Vodopyanova et al., 2017 ; Orlova et al., 2004, 2011). Table 3 shows zooplankton quantitative estimates in the study period.

Table 3. Total zooplankton and copepod abundance, biomass, and daily production in the southern Barents Sea in May 2016

Parameter	$\overline{X}$	SE	Min	Max
Zooplankton/copepod abundance, thousands of ind. per m^2	584 / 521	36 / 29	202 / 178	889 / 802
Zooplankton/copepod biomass, g of dry mass per m^2	16.3 / 15.8	1.1 / 1.1	4.2 / 4.0	25.8 / 25.6
Zooplankton/copepod production, mg of dry mass per m^2	426 / 403	27 / 26	121 / 113	648 / 636

Note: $\overline{X}$ is mean; SE is standard error; min is minimum; max is maximum.

Distribution of total zooplankton biomass and daily production is shown in Fig. 2. For the water area studied (25.8 thousand km^2), total value of zooplankton stock is estimated to be 425 thousand tons of dry mass. In this water area, according to the data calculated, daily zooplankton production was about 10 thousand tons of dry mass.

Cluster analysis revealed presence of four groups of stations (Fig. 3), being quite similar in zooplankton abundance and composition. Minimum similarity between clusters according to Bray – Curtis index was 65 %. The main contribution to the difference between groups of stations was made by *Calanus finmarchicus* (2–8 %), *Oithona similis* (1–4 %), copepod nauplii (2–4 %), Echinodermata larvae (1–5 %), and appendicularian *Fritillaria borealis* (1–3 %). Four clusters significantly differed in structure of zooplankton assemblages, *i. e.* in the ratio of common species (ANOSIM Test, global $R = 0.779$, $p < 0.001$).

The highest zooplankton abundance and biomass were registered at stations of cluster 3, the lowest – at stations of cluster 4. *Calanus finmarchicus*, as well as copepod nauplii, dominated in abundance (on average, 58–63 and 12–18 %, respectively) at stations of clusters 1–3. A peculiar characteristic of cluster 4 was high *Oithona similis* ratio in total zooplankton abundance. In terms of biomass, *Calanus finmarchicus* dominated within all groups of stations, but at stations of cluster 4 its contribution was minimal (< 88 %).

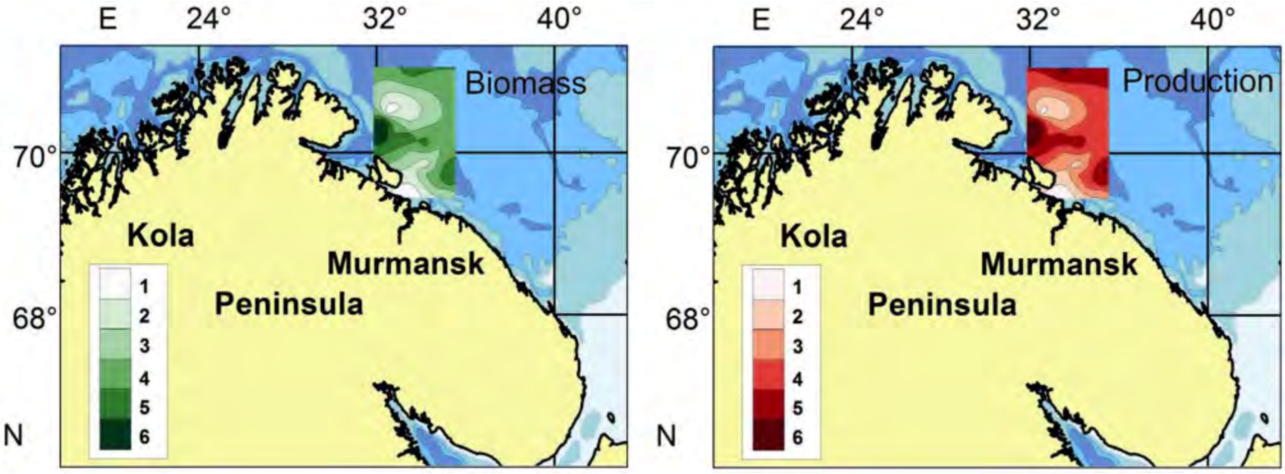

Fig. 2. Distribution of total zooplankton biomass and daily production in the southern Barents Sea in May 2016. Biomass (g of dry mass per m^2): 1 – < 5; 2 – 5–10; 3 – 10–15; 4 – 15–20; 5 – 20–25; 6 – > 25. Daily production (mg of dry mass per m^2 per day): 1 – < 100; 2 – 100–200; 3 – 200–300; 4 – 300–400; 5 – 400–500; 6 – > 500

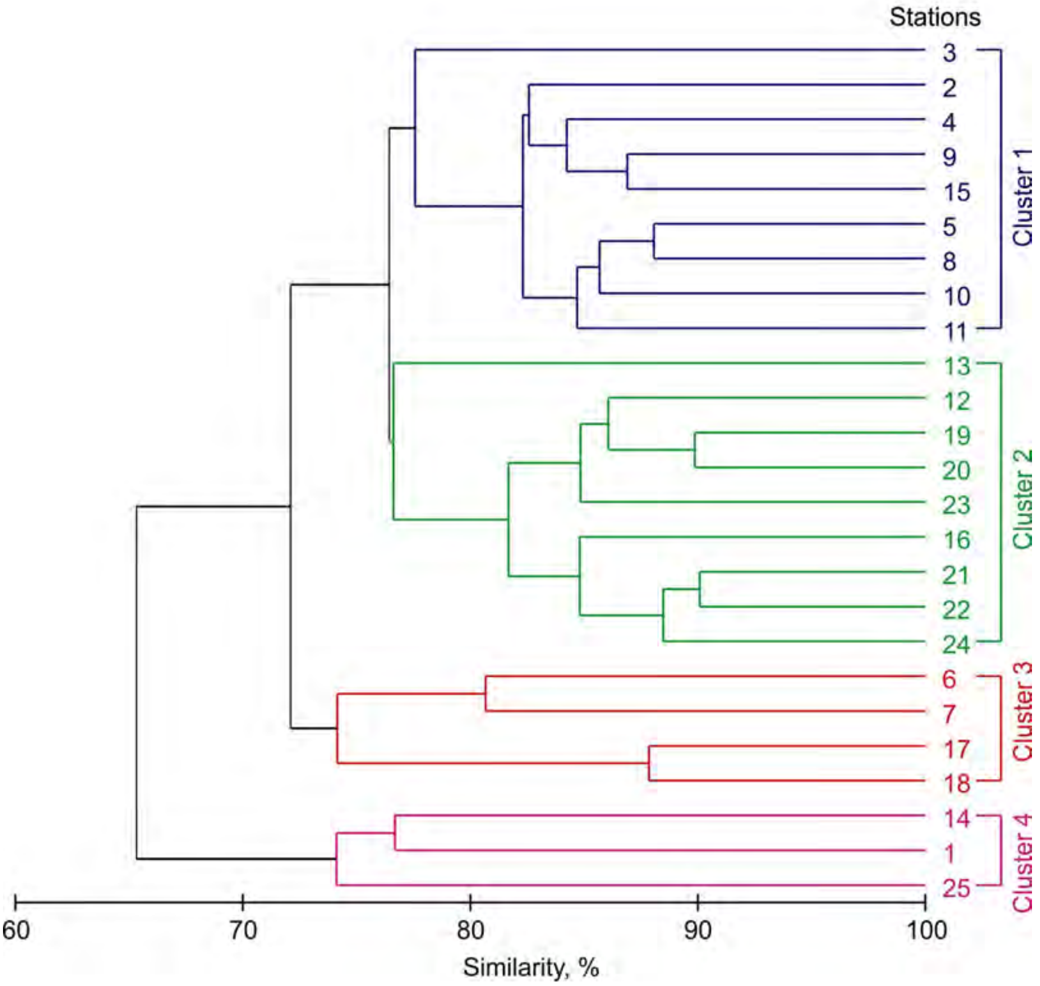

Fig. 3. Dendrogram of stations similarity in terms of zooplankton abundance (Bray – Curtis index, %) in the southern Barents Sea in May 2016

BIO-ENV routine showed as follows: spatial variations in zooplankton abundance were determined by a combination of five factors, being latitude, longitude, station depth (location), bottom temperature, and mean salinity ($r = 0.541$). A similar value of the correlation coefficient ($r = 0.540$) was obtained for a combination of three factors, being latitude, bottom temperature, and mean salinity. For other combinations of factors, values of the correlation coefficients were lower. Hydrological conditions and geographic location are known to effect zooplankton assemblages. These factors are significant in the distribution of planktonic organisms in the Arctic seas (Degtereva, 1973 ; Timofeev, 2000). In our case, zooplankton variations were most likely associated with hydrological factors (temperature and salinity), while latitude indirectly reflected the spatial changes of these two factors. As moving northward, temperature decreased, and salinity increased; these affected the ratio of common species, as well as zooplankton abundance.

We revealed a weak correlation between zooplankton abundance and chlorophyll *a* concentration, the value of which indirectly reflects the state of phytoplankton, being the main zooplankton food resource. It seems to be associated with different periods of phyto- and zooplankton abundance. A peak of phytoplankton bloom in the southern area of the sea is observed in April, but zooplankton was sampled in May, when phytoplankton concentration decreased (Plankton morei…, 1997). Therefore, no direct relationship between phyto- and zooplankton abundance was established (Dvoretsky & Dvoretsky, 2015 ; Timofeev, 2000).

Calanus finmarchicus age structure was characterized by the dominance of early copepodites, which, on average, accounted for more than 60 % of total population abundance. The ratio of adults was lower than 1 % (Fig. 4).

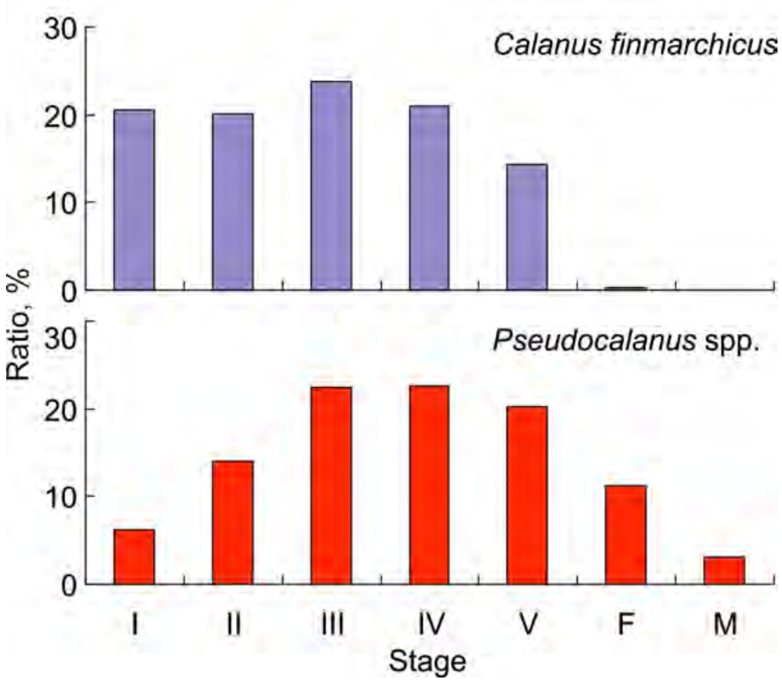

Fig. 4. Age structure of common copepod species (mean ratio in population at all stations) in the southern Barents Sea in May 2016

Such distribution of different age stages indicated recently completed species spawning within the water area studied, which is in good agreement with the general pattern of *Calanus finmarchicus* development in the Barents Sea (Dvoretsky & Dvoretsky, 2015 ; Kamshylov, 1952 ; Plankton morei…, 1997 ; Timofeev, 2000). In *Pseudocalanus* spp. populations, mainly copepodites of stages II–IV were recorded (Fig. 4), with the ratio of adults averaging 10 %, which undoubtedly indicated *Pseudocalanus* spp. continued reproduction (Dvoretsky & Dvoretsky, 2015). In *Oithona similis* population, all developmental stages were found, with the ratio of early ones being low, which is associated with low catchability of the net, used for these age groups. As a rule, in the Barents Sea, small opportunistic species reproduce throughout the year, forming 1–2 generations (Dvoretsky & Dvoretsky, 2011 ; Raymont, 1983).

High values of copepod abundance, biomass, and production created favorable feeding conditions for larvae and early age stages of pelagic fish, as well as for larvae of benthic fish. In general, the analysis carried out indicates a high production potential of zooplankton in the southern Barents Sea in spring.

Conclusions:

1. In the southern Barents Sea, 47 taxa were identified in zooplankton samples. The most common ones were copepods *Calanus finmarchicus*, *Metridia longa*, *M. lucens*, *Microcalanus* spp., *Oithona atlantica*, *O. similis*, *Pseudocalanus* spp., as well as copepod nauplii and ova, cladoceran *Evadne nordmanni*, larvae of Echinodermata and Polychaeta, chaetognath *Parasagitta elegans*, and early stages of euphausiids of the genus *Thysanoessa*. On average, copepods accounted for 90 % of zooplankton abundance, 96 % of biomass, and 94 % of total production.
2. Total zooplankton abundance varied within a wide range, averaging (3012 ± 255) ind.$\cdot$m^{-3}. Mean values of zooplankton dry mass and daily production were (83 ± 7) and (2.17 ± 0.17) mg of dry mass per m^3, respectively. According to our calculations, daily zooplankton production in this water area was about 10 thousand tons of dry mass.
3. Based on zooplankton abundance and composition, cluster analysis revealed four groups of stations. The main contribution to the difference between these groups was made by *Calanus finmarchicus*, Copepoda nauplii, larvae of Echinodermata, *O. similis*, and *Fritillaria borealis*. Spatial variations in zooplankton abundance were determined by a combination of five factors, being latitude, longitude, station depth, bottom temperature, and mean salinity. Of these, the most important ones were temperature, salinity, and latitude.
4. Age structure of common copepod species *Pseudocalanus* spp. and *Calanus finmarchicus* was characterized by predominance of early stages, which indicated *Pseudocalanus* spp. continued reproduction, as well as recently completed *C. finmarchicus* spawning.

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ПРОДУКТИВНОСТЬ ЗООПЛАНКТОНА В ПРИБРЕЖНОЙ ЗОНЕ ЮЖНОЙ ЧАСТИ БАРЕНЦЕВА МОРЯ В ВЕСЕННИЙ ПЕРИОД

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Представлены результаты анализа состояния зоопланктонного сообщества в южной части Баренцева моря. Пробы отобраны в ходе экспедиции НИС «Дальние Зеленцы» в мае 2016 г. Гидрологические условия были типичными для мурманской прибрежной водной массы в этот сезон. Всего обнаружено 47 таксонов зоопланктона. Их количество колебалась по станциям от 18 до 29. Копеподы были доминирующей группой в зоопланктоне. Наиболее часто встречались *Calanus finmarchicus*, *Metridia longa*, *Metridia lucens*, *Microcalanus* spp., *Oithona atlantica*, *Oithona similis*, *Pseudocalanus* spp., науплии и яйца копепод, а также кладоцеры *Evadne nordmanni*, личинки иглокожих, полихет, щетинкочелюстные *Parasagitta elegans*, ювенильные стадии эвфаузиид рода *Thysanoessa*. В составе популяций массовых видов копепод *Pseudocalanus* spp. и *Oithona similis* преобладали младшие возрастные группы, что свидетельствовало о продолжающемся их размножении. Суммарная численность зоопланктона варьировала от 748 до 6576 экз.·м$^{-3}$, составляя в среднем 3012. Общая биомасса колебалась от 17 до 157 мг сухой массы·м$^{-3}$, средняя величина равнялась 83. Полученные величи́ны сопоставимы с данными июля 2008 г. и превышают показатели августа 2007 г., что авторы связали с разными сезонами отбора проб и с различающимися гидрологическими условиями. Суточная продукция зоопланктона колебалась в диапазоне 0,49–4,04 мг сухой массы·м$^{-3}$ при средней величине (2,17 ± 0,17), что примерно в 2 раза выше, чем средние показатели для мурманских прибрежных вод в летний период. Вероятно, эти различия связаны с более высокой концентрацией фитопланктона в весеннее время. Суммарная величина запаса зоопланктона в исследуемой акватории (25,8 тыс. км^2) оценена в 425 тыс. т сухой массы. Кластерный анализ выявил наличие четырёх групп станций, которые различались соотношением *Calanus finmarchicus*, Copepoda nauplii, *Oithona similis*, личинок иглокожих и аппендикулярий *Fritillaria borealis*. Пространственную изменчивость численности зоопланктона определяли местоположение станций (широта, долгота, глубина), а также температура придонного слоя и средняя солёность вод на станции.

Ключевые слова: зоопланктонное сообщество, копеподы, пелагическая экосистема

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GENOTYPING OF BLACK SEA TREMATODES OF THE FAMILY OPECOELIDAE BY MITOCHONDRIAL MARKERS

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Opecoelidae Ozaki, 1925 (Trematoda: Opecoeloidea) is the biggest trematode family in the Black Sea in terms of species and genera number. Maritae of the most common Black Sea Opecoelidae trematodes are well described morphologically; nevertheless, information on their genomes structure is sketchy, and data on mitochondrial genomes are absent. The aim was to study the structure of mitochondrial genome fragments of Black Sea trematode species: *Cainocreadium flesi* Korniychuk & Gaevskaya, 2000, *Gaevskajatrema perezi* (Mathias, 1926) Gibson & Bray, 1982, and *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902. Sequences were made for CO1 (the cytochrome c oxidase subunit I) and 16S mitochondrial genes. To amplify CO1 gene fragment of *Cainocreadium* and *Helicometra* trematodes, primers were developed. Phylogenetic relationships within the analyzed part of the Opecoelidae family were reconstructed on the basis of our data and the corresponding GenBank data by the Maximum Likelihood algorithm, implemented in MEGA X program. To root the phylogenetic trees, the corresponding sequences of the closely related trematode *Brachycladium goliath* (Brachycladioidea: Brachycladiidae) were used. For the first time, nucleotide sequences of CO1 and 16S mitochondrial genes fragments of Black Sea trematodes *C. flesi*, *G. perezi*, and *H. fasciata* from different definitive fish hosts were identified and deposited in GenBank. In case of *C. flesi*, no host-specific lines were found in the structure of CO1 mitochondrial gene fragment, but high CO1 nucleotide diversity was noted. Black Sea *H. fasciata*, parasitizing peacock wrasse, *Symphodus tinca*, were revealed to be a host-specific CO1 haplogroup; its taxonomic status requires further clarification, and ecological and genetic studies of the putative *H. fasciata* species complex from different water areas are needed. No host-specific genetic lines were found when analyzing the sequences of *H. fasciata* 16S rRNA mitochondrial gene fragment. No significant differences in 16S fragment were registered between *G. perezi* trematodes from different Black Sea definitive hosts; however, the intraspecific 16S nucleotide diversity was rather high.

Keywords: Black Sea, Trematoda, Opecoelidae, *Cainocreadium*, *Gaevskajatrema*, *Helicometra*, mitochondrial genes, CO1, 16S rRNA

Opecoelidae Ozaki, 1925 (Trematoda: Opecoeloidea) is the leading Black Sea trematode family in terms of genera and species number (Gaevskaya & Kornyychuk, 2003). Over the past approximately 50 years, it has experienced a progressive decrease in species and genera representation in this water basin (Dmitrieva et al., 2018), and its reasons are not completely clear. To date, maritae of the most widespread Opecoelidae species in the Black Sea (*Cainocreadium flesi* Korniychuk & Gaevskaya, 2000, *Gaevskajatrema perezi* (Mathias, 1926) Gibson & Bray, 1982, and *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902) are detailed only morphologically, by light microscopy methods (Gaevskaya & Solonchenko, 1989 ; Korniychuk, 2009 ; Kornyychuk & Gaevskaya, 2000 ; Opredelitel' parazitov pozvonochnykh…, 1975). The available data on the genomes structure of Opecoelidae species (and generally trematodes), known from the Black Sea, are mainly represented by nucleotide sequences of nuclear DNA (18S rDNA, 28S rDNA, ITS1, and ITS2) (Katokhin & Kornyychuk, 2018 ; Andres et al., 2014 ; Born-Torrijos et al., 2012 ; Bray et al., 2016 ; Jousson & Bartoli, 2001). Few data on mitochondrial genes structure of Opecoelidae genera of the Black Sea were obtained only from the Pacific material (Donald et al., 2004 ; Donald & Spencer, 2016 ; González et al., 2013 ; Lagrue, 2016 ; López et al., 2015 ; Martin et al., 2019 ; Yano & Urabe, 2017); they are of little use for confirming species identification of Black Sea trematodes by molecular genetic methods.

The aim of the study is to obtain the first data on the structure of mitochondrial genome fragments of trematode species, being the most common ones in the Black Sea in the current period (*C. flesi*, *G. perezi*, and *H. fasciata*), for further clarification of their taxonomic status.

MATERIAL AND METHODS

Maritae of Opecoelidae trematodes (*Cainocreadium flesi*, *Gaevskajatrema perezi*, and *Helicometra fasciata*) were obtained from Black Sea fish, caught in Sevastopol and Batiliman water areas, as well as in Karadag nature reserve water area (Table 1). The species were identified by generally accepted morphological criteria (Gaevskaya & Solonchenko, 1989 ; Korniychuk, 2009 ; Kornyychuk & Gaevskaya, 2000 ; Opredelitel' parazitov pozvonochnykh…, 1975).

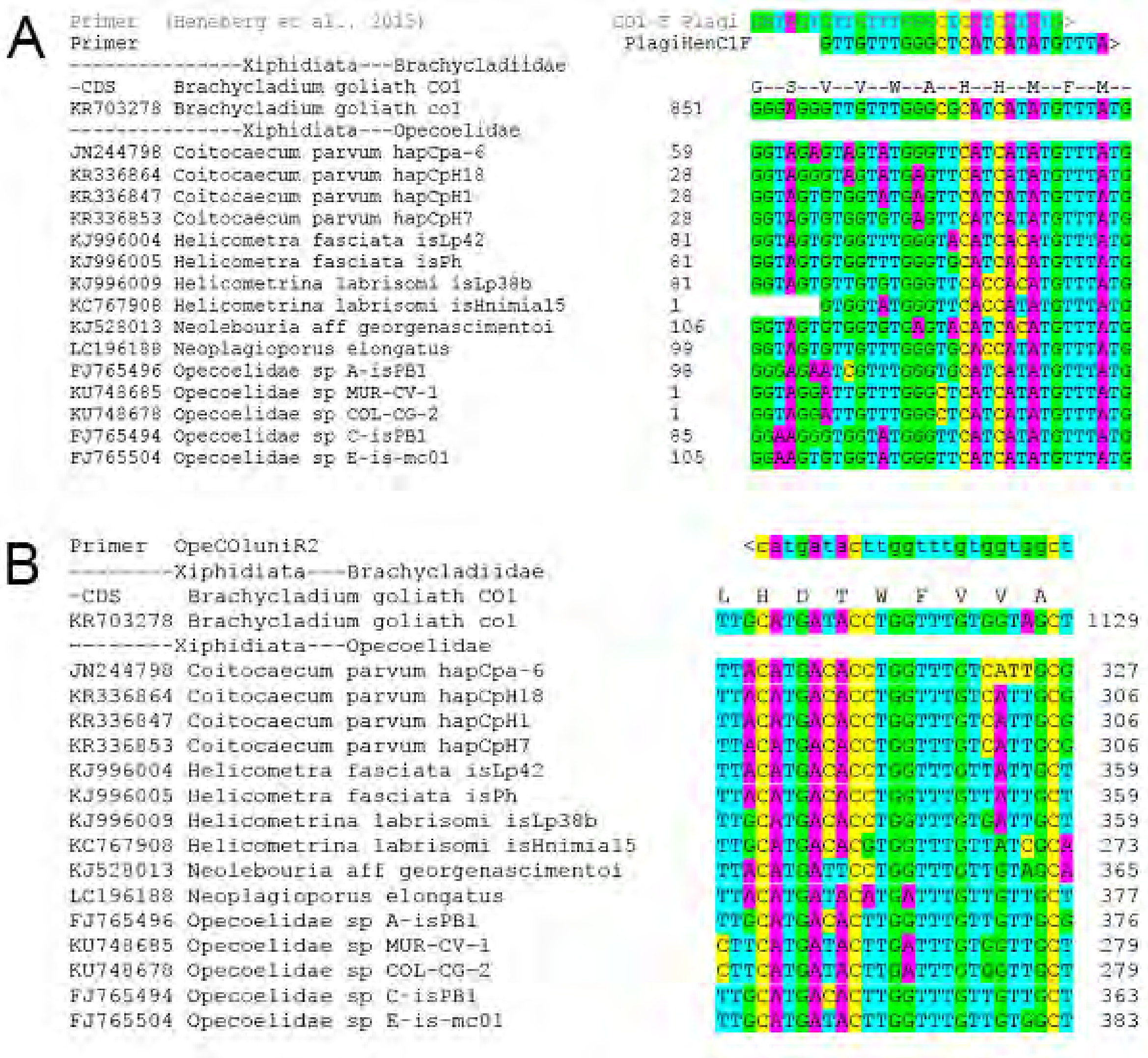
For molecular genetic analysis, trematode mitochondrial genes fragments, encoding 16S rRNA and the cytochrome c oxidase subunit I (CO1), were selected. Total DNA was isolated by the CTAB method (Wilke et al., 2006). To amplify 257-nucleotide 16S rRNA fragment, the primers OMP38 5'-AGACGGAAAGACCCCGAG-3' and OMP04 5'-CTCACGCCGGTCTTAACT-3' were used, as well as the thermal profile of the polymerase chain reaction as follows: denaturation at +94 °C for 3 minutes, 40 cycles (denaturation at +94 °C for 20 seconds; primer annealing at +52 °C for 30 seconds; synthesis at +72 °C for 25 seconds). These primers were developed and used for the reconstruction of opisthorchiid trematodes mitogenomes (Shekhovtsov et al., 2010), and they are also suitable for Opecoelidae species genotyping. Amplification was performed under standard reaction conditions (see http://molbiol.ru/protocol/12_01.html).

To amplify 276-nucleotide CO1 gene fragment, we used the primers PlagiHenC1F 5'-GTTGTTTGGGCTCATCATATGTTTA-3' and OpeCo1uniR2 5'-AGCCACCACAAACCAAGTATCATG-3', as well as the thermal profile of the polymerase chain reaction, described above, but with primer annealing at +54 °C. Since CO1 sequences for *Cainocreadium* trematodes were not known before our study, we used the primer, which was proposed earlier for Prosthogonimidae trematodes (Heneberg et al., 2015), as the basis for the development of the PlagiHenC1F primer. After the alignment with the sequences from GenBank (NCBI), this primer was modified to be suitable for Opecoelidae species genotyping. When developing the OpeCo1uniR2 primer, we used the primer, which was developed and used for opisthorchiid genotyping, as the basis; it was similarly modified to work with Opecoelidae trematodes DNA (Fig. 1).

The amplicons were sequenced by the Sanger method. The analysis of the sequencing reaction products after purification by isopropanol precipitation was carried out in the sequencing core facility in the Institute of Molecular and Cellular Biology of Siberian Branch of RAS on a capillary sequencer ABI 3730xl Genetic Analyzer (Applied Biosystems).

Table 1. Trematoda (Opecoelidae) maritae samples, analyzed in the study

Sample identifier	Trematode species	Fish host species	Sampling area	No. in GenBank	
				16S	CO1
009Cai-PfSKr05-21	*Cainocreadium flesi*	*Platichthys flesus*	Sevastopol	MT472528	MT472167
010Cai-PfSKr05-22				MT472529	MT472168
022Cai-PfSKr05-23				MT472530	MT472169
001Cai-GmSKr05-21		*Gaidropsarus mediterraneus*	Sevastopol	MT472531	–
002Cai-GmSKr05-22				MT472532	–
003Cai-GmSKr05-23				MT472533	MT472170
004Cai-GmSKr05-24				MT472534	–
011Cai-GmKKr18-21			Karadag	MT472535	MT472171
012Cai-GmKKr18-22				MT472536	–
013Cai-GmKKr18-23				MT472537	MT472172
014Cai-GmKKr18-24				MT472538	MT472173
018Cai-GmKKr18-26				MT472539	–
019Cai-GmKKr18-27				MT472540	–
005Hel-SpSKr05-21	*Helicometra fasciata*	*Salaria pavo*	Sevastopol	–	MT472174
006Hel-SpSKr05-22				MT472542	MT472175
015Hel-GmBKr17-22		*Gaidropsarus mediterraneus*	Batiliman	MT472543	MT472176
042Hel-GmKKr18-21			Karadag	MT472548	–
043Hel-GmKKr18-22				MT472549	–
047Hel-GmKKr18-23				MT472551	MT472177
048Hel-GmKKr18-24				MT472552	–
036Hel-AsKKr18-21		*Aidablennius sphynx*	Sevastopol	MT472545	–
037Hel-AsKKr18-22				MT472546	–
040Hel-AsKKr18-25				MT472547	–
049Hel-SpKKr06-21		*Scorpaena porcus*	Karadag	MT472553	MT472178
045Hel-SrBKr02-21		*Symphodus roissali*	Sevastopol	MT472550	–
056Hel-StSKr19-22		*Symphodus tinca*	Sevastopol	MT472554	MT472179
057Hel-StSKr19-23				MT472556	–
041Gae-SsSKr19-21	*Gaevskajatrema perezi*	*Symphodus ocellatus*	Sevastopol	MT472557	–
051Gae-SoSKr19-22				MT472558	–
052Gae-StSKr19-23		*Symphodus tinca*	Sevastopol	MT472559	–
053Gae-StSKr19-24				MT472556	–
–	*Helicometra fasciata*	*Labrisomus philippii*	Pacific Ocean, Chile coast	–	KJ996004 [20]
–	*Helicometra fasciata*	*Paralabrax humeralis*		–	KJ996005; KJ996006 [20]
–	*Brachycladium goliath*	*Balaenoptera acutorostrata*	Atlantic Ocean	KR703278	KR703278 [17]



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A
Primer  (Heneberg et al., 2015)                  CO1-F Plagi GGTAGTGTTGTTTGGGCTCATCATATG>
Primer                                            PlagiHenC1F       GTTGTTTGGGCTCATCATATGTTTA>
---------------Xiphidiata---Brachycladiidae
-CDS     Brachycladium goliath CO1                           G--S--V--V--W--A--H--H--M--F--M--
KR703278 Brachycladium goliath co1                   851     GGGAGGGTTGTTTGGGCGCATCATATGTTTATG
---------------Xiphidiata---Opecoelidae
JN244798 Coitocaecum parvum hapCpa-6                 59      GGTAGAGTAGTATGGGTTCATCATATGTTTATG
KR336864 Coitocaecum parvum hapCpH18                 28      GGTAGGGTAGTATGAGTTCATCATATGTTTATG
KR336847 Coitocaecum parvum hapCpH1                  28      GGTAGTGTGGTATGAGTTCATCATATGTTTATG
KR336853 Coitocaecum parvum hapCpH7                  28      GGTAGTGTGGTGTGAGTTCATCATATGTTTATG
KJ996004 Helicometra fasciata isLp42                 81      GGTAGTGTGGTTTGGGTACATCACATGTTTATG
KJ996005 Helicometra fasciata isPh                   81      GGTAGTGTGGTTTGGGTGCATCACATGTTTATG
KJ996009 Helicometrina labrisomi isLp38b             81      GGTAGTGTTGTGTGGGTTCACCACATGTTTATG
KC767908 Helicometrina labrisomi isHnimial5          1            GTGGTATGGGTTCACCATATGTTTATG
KJ528013 Neolebouria aff georgenascimentoi           106     GGTAGTGTGGTGTGAGTACATCACATGTTTATG
LC196188 Neoplagioporus elongatus                    99      GGTAGTGTTGTTTGGGTGCACCATATGTTTATG
FJ765496 Opecoelidae sp A-isPB1                      98      GGGAGAATCGTTTGGGTGCATCATATGTTTATG
KU748685 Opecoelidae sp MUR-CV-1                     1       GGTAGGATTGTTTGGGCTCATCATATGTTTATG
KU748678 Opecoelidae sp COL-CG-2                     1       GGTAGGATTGTTTGGGCTCATCATATGTTTATG
FJ765494 Opecoelidae sp C-isPB1                      85      GGAAGGGTGGTATGGGTTCATCATATGTTTATG
FJ765504 Opecoelidae sp E-is-mc01                    105     GGAAGTGTGGTATGGGTTCATCATATGTTTATG

B
Primer   OpeCO1uniR2                                   <catgatacttggtttgtggtggct
--------Xiphidiata---Brachycladiidae
-CDS      Brachycladium goliath CO1                  L  H  D  T  W  F  V  V  A
KR703278 Brachycladium goliath co1                   TTGCATGATACCTGGTTTGTGGTAGCT  1129
--------Xiphidiata---Opecoelidae
JN244798 Coitocaecum parvum hapCpa-6                 TTACATGACACCTGGTTTGTCATTGCG  327
KR336864 Coitocaecum parvum hapCpH18                 TTACATGACACCTGGTTTGTCATTGCG  306
KR336847 Coitocaecum parvum hapCpH1                  TTACATGACACCTGGTTTGTCATTGCG  306
KR336853 Coitocaecum parvum hapCpH7                  TTACATGACACCTGGTTTGTCATTGCG  306
KJ996004 Helicometra fasciata isLp42                 TTACATGACACCTGGTTTGTTATTGCT  359
KJ996005 Helicometra fasciata isPh                   TTACATGACACCTGGTTTGTTATTGCT  359
KJ996009 Helicometrina labrisomi isLp38b             TTGCATGACACCTGGTTTGTGATTGCT  359
KC767908 Helicometrina labrisomi isHnimial5          TTGCATGACACGTGGTTTGTTATCGCA  273
KJ528013 Neolebouria aff georgenascimentoi           TTACATGATTCCTGGTTTGTTGTAGCA  365
LC196188 Neoplagioporus elongatus                    TTACATGATACATGATTTGTTGTTGCT  377
FJ765496 Opecoelidae sp A-isPB1                      TTGCATGACACTTGGTTTGTTGTTGCG  376
KU748685 Opecoelidae sp MUR-CV-1                     CTTCATGATACTTGATTTGTGGTTGCT  279
KU748678 Opecoelidae sp COL-CG-2                     CTTCATGATACTTGATTTGTGGTTGCT  279
FJ765494 Opecoelidae sp C-isPB1                      TTGCATGACACTTGGTTTGTTGTTGCT  363
FJ765504 Opecoelidae sp E-is-mc01                    TTGCATGATACTTGGTTTGTTGTGGCT  383
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Fig. 1. Alignment of CO1 gene fragments of Opecoelidae trematodes and *Brachycladium goliath*; position of PlagiHenC1F (A) and OpeCo1uniR2 (B) primers

The sequences obtained were aligned by the Clustal W algorithm; genetic distance calculation and multiple alignment analysis were carried out by the MEGA X program (Kumar, 2018).

Nucleotide sequences of CO1 and 16S mitochondrial gene fragments of Black Sea trematodes, identified in this work, were deposited in GenBank (see Table 1). For phylogenetic analysis, the corresponding 16S and CO1 sequences of Opecoelidae from GenBank were used (Table 1). For *Gaevskajatrema perezi*, CO1 fragment failed to amplify; the possible reason of the failure is excessive polymorphism in template fragments, targeted by primers. Phylogenetic relationships within the analyzed part of the Opecoelidae family were reconstructed on the basis of our data and the corresponding GenBank data by the Maximum Likelihood (ML) algorithm and the HKY nucleotide substitution model, recommended by the Model Test subprogram (the MEGA X program). To root the phylogenetic trees, the corresponding sequences of the closely related trematode *Brachycladium goliath* (van Beneden, 1858) Fraija-Fernández, Aznar, Raga, Gibson & Fernández, 2014 (Brachycladioidea: Brachycladiidae) were used.

Haplotypes were identified and analyzed by the DnaSP 5.10 software (Librado & Rozas, 2009). Haplotype networks were built by the Network 10 program (http://www.fluxus-engineering.com/sharenet.htm) (Bandelt et al., 1999).

RESULTS AND DISCUSSION

The analysis of the genetic distances between Black Sea Opecoelidae trematodes from different genera according to the sequences of 16S and CO1 mitochondrial genes fragments revealed significant quantitative differences between them, with a much lower intraspecific variability of the corresponding trematode genome fragments (Table 2).

Table 2. Estimates of genetic distances (number of base substitutions per site) intra- (in brackets, bold) and between studied Black Sea Opecoelidae trematodes for CO1 (above diagonal) and 16S mitochondrial genes fragments (below diagonal)

CO1 16S	*Cainocreadium flesi* (**0.2175**)	*Helicometra fasciata* (**0.0423**)
Cainocreadium flesi (**0.0031**)		0.3108
Helicometra fasciata (**0.0109**)	0.0719	
Gaevskajatrema perezi (**0,0127**)	0.0688	0.0616

The position of Black Sea Opecoelidae trematode species on the phylogenetic tree, based on 16S rRNA fragment under study (Fig. 2), adequately reflected the assignment of trematode species to genera.

***Cainocreadium flesi* Korniychuk & Gaevskaya, 2000.** As a rule, 16S mitochondrial gene is used to distinguish trematode taxa above the species level (most reliably to distinguish families) (Blasco-Costa et al., 2016). On the intraspecific level, any significant differences in its nucleotide sequences are unlikely. However, in *Cainocreadium* trematodes, six 16S haplotypes were identified, and the transitions between them are clearly illustrated in Fig. 3. The samples analyzed from the European flounder *P. flesus* are represented by three haplotypes (No. 4–6), differing by one nucleotide substitution and/or insertion-deletion. All analyzed *Cainocreadium* maritae samples from shore rockling *G. mediterraneus* are represented by three other haplotypes (No. 1–3), differing by one nucleotide substitution. The most common haplotype is No. 1: it was identified from *Cainocreadium* specimens, sampled in different years, in different areas, and from different fish host specimens. The host specificity of 16S gene haplogroups indicates a certain multidirectionality of microevolutionary processes in relation to Black Sea *Cainocreadium* maritae, parasitizing shore rocklings and European flounders.

The analysis of similarity and difference of *C. flesi* trematodes from various hosts by the structure of CO1 mitochondrial gene fragment (Fig. 4) revealed no host-specific clusters, which is consistent with the previously obtained data on the absence of genetic variability of these trematodes from shore rockling and European flounder by ITS1 nucleotide sequences (Katokhin & Kornyychuk, 2018). Moreover, it apparently may indicate belonging of Black Sea *Cainocreadium* maritae from different definitive fish hosts, having, nevertheless, obvious morphological differences (Kornyychuk, 2008), to one and the same species: *C. flesi*. Meanwhile, in *C. flesi* samples analyzed, a very high CO1 haplotypic diversity was observed (Fig. 4): each of them belonged to a separate haplotype; 14 nonsynonymous substitutions (0.21 %) and 28 synonymous ones (0.42 %) were found.

The analysis by the Network 10 program revealed as follows: CO1 haplotypes of Black Sea *Cainocreadium* are divided into two groups (Hap 1÷4 and Hap 5÷7), separated by 42 mutational events; within the haplogroups, the differences do not exceed 5 mutational steps (Fig. 5). Meanwhile, neither

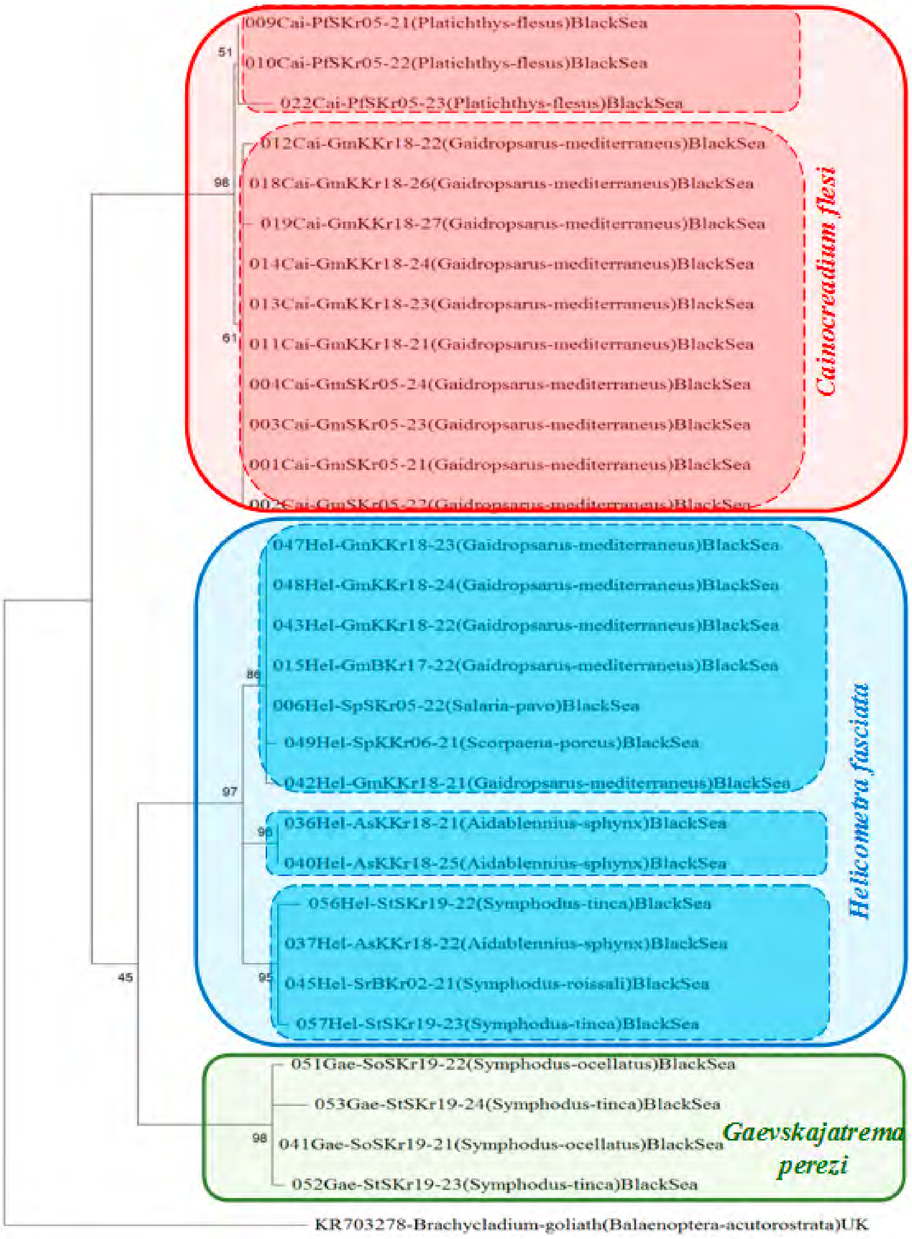

Fig. 2. ML phylogram of Black Sea Opecoelidae trematodes, based on 16S rRNA mitochondrial gene fragment

host nor spatial specificity of CO1 haplogroups was noted (Fig. 4): each of them includes trematodes from both shore rockling and European flounder, caught in two Black Sea water areas, being almost 200 km apart along the coast.

Cainocreadium entered the Black Sea apparently from the Mediterranean Sea and got completely new definitive and intermediate hosts (Kornyychuk, 2008). A new species, *C. flesi*, was formed from trematode ancestral form (Kornyychuk & Gaevskaya, 2000); the adaptation to exploiting new Black Sea hosts is clearly ongoing.

***Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902.** Based on 16S RNA structure, Black Sea *H. fasciata* maritae (see Fig. 2) were divided into three clusters, with none of them reflecting narrow specificity to the definitive host: trematodes from *A. sphynx* both formed a separate cluster and were present in another cluster (from different fish hosts).

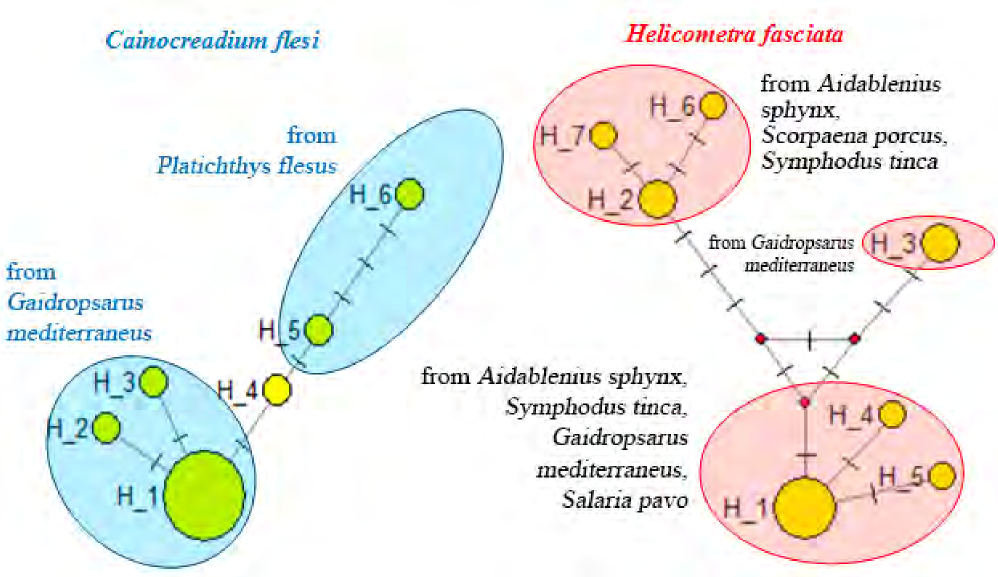

Fig. 3. Haplotype networks of 16S of two Black Sea trematodes

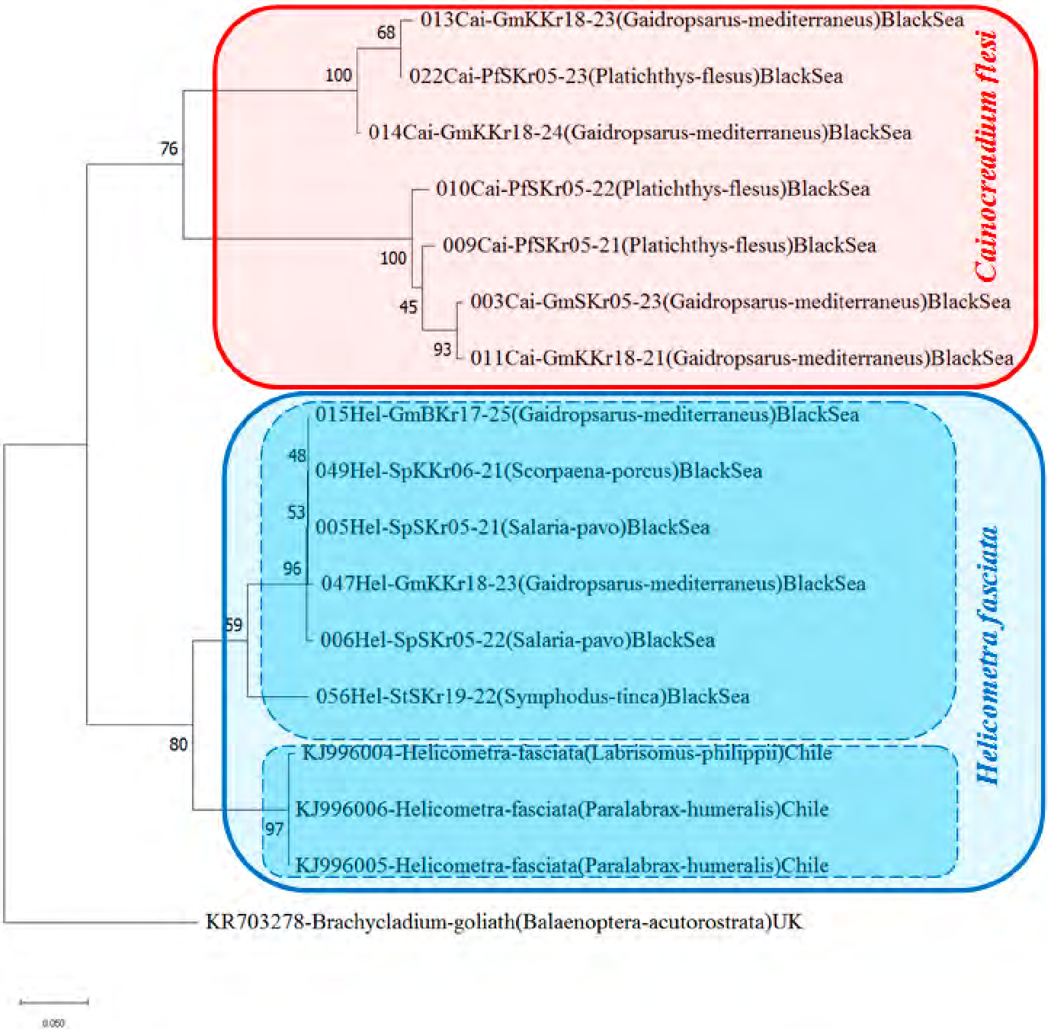

Fig. 4. ML phylogram of Black Sea Opecoelidae trematodes, based on mitochondrial gene fragment, encoding the cytochrome c oxidase subunit I (CO1)

Three groups of *H. fasciata* 16S haplotypes were identified. Hap 4÷7 are confined to Sevastopol region only; Hap 2 and 3 were found in Karadag only; Hap 1 was registered in maritae from fish from geographically significantly distant water areas (Sevastopol, Batiliman, and Karadag; more than 200 km in a straight line between the extreme points). Meanwhile, none of fish hosts is able to move long distances (Svetovidov, 1964).

Black Sea *H. fasciata* maritae studied, parasitizing peacock wrasse, *Symphodus tinca*, significantly differ in CO1 structure from *H. fasciata* maritae from the other fish hosts studied, which are grouped into another cluster on the tree (Fig. 4).

We compared our data on CO1 structure of Black Sea *H. fasciata* with the corresponding GenBank data (see Table 1) on *H. fasciata* trematodes off Chile coast. It turned out that *Helicometra* from this geographically distant area and specimens, parasitizing Black Sea fish from other families, are clustered in different groups.

CO1 haplotypes of *H. fasciata* samples studied were subdivided into three haplogroups (Fig. 5). The haplotypes of *H. fasciata* from the majority of Black Sea fish studied are closely related (Hap 1÷3; average intragroup genetic distance is 0.032). They differ significantly from haplotypes of *H. fasciata* from *S. tinca* (Hap 4; average genetic distance to above described complex of *H. fasciata* is 0.088).

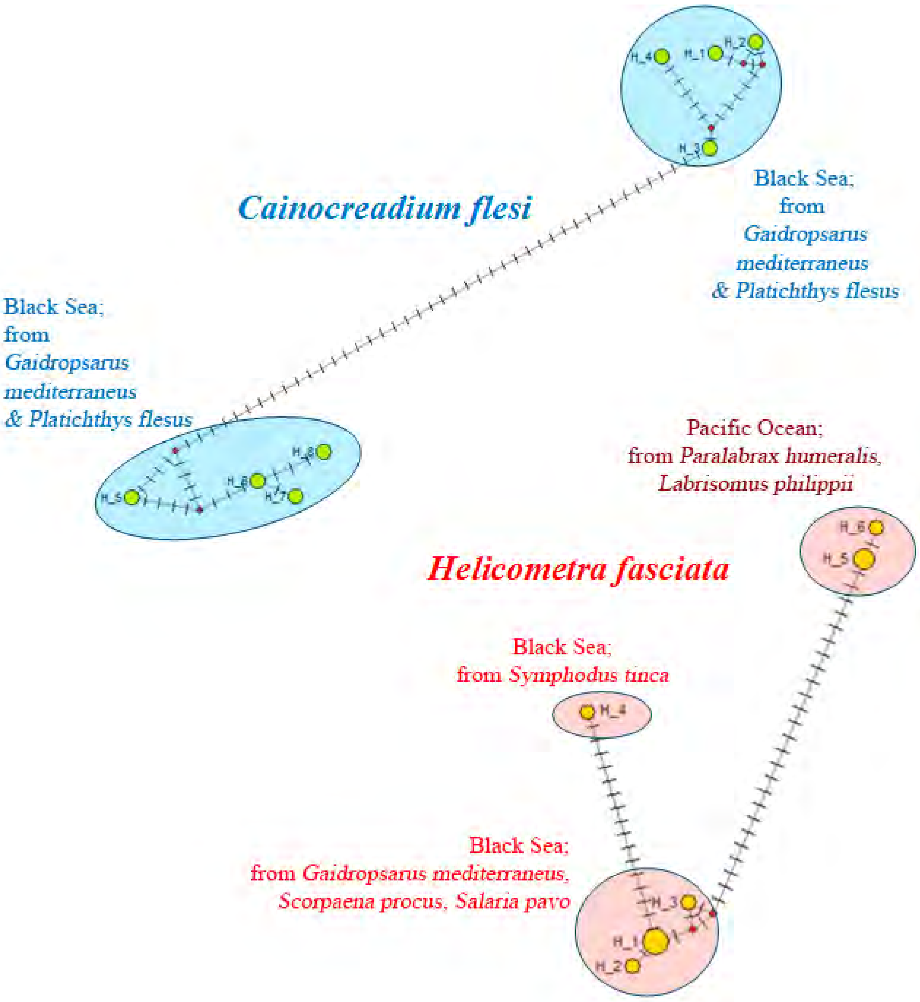

Fig. 5. Haplotype networks of CO1 of Black Sea trematodes

The most distant *H. fasciata* haplogroup (CO1 Hap 5÷6) included Pacific representatives of the species: average genetic distance is 0.154 (range 0.146÷0.158); the distance from Black Sea *H. fasciata* from *S. tinca* is 0.148, from other Black Sea *H. fasciata* – 0.155.

The values of genetic distances between two CO1 haplogroups of Black Sea *H. fasciata* and between each of these groups and Pacific *H. fasciata* are in the range of interspecific CO1 differences for trematodes, established by several researchers (León-Règagnon, 2010 ; Pérez-Ponce et al., 2016 ; Rosser et al., 2017 ; Vilas et al., 2005). These data, as well as previously established morphological peculiarity of Black Sea *H. fasciata* maritae from *S. tinca* (Korniychuk, 2000), allow suggesting as follows: all three CO1 haplogroups of *H. fasciata*, mentioned above, may be taxonomically unequal. Verification of this hypothesis requires a comprehensive study of the putative *H. fasciata* species complex: obtaining more complete information on CO1 haplotypic composition of host maritae groups, studying morphological features of *H. fasciata* of different CO1 haplogroups, and assessing presence of transitional morphotypes.

***Gaevskajatrema perezi* (Mathias, 1926) Gibson & Bray, 1982.** *G. perezi* trematodes are specific only to Labridae fish and are common in these hosts in the Black Sea (Korniychuk, 2001). Studied maritae of this species from two species of Black Sea wrasses (ocellated wrasse and peacock wrasse) appeared to have the close structure of 16S fragment (Fig. 2). It will be interesting to check this conclusion in future, using other markers.

In general, the use of two mitochondrial markers for answering the question of the status of Opecoelidae species seems to be promising. It is clear that the involvement in the analysis of new samples from different hosts and water areas will provide a lot of unique data on species diversity and intrapopulation genetic structure of these trematodes.

The data obtained on the structure of fragments of mitochondrial genes, encoding 16S rRNA and the cytochrome c oxidase subunit I (CO1), of Black Sea trematodes studied were deposited in GenBank (see Table 1) and integrated into the website of parasitic organisms subcollection of IBSS collection of the World Ocean hydrobionts (http://marineparasites.org/taxa/?dna-sequences).

Conclusions:

1. For the first time, the primers PlagiHenC1F and OpeCo1uniR2 were developed to amplify CO1 gene fragments of *Cainocreadium* and *Helicometra* trematodes.
2. For the first time, nucleotide sequences of mitochondrial gene fragments of Black Sea trematodes were identified: 16S gene of *Cainocreadium flesi* Kornyychuk & Gaevskaya, 2000, *Gaevskajatrema perezi* (Mathias, 1926) Gibson & Bray, 1982, and *Helicometra fasciata* (Rudolphi, 182) Odhner, 1902, as well as CO1 gene of *C. flesi* and *H. fasciata* from different definitive fish hosts.
3. In *C. flesi* trematodes, no host-specific groups in the structure of 16S and CO1 mitochondrial genes fragments were found; however, high CO1 nucleotide diversity was revealed.
4. In Black Sea *H. fasciata* maritae from *Symphodus tinca*, CO1 haplotype was identified, being significantly distant from CO1 haplogroup of *H. fasciata* from other fish hosts studied; the taxonomic status of maritae from *S. tinca* requires clarification. When analyzing the sequences of 16S rRNA mitochondrial gene fragment, host-specific genetic lines of *Helicometra* were not found.
5. CO1 haplogroup of Pacific *H. fasciata* is significantly distant from the haplogroups of Black Sea ones, and this requires clarification of the taxonomic status of Pacific *H. fasciata* as well.
6. No differences were found in the structure of studied 16S gene fragment in Black Sea *G. perezi* from different definitive hosts.

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ГЕНОТИПИРОВАНИЕ ЧЕРНОМОРСКИХ ТРЕМАТОД СЕМЕЙСТВА OPECOELIDAE ПО МИТОХОНДРИАЛЬНЫМ МАРКЕРАМ

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Opecoelidae Ozaki, 1925 (Trematoda: Opecoeloidea) — ведущее по числу видов и родов семейство трематод в Чёрном море. Мариты наиболее распространённых видов черноморских опецелидных трематод подробно описаны морфологически, однако сведения о структуре их геномов отрывочны, а данные о митохондриальных геномах отсутствуют полностью. Цель исследования — получить первые сведения о строении участков митохондриального генома представителей наиболее распространённых в Чёрном море в современный период родов трематод семейства Opecoelidae для последующего уточнения их таксономического статуса. Филогенетические отношения внутри анализируемой части этого семейства реконструированы на основе данных, полученных нами, и соответствующих данных из GenBank с помощью алгоритма Maximum Likelihood и модели нуклеотидных замен HKY. Для укоренения филогенетического дерева использованы соответствующие последовательности *Brachycladium goliath* (Brachycladioidea: Brachycladiidae). Поскольку последовательности CO1 — стандартного и наиболее популярного митохондриального маркера — для исследуемых родов опецелид до сих пор не были известны, нами на основе известных соответствующих последовательностей Xiphidiata разработаны праймеры для амплификации фрагмента CO1, чтобы впервые провести соответствующий филогенетический анализ. Впервые определены и депонированы в GenBank нуклеотидные последовательности фрагментов митохондриальных генов CO1 и 16S черноморских трематод *Cainocreadium flesi* Korniychuk & Gaevskaya, 2000, *Gaevskajatrema perezi* (Mathias, 1926)

Gibson & Bray, 1982 и *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902 от разных видов дефинитивных хозяев — рыб. У *C. flesi* не выявлено специфичных к окончательным хозяевам — рыбам линий по структуре фрагмента митохондриального гена CO1, однако отмечено высокое CO1-нуклеотидное разнообразие. У марит черноморских *H. fasciata* определена приуроченная к зеленушкам-руленам *Symphodus tinca* CO1-гаплогруппа, статус которой требует дальнейшего выяснения; необходимы экологические и генетические исследования предполагаемого видового комплекса *H. fasciata* из разных акваторий. При анализе последовательностей фрагмента митохондриального гена 16S рРНК гостальных генетических линий у *H. fasciata* выделить не удалось. У черноморских *G. perezi* не обнаружено значительных различий по фрагменту 16S между трематодами из окончательных хозяев разных видов, однако внутривидовое 16S-нуклеотидное разнообразие оказалось высоким.

Ключевые слова: Чёрное море, Trematoda, Opecoelidae, *Cainocreadium*, *Gaevskajatrema*, *Helicometra*, митохондриальные гены, CO1, 16S рРНК

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IMPACT OF 24-HOUR HYPOXIA ON HEMOCYTE FUNCTIONS OF *ANADARA KAGOSHIMENSIS* (TOKUNAGA, 1906)

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Shellfish farms are usually located in coastal areas, where molluscs can be exposed to hypoxia. Cultivating at low oxygen levels causes general disruptions of growth rate, outbreaks of diseases, and mollusc mortality. Impact of short-term hypoxia on hemocyte functions of ark clam (*Anadara kagoshimensis*) was investigated by flow cytometry. A control group was incubated at 6.7–6.8 mg $O_2 \cdot L^{-1}$, an experimental one – at 0.4–0.5 mg $O_2 \cdot L^{-1}$. Exposition lasted for 24 hours. Hypoxia was created by blowing seawater in shellfish tanks with nitrogen gas. In ark clam hemolymph, 2 groups of hemocytes were identified on the basis of arbitrary size and arbitrary granularity: granulocytes (erythrocytes) and agranulocytes (amebocytes). Erythrocytes were the predominant cell type in *A. kagoshimensis* hemolymph, amounting for more than 90 %. No significant changes in cellular composition of ark clam hemolymph were observed. The production of reactive oxygen species and hemocyte mortality in the experimental group also remained at control level. The results of this work indicate ark clam tolerance to hypoxia.

Keywords: ark clam, marine cultivating, flow cytometry, hematological parameters, reactive oxygen species

Hypoxic zones are widespread on the shelf of marine areas (Dang et al., 2013 ; Diaz & Rosenberg, 2008). Shellfish farms are traditionally located in coastal areas, where they can be exposed to stable or periodic hypoxia. Oxygen deficiency results in disruption of growth rate, outbreaks of diseases on farms, and mass mollusc mortality (Andreyeva et al., 2019 ; De Zwaan et al., 1991 ; Nicholson & Morton, 1998 ; Sussarellu et al., 2013). The latter determines the relevance of the search for cultivation species, being characterized with wide adaptive potential for oxygen deficiency. The main cultivation objects on Black Sea coast are *Mytilus galloprovincialis* Lamarck, 1819 and *Crassostrea gigas* (Thunberg, 1793) (Yakhontova & Dergaleva, 2008).

Physiological parameters, such as filtration intensity, respiration rate, and growth rate, are known to deteriorate when mussels lack oxygen (Wang & Widdows, 1991). Hypoxia has the most detrimental impact on juvenile mussels and oysters, in particular on their growth, dispersal, and survival (Baker and Mann, 1992). This can be explained by the fact that under hypoxia, larvae of these molluscs are forced to reduce energy consumption for the processes of food intake, digestion, and growth, thereby reducing the need for oxygen (Wang et al., 2012 ; Wang & Widdows, 1991).

The bivalve mollusc *Anadara kagoshimensis* (Tokunaga, 1906), belonging to Arcidae family, is considered a promising species for industrial cultivation on Black Sea coast (Yakhontova & Dergaleva, 2008). This is due to ark clam wide habitat, high growth rates, and ability to increase biomass at high stocking density (Yakhontova & Dergaleva, 2008). However, the development of a reproduction technology of cultivation object, being not typical for a region, requires an understanding of its physiological responses to stressful environmental factors, in particular hypoxia.

Physiological state of mollusc can be assessed by the functional state of hemocytes: cells, circulating in hemolymph. Physiological impact of oxygen deficiency is assessed by changes in the ratio of hemocyte types, as well as by indicators of non-specific immune response (production of reactive oxygen species (hereinafter ROS) and phagocytic activity) and hemocyte proliferation (Sussarellu et al., 2013, 2010). There are many studies on the impact of short-term hypoxia on traditional cultivation objects: *M. galloprovincialis* and *C. gigas* (Novitskaya & Soldatov, 2011 ; Sussarellu et al., 2012, 2013, 2010 ; Wu, 2002). As a result of hypoxia, some physiological disorders occur at the cellular level in oysters: intensity of mitochondrial respiration decreases (Sui et al., 2016), and expression of antioxidant enzyme genes increases (Sussarellu et al., 2013). Moreover, cases of cell damage are recorded, in particular hemocytes, circulating in the hemolymph (Hermes-Lima, 2015). The latter may result in suppression of immune functions (Donaghy et al., 2013). At the same time, total number of hemocytes increases, which may be due to their proliferative activity under hypoxia (Sussarellu et al., 2010). In mussels lacking oxygen, number of granulocytes increases, while number of agranulocytes, on the contrary, decreases (Andreyeva et al., 2019). Decreased production of ROS in oyster and mussel hemocytes negatively affects their protective functions (Andreyeva et al., 2019 ; Boyd et al., 1999).

Ark clam *A. kagoshimensis* is considered a species, being tolerant to oxygen deficiency due to hemoglobin in pigmented hemocytes – erythrocytes (Soldatov et al., 2010, 2018). It is able to survive for several weeks under deep hypoxia (Holden et al., 1994 ; Mydlarz et al., 2006). Tolerance for oxygen deficiency is ensured due to ability to efficient anaerobic metabolism (Novitskaya & Soldatov, 2011 ; Cortesi et al., 1992 ; Isani et al., 1986 ; Miyamoto & Iwanaga, 2012). It is known that even under acute hypoxia, ark clam is able to maintain intracellular energy balance at a relatively high level (Cortesi et al., 1992 ; Novitskaya & Soldatov, 2013). Moreover, hypoxia does not lead to lysis of ark clam erythrocytes (Andreyeva et al., 2019 ; Zwaan et al., 1995). However, it was noted that after 3 days of incubation under hypoxia, morphological parameters of erythrocytes change (Wang & Widdows, 1991). The impact of hypoxia on the parameters of *A. kagoshimensis* hemocyte cell immunity is not known so far. Based on the available data, it is impossible to draw a conclusion about the immune status of ark clam hemocytes under hypoxia. At the same time, it is the suppression of immune functions that can lead to mass mollusc mortality (Wang & Widdows, 1991 ; Widdows et al., 1989), which creates problems for shellfish farms.

The aim of this work is to study the impact of 24-hour hypoxia on the functional parameters of hemocytes of the bivalve mollusc *A. kagoshimensis* during *in vivo* experiments.

MATERIAL AND METHODS

Bivalve molluscs *A. kagoshimensis* (shell length (15.2 ± 6.1) mm; weight (30.6 ± 2.8) g; $n = 20$) were collected in the Sevastopol Bay (Sevastopol) in June 2019. To relieve the stress, caused by catching and transportation, ark clams were kept in tanks with running seawater at the rate of 3–5 L per individual; oxygen concentration was maintained at 6.7–6.8 mg $O_2 \cdot L^{-1}$ for a week. Hypoxia was created by blowing seawater in shellfish tanks with nitrogen gas, until a concentration of 0.4–0.5 mg $O_2 \cdot L^{-1}$ was reached. Ark clams were kept under hypoxia for 24 hours. The control group ($n = 10$) was maintained at an oxygen concentration of 6.7–6.8 mg $O_2 \cdot L^{-1}$.

Hemolymph was collected with a sterile syringe from the extrapalial space. The remaining cells were resuspended in sterile seawater (hemocyte concentration was from $1 \cdot 10^6$ to $2 \cdot 10^6$ cells$\cdot$mL^{-1}). Hemocytes functional characteristics were analyzed on a Beckman Coulter FC 500 flow cytometer. To identify cell types and to assess the DNA content, the prepared hemocyte suspension was stained with a SYBR Green I DNA dye (final concentration in the sample was 10 μmol; incubation period was 30 minutes in the dark). The DNA content in mollusc hemocytes was analyzed on the basis of histograms of the dye fluorescence distribution in the FL1 channel by the Flowing Software 5.2. The DNA content in the cells is on the abscissa axis of the histogram, and number of cells is on the ordinate axis.

The ability of hemocytes to spontaneously produce ROS was assessed by flow cytometry using fluorescence of the 2’,7’-dichlorofluorescein diacetate (DCF-DA) dye: 1 mL of hemocyte suspension was incubated with 10 μL of DCF-DA solution for 30 minutes in the dark. Final dye concentration in the sample was 10 μmol. Dye fluorescence was analyzed in the FL1 channel of the flow cytometer (green area of the spectrum).

Hemocytes mortality rate was determined using the fluorescent dye of propidium iodide (PI). To 1 mL of hemocyte suspension, 10 μL of PI solution (Sigma Aldrich) were added and incubated in the dark for 30 minutes at +4 °C. The ratio of dead hemocytes in total number of hemocytes was estimated by the histograms of PI fluorescence in the FL4 channel (red area of the spectrum).

Protocol calibration for the analysis of the arbitrary cell size was performed by fluorescent microspheres with a diameter of 0.9; 2.0; 4.2; 5.7; and 9.0 μm. Normality of distribution was checked by the Shapiro – Wilk test. The statistical significance of the differences was assessed by the Student’s *t*-test at $p \leq 0.05$. The results are presented as mean values and their errors (mean ± *SE*).

RESULTS

Fluorescence peak of SYBR Green I dye was heterogeneous in hemocytes; it was characterized by a relatively high coefficient of variation (*CV*) both under normoxia [(21.6 ± 1.4) %] and hypoxia [(21.3 ± 1.1) %] (Fig. 1). Hemolymph samples analyzed contained small number of dead cells: their ratio in the control and experimental samples did not exceed 1.5 %.

The analysis of cells distribution by forward and side scattering (FS and SS, respectively) allowed identifying two cell subpopulations with different arbitrary size and granularity level (Fig. 2). The differences were statistically significant. Subpopulation 1 was characterized by high values of arbitrary size [(1282.7 ± 89.3) arbitrary units] and granularity [(199.2 ± 21.7) a. u.]. According to the data of protocol calibration with latex microparticles, average cell diameter was 14–15 μm. Probably, the elongation of cell distribution along the SS axis was due to differences in nucleus size and number of granules in the cytoplasm. Subpopulation 2 was heterogeneous by FS and SS values [(181.8 ± 18.4) a. u.] and had relatively low values of arbitrary size [(392.8 ± 36.1) a. u.], which corresponded to average cell diameter of 7–8 μm. The heterogeneity of cell distribution indicates presence of several subtypes of cells among amebocytes, but it is impossible to identify these subtypes using existing methods. Based on hemocytes classification (Dang et al., 2013), subpopulation 1 was identified as erythrocytes, and subpopulation 2 – as amebocytes.

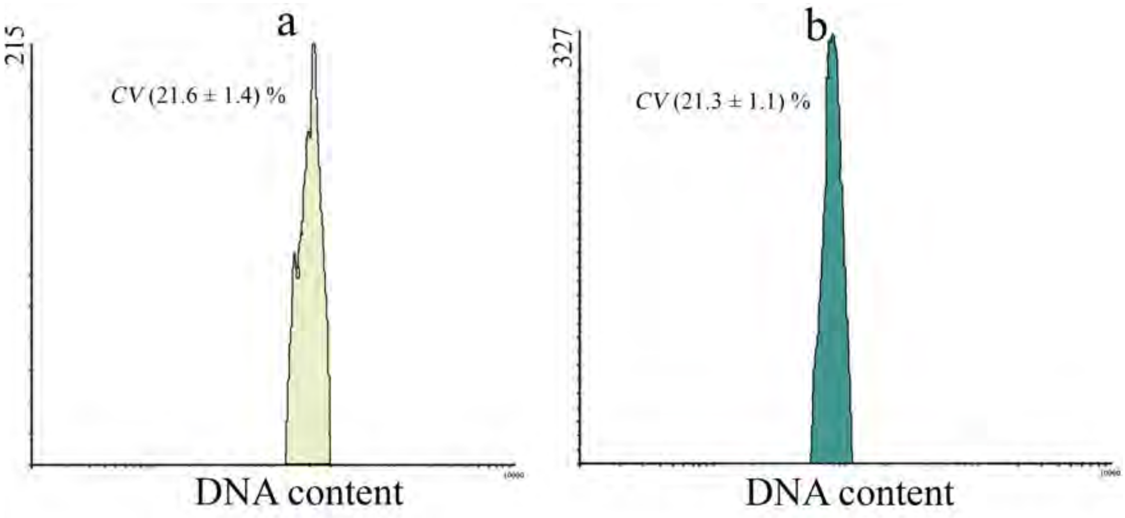

Fig. 1. DNA content in ark clam hemocytes: a – normoxia; b – hypoxia

Cellular composition of *A. kagoshimensis* hemolymph underwent no changes after 24-hour incubation under hypoxia (Fig. 3a). The ratio of granular cells – erythrocytes – in hemolymph of molluscs from the experimental and control groups actually coincided, being (92.6 ± 0.9) and (93.9 ± 1.9) %, respectively. It was similar in relation to agranular cells – amebocytes: (7.4 ± 0.9) % – normoxia; (6.3 ± 1.9) % – hypoxia.

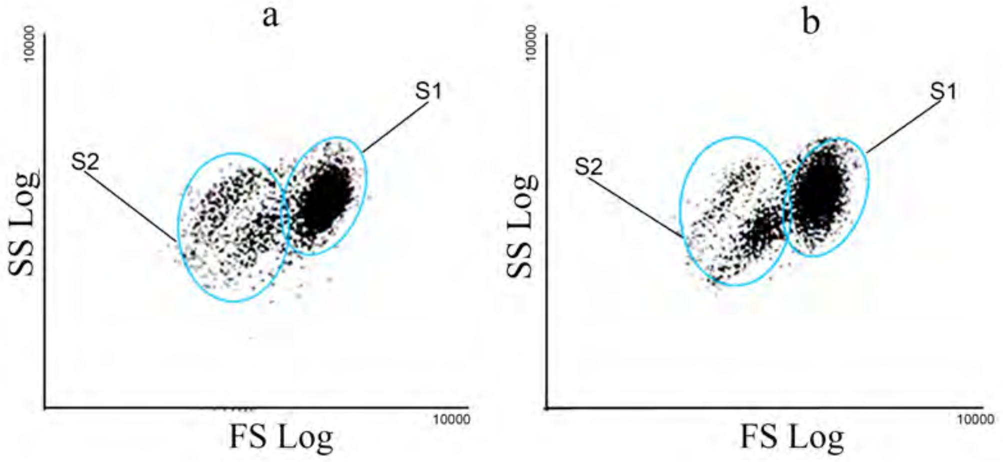

Fig. 2. Cellular composition of ark clam hemolymph. S1 – erythrocytes; S2 – amebocytes; a – normoxia; b – hypoxia

Both cell subpopulations were characterized by pronounced fluorescence of the DCF-DA dye under normoxia: (2439.5 ± 189.0) a. u. for large cells and (4104.3 ± 556.7) a. u. for small ones, which indicates their active production of ROS. Differences in dye fluorescence are not statistically significant. Hypoxia for 24 hours did not lead to statistically significant changes in DCF-DA fluorescence in *A. kagoshimensis* (Fig. 3b).

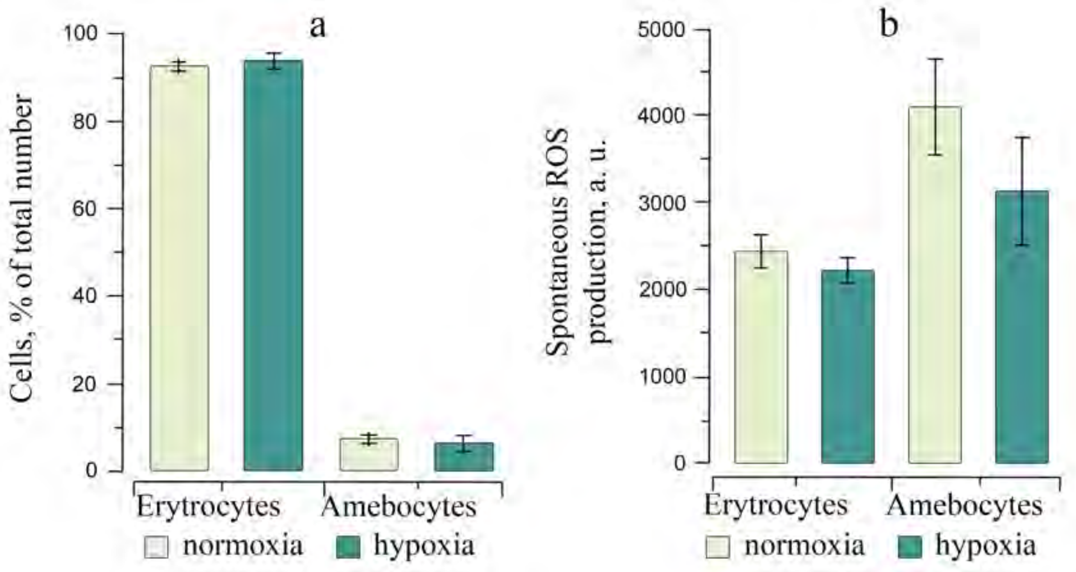

Fig. 3. Hypoxia impact on cellular composition of ark clam hemolymph (a) and hemocytes capacity to produce reactive oxygen species (b)

DISCUSSION

It is known that incubation under oxygen deficiency can induce a decrease in total number of hemocytes (Mydlarz et al., 2006). It was shown as follows: in *Mytilus coruscus* and *Perna viridis*, after incubation under hypoxia, the ratio of dead cells increased (Sui et al., 2016 ; Wang et al., 2012), while in *M. galloprovincialis* their number did not change (Andreyeva et al., 2019). We did not determine absolute number of hemocytes. In our study, hypoxia did not lead to an increase in cell mortality, which probably indicates normal functioning of ark clam hemocytes under short-term hypoxia. The differences can be explained by exposure duration and level of species tolerance to oxygen deficiency.

By flow cytometry, two cell subpopulations with different arbitrary size and granularity level were identified, which, in general, is consistent with data, obtained on other species of bivalve molluscs (Andreyeva et al., 2019 ; Sussarellu et al., 2013 ; Wang et al., 2012). Subpopulation 1 was identified as cells of the granular type – erythrocytes, according to the classification of Dang et al. (2013). Subpopulation 2 was identified as cells of the agranular type – amebocytes (Dang et al., 2013). Both cell subpopulations show pronounced DCF-DA fluorescence, which is not typical for other species of bivalve molluscs (*M. galloprovincialis*, *O. edulis*, and *C. gigas*). It is believed that granular hemocytes are not only responsible for gas transport function, but are also more active, in comparison with agranular ones, in the manifestation of immune reactions: phagocytosis, as well as production of protective peptides and ROS. The generation of an oxidative burst plays an important role in protection against microorganisms, since ROS, in combination with lysosomal enzymes, take part in the phagocytosis reaction, contributing to destruction of opportunistic objects (Sussarellu et al., 2013). The absence of differences in the ability to produce ROS between agranular and granular cells may indicate the absence of functional differentiation of ark clam hemocytes. The latter is consistent with data of previous studies on representatives of Arcidae family (Dang et al., 2013).

The ability to produce ROS directly depends on oxygen content in the environment, since the main source of free radicals is mitochondria and endoplasmic reticulum (Wang et al., 2012). Many authors believe that hypoxia causes a decrease in the ability to produce ROS in other species of bivalve molluscs, suppressing oxidative protection (Sui et al., 2016 ; Widdows et al., 1989). The mechanism, underlying the maintenance of production of ROS by hemocytes under oxygen deficiency, is not fully understood. It is believed that hypoxia can induce oxidation-reduction changes at the level of electron carriers, which leads to the generation of an oxidative burst at the level of mitochondrial complex III (Chandel et al., 2000). Hemoglobin can participate in this (Jiang et al., 2007). It was shown that after deoxygenation, hemoglobin acquires pseudoperoxidase activity, which can catalyze production of superoxide ion (Kawano et al., 2002). Consequently, peroxidase activity of ark clam hemoglobin can induce production of ROS (Bao et al., 2016), maintaining the ability to immune response under hypoxia. In our case, 24-hour hypoxia does not induce changes in DCF-DA fluorescence in *A. kagoshimensis*. The absence of these changes in our work may indicate sufficient compensatory mechanisms of ark clam to maintain the normal functional state of the endoplasmic reticulum and mitochondria under 24-hour hypoxia.

Conclusion. By flow cytometry, two groups of cells were identified in *Anadara kagoshimensis* hemolymph: granulocytes (represented by erythrocytes) and agranulocytes (amebocytes). The ratio of granulocytes in ark clam hemolymph exceeds 90 % of total number of cells. Hypoxia for 24 hours does not lead to changes in cellular composition of ark clam hemolymph. The ability to produce reactive oxygen species and the mortality rate of hemocytes in molluscs of the experimental group remain at the level of control values (normoxia). The results of this study indicate *A. kagoshimensis* tolerance to acute oxygen deficiency.

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ВЛИЯНИЕ СУТОЧНОЙ ГИПОКСИИ НА ФУНКЦИОНАЛЬНЫЕ ПОКАЗАТЕЛИ ГЕМОЦИТОВ *ANADARA KAGOSHIMENSIS* (TOKUNAGA, 1906)

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Марикультурные хозяйства традиционно расположены в прибрежных участках, где моллюски могут подвергаться воздействию гипоксии. Культивирование в условиях дефицита кислорода приводит к снижению темпов роста, вспышкам заболеваний на фермах и массовой гибели моллюсков. Методом проточной цитометрии исследовано влияние краткосрочной гипоксии на функциональные показатели гемоцитов анадары (*Anadara kagoshimensis*). Контрольную группу содержали при 6,7–6,8 мг $O_2 \cdot л^{-1}$, опытную — при 0,4–0,5 мг $O_2 \cdot л^{-1}$. Экспозиция — 24 часа. Содержание кислорода в воде снижали, продувая её газообразным азотом. В гемолимфе моллюска на основании относительного размера и относительной гранулярности идентифицировано две группы гемоцитов: гранулоциты (эритроциты) и агранулоциты (амёбоциты). Эритроциты — преобладающий тип клеток в гемолимфе *A. kagoshimensis*: их доля составила более 90 % от общего числа клеток. Суточная гипоксия не привела к изменениям клеточного состава гемолимфы анадары. Способность к продукции активных форм кислорода и уровень смертности гемоцитов моллюсков экспериментальной группы также остались на уровне контрольных значений. Результаты проведённого исследования свидетельствуют о толерантности анадары к условиям острого дефицита кислорода.

Ключевые слова: анадара, марикультура, проточная цитометрия, гематологические показатели, активные формы кислорода

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BIOGEOCHEMICAL CHARACTERISTICS OF SHALLOW METHANE SEEPS OF CRIMEAN COASTAL AREAS IN COMPARISON WITH DEEP-SEA SEEPS OF THE BLACK SEA

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Methane gas bubble emissions (seeps) are widespread phenomenon in the World Ocean, *inter alia* in Black Sea basin. The relevance of the research of methane seeps is due to their important role as a source of methane – greenhouse and environment-forming gas – for water column and atmosphere. The article presents a comparative analysis of the data from our biogeochemical 10-year studies of shallow gas seeps of the Crimean Peninsula and data on deep-sea gas seeps of the Black Sea. During 10-year period, apart from carrying out hydroacoustic research, the following parameters were determined: bubble gas component composition, methane carbon isotopic composition, microbial community structure of bacterial mats, covering gas bubble emission sites, and gas fluxes from separate seeps. During long-term monitoring, 14 separate gas bubble emission sites were detected and described in Crimean coastal areas; they were located from Cape Tarkhankut in the west of the peninsula to the Dvuyakornaya Bay in the southeast. Crimean coastal seeps were mostly of biogenic origin, with a seasonal type of gas bubble emission. Laspi Bay seeps were classified as emissions of deep gas of thermocatalytic genesis. A significant variation was recorded in values of isotopic composition of methane carbon $\delta^{13}C\text{-}CH_4$ of bubble gas in coastal shallow areas (−94…−34 ‰), which indicates different conditions for bubble gas generation and maturation in seabed sediments. Similar to deep-sea seeps, coastal gas bubble emissions were accompanied by bacterial mats of diverse structure, with different dominating species. As shown, formation of stable bacterial biomass, usually consisting of sulfide- and sulfur-oxidizing bacteria, requires a fluid flux of reduced dissolved gases, while pointwise bubble gas discharge does not provide sufficient concentration gradients and can mechanically disrupt community structure. Various methods were used to estimate the size spectra of bubbles, as well as fluxes from separate seeps. Gas flux values varied from 1.8 $L \cdot day^{-1}$ (the Martynova Bay) to 40 $L \cdot day^{-1}$ (the Laspi Bay). The environment-forming effects, related to gas bubble emission in coastal areas, are discussed: effect of seeps on oxygen conditions in seabed sediments and in water column above gas emission sites, vertical water mixing due to gas lift effect, and fluid discharge at gas emission sites.

Keywords: methane seeps, genesis, isotopic composition, bacterial mats, hydroacoustic methods, environment-forming gas, Crimean shelf, Black Sea

Methane gas bubble emissions are a widespread phenomenon in the World Ocean; according to geological classification, these are “cold seeps” (Judd & Hovland, 2007). The relevance of the research of methane seeps is due to their important role as a source of methane – greenhouse and environment-forming gas – for water column and atmosphere. Assessing the contribution of marine methane to global warming is a challenging task due to the non-uniformity of distribution of these sources and high sporadicity of gas influx. Recently, special attention has been paid to studies of methane of polar regions due to their sensitivity to the processes of global climatic change (Römer et al., 2014 ; Sergienko et al., 2012). Thus, on Siberian Arctic shelf, many gas emission sites have been discovered; they are considered to be related to thawing of permafrost zones and "unpacking" of gas hydrates, buried there. At the same time, new seep sites are found in the southern areas at shallow depths (Pimenov et al., 2013 ; Shik, 2006 ; Sciarra et al., 2019 ; Tarnovetskii et al., 2018).

In the Black Sea, methane seeps were found in all areas from shallow coastal ones to a depth of 2084 m (Egorov et al., 2011, 2003 ; Naudts et al., 2006). Geographic coordinates of more than 4380 sites of bottom bubble methane discharge have been established, and it was shown that separate streams can produce gas fluxes of up to 510 $\text{L}\cdot\text{min}^{-1}$ (Egorov et al., 2011). It was determined that gas bubble streams can significantly affect concentration of dissolved methane in water column and result in gas lift upwelling (Egorov et al., 1999). As shown, production of organic matter by methanotrophic bacteria can reach tens of percent of primary production and affect biological productivity of marine ecosystems (Egorov et al., 2011). The downward transport of bottom water into seabed sediments, resulting from fluid advection, turbulent diffusion, convection, and bioirrigation, is also an important process in "cold seeps", since it transports oxidized components, capable to act as electron acceptors in the early diagenesis, to the reduced environment. At active methane emission sites in the oxic zone of the Black Sea, bacterial mats were found at the seabed, whereas in the deep-sea reduced zone, carbonate structures of up to 4 m high were recorded (Gulin et al., 2005 ; Michaelis et al., 2002), with their genesis being related to the functioning of microbial consortia of methanotrophic archaea and sulfate-reducing bacteria (Boetius et al., 2000). It should be noted that gas emissions can cause potential environmental adverse effects, *e. g.* lead to methane ignition in case of natural disasters or even losing buoyancy of ships (Egorov et al., 2005 ; Shnyukov, 2005).

Interest in the studying shallow seeps is due to the fact that bubble gas, unlike deep-sea one, is not completely dissolved in water column and enters the atmosphere. Another difference between shallow Black Sea seeps and deep-sea ones is an oxidized environment, where gas emission and accompanying biogeochemical processes occur. The geochemical gradients are formed at the interface between seabed sediments and water column, and this establishes conditions for growth of aerobic chemolithotrophs. On the other hand, the sites of bubble gas discharge off the Crimean coast can be potentially hazardous areas, since with an increase in seismic activity of the area, gas emission volume can increase manifold (Shnyukov, 2005).

The aim of the research was to generalize and analyze current data on localization and biogeochemical characteristics of shallow seeps of Crimean coastal areas in comparison with deep-sea seeps of the Black Sea.

MATERIAL AND METHODS

Our data of 2010–2019 was analyzed, and literature data, concerning the problem under study, was reviewed. During 10-year period, the authors carried out hydroacoustic research, isotopic studies of bubble gas, phylogenetic studies of microbial communities of bacterial mats, covering shallow gas emission sites in Crimea, and assessment of gas fluxes from separate seeps.

The search for gas bubble emissions in coastal areas was carried out on small vessels using echo sounders JFC-46, SeaCharter 480 DF, and Lowrance Elite-7 Ti at operating frequency of 200 kHz, as well as a Garmin-300 echo sounder at 210 kHz. To process acoustic data and form a database of electronic echograms of methane seeps, a licensed WaveLens software was used (Artemov, 2006). Visual observations and filming were carried out to assess size ranges of bubbles and fluxes from separate streams, with an underwater controlled TV camera MiniRover MK-II, as well as a GoPro 3/4 video camera, with the help of deep divers and surface-supplied divers (Malakhova et al., 2015). Bubble gas samples for subsequent component and mass spectral analysis were collected with cone traps and stationary pyramidal bottom traps (Malakhova, 2014). Bubble gas fluxes were assessed by various methods: trap one (Malakhova, 2014), as well as active (Malakhova et al., 2015) and passive hydroacoustics (Budnikov et al., 2020). Seabed sediment sampling was carried out by divers directly at gas emission sites using an acrylic ground tube with a vacuum seal, allowing to sample the surface layer of seabed sediments without disrupting its structure. Methane content in bottom water, seabed sediments, and bubble gas was measured using HP-5890 and "Kristall-2000" gas chromatographs (Russia) with a flame ionization detector after phase equilibrium degassing of the samples (Bol'shakov & Egorov, 1987). Methane δ^{13}C value was measured using a TRACE GC gas chromatograph (Germany), combined with a Delta Plus mass spectrometer (Germany). The composition of microbial communities was determined by high-throughput sequencing of 16S rRNA genes (Bryukhanov et al., 2018 ; Pimenov et al., 2018).

RESULTS AND DISCUSSION

Localization and edaphic characteristics of shallow seep sites. Localization of gas bubble emission sites off the Crimean coast is shown in Fig. 1. Coordinates, depth, sediment type, and presence of bacterial mats in the areas of recorded seeps are listed in Table 1.

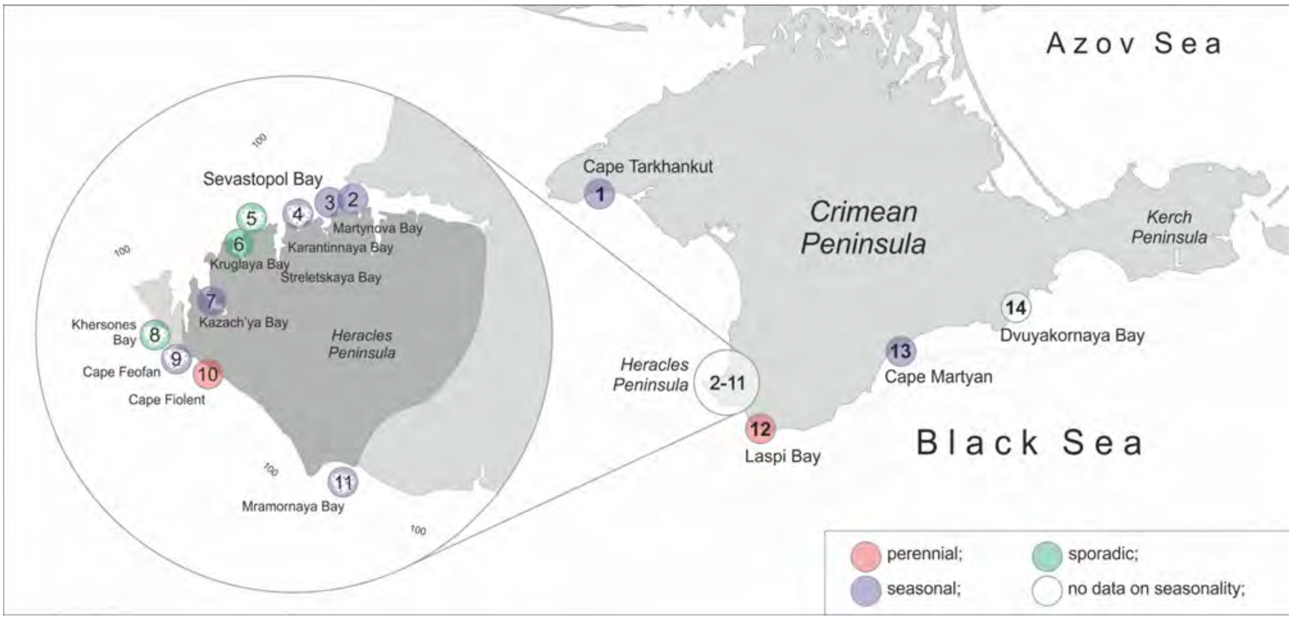

Fig. 1. Map of methane seep sites off the Crimean coast; white markers within the circles indicate presence of bacterial mats at the sites

Table 1. Localization and depth of gas emission sites, seep types, seabed sediment types, and characteristics of microbial communities

Station No.	Research area	Coordinates	Depth, m	Seep type	Sediment type	Microbial communities	References
1	Cape Tarkhankut	45°21′45″N, 32°44′57″E	4	Seasonal	Dark gray / black silt	No data	[49]
2	Sevastopol Bay	44°37′19″N, 33°30′38″E	17–18	Seasonal	Silted sand	No data	[9 ; 19]
3	Martynova Bay	44°36′57″N, 33°30′11″E	5	Seasonal	Gas-saturated detrital sediment	+	[37 ; 38]
4	Karantinnaya Bay	44°36′37″N, 33°29′44″E	0,5	Seasonal	Dark gray reduced sand	++	New data
5	Streletskaya Bay open area	44°36′40″N, 33°27′42″E	10	Sporadic	Dark gray reduced silt under a curd-type white mat	+++	[32]
6	Kruglaya Bay	44°35′57″N, 33°26′49″E	1,5	Sporadic	Detrital sediment, covered with macrophytes	+	New data
7	Kazach’ya Bay	44°33′57″N, 33°24′39″E	1,5	Seasonal	Detrital sediment, covered with macrophytes	+	[38]
8	Khersones Bay	44°33′53″N, 33°23′57″E	5	Sporadic	Local patches of highly reduced silty gas-saturated sand	++	[31 ; 38]
9	Cape Feofan	44°33′34″N, 33°24′01″E	10	Seasonal	Gas-saturated detrital sediment at the bottom of a rocky crack, covered with a biofilm	+++	New data
10	Cape Fiolent	44°31′21″N, 33°28′01″E	2	Year-round	Coarse sand with pebbles	+	New data
11	Mramornaya Bay	44°30′03″N, 33°30′51″E	6	Seasonal	Gas-saturated detrital sediment at the bottom of a rocky depression, covered with a biofilm	++	[42]
12	Laspi Bay	44°25′15″N, 33°42′25″E	3	Year-round	Bedrock, covered with coarse sand	–	[1 ; 16 ; 23 ; 38 ; 40]
13	Cape Martyan	44°30′15″N, 34°14′02″E	2,5	Seasonal	Coarse sand with pebbles	+	[5]
14	Dvuyakornaya Bay	44°59′00″N, 35°21′18″E	2,5–4	No data	Sand, covered with a layer of detritus-bacterial mat	+++	[12 ; 22]

Note:
– indicates, that bacterial mats were not found;
+ indicates traces of bacterial communities in the form of the finest whitish films;
++ indicates distinct mats, covering a small area, with a significant amount of biomass;
+++ indicates abundant bacterial mats, covering a large area.

Association of seeps with seabed morphometric and geological structures. Previously, the relationship between gas bubble emissions and morphometric and geological structures of the seabed was shown (Shnyukov et al., 2005 ; Artemov et al., 2007 ; Römer et al., 2012). For example, for deep-sea seeps of biogenic origin in Kerch area, several patterns of spatial distribution were identified, depending on geomorphological structure. In the area of the upper continental margin, being steep and cut by ravines, seeps were found mainly along the ridges, oriented down the slope. In paleo-Don valley area, the slope was affected by extensive landslides. Gas emissions were associated there with areas of ruptured sediments; they were detected mainly along the ledges of underwater landslides (Zander et al., 2020). Same as in Kerch area, in paleo-Dnieper water area, gas emissions were found mostly at the tops of mountain ranges, formed by sedimentary rocks, on canyon walls, dotted with ditches, and on the slopes of underwater landslides (Artemov et al., 2007). At the same time, a few seeps were detected at the bottom of canyons (Egorov et al., 2011). A thorough analysis of geological patterns of active methane gas emission sites, performed in (Naudts et al., 2006), showed as follows: gas bubble emissions in paleo-Dnieper area do not depend on the system of faults, as stated in (Kruglyakova et al., 2004); they depend on stratigraphic and sedimentary factors.

The seeps, in turn, can affect small-scale morphology of seabed, creating specific structures during gas emission; these occur either as local depressions (pockmarks) or, conversely, as elevations of sediment level due to excessive pressure of gas, accumulating under the surface. Pockmarks are caused by fine sediments dispersion, resulting from gas seepage at fluid and gas bubble emission sites, whereas the increase in sediment level is caused by bubble gas, mainly of microbial origin, accumulating under “dome” surface. Such geomorphological peculiarities of seabed can serve as distinguishing features during prospecting and exploration.

A distinctive feature of coastal shallow seeps is a thin layer of sedimentary material, often being sandy sediments or silted sand (Table 1). Low thickness of these sediments, insignificant amount of organic matter in them, and, as a consequence, low intensity of microbial processes mostly preclude identification of such upper sedimentary layers as genetically related to gas emission occurrences. Despite the biogenic origin of most Crimean coastal seeps, their source was located in the underlying layers, as shown in (Egorov et al., 2012).

In this regard, the Sevastopol Bay stands out, with its morphology, contributing to the accumulation of a sedimentary layer with the thickness ranging from 28 m in the apex to 40 m in the mouth (Bondarev et al., 2015). On the roadstead, more than 20 sites of periodically active gas bubble streams were recorded; most of them were detected in the areas of geodynamic nodes (Eremeev et al., 2007 ; Malakhova et al., 2020a, 2015). Echograms of the seeps, found in this and other areas of the Crimean shelf, are shown in Fig. 2.

It is important to note that not all geological blocks possess the necessary and sufficient conditions for formation of gas and fluid emissions. The main conditions are as follows: lack of degassing of underlying horizons, lack of gas traps, and either low or extremely high degree of crustal fragmentation, preventing formation of visible gas emission fluxes. Thus, repeated hydroacoustic surveys of Karan block seaward part, located along the Georgievsky fault (one of the largest tectonic faults), did not reveal any gas bubble emissions in the area. This is probably due to a combination of the conditions, mentioned above.

In general, the locality of coastal gas emissions, their association with the mapped fault structures, and gas discharge occurrence in the surf zone from the sediments, depleted in organic matter, where stationary anaerobic conditions are not maintained due to mixing, indicate a certain trigger, starting the mechanism of gas bubble emission. Such a trigger can be submarine discharge of fresh groundwater (Kravchenko, 2008 ; Whiticar, 2002), resulting in the rise of fluid fluxes, enriched in nutrients and reduced gases, from the aquifers along the system of micro-faults. At local sites, with especially intense fluxes, strongly reducing conditions are created; these, with temperature along, contribute to development of anaerobic microbial communities and formation of gas-saturated silts.

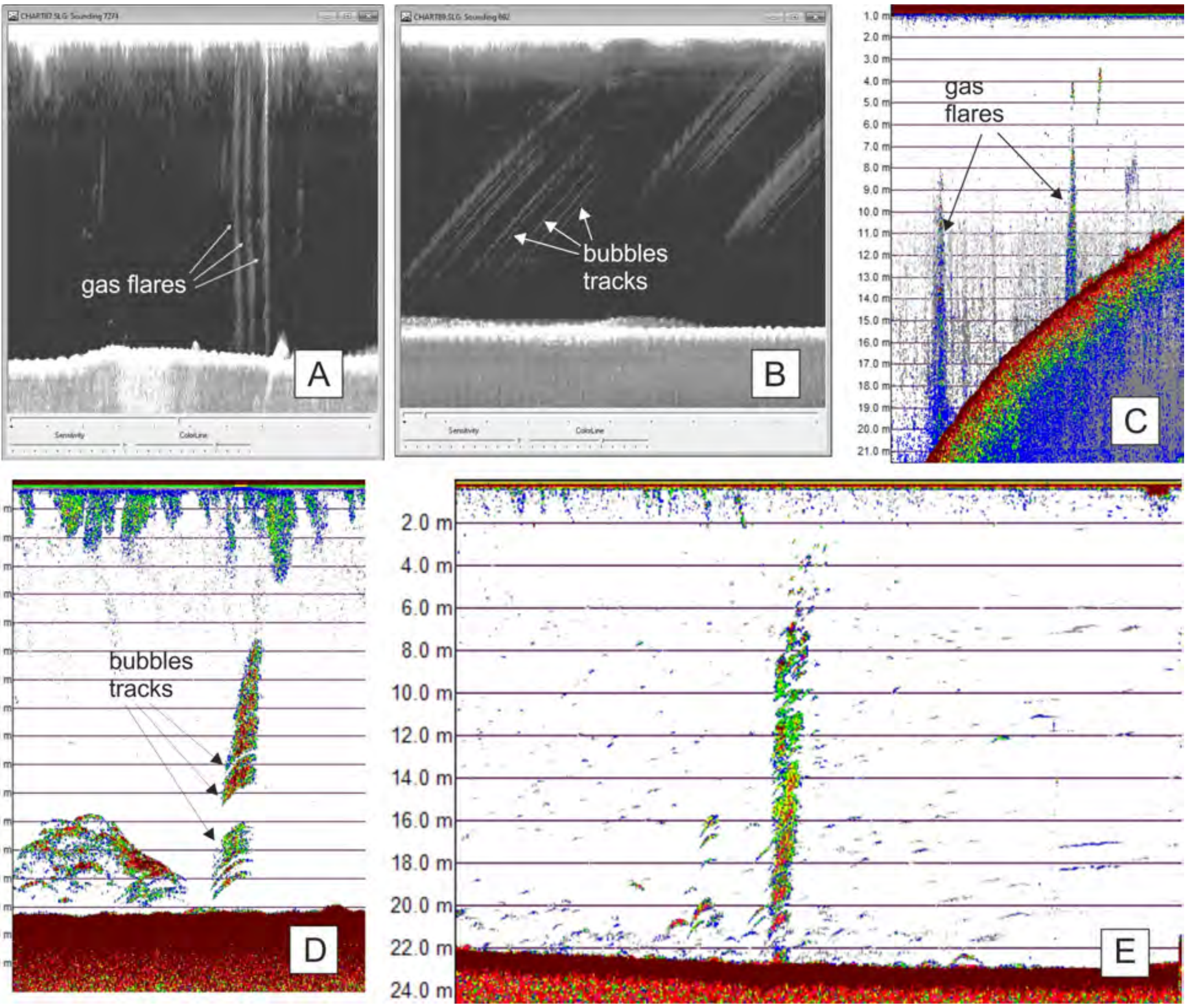

Fig. 2. Echograms of methane gas bubble emissions in Crimean Peninsula coastal areas: A and B – the Sevastopol Bay, October 2011 (SeaCharter 480 DF echo sounder); C – the Laspi Bay, July 2017 (SeaCharter 480 DF); D – sea area of the specially protected natural area “Cape Martyan”, September 2019 (Lowrance Elite-7 Ti echo sounder); E – the Streletskaya Bay, September 2006 (SeaCharter 480 DF)

Frequency and intensity of Crimean coastal seep fluxes. Long-term monitoring of Crimean coastal seeps made it possible to identify several types of their activity. By gas emission frequency, seeps can be divided into seasonal, year-round, and sporadic ones (see Table 1).

The seeps of the Heracles Peninsula and Cape Tarkhankut (Tarnovetskii et al., 2018) were classified as **seasonal** ones, with both gas emissions and adjacent bacterial mats recorded during the warm season: from June to October. The highest spatial density of methane gas bubble emissions was registered in Sevastopol Bay alignment: in the area of about 500 m^2, by the acoustic method, 23 gas flares at depths of 17–18 m were detected in October 2011 (Egorov et al., 2012). Regular acoustic observations, carried out in the area of Sevastopol bays in different years in winter and spring, did not result in revealing presence of gas flares. As shown, these gas emissions were caused by bubble methane discharge into surface horizons of sediments from deeper layers of sedimentary strata and did not originate from the upper sediment layer (Egorov et al., 2012). The potential forecast for methane occurrence transformation in this area is associated with development of a layer of gas-saturated sediments, charged by bay outflows, *inter alia* Chernaya River runoff, and with formation of conditions for all-season activity of methane seeps, similar to that observed in paleo-Dnieper area: one of the world's most intense sites of bubble methane discharge, located 105–125 km northwest of the area described. There, more than 2200 permanent local methane gas bubble emission sites in the depth range of 35–835 m have been detected; their spatial density distribution on the shelf (depths up to 90 m) can reach 300 km^{-2} (Egorov et al., 2011).

The seeps at Cape Fiolent and in the Laspi Bay were classified as **year-round** ones, with gas occurrences recorded both in the warm and cold seasons since 2004 (Shik, 2006) (Fig. 3C). In the Laspi Bay, more than 20 separate gas bubble emission sites were detected both hydroacoustically and visually (Malakhova et al., 2015).

Sporadic seeps were recorded only once (they were not detected during subsequent monitoring). Thus, gas bubble emissions in the Kruglaya and Khersones bays were of irregular character and weak activity. Subsequently, in these areas, local sites of gas-saturated sediments were recorded, with bubble gas, emitting under mechanical action on them (Fig. 3D). In the center of such sites, highly reduced conditions were formed (Eh = −330…−245 mV) in seabed sediments, resulting in active sulfate reduction and methanogenesis, as well as in formation of stable fluxes of hydrogen sulfide and methane into seabed layers of water column (Bryukhanov et al., 2018). It was determined that formation of such areas of gas-saturated sediments (sulfurets) and scale of gas occurrences depend on the ambient temperature. Thus, a decrease was recorded in the area of seabed coverage with sulfurets in the Khersones Bay, as well as their formation only by the end of the summer season of the abnormally cold 2018.

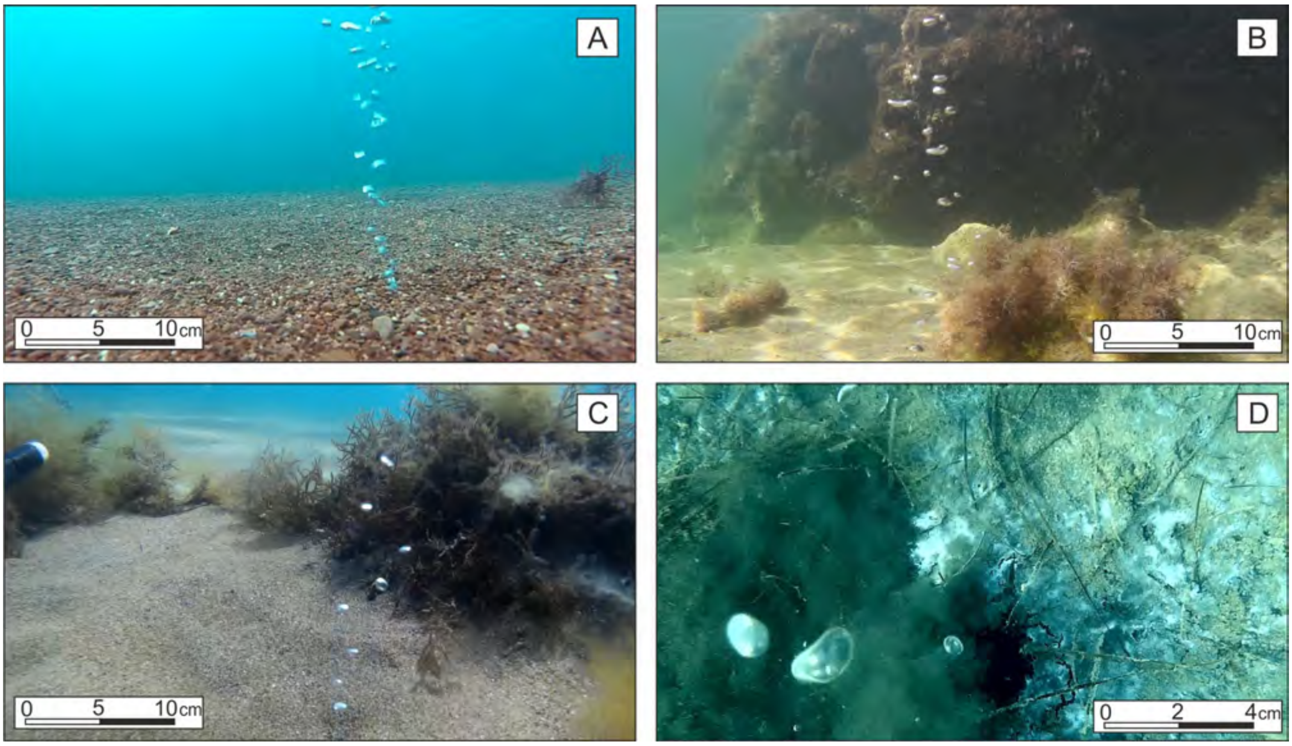

Fig. 3. Underwater photos of gas bubble emissions of Crimean coastal areas: A – Cape Martyan (2019); B – Cape Fiolent (2019); C – the Laspi Bay (2019); D – the Kruglaya Bay (2018)

Quantitative estimates of bubble gas volumes, reaching the surface and entering the atmosphere, are of importance for analyzing the contribution of greenhouse gases to the pool. The assessment was performed in the Martynova Bay in July 2009, using a pyramidal trap to collect bubble gas from seabed sediments. Time dynamics of gas volumes, emerged from Martynova Bay seabed, is shown in Fig. 4. Bubble gas flux during the exposure varied from 1.8 to 14 L·day^{-1}, averaging 4.5 L·day^{-1} (Malakhova, 2014).

In the Laspi Bay, to estimate the daily fluxes of gas bubble emission, a passive acoustic method, proposed by the authors, was used. It is based on the relationship between the frequency of the audio signal, produced by a gas bubble, when emerging from the outlet underwater channel, and bubble size (Budnikov et al., 2020). As shown for two seeps studied, the average bubble diameters were 7 and 5 mm. Given the intensity of bubble gas discharge, the calculated gas flux at these sites was 40 and 6 L·day^{-1}, respectively (Budnikov et al., 2020).

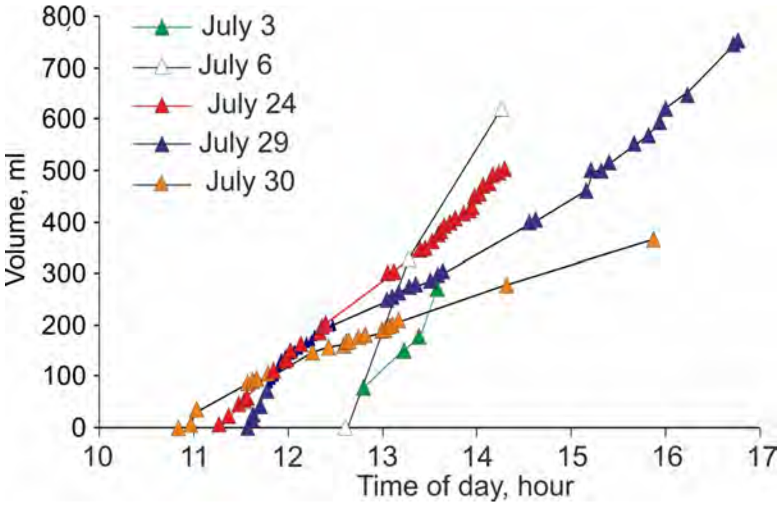

Fig. 4. Volume of bubble gas, emerged from 1 m^2 of seabed sediments in the Martynova Bay, measured by the trap method (July 2009)

Bubble gas hydrocarbon composition and genesis. There are two points of view on the source of methane of gas bubble emission. According to a geological hypothesis, degassing of Black Sea seabed passes through a system of geological faults and/or through an intermediate link: gas hydrate deposits in seabed sediments, resulting from gas discharge of hydrocarbon deposits. The arguments of geological hypothesis supporters are as follows: forecast estimates of high total prospective gas content in the area, presence of detected gas fields on Black Sea shelf in close proximity to methane gas bubble emission sites, and estimates of methane flux from gas emission sites (Shnyukov, 2005 ; Shnyukov et al., 2005). According to a biogenic hypothesis, the source of methane is microbial production in seabed sediments, which is confirmed by carbon stable isotopic composition of Black Sea methane, sampled at many gas-producing areas, such as paleo-Don and paleo-Dnieper ones (Lein & Ivanov, 2009).

Methane predominated in component composition of bubble gas of Crimean coastal areas (Table 2), but its amount was much lower than in bubble gas of deep-sea areas of the Black Sea. In the Kruglaya Bay, for example, methane in bubble gas accounted for 55 %, while at Cape Martyan it accounted only for 38 %. A significant amount of methane homologues was found only in Laspi Bay seeps.

One of the indicators of low-temperature (microbial) or high-temperature genesis of methane is the isotopic composition of its carbon and hydrogen (Whiticar, 1999). Of particular interest is a considerable variation of values in the isotopic composition of methane carbon $\delta^{13}C\text{-}CH_4$ in bubble gas, ranging from −94 to −34 ‰ in shallow coastal areas (Table 2, Fig. 5). A wide range of values is recorded both for gas samples of different areas and for gas samples of one area, *e. g.* for seeps of the Heracles Peninsula (−84…−58 ‰ $\delta^{13}C\text{-}CH_4$). High variability of $\delta^{13}C\text{-}CH_4$ was also established for bubble gas of Cape Tarkhankut: in different years, it ranged from −65 to −48 ‰ (Tarnovetskii et al., 2018). The variation may be explained by several factors. Microbial methane oxidation in the upper horizons of the sedimentary layer results in the change of $\delta^{13}C\text{-}CH_4$ isotopic composition due to selective consumption of methane with the lighter carbon isotope ^{12}C by methanotrophs (Whiticar, 1999). On the other hand, the cause may be the mixing of isotopically heavy gas from deep layers with a near-surface isotopically light gas of microbial origin, so that the isotopic ratio of $\delta^{13}C\text{-}CH_4$ depends on the contribution of these two sources (Pape et al., 2010).

Table 2. Hydrocarbon (CH_4, %; C_1/C_{2+}) and isotopic ($\delta^{13}C\text{-}CH_4$, ‰ PDB; $\delta D\text{-}CH_4$, ‰ SMOW) composition of bubble gas of Crimean coastal areas and deep-sea areas of the Black Sea

Station No.	Research area	CH_4, %	C_1/C_{2+}	$\delta^{13}C\text{-}CH_4$, ‰ PDB	$\delta D\text{-}CH_4$, ‰ SMOW	Reference
Crimean coastal areas						
1	Cape Tarkhankut	n. d.*	n. d.	−65…−48	n. d.	[49]
3	Martynova Bay	57	$1.7 \cdot 10^4$	−56.7	−340.1	our data
4	Karantinnaya Bay	73	$21 \cdot 10^4$	−58	n. d.	-”-
6	Kruglaya Bay	54–55	n. d.	−94.5…−92.4	n. d.	-”-
8	Khersones Bay	66–72	10^4	−84…−58	n. d.	-”-
9	Cape Feofan	68.5–75.5	10^4	−83.4…−67.2	n. d.	-”-
10	Cape Fiolent	n. d.	n. d.	−60.3	n. d.	-”-
11	Mramornaya Bay	n. d.	n. d.	−67.54…−67.1	n. d.	-”-
12	Laspi Bay	92	31	−43…−36	n. d.	-”-
13	Cape Martyan	38	10^4	−89…−84	n. d.	-”-
Deep-sea areas of the Black Sea						
	Vodyanitsky Mud Volcano	99.8	n. d.	−61	−170.8	[46]
	Dvurechensky Mud Volcano	n. d.	n. d.	−66…−62	−209…−185	[29]
	Helgoland Mud Volcano	n. d.	n. d.	n. d.	−217.4	our data**
	Kerch seep	99.2	2372	−66.6	−248	[44]
	Batumi seep	99.9	4267	−53.5	−175	[41]
	Kolkheti seep	n. d.	566	−51.1…−45.0	−192	[43]
	Pechori Mound	n. d.	299	−52.2…−45.8	−224…−216	[43]
	Ordu Ridge	n. d.	1998	−72.6…−68.5	−224.2…−221.5	our data

Note:

[*] – no data;

[**] – data for deep-sea areas of the Black Sea was obtained during the Summer Student Fellowship 2011 at the MARUM University (Bremen, Germany).

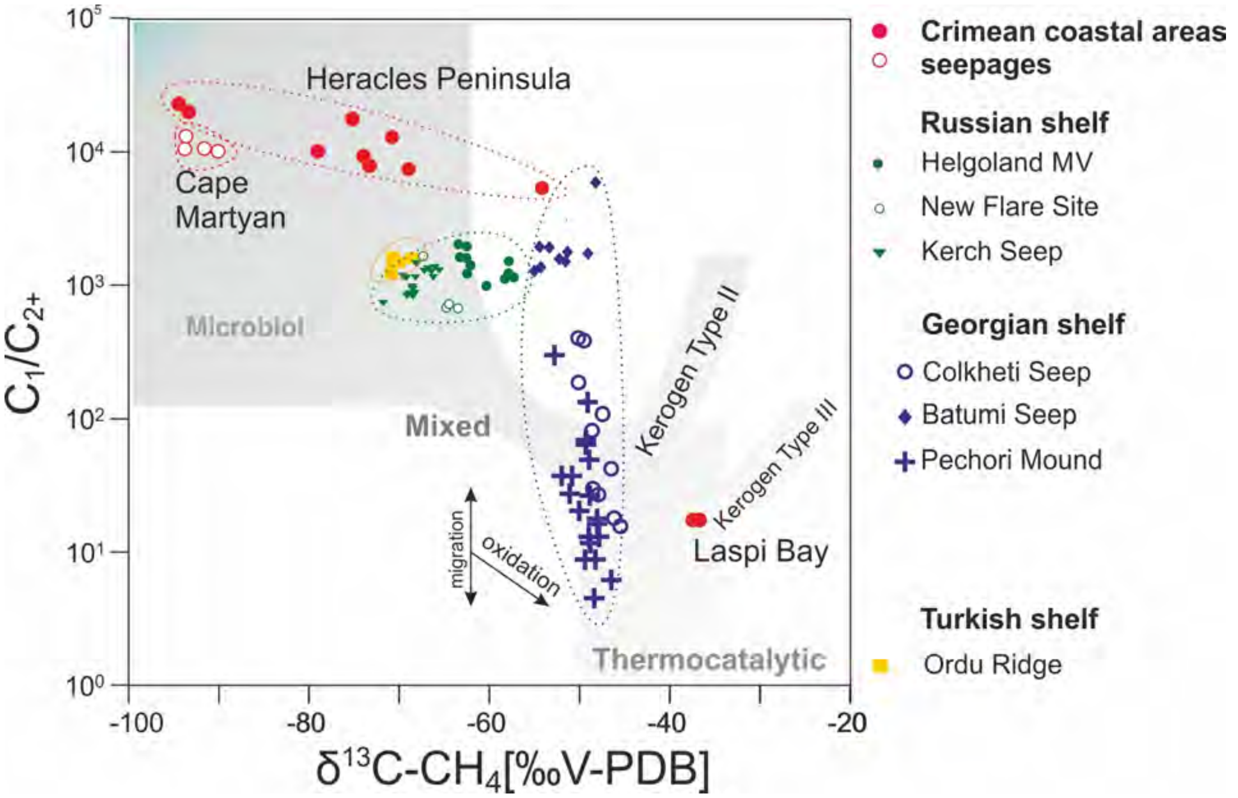

Fig. 5. Bernard diagram (Bernard et al., 1976), illustrating hydrocarbon (C_1/C_{2+}) and isotopic ($\delta^{13}C\text{-}CH_4$) ratios of bubble gas of Crimean coastal areas and deep-sea areas of the Black Sea

The ratio of methane homologues in seeps to the ratio of heavy ^{13}C carbon isotope in them ($C_1/C_{2+}/\delta^{13}C\text{-}CH_4$) shows that methane of most seeps of Crimean coastal areas, except for Laspi Bay gas, is of biogenic origin (Fig. 5). To date, sedimentary strata depth is unclear, from which isotopically light microbial methane is discharged in the areas like Cape Martyan and Cape Fiolent, where the upper layer of sandy sediments is characterized by low methane content and relatively low rates of microbial processes (Malakhova et al., 2015).

Bernard diagram (Fig. 5) illustrates as follows: methane from deep-sea areas of the Russian shelf (Kerch seeps, Sorokin Trough mud volcanoes) is in the sector, which characterizes the gas as microbial one, whereas methane from the Georgian shelf (Pechori Upland, Kolkheti seep) is either within thermocatalytic methane sector or in close proximity to it. The character of values distribution on the diagram indicates as follows: for the gas of the Georgian shelf, the change in its hydrocarbon composition is most likely caused by fractionation of homologues during gas migration through the sediment. For coastal seeps and seeps of the Russian shelf, the isotopic ratio $\delta^{13}C\text{-}CH_4$ changes more, which indicates microbial oxidation.

The ratio of stable hydrogen isotopes δD of methane in combination with $\delta^{13}C$ provides additional information on the type of gas formation (Whiticar, 1999). Values of $\delta D\text{-}CH_4$ of deep-sea gas samples ranged −248…−170 ‰ (see Table 2). According to the typification, proposed in (Whiticar, 1999), biogenic methane, sampled in Kerch seeps area (Russian shelf) and Ordu Ridge area (Turkish shelf), is formed *via* the hydrogenotrophic pathway ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$). This type of methane generation is dominant in marine ecosystems, while the acetoclastic pathway, characterizing the seep in the Martynova Bay (δD-CH_4 −340.1 ‰, see Table 2), is more common in freshwater and in the sediments of highly contaminated areas, where a significant amount of acetate ions accumulates in pore water during organic matter decomposition by primary destructors (Whiticar, 1999).

Bacterial mats and phylogenetic variety of microorganisms of gas emission areas. Long-term observations of the seeps of the Heracles Peninsula and Cape Tarkhankut showed that most of gas emissions, as well as accompanying bacterial mats, were of seasonal type (Table 1). A noticeable bacterial mass was usually accumulated in the second half of June and remained, as a rule, until October. The thickness of seabed coverage with bacterial films varied, increasing towards the end of the summer and sometimes reaching tens of square meters. Photographs of bacterial fouling in the gas-emitting areas of the Crimean coast are shown in Fig. 6.

Fig. 6. Underwater photos of bacterial mats in Heracles Peninsula water area, detected in 2017–2018. A – bacterial biofilm in a rocky depression in the Mramornaya Bay (Pimenov et al., 2018); B – filamentous fouling on living algae thalli, Cape Feofan; C – curd-type bacterial mat on sandy sediments in the Khersones Bay (Bryukhanov et al., 2018); D – massive bacterial mats, detected in Streletskaya Bay open area in July 2018 (Budnikov et al., 2019)

It was noted that in areas of intense gas bubble emissions (the Laspi Bay, Cape Fiolent), there were either no bacterial films, or only their traces, in the form of the finest whitish films (Table 1). Apparently, formation of a stable bacterial biomass requires a stable fluid flux, containing dissolved gases, with obligatory presence of reduced sulfur, same as in the case of mats in the Mramornaya Bay (Fig. 6A). Here, in rocky depressions, filled with dispersed detrital gas-saturated sediments, white bacterial mats with a characteristic cotton-like structure developed, with the basis, formed by sulfide-oxidizing epsilonproteobacteria of the genus *Arcobacter* (Pimenov et al., 2018). At the same time, bubble gas, emitting pointwise from the seabed, was not creating sufficient concentration gradients and could mechanically disrupt the structure of the forming community. Hence, for the entire observation period (since 2012), we did not register any biofilm formation in Laspi Bay gas emission area. However, by high-throughput 16S rRNA gene sequencing in the surface horizons of Laspi Bay sediments, we identified sulfur-oxidizing epsilonproteobacteria of the family Thiovulaceae (8.2 %). Deeper, in the horizons of 10–15 cm, archaea of the phylogenetic cluster ANME-2a/b (19.2 %) and bacteria of the family Desulfobacteraceae (12.2 %), mediating anaerobic methane oxidation, were the most numerous ones. Bacterial mats in the Khersones Bay, represented by white flocs, developing on gas-saturated sands, had the largest coverage area (Fig. 6C, D). According to the results of electron microscopic and molecular biological studies, the basis of bacterial mats was formed by filamentous sulfur bacteria of the family Thiotrichaceae and epsilonproteobacteria of the family Helicobacteraceae (Bryukhanov et al., 2018).

Environment-forming effect of gas bubble emissions. The environment-forming and ecological effects, related to Black Sea methane seeps, include the effect of bubble methane flux on water gas composition, water hydrochemical structure, and formation of carbonate edifices, as well as the effect of methane seeps on microbial processes and microbial community structure (Egorov et al., 2011). The environment-forming roles of deep-sea and shallow seeps are similar, but there are some differences.

The defining difference between shallow and deep-sea seeps of the Black Sea is an oxidized environment, where gas emission and accompanying biogeochemical processes occur. In hydrogen sulfide zone, gas emission sites are a kind of oases of life due to matter and energy properties of methane for microbial trophic chains, whereas in coastal oxidizing conditions, on the contrary, they are zones of inhibition. Thus, as shown in (Ivanova, 2017), in seep areas at Cape Tarkhankut and in the Dvuyakornaya Bay, the meiofauna was characterized by considerably lower abundance and differed in taxonomic composition from the population of surrounding sands due to hydrogen sulfide contamination and acute hypoxia / anoxia.

It has been established that not only in seabed sediments, but also in water above gas emission sites, a significant decrease in O_2 concentration can be observed. Vertical profiling of water column above gas emission site at Cape Feofan showed a notable decrease in O_2 content in the seabed water layer, with a minimum reaching 0.2 $\mathrm{mg \cdot L^{-1}}$ (Malakhova et al., 2020a). Minimum salinity values were revealed as well, which may indicate seep-related pulse-wise freshwater discharge. It was recorded that these patterns of distribution of hydrological indicators are determined by presence of gas bubble emissions and geomorphology of the area studied. The key factors of the intensive development of hypoxic phenomena in the seabed water layer are the high degree of isolation of underwater canyons, representing Cape Feofan underwater relief, and, as a result, the slowed down water exchange with the open area (Malakhova et al., 2020a).

Previously, it was calculated that energy dissipation above gas bubble emission sites can be the cause of a micro-upwelling effect (Egorov et al., 2011). Thus, the effect of bubble flux was shown on temperature distribution in water column above gas emission sites in paleo-Dnieper area (Egorov et al., 2011).

In 2018, the authors of this article carried out an experiment in coastal water area, aimed at simulating the effect of gas bubble emissions on stratified layers of water column (Ivanova et al., 2018). The results showed as follows: the generated gas stream, with the flux of several liters per minute, transports colder layers of water from the seabed almost to the surface (Ivanova et al., 2018).

Of particular interest is the assessment of the contribution of fluid emission at methane gas emission sites to total gas flux. It should be noted that the quantitative data on methane influx from seabed sediments in the form of fluid discharge or diffusion fluxes is still not sufficiently complete. Data of direct *in situ* measurements is particularly scarce, which is due to the difficulty of experiments in sea area.

In 2019, the trap method was used to measure methane fluid discharge from seabed sediments in the Laspi Bay near the area of gas bubble emissions; the values reached 74.3 mmol·m^{-2}·day^{-1} (Malakhova et al., 2020b). According to the calculations, the integral fluid discharge in the Laspi Bay can be comparable to the flux from gas bubble emissions, since the maximum fluid flux per 1 m^2 of the seabed is only 3.5 times lower than the flux from a single seep (Malakhova et al., 2020b).

Conclusion. A comparative analysis of our data on the biogeochemical research of shallow seeps of the Crimean Peninsula and data on deep-sea gas bubble emissions of the Black Sea is presented. It is shown that gas bubble emissions are widespread in Crimean coastal areas: they are located within the territory from Cape Tarkhankut in the west of the peninsula to the Dvuyakornaya Bay in the southeast.

Long-term monitoring of the activity of coastal gas emissions made it possible to distinguish seasonal, year-round, and sporadic seeps. Most of Crimean coastal seeps were of a biogenic origin, with the source of gas emission both in the upper layers of seabed sediments and much deeper. Methane predominated in component composition of bubble gas in Crimean coastal areas. However, compared to its content in deep-sea areas of the Black Sea, exceeding 99 %, its content in coastal gas emissions was significantly lower (55 % in the Kruglaya Bay and 38 % near Cape Martyan). A significant number of homologues were found only in Laspi Bay seeps, classified, according to their isotopic and hydrocarbon composition, as those producing gas of thermocatalytic origin, in contrast to other seeps, where the main amount of methane is formed as a result of methanogenic archaea activity. A significant variation was observed in values of isotopic ratio of methane carbon $\delta^{13}C\text{-}CH_4$ of bubble gas in shallow coastal areas (from −94 to −34 ‰); this confirms the assumption about different conditions for bubble gas generation and maturation of seabed sediments, as well as different rates of microbial oxidation and methane formation at separate gas bubble emission sites.

Like deep-sea seeps, coastal ones were often accompanied by bacterial mats. In the areas with bubble gas, freely emitting from the sand, there were either no bacterial films, or only their traces, in the form of the finest whitish films. It was shown as follows: formation of stable bacterial biomass, usually consisting of sulfide- and sulfur-oxidizing bacteria, requires a fluid flux of reduced dissolved gases, while pointwise bubble gas discharge does not provide sufficient concentration gradients and can mechanically disrupt the structure of the forming community.

Various methods were used to estimate the size spectra of bubbles, as well as fluxes from separate streams. Gas flux values varied from 1.8 L·day^{-1} (the Martynova Bay) to 40 L·day^{-1} (the Laspi Bay).

The environment-forming effects, related to gas bubble emissions in coastal areas, are described: oxygen conditions in both seabed sediments and water column above gas emission sites, vertical water mixing due to gas lift effect, and volumes of fluid discharge at gas emission sites.

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БИОГЕОХИМИЧЕСКИЕ ХАРАКТЕРИСТИКИ МЕЛКОВОДНЫХ СТРУЙНЫХ МЕТАНОВЫХ ГАЗОВЫДЕЛЕНИЙ В ПРИБРЕЖНЫХ РАЙОНАХ КРЫМА В СРАВНЕНИИ С ГЛУБОКОВОДНЫМИ СИПАМИ ЧЁРНОГО МОРЯ

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Струйные метановые газовыделения (сипы) — широко распространённый феномен в Мировом океане, в том числе в бассейне Чёрного моря. Актуальность исследований метановых сипов обусловлена их важной ролью в качестве источника метана как парникового и средообразующего газа для водной толщи и атмосферы. В работе представлен сравнительный анализ данных собственных биогеохимических исследований мелководных сипов Крымского полуострова, охватывающих последние десять лет, и материалов, посвящённых глубоководным газовыделениям Чёрного моря. В этот период были проведены поисковые гидроакустические исследования, выявлен компонентный состав пузырькового газа, измерен изотопный состав углерода метана, а также молекулярно-биологическими методами определена структура микробного сообщества бактериальных матов, покрывающих площадки газовыделений, и выполнена оценка газовых потоков от отдельных сипов. В течение многолетнего мониторинга обнаружено и описано 14 отдельных газовыделяющих площадок в прибрежных районах Крыма, которые располагались от мыса Тарханкут на западе полуострова до бухты Двуякорная на юго-востоке. Превалирующая часть прибрежных сипов Крыма имела биогенную природу и сезонный характер газовыделений. К глубинному газу термокаталитического генезиса отнесены сипы в бухте Ласпи. Наблюдался значительный разброс величин изотопного состава углерода метана $\delta^{13}C\text{-}CH_4$ пузырькового газа прибрежных мелководных районов (−94…−34 ‰), что указывает на разные условия его генерации и созревания в донных отложениях. Так же, как и глубоководным сипам, прибрежным струйным газовыделениям сопутствовали бактериальные маты разной структуры с различными доминирующими видами. Показано, что для формирования устойчивой бактериальной биомассы, основу которой составляли, как правило, сульфид- и сероокисляющие бактерии, необходим флюидный поток восстановленных растворённых газов, тогда как точечная разгрузка пузырькового газа не обеспечивает достаточных градиентов концентрации и может механически разрушать структуру образующегося сообщества. Различными методами сделаны оценки размерных спектров пузырьков и потоков от отдельных струй. Диапазон значений газового потока варьировал от 1,8 л·сут$^{-1}$ (бухта Мартынова) до 40 л·сут$^{-1}$ (бухта Ласпи). Проанализированы средообразующие эффекты, связанные с выделением пузырькового газа в прибрежных районах: влияние сипов на кислородный режим в донных осадках и в толще воды́ над точками газовыделений; вертикальное перемешивание вод за счёт газлифтового эффекта; флюидная разгрузка на площадках струйных газовыделений.

Ключевые слова: метановые сипы, генезис, изотопный состав, бактериальные маты, гидроакустические методы, средообразующий газ, крымский шельф, Чёрное море

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HEAVY METALS IN SURFACE WATER OF THE ATLANTIC SECTOR OF THE ANTARCTIC DURING THE 79TH CRUISE OF THE RESEARCH VESSEL “AKADEMIK MSTISLAV KELDYSH”

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Relevance of monitoring heavy metals content in the water of the Atlantic sector of the Antarctic is due to the need for a current assessment of quality of the marine environment for making responsible decisions on the conservation of marine living resources in this unique area of the World Ocean. The aim of the study was to obtain new data on levels and spatial distribution of concentrations of trace elements, mainly heavy metals, in surface water. Sampling of surface seawater was carried out during the Antarctic expedition of the 79th cruise of the RV “Akademik Mstislav Keldysh” at 21 stations in the area of the Drake Passage, the Bransfield Strait, and the Antarctic Sound, as well as in Weddell and Scotia seas. Extracting and concentrating of dissolved form of 13 trace elements (Be, Se, Sb, Tl, V, Pb, Cd, Cu, Zn, Ni, Mo, Co, and Fe) were performed using sodium diethyldithiocarbamate and carbon tetrachloride (CCl_4). The elements were measured by mass spectrometry. Among all trace elements content, only Mo concentration in seawater at 9 stations, located in the Drake Passage, the Bransfield Strait, northern Weddell Sea, and off the southern coast of Tierra del Fuego Island, exceeded 1.2–2.8 times maximum permissible concentration of trace elements in fishery water bodies of the Russian Federation (MPC_F). According to international regulatory legal acts, such as “Dutch sheets”, there were single cases of exceeding MPC (maximum permissible concentration under short-term exposure) for Cd and Zn, as well as exceeding TV (target value under chronic exposure) for Cu, Pb, Cd, Zn, Se, and Co at several stations. The research has shown as follows: despite limited anthropogenic pressure on this area of the Southern Ocean, in seawater of some regions of the Atlantic sector of the Antarctic, increased concentrations of several trace elements, *inter alia* heavy metals, are recorded. Further study of the sources of trace elements intake and the peculiarities of their distribution in seawater of the Atlantic sector of the Antarctic is required in order to account for ongoing processes, take measures for rational management, and provide ecologically acceptable use of natural resources in the Antarctic.

Keywords: heavy metals, surface seawater, Atlantic sector of the Antarctic

The 79th cruise of the RV “Akademik Mstislav Keldysh” to the Atlantic sector of the Antarctic was held on 30.11.2019–08.05.2020 (Morozov et al., 2020) within the framework of international obligations of the Russian Federation as a party to the Antarctic Treaty (Dogovor ob Antarktike…., 2020) and the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR)

(Commission for the Conservation…, 2020). Expedition was organized and managed by P. P. Shirshov Institute of Oceanology. During the cruise, several fundamental tasks, assigned to the Russian scientists in a number of strategic documents, were solved (Strategiya nauchno-tekhnologicheskogo razvitiya…, 2016 ; Strategiya razvitiya morskoi deyatel'nosti…, 2010 ; Strategiya razvitiya deyatel'nosti…, 2010). They indicate the need to obtain new results for a comprehensive assessment of the state of Antarctic ecosystems, as well as the effect of climate change and other factors, including limited anthropogenic impact, on them.

Trace elements are known to be among the most important components of the marine environment, playing an essential role in the functioning of living organisms (Bowen, 1988 ; Mertz, 2013). However, exceeding a certain limit for the content of these substances can lead to toxic effects (Fuentes et al., 2016 ; Rainbow, 2002). For example, V, Co, Ni, Cu, and Zn are moderately hazardous toxicants, while Cd and Pb are highly hazardous ones (Kharakteristika zagryaznyayushchikh veshchestv…, 2016). Hence, these chemical elements and a number of other heavy metals are included in the list of substances, the amount of which in natural environments, *inter alia* oceanic water and seawater, is subject to regular monitoring (Klenkin et al., 2007). The issue of studying both levels and redistribution of heavy metals is also relevant for the Antarctic. According to international agreements (Dogovor ob Antarktike.…, 2020 ; Commission for the Conservation…, 2020), limited anthropogenic activity is allowed on the Antarctic continent and in the waters of the Southern Ocean. This applies to members of research expeditions, station personnel, and crews of ships, fishing for seafood and ensuring tourism functioning. Previous studies have shown that an increase in heavy metals concentration in Antarctic waters can also result from natural processes (Ahn et al., 1996, 2004 ; Samyshev & Minkina, 2019). They include, in particular, the washout of tectonic elements from the mainland as a result of the increased glaciers melting, caused by global warming in recent decades (Ahn et al., 1996, 2004 ; Samyshev & Minkina, 2019 ; Sanchez et al., 2019), and the rise of deep waters (Honda et al., 1987).

The relevance of heavy metals monitoring in the waters of the Atlantic sector of the Antarctic is mainly due to the fact as follows: in this area, favorable conditions are formed for stable aggregations of Antarctic krill (*Euphausia superba* Dana, 1852), with its highest biomass density in the Southern Ocean (Bykova et al., 2004 ; Samyshev & Minkina, 2019). This area is currently the site of krill fishery. Many representatives of aquatic biota, including krill, accumulate heavy metals to concentrations, being many times higher than their content in water (Polikarpov et al., 1986 ; Chudinovskikh, 2016 ; Honda et al., 1987 ; Mertz, 2013). At the same time, they can not only be affected by toxicants themselves (especially at their most sensitive stages of development: eggs, larvae, and juveniles), but also serve as a "transport link" for toxic substances along trophic chains up to the human, consuming seafood (Casas et al., 2008 ; Samyshev & Minkina, 2019). Therefore, monitoring of concentration levels of heavy metals in the waters of the Southern Ocean is of considerable scientific and practical interest.

The aim of this study was to obtain new data on the levels and spatial distribution of trace elements, mainly heavy metals, in surface water in order to assess the current quality of the marine environment of natural complexes in the Atlantic sector of the Antarctic.

MATERIAL AND METHODS

Research areas and material. Seawater sampling, aimed at determining the content of dissolved forms of trace elements, *inter alia* heavy metals, was carried out from 10.02.2020 to 01.03.2020 during the Antarctic expedition of the 79th cruise of the RV "Akademik Mstislav Keldysh" in the Atlantic sector of the Antarctic at 21 stations in the area of the Drake Passage, the Bransfield Strait, and the Antarctic Sound, separating the Antarctic Peninsula from the Joinville group of islands, as well as in the Weddell and Scotia seas (Fig. 1). Coordinates of sampling stations and characteristics of the marine environment (temperature and salinity) are shown in Table 1.

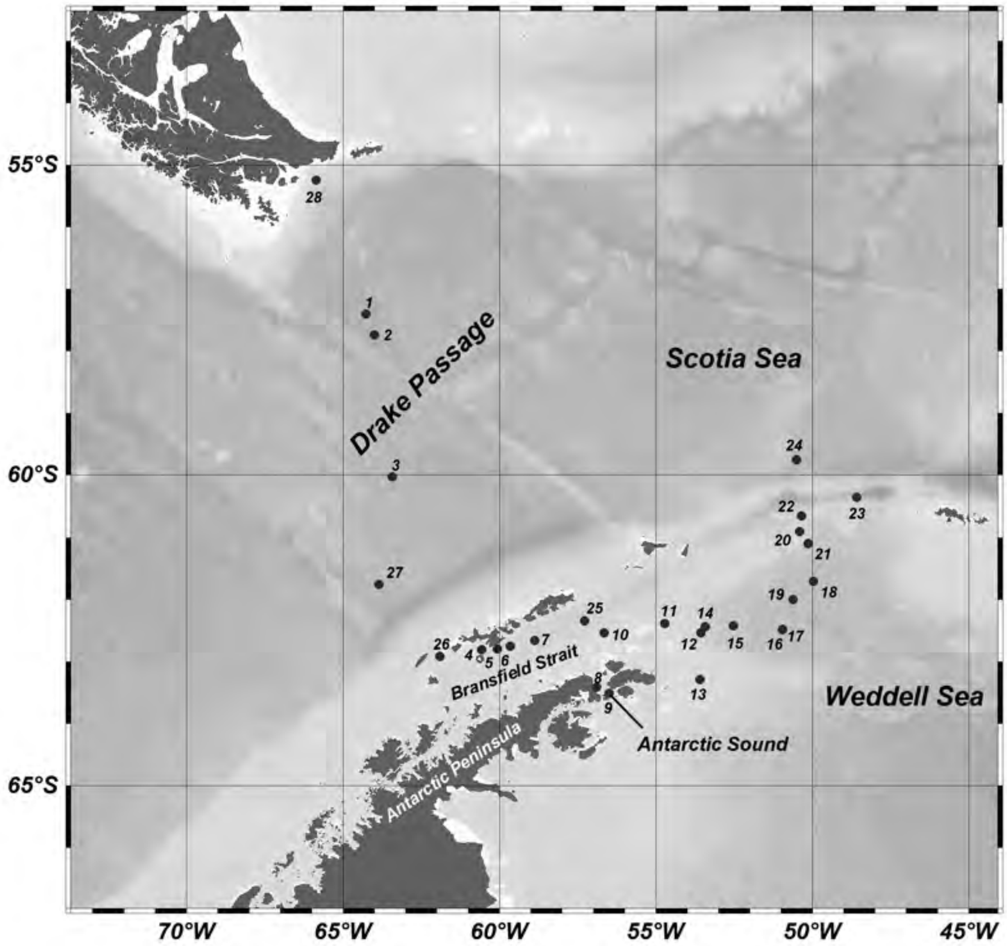

Fig. 1. Map of studied area and location of sampling stations

Methods for trace elements determination. To determine trace elements, including heavy metals, 21 samples of surface (0–5 m) seawater were collected from the marine environment of the studied area (Fig. 1, Table 1). Water temperature and salinity (see Table 1) were measured automatically by a SBE 911plus CTD probe with a cassette, containing 21 5-L bottles. In water samples, 13 trace elements were identified: Be, Se, Sb, Tl, V, Pb, Cd, Cu, Zn, Ni, Mo, Co, and Fe. Samples were processed in the onboard laboratory in accordance with the guiding document RD 52.10.243-92 (Rukovodstvo po khimicheskomu analizu…, 1993). Sample preparation method was based on extracting and concentrating trace amounts of metals using sodium diethyldithiocarbamate as a complexing agent and carbon tetrachloride (CCl_4) as extractant, with the following acid re-extraction. Nitric acid solutions of the extracts were delivered to the onshore laboratory; measurements of the studied elements were performed at IBSS "Spectrometry and Chromatography" core facility, using inductively coupled plasma mass-spectrometer PlasmaQuant MS Elite (Analytik Jena AG) (GOST R 56219-2014. Water…, 2015 ; Mass-spektrometriya…, 2014). The spectrometer was calibrated with a standard solution "Multi-element calibration standard IV-28, HNO_3/HF, 125 mL" (Inorganic Ventures). Measurement mode on the mass-spectrometer included 7 replicates of 10 scans for each identified element from 10,000 to 100,000 µs, depending on its expected concentration. Calculation and registration of measurement results were carried out in accordance with the all-union state standard GOST R 56219-2014 and guiding document RD 52.10.243-92 (GOST R 56219-2014. Water…, 2015 ; Rukovodstvo po khimicheskomu analizu…, 1993). The average relative determination error was not higher than ± 10 %.

Table 1. Metadata of sampling stations

Station number	Sampling date	Coordinates		Ocean depth, m	Water temperature, °C	Salinity, PSU
		S	W			
2	10.02.2020	57°44.4074′	63°59.9839′	4130	+8.62	33.92
3	11.02.2020	60°01.5902′	63°25.3325′	3778	+5.73	33.63
4	12.02.2020	62°48.3626′	60°33.7133′	373	+3.16	34.11
7	12.02.2020	62°39.3628′	58°52.8365′	1574	+4.19	34.14
8	13.02.2020	63°24.4219′	56°53.5885′	187	–0.91	34.28
9	13.02.2020	63°30.5974′	56°29.7344′	736	+0.64	34.27
10	14.02.2020	62°32.0215′	56°39.2660′	345	+1.64	34.04
11	14.02.2020	62°23.0395′	54°43.0033′	362	–0.72	34.21
12	15.02.2020	62°32.2922′	53°33.7689′	1030	–0.67	34.32
13	15.02.2020	63°17.4354′	53°35.9791′	362	+0.25	33.88
14	16.02.2020	62°26.0611′	53°25.7918′	1383	–0.02	33.66
16	17.02.2020	62°29.0724′	50°57.3809′	3290	–0.42	33.27
18	18.02.2020	61°42.2470′	49°58.2523′	1743	+0.26	32.74
19	19.02.2020	61°59.7085′	50°37.6094′	700	+1.00	33.97
20	19.02.2020	60°53.9809′	50°24.0082′	940	+0.89	34.03
21	20.02.2020	61°05.5251′	50°08.3302′	2766	+0.31	32.99
24	24.02.2020	59°45.2400′	50°30.7537′	3787	+2.94	34.12
25	26.02.2020	62°20.7280′	57°16.5980′	1331	+3.22	34.12
26	27.02.2020	62°55.1235′	61°54.2073′	800	+2.61	33.8
27	27.02.2020	61°45.3571′	63°50.9526′	3622	+4.17	33.64
28	28.02.2020	55°14.6157′	65°51.9639′	1098	+10.64	33.46

RESULTS

Among the studied heavy metals, as already noted, the most toxic pollutants are copper, zinc, cadmium, and lead (Klenkin et al., 2007). The results of measuring their concentrations are shown in Fig. 2.

Trace elements beryllium and thallium are also highly toxic substances (Kharakteristika zagryaznyayushchikh veshchestv…, 2016), but their concentrations in seawater samples from all the stations were very low: they did not exceed 0.005 µg·L^{-1} (Be) and 0.001 µg·L^{-1} (Tl). The measured values were 60 times lower than maximum permissible concentration (hereinafter MPC) of trace elements in fishery water bodies of the Russian Federation (MPC$_F$) for Be and 1000 times lower than MPC in water bodies for drinking and cultural-domestic water use for Tl (Kharakteristika zagryaznyayushchikh veshchestv…, 2016). Molybdenum, cobalt, and nickel are toxic substances, but they are also characterized as having carcinogenic and mutagenic effects on living organisms (Kharakteristika zagryaznyayushchikh veshchestv…, 2016). The results of determining these trace elements distribution are shown in Fig. 3.

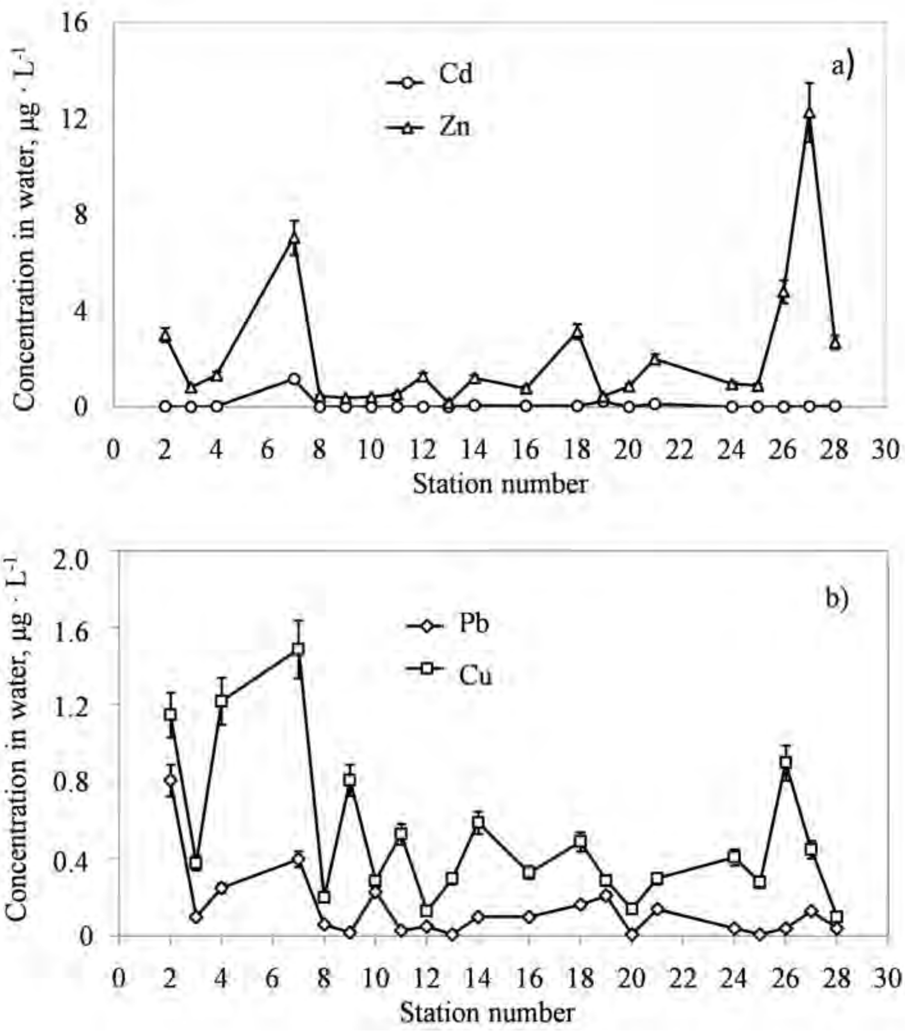

Fig. 2. Concentration of Cd and Zn (a) and Pb and Cu (b) in surface seawater in the studied areas of the Antarctic

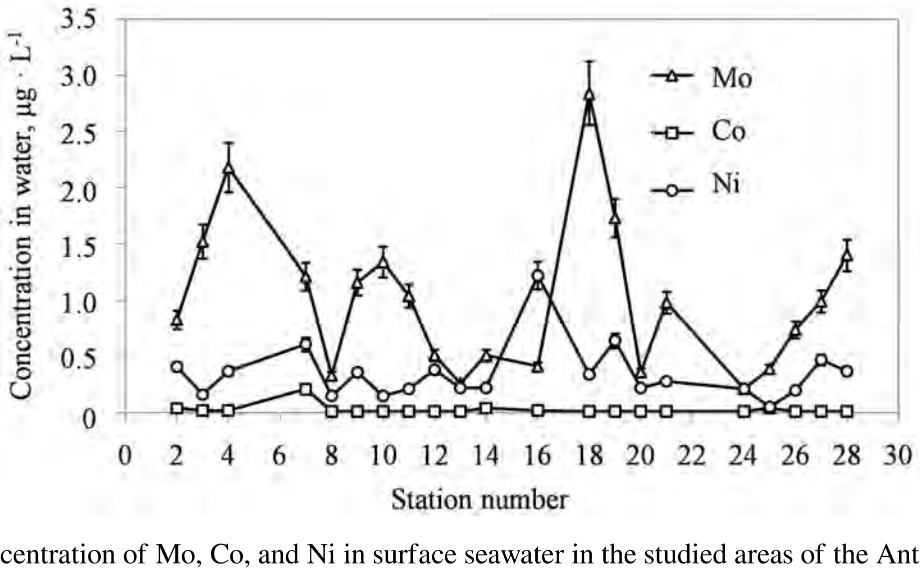

Fig. 3. Concentration of Mo, Co, and Ni in surface seawater in the studied areas of the Antarctic

Among trace elements studied, there were toxic substances selenium and vanadium, as well as a toxic hazardous substance antimony and less toxic iron (Kharakteristika zagryaznyayushchikh veshchestv…, 2016). Data on changes in concentrations of these chemical elements in surface seawater are shown in Fig. 4.

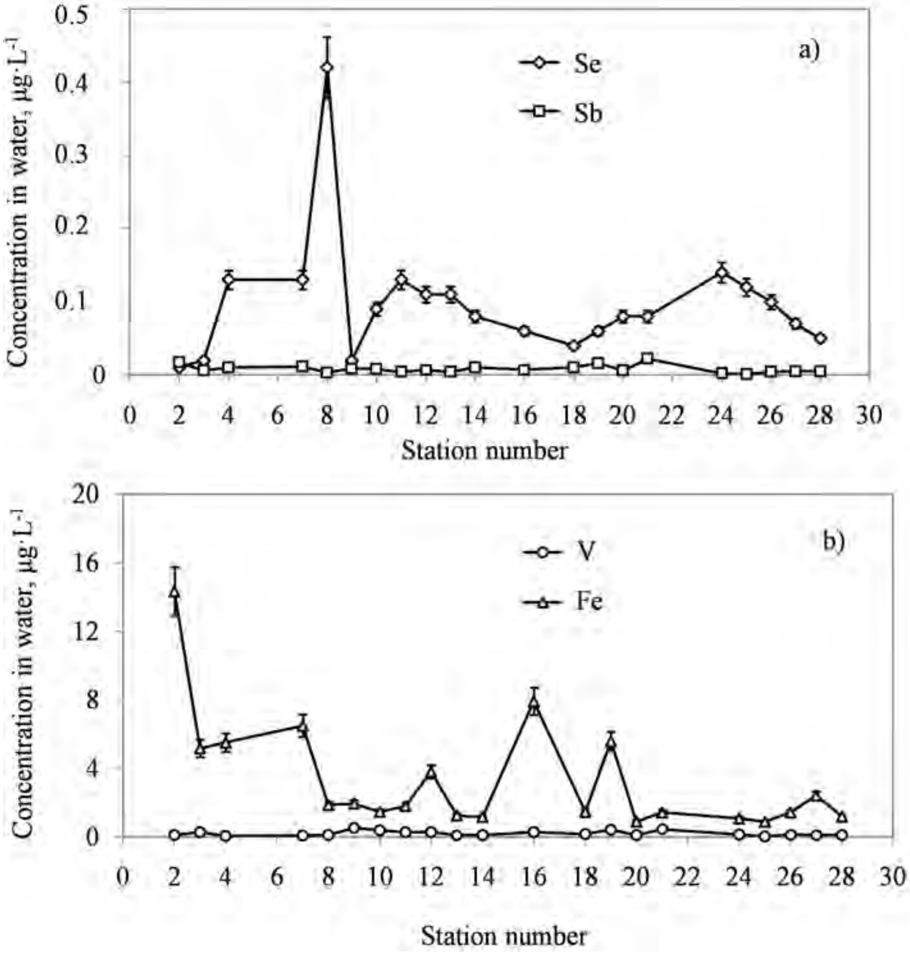

Fig. 4. Concentration of trace elements Se and Sb (a) and V and Fe (b) in surface seawater in the studied areas of the Antarctic

Since studied water areas belong to Antarctic krill fishery area, the data were compared with MPC_F in order to estimate the current ecological state of Antarctic waters (Kharakteristika zagryaznyayushchikh veshchestv…, 2016). Moreover, the results were compared with the reference values given in "Dutch sheets": with MPC (maximum permissible concentration of dissolved form of a pollutant under short-term exposure) and with TV (target value of the inactive concentration for dissolved form of a pollutant under chronic (long-term) exposure in surface water) (Warmer & van Dokkum, 2001), as well as with background concentrations of the studied elements in surface seawater (Crommentuijn et al., 1997) and in surface water in the open ocean (Israel & Tsyban, 2009).

DISCUSSION

The analysis of the results obtained (Figs 2, 3, and 4) indicates relatively low levels of trace elements concentrations in surface seawater in studied water areas of the Antarctic. However, when comparing our data with these trace elements content in waters of the open ocean (Israel & Tsyban, 2009), it was recorded as follows: maximum concentrations of most chemical elements studied (Pb, Cd, Zn, Cu, Be, Mo, Co, and V) in the Antarctic were higher than those, typical for the waters of open ocean: in the range from 2.3 times for Se to 211.3 times for Zn (Table 2).

Table 2. Comparison of the measured values of trace element concentrations in surface ocean waters in the Antarctic with their background and normatively established concentrations, accepted in the Russian Federation and given in “Dutch sheets” ($\mu g \cdot L^{-1}$)

Chemical element	Range of measured values of concentration of dissolved form of the element in water	Background concentrations		Normatively established reference values for chemical elements		
		$C_{open}{}^{*}$	$C_{SWB}{}^{**}$	$MPC_F{}^{***}$ for fishery water bodies	MPC^{****} – MPC in “Dutch sheets”	TV^{*****} – target value according to “Dutch sheets”
Pb	0.01…0.90	$\frac{0.001}{0.034}$	0.02	10	11	0.3
Cd	< 0.002…1.17	$\frac{0.00010}{120}$	0.025	10	0.4	0.08
Zn	0.39…12.26	$\frac{0.003}{0.058}$	0.35	50	9.4	2.9
Cu	0.10…1.49	–	0.25	5	1.5	0.5
Tl	< 0.01	–	–	–	1.6	0.06
Be	< 0.005	–	–	0.3	0.2	0.02
Mo	< 0.21…2.84	–	–	1	290	4.3
Co	0.003…0.21	–	–	5	2.8	0.2
V	< 0.01…0.67	$\frac{1.02}{1.78}$	–	1	4.3	0.9
Ni	0.05…1.22	$\frac{0.116}{0.70}$	–	10	5.1	3.3
Se	< 0.01…0.419	$\frac{0.04}{0.18}$	–	2	5.3	0.09
Sb	< 0.001…0.02	–	–	–	0.4	1.6
Fe	0.76…14.33	$\frac{0.005}{0.140}$	–	50	–	–

Note:

[*] C_{open} is concentration of an element in surface water of the open ocean (background) (minimum value is above the line, maximum value is below the line) (Israel & Tsyban, 2009);

[**] C_{SWB} is background concentration of an element in surface seawater (Crommentuijn et al., 1997);

[***] MPC_F is maximum permissible concentration of an element for fishery water bodies, accepted in the Russian Federation (Kharakteristika zagryaznyayushchikh veshchestv…, 2016);

[****] MPC is maximum permissible concentration for dissolved form of an element in surface water (under short-term exposure) (Warmer & van Dokkum, 2001);

[*****] TV is target value of concentration for dissolved form of an element in surface water considered safe under chronic (long-term) exposure (Warmer & van Dokkum, 2001).

Studied water areas belong to Antarctic krill fishery area; so, the data obtained were compared with $\mathrm{MPC_F}$, established for fishery water bodies (Kharakteristika zagryaznyayushchikh veshchestv…, 2016). The comparison showed that concentrations of Pb, Cd, Zn, Cu, Co, Be, Ni, V, Se, and Fe did not reach $\mathrm{MPC_F}$ (Figs 2, 3, and 4). Their content in all studied areas was many times lower than $\mathrm{MPC_F}$ values (Table 2). The only exception was obtained for Mo, with exceeding $\mathrm{MPC_F}$ 1.2–2.8 times at nine stations (Fig. 3). Thus, increased Mo content was recorded in the central area of the Drake Passage (st. 3), along the Bransfield Strait (st. 4, 7, 10, and 11), and along the northern coast of the Weddell Sea (st. 9, 18, and 19) (Figs 1 and 3). Exceeding $\mathrm{MPC_F}$ value for molybdenum was also recorded off the southern coast of Tierra del Fuego Island (st. 28) (Figs 1 and 3). According to “Dutch sheets” (Table 2), Mo concentrations did not reach MPC and TV, established for acute and chronic exposure of the pollutant (Warmer & van Dokkum, 2001).

We assume that intake of a certain amount of Mo into the Atlantic sector of the Antarctic may have South American origin, since Chile ranks third in the world in terms of molybdenum reserving and mining (National Minerals Information Center…, 2020). As known (Medenosnyi poyas Yuzhnoi Ameriki…, 2004), continental surface water with possible admixtures of molybdenum and other metals, coming from the regions of development of South America copper belt, enters Pacific Ocean surface layer from the territory of Chile. Copper reserves in Chile account for 82 % of the total inventory in South America; for example, the El Teniente copper mine has been developed since 1904, and Chuquicamata – since 1915 (Medenosnyi poyas Yuzhnoi Ameriki…, 2004). Molybdenum reserves in molybdenum ores in Chile are estimated at 2.3 million tons, and reserves of Mo as a by-product of copper ore processing are estimated at 2.5 million tons (13 % of global reserves) (Medenosnyi poyas Yuzhnoi Ameriki…, 2004 ; Mirovoi rynok molibdena…, 2020). Molybdenum, entering oceanic water, is afterwards transported by currents and possibly further distributed in seawater, *inter alia* along Antarctic coast. The highest Mo concentrations were recorded at stations in the central area of the Bransfield Strait (st. 7) and in the northeastern Weddell Sea (st. 18) (see Fig. 1). This peculiarity can also be explained by molybdenum intake with meltwater of continental Antarctic ice into the marine environment (Samyshev & Minkina, 2019), as well as by possible effect of hydrological processes on the distribution of dissolved elements, in particular transfrontal water masses transport from the north by powerful synoptic eddies, observed in the Drake Passage (Koshlyakov & Tarakanov, 2011). In this publication, the authors identified water transport by eddies to the south only in the thermocline, without considering the upper layer separately.

In the Drake Passage, the powerful Antarctic Circumpolar Current transports surface water from the Pacific Ocean to the east. Due to the presence of significant synoptic eddies in the Drake Passage, surface water can also be transported to its southern area. The current in the Bransfield Strait, flowing northeastward along the South Shetland Islands, is the southernmost stream of the Antarctic Circumpolar Current.

The group of stations 13–17 is located in the western Weddell Sea. There, the cyclonic (clockwise) circulation in the Weddell Sea carries waters from its southern area, and the content of metals is low, since there is no industrial metal mining on the Antarctic continent. The authors of (Sanchez et al., 2019) have shown as follows: along the eastern border of the Antarctic Peninsula, the currents carry to the north iron compounds, entering from a natural source in the south. This was confirmed by our measurements at st. 16 and 19. On the border of the Scotia and Weddell seas (in the Weddell-Scotia confluence zone), waters of the Antarctic Circumpolar Current merge with waters of the cyclonic circulation of the Weddell Sea. This leads to the inflow into the area of both Pacific Ocean and Weddell Sea waters, with their impurities of metals.

According to "Dutch sheets", for Tl, Sb, Be, Ni, V, and Fe, the measured concentrations were significantly lower than MPC and TV (Warmer & van Dokkum, 2001) (see Table 2) at all stations in the Atlantic sector of the Antarctic. In studied water areas, according to "Dutch sheets" (Warmer & van Dokkum, 2001), exceeding MPC was registered for Cd at st. 7 and for Zn at st. 27 (Fig. 2a); exceeding TV was observed for Cu at seven stations, for Zn – at three, for Cd and Pb – at two, for Se – at nine, and for Co – at one station (Table 2, Figs 2, 3, and 4).

The importance of hydrological processes, primarily currents, in the distribution of increased concentrations of dissolved forms of trace elements in Antarctic water is proved by the similarity of the distribution of the studied trace elements in seawater at sampling stations (Figs 2, 3, and 4). The coastal boundary current in the Bransfield Strait is known to be an important factor in water circulation in this area. Together with the Antarctic Circumpolar Current in the Drake Passage, it plays an essential role in water transport to the east (Morozov, 2007).

It is difficult to establish the main sources of trace elements intake into the Antarctic water by the data of one study. The intake can result from both anthropogenic and natural processes on the mainland, in the water column, and in different areas of the planet; moreover, trace elements can reach the Antarctic as a result of transboundary transport of substances (Klenkin et al., 2007 ; Kharakteristika zagryaznyayushchikh veshchestv…, 2016 ; Fuentes et al., 2016 ; Samyshev & Minkina, 2019 ; Sanchez et al., 2019).

Conclusion. New data on the current levels and spatial distribution of 13 trace elements (Be, Se, Sb, Tl, V, Pb, Cd, Cu, Zn, Ni, Mo, Co, and Fe), including heavy metals, in the Antarctic have been obtained. Only Mo concentrations exceeded MPC_F at nine stations.

According to "Dutch sheets", there were single cases of exceeding MPC for Cd and Zn, as well as exceeding TV for Cu, Pb, Cd, Zn, Se, and Co at several stations.

The observed levels of trace elements content in seawater of the Antarctic indicate the presence of increased concentrations of some heavy metals, despite limited anthropogenic pressure on that area of the Southern Ocean.

Increased concentrations of elements in comparison with established MPC_F, MPC, and TV in the northern Drake Passage are likely to be related to a water runoff from industrial enterprises in Chile, mining and processing copper and molybdenum for more than a hundred years. The impact of natural sources cannot be excluded as well. Increased iron content in the northern Weddell Sea (the Powell Basin) is most likely determined by the intake of iron compounds from natural sources in the western Weddell Sea, which is consistent with earlier studies.

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ТЯЖЁЛЫЕ МЕТАЛЛЫ В ПОВЕРХНОСТНОЙ ВОДЕ АТЛАНТИЧЕСКОГО СЕКТОРА АНТАРКТИКИ В 79-М РЕЙСЕ НАУЧНО-ИССЛЕДОВАТЕЛЬСКОГО СУДНА «АКАДЕМИК МСТИСЛАВ КЕЛДЫШ»

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Актуальность мониторинга содержания тяжёлых металлов в водах Атлантического сектора Антарктики определяется необходимостью современной оценки качества водной среды для принятия ответственных решений по сохранению морских живых ресурсов в этом уникальном регионе Мирового океана. Цель проводимых исследований — получить новые данные об уровнях и пространственном распределении концентрации микроэлементов, преимущественно тяжёлых металлов, в поверхностной воде. Отбор проб поверхностной морской воды производили в антарктической экспедиции 79-го рейса НИС «Академик Мстислав Келдыш» на 21 станции в районе проливов Дрейка, Брансфилда, Антарктика, а также в морях Уэдделла и Скотия. Экстракцию и концентрирование растворённой формы 13 микроэлементов (Be, Se, Sb, Tl, V, Pb, Cd, Cu, Zn, Ni, Mo, Co и Fe) осуществляли с помощью диэтилдитиокарбамата натрия и четырёххлористого углерода (CCl_4). Измерение элементов проводили масс-спектрометрическим методом. Только для Mo на девяти станциях, расположенных в проливах Дрейка и Брансфилда, в северном районе моря Уэдделла, а также возле южного побережья острова Огненная Земля, отмечали превышение его концентрации в морской воде в 1,2–2,8 раза по отношению к ПДК микроэлементов в воде рыбохозяйственных объектов РФ ($ПДК_{РХ}$). Согласно международным нормативно-правовым актам, таким как «Голландские листы», зарегистрированы единичные случаи превышения MPC (maximum permissible concentration — ПДК при кратковременном воздействии) для Cd и Zn, а также превышение TV (target value — контрольные уровни при хроническом воздействии) для Cu, Pb, Cd, Zn, Se и Co на нескольких станциях. Исследования показали, что, несмотря на ограниченный режим антропогенной нагрузки в этом регионе Южного океана, в морской воде отдельных районов Атлантического сектора Антарктики в современный период зафиксированы повышенные концентрации некоторых микроэлементов, включая тяжёлые металлы. Необходимо дальнейшее изучение источников поступления и особенностей распределения микроэлементов в морских водах Атлантической части Антарктики для объяснения происходящих процессов, а также для принятия мер по рациональному управлению и экологически приемлемому природопользованию в Антарктическом регионе.

Ключевые слова: тяжёлые металлы, поверхностная морская вода, Атлантический сектор Антарктики

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SPECTRAL BIO-OPTICAL PROPERTIES OF WATER OF ATLANTIC SECTOR OF ANTARCTIC

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Studies of variability of spectral bio-optical properties of water of Atlantic sector of Antarctic were carried out during the 79th cruise of the RV "Akademik Mstislav Keldysh" (11.01.2020–04.02.2020). Chlorophyll *a* and phaeopigment concentration varied in the layer studied from 0.1 to 1.8 $mg \cdot m^{-3}$, except for two stations with content reaching 2.2 and 4.4 $mg \cdot m^{-3}$. The relationship was revealed between light absorption coefficient by phytoplankton and chlorophyll *a* concentration at a wavelength, corresponding to spectrum maxima: $a_{ph}(438) = 0.044 \times C_a^{1.2}$, $r^2 = 0.84$ ($n = 117$); $a_{ph}(678) = 0.021 \times C_a^{1.1}$, $r^2 = 0.89$ ($n = 117$). Spectral distribution of light absorption coefficient by non-algal particles and colored dissolved organic matter was described by exponential function. Absorption parameterization coefficients were retrieved: (1) light absorption coefficient by non-algal particles (0.001–0.027 m^{-1}) and by colored dissolved organic matter (0.016–0.19 m^{-1}) at a wavelength of 438 nm; (2) spectral slope coefficients of these components (0.005–0.016 and 0.009–0.022 nm^{-1}, respectively).

Keywords: chlorophyll *a*, spectral light absorption coefficient, phytoplankton, non-algal particles, colored dissolved organic matter, Antarctic

The study of spectral bio-optical properties of water is necessary for understanding the regularities of the formation of optical signal, recorded by satellite scanners, and for solving the problem of retrieving water biological characteristics, based on Earth remote sensing data from space (IOCCG. Uncertainties in Ocean…, 2019). Spectral optical properties of suspended and dissolved organic matter in water, *i. e.* their capacity to scatter light and, to a greater extent, to absorb it, are determined by the formation of a light field in the sea, as well as water leaving radiance, recorded by satellite optical scanners (Kirk, 2011 ; Reynolds et al., 2001). For the correct transformation of satellite data (remote sensing reflectance Rrs) into water quality and productivity indicators, regional algorithms are required. These algorithms must be developed on the basis of empirical regularities of variability of spectral light absorption coefficient by phytoplankton pigments, non-algal particles, and colored dissolved organic matter, as well as their relationship with concentration of chlorophyll *a* (the main photosynthetic pigment).

The studies of Antarctic water, aimed at understanding the effect of the content of optically active components of the environment and their spectral properties on the formation of a signal, "visible" for remote scanners, have evolved from relatively simple ones, based on the analysis of relationships between chlorophyll *a* concentration and spectral coefficients of light attenuation, as well as remote sensing reflectance (Dierssen & Smith, 2000 ; Figueroa, 2002 ; Mitchell & Holm-Hansen, 1991), to comprehensive ones: analysis of spectral absorption and scattering coefficients by all optically active components (Ferreira et al., 2018, 2017). To date, only single complex researches are known (Ferreira et al., 2018, 2017), carried out in the water area west of the Antarctic Peninsula.

In this regard, the aim of our work was to study the variability of spectral light absorption coefficients by suspended and dissolved organic matter in water of Atlantic sector of Antarctic in summer, based on the results, obtained in the research cruise (11.01.2020–04.02.2020).

MATERIAL AND METHODS

The studies were carried out during the 79th cruise of the RV "Akademik Mstislav Keldysh" in water of Atlantic sector of Antarctic from 11.01.2020 to 04.02.2020. Most stations were carried out in the Bransfield Strait (Fig. 1A) and in the Powell Basin (Fig. 1B).

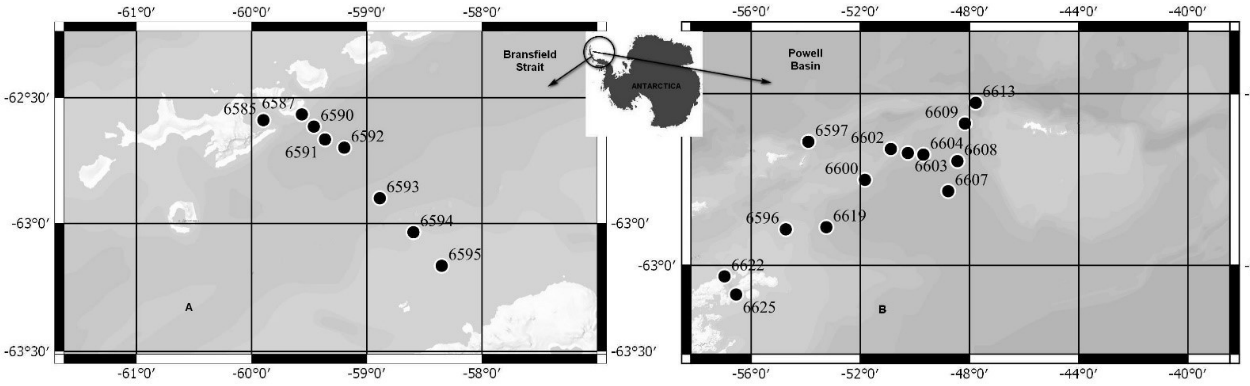

Fig. 1. Map of stations (●) of bio-optical studies, carried out in the 79th cruise of the RV "Akademik Mstislav Keldysh" in the Bransfield Strait (A) and the Powell Basin (B) (11.01.2020–04.02.2020)

The measurement of photosynthetically active radiation (hereinafter PAR), falling on the sea surface, and of its change with a depth of up to 100 m was carried out using Li-Cor deck and submersible devices. Vertical profiles of changes in temperature and chlorophyll *a* fluorescence intensity were recorded by a PUM-200 transparent probe. Samples were taken by a cassette of bathometers at the horizons, selected on the basis of the measured profiles of hydrophysical indicators.

Euphotic zone depth Z_{eu} was taken to be equal to penetration depth of 1 % of PAR, falling on the sea surface.

Chlorophyll *a* and phaeopigment concentration C_a was determined by the standard spectrophotometric method (Jeffrey & Humphrey, 1975 ; Lorenzen, 1967). Water samples (1.5–2 L) were filtered through glass microfiber filters (GF/F, Whatman) at low vacuum (< 0.2 atm). The filters were foil-wrapped and stored in liquid nitrogen in a cryogenic storage dewar, until measurements in the laboratory were carried out.

Light absorption by suspended matter $a_p(\lambda)$ was measured according to the NASA protocol (IOCCG. Ocean Optics…, 2019). Optical densities were measured at a wavelength range 350–750 nm with a step of 1 nm by a double-beam spectrophotometer Lambda 35 (PerkinElmer), equipped with an integrating sphere. The division of $a_p(\lambda)$ into light absorption by phytoplankton pigments $a_{ph}(\lambda)$ and by non-algal particles $a_{NAP}(\lambda)$ was carried out according to (Kishino et al., 1985). Absorption correction (β-correction) was performed in line with (Mitchell, 1990). Correction for non-specific absorption was made by "zeroing" by the mean value for 700–750 nm.

Light absorption of colored dissolved organic matter $a_{CDOM}(\lambda)$ was measured according to the NASA protocol (IOCCG. Ocean Optics…, 2019). Water samples were filtered through a nucleopore filter (Sartorius, 0.2 μm), previously rinsed with deionized water. For prefiltration, GF/F filters were used. Optical densities were measured at a wavelength range 250–750 nm with a step of 1 nm by a spectrophotometer Lambda 35.

Spectral distribution of $a_{NAP}(\lambda)$ and $a_{CDOM}(\lambda)$ was parameterized. Thus, the data were fitted to exponential function at the spectral ranges 400–700 and 350–500 nm, respectively:

$$a_i(\lambda) = a_i(\lambda_{ref}) \times e^{-S_i \times (\lambda - \lambda_{ref})} , \tag{1}$$

where i is non-algal particles (NAP) or colored dissolved organic matter (CDOM);

λ_{ref} is reference wavelength (in our case, λ_{ref} = 438 nm);

S_i is spectral slope coefficient, nm^{-1}.

RESULTS

During the study period, surface temperature in the Bransfield Strait varied from −0.47 to +3.2 °C, averaging +1.3 °C. In the Powell Basin, it varied from −0.77 to +2.0 °C, averaging +0.72 °C.

In the study area, high non-uniformity in water hydrological structure was noted (Fig. 2). At most stations, the temperature was almost equal within the layer studied. At single stations (6591, 6592, and 6593), an upper mixed layer with a thickness of 7–47 m was formed. The complicity and non-uniformity of water hydrological structure was accompanied by a similar non-uniformity in the vertical profile of chlorophyll *a* fluorescence (Fig. 2).

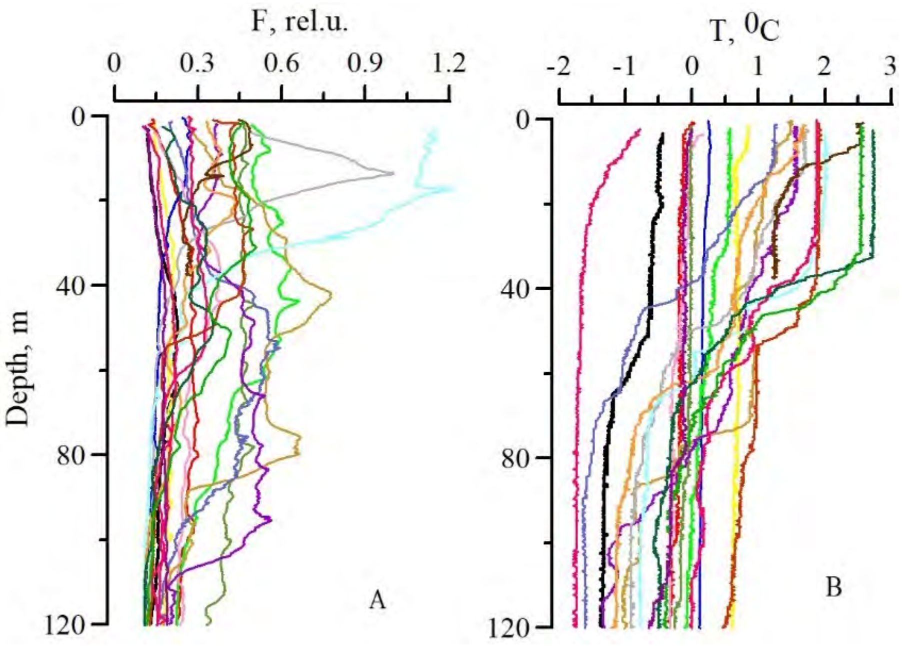

Fig. 2. Vertical profiles (n = 26) of chlorophyll *a* fluorescence intensity (A) and water temperature (B) of Atlantic sector of Antarctic (11.01.2020–04.02.2020)

Euphotic zone depth varied between stations in a narrow range: from 50 m (st. 6614) to 83 m (st. 6619), except for two stations (st. 6609 and 6613), where lower water transparency was registered. On these stations, Z_{eu} was of 28 and 35 m, respectively. On average, Z_{eu} was of (64 ± 12) m.

An important indicator of water productivity and quality is concentration of chlorophyll *a* as the main photosynthetic pigment.

Chlorophyll *a* and phaeopigment concentration in the surface layer in the study area – the Bransfield Strait and the Powell Basin – changed significantly: from 0.25 to 4.4 $mg{\cdot}m^{-3}$ (Fig. 3A). The maximum values were obtained at st. 6609 ($C_a = 4.4\ mg{\cdot}m^{-3}$) and st. 6613 ($C_a = 2.2\ mg{\cdot}m^{-3}$) in the Powell Basin. In this case, the mean C_a value in the surface layer for all stations, except for st. 6609 and 6613, was (0.72 ± 0.35) $mg{\cdot}m^{-3}$. Within the euphotic layer, C_a values varied from 0.1 to 1.8 $mg{\cdot}m^{-3}$, except for st. 6609 and 6613. For the whole layer, C_a averaged (0.61 ± 0.35) $mg{\cdot}m^{-3}$.

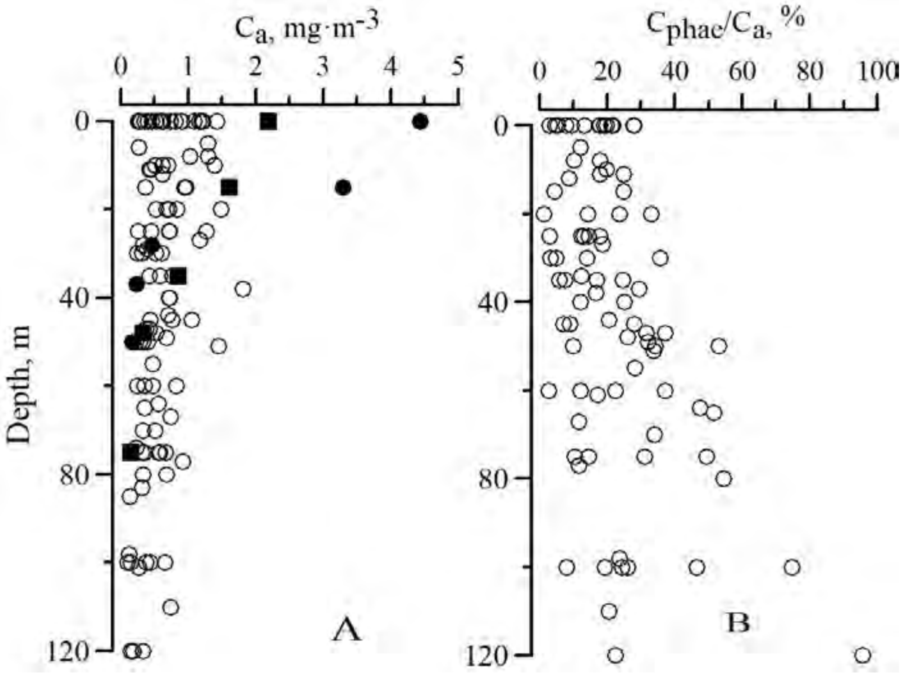

Fig. 3. Vertical profiles of chlorophyll *a* and phaeopigment concentration (C_a) at st. 6609 (●), at st. 6613 (■), and at other stations (○) (A); vertical profiles of relative phaeopigment concentration (C_{phae}/C_a) (B) in water of Atlantic sector of Antarctic (11.01.2020–04.02.2020)

There are three types of C_a vertical distribution (Fig. 4):

1) uniform C_a distribution within the euphotic layer (for example, at st. 6602);
2) with a maximum of C_a in the lower euphotic layer (~ 1–0.1 % PAR) (for example, at st. 6604);
3) C_a decrease with depth within the layer studied (for example, at st. 6614).

Profiles of the third type, characterized by C_a decrease with depth, were the most common ones. They were registered at 16 stations out of 26.

Phaeopigment ratio in chlorophyll *a* and phaeopigment concentration varied from 1 to 96 %, averaging (22 ± 16) % (Fig. 3B). The maximum values (47–96 %) were registered in the layer deeper than 50 m. In the upper, lighted layer (0 to 50 m), phaeopigment ratio did not exceed 30 %. This indicates a good physiological state of phytoplankton in this layer.

In the study area, high variability of spectral coefficients $a_{ph}(\lambda)$ and $a_{NAP}(\lambda)$ was recorded (Fig. 5).

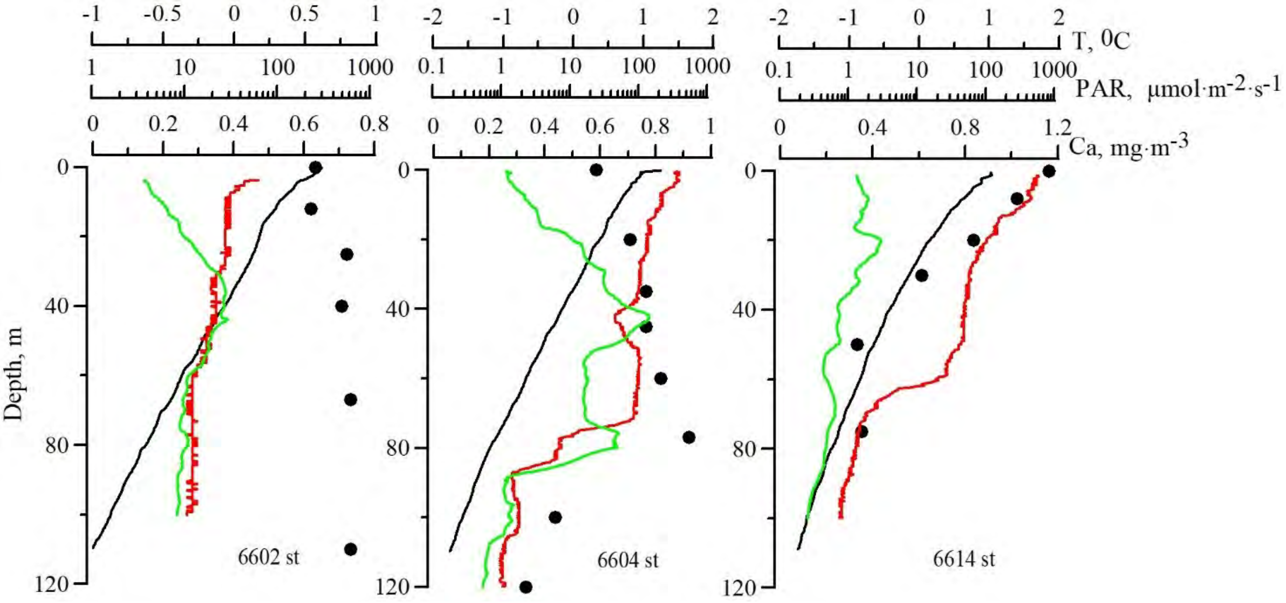

Fig. 4. Examples of typical vertical profiles of temperature (T, red line), chlorophyll *a* fluorescence intensity (F, green line), photosynthetically active radiation (PAR, black line), and chlorophyll *a* and phaeophytin concentration (C_a, ●) at single stations in water of Atlantic sector of Antarctic (11.01.2020–04.02.2020)

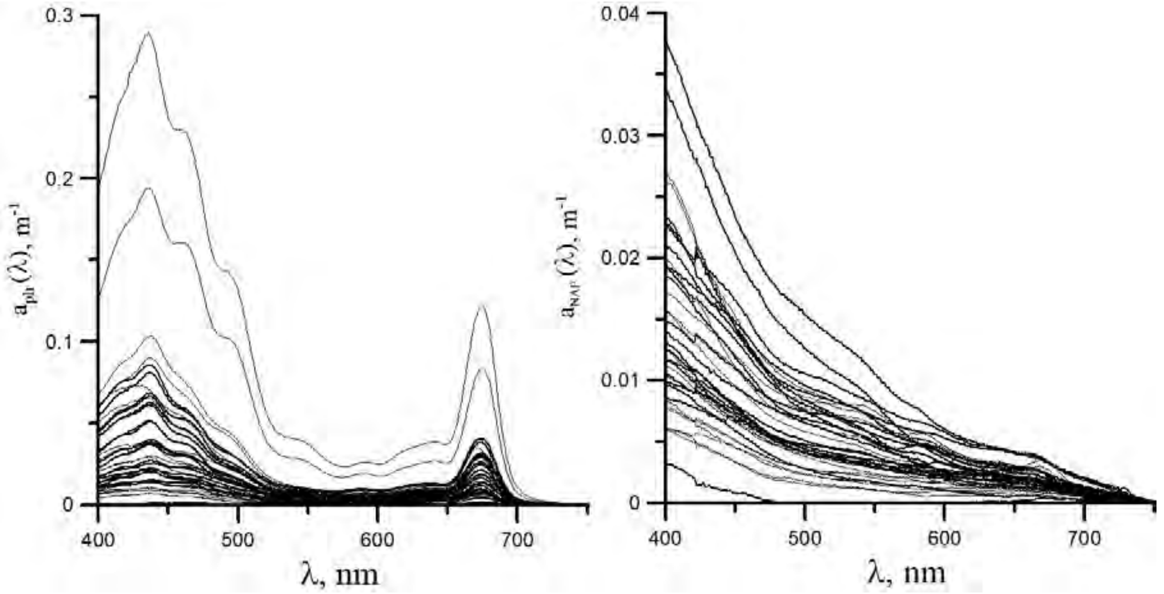

Fig. 5. Light absorption spectra of phytoplankton pigments $a_{ph}(\lambda)$ and non-algal particles $a_{NAP}(\lambda)$ in water of Atlantic sector of Antarctic (11.01.2020–04.02.2020)

The $a_{ph}(\lambda)$ spectra have two main absorption maxima: in the blue and red spectral domains (at ~ 438 and ~ 678 nm, respectively). For the entire data array, the ratio between the coefficients in these spectra peaks (R) varied in a range 1.1–2.8, with a trend towards a decrease with depth. R value varied from 2.8–2.3 in the surface layer to 2.1–1.1 in the end of the layer studied. Values in the blue [$a_{ph}(438)$] and red [$a_{ph}(678)$] maxima varied 0.001–0.29 and 0.001–0.12 m^{-1}, respectively. The highest values were recorded at the stations with high chlorophyll *a* concentration (st. 6609 and 6613), the lowest ones – in the lower euphotic layer.

A relationship was established between C_a and $a_{ph}(\lambda)$ at single wavelengths, corresponding to the spectrum maxima: $a_{ph}(438)$ and $a_{ph}(678)$ (Fig. 6).

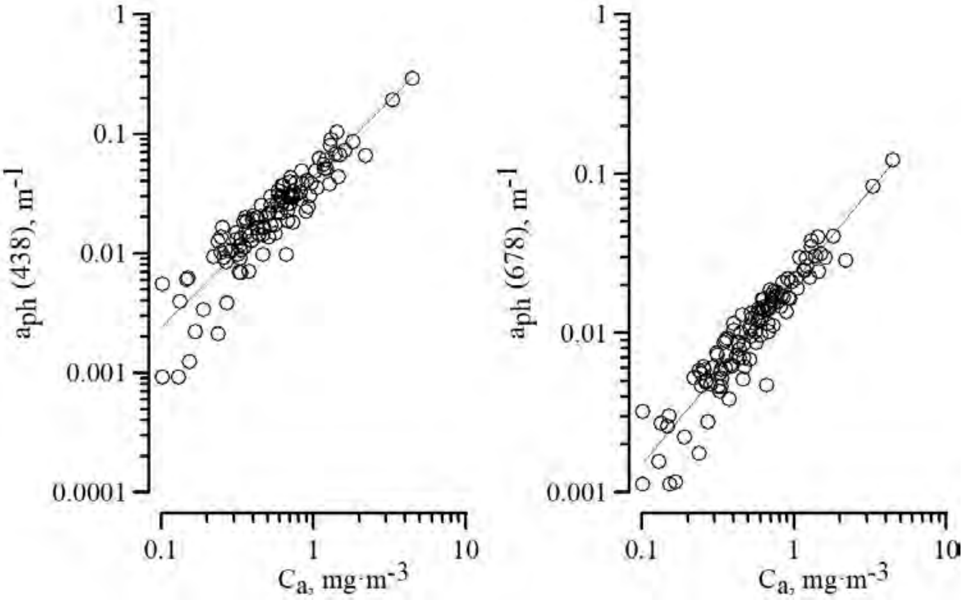

Fig. 6. Dependence of light absorption coefficient by phytoplankton pigments at wavelengths of 438 nm [$a_{ph}(438)$] and 678 nm [$a_{ph}(678)$] on chlorophyll *a* and phaeopigment concentration (C_a) in water of Atlantic sector of Antarctic (11.01.2020–04.02.2020)

These relationships are described by exponential functions with high coefficients of determination:

$$a_{ph}(438) = 0.044 \times C_a^{1.2}, r^2 = 0.84, n = 117\ , \tag{2}$$

$$a_{ph}(678) = 0.021 \times C_a^{1.1}, r^2 = 0.89, n = 117\ . \tag{3}$$

Light absorption coefficients at a wavelength of 438 nm by non-algal particles [a_{NAP} *(438)*] and colored dissolved organic matter [a_{CDOM} *(438)*] varied from 0.001 to 0.027 m^{-1} and from 0.016 to 0.19 m^{-1}, respectively. Spectral slope coefficients S_{NAP} and S_{CDOM} ranged 0.005–0.016 nm^{-1} and 0.009–0.022 nm^{-1}, respectively. The mean values were (0.010 ± 0.002) nm^{-1} for S_{NAP} and (0.013 ± 0.003) nm^{-1} for S_{CDOM}.

For the surface layer (0–1 m), relative contribution of all optically active components to total light absorption coefficients at 438 nm was estimated. The largest one was made by colored dissolved organic matter: from 6 to 88 %, averaging (54 ± 22) %. Phytoplankton contribution was smaller: from 7 to 78 %, averaging (36 ± 19) %. The smallest one was registered for non-algal particles: from 4 to 20 %, averaging (10 ± 5) %.

DISCUSSION

During the study period, different types of vertical profiles of chlorophyll *a* fluorescence were registered (see Fig. 2): uniform distribution; profiles with maximum at different depths; and fluorescence decrease with depth. This is probably associated with vertical distribution of water temperature and density. Similar types of distribution of chlorophyll *a* fluorescence were recorded earlier in Antarctic Peninsula water (Figueiras et al., 1999).

The depth of the euphotic zone was comparable to that of the upper mixed layer, except for several stations, where temperature stratification of water within the euphotic zone was recorded (see Fig. 2). The shape of $a_{ph}(\lambda)$ spectrum reflects cell pigment composition, which, in turn, depends on phytoplankton species composition (Churilova et al., 2008 ; Ciotti et al., 2002 ; Morel & Bricaud, 1981). Chlorophyll-*a*-specific light absorption coefficient by phytoplankton pigments is determined by intracellular pigment content, as well as by shape and size of phytoplankton cells (Bricaud et al., 1995 ; Morel & Bricaud, 1981).

The exponential functions obtained, reflecting the relationship between light absorption coefficient by phytoplankton pigments at 438 and 678 nm and chlorophyll *a* and phaeopigment concentration, can help in calculating $a_{ph}(438)$ and $a_{ph}(678)$ on the basis of C_a data. In these equations, the power coefficient is higher than 1. This indicates a slight increase in C_a-specific absorption coefficients with increase in C_a content in water, which reflects a decrease in the degree of pigment packing in phytoplankton cells (Morel & Bricaud, 1981). This is probably due to the fact that in more trophic water, *i. e.* with higher C_a values, phytoplankton is represented by smaller cell species.

The $a_{ph}(\lambda)$ spectra in the lower euphotic zone (at penetration depth of 1–0.1 % of PAR) were smoother than in the upper one, with minimum R values registered. In the blue spectral domain, accessory pigments and chlorophyll *a* absorb light (Phytoplankton Pigments in Oceanography…, 1997). The red maximum of the spectrum (at 678 nm) is formed due to light absorption by chlorophyll *a* and phaeopigments. A decrease in R value with depth indicates a decrease in the ratio of accessory pigments relative to chlorophyll *a* concentration.

At all stations, except for st. 6609 (at depths of 0 and 15 m), in the obtained light absorption spectra by phytoplankton pigments, no local maximum was registered at a wavelength of 544 nm, corresponding to phycoerythrin absorption band (Ting et al., 2002).

Our values of $a_{NAP}(438)$, $a_{CDOM}(438)$, S_{NAP}, and S_{CDOM} corresponded to those recorded earlier in Antarctic Peninsula water (Ferreira et al., 2018). They were also similar to those registered for the Black Sea in winter (Churilova et al., 2017).

Relative contributions of optically active components of the environment to total light absorption in the surface layer are not the same. Thus, light was mostly absorbed by non-algal component. It is planned to carry out a detailed analysis of the factors, affecting the ratio between all optically active components, in order to use it in development of a refined algorithm for C_a recovery by satellite data.

The data array obtained makes it possible to analyze the variability of absorption coefficients depending on the hydrophysical and hydrochemical conditions in the environment. Moreover, the data array makes it possible to identify regularities that can be helpful in further works on studying the effect of suspended and dissolved organic matter on spectral properties of PAR in the sea, as well as on studying phytoplankton capacity to use it in the photosynthesis. These regularities can also be helpful in research on modeling water quality and productivity indicators, based on Earth remote sensing data from space.

Conclusions:

1. New data were obtained on the variability of chlorophyll *a* and phaeopigment concentration and spectral light absorption coefficients by all optically active components of the environment in the Bransfield Strait and the Powell Basin.
2. Non-uniformity in vertical distribution of chlorophyll *a* concentration was registered. High variability (of more than an order of magnitude) of spectral light absorption coefficient by phytoplankton pigments, non-algal particles, and colored dissolved organic matter was recorded.

3. Parameterization of light absorption by suspended and dissolved matter was performed. By the coefficients obtained, it is possible to recover light absorption spectra by these components.

This work has been carried out within the framework of IBSS government research assignments "Study of the spatiotemporal organization of aquatic and terrestrial ecosystems in order to develop an operational monitoring system, based on remote sensing data and GIS technologies" (No. AAAA-A19-119061190081-9) and "Comprehensive studies of the current state of the ecosystem of the Atlantic sector of the Antarctic" (No. AAAA-A19-119100290162-0), as well as with the support of government research assignment No. 0128-2019-0008. Optical data analysis was carried out with the support of the RFBR grant No. 19-55-45024 ИНД_а.

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СПЕКТРАЛЬНЫЕ БИООПТИЧЕСКИЕ ПОКАЗАТЕЛИ ВОД АТЛАНТИЧЕСКОГО СЕКТОРА АНТАРКТИКИ

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Исследования изменчивости спектральных биооптических показателей вод Атлантического сектора Антарктики проводили в 79-м рейсе НИС «Академик Мстислав Келдыш» в период с 11.01.2020 по 04.02.2020. Получено, что концентрация хлорофилла *a* в сумме с феопигментами изменялась в исследованном слое на большинстве станций от 0,1 до 1,8 мг·м$^{-3}$, за исключением двух станций, где достигала 2,2 и 4,4 мг·м$^{-3}$. Установлена связь показателей поглощения света пигментами фитопланктона с концентрацией хлорофилла *a* на длинах волн, соответствующих основным максимумам спектра: $a_{ph}(438) = 0{,}044 \times C_a^{1,2}$, $r^2 = 0{,}84$ ($n = 117$); $a_{ph}(678) = 0{,}021 \times C_a^{1,1}$, $r^2 = 0{,}89$ ($n = 117$). Спектры показателей поглощения света неживым взвешенным веществом и окрашенным растворённым органическим веществом описаны экспоненциальной зависимостью. Подобраны коэффициенты параметризации: (1) показатели поглощения света неживым взвешенным (0,001–0,027 м$^{-1}$) и растворённым органическим веществом (0,016–0,19 м$^{-1}$) на длине волны 438 нм; (2) коэффициенты наклона экспоненты спектров этих компонент (0,005–0,016 и 0,009–0,022 нм$^{-1}$ соответственно).

Ключевые слова: хлорофилл *a*, спектральный показатель поглощения света, фитопланктон, неживое взвешенное вещество, окрашенное растворённое органическое вещество, Антарктида

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CESTODES OF ANTARCTIC AND SUBANTARCTIC FISH: HISTORY AND PROSPECTS OF RESEARCH

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The first information about cestodes of Antarctic and Subantarctic fish appeared at the beginning of the XX century: a cestode *Phyllobothrium dentatum* from an unknown shark was described. Peak of activity of studying Antarctic cestodes fell on 1990–2006. During this period, significant works were published, devoted to description of new species, their life cycles, host specificity of cestodes – fish parasites, and their geographical distribution. A notable contribution to the study of elasmobranch cestodes was made by a group of Polish scientists, headed by Wojciechowska (Rocka). Systematic position of 21 cestode species from 13 genera of 8 families of 6 orders was analyzed. Cestode fauna has been studied in less than 7 % of the total ichthyofauna of this area, while potential definitive and intermediate hosts remain unexplored. The largest number of cestode species (12) was recorded in four ray species of the family Rajidae. Eight cestode species, reaching sexual maturity, have been registered in intestines of teleosts: *Bothriocephalus antarcticus*, *B. kerguelensis*, *Bothriocephalus* sp., *Parabothriocephalus johnstoni*, *P. macruri*, *Clestobothrium crassiceps*, *Neobothriocephalus* sp., and *Eubothrium* sp. Larvae of five cestode species (*Onchobothrium antarcticum*, *Grillotia (Grillotia) erinaceus*, *Lacistorhynchus tenuis*, *Calyptrobothrium* sp., and *Hepatoxylon trichiuri*), ending their development in elasmobranchs, were found in teleosts. Systematic position of 5 cestode species out of 12, found in rays, is unidentified. Cestode fauna is characterized by a high level of endemism: 67 % of the total cestode fauna is not found to the north of Subantarctic. Coastal areas, mostly covered by research, are those in the Atlantic and Indian sectors of Antarctic. The biodiversity of elasmobranch cestodes, inhabiting Antarctic and Subantarctic, is underestimated, since only one third of species of these fish have been studied so far. Genetic studies of Antarctic cestodes have just begun to develop. Ribosomal sequences from D1–D3 fragments of 28S rDNA are known for 2 species only: *Onchobothrium antarcticum* from the second intermediate (*Notothenia rossii* and *Dissostichus mawsoni*) and definitive hosts (*Bathyraja eatonii*), as well as larvae of *Calyptrobothrium* sp. from the second intermediate hosts (*D. mawsoni* and *Muraenolepis marmorata*). The main directions of further research on cestode fauna should be developed in combination with morphological, faunistic, genetic, and ecological studies.

Keywords: cestodes, fish, fauna, taxonomy, endemism, Antarctic, Subantarctic

Parasitic organisms are representatives of various systematic groups of invertebrates; they form a significant part of the ecosystem's species diversity (Fonseca et al., 2010). Some current calculations show as follows: there are at least 50 % more parasites (75 thousand species have been described) than free-living animals (Poulin & Morand, 2000, 2004). According to preliminary calculations, with an increase in the intensity of studying parasites, inhabiting vertebrates, their number may reach 300 thousand (de Meeus & Renaud, 2002 ; Dobson et al., 2008). The effect of parasites on their hosts spreads both up and down the food webs, and this affects all the elements of the ecosystem. Like free-living organisms, parasites are affected by biotic and abiotic environmental factors; moreover, they are effective indicators of many aspects of host biology and can act as markers of the state of the free-living biota of the community. So, parasites have to be accounted when studying the state of diversity of any biological community.

Antarctic is a unique and poorly explored region, with a large number of endemics recorded. Peculiarities of Antarctic water (stable low temperature and, as a consequence, good solubility of oxygen in water; narrow shelf; and seasonal fluctuations in illumination) contributed to an emergence of unique adaptations and a high degree of endemism both among representatives of local fauna and among their parasites (Bargelloni et al., 1994 ; Eastman, 1993 ; Klimpel & Pal, 2011 ; Kock, 1992 ; Rocka, 2006). Due to unfavorable climatic conditions during most of the year, Antarctic marine fauna, *inter alia* parasite fauna, has not yet been studied in many aspects.

According to current data, ichthyofauna within the Antarctic Convergence zone is represented by 374 species from 47 families, including 14 elasmobranch species (Chondrichthyes) (Duhamel et al., 2014 ; FishBase…, 2019). More than 115 species out of the entire diversity of Antarctic fish belong to the endemic family Nototheniidae Günther, 1861. In this zone, elasmobranchs are represented by sharks (5 species from 3 families) and rays (10 from 2). Sharks prefer warm water; they are mainly captured in the northern area of the Southern Ocean, in the demersal zone of the islands. So far, only one shark capture is known in the southern Antarctic: in the Ross Sea (Rocka, 2003).

The aim of this work was to evaluate the diversity of cestode fauna of Antarctic and Subantarctic fish, the degree of cestodes studying, the level of endemism of cestode fauna, and the prospects for their further research.

RESULTS AND DISCUSSION

In this study, we tried to cover as much as possible the most significant works on cestodes of Antarctic and Subantarctic fish, to evaluate the diversity of these helminths in different hosts, and to highlight promising directions of research. For simplicity of perception, we give a combined list of cestodes species in definitive fish hosts (Table 1), except for cestode larvae of various structures, found in teleosts of this area: they are fully presented in the annotated work (Oğuz et al., 2015, see Table 1).

Study areas of fish cestodes are the Atlantic, Indian, and Pacific sectors of Antarctic and Subantarctic (Fig. 1). Systematic position of 21 cestode species from 13 genera of 8 families of 6 orders, as well as their vertebrate hosts, was analyzed according to WoRMS (http://www.marinespecies.org) and other sources (Kvach & Kuzmina, 2020 ; Klimpel et al., 2017 ; Kuzmina et al., 2020 ; Muñoz & Cartes, 2020 ; Oğuz et al., 2015).

History of development of fish cestodes study. According to Southwell's summary (1925), the first data on cestodes of Antarctic and Subantarctic fish appeared in the early XX century. This applies to the description of *Phyllobothrium dentatum* Linstow, 1907 from an unknown shark, captured off South Georgia coast during the Scottish National Antarctic Expedition (1902–1904). Current systematic position of this species is ambiguous. Southwell (1925), Yamaguti (1959), and Rocka (2003 ; 2006) consider it *species inquirenda*. In Ruhnke's monograph (2017), this species is absent from the list of valid taxa and species of unclear systematic position of the order Phyllobothriidea.

Table 1. Fauna of mature cestodes of Antarctic and Subantarctic fish

Cestode species	Species status	Host	Finding areas	Authors
“Tetraphyllidea” Van Beneden, 1850 relics				
Dinobothrium septaria Van Beneden, 1889	valid	*Lamna nasus* (Bonnater, 1788)	SG, Kerguelen Islands	[45]
Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014				
Onchobothrium antarcticum Wojciechowska, 1990	valid	*Bathyraja eatonii* (Günther, 1876), *B. maccaini* S. Springer, 1971	SSI	[61]
			Weddell Sea	[48]
Phyllobothriidea Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014				
*Phyllobothrium georgiense** Wojciechowska, 1991	*incertae sedis*	*Amblyraja georgiana* (Norman, 1938)	SG shelf	[55]
Ph. siedleckii Wojciechowska, 1991		*B. eatonii*, *B. maccaini*	SSI	
			Weddell Sea	[48]
Ph. rakusai Wojciechowska, 1991		*B. maccaini*	SSI	[55]
Ph. arctowskii Wojciechowska, 1991		*Bathyraja* sp. 2		
			Weddell Sea	[48]
Phyllobothrium sp.	–	*B. eatoni*	Heard Island plateau	[61]
Guidus antarcticus (Wojciechowska, 1991)	valid	*B. eatonii*, *B. maccaini*	SSI	[56]
G. awii (Rocka & Zdzitowiecki, 1998)	valid	*B. maccaini*	Weddell Sea	[48]
Rhinebothriidea Healy, Caira, Jensen, Webster & Littlewood, 2009				
Notomegarchynchus shetlandicum (Wojciechowska, 1990)	valid	*B. eatonii*, *B. maccaini*	Admiralty Bay, Elephant Island	[25]
Pseudanthobothrium minutum Wojciechowska, 1991	valid	*B. eatonii*	SSI	[56]
Ps. notogeorgianum Wojciechowska, 1990	valid	*A. georgiana*	SG shelf, Joinville Island, SSI	[54]
Diphyllidea Van Beneden in Carus, 1863				
Echinobothrium acanthocolle Wojciechowska, 1991	valid	*A. georgiana*	SG shelf	[45 ; 56]
Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008				
Bothriocephalus kerguelensis Prudhoe, 1969	valid	*Notothenia cyanobrancha* Richardson, 1844, *N. rossi* Richardson, 1844	Kerguelen subregion	[43]
B. antarcticus Wojciechowska, Pisano & Zdzitowiecki, 1995	valid	*Champsocephalus gunnari* Lönnberg, 1905, *Channichthys rhinoceratus* Richardson, 1844	Kerguelen subregion, Heard Island	[61]
Bothriocephalus sp.	–	No data	Kerguelen subregion	[5 ; 35]
			No data	[61]

Continue on the next page…

Cestode species	Species status	Host	Finding areas	Authors
Parabothriocephalus macruri Campbell, Correia & Haedrich, 1982	valid	*Macrourus berglax* Lacépède, 1801	No data	[14]
		Mac. carinatus (Günther, 1878)	Falkland Islands	[1]
Parabothriocephalus johnstoni Prudhoe, 1969	valid	*Mac. whitsoni* (Regan, 1913), *Mac. holotrachys* Günther, 1878	Indian sector of the Southern Ocean	[43]
			Weddell Sea	[48]
			Heard Island, Kerguelen Islands	[30]
		Mac. whitsoni	Weddell Sea, SSI	[52]
			King George Island, Elephant Island	[37]
Clestobothrium crassiceps (Rud., 1819)	valid	*Dissostichus eleginoides* Smitt, 1898	Falkland Islands	[12]
			SG	[3]
		Merluccius hubbsi Marini, 1933	Patagonian shelf	[44]
Neobothriocephalus sp.	–	No data	Kerguelen subregion	[5]
Eubothrium sp.	–	*N. rossii*	Crozet Islands, Ob Bank	[9]
			Heard Island, Crozet Islands	[8]
		Lepidonotothen squamifrons (Günther, 1880)	Crozet Islands, Skif, Ob, and Lena banks	[8 ; 9]

Note: * taxonomy of cestodes of the genus *Phyllobothrium* according to (Wojciechowska, 1993c); SG is South Georgia Island; SSI is South Shetland Islands.

The most significant contribution to the development of studies of elasmobranch cestodes of the area was made by Wojciechowska (1990a ; 1990b ; 1991a ; 1991b ; 1993a ; 1993b ; 1993c) with co-authors (1994 ; 1995). She has described ten new cestode species from four ray species from the family Rajidae Blainville, 1816; currently, six species are considered valid (Table 1). Of her later studies, published under the name of Rocka (2014 ; 2005 ; 2007 ; 2003 ; 2006 ; 2017 ; 1998), the work (2006) is of particular interest. It is devoted to study of life cycles, specificity, and geographic distribution of helminths of Antarctic fish.

The first works on cestodes of Antarctic and Subantarctic teleosts were published in the late 1960s. Prudhoe (1969) has described *Parabothriocephalus johnstoni* Prudhoe, 1969 from macrourids and *Bothriocephalus kerguelensis* Prudhoe, 1969 from notothenium fish in Kerguelen Island area. Later, Reimer and Jessen (1974) found *Clestobothrium crassiceps* (Rud., 1819) in *Merluccius hubbsi* Marini, 1933 on the Patagonian shelf. The description of another species – *Parabothriocephalus macruri* Campbell, Correia & Haedrich, 1982 from *Macrourus berglax* Lacépède, 1801 – marked the 1980s (Campbell et al., 1982). This discovery was re-confirmed by Gaevskaya and Rodyuk in another host species: *Macrourus carinatus* Günther, 1878 (Gaevskaya & Rodyuk, 1988).

In the same period, in a series of works by Parukhin (1981 ; 1982) and Lyadov (1981 ; 1985), mature cestodes, not identified to species level, were found in Kerguelen Island area: *Bothriocephalus* sp., *Neobothriocephalus* sp., and *Eubothrium* sp. The last species of Antarctic cestodes

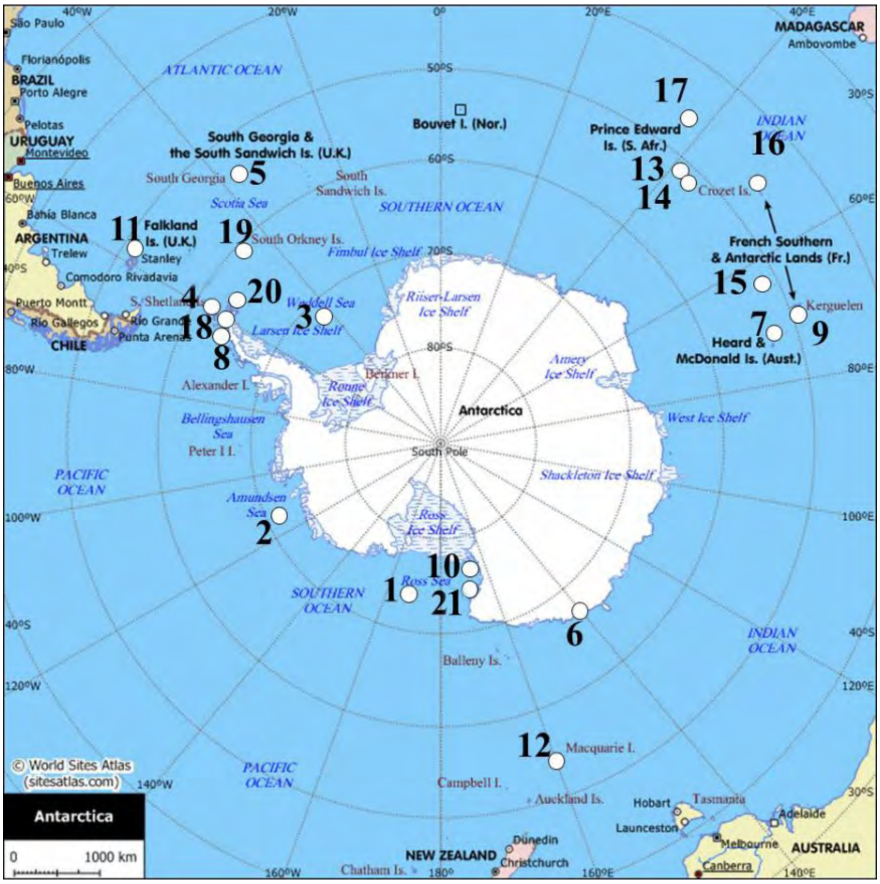

Fig. 1. Sampling localities of cestodes of Antarctic and Subantarctic fish: the Atlantic (3–5, 8, 11, and 18–20), Indian (6, 7, 9, and 13–17), and Pacific (1, 2, 10, 12, and 21) sectors. 1 – Ross Sea; 2 – Amundsen Sea; 3 – Weddell Sea; 4 – South Shetland Islands (Deception Island, King George Island, Admiralty Bay, and Elephant Island); 5 – South Georgia (Cumberland Bay); 6 – Adélie Land; 7 – Heard Island; 8 – Archipelago Melchior; 9 – Kerguelen Island; 10 – McMurdo Sound; 11 – Falkland Islands; 12 – Macquarie Island; 13 – Ob Bank (guyot); 14 – Lena Bank; 15 – Skif Bank; 16 – Crozet Islands; 17 – Prince Edward Islands; 18 – Bransfield Strait; 19 – South Orkney Islands (Signy Island); 20 – Joinville Island; 21 – Terra Nova Bay

described – *Bothricephalus antarcticus* Wojciechowska, Pisano & Zdzitowiecki, 1995 – was registered by Wojciechowska and co-authors (1995) from *Champsocephalus gunnari* Lönnberg, 1905 and *Channichthys rhinoceratus* Richardson, 1844, captured in Heard Island area (the Indian sector of Antarctic).

Since 1965, a large number of articles have been published on the finding of various cestode larvae in teleosts of this area. In the general number of publications, the works of Polish scientists stand out, in particular a series of articles by Wojciechowska with co-authors (1993a ; 1993b ; 1993c ; 1994), in which variations in the forms of cestode larvae from the orders Tetraphyllidea Carus, 1863 and Tetrabothriidea Baer, 1954 were studied. The authors tried to systematize the variety of forms of cestode larvae of these orders, since in most cases, morphology of larval stages differs significantly from morphology of mature cestodes. To date, three more orders have been isolated from Tetraphyllidea (Caira et al., 2014): Onchoproteocephalidea, Phyllobothriidea, and Rhinebothriidea. Therefore, Wojciechowska and co-authors (1993a ; 1993b ; 1993c ; 1994) analyzed the diversity of morphology of cestode larvae of five orders. The works of Zdzitowiecki with co-authors (2001 ; 2004 ; 1999 ; 1997 ; 1998) and Laskowski with co-authors (2005 ; 2007) are devoted to the study of helminth fauna of certain fish species, in which cestode larvae are registered, being identified only to order level, as well as to the study of indicators of their abundance. Of domestic studies, the publications of Parukhin and Lyadov (1981 ; 1986 ; 1981 ; 1982 ; 1985), as well as those of Gaevskaya with co-authors (1988 ; 1987 ; 1990), have to be highlighted. These works are dedicated to the study of fish helminths, *inter alia* matures and cestode larvae, in the Kerguelen subregion, Falklands-Patagonian region, South Georgia Island, and Ob and Lena banks.

The work of Laskowski and Rocka (2014) on the molecular identification of plerocercoids from *Notothenia rossii* Richardson, 1844 is of significant interest. The authors have shown the co-specificity of plerocercoids and matures of *Onchobothrium antarticum* Wojciechowska, 1990 from stingray *Bathyraja eatonii* (Günther, 1876), caught in the Atlantic sector of Antarctic. The publications of Gordeev and Sokolov (2016 ; 2017) contain data on the helminth fauna of teleosts *Dissostichus mawsoni* Norman, 1937 and *Muraenolepis marmorata* Günther, 1880 from the Pacific sector of Antarctic. The authors confirmed, *inter alia* by molecular genetics methods, that the plerocercoids found belong to the species *Onch. antarticum*. These data expanded information on species composition of the second intermediate hosts of *Onch. antarticum* cestodes in Antarctic. Moreover, this is of particular interest in terms of the first finding of *Calyptrobothrium* sp. cestode larvae in *D. mawsoni* and *M. marmorata*, confirmed by molecular genetic data. At the same time, mature *Calyptrobothrium* Monticelli, 1893 have not yet been found in Antarctic and Subantarctic elasmobranchs. It should be emphasized that *Calyptrobothrium* spp. are cosmopolitans, parasites of rays Torpedinidae Henle, 1834, found in temperate and tropical seas.

In recent years, generalizing publications appear, devoted not only to helminth fauna of certain teleost species, but also to their role in the transmission of helminth larvae of different taxa to definitive hosts (fish, birds, and mammals), community structure, and pathogenic effect of helminths on their hosts. Thus, Brickle and co-authors (2005 ; 2006) have studied changes in fauna and structure of parasite communities of the Patagonian toothfish *Dissostichus eleginoides* Smitt, 1898, depending on body length, sex, season, and depth of the host habitat; the researchers have used the example of various helminths, *inter alia* trypanorhynches larvae (*Grillotia (Grillotia) erinaceus* (Van Beneden, 1858) Guiart, 1927 and *Hepatoxylon trichiuri* (Holten, 1802) Bosc, 1811), diphyllobothriids, and tetraphyllids.

The study of parasite communities of *Notothenia coriiceps* J. Richardson, 1844, depending on its size, was continued by Kuzmina and co-authors (2020). In addition to the previously identified helminths, the researchers have found in this host cestode larvae of the orders Diphyllobothriidea Kuchta, Scholz, Brabec & Bray, 2008 (*Diphyllobothrium* sp.) and Tetraphyllidea (three morphotypes: with monolocular, bilocular, and trilocular bothridia). Out of the identified cestode larvae, the dominating ones in terms of abundance were *Diphyllobothrium* sp., whose definitive hosts are marine mammals. It was found that the intensity of infestation with *Diphyllobothrium* sp. and tetraphyllide larvae increases with fish age.

Palm with co-authors (1998 ; 2007) have studied possible life cycles of some helminths in Antarctic. It was revealed that larvae of mammalian parasites, for example cestode larvae of the order Diphyllobothriidea, use teleosts as paratenic hosts, especially those from the families Nototheniidae and Channichthyidae T. N. Gill, 1861. The authors claim that some teleost helminths, *e. g.* cestode larvae of the order Tetraphyllidea, have concurrently adopted different benthic host systems in order to reach their definitive hosts: elasmobranchs. Walter and co-authors (2002) analyzed species composition of parasites in three macrourus species from two Antarctic areas and identified factors, contributing to the similarity of parasite faunas: macrourus nutrition, migration, and close phylogenetic relationships. Santoro with co-authors (2013) studied pathological changes in five teleost species as a result of parasitizing of Diphyllobothriidea and Tetraphyllidea larvae (with monolocular and bilocular bothridia).

Muñoz and Cartes (2020) have analyzed the diversity and abundance of endoparasites of Antarctic and Subantarctic fish. It was revealed that a number of parasite species in Antarctic fish is larger. The authors suggested as follows: high abundance of parasites in Antarctic fish is caused by sympatric speciation in certain parasitic lines or by exploitation of new resources, contributing to the appearance of more parasite species than in Subantarctic environments.

Based on the foregoing, we summarize that the Atlantic and Indian sectors of Antarctic are more fully covered by research than the Pacific one. The largest number of mature cestodes of teleosts was recorded in the Indian sector (Table 1). All this indicates the fragmental character of the ongoing studies of Antarctic cestodes and the need for more thorough and systematic work to obtain a complete faunistic picture.

Biodiversity of cestodes of Antarctic and Subantarctic fish. Parasites of Antarctic and Subantarctic are represented by various taxa among invertebrates and vertebrates. Most of marine vertebrates' fauna in this area is represented by fish: 374 species have been recorded (Duhamel et al., 2014 ; Eastman, 1993). Currently, 135 species of Antarctic fish have 189 species of parasitic organisms from 11 taxonomic groups: Coccidia – 6 species; Microsporidia – 2; Myxosporea – 13; Monogenea – 23; Trematoda – 65; Cestoda – 21; Nematoda – 14; Acanthocephala – 19; Hirudinea – 16; Copepoda – 7; and Isopoda – 3 species. Thus, a little more than a third of the total ichthyofauna of this area was examined for the presence of parasites. The ratio of cestodes, recorded in Antarctic and Subantarctic fish, is 11 % of the parasite species, known for this area. This indicates lack of knowledge of these helminths' fauna.

According to the recent report on Antarctic cestodes (Rocka, 2017), 12 species from 5 orders were found in spiral intestines of 5 elasmobranch species (Table 1). The highest species richness was recorded for the orders Phyllobothriidea (*Phyllobothrium* Van Beneden, 1850 – 4 species; *Guidus* Ivanov, 2006 – 2) and Rhinebothriidea (*Pseudanthobothrium* Baer, 1956 – 2; *Notomegarhynchus* Ivanov & Campbell, 2002 – 1). The smallest number of cestode species (one species each) was registered for the orders Onchoproteocephalidea (*Onchobothrium* de Blainville, 1828) and Diphyllidea (*Echinobothrium* Van Beneden, 1849). Out of 12 cestode species, only 7 have a recognized taxonomy (Table 1); the status of the rest is *incertae sedis* (Ruhnke et al., 2017).

Eight cestode species from five genera of the order Bothriocephalidea (Table 1) reach maturity in intestines of Antarctic and Subantarctic teleosts (Parukhin & Lyadov, 1981, 1982 ; Rocka, 2017). Three cestode species, identified in teleosts, were representatives of the genus *Bothriocephalus* Rudolphi, 1808; two cestode species were of the genus *Parabothriocephalus* Yamaguti, 1934; one species each were identified from the genera *Clestobothrium* Lühe, 1899, *Neobothriocephalus* Mateo & Bullock, 1966, and *Eubothrium* Nybelin, 1922. The findings of mature cestodes of the genus *Eubothrium* (Table 1) in intestines of Antarctic notothenium fish (Parukhin & Lyadov, 1981, 1982) are unique: these cestodes were not repeatedly recorded in fish of this family (Kuchta & Scholz, 2017). To date, only six species of the genus *Eubothrium* have a recognized taxonomy; they are found in Salmonidae G. Cuvier, 1816, Gadidae Rafinesque, 1810, Acipenseridae Bonaparte, 1831, Clupeidae Cuvier, 1817, Zoarcidae Swainson, 1839, Osmeridae Jordan, 1923, Cyprinidae Rafinesque, 1815 (*Phoxinus* L., 1758), and Trichodontidae Bleeker, 1859 (Kuchta & Scholz, 2017). In addition to mature cestodes, larvae of unclear species identity and of various morphology were found in intestines of teleosts (Oğuz et al., 2015). Only five species of cestode larvae, whose definitive hosts are elasmobranchs, were identified: *Onchobothrium antarticum*, *Calyptrobothrium* sp., *Grillotia (Grillotia) erinaceus*, *Lacistorhynchus tenuis* (Van Beneden, 1858) Pintner, 1913, and *Hepatoxylon trichiuri* (Gaevskaya & Rodyuk, 1988 ; Gaevskaya et al., 1990 ; Brickle et al., 2005 ; Brickle, 2006 ; Gordeev & Sokolov, 2016, 2017).

In addition to fauna, life cycles, and ecology of cestodes of Antarctic and Subantarctic hydrobionts, authors began studying phylogeny of these helminths. The research of genetic diversity of Antarctic cestodes just started developing. To date, data on ribosomal sequences from D1–D3 fragments of 28S rDNA are known for 2 species only: *Onchobothrium antarcticum* from the second intermediate (*Notothenia rossii* and *Dissostichus mawsoni*) and definitive hosts (*Bathyraja eatonii*) and larvae of *Calyptrobothrium* sp. from the second intermediate hosts (*D. mawsoni* and *Muraenolepis marmorata*) (Gordeev & Sokolov, 2016, 2017 ; Laskowski & Rocka, 2014).

The biodiversity of cestodes of Antarctic and Subantarctic elasmobranchs has been studied fragmentarily. Within the Antarctic Convergence zone, there are 15 elasmobranch species (5 shark and 10 ray ones) (Duhamel et al., 2014 ; FishBase…, 2019), and only for 5 species (4 ray and 1 shark one) there are data about their infestation with cestodes. Systematic position of 5 cestode species out of 12, found in rays, is unidentified. Taxonomic identification of these helminths is often difficult due to their poor morphological preservation and unsuitability for genetic research.

Endemism of cestode fauna of Antarctic and Subantarctic fish. Endemism of parasites of Antarctic and Subantarctic invertebrates and vertebrates is studied insufficiently. According to Rocka (2006), all cestodes, found in elasmobranchs, are endemic to this area, except for *Dinobothrium septaria*, registered also in the great white shark *Carcharodon carcharias* (L., 1758) in the northwestern Atlantic, Woods Hole, Massachusetts (Dailey & Vogelbein, 1990). It should be mentioned that *Ph. georgiense* was previously considered an Antarctic endemic. Meanwhile, it was recently found in the ray *Bathyraja sexocuata* Mysawa, Orlov, Orlova, Gordeev, Ishihara, 2020 in Simushir Island water in the northwestern Pacific (Gordeev & Polyakova, 2020), which allows us to consider its distribution bipolar. Consequently, 11 cestode species are endemic to elasmobranchs (Table 1).

In teleosts, endemics are represented by mature cestodes: Antarctic species *Parabothriocephalus johnstoni* and Subantarctic ones *Bothriocephalus kerguelensis* (Prudhoe, 1969) and *B. antarcticus* (Wojciechowska et al., 1995). Moreover, in teleosts of this area, bipolar species *Parabothriocephalus macruri* and cosmopolitan *Clestobothrium crassiceps* can be found (Wojciechowska et al., 1995).

The analyzed data indicate a high endemism of cestode fauna of Antarctic and Subantarctic fish (67 % of all cestode fauna): out of 21 cestode species, 14 are not found to the north of this area.

Study of larval stages of cestode development in Antarctic and Subantarctic fish. Cestode larvae of different structure are often found in Antarctic teleosts (Rocka, 2003, 2006, 2017). It is difficult to identify systematic position of these larvae, parasitizing at procercoid and plerocercoid stages of crustaceans and teleosts, since their scolices differ significantly in structure from those of matures. To date, many various forms of cestode larvae have been identified on the basis of different scolex structure (cercoid I, II, III, IV, V, VI; cercoid VIII (without bothria); monolocular, bilocular and others) (Wojciechowska, 1993a, b, c ; Wojciechowska et al., 1994). A complete list of all morphological variations of cestode larvae with an indication of the hosts, in which they were found, is given in the annotated list of parasites of Antarctic hydrobionts, complied by Oğuz with co-authors (2015, see Table 1). It should be mentioned that such classification by different researchers of cestode larvae without establishing their taxonomic affiliation, in particular on the basis of molecular genetic data, leads to considerable confusion.

Researchers attempted to systematize and identify various cestode larvae in teleosts of the area. The most recognized works are those of Rocka (2003 ; 2006), in which five larvae morphotypes were distinguished: cercoids with bothridia, subdivided into one, two, and three loculi; cercoids with undivided bothridia with hook-like projections; and cercoids with subcylindrical bothridia. Rocka suggested as follows: cercoids with undivided bothridia are representatives of the genus *Anthocephalum* Linton, 1890, and cercoids with bilocular bothridia are cestode larvae from the genera *Pseudanthobothrium* Baer, 1956, *Notomegarhynchus* Ivanov & Campbell, 2002, and *Anthobothrium* Van Beneden, 1850. It should be noted that mature cestodes of the genera *Anthocephalum* and *Anthobothrium* have not yet been found in Antarctic and Subantarctic rays; therefore, it is impossible to confirm this assumption.

By molecular genetic methods (Laskowski & Rocka, 2014), it was proved that cercoids with trilocular bothridia, parasitizing teleosts, belong to the species *Onchobothrium antarcticum* (Oncobothriidae) from spiral intestine of rays. Also, Rocka suggested that cercoids with undivided bothridria with hook-like projections are of the species *Dinobothrium septaria*: a parasite of the pelagic shark *Lamna nasus* (Bonnaterre, 1788). Finally, cercoids with subcylindrical bothridia are probably representatives of the genus *Guidus* Ivanov, 2006 (syn. *Marsupiobothrium* Yamaguti, 1952). This assumption is confirmed by the presence of two cestode species of this genus in Antarctic rays (Table 1). Other common larval form of cestodes, found in teleosts, is larvae of Diphyllobothriidae, whose definitive hosts are seals and birds (Rocka, 2006, see Table V). Plerocercoids without bothridia, registered in intestines of teleosts in South Georgia Island water, are similar to plerocercoids of the family Tetrabothriidae: common parasites of Antarctic birds and mammals (Rocka, 2006). Rocka suggested that the presence of different cestode larvae in teleosts, being endemic to Antarctic, indicates that full life cycles of most cestodes are realized in this area (Rocka, 2006).

Conclusion. Faunistic and taxonomic studies of Antarctic cestodes are still sporadic, despite diversity of these helminths in Antarctic and Subantarctic water. In 25 Antarctic and Subantarctic fish species, 21 cestode species are found at different stages of their development. Cestode fauna has been studied in less than 7 % of fish of this area, while potential definitive and intermediate hosts remain unexplored. Of Antarctic and Subantarctic fish, the highest cestode species richness (12 species) was registered in 4 ray species of the family Rajidae. In teleosts, 8 species of mature cestodes and 5 species of cestode larvae were found. The most intensive studies of fish cestodes were carried out in the Atlantic and Indian sectors of Antarctic. The Pacific sector, due to the low degree of study of fish cestode fauna, is the most promising one. Biodiversity of elasmobranch cestodes is underestimated. Out of 15 ray and shark species, cestodes has been studied in 5 species only, *i. e.* one third of the total elasmobranch fauna has been examined for the presence of cestodes. Systematic position of 5 cestode species out of 12, found in rays, is unidentified. Cestode fauna is characterized by a high level of endemism: 67 % of their species are not registered to the north of Subantarctic. Despite the facts that faunistic and ecological studies of cestodes of Antarctic and Subantarctic fish have been carried out for more than 100 years and a significant amount of knowledge has been accumulated, so far little has been done to genetically confirm the systematic affiliation of previously discovered species. Genetic researches on cestodes has just begun to develop. Ribosomal sequences are known only for 2 out of 21 cestode species, *i. e.* molecular genetic data are available for less than 10 % of the total cestode fauna of Antarctic and Subantarctic. The main directions of further research on cestode fauna should be developed in combination with morphological, faunistic, genetic, and ecological studies. Ecological and genetic directions are especially promising, since there are no data on host – parasitic relationships of cestodes with definitive hosts: elasmobranchs.

There is no information on the effect of physiological characteristics of definitive hosts (sex, size/age, age-related changes in nutrition, and degree of maturity) on cestode number and its seasonal dynamics, on their inter- and intraspecific relationships, *etc.* Moreover, molecular data on the latent biodiversity of parasites, in particular cestodes, can be useful for studying speciation and geographic distribution of cryptic parasite species.

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ЦЕСТОДЫ РЫБ АНТАРКТИКИ И СУБАНТАРКТИКИ: ИСТОРИЯ И ПЕРСПЕКТИВЫ ИССЛЕДОВАНИЯ

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Первые сведения о цестодах рыб Антарктики и Субантарктики появились в начале XX века: от неизвестной акулы была описана цестода *Phyllobothrium dentatum*. Пик активности изучения антарктических цестод пришёлся на 1990–2006 гг. В этот период опубликованы значимые работы, посвящённые описанию новых видов, изучению их жизненных циклов, гостальности цестод — паразитов рыб, их географическому распространению. Существенный вклад в изучение цестод хрящевых рыб внесла группа польских учёных во главе с А. Войцеховской (Рока). Проанализировано систематическое положение 21 вида цестод из 13 родов 8 семейств 6 отрядов. Фауна цестод изучена менее чем у 7 % от ихтиофауны данного региона, в то время как потенциальные окончательные и промежуточные хозяева остаются неисследованными. Наибольшее количество видов цестод (12) зарегистрировано у четырёх видов скатов семейства Rajidae. В кишечнике костистых рыб обнаружено восемь видов цестод, достигающих половой зрелости: *Bothriocephalus antarcticus*, *B. kerguelensis*, *Bothriocephalus* sp., *Parabothriocephalus johnstoni*, *P. macruri*, *Clestobothrium crassiceps*, *Neobothriocephalus* sp. и *Eubothrium* sp. В костистых рыбах зарегистрированы личинки пяти видов цестод (*Onchobothrium antarcticum*, *Grillotia (Grillotia) erinaceus*, *Lacistorhynchus tenuis*, *Calyptrobothrium* sp. и *Hepatoxylon trichiuri*), заканчивающих своё развитие в хрящевых рыбах. Из 12 видов цестод, обнаруженных у скатов, для пяти не установлено систематическое положение. Фауна цестод характеризуется высоким уровнем эндемизма: 67 % от всей фауны не встречается севернее Субантарктики. В наибольшей степени исследованиями охвачены прибрежные области в Атлантическом и Индийском секторах Антарктики. Разнообразие цестод хрящевых рыб, обитающих в Антарктике и Субантарктике, недооценено: к настоящему времени изучена всего треть видов этих рыб. Генетические исследования антарктических цестод только начали развиваться. Известны рибосомальные последовательности из области D1–D3 рДНК 28S лишь для двух видов — *Onchobothrium antarcticum* от вторых промежуточных (*Notothenia rossii* и *Dissostichus mawsoni*) и окончательного хозяев (*Bathyraja eatonii*), а также личинок цестоды *Calyptrobothrium* sp. от вторых промежуточных хозяев (*D. mawsoni* и *Muraenolepis marmorata*). В дальнейшем основные направления изучения фауны цестод следует развивать в сочетании с морфологическими, фаунистическими, генетическими и экологическими исследованиями.

Ключевые слова: цестоды, рыбы, фауна, систематика, эндемизм, Антарктика, Субантарктика

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SOME PECULIARITIES IN VERTICAL DISTRIBUTION OF METAZOAN MICROZOOPLANKTON IN THE BLACK SEA IN SPRING

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Based on material, received in the 84th and 93rd cruises of the RV “Professor Vodyanitsky”, vertical distribution of microplankton fraction of metazooplankton (MM) in the Black Sea in spring was analyzed. A total of 27 stations were examined both in the coastal zone and in the deep sea. The 10-L bottles of the CTD probes “Mark-III Neil Brown” and “Sea Bird 911” were used to collect 4–6 L of water from 4–11 horizons of the water column. The samples obtained were concentrated by the reverse filtration through the plankton net with the mesh size of 10 μm. Quantitative and systematic analysis of all samples was carried out totally in the Bogorov chamber using an MBS-9 stereo microscope. The main factors determining nature of the distribution are MM species composition, physical structure of the water column, and hydrodynamic processes affecting its stability/instability. Nauplii of Black Sea Copepoda and veligers of Bivalvia were the most numerous systematic groups in “spring” MM. Mollusc veligers determined abundance maxima in the lower layers of shallow water habitats, while copepods prevailed over large depths and determined total abundance peaks in the upper and middle water layers. Daily time series experiment showed that advective hydrodynamic processes can significantly affect MM vertical distribution, changing physical structure of the water column. For some species, in most cases, a correlation of their distribution with vertical profiles of temperature and salinity was revealed, which rarely manifested at total MM abundance level. A comparison of two spring seasons (2016 and 2017) showed the relationship between vertical distribution of MM abundance and temperature to be more pronounced in cases of low temperature. A change in the sign of correlation with temperature was detected during spring season for *Oithona similis*: an initially cold-loving species of Black Sea copepods. This revealed in a more superficial distribution of the maxima abundance of this species at lower seasonal temperatures, which could reflect a shift in temperature optimum for the species population and play the role of an adaptive reaction in conditions of seasonal changes in sea thermal characteristics.

Keywords: metazoan microzooplankton, abundance and species composition, vertical distribution, correlation with temperature and salinity, Black Sea

The assessment of trends in vertical distribution of abundance, biomass, and species diversity of zooplankton is important due to the role this group plays in matter and energy transfer from the surface to the depth in marine ecosystems. This transfer mediates availability of food resources and structure of trophic relationships over depth.

A pattern of vertical distribution of zooplankton as a complex organism with different biology is very complicated and requires its interpretation in the study of individual components of plankton community. As an ecological group, the zooplankton is rich by taxa, and its vertical distribution varies gradually.

As far as the Black Sea is concerned, the issue of zooplankton vertical distribution has been studied by a number of researches (Delalo et al., 1965 ; Petipa et al., 1963 ; Vinogradov et al., 1987). These studies and some others, including the more recent ones, were focused on the distribution of so-called net zooplankton, in which meso- and macrozooplankton fractions dominate, while the issue of distribution of metazoan microzooplankton (hereinafter MM) has not been studied. MM fraction is represented primarily by copepod naupliar stages; they used to be sampled by Niskin bottles (Seregin & Popova, 2016a ; Takahashi & Uchiyama, 2008 ; Ueda, 1987), by specialized plankton nets with a mesh size of less than 60–70 µm (Uye & Sano, 1995), or by other devices (Kršinić & Grbec, 2012). In general, crustaceans could contribute up to 90 % of total MM abundance. This group mediates the survival rate of many commercial fish species in the region (Klimova & Vdodovich, 2011).

MM habitat of holo- and meroplanktonic organisms in the Black Sea is constrained by the upper 50–100-m layer. Vertical distribution varies for different zooplankton species, sizes, and ontogenetic fractions (Kovalev, 1967 ; Shmeleva & Zaika, 1973 ; Takahashi & Uchiyama, 2008 ; Trudnowska et al., 2015 ; Ueda, 1987). It is influenced by season and hydrographic conditions (Takahashi & Uchiyama, 2008 ; Trudnowska et al., 2015), including the presence of thermo-, halo-, and pycnoclines (Landaeta et al., 2013 ; Lougee et al., 2002), wind mixing (Lagadeuc et al., 1997), and many other factors.

There are very few data on MM vertical distribution in the Black Sea. In Crimean waters in winter, abundance peaks were located at 5–15 and 40–60 m, whereas the biomass was distributed without clear peaks in the water column (Pavlovskaya, 1976). In summer, being the period of developed thermal stratification of the water column, the subsurface maximum of MM abundance is formed in the thermocline or beneath it (Seregin & Popova, 2010). MM vertical distribution spring pattern is yet to be defined. So, the aim of the present study was to assess metazoan microzooplankton spring vertical pattern, as well as to analyze environmental factors mediating this pattern.

MATERIAL AND METHODS

In total, 44 and 147 MM samples were collected in April 2016 and 2017, respectively, during two cruises of the RV "Professor Vodyanitsky". In 2016, 10 stations were located along Crimean Peninsula shelf, 12 miles offshore. In 2017, the sampling area was extended up to the central part of the Black Sea (Fig. 1, Table 1). Along with that, in order to reveal a diel variation of MM abundance, a daily time series experiment was carried out in the southern region, on the station in front of the Laspi Bay.

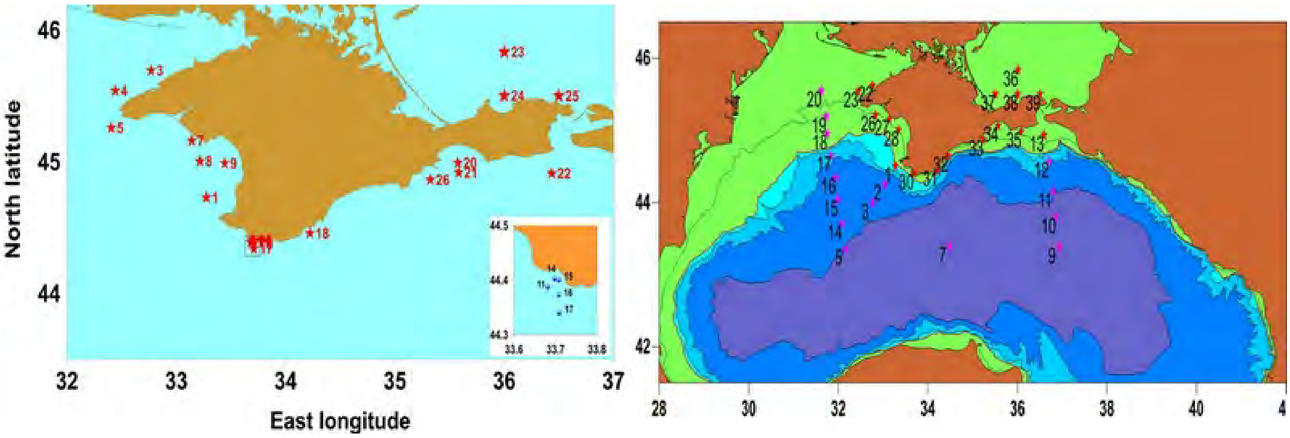

Fig. 1. Map of the stations of the 84th (left) and the 93rd (right) cruises of the RV "Professor Vodyanitsky" in the Black Sea and the Sea of Azov (2016 and 2017)

Table 1. Station numbers, coordinates, depths, and ranges of temperature (T) and salinity (S) changes on the sampling horizons (2016 and 2017)

Year	Station*	North lat., east long.	Total depth, m	Range of changes**		Sampling horizons, m
				T, °C	S, ‰	
2016	1	44°43.7′, 33°16.5′	97	11.5 / 10.3	18.21 / 18.17	0, 10, 20, 30
	3	45°41.6′, 32°46.1′	25.5	11.1 / 10.5	18.37 / 18.35	0, 5, 10, 18
	4	45°32.3′, 32°26.5′	38	11.0 / 8.5	18.38 / 18.31	0, 5, 15, 32
	7	45°09.5′, 33°08.5′	24	11.5 / 10.0	18.36 / 18.25	0, 8, 15
	11	44°23.3′, 33°40.9′	90	10.1 / 9.5	18.25 / 18.32	0, 10, 20, 30, 40, 50
	17	44°20.4′, 33°42.5′	93	10.9 / 10.3	18.21 / 18.21	0, 5, 10, 20, 30
	18	44°27.4′, 34°13.3′	86	11.0 / 10.4	18.18 / 18.00	0, 5, 15, 25, 30
	20	44°59.5′, 35°34.5′	26	10.6 / 9.6	18.25 / 18.17	0, 10, 21
	21	44°55′, 35°34.9′	39	11.9 / 11.0	18.13 / 18.09	0, 10, 20, 30
	26	44°51.7′, 35°19.4′	51	12.1 / 9.0	18.18 / 18.24	0, 10, 20, 30, 40
2017	1	44°30.2′, 33°16.3′	112	10.1 / 8.2	18.29 / 18.34	0, 5, 15, 30, 50
	3	43°59.4′, 32°45.9′	1796	10.1 / 8.2	18.13 / 18.52	0, 15, 28, 50
	5	43°21.2′, 32°09.9′	2030	9.7 / 8.6	18.69 / 20.39	0, 5, 10, 20, 30, 40, 50
	7	43°23.4′, 34°29.6′	2200	9.1 / 8.8	18.53 / 20.99	0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 116
	11	44°08.8′, 36°47.3′	1600	10.5 / 8.6	18.12 / 19.59	0, 10, 15, 25, 40, 60
	13	44°55.8′, 36°35.5′	43	9.4 / 8.8	18.24 / 18.24	0, 5, 15, 40
	14	43°42.3′, 32°05.1′	1894	9.4 / 7.5	18.57 / 18.76	0, 5, 10, 20, 30, 45
	15	44°02.0′, 31°59.0′	1515	9.6 / 8.3	18.46 / 18.49	0, 5, 15, 25, 45
	16	44°20.5′, 31°56.3′	1370	9.7 / 8.0	18.32 / 18.36	0, 8, 16, 32, 48
	18	44°57.1′, 31°44.9′	62	9.5 / 7.0	18.37 / 18.39	0, 8, 15, 36, 45
	20	45°33.0′, 31°37.3′	48	8.2 / 6.1	18.43 / 18.43	0, 7, 15, 36
	30	44°24.2′, 33°40.9′	70	9.0 / 7.7	18.15 / 18.41	0, 5, 12, 24, 40
	31	44°26.6′, 34°12.9′	86	9.2 / 8.0	18.26 / 18.39	0, 5, 14, 30, 55
	33	44°52.2′, 35°14.3′	44	8.7 / 8.2	18.02 / 18.19	0, 12, 20, 40

Note: * station numbers in 2016 and 2017 do not coincide; ** values in the upper / lower sampling horizon.

The 10-L bottles of the CTD probes "Mark-III Neil Brown" and "Sea Bird 911" were used to sample 4–6 L of water, which was filtered by the reverse filtration through the plankton net with the mesh size of 10 μm. Samples were preserved with 4 % buffered formaldehyde and kept in the freezer. Organisms were counted under the microscope MBS-9, with the magnification ×32. The Bogorov chamber and the ICES Zooplankton Methodology Manual (Harris et al., 2000) were used to count organisms. According to previous researches (Denda et al., 2017 ; Kovalev, 1980 ; Zaika et al., 1976), all individuals, fitting the size range of 50 to 500 μm, were treated as MM. Copepods were identified to species level, including naupliar stages (Opredelitel' fauny…, 1969 ; Sazhina, 1985).

All nauplii of the genus *Acartia* were identified as *Acartia clausi* Giesbrecht, 1889, since the related species *Acartia tonsa* Dana, 1849, also inhabiting the Black Sea, is present in its coastal water plankton in summer and autumn (Zaremba, 2017). This species sharply reduces its fecundity at temperatures below +20 °C (Peck et al., 2015), develops in shallow waters (Paffenhöfer & Stearns, 1988 ; Tester & Turner, 1991), predominantly in bays, where the water warms up to the bottom (Gubanova, 2000), and experiences low temperatures as dormant delayed-hatching eggs (Marcus & Lutz, 1994).

To identify the relationship of MM vertical distribution with water physical parameters, the value of the correlation coefficient was used (Urbakh, 1975). Temperature and salinity were used as the main factors reflecting hydrological structure of the water column. Water temperature and salinity were measured every time when sampling by CTD probes was carried out. The correlation was calculated for total number of MM, its groups (non-crustacean plankton, Copepoda, Bivalvia, etc.), and separate species (*Oikopleura dioica* Fol, 1872, various copepod species). The Sigmaplot 12.5v software was used to estimate the Pearson correlation coefficients and their reliability, as well as to construct plots of vertical distribution. The Golden Software Surfer 9v software was applied to map the water areas sampled and the spatial distribution of temperature.

RESULTS AND DISCUSSION

In 2016, average temperature of the surface layer of the water areas studied was +11.19 °C, and average salinity was 18.22 ‰. Vertical distribution of temperature and salinity in western shallow coastal regions (20–40 m) was relatively homogeneous in the upper 15-m mixed layer, with a subsequent gradual decline beneath it (Fig. 2). In response to this thermohaline structure, MM maximum was observed in the thermo- and halocline (stations 3 and 4) and contributed by bivalve larvae, predominantly. However, at st. 7, MM maximum was located in the upper mixed layer. This maximum was formed by *A. clausi*, while bivalve mollusc larvae were abundant in the thermocline.

As far as general trends of vertical distribution are concerned, the dominance of crustaceans (contributed up to 81 % to MM in the upper 5-m layer) was substituted by that of non-crustacean fraction, with the predomination of bivalve larvae. On species level, *A. clausi* dominated in the upper mixed layer, while *Paracalanus parvus* (Claus, 1863) and *Oithona similis* Claus, 1866 formed abundance maxima in deep layers.

In the southeastern coastal region, MM abundance reached 37 000 ind.·m^{-3}. The thermocline and halocline were not pronounced in shallow waters, so MM abundance declined from the surface to the bottom (see Fig. 2, st. 26). Bivalve larvae were numerous near the bottom (st. 22), while copepods dominated in the upper and middle layers, especially on “deep” stations.

The thermohaline characteristics of the southern coastal region were different compared to those of western and eastern regions. At st. 1, 11, and 17, the low-saline lenses were traced at 7–12-m depth. In response to these lenses, MM abundance declined, especially for *Acartia* and *Paracalanus* species (Fig. 2, st. 11 and 17). Maximum MM abundance (15 000 ind.·m^{-3}), contributed by *O. similis*, *P. parvus*, and *Pseudocalanus elongatus* (Brady, 1865), was observed at 30 and 50 m, while *A. clausi* was the most abundant in the upper mixed layer.

In 2016, no correlations in the distribution of total MM abundance, temperature, and salinity were found. However, the correlation between these parameters have persisted on species level. For instance, negative correlation with temperature was noticed in vertical distribution of *Oithona*, *Pseudocalanus*, and bivalve larvae abundance at st. 1, 17, and 26, while positive correlation was recorded for *Acartia* and *Paracalanus* abundance. At st. 11, abundance of *Oikopleura dioica* correlated positively with temperature and negatively with salinity (Table 2).

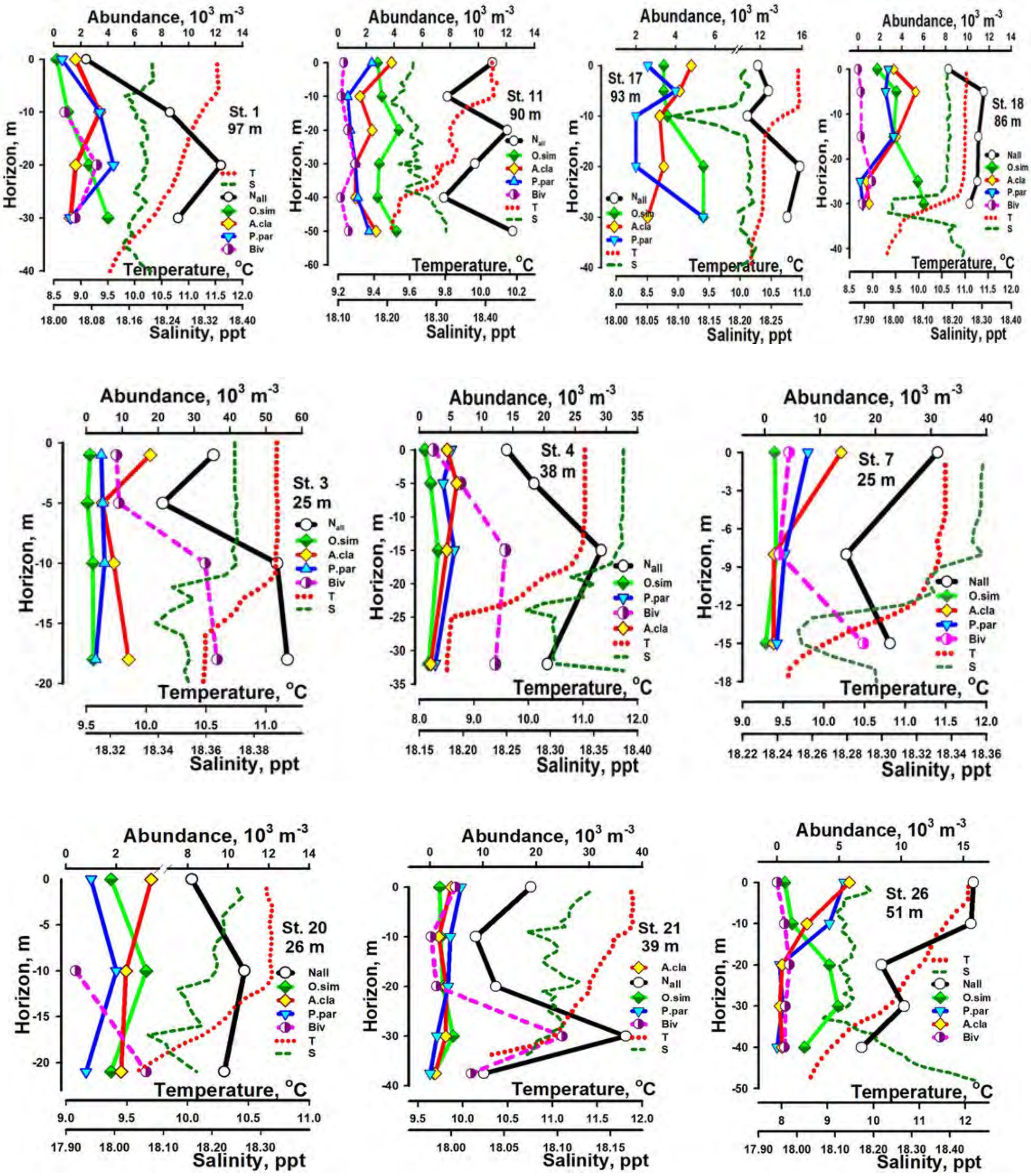

Fig. 2. Vertical distribution of water temperature (T), salinity (S), abundance of total metazoan microzooplankton (N_{all}), *Oithona similis* (O. sim), *Acartia clausi* (A. cla), *Paracalanus parvus* (P. par), *Pseudocalanus elongatus* (P. el), Bivalvia veligers (Biv) in the western (top row), southern and southwestern (middle row), and southeastern (bottom row) coastal waters of Crimea in spring 2016

Table 2. Correlation between metazoan microzooplankton abundance and temperature (T) and salinity (S) in the water column in spring 2016 and 2017

Station	Number of horizon	Correlation between total abundance and				Significant correlations on species level: *r* – *P*
		temperature		salinity		
		C_{corr}, *r*	Significance level, *P*	C_{corr}, *r*	Significance level, *P*	
2016						
1	4	–0.66	0.342	–0.45	0.550	*O. similis* – T: –0.996 – 0.004**
3	4	–0.61	0.388	–0.60	0.405	*P. parvus* – S: 0.95 – 0.048*
4	4	–0.03	0.968	–0.08	0.917	
7	3					
11	6	–0.16	0.761	0.45	0.377	*O. dioica* – T: 0.86 – 0.028* *O. dioica* – S: –0.92 – 0.010**
17	5	–0.74	0.156	0.69	0.193	*O. similis* – T: –0.96 – 0.01* *P. elongatus* – T: –0.93 – 0.023* Bivalvia – T: –0.997 – 0.0002*
18	5	0.02	0.979	–0.02	0.972	
20	3	–0.09	0.940	–0.62	0.574	
21	4	–0.04	0.946	0.17	0.782	Copepoda – T: 0.88 – 0.048* Copepoda – S: 0.92 – 0.028* *P. parvus* – T: 0.95 – 0.011* *A. clausi* – S: 0.95 – 0.015*
26	5	0.86	0.061	–0.25	0.681	*P. elongatus* – T: –0.89 – 0.043*
2017						
1	5	0.43	0.470	–0.77	0.129	*A. clausi* – T: 0.97 – 0.005* *P. elongatus* – T: –0.92 – 0.026*
3	4	–0.41	0.59	0.55	0.451	*P. elongatus* – S: 0.99 – 0.042*
5	7	0.31	0.493	–0.86	0.014*	Copepoda – S: –0.86 – 0.013* *P. parvus* – S: –0.98 – 0.016*
7	11	–0.12	0.78	–0.88	0.0041**	*O. similis* – T: 0.89 – 0.016* *P. elongatus* – T: –0.85 – 0.008** Copepoda – S: –0.88 – 0.004** *P. parvus* – S: –0.93 – 0.02*
11	6	0.99	0.001**	–0.90	0.039	Copepoda – T: 0.99 – 0.0009** Copepoda – S: –0.89 – 0.045* *A. clausi* – T: 0.99 – 0.0056** *A. clausi* – S: –0.97 – 0.028* *O. similis* – T: 0.93 – 0.021*
13	4	–0.78	0.217	0.75	0.252	
14	6	–0.64	0.171	0.42	0.404	*P. elongatus* – T: –0.98 – $3.3\cdot10^{4}$** *P. elongatus* – S: 0.92 – 0.009**
15	5	–0.58	0.305	0.44	0.458	
16	5	0.51	0.376	–0.78	0.123	
18	5	–0.57	0.313	0.03	0.961	

Continue on the next page…

Station	Number of horizon	Correlation between total abundance and				Significant correlations on species level: *r* – *P*
		temperature		salinity		
		C_{corr}, *r*	Significance level, *P*	C_{corr}, *r*	Significance level, *P*	
20	4	0.76	0.241	0.08	0.916	*P. elongatus* – T: –0.99 – 0.011*
30	5	0.92	0.027*	–0.90	0.037*	Copepoda – T: 0.98 – 0.003*
						Copepoda – S: –0.98 – 0.004* *O. similis* – T: 0.90 – 0.037* *O. similis* – S: –0.92 – 0.027* *P. elongatus* – S: 0.89 – 0.041*
31	5	–0.07	0.906	–0.03	0.957	
33	4	0.90	0.097	–0.87	0.125	*A. clausi* – T: 0.96 – 0.045* *A. clausi* – S: –0.97 – 0.029* *P. elongatus* – T: –0.96 – 0.040* *P. elongatus* – S: 0.96 – 0.042*

Note: correlations are significant at $P \leq 0.05$ (*) and $P \leq 0.01$ (**).

April 2017 in the Black Sea was characterized by the lowest monthly average water temperature over the last 10 years of meteorological observations (WeatherArchive official site, 2020). Our data is consistent with this: surface temperature averaged +9.21 °C; temperature in various water areas was 1.5–3 degrees lower than in 2016 (Fig. 3). Salinity of the surface layer was slightly higher than in 2016 and averaged 18.34 ‰.

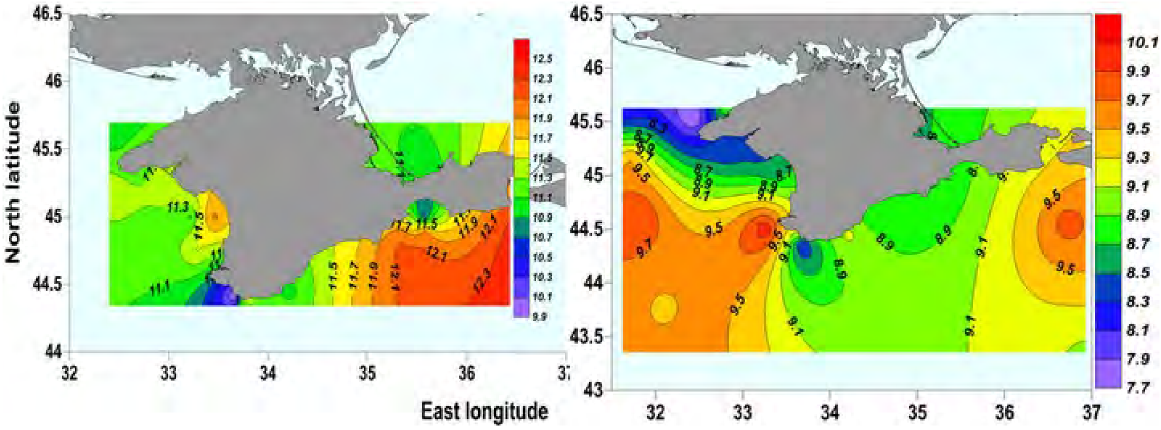

Fig. 3. Temperature of the surface layer of Crimean Peninsula Black Sea waters in spring 2016 (left) and 2017 (right)

Some examples of MM vertical distribution over three coastal regions are shown on Fig. 4. For interannual comparison, only matching stations were selected. In the southern region (in front of Sevastopol, st. 1), relatively invariant thermohaline structure of 110-m water column was observed over years. The upper mixed layer was confined by 5-m isobath, with a smooth temperature decline underneath it. Vertical distribution of MM abundance was similar to that in 2016. The subsurface total abundance maximum was located at 20–30 m. However, some differences persisted on species level. For instance, *A. clausi* abundance peak was observed on the surface in 2017, which was not the case a year earlier. Bivalve larvae were numerous in 2016, but their abundance was very low in 2017.

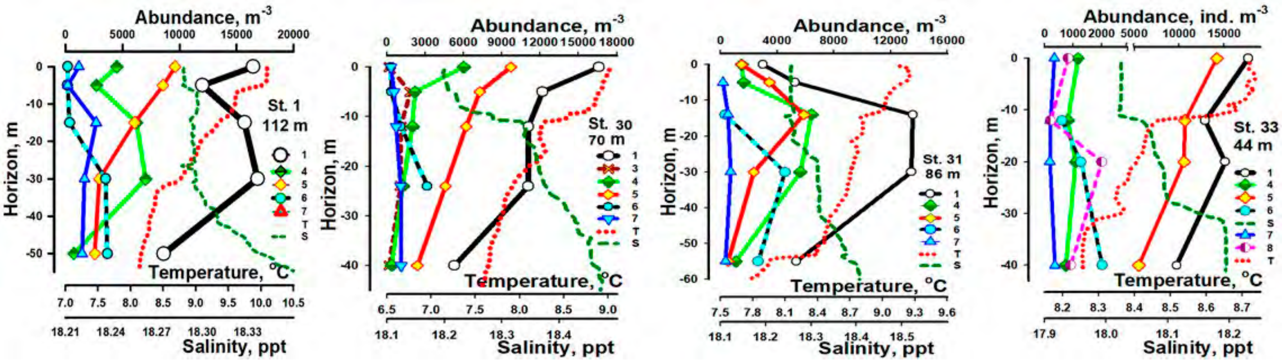

Fig. 4. Vertical distribution of water temperature (T), salinity (S), total abundance of metazoan microzooplankton (1) and non-crustacean plankton (3), abundance of copepods *O. similis* (4), *A. clausi* (5), *P. elongatus* (6), *P. parvus* (7), and Bivalvia veligers (8) in waters of the Sevastopol coastal zone (station 1), the southern (st. 30 and 31) and southeastern (st. 33) coast of Crimea in spring 2017

In the southern region (in front of the Laspi Bay) in 2016, total MM abundance maximum (16 000 ind.·m^{-3}) was located under the thermocline (Fig. 2, st. 11 and 17), whereas in 2017, the maximum (14 000 ind.·m^{-3}) was observed in the surface (Fig. 4, st. 30) or in 20–30-m upper part of the water column (Fig. 4, st. 31).

In the southeastern region (in front of Karadag), vertical distribution of MM abundance over two sampling years was similar for both near-shore and deeper stations. On species level, a more shoaled position of *O. similis* peaks was observed in 2017 compared to 2016.

In 2017, positive correlation between total MM abundance and temperature was observed at st. 11 and 30. Negative correlation between total MM abundance and salinity was registered at st. 5, 7, 11, and 30. If comparing all stations by species distribution, *O. similis* and *A. clausi* had positive correlation with temperature but negative with salinity. *P. parvus* abundance correlated negatively with salinity, whereas *P. elongatus* abundance correlated positively with salinity and negatively with temperature (see Table 2). Interestingly, *Oithona similis* had negative correlation with temperature in 2016, but positive one in 2017. Along with that, the subsurface abundance maximum of these species shoaled over two years.

The daily time series experiment, carried out in the southern region (in front of the Laspi Bay), enabled a short-term interplay between MM abundance and thermohaline characteristics to be analyzed. In the morning (8:00–12:00 a. m.), the upper mixed layer was poorly developed, so temperature declined almost linearly over depth. MM abundance peak was located on the surface (Fig. 5). In the afternoon, an upper mixed layer of 15–25 m was formed. MM abundance responded to that by descending to the lower boundary of the mixed layer and formed the subsurface maximum there (Fig. 5). So, the monotonic declining trend of MM abundance has transformed into a nonlinear vertical distribution with multiple maxima and minima.

It should be emphasized that temperature changes observed were not mediated by solar insulation of the upper layer. These changes were associated with horizontal advection of waters, which was reflected in transformations of temperature and salinity temporal profiles in the upper horizons of the water column (Fig. 6). These transformations indicated the penetration of cold and saline deep waters: an indicative of coastal upwelling, mediated by regional winds (Repetin, 2012).

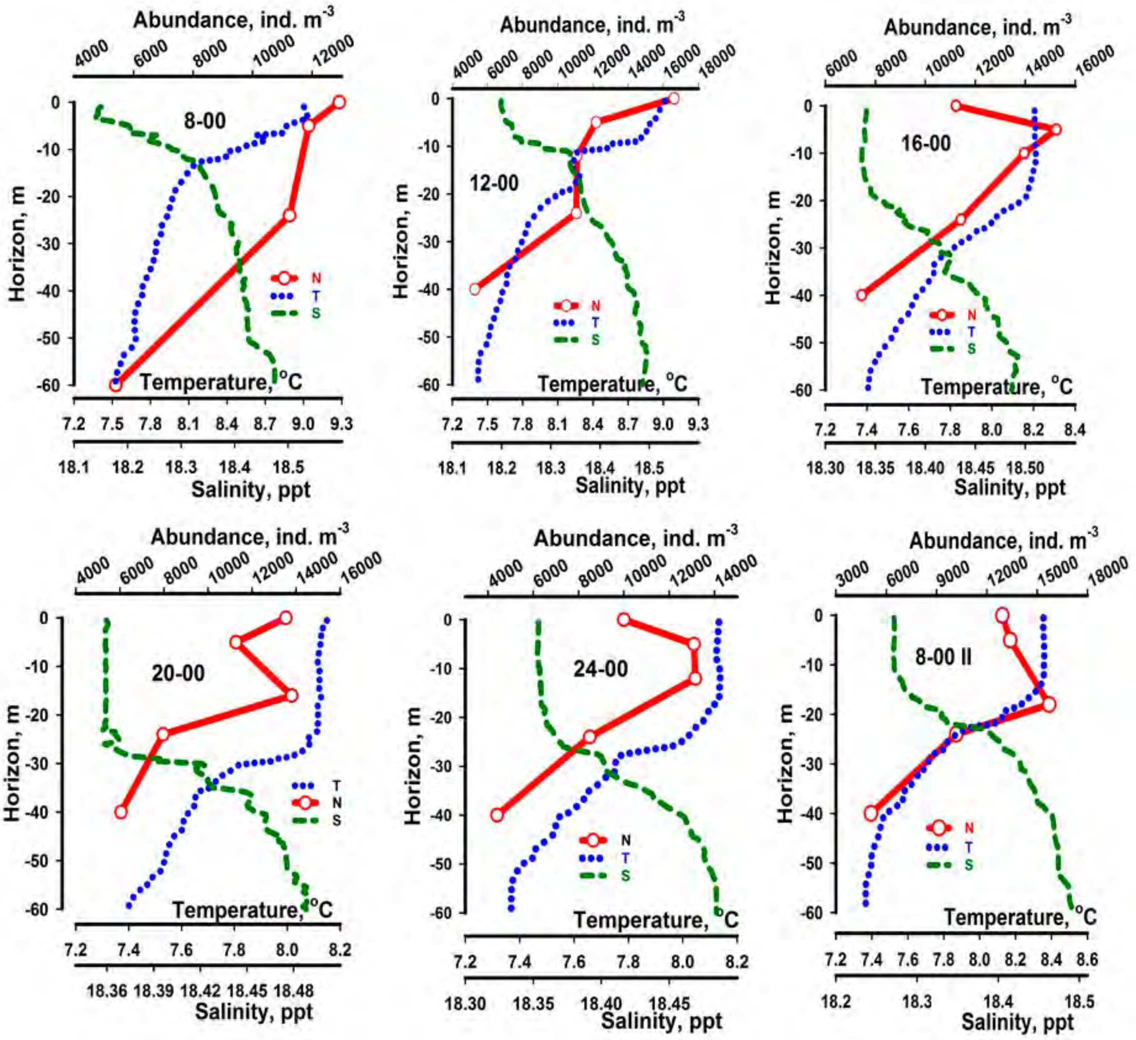

Fig. 5. Vertical distribution of temperature, salinity, and total metazoan microzooplankton abundance during the daily time series experiment at station 30 (Laspi Bay traverse, 30–31 March 2017)

During the time series experiment, the correlation between MM abundance and temperature was positive (0.7–0.9; $P < 0.05$), while the correlation between MM abundance and salinity was negative.

On the one hand, this experiment confirmed the stability of the correlations between MM distribution and physical characteristics of the water column in the temporal aspect. On the other hand, this experiment revealed the importance of hydrodynamic factor, capable of modifying hydrological structure of the water column in a short period of time.

Total MM abundance during two spring researches (2016 and 2017) was contributed mainly by copepod naupliar stages and bivalve larvae. Gastropoda larvae, *O. dioica*, and Rotatoria and Polychaete larvae were much less abundant. As known, temperature is one of the most critical abiotic factors, influencing biological functions of organisms at all levels (Hochachka & Somero, 2002). With regard to this factor, Black Sea copepods were divided into three groups: the first one – preferring low temperature, the second one – preferring high temperature, and the third one – being tolerant to a wide range of temperature changes. High temperatures are preferred by *Acartia tonsa*, *Oithona davisae*, and *Centropages ponticus* Karavaev, 1894. The first two species were not observed in our samples, while the third one contributed 1.5 % to total MM abundance in 2016, but was almost absent in samples a year later.

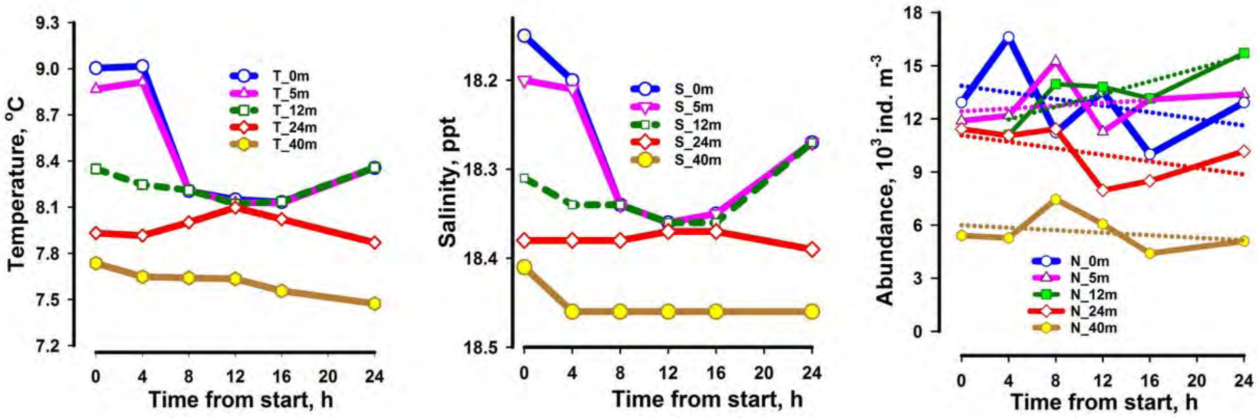

Fig. 6. Daily dynamics of temperature, salinity, and total metazoan microzooplankton abundance on sampling depths (surface, 5, 12, 24, and 40 m) on Laspi Bay traverse

Cold-loving species were represented by *Oithona similis*, *Pseudocalanus elongatus*, and *Calanus euxinus* Hulsemann, 1991. Species with a wide range of thermal tolerance were represented by *Acartia clausi* and *Paracalanus parvus*.

The cited diversity of thermal preferences explains low correlations between total MM abundance and temperature over stations sampled, since temperature preferences of cold-loving and more thermophilic species compensate each other. Another possible reason for the lack of correlations at some stations is the small number of horizons studied. Thus, all cases of statistically significant correlation between total abundance and temperature/salinity occurred at stations with a greater number of samples taken (horizons studied) (st. 5, 7, 11, and 30 in 2017).

On species level, certain correlations with temperature were observed. For instance, cold-loving *P. elongatus* has negatively correlated with temperature, while eurythermic *A. clausi* showed an opposite trend. Due to this, *Pseudocalanus* formed its maximum in deeper layers, while *Acartia* abundance maximum was observed on the surface.

Copepod fraction of MM showed different vertical patterns during summer and spring seasons. For instance, *O. similis* was completely absent in the surface waters at summer and lived mainly under the thermocline and in the bottom layers (Seregin & Popova, 2016b), whereas in mid-spring this species peaks were associated both with the lower horizons at +9.5…+10.3 °C and with the upper layers at +10.9…+11.0 °C (Seregin & Popova, 2019). Similar spring *vs* summer difference was observed in *P. elongatus* distribution, which preferred cold temperatures as well (Seregin & Popova, 2016b). *O. similis* summer pattern corresponds to the cold-loving status of this species, distributed over the World Ocean Arctic and temperate waters (Wend-Heckmann, 2013). For Black Sea waters, this species is a North Atlantic immigrant. *O. similis*, along with *C. euxinus* and *P. elongatus*, forms a deep-sea complex of cold-water copepods (Nikitin, 1926) and can be found year-round (Yildiz & Feyzioğlu, 2014). Adult forms and copepodites prefer summer layers with temperature about +8 °C (Kovalev, 1967). At lower temperatures in early spring, we registered further changes in vertical distribution of *O. similis* juvenile stages: an even more superficial distribution of this species abundance. As a result, there was a change in the sign of correlation with temperature from negative to positive, which suggests a corresponding change in species status (temporary) from cold-loving to somewhat more thermophilic. Such phenomena may reflect a shift in temperature optimum of a population (Verbitsky, 2012) and play the role of an adaptive reaction in conditions of seasonal changes in sea thermal characteristics. The mechanism of such reaction can be a fine-scale behavioral selection of preferable water characteristics by different zooplankton species and life stages (Trudnowska et al., 2015).

A. clausi was abundant in the upper mixed layer of the water column during both seasons. Despite the status of eurythermic, this species manifested itself rather as a warmth-loving species at spring temperatures. Therefore, correlation of *Acartia* with temperature was always positive. Along with *O. similis*, which has changed its temperature status, *A. clausi* was the most abundant species in 2017. So, these two species mediated a positive correlation of total MM with temperature.

Conclusion. Hydrological parameters of the environment (temperature and salinity) and species composition in specific habitats were the main drivers of vertical distribution of metazoan microzooplankton abundance. In addition, hydrodynamic processes (for instance, horizontal advection of waters) can significantly affect hydrological structure of the water column and MM distribution pattern.

The correlation between MM species abundance, temperature, and salinity was elucidated for most sampled stations during spring season. In particular, *A. clausi* abundance always correlated positively with temperature and negatively with salinity. This species manifested itself rather as a warmth-loving one at spring temperatures. A reversed correlation pattern was observed in *P. elongatus* abundance. On total MM abundance level, correlations with temperature and salinity were weak and observed in early spring in 2017; thus, the relationship between vertical distribution of MM abundance and temperature is more pronounced in cases of low temperature.

A change in the sign of correlation with temperature during spring season was determined for *O. similis*, an initially cold-loving species of Black Sea copepods. This manifested in a more superficial distribution of maximum abundance of this species at lower seasonal temperatures, which could reflect a shift in temperature optimum of species population and play the role of an adaptive reaction in conditions of seasonal changes in sea thermal characteristics.

Highlights:

1. Correlations with temperature were weak on total abundance level and were only observed in early spring period with lower temperatures.
2. On species level, correlation between abundance, temperature, and salinity was elucidated for most cases.
3. Different species exhibited different correlation with temperature: both positive (for instance, *Acartia clausi*) or negative (*e. g.*, *Pseudocalanus elongatus*). Some cold-loving species (*Oithona similis*) exhibited the change in the sign of correlation with temperature during a subsequent seasonal warming.

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НЕКОТОРЫЕ ОСОБЕННОСТИ ВЕРТИКАЛЬНОГО РАСПРЕДЕЛЕНИЯ МЕТАЗОЙНОГО МИКРОЗООПЛАНКТОНА В ЧЁРНОМ МОРЕ В ВЕСЕННИЙ ПЕРИОД

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По материалу, полученному в 84-м и 93-м рейсах НИС «Профессор Водяницкий», проанализировано вертикальное распределение микропланктонной фракции метазоопланктона (ММ) в Чёрном море в весенний период. Суммарно обследовано 27 станций как в прибрежной, так и в глубоководной частях мóря. Пробы воды для учёта численности ММ объёмом 4–6 л отбирали 10-литровыми батометрами зондов Mark-III Neil Brown или Sea Bird 911 (США) с 4–11 горизонтов водного столба. Полученные пробы концентрировали при помощи воронок обратной фильтрации и планктонного сита с размером пор 10 мкм. Количественный и систематический анализ всех проб проводили тотально в камере Богорова при помощи стереомикроскопа МБС-9. Основными факторами, определяющими характер распределения, являлись видовой состав ММ, физическая структура водного столба, а также гидродинамические процессы, воздействующие на его стабильность/неустойчивость. Наиболее многочисленными систематическими группами в «весеннем» ММ были науплиусы черноморских Copepoda и велигеры Bivalvia. Велигеры моллюсков обуславливали максимумы численности в нижних слоях мелководных местообитаний; над большими глубинами в составе ММ превалировали копеподы, определявшие пики общей численности в верхних и средних слоях воды. Суточный эксперимент по выявлению динамики вертикального распределения ММ показал существенное влияние на него адвективных гидродинамических процессов, воздействующих на физическую структуру водного столба. Для отдельных видов ММ в большинстве случаев выявлена корреляция их распределения с вертикальными профилями температуры и солёности, что редко проявлялось на уровне общей численности ММ. Сравнение двух весенних сезонов (2016 и 2017) показало, что связь вертикального распределения ММ и температуры проявлялась сильнее при более низких её значениях в море. Для исходно холоднолюбивого вида черноморских копепод, *Oithona similis*, обнаружена смена знака корреляции с температурой воды́ в процессе сезонного её изменения. В результате зарегистрировано более поверхностное распределение основных максимумов численности в более холодных условиях, что могло отражать изменение температурного оптимума вида и играть роль адаптивной реакции популяции при сезонных изменениях термальных характеристик моря.

Ключевые слова: метазойный микрозоопланктон, обилие и видовой состав, вертикальное распределение, корреляция с температурой и солёностью, Чёрное море

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CHRONICLE AND INFORMATION

TO THE JUBILEE OF D. SC. NELLI SERGEEVA

November 20, 2020 is a significant date in the life of Nelli Sergeeva, D. Sc., chief researcher of A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS.

Nelli Sergeeva was born in Ashgabat (Turkm.SSR). After finishing school in Alma-Ata (Kaz.SSR), she entered the faculty of biology and soil science of S. M. Kirov Kazakh State University (now Al-Farabi Kazakh National University). When being a student, she completed both course and pre-diploma internships in benthos department of IBSS of the Academy of Sciences of the Soviet Union, under the guidance of PhD Marta Kisseleva and PhD Iraida Greze. N. Sergeeva received a degree in biology, hydrobiology, and ichthyology and was assigned to work in the Kazakh Research Institute of Fisheries (the city of Balkhash). She was involved in assessing the effect of Balkhash Mining and Metallurgical Combine effluents on development and distribution of plankton and benthos in Lake Balkhash. She also focused on the formation of benthos as a food base for valuable commercial fish species in the Chardara Reservoir, artificially created in 1966 on the Syr Darya River.

In 1968–1972, Nelli Sergeeva studied in the PhD graduate school in hydrobiology in IBSS under the supervision of Vladimir Vodyanitsky, director of the Institute, corresponding member of the Academy of Sciences of the Soviet Union. Having successfully completed PhD graduate studies, she started working in IBSS benthos department, where defended PhD thesis “Fauna and some questions of ecology of free-living nematodes of the Black Sea” (1974). Her further research in the area of diversity and properties of life in the Black Sea resulted in obtaining a very wide range of new data. In 2000, N. Sergeeva defended her D. Sc. dissertation “Zonal distribution of meiobenthos and its most important component – free-living nematodes in the Black Sea”, with the advisory assistance of Victor Zaika, the corresponding member of the National Academy of Sciences of Ukraine. In this work, she described 4 genera and 27 new species of free-living nematodes; 30 species and 28 genera of nematodes were indicated for the Black Sea for the first time.

Nelli Sergeeva and Irina Yakusheva, Barcelona (1974)

Participants of the 102nd cruise of the RV “Akademik Kovalevsky” under the guidance of N. Sergeeva

Being in love with science, N. Sergeeva permanently broadens the scope of her interests. She is studying taxonomy and ecology of various marine meiofauna groups, their significance for bioindication, structure of meiobenthos, and its role in benthic ecosystems. She has made an inventory of the meiobenthos composition (more than 500 species) of the Black Sea.

She has shown particular interest in studying specific Black Sea benthic communities, formed under extreme conditions: hypoxia, anoxia, hydrogen sulfide contamination, anthropogenic pollution, and biotopes of methane gas bubble streams. Being thoughtful and scrupulous, she has discovered unknown to science living unicellular organisms (ciliates, soft-shelled foraminifera, and gromiids) and multicellular organisms (rotifers, nematodes, oligochaetes, polychaetes, and tardigrades), as well as unknown to the Black Sea species and genus of gastrotrichs. These discoveries were made in Black Sea anoxic zone – at the depths, previously considered lifeless (except for the bacterial flora) due to hydrogen sulfide contamination. She described live Cladocera specimens, raised from the depths of 1900 and 2140 m, as the new genus *Pseudopenilia* Sergeeva, 2004 and species *P. bathyalis* Sergeeva, 2004. In co-authorship with D. Sc. N. Korovchinsky, she substantiated the family Pseudopenilidae Korovchinsky & Sergeeva, 2008, new to science, in the order Ctenopoda (Crustacea). During a cruise of the RV “Maria S. Merian” (Germany), Nelli Sergeeva found specific representatives of epibiotic ciliates on oligochaetes and harpacticoids, inhabiting Black Sea area of permanently anoxic and hydrogen sulfide conditions (depth over 250 m). Later, D. Sc., Prof. I. Dovgal has identified them as three species from the subclass Peritrichia and class Suctoria. Direct microscopic observations of active ciliates in hydrogen sulfide environment indicate the vital activity of benthic ciliates and their multicellular hosts under these extreme conditions.

Research of N. Sergeeva expanded the list of benthos faunal groups in the Black Sea. She was the first to discover gromiids (Gromiida) and soft-shelled foraminifera (Allogromiida) in Black Sea benthos communities and to describe their distribution. Together with O. Anikeeva, she has published the monograph with the description of 13 valid species and more than 90 unknown morphospecies (*incertae sedis*), inhabiting the Black Sea (https://doi.org/10.21072/978-5-907118-84-3). To date, within the framework of this group, she and her co-authors have described 3 genera and 7 new species of allogromiids; 13 species and 8 genera have been indicated

N. Sergeeva among the participants of the 53rd cruise of the RV “Professor Vodyanitsky” (1999) for the Black Sea for the first time. Together with T. Revkova, she continues studying free-living nematode species, new to science and the Black Sea. According to Nelli Sergeeva, systematic status of at least 250 species of this group requires clarification. The interest of N. Sergeeva and her student Kh. O. Kharkevich in a poorly studied group of tardigrades (Tardigrada) resulted in discovering two species, new to the Black Sea, in Bosphorus Strait area.

Nelli Sergeeva made a significant contribution to the development of the concept of vertical zoning of life in the Black Sea. Based on new data on the diversity and bathymetric distribution of benthic fauna in the Black Sea, N. Sergeeva has revealed two belts of Black Sea benthal. Thus, the area from the water edge to 120–150 m is inhabited by organisms of macro-, meio-, and microbenthos, while the area from 120–150 m to maximum depths is inhabited by organisms of meio- and microbenthos. In fact, this created a new scientific direction in the research of benthos of deep-water periazoic areas of the Black Sea. Nelli Sergeeva considers that the study of various morphotypes of benthic organisms, common for bottom sediments at the extreme depths of the continental slope and deep-sea bed of the Black Sea, may be useful both for understanding the processes of modern sedimentation in the sea and for deciphering paleoclimatic changes.

Participants in the celebration of the anniversary of IBSS shelf ecosystems department (2006)

N. Sergeeva has taken part in 17 scientific cruises, *inter alia* international ones. As a hydronaut-researcher, she dived to a depth of 110 m on an underwater vehicle “Benthos-300” in Caucasus and Crimea area. She acted as a coordinator, manager, and responsible executor of several international (HERMES, HYPOX, CoCoNet, PERSEUS, TÜBITAK-NASU, and EPA USA-STCU) and national projects, as well as of RFBR grants. She has published over 200 scientific works, *inter alia* sections in nine collective national and foreign monographs. The results of her research are widely cited by scientists all over the world. Zoologists highly appreciated the contribution of Nelli Sergeeva to taxonomy by naming in her honor two new species of free-living nematodes, *Leptolaimus sergeevae* (Ürkmez & Brennan, 2013) Holovachov & Boström, 2013 and *Microlaimus sergeevae* Revkova, 2020, as well as the genus and species of soft-shelled foraminifera, *Nellia* Gooday, Anikeeva & Pawlowski, 2011 and *Nellia rugosa* Gooday, Anikeeva & Pawlowski, 2011.

N. Sergeeva successfully combines intensive scientific work with organizational one: she was a secretary and a member of the specialized council for the defense of PhD and D. Sc. dissertations; she was the head of IBSS shelf ecosystems department (2000–2013); she participated in examination commissions, supervised the work of graduate students and applicants, and was the advisor at IBSS directorate (2017–2018). For many years of fruitful work and significant contribution to the development of national science in the field of marine hydrobiology, she was listed on the city Board of Honor (2019) and awarded the Certificate of Honor of the Russian Academy of Sciences (2020).

Nelli Sergeeva is immensely hard-working; she gets interested in anything new and unknown and tries to inspire her colleagues with own example. Having vast experience and scientific intuition, she is approached for consultations and proposals for joint research by bentologists. Her whole life is devoted to science. In her free time, she enjoys travelling; she is fond of classical music, painting, and theater. For the family, she is a loving mother and a caring grandmother.

We sincerely congratulate Nelli Sergeeva on her jubilee! We wish her new scientific discoveries and achievements, talented students, happiness, health, and family well-being.

Colleagues from IBSS benthos ecology department

К ЮБИЛЕЮ ДОКТОРА БИОЛОГИЧЕСКИХ НАУК НЕЛЛИ ГРИГОРЬЕВНЫ СЕРГЕЕВОЙ

В ноябре 2020 г. отметила юбилей Нелли Григорьевна Сергеева — главный научный сотрудник ФИЦ ИнБЮМ, доктор биологических наук. Н. Г. Сергеева является известным в мире специалистом-мейобентологом, автором более чем 200 работ и соавтором 9 монографий.

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TO THE MEMORY OF VALERY EREMEEV
(12.01.1942 – 31.10.2020)

On 31 October, 2020, Academician Valery Eremeev, who headed A. O. Kovalevsky Institute of Biology of the Southern Seas in 1999–2015, passed away.

V. Eremeev was born on 12 January, 1942, in Saratov, where his parents were evacuated at the beginning of the Great Patriotic War. He spent his post-war childhood in Rostov-on-Don. His parents instilled in him a taste for art, culture, and freedom of thought, but at the same time he considered compliance with the laws as a cornerstone duty: mostly thanks to his mother Kaleriya, who was an honored lawyer of the Russian Federation. After finishing school, he entered Rostov-on-Don State University and received a degree in atomic physics.

After graduating from the University, Valery Eremeev started working on assignment: at the Marine Hydrophysical Institution (MHI) in Sevastopol. Later, he served as a sailor in the Red Banner Black Sea Navy. After returning from the service, he resumed scientific activity. In 1968, he started PhD graduate studies at MHI, specializing in marine physics. Six years of hard work on the PhD thesis “Peculiarities of Precision Mass Spectrometric Isotope Analysis of Water Oxygen and Some Issues of ^{18}O Distribution in Atlantic Ocean Waters” resulted in its successful defense at the P. P. Shirshov Institute of Oceanology.

In 1975, at the age of 33, V. Eremeev was appointed MHI scientific secretary. He went on with scientific work and participated in many research cruises. Fortunately, Soviet oceanology provided an opportunity to work on specialized research vessels in various areas of the World Ocean. He was the head and a participant of expeditions to the Atlantic, Indian, and Pacific oceans and visited many countries, such as Sri Lanka and Seychelles, India, Singapore, Egypt and Turkey, Costa Rica and Brazil, Spain, Italy, Yugoslavia, Morocco, and the Republic of Guinea: there were more and more points on the world map, where his scientific curiosity found research objects. A lot of new data, resulting from his research, were obtained in the field of hydrochemistry, ocean geochemistry, hydrophysics, marine ecology, and scientific instrumentation.

In 1982–1992, he headed the Soviet-Guinean research program in oceanography, heliophysics, and testing of structural materials under the conditions of tropical climate, working at the large research center CERESCOR (Conakry Rogbanè), built by the Soviet Union. In 1988, Valery Eremeev was awarded a Certificate of Honor of the Secretariat for Scientific Research and Education by the Republic of Guinea. Later, he said that the time, spent in Guinea, was one of the happiest in his life, and the reverent attitude towards Africa remained forever.

In 1982–1992, V. Eremeev was a member of the Scientific Council of the USSR State Committee for Science and Technology on the problems of studying seas and oceans. In 1982, he was awarded the diploma of the Presidium of the Supreme Soviet of the Ukrainian SSR for achievements and application of scientific developments in practice. In 1983, he was elected a member of the Ukrainian Geographical Society.

After five years of work as a MHI deputy director for scientific work (1980–1985), at the age of 43, Valery Eremeev became the director of MHI. Three years later, he summarized the accumulated data and defended D. Sc. dissertation "Oceanographic Tracers: Dynamics Simulation, Transport Parameterization, and Practical Applications" at the Institute of Geophysics of the Academy of Sciences of the USSR, in the specialty "Geophysics" and "Marine Physics". In 1989, the Academy of Sciences noted the importance of the results, obtained under his leadership in the study of mechanisms of transport of substances and physicochemical fractionation of impurities in Atlantic Ocean and Black Sea waters and at the boundaries of the marine environment with the atmosphere and the seabed. He became the Academician V. I. Vernadsky Prize winner in Geology, Geochemistry, Geophysics, and Hydrochemistry.

In 1991, by the decision of the Higher Attestation Commission under the USSR Council of Ministers, V. Eremeev was awarded the academic title of Professor. By the end of the year, the collapse of the USSR was over. A new stage of Valery Eremeev's life began.

In 1992, he became the scientific director of both the State Scientific and Technical Program of Research and Use of the World Ocean in the Interests of Science, National Economy, and Defense of Ukraine and the National Program of Research and Use of Resources of the Sea of Azov – Black Sea Basin. In the same year, the Academy of Technological Sciences of Ukraine elected him the Academician in geotechnology, and the National Academy of Sciences of Ukraine (the NAS of Ukraine) – a corresponding member in chemical oceanography. Moreover, he became the vice president of the Mediterranean Science Commission for three years. In 1997, V. Eremeev was elected the Academician of the NAS of Ukraine in oceanology.

Since 1999, he headed the Oceanological Center of the NAS of Ukraine, organized on his initiative. Its objectives were to create favorable conditions for the development of fundamental and applied oceanography, as well as to develop a unified concept of rational marine nature management and use of scientific and technical potential of marine institutions and technical means of marine environment state monitoring.

Seemingly, brilliant career and clear prospects. But then Boris Paton, the President of the NAS of Ukraine, urged Valery Eremeev to act as a crisis manager and temporarily director of the A. O. Kovalevsky Institute of Biology of the Southern Seas (IBSS), which was going through a difficult time.

On 8 December, 1999, by the decision of the Presidium of the NAS of Ukraine, the duties of IBSS director were assigned to V. Eremeev (temporarily, for the period *prior* elections). In January 2000, elections were announced, and Institute staff nominated seven candidates, with Valery Eremeev not present among them.

Understanding that there is no obvious leader and the probability of any candidate receiving the required half of scientists' votes is minimal, in March the Presidium of the NAS of Ukraine decided to postpone the elections for a year, with a diplomatic formulation "proceeding from the necessity to avoid complicating the situation in the Institute and to strengthen its management". Boris Paton was sure, that V. Eremeev only, one of the best directors of the institutions in the system of the NAS of Ukraine, would be able to get the accumulated problems sorted out. It was impossible to refuse, and the duties of IBSS director were assigned to Valery Eremeev again.

The entrusted "household" definitely was not in the best condition. Everything was in chaos. The Institute was barely paying part-time wage, having no money for more. The opportunities to collect samples were minimal: the leased RV "Professor Vodyanitsky" was carrying "shuttle traders" to Istanbul; the researchers earned part-time money on loading and unloading the vessel and worked during these cruises as service personnel. Not surprisingly, dissertations were defended only once every few years. Since there was no money for the maintenance of Aquarium inhabitants, it was also in a long-term lease. Boat houses, built at the end of the Soviet regime for small fleet vessels, were leased as well; "Konstantinovskiye saunas" were housed there. The RV "Akademik Kovalevsky" was sold to Turkey in hope of paying off wage arrears. On the day the new director first came to the Institute, there were messages on the notice board about the upcoming sale of small Soviet-built IBSS vessels, to pay debts.

V. Eremeev managed to achieve an increase in IBSS budget financing; soon after his assignment, the directorate began dealing with a matter, long forgotten in the maelstrom of perestroika years: planning of the expeditionary research within the framework of the future Marine Program of the NAS of Ukraine. Since that time, not a single meeting of IBSS Scientific Council was held without a scientific report: Valery Eremeev considered collective discussion of research results the most important factor of science support and planning. In subsequent years, the Institute participated in various academic programs of the departments of General Biology and the Department of Geosciences of the NAS of Ukraine. With the director's support, the Institute was involved in the projects under UNESCO programs, as well as in the 5th, 6th, and 7th Framework Programmes of the European Union. IBSS became one of the leaders in international cooperation among the institutions of the Department of General Biology of the NAS of Ukraine.

Through his efforts, the RV "Professor Vodyanitsky" was returned from lease and later repaired. In 2010 (after more than a 15-year break), research cruises were resumed. Comprehensive monitoring of geological and marine environment state and biota of the Black Sea was restored, using modern technologies of ship measurements, satellite information, and specialized databases.

In 2000, V. Eremeev, with a team of authors, became a laureate of the State Prize of Ukraine in science and technology for the series of works "Regional Oceanology: State of the Environment and Mineral Resources of the Atlantic, Indian, and Southern Oceans and Their Seas". Three years later, he was awarded the title of Honored Worker of Science and Technology of Ukraine. He was a member of the Committee on the State Prizes of Ukraine in science and technology. He was convinced that scientific achievements only become noticeable when they are loudly announced. Therefore, he did his best for the merits of scientists of the Institute in Sevastopol and its branch in Odessa to be known and recognized. At the insistence of Valery Eremeev, scientists of IBSS and its branch applied four times to the Committee on the State Prizes of Ukraine. During the period he was the director of the Institute, such prizes were deservedly awarded to 10 IBSS hydrobiologists: in 2007 – for a series of scientific works "Productivity, Biodiversity, and Ecological Safety of Black Sea Ecosystems and World Ocean Areas, Promising for Ukraine", and in 2011 – for the development and creation of the National Collection of Nautical Charts and the Oceanographic Atlas of the Black Sea and the Sea of Azov. He was especially proud of the Atlas and kept this beautiful huge blue book in the place of honor of his office: in the "exhibition" of IBSS monographs.

In spite of numerous scientific titles, unusually broad scientific outlook, membership in several dissertation councils, and chairmanship of MHI dissertation council, V. Eremeev refused to be a chairman of IBSS dissertation council: being an oceanologist, he considered himself not competent enough to assess hydrobiological works. When preparing director's reports for the Presidium of the NAS of Ukraine, he was never ashamed to ask narrow specialists for clarification, if he did not understand something in their scientific results. As colleagues joked, he did not wear the "halo of the Academician" at work. The opinion of IBSS scientists was always important to him when solving the pressing issues; he listened to everyone and tried to prevent conflicts.

His research interests covered the key problems of oceanography: experimental and nuclear hydrophysics, isotope oceanography, geophysical hydrodynamics, and ocean hydrochemistry. His works in isotope oceanography were related to studying the fundamental regularities of the spatial formation and dynamics of artificial and natural radioactivity areas of the World Ocean and its seas, development of general theoretical approaches to the analysis and forecast of possible consequences of marine disasters of natural and anthropogenic nature, and investigating the problems of radioactive waste disposal and use of water column as a global radiation indicator of cosmic radiation.

Valery Eremeev worked fruitfully on the formation and development of geophysical direction in marine ecological research: studying of physical and chemical aspects of interaction between the ocean and the atmosphere and the development of general concept and analytical technologies for investigating water dynamics and substance transport in marine ecosystems by natural and artificial oceanographic tracers. His most significant results are description of dynamics, interannual and seasonal variability of characteristics of Black Sea hydrogen sulfide zone, oxygen and hydrogen sulfide interaction layer, as well as the influence of various physical, chemical, and hydrodynamic factors on migration and redistribution of elements of basic salt and microelement composition of waters on Black Sea shelf and in depths.

He established a new scientific direction: "operational oceanology", with the subject being methods and technologies for complex research of the state and dynamics of complex marine systems, aimed at predicting their variability under the influence of natural and anthropogenic factors and developing technologies for sustainable use of the marine environment and resources, as well as theory and methods for modeling marine ecological and economic systems for integrated coastal zone management.

The practical value of V. Eremeev's results is determined by their direct relation to the problem of radioactive pollution of the marine environment and development of the nuclear navy. These data were used, in particular, to monitor the nuclear powers' compliance with the Treaty of Moscow, banning nuclear weapon tests in the atmosphere, outer space, and under water, and to establish the principles of organizing the global system for monitoring nuclear tests and preparing the Comprehensive Nuclear-Test-Ban Treaty. Great attention in such research was given to the study of Chernobyl disaster consequences for the ecosystem of the Black Sea and other World Ocean seas. His works significantly contributed to the formation of scientific bases for various conventions for marine environment protection and to the development and implementation of several national and international oceanographic programs. Highly appreciating Valery Eremeev's scientific achievements, in 2005, the Department of Earth Sciences of the Russian Academy of Sciences awarded him the title of doctor *honoris causa* of RAS. Three years later, he became a laureate of the State Prize of the Russian Federation in science and technology: for creation and implementation of a multicomponent system for obtaining reliable data of ecological estimation and forecast of marginal seas of Russia. In 2007, he was awarded the Order of Ukraine "For Merit" of the III degree.

Theoretical foundations, methods, and means of the global oceanographic observation system, developed under the leadership of V. Eremeev, were the basis of the corresponding programs of the Intergovernmental Oceanographic Commission (IOC) of UNESCO and of several countries of the Black Sea basin. Since 1994, he was a member of the National Commission of Ukraine for UNESCO. Over the years, he was the representative of Ukraine on the Executive Council of IOC of UNESCO and the chairman of its Black Sea Regional Committee, as well as the chairman of the Steering Committee of the Global Ocean Observing System for the Black Sea. In 2011, recognizing his outstanding contribution to the development of marine science and technology, the IOC of UNESCO awarded him a commemorative medal.

Valery Eremeev was the author of more than 500 scientific works, *inter alia* 15 monographs and 3 atlases of the Sea of Azov – Black Sea basin, organizer and participant of numerous oceanographic expeditions, and Editor-in-Chief of "Marine Hydrophysical Journal" (MHI) and "Marine Ecological Journal" (IBSS). An incredible workload did not stop him from working for the good of the city: in 1985–1992, he was a deputy of the Sevastopol City Council of People's Deputies. In 2004, he was awarded the sign "For Services to the Hero City of Sevastopol".

Outstanding scientist and talented leader, he worked until the very end. After leaving IBSS in 2015 and moving to Kyiv, he remained an advisor to the Directorate of the Scientific Hydrophysical Center of the NAS of Ukraine.

The merits of V. Eremeev as a researcher and organizer of science are numerous; his honorary titles and awards are a sign of recognition of undoubted achievements. Among his students, there is one Academician, seven D. Sc., and more than twenty PhDs. But could his destiny be different, if he was nicknamed "professor" by friends in his early childhood?

The staff of the A. O. Kovalevsky Institute of Biology of the Southern Seas is deeply grateful to Valery Eremeev for raising IBSS from the ruins in the 1990s, resuming its marine expeditionary practice, and putting the RV "Professor Vodyanitsky" back at the service of science. For 14 years, he ensured the smooth movement of IBSS to new scientific achievements, with a sense of tact of a talented leader, subtle intelligence, erudition of a 6prominent scientist, and benevolent humaneness.

Blessed memory to Valery Eremeev, endless gratitude, and respect.

Staff of IBSS

ПАМЯТИ ВАЛЕРИЯ НИКОЛАЕВИЧА ЕРЕМЕЕВА
(12.01.1942 – 31.10.2020)

Ушёл из жизни академик Валерий Николаевич Еремеев, возглавлявший ИнБЮМ в 1999–2015 гг. Он автор более чем 500 научных трудов, среди которых 15 монографий и 3 атласа Азово-Черноморского бассейна. В. Н. Еремеев был организатором и участником многочисленных океанографических экспедиций, академиком НАН Украины и почётным доктором РАН.

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GLOBAL PROBLEMS OF THE WORLD OCEAN: RESULTS OF THE XI ALL-RUSSIAN SCHOOL-SEMINAR FOR YOUNG SCIENTISTS


Федеральный исследовательский центр
Институт биологии южных морей имени А. О. Ковалевского
Российской академии наук

СОВРЕМЕННАЯ ГИДРОБИОЛОГИЯ:
ГЛОБАЛЬНЫЕ ПРОБЛЕМЫ
МИРОВОГО ОКЕАНА

Материалы XI Всероссийской онлайн-школы-семинара
для молодых ученых, студентов и аспирантов

Севастополь
28 сентября – 2 октября 2020 года

The XI All-Russian school-seminar for young scientists, students, and postgraduates “Modern hydrobiology: Global problems of the World Ocean”, organized by IBSS Council of young scientists, took place on 28 September – 02 October 2020. Since 2001, the Institute has held 11 schools-seminars for young scientists. However, this year alone, all the events took place online: on the platform https://webinar.ru/ and on the VK social network.

In the VK group, sessions of oral and poster presentations were organized for the participants to discuss global problems of the World Ocean and possible ways of solving them. The organizing committee of the school-seminar assessed the relevance and scale of scientific research, as well as the presentation level. The first place among oral presentations was taken by the report of A. A. Tkachuk (Sevastopol) “Influence of plastic decomposition products in the ocean (phthalates) on the functional parameters of the Black Sea mussel (*Mytilus galloprovincialis*)”. The first place among posters was shared by reports of A. I. Ilyasova (Kazan) “Additional nidamental glands of three species of the genus *Rossia* (Cephalopoda, Sepiolida) from the Barents Sea” and A. A. Paraskiv (Sevastopol) “Study of sedimentation processes in the Black Sea using plutonium radioisotopes”.

Based on the results of the work of the sections, a book of proceedings was prepared. It is uploaded in the national bibliographic database Russian Science Citation Index (https://www.elibrary.ru/item.asp?id=44077021), as well as in IBSS Open Access Repository (https://doi.org/10.21072/978-5-6044865-2-8). It includes materials of reports, revealing various aspects of modern marine and freshwater hydrobiology and hydroecology. The publications of young scientists from Russia and Uzbekistan highlight the results of scientific research in the field of dynamics of number of hydrobionts and peculiarities of their life cycles. Some works consider mechanisms of hydrobiont adaptation to abiotic and anthropogenic environmental factors, as well as hydrobiology application areas.

Online participants got acquainted with the research of leading specialists on fundamental bases of hydrobionts adaptation to environmental changes, problems of conservation and rational use of marine biological resources, perspective directions of marine biotechnology and aquaculture, and methodology and organization of operational control of Black Sea biota and coastal ecosystems. Online master classes were organized by IBSS Council of young scientists in every direction presented. The participants got acquainted with the application of flow cytometry and chromatographic and molecular-genetic methods in hydrobiological studies, as well as with carotenoid production technology. Lectures are available even after the end of the school-seminar, since using the platform https://webinar.ru/ made it possible to record all the reports and to post them on the VK social network.

Thus, the restrictions, imposed by the distance format, did not affect the process of scientific communication during the school-seminar. Moreover, online broadcasts organization and reports recording can increase the number of participants and the quality of offline conferences.

E. S. Kladchenko, IBSS

ГЛОБАЛЬНЫЕ ПРОБЛЕМЫ МИРОВОГО ОКЕАНА: ИТОГИ XI ВСЕРОССИЙСКОЙ ШКОЛЫ-СЕМИНАРА ДЛЯ МОЛОДЫХ УЧЁНЫХ

XI Всероссийская школа-семинар для молодых учёных, студентов и аспирантов «Современная гидробиология: глобальные проблемы Мирового океана», организованная советом молодых учёных ФИЦ ИнБЮМ, прошла с 28 сентября по 2 октября 2020 г. Все мероприятия состоялись в онлайн-формате. Участники ознакомились с исследованиями по фундаментальным основам адаптации гидробионтов к изменениям окружающей среды, а также по проблемам сохранения и рационального использования морских биологических ресурсов, перспективным направлениям морской биотехнологии и аквакультуры, методологии и организации оперативного контроля состояния биоты Чёрного моря и прибрежных экосистем. В мероприятиях приняло участие более 30 докладчиков и слушателей. По результатам работы школы-семинара подготовлен сборник, размещённый в национальной библиографической базе данных РИНЦ.

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ONLINE CONFERENCE "ACTUAL PROBLEMS OF RESEARCH OF BLACK SEA ECOSYSTEMS – 2020"


ВСЕРОССИЙСКАЯ ОНЛАЙН-КОНФЕРЕНЦИЯ
АКТУАЛЬНЫЕ ПРОБЛЕМЫ ИЗУЧЕНИЯ ЧЕРНОМОРСКИХ ЭКОСИСТЕМ
2020
ТЕЗИСЫ ДОКЛАДОВ
19–22 октября 2020 г.
г. Севастополь, Российская Федерация

On the basis of A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS, an online conference "Actual problems of research of Black Sea ecosystems – 2020" was held on 19–22 October 2020, with the financial support of Russian Foundation for Basic Research (project No. 20-04-22006). The scientific forum was organized by IBSS, Marine Hydrophysical Institute of RAS, Institute of Natural and Technical Systems of RAS, Sevastopol State University, Sevastopol Branch of Lomonosov Moscow State University, and Department of Education and Science of Sevastopol.

More then 140 researchers, representing 15 scientific and educational institutions from Sevastopol, Moscow, Yalta, Simferopol, Voronezh, Rostov-on-Don, and Novosibirsk, took part in the scientific forum. Using TrueConf video conferencing software, 24 oral reports and 16 poster ones were made. Most of the reports were prepared based on the results of the authors' work on projects, supported by RFBR grants, *inter alia* on the results of investigations, which were carried out under grants from regional competitions for the best fundamental scientific research projects, conducted by RFFR and the city of Sevastopol.

The participants of the online conference presented the results of scientific research in marine biology and ecology of aquatic and coastal systems and discussed the prospects of their practical use for the development of innovative technologies, environmental protection, rational use of natural resources, reproduction of biological resources, and aquaculture. Methods, technologies, and means were discussed of technical support of ecological monitoring of aquatic and coastal ecosystems, information technologies for managing sustainable development of an ecological and economic system coast – sea of Sevastopol region; they allow establishing the permissible volumes of marine resources withdrawal. Applied problematics of the reports is of considerable importance for planning sustainable development of Sevastopol coastal areas, monitoring the state of the abiotic component of aquatic and coastal ecosystems, and increasing the reliability of marine forecasts.

A project of a new methodology was presented of system modeling for managing scenarios of nominal seafood production volumes in a natural and economic complex coast – the Sevastopol Bay. The need was shown to use new models and digital information technologies for managing assimilation resources of the Sevastopol Bay and recreational potential of resort facilities in the Sevastopol region; indicators of pollution and biodiversity were proposed as criteria for assessing the ecological state of marine ecosystem, as well as level of ecological safety of marine environment for recreation. For rational planning of sustainable development of agriculture in Crimea and Sevastopol, the speakers analyzed the effect of climatic conditions on viniculture by agroclimatic indicators; work in this field will help in predicting conditions for grapes growth throughout the life of vines. The mechanisms were studied of the effect of penalties for marine environment pollution of Sevastopol inner bays on the efficiency of economic systems, operating in coastal area. Based on bioindication methods, approaches were presented to ecological monitoring of the state of marine environment, aimed at assessing marine environment quality by biochemical response of fish blood parameters, as well as bioluminescence indicators of gelatinous animals. Such approaches expand the prospects of a comprehensive assessment, *inter alia* operational one, of the state of coastal area.

The need was substantiated to carry out microbiological research of aquaculture of Black Sea bivalve molluscs; methods were proposed of phage detection and phage identification of infections, caused by halophilic vibrios, which are pathogenic to Pacific oyster cultivated. The development of this research direction helps to solve the urgent problem of containing the spread of infectious diseases of molluscs during their industrial cultivation.

The current ecological state of Sevastopol coastal areas, water areas, and shelf zones, as well as their suitability for recreation were assessed by analysis of satellite data and using unmanned aerial vehicles. The results were presented of mathematical modeling of currents and eddies in the Kalamitsky Bay and Sevastopol area, as well as level of precipitation, *inter alia* extreme one, in Crimea and monitoring of coastal water subsequent pollution by storm runoffs, performed both by a surface unmanned vehicle and by a working model of an autonomous miniship with a set of sensors for physical and chemical indicators of marine environment and an intelligent control system. The assessment was carried out of changes and variability of anticyclonic activity indicators in Black Sea area. These works are of great applied importance; moreover, they serve as a key part of the system for forecasting atmospheric effects on hydrological situation. Experimental models of movement of landslide blocks were proposed; a project for reclamation of landslide-prone sectors of Sevastopol coastal area was presented.

Studies of fish taxonomic characters and biological peculiarities were carried out; the data obtained were used, *inter alia*, to form the basis of computer taxonomic guides. Modern data are presented, describing the state of taxonomic diversity and quantitative development of several groups of Black Sea hydrobionts (diatoms and cyanobacteria – foulers of polymer substrates on marine farms, as well as polychaetes, ascidians, molluscs and crustaceans, soft-shelled foraminifera and gromiids, trematodes, and phytoplankton complex of Black Sea pelagic zone). Current molecular genetic approaches were presented to the analysis of taxonomy and phylogeny of Black Sea hydrobionts, with the corresponding results. The obtained data on AqE gene representation, structure, and evolution in aquatic vertebrates help in analyzing its differential expression under various conditions, and this is to contribute to a better understanding of mechanisms of hydrobiont resistance to stress.

The participants of the online conference presented the results of theoretical and experimental studies of the state of the Black Sea and the atmosphere above it in the Crimea region. A method was proposed of environmental air monitoring for meteorological monitoring of coastal areas and, as a part of gas analytical complexes, for atmosphere monitoring at toxic and explosive enterprises.

Analysis of the dynamics of ecosystem abiotic parameters of Sea of Azov coastal area helps to make a near-term forecast of their changes in relation to the effects of global warming. Reduce of the volume of Don River annual runoff, changes in water hydrochemical composition, and radical transformations of its intra-annual distribution are considered the main factors of a possible ecological disaster in Tsimlyansk Reservoir area and in Lower Don basin. The need was emphasized to make a state comprehensive target programme on the recovery and development of the water management complex of the Don River and Sea of Azov to ensure food security in Russia. The study of the long-term dynamics of the hydrochemical indicators of Sea of Azov water shows a persistent tendency towards its significant salinization.

A new approach was presented to the development of wireless power transmission systems when charging batteries of autonomous electric vehicles; this assumes a complete absence of spurious radiation, and, accordingly, of a harmful effect on human and the environment. The use of wireless methods of energy transmission is very promising for the development of urban transport infrastructure, especially in coastal areas (recreation-oriented ones).

Several reports were focused on the results of biogeochemical studies. An original technique of sorbent production for lead extraction from seawater was presented. Results of field measurements of cosmogenic phosphorus and bismuth in Sevastopol area seawater were shown. Taking into account the relevance of studying alternative sources of water supply in the region, the researchers presented the results of the analysis of submarine groundwater discharge volumes in one of Sevastopol coastal areas and of water quality by a number of physical and chemical indicators. A balance estimate was carried out of the inflow into the Black Sea with Chernaya River water of biogenic elements, organochlorine compounds, trace elements, and anthropogenic radionuclides. The biogeochemical role of suspended matter was studied; as shown, it can significantly accelerate the biogeochemical cycles of turnover of heavy metals in the marine environment, as well as promote water self-purification.

The participants noted high methodological level of the works presented, good theoretical basis of the studies, their practical significance, and the need to coordinate research and practical activities of scientists to solve the pressing problems of Sevastopol. The participants agreed on the importance of regular scientific conferences.

The abstracts of the reports can be found in the conference proceedings: https://elibrary.ru/item.asp?id=44110968. Videos of oral and poster presentations are available on the scientific forum webpage: http://ibss-ras.ru/science/scientific-activities/actual-problems-of-research-of-black-sea-ecosystems-2020/.

Kornyychuk Yu. M., Pospelova N. V., and Velichko N. V.

ОНЛАЙН-КОНФЕРЕНЦИЯ «АКТУАЛЬНЫЕ ПРОБЛЕМЫ ИЗУЧЕНИЯ ЧЕРНОМОРСКИХ ЭКОСИСТЕМ — 2020»

Приведены результаты работы онлайн-конференции «Актуальные проблемы изучения черноморских экосистем — 2020», состоявшейся 19–22 октября 2020 г. на базе ФИЦ ИнБЮМ. В форуме приняли участие более 140 исследователей, представлявших 15 российских научных и образовательных учреждений.

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