

RESEARCH ARTICLE

The qualitative and quantitative characteristics of the benthic diatoms near Kazantip Cape of the Sea of Azov

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Abstract

This is the first record of species composition and quantitative characteristics of diatom communities on epiphyton and epilithon present near Kazantip Cape, including Kazantip Nature Reserve, located in the Crimean coast of the Sea of Azov for several periods between 2005 and 2014 (2005, 2006, 2008, 2010, 2011, 2014). Ninety-two taxa of Bacillariophyta were found, belonging to three classes, Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae (9, 19 and 64 taxa, respectively), 18 orders, 26 families, 41 genera. 17 new species were indicated for the Sea of Azov. The number of species found varied seasonally: 12 diatoms were seen in winter, 54 in spring, 41 in summer and 45 in autumn. The epiphyton of macrophytes contained 79 taxa and showed spring maximal number of 52 species, and the epilithon – 44, with the autumn maximum of 25 species. Applied to diatoms from the two ecotopes, Czekanowski-Sørensen index of species similarity was estimated 52%. Quantitative analysis for two ecotopes of diatoms was carried out; the epiphyton abundance ($N = 1171, 5 \cdot 10^3 \text{ cells} \cdot \text{cm}^{-2}$) was largest in September (19.3°C) and the biomass ($B = 0.142 \text{ mg} \cdot \text{cm}^{-2}$) in April (10°C) in 2006. For epilithon the maximum abundance and biomass were evaluated as $N = 72.6 \cdot 10^3 \text{ cells} \cdot \text{cm}^{-2}$ in April and $B = 0,008 \text{ mg} \cdot \text{cm}^{-2}$ in September. Epiphyton diatoms showed the averages of minimal and maximal abundance 2.4 and 16 times larger than in the epilithon.

Keywords: Biomonitoring, diatoms, microphytobenthos, Crimean coast, Kazantip Cape, Kazantip Nature Reserve, the Sea of Azov

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Introduction

The Sea of Azov belongs to the Mediterranean basin of the Atlantic Ocean having water exchange with the Black Sea through the Kerch Strait. Low salinity (11–13 ‰), a wide temperature range (-0.5°C to +30°C) and well-illuminated (to 15 m deep) water column are the features typical of the sea (Bronfman and Khlebnikov 1985). The sea bed actively accumulates auto- and allochthonous substances.

Growing anthropogenic load enhances nutrient regeneration at the seabed/water interface and, concurrently, bacterial and microalgal growth (Studenikina *et al.* 2002) thereby giving rise to high primary production and rich nutritive base for the invertebrates and fish. In the recent decade anaerobic destruction evaluated as 76–81% of the total decomposition of organic matter in the benthic sediment leads to hypoxic areas enlarging over the Sea of Azov.

Not only phytoplankton but also microphytobenthos play an important role in marine ecosystem. However diatoms have long been used as a leading group of organisms as environment indicators. Microphytobenthic diatoms are reliable test objects for measuring the level of biocontamination of seas, especially in coastal ecosystems. Therefore a study of qualitative and quantitative distribution of the benthos can substantially contribute to understanding of general tendency underlying the interaction with the environment. On the basis of species composition and quantitative characteristics of microphytobenthos, it is possible to assess the quality of the marine environment. It is becoming increasingly important under the conditions of growing anthropogenic load on the Sea of Azov ecosystem, which is the result of economical activities, including recreational activity.

The floristic studies of microphytobenthos diatoms which are presently carried out in different parts of the Sea of Azov are confined mainly to reviewing the species composition and for compiling a list of species (Borysiuk 2001, 2002; Kovaleva 2008; Bondarenko and Ryabushko 2008; Ryabushko and Bondarenko 2011; Bondarenko 2012). Surprisingly, however, the microphytobenthos diatoms of the Mediterranean basin also are poorly understood (Aleem 1950, 1951; Mazzella *et al.* 1978; Foged 1985; Ryabushko 1999; Sabanci 2010, 2012, Sabanci and Koray 2010).

Meanwhile, a little is known about benthic Bacillariophyta species and their quantitative distribution on different substrates in the Sea of Azov, including the coastal water of Kazantip Cape and Kazantip Nature Reserve (KNR). It was created in 1998 and now it is a single marine reserve in the Crimean coast of Azov, where these algae had never been studied.

Besides, periodical high abundance of some microalgae is the cause of bloom and red tides in the sea. Disturbing the regional natural ecosystem equilibrium and worsening the environmental quality, these phenomena bring about mass mortality of fish and invertebrates (Ryabushko 2003, 2008; Maltsev and Kluchnikov 2004). From 1988 to 2002, over 20 mass mortalities of fish were registered in the Sea of Azov, among which more than 400 t of dead fish were found on a 45 km long stretch of the Crimean shoreline in September 2003 (Maltsev and Kluchnikov 2004). Mass mortality of marine organisms observed in seas is explained by mass growth of toxic microalgae among which are diatoms genus *Pseudo-nitzschia* also dwelling in the Sea of Azov (Kovaleva

2008; Ryabushko and Bondarenko 2011; Ryabushko *et al.* 2015). The toxin of domoic acid in the diatom cells of this genus causes amnesic shellfish poisoning (ASP) in humans eating poisoned mollusks (Maranda *et al.* 1990), and each cell of a diatom *Pseudo-nitzschia calliantha* from the Crimean coast of the Black Sea has 0,43 ng of domoic acid on average (Besiktepe *et al.* 2008).

In connection with problems related to microalgae, there is need for studies on their qualitative and quantitative characteristics in different environmental conditions, and also on comparison of polluted areas in the Sea of Azov with waters of KNR (Ryabushko *et al.* 2015).

The aim of this work is to investigate the species composition and quantitative distribution of the benthic diatoms inhabiting epiphyton and epilithon in the coast of the Sea of Azov near Kazantip Cape, including Kazantip Nature Reserve.

Materials and methods

The samples were collected on eight stations near Kazantip Cape (45°28' N, 35° 52' E) of the Sea of Azov, including KNR (stations 5–8) (Figure 1) from 0.3 to 1.5 m depths in two ecotopes: epilithon of stones (40 samples) and epiphyton of macrophytes (40 samples).

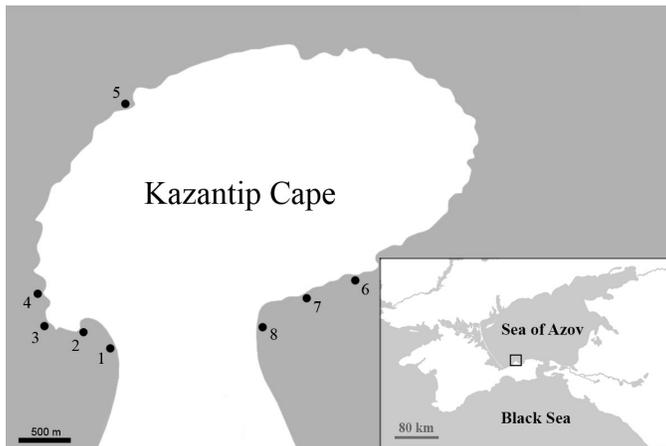


Figure 1. Sampling stations (1 – 8) along the shoreline of Kazantip Cape (including Kazantip Nature Reserve) in the Crimean coast of the Sea of Azov

The samples (macrophytes and stones) were collected in different seasons in 2005, 2006, 2008, 2010, 2011, and 2014. The seawater temperature ranged from -0.5°C in February to +27.5°C in August; the salinity was 11.5‰. During winter and in March the shores of Kazantip Cape is covered by ice. The sea bed is

largely Sarmatian clays and rift limestone, with patches of shell debris. The collected macrophytes and stones (6-10cm) were carefully cleaned by a scraper to collect suspensions. Samples of the suspension from live algae were used for the taxonomic study of diatoms and the samples fixed by 2% formaldehyde for the quantitative assessment. Light microscopes "BIOLAM-212" and "Axioskop 40" with oculars (x 20 and x 40) and oil immersion (x 90 and x 100), respectively provided 400–2500 magnification. Diatom cells were counted in three replications in the 0,0009-ml counting chamber.

In our work we used original methods and techniques, which were designed during investigations of quantitative distribution of benthic diatoms in the Black Sea, the Sea of Japan and the Sea of Azov (Ryabushko and Ryabushko 1991; Bondarenko and Ryabushko 2010; Ryabushko 2013). Abundance (N) and biomass (B) of diatoms in the epilithon and epiphyton were calculated by formulas V. Ryabushko (Ryabushko *et al.* 2003). Surface area of each stone was determined by a formula developed by Graham *et al.* (1988). Surface area of macroalgae was calculated by the equation that G.G. Minicheva (1992) proposed for describing allometric relationship between specific surface area of the macroalgae and the diameter of thallus: $S/W = 3334/d$, where W – wet weight, d – diameter. Diatom community structure was estimated applying indices of Shannon-Weaver (1949), Pielou (1966) and Czekanowski-Sørensen (1948). In rating the dominance of diatoms which had largest numbers in the community we used Berger-Parker index (Berger-Parker, 1970): $D_{BP} = N_{max} / N$, where N_{max} is the number of individuals of the most abundant species, N – the total number of diatoms.

Results and Discussion

Qualitative characteristics of the benthic diatoms were studied in the epiphyton of macrophytes *Cladophora* Kütz., *Entheromorpha* Link, *Blidingia* Kylin, *Ulothrix* Kütz., *Ulva* L., *Ceramium* Roth, *Polysiphonia* Grev., *Cystoseira* Agardh and on the leaves of *Zostera noltei* Hornem, and quantified only in the epiphyton of green alga *Cladophora* sp.

Earlier in the study of benthic microalgae in different ecotopes (including epipsammon), regions and periods in the Crimean coast of the Sea of Azov: Kerch Strait (I) between the Black and Azov Seas, Kazantip Cape (II), including KNR, and Sivash (III), we discovered 200 taxa from 6 phylums of microalgae, including 157 species of diatoms, from them 102 were typical benthic species. It was found 12 species of the potentially harmful algae, including 8 species of diatoms (not published). On the whole Czekanowski-Sørensen index similarity microalgae species values for the three areas of research of the Sea of Azov was 55% between (I + II) and 27 % for (I + III) and (II + III) areas (Bondarenko 2012).

In this work we discuss only diatoms present in two ecotopes of Kazantip Cape. It was found 92 taxa of Bacillariophyta, which related to three classes: Coscinodiscophyceae (9 taxa), Fragilariophyceae (19 taxa) and Bacillariophyceae (64 taxa), 18 orders, 26 families, 41 genera in microphytobenthos near Kazantip Cape (Table 1), 46 taxa from them were found immediately in the waters of KNR.

Table 1. The taxonomic structure of Bacillariophyta microphytobenthos of Kazantip Cape in the Sea of Azov

Class	Number of diatom taxa			
	Order	Family	Genus	Species
Coscinodiscophyceae	6	6	6	9
Bacillariophyceae	7	15	24	64
Fragilariophyceae	5	5	10	19
Total	18	26	40	92

There were 17 taxa new for the Sea of Azov: *Amphora parvula* Proschk.-Lavr., *Cocconeis costata* Greg., *L. abbreviata*, *L. oedipus* (Kütz.) Grun., *Mastogloia kariana* Grun., *Navicula palpebralis* Bréb. ex W. Smith, *Nitzschia hybrida* f. *hyalina* Proschk.-Lavr., *N. lanceolata* W. Smith, *N. spathulata* Bréb. ex W. Smith, *P. delognei*, *P. delognei* var. *remotiva* (Proschk.-Lavr.) L.I. Ryab., *Pleurosigma aestuarii* (Bréb. ex Kütz.) W. Smith, *Pl. nubecula* W. Smith, *Pseudostaurosira brevistriata* (Grun.) Will. et Round, *Rh. flexa*, *S. curvata*, among them potentially toxic *Pseudo-nitzschia prolongatoides* which, like *P. pseudodelicatissima* (Hasle) Hasle and *P. pungens* (Grun. ex Cleve) Hasle, were detected in this sea for the first time (Bondarenko and Ryabushko 2008; Kovaleva 2008; Ryabushko and Bondarenko 2011).

As a basic contributor (70%) to the total number of diatom species of class Bacillariophyceae underlies the taxonomic diversity of diatoms, typical for the benthos in seas. Order *Naviculales* Bessey, represented by 6 families, 10 genera, shows the highest species richness (45%). Genus with the largest number of species were *Navicula* Bory (12), *Nitzschia* Hasall (9), *Pleurosigma* W. Smith (6), *Licmophora* Agardh (5) and *Cocconeis* Ehrenb. (4). Diatoms in both ecotopes were mainly benthic (61%), planktonic species, which periodically settle onto different substrates at shallow depths in the sea, were 13%. Some diatoms, namely *A. brevipes*, *B. rutilans*, *C. scutellum*, *N. ammophila* var. *intermedia*, *Rh. abbreviata*, *Rh. marina*, *T. parva*, *T. tabulata*, occurred in both ecotopes throughout the year regardless of season.

In total, 79 taxa of diatoms were detected in the epiphyton of macrophytes and 44 on the epilithon of stones; the former had maximal diversity in spring and the latter in autumn (52 species and 25 species, respectively) (Table 2).

Table 2. The number of diatom species present on the epiphyton and epilithon of Kazantip Cape in the Sea of Azov in different seasons

	Winter	Spring	Summer	Autumn	Total
Water temperature (°C)	-0.5	10–18	20–27.5	9–19.3	
Ecotopes	The number of diatom species				
Epiphyton	–	52	34	36	79
Epilithon	12	11	23	25	44
Total	12	54	41	45	92

The epiphyton presented mainly colonial diatoms *Licmophora* Agardh, *Fragilaria* Lyngb., *Melosira moniliformis* (O.F. Müll.) C. Agardh, *Pl. hauckianum*, *S. delicatula*, and solitary *S. curvata*, *Undatella lineolata* (Kütz.) L.I. Ryab. Diatoms in the epilithon were of genera *Amphora* Ehrenb. ex Kütz., *Halamphora* Levkov, and species *H. capitata*, *Diploneis didyma* (Ehrenb.) Ehrenb., *Haslea spicula* (Hickie) Bukht. and *N. palpebralis*.

Analysis of epiphyton and epilithon diatoms showed that marine and marine-brackish species predominated in the structure of the microphytobenthos. Due to the low salinity in the areas of research 9 freshwater species were met (Figure 2). According to phytogeographic survey, both in the epiphyton and epilithon in the shallow sea at Kazantip, 27 cosmopolitan species were the major fraction which are widespread species throughout the world oceans. The most minor ones were arctic-boreal species (Figure 3).

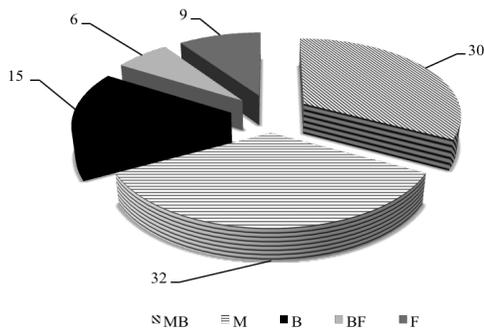


Figure 2. The number of diatom species depending on salinity near Kazantip Cape in the Sea of Azov: M – marine; MB – marine-brackishwater; B – brackishwater; BF – brackish-fresh; F – freshwater

In winter, as the temperature decreased below zero in the shallow water, macrophytes were absent, the research of diatoms was conducted only with stones. Twelve species of diatoms were found during these periods on the epilithon (Table 2) with dominant colonial species *H. capitata*, which was the key contributor common at all sampling stations. Other colonial diatoms, though abundant in other seasons of a year, in winter were seen only solitary.

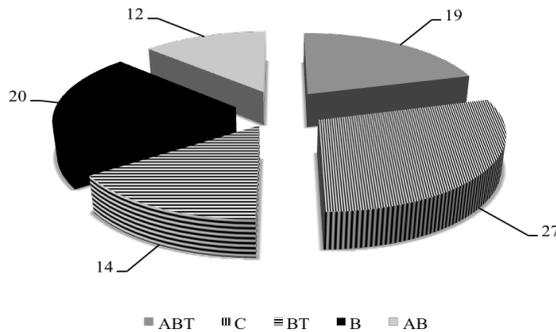


Figure 3. The number of phytogeographical elements of diatoms near Kazantip Cape: ABT – arctic-boreal-tropical; C – cosmopolitan; BT – boreal-tropical; B – boreal; AB – arctic-boreal forms

In spring the number of diatom species in the epiphyton of macrophytes rose to its maximum (Table 2).

In summer and autumn, in spite of the high species diversity for both habitats, most of benthic diatoms preferred macrophytes. The whole Czekanowski-Sørensen index was 52% similarity for diatoms of inhabiting two compared ecotopes.

Amount of the biomass depends on the number of diatom cells and their morphometric parameters. In the epiphyton of *Cladophora* sp., on which colonial benthoplanktonic species *Th. nitzschioides* and benthic diatom *B. rutilans* predominated, abundance and biomass of diatom community were the largest, $1171.5 \cdot 10^3$ cells·cm⁻² (April, 10°C) and 108mg·cm⁻² (September, 19.3°C), respectively (Table 3).

The abundance of diatom community in epilithon was $72.6 \cdot 10^3$ cells·cm⁻² in April, followed by September, but due to the small size of diatom, their biomass in both biotopes was low, and dominate diatom species in both ecotopes remained similar throughout the year, regardless of season (Table 3). In both ecotopes dominated mainly colonial species, but living singly cosmopolitan *C. sculellum*, prefers rocky substrates and bottom vegetation is marked in epilithon in November. Diatoms of the epiphyton had the minimal and maximal abundance respectively 2.4 times and 16 times larger than the epilithon.

Comparative analysis of indices applied for determining marine ecosystem stability and diatom community's structure of the epiphyton and epilithon showed that, despite some structural difference, absolute values were relatively close (Table 3). Species diversity index for diatoms was larger in summer: $H =$

2.81 on the epiphyton of the macrophytes with the dominant *T. tabulata* and $H = 3.05$ on the epilithon – *Rh. abbreviata*. The diversity species of diatoms in the epiphyton showed three peaks, in April, August and September, at high values of H and e (Table 3).

Table 3. The abundance (N , 10^3 cells·cm⁻²), biomass (B , mg·cm⁻²), Shannon diversity index (H), Pielou evenness index (e), Berger-Parker dominance index (D_{BP} , %), species richness (S) and dominant species of diatoms on the epiphyton and epilithon at Kazantip Cape in the Sea of Azov, 2006

Ecotopes	Month	N	B	S	H	e	D_{BP}	Dominant species
Epiphyton	April	1012 ± 20.74	0.142 ± 0.023	17	2.75 ± 0.18	0.671 ± 0.03	27.5	<i>Th. nitzschioides</i> <i>B. rutilans</i>
	May	41.9 ± 2.93	0.005 ± 0.001	8	2.1 ± 0.29	0.693 ± 0.09	41.8	<i>T. tabulata</i> <i>D. tenuis</i>
	July	18.3 ± 1.89	0.003 ± 0.0004	7	1.93 ± 0.32	0.684 ± 0.07	48.5	<i>Rh. abbreviata</i>
	August	670.2 ± 17.84	0.109 ± 0.012	15	2.81 ± 0.05	0.715 ± 0.01	23.1	<i>T. tabulata</i>
	September	1171.5 ± 59.89	0.108 ± 0.01	15	2.56 ± 0.08	0.656 ± 0.03	41.5	<i>P. brevistriata</i>
	November	246 ± 6.74	0.031 ± 0.002	14	1.88 ± 0.02	0.497 ± 0.02	47.2	<i>Rh. abbreviata</i>
Epilithon	April	72.6 ± 5.2	0.007 ± 0.0001	11	2.78 ± 0.28	0.804 ± 0.05	27.8	<i>Th. nitzschioides</i> <i>B. rutilans</i>
	May	7.5 ± 0.14	0.001 ± 0.0001	4	1.75 ± 0.25	0.973 ± 0.03	-	<i>Rh. abbreviata</i>
	July	16.2 ± 1.94	0.002 ± 0.0001	11	2.98 ± 0.07	0.88 ± 0.001	33.2	<i>Rh. abbreviata</i>
	August	60.2 ± 4.31	0.007 ± 0.0003	12	3.05 ± 0.05	0.866 ± 0.001	27.7	<i>Rh. abbreviata</i>
	September	70.3 ± 5.56	0.008 ± 0.0006	11	2.95 ± 0.03	0.854 ± 0.01	20.3	<i>Rh. abbreviata</i> <i>T. tabulata</i>
	November	34.4 ± 3.6	0.004 ± 0.0003	7	2.51 ± 0.04	0.893 ± 0.01	30.3	<i>C. sculellum</i>

Determining species evenness in the community, we used Pielou index (e) calculated as based on index H , the values of which were maximum in July in the epiphyton and in May in the epilithon and had nearly similar in all seasons. Determining species dominance, Berger-Parker index (D_{BP}) was applied. Its values, as large as 48.5% for the epiphyton and 33.2% for the epilithon in July (Table 3) and low in April, suggest relative species evenness in the diatom community. Diatoms had similarly moderate species diversity in May and in July, when their species richness was minimal and dominance index high. In November, when as many as 14 species were seen in the diatom assemblages, dominant species had abundance so huge that it suppressed the share of other diatoms thereby limiting species diversity ($H = 1.88$) (Table 3).

The indices H , e , D_{BP} characterized structure of the epilithic diatom community as largely homogeneous over the year except for May, when the diversity is minimal and the abundance low. Generally, the epiphytic diatoms had greater

diversity, abundance and biomass. It is noted that in sheltered places seawater both ecotopes showed higher estimates of these parameters than in the open sea.

The survey of references on diatoms of Mediterranean microphytobenthos found in the Aegean, Black Sea and the Sea of Azov showed that there had been few studies and almost complete absence of quantitative data on addressed ecotopes (Ryabushko 2013). These three seas of the Mediterranean Basin have a portion of common diatom species. Mass species of *Licmophora*, *Cocconeis*, and *Tabularia* (Kütz.) Will. et Round, which occur in coastal waters of the temperate latitudes, were inhabitants of epiphyton macrophytes growing on the banks of the Aegean Sea deeper than 80 m (Ryabushko 1999).

The discord in species composition can be due to different salinity of these seas. Four of nine species attributed to *Nitzschia* Hassall and known from the Mediterranean Basin have been detected in benthos of the Black Sea, namely *N. microcephala* Grunow, *N. scalpelliformis*, *N. vermicularis* (Kütz.) Hantzsch, and *N. vidovichii* Grunow. Except for the first, three other diatoms dwell in the Sea of Azov near the Crimean shore. Members of this genus, like many other diatoms, are good indicators of organic contamination, therefore their significance at conducting monitoring of marine environment quality (Ryabushko 2013).

Over 30 of 60 species of Bacillariophyta detected in the benthos in the port of Ischia, western Italy (Mazzella *et al.* 1978), are similarly present in the Black Sea and eight species were common in the Sea of Azov. Diatoms dwelling in the sea by Cape Kazantip were represented by 92 species; 81 of them, including 51 from the epilithon and the epiphyton, were inhabitants of the Black Sea. Epiphyton of the Turkish Aegean Sea provides habitats for 67 diatoms (Sabanci 2012), only eight of which could be seen in the shallow sea of Kazantip Cape.

Among pioneers who initiated studying diatoms of benthos in the Sea of Azov was C. Mereshkowsky (1902); having detected 64 taxa in Genichesk Bay, he noted unique character of this flora, different from typical of the Black Sea. In his work described over 10 species common for the Black Sea and the Sea of Azov. According to our observations, over 20 species of diatoms in the seawaters of Kazantip Cape are dwellers of these two seas. Species similarity of the epiphyton and epilithon in the shallow waters of Crimean coast of Kazantip Cape in the Sea of Azov and in the Black Sea was evaluated by Czekanowski-Sørensen index as 52% and 54%, respectively.

Because of the lack of quantitative data about diatoms of epiphyton of green macroalgae in the Black Sea, we provided information on diatoms of epilithon of the two seas. It was found that the close values of the water temperatures 9-10°C, and at the same depth (0.3-0.5 m) the abundance of diatom populations in the Black Sea was higher in 3.5 times, and the biomass was higher in 28 times

than in the Sea of Azov. The differences in species composition of the seas can be explained by different methods of collecting samples and microphytobenthos difference in environmental conditions (salinity, depth, temperature, etc.).

Conclusion

For the first time the survey of species composition and quantitative distribution of diatoms in microphytobenthos of Kazantip Cape on the Crimean coast of the Sea of Azov, including KNR, was presented. Ninety-two detected species of Bacillariophyta belong to three classes Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae (9, 19 and 64 species, respectively), 18 orders, 26 families, 41 genera. Seventeen species were new for the Sea of Azov. Forty-six species inhabited the waters of KNR. Seasonally, 12 species were found in winter, 54 in spring, 41 in summer and 45 in autumn. Marine and brackish-water diatoms were the major contributors, cosmopolitan, arctic-boreal-tropical species were 84 and 50%, respectively. Species similarity Czekanowski-Sørensen index for epiphyton and epilithon of Kazantip Cape was 52%.

Diatoms of epiphyton had the largest quantitative two peaks in 2006; in September (19.3°C) and April (10°C), $1171.5 \cdot 10^3$ cells·cm⁻² and $1012 \cdot 10^3$ cells·cm⁻², respectively, with the biomass maximum of 0.142 mg·cm⁻². Diatom abundance of epilithon was greatest as $70 \cdot 10^3$ cells·cm⁻² and biomass 0,008 mg·cm⁻², respectively, also in April and September. In epiphyton diatoms showed the averages of minimal and maximal abundance 2.4 and 16 times larger than in epilithon.

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