

Ecologica Montenegrina 76: 154-167 (2024) This journal is available online at: www.biotaxa.org/em https://dx.doi.org/10.37828/em.2024.76.9

Species assemblages and spatial organization of phytoperiphyton on the surface of nylon halyards in the Eastern Caspian (Aktau Region, Kazakhstan)

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Received 12 November 2024 │ Accepted by *V. Pešić*: 4 December 2024 │ Published online 7 December 2024.

Abstract

Any objects in natural water bodies are gradually colonized by hydrobionts such as algae and microorganisms. This is how phytoperiphyton is formed. In recent decades, more and more plastic objects have ended up in seas and lakes. They are substrates potentially suitable for colonization by aquatic biota. In the coastal Kazakhstan part of the Caspian Sea, no studies have yet been carried out on the microfouling of artificial polymer substrates. At the same time, as our observations have shown, such fouling can form extensive, clearly visible phenomena on a macroscopic scale. In 2023-2024, we conducted studies of phytoperiphyton foulings on nylon halyards anchored at rocky bottom at a depth of 15 m, at two different locations at distances at least 2 nautical miles from the shore. The 8 mm diameter halyards were stretched from anchors on the bottom to floating buoys at the sea surface, where floating buoys were suspended from them. These two halyards originally served to hold instruments deployed to measure coastal currents (Zavialov et al., 2024). In the first deployment, the halyards were exposed for 8 months (September 2023 - April 2024), in the second one - 4 months (June 2024 - September 2024). In both cases, multispecies phytoperiphyton was formed, whose specific features of composition and spatial organization are described in detail in this article.

Key words Eastern Caspian Sea, Aktau Region, Phytoperiphyton, Algal-bacterial communities, Colonial diatoms, Cyanoprokariotes, Rodophyta, Nylon halyards, Fouling of aquatic plastic.

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Introduction

This paper is focused on field experiments and laboratory analysis of phytoperiphyton on synthetic polimers (nylon halyards), exposed in the Eastern Caspian Sea coastal zone within the City of Aktau area in the autumn-winter-spring period (September 2023 – April 2024) and summer period (May-September 2024).

The Caspian Sea is a water body with very different conditions for the formation of micro- and macroalgae communities (Stepanian, 2016). Phytoperiphyton is a specific but rather variable ecological group of communities developing at the boundary of the liquid (water) and solid (substrate) phases. In the structure of these communities, the main roles are played by attached forms of organisms. Such assemblages are an important component of aquatic ecosystems, making a significant contribution to the total primary production of coastal zones and shallow areas of many water bodies. The value of this contribution reaches 50% or more (Trifonova et al., 1998; Wetzel, 1964, 1990).

Among the periphyton communities, there are several main types, depending on the substrates on which they develop. These include such algocenoses as epilithon (on stones), epiphyton (on living or dead aquatic plants), epipelon (on the surface of silts), epipsammon (on sand). In 2021, a new type of periphyton microorganism communities developing on the surface of plastic was identified microplaston (Sapozhnikov et al., 2021). However, phytoperiphyton in the biotopes of such a huge sealake as the Caspian Sea has so far been studied to a relatively small extent along the western coast (Abdurakhmanova et al., 2012, 2023; Amaeva et al., 2022; Barkhalov et al., 2019; Osmanov et al., 2019, 2022) and fragmentarily along the eastern coast of the sea (Sapozhnikov et al., 2021, 2023).

According to the results of analyses conducted by N.Yu. Andrulionis (Shirshov Institute of Oceanology, Moscow, Russia), in the coastal zone near the City of Aktau, the salinity of waters currently varies seasonally within the range of 12.0-12.9 ppt. In this salinity range, which is within the mesohaline zone (Nevesskaya, 1998), hydrobionts already live outside the lower chorohaline zone, characterized for the Caspian Sea in the range of 7-11 ppt (Khlebovich, 1974; Aladin, 1989). This implies the possibility of the presence in communities of a significant number of micro- and macrophyte species with their relatively high diversity.

In turn, the fouling of aquatic plastic in recent decades has been considered by most researchers as plastisphere communities (Amaral-Zettler et al., 2020). They represent a fundamentally new type of community for ecosystems that can form on the surfaces of synthetic polymers. The bulk of the creatures that form the plastisphere are microorganisms (Du et al., 2022). The features of these assemblages consist, in particular, in the fact that the plastisphere selectively forms compositions of microorganisms that differ from those for natural substrates, and new patterns of microbial coexistence are formed in the structure of the plastisphere (Li et al., 2023).

Experiments related to the fouling of synthetic polymers in a large layer of water (15 m) in the coastal-marine zone of the Eastern Caspian were carried out as an accompanying study in relation to seasonal hydrological and hydrophysical measurements carried out in the Aktau area (Western Kazakhstan) in 2023-2024. In turn, these works became a continuation of the studies of coastal currents (Zavialov et al., 2024) and of epilithic biofilms (Sapozhnikov et al., 2023) successfully carried out in 2022-2023. The motivation for studying phytoperiphyton was the complete lack of data on the forms of fouling of polymeric materials in the Middle Caspian in the modern scientific literature. Also, interest in the organization of these communities was due to the fact that their architecture could be formed with the joint participation of both conditionally freshwater, and brackish-water, and marine species. In this regard, the Middle Caspian is a unique biotope with a giant coastal zone, providing conditions for the joint development of such species and the construction of complexly organized communities by them, the very existence of which is impossible for other reservoirs - freshwater, marine and most brackish. At the same time, phytoperiphyton, which is capable of developing on nylon halyards in the coastal zone, can serve as a food base for commercial herbivorous fish. In light of this useful feature of such communities, the study of their organization in this region opens up prospects for the organization of mariculture farms where these commercial fish could be fattened at a certain distance from the shore.

Data and Methods

The experiments were carried out using nylon halyards with a diameter of 8 mm, located under low tension in the water column. They covered the water layer from the flattened rocky bottom (15 m depth) to the sea surface. These halyards - 1 per observation point - were buoy (signal) ends of the bottom autonomous anchored current meter stations. Using buoys tied to their upper ends, they marked the location of the stations in the coastal waters of the Caspian Sea. One station (hereinafter referred to as the near one) was located approximately on the traverse of Cape Melovoy offshore the City of Aktau, and the second (hereinafter referred to as the far one) was installed to the South outside of it (in 2023) or to the North of the city (in 2024). The first two stations were anchored in early September 2023 and raised in April 2024, meaning that the halyards were exposed in the water column for about 7 months. The second two stations were installed at the bottom at the end of May 2024 and raised in September the same year – their exposure took about 4 months. The coordinates of the stations, the dates of their installation and lifting from the bottom, as well as the time of halyard exposure (in days) in the water are given in Table 1. The layout of the stations on the map of the area is shown in Fig. 1.

Table 1. Data for the halyards installations (stations) in different periods of the year.

Figure 1. Scheme of location of nylon rope installations (stations) in the water area near the City of Aktau. A – from September 2023 to April 2024, B – from May to September 2024. Station marking "aws" means that they were exposed in the cold period of the year (autumn – winter - spring), "som" - in the warm period (summer - early autumn).

When installing the bottom moored instrument stations on September 12, 2023, the shortest distance from the shore to station "1 aws" (far) was 4260 m, and to station "2 aws" (near) - 2028 m. In turn, when installing the stations on May 31, 2024, the shortest distance from station "1 som" (near) to the shore was 2030 m, and from station "2 som" (far) - 2714 m. In all cases, the distances were from about 1 nautical mile or more, which excluded the intense impact of coastal stirring of soft bottom sediments on the surface of the halyards. Such stirring, occurring during strong storms, can heavily litter the surface of the halyards, thereby reducing its availability for colonization. In these experiments, we monitored the overall result of each of the two periods of halyard colonization by phytoperiphyton. The first period, which covered the autumn-winter-spring colonization, we will designate as cold. The second one which covered the summer and early autumn, we will designate as warm.

In all cases, the bottom stations were installed and lifted to the surface from a small boat. The layer of phytoperiphyton that had grown during the exposure period was scraped off the surface of the halyard using a rubber-coated glove. Removing the fouling with such a glove allowed scraping off the maximum amount of the living layer, since the surface of the rubber coating was rough. All material removed from the halyard was immediately fixed with an ethyl alcohol solution and stored in a dark place to prevent exposure to strong sunlight and heating on the protoplasts of the fixed cells and the matrices of the microorganism colonies.

The material was analyzed in its raw form in the laboratory of the P.P. Shirshov Institute of Oceanology of the Russian Academy of Sciences (Moscow, Russia). In all cases, the fouling looked like fibrous strands ranging in size from 3 to 15-18 mm. 1-2 such strands were taken from the integral sample and carefully straightened in a drop of clean water on a glass slide. Then they were covered with a cover glass on top so that the straightened fragments of the strand did not overlap each other. These procedures facilitated the process of studying the preparations using Leica DMLS and Leica DM2500 light microscopes, since light penetrated better through the resulting thin layer of strand. Photographic documentation of the material was performed using digital cameras (Leica, Blackview, and Canon), at magnifications of x200, x400, and x1000. In this case, when studying the material at low magnification (x200), attention was paid to the macroscopic details of the organization of the fibers that made up the strand. Identification of microphytes and filamentous macroalgae, from which these fibers were constructed, as well as an assessment of their roles in the structuring of fouling, was carried out using digital images taken at magnifications of x400 and x1000. To identify the species of diatoms, permanent preparations of valves were made, enclosed in the light-refracting medium "Canadian balsam". The abundance of species in the cenosis structure was taken into account by ranks: single - 1-2 specimens per preparation; rare - 1-2 per 3 visual fields of the microscope at a magnification of x400; frequent - 1- 2 in each visual field; mass - 3-5 in each visual field or in groups/colonies often in visual fields, but not in each of them; dominant - (6) 8-15 and more in most visual fields. The material was removed from the halyard in total, without dividing into segments by depth horizons. For this reason, fouling strands were taken from different places of the integral sample for analysis, a total of 30-35 on each halyard.

When establishing the species affiliation of microphytes and filamentous algae, we used a wide range of modern atlases and reference books (Witkowski et al., 2000; Krammer, Lange-Bertalot, 2004; Spaulding, Edlund, 2008a, b, c, d; Levkov, 2009; Spaulding, 2011a, b; Komárek, Anagnostidis, 1989, 1998, 2005; Komárek, 2013; Kulikovsky et al., 2016). As to the current taxonomic status of microphytes, we also used periodically updated materials from the World Register of Marine Species (WoRMS Editorial Board, 2023) and AlgaeBase (Guiry, Guiry, 2022) portals.

The salinity of the Caspian Sea water samples being studied was determined by summing up the concentrations of the major ions. Water samples were collected during two field studies conducted from September 2023 to September 2024 and delivered to the laboratory. Water samples were filtered through a 0.7 μm GF/F Whatman membrane filter to remove suspended matter and organic matter and placed in 100˗250 ml glass containers. The major ion content of the Caspian Sea waters was analyzed in accordance with methods originally selected for the analysis of hypersaline waters and described in (Andrulionis, Zavialov, 2019), but taking into account the Caspian Sea water salinity. Concentrations of chlorides, sulfates, calcium, magnesium, total inorganic carbon, and total alkalinity were measured using potentiometric titration on a Metrohm 905 Titrando automatic titrator, with indicator electrodes selected based on the reaction type and ion under analysis. Total dissolved inorganic carbon was determined in mmol/kg and converted to g/kg as hidrocarbonation. The concentration of potassium ions was determined gravimetrically. Sodium was determined by the difference between the total anions and cations in molar equivalent, then recalculated to g/kg.

Results

An analysis of the total concentration of salts in coastal waters in the Aktau Region, on the traverse of Cape Melovoy, showed that at the beginning of December 2023 it was 12.47 g/kg, and at the end of May 2024 – 13 g/kg.

Two multi-season experiments showed that a total of 86 species and subspecies of microphytes and filamentous macroalgae lived on the surface of nylon halyards in the coastal zone of the Middle Caspian, in the Aktau Region, at different times and in different locations. Of these, diatoms made up the majority – 79, or 91.86% of the total number of species. All the noted filamentous algae belonged to red algae (Rhodophyta). The general list of phytoperiphyton species noted based on the results of the experiments is presented in Table 2.

Table 2. General list of species and subspecies of microphytes and filamentous macroalgae noted in experiments with fouling of nylon halyards in the Middle Caspian Sea near the City of Aktau, for the period from September 2023 to September 2024. For the community of each station, the roles of species in the abundance hierarchy are noted. Species that played structuring roles in the communities are marked in bold.

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At the end of the cold period of exposure of the nylon halyards, the total number of species on them was 73, of which 69 (94.52%) were diatoms. In turn, in the composition of the fouling formed during the warm period, a total of 48 species and subspecies were noted, among which 41 were diatoms (85.41%). Thus, according to the data we obtained, the cold-period phytoperiphyton was 1.52 times richer than the warm-period one. At some stations, the total number of species was also higher at the end of the cold season (Table 3): 48-54 against 37, that is, 1.3-1.46 times more. The main contribution to these differences was made by the species of mobile diatoms, which were represented in a significantly more diverse manner in the cold period than in the warm one (Table 3). Moreover, the number of species of attached diatoms, including colonial forms, was the same at the stations at the end of the cold period, and almost the same – and higher – at the stations at the end of the warm period.

Table 3. Species richness of phytoperiphyton (S total) recorded at the end of the experiments at different stations. For each location, the total number of diatom species (S diatoms), the number of attached diatom species (S attached diatoms), and the number of motile diatom species (S motile diatoms) are presented, as well as the contributions (in %) of all recorded diatoms, attached and motile forms, to the total species richness.

Stations	1 aws	2 aws	1 som	2 som
S total	54	48	37	37
S diatoms	50	47	31	33
% S diatoms from S total	92.59	97.92	83.78	89.19
S attached diatoms	22	22	26	27
% S attached diatoms from S total	40.74	45.83	70.27	72.97
S motile diatoms	28	25	5	6
% S motile diatoms from S total	51.85	52.08	13.51	16.22

Let us now describe the spatial organization of communities. In different locations and according to the results of different seasons, the main structuring roles in cenoses were played by filamentous red algae and colonial species of diatoms. It should also be noted that many preparations studied for each community did not show significant differences in the composition and structure of fibrous strands. In fact, phytoperiphyton changed little in depth within the length of one filament.

At Station 1 aws, where the colonization of the halliard occurred in the cold season of the year, the main structuring role in the formed phytoperiphyton layer belonged to the colonies of the diatom *Berkeleya micans*. The branching biopolymer tubes of these colonies, containing 3-4 rows of elongated lanceolate cells inside (Fig. 2, a, e), densely penetrated the entire layer of golden-brown fouling. The second of the main structuring components were thin filamentous thalli of the red algae *Kylinia virgatula*. Also, a significant role in organizing the architecture of the fouling layer was played by branching tubular colonies of the diatom *Navicula* cf. *rusticensis* (Fig. 2, b, c, d, f). Here, in smaller quantities, thin tubular colonies of *Berkeleya fragilis* and *Berkeleya scopulorum* developed. On the surface of the biopolymer tubes of different species of *Berkeleya*, extensive zigzag branching colonies of *Grammatophora marina* often developed, as well as small ribbon-shaped colonies of *Achnanthes longipes* (Fig. 3, a, b), fan-shaped cells of *Licmophora dalmatica* and radial bundles of needle-shaped cells of *Tabularia fasciculata*.

Figure 2. Tubular colonies of diatoms *Berkeleya micans* (a, e) and *Navicula* cf. *rusticensis* (b, c, d, f). Photos by Philipp Sapozhnikov.

The cells of the sedentary diatom *Rhopalodia gibberula*, which leads a predominantly attached lifestyle, formed a phenomenon here that we noted for this species for the first time. Firstly, the cells of this species, which usually attach to a plant or other substrate with the entire surface of both valves, here attached to the *Berkeleya* tubes with only one end of the cell (Fig. 3, c). Secondly, they secreted a large amount of loose exopolymer, in which, like in jelly, they were located at different angles to the surface of the tube. Such growths of epiphytic *Rhopalodia* in this cenosis were frequent, but still point-like. Young sprouts of the red algae *Ceramium diaphanum* often developed among the accumulation of such a gelatinous matrix. In the lowest layer of fouling, directly adjacent to the nylon fibers, cells of *Cocconeis scutellum* and *Cocconeis scutellum* var. *parva* were often encountered. It is possible that it

was from them, as well as small branching shoots of the red algae *Colaconema daviesii*, that the colonization of the surface of the halliards in this location began.

Figure 3. a, b: Large cells of the colonial diatom *Achnanthes longipes* (Ach. long). c: cells of epiphytic diatoms *Rhopalodia gibberula* (Rh. gib), *Rh. parallela* (Rh. par.) and *Epithemia sorex* (Ep. sor.) in a "cloud" of extracellular matrix on the surface of the cortex of *Polysiphonia* sp. 1. Photos by Philipp Sapozhnikov.

In the fouling at Station 2 aws, the main structuring function was performed by abundantly branching tubular colonies of *N.* cf. *rusticensis*, thin tubes of *Berkeleya scopulorum* colonies, and often located on them bundles of *R. gibberula* cells in a loose matrix. Here, mobile diatoms *Navicula* cf. *recens* and *Navicula vekhovii* were also encountered in large numbers. Along with a large number of taxa of other mobile diatoms, which had the status of frequently encountered, rare and single, these two species actively loosened the thickness of the fouling, pushing apart clumps of fine mineral-organic dust and aerating the entire living layer. These viscous dust accumulations are constantly formed from suspended nanoparticles entangled in the interweaving of tubular colonies and among accumulations of gelatinous matrices of microphytes. A similar function was performed by numerous mobile species with low abundance statuses in the community of Station 1 aws. Among the dust clots in the communities of both stations, sprouts of thalli of the red algae *Polysiphonia* sp. 1 were occasionally found.

The architecture of communities formed by the end of the warm season was much more complex, but less diverse. Nevertheless, these cenoses had a higher degree of structural similarity than those that developed during the cold season.

In the cenosis at Station 1 som, the first structuring dominant was the filamentous red algae *Polysiphonia* sp. 1. The branching shoots of this macrophyte reached a height of several millimeters here, and carried a thick layer of microepiphyton. Directly on the cortex cells of *Polysiphonia*, a mosaic of different density was formed from attached cells of *Epithemia sorex* (Fig. 4, a, b), alternating with rarer *Epithemia adnata* var. *proboscidea* and *Epithemia turgida*. A higher layer – there, on the bark – densely sat the cells of *Rh. gibberula* and *Rhopalodia parallela* (Fig. 4, b; Fig. 5), attached to it by their ends and secreting an abundant loose matrix around themselves. In turn, colonies of *Mastogloia pusilla*, *Mastogloia ohridana* and, less frequently, *Mastogloia elliptica* and *Mastogloia pumila* developed in abundance among the "clouds" of the matrix. In these colonies, lanceolate cells of diatoms were located inside multilayer exopolymer capsules (Fig. 6). These capsules were highly transparent. A feature of *M. pusilla* colonies was the formation of long wriggling ribbons during cell division (Fig. 7, a). *M. ohridana* colonies were also distinguished by the development of short straight ribbons of tightly closed cells. Also, colonies of *Rhoicosphenia linearis* developed in mass on the bark of *Polysiphonia*, in which long curved cells sat on thin branching polymer stalks.

Figure 4. a: A group of cells of the epiphytic diatom *Epithemia sorex* (Ep. sor.) on the bark of *Polysiphonia* sp. 1. b: A mixed colonial settlement of *Rhopalodia gibberula* (Rh. gib), *Rh. parallela* (Rh. par.), *Epithemia sorex* (Ep. sor.) and *Epithemia turgida* (Ep. tur.). Photos by Philipp Sapozhnikov.

Figure 5. Mixed colonial settlements of Rhopalodia gibberula (Rh. gib), Rh. parallela (Rh. par.). Photos by Philipp Sapozhnikov.

Figure 6. Colonies of different *Mastogloia* species. Cells are surrounded by transparent capsules. Photos by Philipp Sapozhnikov.

Abundantly branching tubular colonies of *B. scopulorum* and *B. micans* also dominated this community. Tree-like colonies of *N.* cf. *rusticensis* and *Berkeleya rutilans* were found here in large numbers. Intertwining, these four species formed powerful branching fibers, also densely dotted along the surfaces of the tubes with loose colonial settlements of *Rhopalodia* and encapsulated colonies of *Mastogloia*. Short colonies of *Achnanthes longipes* often developed on the tubes, as well as abundantly branching chains of *Grammatophora oceanica* (Fig. 7, b) and *G. marina*. Long narrow cells of *Nitzschia sigmaformis* crawled in mass among the "clouds" of the matrix and accumulations of settled nanosuspension.

In the cenosis at Station 2 som, the main structuring function was also performed by *Polysiphonia* sp. 1, the first dominant of this community. On its shoots, all the same species that characterized the cenosis at Station 1 som developed in the status of dominants and mass species. However, in the structure of fibers formed by tubular colonies of diatoms, the main skeletal functions were performed by *N.* cf. *rusticensis*, *B. rutilans*, and *B. micans*, while *B. scopulorum* moved into the category of a frequently encountered species. Among the *Grammatophora* species, only colonies of *G. oceanica* were distinguished by their mass occurrence.

Other subtle differences included a lower abundance of *Rh. parallela*, *M. pusilla*, and *E. sorex*, and a higher abundance of *M. elliptica*. While the fouling at Station 1 som occasionally contained filamentous thalli of *K. virgatula*, *C. daviesii* and *C. diaphanum*, the cenosis at Station 2 som contained rare filaments of only the first two species. The role of mobile *Nitzschia sigmaformis* in aerating the living layer was less noticeable, and the layer itself appeared looser.

Figure 7. a: Wriggling ribbon-shaped colony of *Mastogloia pusilla* in a transparent capsule. b: Colony of *Grammatophora oceanica*. Photos by Philipp Sapozhnikov.

Conclusions

Phytoperiphyton formed on the surface of nylon halyards in locations of the Eastern Caspian Sea coastal waters, located from the shore at a distance of 1-2 n.m., was distinguished by significant species richness and diversity of life forms. Communities developed on this synthetic polymer material in the cold season of the year looked more diverse and were richer in species than summer assemblies. This was facilitated by a higher diversity of mobile forms of diatoms. At the same time, in the warm season, the fouling layer on these substrates was structured to a greater extent. In the summer-autumn period, the architectural uniformity of the cenoses also increased. This was achieved due to the development of *Polysiphonia* sp. 1 thalli and composite fibers from tubular colonies of different species of *Berkeleya* and *N.* cf. *rusticensis*. Both thalli of red algae and branching fibers from biopolymer tubes served as the basis for the development of a mosaic layer of microepiphyton, represented by colonies of different species of *Mastogloia*, colonial settlements of *Rhopalodia* spp. and *Epithemia* spp. The formation of colonial settlements of cells associated with the substrate surface by only one of the valve poles was noted for the first time in *R. gibberula* and *R. parallela*. Likewise, the secretion of abundant "cloud" matrix by the cells of these species has not been described previously. The formation of extended wriggling ribbons developing inside large transparent capsules was described for the first time in *M. pusilla* colonies. Also new is the discovery of dense ribbons of cells in *Mastogloia ohridana* colonies. From our observations on the rocky coast of the Eastern Caspian, it follows that the cells of these species usually form colonies of the capsule type of 4-8 cells located in pairs.

The experimental sites during the cold season were located in the area Cape Melovoy, offshore the City of Aktau, and in the southern suburbs. In turn, the locations for the warm season experiments were located near Cape Melovoy and in the northern suburbs of Aktau. It can be assumed that a higher degree of structural differences between cenoses in the cold period than in the warm one was due to these differences in the location of the stations. However, as recent hydrological and hydrophysical studies have shown (Zavialov et al., 2024), the alongshore current in the coastal zone of the Eastern Caspian near the City of Aktau non-periodically changes direction to the opposite. Thus, communities developing on the surface of halyards stretched from the surface to the bottom at a depth of 15 m at a significant distance from the shore will be under the non-periodic influence of water masses moving either from north to south or in the opposite direction. This levels out the existing differences in their localization along the coast. Nevertheless, it should be noted that in the area of Cape Melovoy the communities were organized more densely and were distinguished by a higher mass of a number of diatom species that form colonies and colonial settlements. This may indicate a local influence of eutrophicating urban runoff, which has a less strong effect within the suburbs.

Acknowledgements

The study was supported by the Russian Science Foundation grant No. 23-17-00167 [\(https://rscf.ru/project/23-17-00167/\)](https://rscf.ru/project/23-17-00167/), and with the support of the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP23490657 "Study of the ecological state and dynamics of the waters of the Kazakhstan sector of the Northern Caspian"). Within the framework of Grant No. AP23490657 field experiments were carried out in the Caspian Sea, material was collected and characteristic features of the community structure were photographed. Within the framework of the RSF grant No. 23-17-00167, material was analyzed using light microscopy methods, as well as a description of the spatial structure of communities, similarities and differences in their organization. The team of scientists expresses gratitude to the chairman of the yacht club "Breeze" (Aktau, Republic of Kazakhstan), Alexey Oskin, for assistance in organizing research trips to the coastal waters of the Eastern Caspian and providing a boat for these expeditions.

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