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Benthic diatom species richness at Dvuyakornaya Bay and other coastal sites of Crimea (Black Sea) under various environmental conditions

Elena L. NEVROVA and Alexei N. PETROV

2, Nakhimov ave., 299011 Sevastopol, Russian Federation Benthos Ecology Department, A.O. Kovalevsky Institute of Marine Biological Research RAS

Corresponding author: el nevrova@mail.ru

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Abstract

Initial research of the pristine waters of Dvuyakornaya Bay (South-eastern Crimea, Black Sea) revealed a high taxonomic richness in benthic diatoms (Bacillariophyta). A total of 304 species, 78 genera, 37 families, and 20 orders were identified. Among these, 68 novel species for the Black Sea were noted. The largest number of species was identified in the genera *Navicula* (41 species), followed by *Nitzschia* (29), *Amphora* (25), *Cocconeis* (20), *Diploneis* (16), *Lyrella, Fallacia*, and *Planothidium* (10 species each, respectively). An inter-regional comparative analysis of diatom species richness was performed using Bray-Curtis similarity coefficient and Venn diagrams. The diatom taxocene of Dvuyakornaya Bay (DB) was compared with Bacillariophyta flora from several Crimean coastal sites under different levels of anthropogenic impact, i.e. relatively pristine waters near Cape Fiolent (CF) and heavy polluted Sevastopol Bay (SB) and Balaklava Bay (BB). The highest species composition similarity (58%) was registered between intact biotopes (DB vs CF) and between heavily polluted areas (SB vs BB), despite the geographical remoteness of the compared areas as well as differences in their hydrological-hydrochemical conditions and bottom substrates patterns. The lowest diatom taxocene species similarity was observed between SB and CF (32.3%) and between SB and DB (36.5%). It was concluded that the content of technogenic pollutants (trace metals, PCBs, PAHs, and pesticides) in the bottom sediments, as well as the heterogeneity of microbiotopes, represent major factors influencing the species composition of benthic Bacillariophyta in the investigated coastal areas.

Keywords: Bacillariophyta; diatoms; taxocene; species richness; technogenic pollution; Black Sea.

Introduction

The study of the benthic diatom taxocene structure in various marine biotopes is highly relevant due to the key role of Bacillariophyta in coastal ecosystem functions. Evaluation of diatom species richness represents an important task for bioindication purposes and to determine species conservation priorities (Keck et al., 2016; Hafner et al., 2018; Nevrova et al., 2015; Stenger-Kovacs et al., 2014, 2016). With coastal marine ecosystems being under increasing anthropogenic impact, a primary task involves the assessment of taxonomical diversity and comparative evaluation of taxocene structure in biotopes with varying levels of pollution. These studies allow researchers to identify the peculiarities of diatom taxocene structures and create recommendations for biodiversity maintenance. Comparisons of diatom taxocene structures in pristine coastal habitats and anthropogenically perturbed biotopes facilitate the identification of various aspects of the formation and sustainability of Bacillariophyta diversity under changing environmental conditions (Heino *et al.*, 2007; Leira *et al.*, 2009; Nevrova, 2015; Petrov *et al.*, 2010; Stenger-Kovacs *et al.*, 2014, 2016).

In the Black Sea, studies of diatoms species richness in various environments is particularly important due to the intensive anthropogenic impact upon the marine environment and the necessity to apply the bioindication methods to assess the current state of nearshore waters (Nevrova et al., 2015; Petrov & Nevrova, 2004; Petrov et al., 2005). A comprehensive generalisation of the results obtained using both floristic studies and computative methods is required to research different aspects of microalgae species diversity, particularly the analysis of diatom taxocene structure in ecologically heterogeneous biotopes (Hafner et al., 2018; Heino et al., 2005, 2007; Petrov & Nevrova, 2007, 2014; Petrov et al., 2010; Stenger-Kovacs et al., 2016). The present work focuses on the taxonomic composition of benthic diatoms in Dvuyakornaya Bay (Eastern Crimea, Black Sea) – a pristine marine area that has not been previously investigated – and on a comparative

analysis of species richness between the previously studied coastal locations of South-western Crimea that suffer from various levels of anthropogenic pollution.

Materials and Methods

Benthic diatom taxocenes of the Crimean coast eastwards from the Karadag State Reserve have not been previously investigated. Bacillariophyta species richness in Dvuyakornaya Bay (DB) (translation: "Two-anchored Bay") – which remains practically free of anthropogenic pollution – was studied for the first time in contrast to other locations of the Crimean coast (Fig. 1), such as the waters around Cape Fiolent (CF) (Nevrova, 2016), Balaklava Bay (BB) (Nevrova, 2014; Petrov *et al.*, 2010), and SB (SB) (Nevrova, 2013; Petrov *et al.*, 2005).

Dvuyakornaya Bay is an "open type" bay (S = 6.4 km²) surrounded on the Northeast by Cape St. Ilya and from the South-west by Cape Kiik-Atlama. The steep coast consists of Jurassic conglomerates, clays, limestones, and siderite. Until the early 2000s, the bay was used as a military training area and was completely withdrawn from any municipal or recreational exploitation. As a result, the natural conditions of the area were preserved. Currently, the waterfront of this bay is being developed with yachting and recreation centres, which may adversely affect the ecological status of this area and the state of benthic communities in the coming years.

Sampling of bottom sediments in DB (sand with clay admixture) was performed in August 2008 at nine sampling sites (N 44°59'09", E 35°23'05") within a depth range of 2–10 m. Samples were taken by a diver in two replicates from the upper (2–4 cm) layer of soft bottom sediments using a meiobenthic tube with a surface area 16 cm². Sediment grain-size composition (as percentage of sandy, silty, and clay fractions) was determined by wet sieving and gravimetric sedimentation. Samples of bottom sediments were taken simultaneously from each site for the chemical analysis of inorganic and organic contaminants, and included measurements of 12 parameters: metals (Cu, Zn, Ni, Cr, Pb, Cd, and Hg), polychlorinated

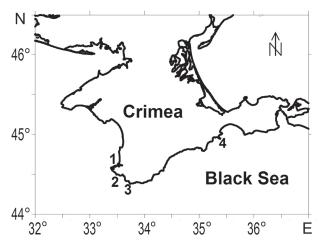


Fig. 1: Schematic map of Dvuyakornaya Bay (4) and other sampling sites along the coast of Crimea (Black Sea): 1-Sevastopol Bay, 2-Cape Fiolent, 3-Balaklava Bay.

biphenyls (PCB, sum of four congeners), pesticides (4-4'-DDTs and metabolites), and the sum of polyaromatic hydrocarbons (PAHs). The content of silt+clay fractions and total organic carbon (C_{org}) of the sediments was also determined. Furthermore, chemical analysis of soft bottom sediments was conducted by colleagues from Institute of Colloid Chemistry and Water Chemistry NASU, Kiev (ICCWC) using previously described techniques (Burgess *et al.*, 2009, 2011; Petrov *et al.*, 2010).

Samples were initially treated in an ultrasonic bath for 20 min, then diatom valves were cleaned by HCl and H₂SO₄ with addition of K₂Cr₂O₇ (Proshkina-Lavrenko, 1974; Nevrova et al., 2015). Slides for light microscopy (LM) were created using Meltmount®. Sampling surveys and treatments were performed at the Institute of Marine Biological Research (IMBR). LM micrographs were taken using a Nikon Eclipse E600 with a PlanAPO 100× objective lens (Institute of Marine Sciences, University of Szczecin, Poland). Scanning electron microscopy (SEM) was performed using a Hitachi S-4500 (Goethe University, Frankfurt-am-Main, Germany). Slides for LM were stored in the collections of Dr. E. Nevrova (IMBR) and Prof. Dr. A. Witkowski (Institute of Marine Sciences, SZCZ). Stubs for SEM were deposited in the collection of Prof. Dr. H. Lange-Bertalot (Goethe University, FR). Valve measurements were made from digital images using ImageJ 1.4.3.67 (ImageJ, 2014).

For Bacillariophyta classification, the taxonomic system according to Round *et al.*, (1990) with the latest update (Witkowski *et al.*, 2000; Levkov, 2009 *etc.*) was used. Nomenclature taxa citation follows Fourtanier & Kociolek (1999, 2011) and the International Plant Names Index (2004).

The diatom taxocene species similarities between DB and other biotopes of the Crimean coast (CF, SB, and BB) were estimated based on the Bray-Curtis similarity coefficient (for presence/absence data) using PRIMER v5.2 software (Clarke & Gorley, 2001). Computation the expected number of species (S_{exp}) that can be found under equal sampling efforts for all compared locations (i.e. in 8-9 samples) was conducted using an extrapolative model of the DIVERSE routine (PRIMER), and data were averaged over 1000 random permutations.

Moreover, in order to visualise regional differences in the species structure of benthic diatom assemblages, a Venn diagram analysis was also applied (Venn diagrams, 2019).

Results

Pollutants in bottom sediments of Dvuyakornaya Bay

The analysis of technogenic pollutants content in soft sediments of DB confirmed the high ecological quality of this area, which was previously closed to any civil activity (Table 1). Very low content of the trace metals (Cu, Zn, Ni, Cr, Pb, Cd, Ag, and Hg) was detected, and the main classes of organic pollutants (PCBs, PAHs, and pesticides) found did not exceed average levels for the soft bottoms of environmentally intact offshore areas of

Table 1. Median values and range (in brackets) of metals, organic contaminants, and elemental concentrations in surface sandy/muddy bottom sediments and the near-bottom water layer of Dvuyakornaya Bay and other coastal waters of Crimea (Black Sea).

| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$ | Site Analyte | Dvuyakornaya Bay | Cape Fiolent | Sevastopol Bay | Balaklava Bay | Background level for coastal zone* | Background level for coastal zone** |
|--|-----------------|----------------------------|-----------------------------|--------------------------------------|---------------------------|---------------------------------------|--|
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | | | N | letals $(\mu g \times g^{-1} dw)$ | | | |
| 98,9 $(96,8+100,2)$ $124,4 (124,0+125,3)$ $266 (107+788)$ $202 (111+431)$ $10,1$ 0,8 $(0,4+1,0)$ 5,5 $(5,2+5,8)$ 43 $(3+62)$ 39 $(32+30)$ $1,6$ 2,5 $(2,2-2,8)$ 0,5 $(0,4+0,8)$ 46 $(26+82)$ 33 $(20+72)$ $1,6$ 45.2 $(43,5+46,8)$ 21,8 $(20,8+22,8)$ 290 $(86+1362)$ 226 $(87+510)$ $9,7$ 0,022 $(0,018+0,028)$ 0,048 $(0,044+0,050)$ 0,95 $(0,22+6,25)$ 0,21 $(0,11+0,36)$ $0,15$ 0,05 $(0,04+0,08)$ 0,02 $(0,014+0,020)$ 0,95 $(0,22+6,25)$ 0,21 $(0,11+0,36)$ $0,15$ 0,06 $(0,04+0,08)$ 0,02 $(0,014+0,020)$ 0,95 $(0,024+0,03)$ 1,03 $(0,04+7,61)$ 0,26 $(0,46+1,42)$ 0,01 0,02 $(0,017+0,032)$ 0,02 $(0,014+1,03)$ 1,03 $(0,04+7,61)$ 0,96 $(0,46+1,42)$ 0,146 $(44-1,42)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ 0,04 $(14-1,03)$ | Cu | 13,2 (12,5÷13,5) | 22,8 (22,0÷23,5) | 153 (58÷418) | 142 (63÷390) | 13 | <20 (18÷30) |
| $0.8 (0,4+1,0) \qquad 5.5 (5.2+5.8) \qquad 44 (31+62) \qquad 39 (32+50) \qquad 1.6$ $2.5 (2.2+2.8) \qquad 0.5 (0,4+0.8) \qquad 46 (26+82) \qquad 33 (20+72) \qquad 1.5$ $45.2 (43.5+46.8) \qquad 21.8 (20.8+22.8) \qquad 290 (86+1362) \qquad 226 (87+510) \qquad 9.7$ $0.0022 (0.018+0.028) \qquad 0.048 (0.044+0.050) \qquad 0.95 (0.22+6.25) \qquad 0.21 (0.11+0.36) \qquad 0.15$ $0.002 (0.017+0.032) \qquad 0.020 (0.015+0.022) \qquad 0.50 (0.14+2.30) \qquad 0.29 (0.14+1.05) \qquad 0.01$ $0.002 (0.017+0.032) \qquad 0.020 (0.015+0.022) \qquad 0.50 (0.14+2.30) \qquad 0.29 (0.14+1.05) \qquad 0.01$ $0.002 (0.017+0.032) \qquad 0.020 (0.015+0.022) \qquad 1.03 (0.04+7.61) \qquad 0.96 (0.46+1.42) \qquad 0.04$ $0.03 (0.81+1.05) \qquad 1.08 (0.73+1.20) \qquad 1.68 (8+2198) \qquad 30 (10+64) \qquad 1.42$ $40 (32+51) \qquad 1.08 (0.73+1.20) \qquad 1.88 (8+2198) \qquad 30 (10+64) \qquad 1.42$ $1.5 (3+9) \qquad 8.6 (7+10) \qquad 5.2 (3+8) \qquad 5.4 (3+7) \qquad 8.4$ $1.7.5 (3+9) \qquad 8.6 (7+10) \qquad 5.2 (3+8) \qquad 5.4 (3+7) \qquad 1.84$ $1.7.8 \qquad 1.8.2 \qquad 1.7.2 \qquad 1.8.1 \qquad 1.8.4 (1.7.3+19.1)$ $0.5 \qquad 0.5 \qquad 6.0 (26+17.4) \qquad 8.1 (0+92)*****$ $1.7.2 \qquad 1.8.2 \qquad 1.7.2 \qquad 1.8.1 \qquad 1.3 (0+6.3) ******$ $1.7.2 \qquad 0.5 \qquad 0.5 \qquad 6.0 (26+11.4) \qquad 2.4 (1.6+3.0) \qquad 1.3 (0+6.3) ******$ $1.2.4 \qquad 2.2.4 \qquad 2.2.2**** \qquad 1.7.2 \qquad 1.8.1 \qquad 1.8.4 (1.7.3+19.1)$ $1.7.8 \qquad 1.8.2 \qquad 1.7.2 \qquad 1.8.1 \qquad 1.3 (0+6.3) *******$ $1.7.2 \qquad 0.5 \qquad 0.5 \qquad 6.0 (26+11.4) \qquad 2.4 (1.6+3.0) \qquad 1.3 (0+6.3) *********$ $1.2.4 \qquad 2.2.4 \qquad 2.2.2**** \qquad 2.1 (0.45-4.4)***********************************$ | Zn | $98,9 (96,8 \div 100,2)$ | 124,4 (124,0÷125,3) | 266 (107÷758) | 202 (111÷431) | 10,1 | <50 (44÷94) |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$ | ïZ | $0.8 (0.4 \div 1.0)$ | 5,5 (5,2÷5,8) | 43 (31÷62) | 39 (32÷50) | 1,6 | <40 (33÷67) |
| $\begin{array}{llllllllllllllllllllllllllllllllllll$ | Cr | $2.5(2.2\div2.8)$ | $0.5(0.4 \div 0.8)$ | 46 (26÷82) | 33 (20÷72) | 1,5 | <45 (35÷84) |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | Pb | 45,2 (43,5÷46,8) | 21,8 (20,8÷22,8) | 290 (86÷1362) | 226 (87÷510) | 7,6 | <15 (10÷25) |
| 0,06 (0,04÷0,08) 0,02 (0,015÷0,022) 0,50 (0,14÷1,05) 0,20 (0,14÷1,05) 0,04 (0,04÷7,61) 0,05 (0,14÷1,05) 0,04 (0,14÷1,02) 0,04 (0,14÷1,02) 0,04 (0,14÷1,42) 0,04 1,03 (0,04÷7,61) 0,96 (0,46÷1,42) 0,04 1,03 (0,04÷7,61) 1,04 (0,04÷1,42) 0,04 1,2854 (790÷41269) 4166 (1475÷15280) 1,42 1,08 (0,73÷1,20) 168 (8÷2198) 30 (10÷64) 1,42 1,66 (14+20) 1897 (142±12690) 594 (196÷4202) 4,5 1,87 (142±12690) 594 (196÷4202) 4,5 1,87 (142±12690) 594 (196÷4202) 4,5 5,2 (3÷8) 5,4 (3÷7) 4,5 1,58 (16,6±17,4) 18,4 (17,3÷19,1) 1,4 | Cd | $0,022 (0,018 \div 0,028)$ | $0.048 (0.044 \div 0.050)$ | $0.95 (0.22 \div 6.25)$ | $0,21 \ (0,11 \div 0,36)$ | 0,15 | <0,5 (0,1+1,0) |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$ | Ag | $0,06 (0,04 \div 0,08)$ | $0.02 (0.015 \div 0.022)$ | $0,50 (0,14 \div 2,30)$ | $0,29 (0,14 \div 1,05)$ | | |
| Organic pollutants (tgx xg¹ dw) <5 <5 12854 (790+41269) 4166 (1475+15280) 0,93 (0,81+1,05) 1,08 (0,73+1,20) 168 (8+2198) 30 (10+64) 1,42 40 (32+51) 16 (14+20) 1897 (142+12690) 594 (196+4202) 4,5 7,5 (3+9) 8,6 (7+10) 5,2 (3+8) 5,4 (3+7) 4,5 22,4 21,3 16,8 (16,6-17,4) 18,4 (17,3+19,1) 17,2 17,8 18,2 17,2 18,1 14,1 4,5 ** 7,4 5,5 (4,8+6,7) 6,5 (4,7+8,2) 6,5 (4,7+8,2) 6,8 7,4 5,5 (4,8+6,7) 6,5 (4,7+8,2) 8,1 (0+52)***** 4,6*** 2,2**** 7,5 (2+22)**** 8,1 (0+52)***** 0,9*** 0,7**** 2,1 (0,4-5,4)***** 1,3 (0+6)***** 0,9*** 2,1 (0,4+5,4)**** 1,3 (0+6)***** 8,2*** 1,2,5 (1,2+33)**** 1,9 (0+40)***** 8,2*** 1,2,5 (1,2+33)**** 1,5 (30+6)***** | Hg | $0.02 (0.017 \div 0.032)$ | $0,02 \ (0,015 \div 0,030)$ | $1,03 (0,04 \div 7,61)$ | $0.96(0.46 \div 1.42)$ | 0,04 | 0,04 |
| <5 <5 12854 (790+41269) 4166 (1475+15280) 0,93 (0,81+1,05) 1,08 (0,73+1,20) 168 (8+2198) 30 (10+64) 1,42 40 (32+51) 16 (14+20) 1897 (142+12690) 594 (196+4202) 4,5 7,5 (3+9) 8,6 (7+10) 5,2 (3+8) 5,4 (3+7) 22,4 21,3 16,8 (16,6+17,4) 18,4 (17,3+19,1) 17,8 18,2 17,2 18,1 +237 +285 -296 (-152384) -167 (-112320) 6,8 7,4 5,5 (4,8+6,7) 6,5 (4,7+8,2) 0,5 0,5 6,0 (2,6+11,4) 2,4 (1,6+3,0) 4,6*** 2,2**** 7,5 (2+22)**** 8,1 (0+92)***** 0,9*** 0,7**** 2,1 (0,4+5,4)**** 1,3 (0+6)***** <0,9*** | | | Organic | : pollutants $(ng \times g^{-1} dw)$ | | | |
| 0,93 (0,81+1,05) 1,08 (0,73+1,20) 168 (8+2198) 30 (10+64) 1,42 40 (32+51) 16 (14+20) 1897 (142+12690) 594 (196+4202) 4,5 7,5 (3+9) 8,6 (7+10) 5,2 (3+8) 5,4 (3+7) 4,5 22,4 21,3 16,8 (16,6+17,4) 18,1 (17,3+19,1) 18,1 17,8 18,2 17,2 18,1 18,1 +237 +285 -296 (-152384) -167 (-112320) 6,5 (4,7+8,2) 6,5 (4,7+8,2) 6,5 (4,7+8,2) 6,5 (4,7+8,2) 8,1 (0-92)***** 0,5 0,5 6,0 (2,6+11,4) 2,4 (1,6+3,0) 8,1 (0-92)***** 8,1 (0-92)***** 1,2,*** 0,7**** 2,1 (0,4+5,4)***** 1,3 (0+6)***** 8,0 (0+40)****** 0,9*** 0,7**** 12,5 (1,2+33)**** 8,9 (0+40)****** 8,0 (0+40)****** 8,2*** 12,5 (1,2+33)**** 15,9 (0+50)***** 15,9 (0+50)***** | Total PAHs | \$ | \$ | 12854 (790÷41269) | 4166 (1475÷15280) | | |
| 40 (32 \pm 51) 16 (14 \pm 20) 1897 (142 \pm 12690) 594 (196 \pm 4202) 4,5 7,5 (3 \pm 9) 8,6 (7 \pm 10) 5,2 (3 \pm 8) 5,4 (3 \pm 7) 4,5 22,4 21,3 16,8 (16,6 \pm 17,4) 18,4 (17,3 \pm 19,1) 18,1 17,8 18,2 17,2 18,1 +237 +285 -296 (-152384) -167 (-112320) 6,8 7,4 5,5 (4,8 \pm 6,7) 6,5 (4,7 \pm 8,2) 0,5 6,0 (2,6 \pm 11,4) 2,4 (1,6 \pm 3,0) 4,6*** 2,2**** 7,5 (2 \pm 22)**** 8,1 (0 \pm 9,2)***** 0,9*** 0,7**** 2,1 (0,4 \pm 5,4)**** 1,3 (0 \pm 6,0**** 82*** 65**** 12,5 (1,2 \pm 3)**** 8,9 (0 \pm 40)***** | Total DDTs | $0.93 (0.81 \div 1.05)$ | $1,08 (0,73 \div 1,20)$ | 168 (8÷2198) | 30 (10÷64) | 1,42 | $0,03 \div 0,1$ |
| Natural environmental parameters 7,5 (3÷9) 8,6 (7÷10) 5,2 (3÷8) 22,4 21,3 16,8 (16,6÷17,4) 17,8 18,2 17,2 +237 +285 -296 (-152384) 6,8 7,4 5,5 (4,8÷6,7) 0,5 0,5 6,0 (2,6÷11,4) 4,6*** 2,2**** 7,5 (2÷22)**** 7,2*** 6,4**** 27,6 (3÷86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** <a block"="" href="text-align: red and expectations of the color of the color</td><td>Total PCBs</td><td>40 (32÷51)</td><td><math>16 (14 \div 20)</math></td><td>1897 (142÷12690)</td><td>594 (196÷4202)</td><td>4,5</td><td>20÷60</td></tr><tr><td>7,5 (3÷9) 8,6 (7÷10) 5,2 (3÷8) 22,4 21,3 16,8 (16,6÷17,4) 17,8 18,2 17,2 +237 +285 -296 (-152384) 6,8 7,4 5,6 (3÷6,7) 0,5 0,5 6,0 (2,6÷11,4) 4,6*** 2,2**** 7,5 (3÷86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** 6,3*** 65**** 182 (44÷697)****</td><td></td><td></td><td>Natural 6</td><td>nvironmental parameters</td><td></td><td></td><td></td></tr><tr><td>22,4 21,3 16,8 (16,6÷17,4) 17,8 18,2 17,2 +237 +285 -296 (-152384) 6,8 7,4 5,5 (4,8÷6,7) 0,5 0,5 6,0 (2,6÷11,4) 4,6*** 2,2**** 7,5 (2÷22)**** 7,2*** 6,4**** 27,6 (3÷86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** 82*** 65**** 182 (44÷697)****</td><td>Water transparency, m</td><td>7,5 (3÷9)</td><td>8,6 (7÷10)</td><td>5,2 (3÷8)</td><td>5,4 (3÷7)</td><td></td><td></td></tr><tr><td>17,8 18,2 17,2 +237 +285 -296 (-152384) 6,8 7,4 5,5 (4,8±6,7) 0,5 0,5 6,0 (2,6±11,4) 4,6*** 2,2**** 7,5 (2±22)**** 7,2*** 6,4**** 27,6 (3±86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** <3***</td> <2****</td> 12,5 (1,2÷33)**** 82*** 65**** 182 (44÷697)****</td><td>Near bottom temperature, <sup>o</sup>C</td><td>22,4</td><td>21,3</td><td><math>16.8 (16.6 \div 17.4)</math></td><td><math>18,4 (17,3 \div 19,1)</math></td><td></td><td></td></tr><tr><td>+237 +285 -296 (-152384) 6,8 7,4 5,5 (4,8÷6,7) 0,5 0,5 6,0 (2,6÷11,4) 4,6*** 2,2**** 7,5 (2÷22)**** 7,2*** 6,4**** 27,6 (3÷86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** <3***</td> <2****</td> 12,5 (1,2÷33)**** 82*** 65**** 182 (44÷697)****</td><td>Salinity, psu</td><td>17,8</td><td>18,2</td><td>17,2</td><td>18,1</td><td></td><td></td></tr><tr><td>6,8 7,4 5,5 (4,8÷6,7) 0,5 0,5 6,0 (2,6÷11,4) 4,6*** 2,2**** 7,5 (2÷22)**** 7,2*** 6,4**** 27,6 (3÷86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** <3*** 65**** 182 (44÷697)****</td><td>Eh, mV</td><td>+237</td><td>+285</td><td>-296 (-152384)</td><td>-167 (-112320)</td><td></td><td></td></tr><tr><td>0,5 0,5 6,0 (2,6÷11,4) 4,6*** 2,2**** 7,5 (2÷22)**** 7,2*** 6,4**** 27,6 (3÷86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** <3***</td> <2****</td> 12,5 (1,2÷33)**** 82*** 65**** 182 (44÷697)****</td><td><math>O_2</math> in near bottom layer, ml <math>\times</math> l<sup>-1</sup></td><td>8,9</td><td>7,4</td><td>5,5 (4,8÷6,7)</td><td><math>6.5(4.7 \div 8.2)</math></td><td></td><td></td></tr><tr><td>4,6*** 2,2**** 7,5 (2÷22)**** 7,2*** 6,4**** 27,6 (3÷86)**** 0,9*** 0,7**** 2,1 (0,4÷5,4)**** <3***</td> <2****</td> 12,5 (1,2÷33)**** 82**** 65**** 182 (44÷697)****</td><td><math>C_{ m org}</math> , %</td><td>0,5</td><td>0,5</td><td><math>6,0 (2,6 \div 11,4)</math></td><td><math>2,4 (1,6\div3,0)</math></td><td></td><td></td></tr><tr><td>7,2*** 6,4**** 0,9*** 0,7*** <pre></td><td><math>PO_4^{3}</math></td><td>4,6***</td><td>2,2***</td><td>7,5 (2÷22)***</td><td>8,1 (0÷92)****</td><td></td><td></td></tr><tr><td>0,9***</td><td><math>NO_3</math> -</td><td>7,2**</td><td>6,4***</td><td>27,6 (3÷86)***</td><td>15,7 (0÷57)****</td><td></td><td></td></tr><tr><td><pre><3*** <2***</td><td><math>NO_2</math> -</td><td>***6,0</td><td>0,7***</td><td>2,1 (0,4.5,4)****</td><td>1,3 (0.6)****</td><td></td><td></td></tr><tr><td>82*** 65****</td><td><math display=">\mathrm{NH_4}^{+} | <3 ** | <2>** | 12,5 (1,2÷33)*** | 8,9 (0÷40)**** | | | |
| | Si | 82*** | ****9 | 182 (44÷697)*** | 159 (30÷540)**** | | |

Note: *Samyshev et al., 2014; **Mitropolsky et al., 2006; ****Kovrigina et al., 2009, ****Kovrigina et al., 2016; *****Kovrigina et al., 2010.

the Black Sea shelf (Mitropolsky *et al.*, 2006; Burgess *et al.*, 2009, 2011; Samyshev *et al.*, 2014). It has previously been shown that this set of pollutants can exert a strong influence on the quantitative development and spatial distribution of benthic diatoms (Petrov & Nevrova, 2004; Petrov *et al.*, 2005, 2010). According to the low concentrations of pollutants observed in the sediments (Table 1), DB is characterised as formally healthy with an undisturbed diatom taxocene species structure.

Taxonomic composition of diatoms of Dvuyakornaya Bay

As is known, the estimated diatom species richness usually depends on the sampling efforts (number of samples). The results of the prognostic modelling obtained for several nearshore Crimean locations have shown that the generalized relationship between the number of samples (X) and the number of observed species (Y) was reliably described by the log-equation $Y=79 \ln (X) + 35 (R^2 = 0.97)$ (Petrov & Nevrova, 2012, 2013, 2014).

Based on this randomised curve, the number of species revealed in nine samples from DB represents 218 (73%) of the actual registered number (304). These results indicate that the actual diatom species richness of DB may significantly exceed the expected average level of richness, which can be revealed after the same sampling efforts throughout the coast of Crimea (Petrov & Nevrova, 2013). In total, 304 species (including 5 intraspecific taxa) belonging to 299 species, 78 genera, 37 families, 20 orders, and 3 classes of Bacillariophyta were identified. Among these, 68 novel Black Sea diatom flora taxa were revealed (Table 2). Moreover, eight species and

taxonomic combinations which are described as new to science were also identified: Lyrella abruptapontica Nevrova, Witkowski, Kulikovskiy & Lange-Bertalot, L. pontieuxini Nevrova, Witkowski, Kulikovskiy & Lange-Bertalot, L. pseudolyra Nevrova, Witkowski, Kulikovskiy & Lange-Bertalot, Navicula parapontica Witkowski, Kulikovskiy, Nevrova & Lange-Bertalot, N. pontica Witkowski, Kulikovskiy, Nevrova & Lange-Bertalot (Nevrova et al., 2013; Witkowski et al., 2010). Additionally, four diatom species that were not registered in the Black Sea were also recorded: Navicula glabriuscula var. elipsoidales Proshk.-Lavr, N. petrovii Nevrova, Witkowski, Kociolek & Lange-Bertalot = Syn.: N. scabriuscula (Cleve et Grove) Mereschk. (Fig. 2: 40, Fig. 4: 29), Toxonidea insignis Donkin (Fig. 3: 26), and Pinnularia trevelyana (Donkin) Rabenh. (Fig. 3: 27) (Nevrova, 2015; Witkowski et al., 2014) (Table 2).

Representatives of four genera recently identified for Black Sea flora were found, including Amicula (Witkowski) Witkowski 2000, Astartiella Witkowski, Lange-Bertalot & Metzeltin 1998, Chamaepinnularia Lange-Bertalot & Krammer 1996, and Cocconeiopsis Witkowski, Lange-Bert & Metzeltin 2000 (Nevrova, 2015). Furthermore, the surveys also identified Amicula specululum (Witkowski) Witkowski (Fig. 4: 30), Astartiella bahusiensis (Grunow) Witkowski, Lange-Bertalot & Metzeltin (Fig. 2: 9; Fig. 4: 16), Astartiella producta Witkowski, Lange-Bertalot & Metzeltin (Fig. 4: 17), Chamaepinnularia sp. 1, Cocconeiopsis breviata (Hust.) Witkowski, Lange-Bertalot & Metzeltin (Fig. 2: 3), and Cocconeiopsis pullus (Hust.) Witkowski, Lange-Bertalot & Metzeltin (Fig. 4: 3). Several taxa were not identified at the species level but were included in the checklist since they have profound morphological differences from the registered species and were recorded on microphotographs. These

Table 2. Novel taxa for the Black Sea diatom flora in the Dvuyakornaya Bay area.

Designations: * newly discovered species for the Black Sea; ** recently described species; *** species not found in the Black Sea over the last 50 to 100 years of research; **** unidentified taxa that have profound differences to registered species.

| Coscinodiscophyceae | Fragilariophyceae |
|---|--|
| Actinoptychus senarius (Ehrenb.) Ehrenb. | Delphineis surirella (Ehrenb.) Andrews |
| Aulacoseira granulata (Ehrenb.) Simonsen | Diatoma vulgare Bory f. breve (Grunow) Bukht. |
| Auliscus sculptus (W. Sm.) Ralfs | Diatoma vulgare f. lineare (Grunow) Bukht. |
| Coscinodiscus radiatus Ehrenb. | Fragilaria atomus Hust. |
| Cyclostephanos dubius (Friske) Round | Fragilaria pulchella (Ralfs) Lange-Bert. |
| Cyclotella caspia Grunow | Fragilaria vaucheriae (Kütz.) Boey-Petersen |
| Cyclotella meneghiniana var. kuetzingiana (Thwaites) Playfair | Grammatophora marina (Lyngb.) Kütz. |
| Cyclotella ocellata Pant. | Hyalosira delicatula Kütz. |
| Cyclotella operculata (C. Agardh) Kütz. | Licmophora gracilis (Ehrenb.) Grunow |
| Cyclotella striata Grunow | <i>Opephora gunter-grassii</i> (Witkowski & Lange-Bert.) Sabbe & Vyverman* |
| Dimeregramma minor (W. Greg.) Ralfs | Opephora marina (W. Greg.) P. Petit |
| Dimeregramma sp.1 | Opephora minuta (Cleve-Euler) Witkowski, Lange-Bert. & Metzeltin* |

Continued

Glyphodesmis distans (W. Greg.) Grunow Opephora mutabilis (Grunow) Sabbe & Vyverman*

Hyalodiscus ambiguus (Grunow) Temp. & H. Perag.Opephora pacifica (Grunow) P. Petit*Hyalodiscus scoticus (Kütz.) GrunowPteroncola hyalina (Kütz.) Gusl.Plagiogramma tenuissima Hust.*Tabularia gaillonii (Bory) Bukht.

Puncticulata radiosa (Lemmerm.) Håk. Tabularia tabulata (C. Agardh) P.J.M. Snoeijs Stephanodiscus hantzschii Grunow Thalassionema nitzschioides (Grunow) Mereschk.

Thalassiosira baltica (Grunow) Ostenfeld Toxarium undulatum J.W. Bailey

Thalassiosira parva Proshk.-Lavr. Trachysphenia australis (H.L. Smith) Cleve*

Thalassiosira parvula Makarova Ulnaria ulna (Nitzsch.) Compère

Triceratium antediluvianum (Ehrenb.) Grunow

Bacillariophyceae

Achnanthes brevipes C. Agardh

Achnanthes vistulana Witkowski*

Achnanthes fimbriata (Grunow) R. Ross

Achnanthes islandica Østrup*

Achnanthes longipes C. Agardh

Achnanthes mercurii Witkowski, Metzeltin & Lange-Bert.*

Hippodonta sp.3

Hippodonta sp.5

Hippodonta sp.6

Hippodonta sp.8

Hippodonta sp.D5

Achnanthes placentuloides (Gusl.) Witkowski & Lange-Bert.

Lyrella abruptapontica Nevrova, Witkowski, Kulikovskiy & Lange-Bert.

Lange-Bert.**

Achnanthes sp.B06 Lyrella aestimata (Hust.) Nevrova, Kulikovskyi, Witkowski &

Lange-Bert.*

Achnanthidium glyphos Riaux-Gob., Compère & Witkowski* Lyrella hennedyi (W. Sm.) A. Stickle & D.G. Mann

Achnanthidium minutissimum (Kütz.) Czarn. Lyrella lyroides (Hendey) D.G. Mann Amicula specululum (Witkowski) Witkowski* Lyrella majuscula (Hust.) Witkowski*

Amphora arcus W. Greg.

Lyrella pontieuxini Nevrova, Witkowski, Kulikovskiy & Lange-

Bert.**

Amphora aspera P. Petit

Lyrella pseudolyra Nevrova, Witkowski, Kulikovskiy & Lange-

Bert.**

Amphora jostesorum Witkowski, Metzeltin & Lange-Bert.*

Lyrella rudiformis (Hust.) Nevrova, Witkowski, Kulikovskiy &

Lange-Bert.

Amphora eunotia Cleve Lyrella spectabilis (W. Greg.) D.G. Mann Amphora exilitata Giffen* Mastogloia cuneata (Meister) Simonsen*

Amphora graeffeana HendeyMastogloia exigua LewisAmphora helenensis Giffen*Mastogloia paradoxa Grunow

Amphora hyalina Kütz. Mastogloia pumila (Cleve & Möller) Cleve

Amphora inconspicua Proshk.-Lavr. Mastogloia tenera Hust.

Amphora marina W. Sm. Mastogloia sp.3

Amphora obtusa W. Greg.

Navicula aleksandrae Lange-Bert., Witkowski, Bogaczewicz-

Amphora obtisti W. Gleg.

Adamczak & Zgrundo*

Navicula arenaria Donkin*

Amphora ostrearia Bréb.

Navicula besarensis Giffen*

Amphora ovalis (Kütz.) Kütz.

Navicula cancellata Donkin

Amphora parvula Proshk.-Lavr.

Navicula bozenae Lange-Bert., Witkowski, Bogaczewicz-

Amphora pediculus (Kütz.) Grunow

Amphora proteus W. Greg.

Adamchak & Zgrundo*

Navicula cf. fauta Hust.*

Navicula cf. lusoria Giffen*

Amphora pusio Cleve*Navicula cf. mahoodii Witkowski & Witon*Amphora staurophora Jahlin-DannfeltNavicula cf. opima (Grunow) Grunow*Amphora subacutiuscula SchoemannNavicula distans (W. Sm.) Ralfs

Continued

Metzeltin*

Amphora turgida W. Greg. Navicula erifuga Lange-Bert.* Amphora wisei (Salah) Simonsen Navicula flanatica Grunow

Navicula germanopolonica Lange-Bert., Witkowski, Amphora sp.168-1W Bogaczewicz-Adamchak & Zgrundo*

Navicula glabriuscula var. elipsoidales Proshk.-Lavr.*** Amphora sp.2F

Anorthoneis excentrica (Donkin) Grunow

Navicula gregaria Donkin

Astartiella bahusiensis (Grunow) Witkowski, Lange-Bert. & Navicula northumbrica Donkin*

Astartiella producta Witkowski, Lange-Bert. & Metzeltin* Navicula palpebralis Bréb.

Astartiella sp. AW B362 1015* Navicula palpebralis var. angulosa (W. Greg.) Van Heurck Navicula palpebralis var. semiplena (W. Greg.) Cleve Bacillaria paxillifera (O.F. Müll.) Hendey

Navicula parapontica Witkowski, Kulikovskiy, Nevrova & Berkeleya rutilans (Trentep.) Grunow

Lange-Bert.**

Berkeleya scopulorum (Bréb. & Kütz.) E.J. Cox Navicula perminuta Grunow

Berkeleya sp. Navicula cf. perminuta Grunow

Navicula pontica Witkowski, Kulikovskiy, Nevrova & Lange-Biremis ambigua (Cleve) D.G. Mann

Biremis lucens (Hust.) Sabbe, Witkowski & Vyverman* Navicula radiosa Kütz.

Biremis ridicula (Giffen) D.G. Mann* Navicula ramosissima (C. Agardh) Cleve

Caloneis bacillum (Grunow) Cleve Navicula salinarum Grunow Caloneis densestriata (Proshk.-Lavr.) Gusl. Navicula salinicola Hust.

Caloneis lancettula (Schulz) Lange-Bert. & Witkowski* Navicula cf. salinicola Hust.

Navicula petrovii Nevrova, Witkowski, Kociolek & Lange-Caloneis liber (W. Sm.) Cleve Bert.** (Syn.: N. scabriuscula (Cleve & Grove) Mereschk.***)

Caloneis schumanniana var. biconstricta (Grunow) Reichelt Navicula veneta Kütz.

Navicula viminoides var. cosmomarina Lange-Bert., Witkowski, Caloneis sp.1

Bogaczewicz-Adamchak & Zgrundo*

Caloneis sp.2 Navicula sp.10 Campylodiscus thuretii Bréb. Navicula sp.135/14 Chamaepinnularia sp.1 Navicula sp.146/2

Cocconeiopsis breviata (Hust.) Witkowski, Lange-Bert. & Navicula sp.2

Metzeltin*

Cocconeiopsis pullus (Hust.) Witkowski, Lange-Bert. &

Navicula sp.3 Metzeltin*

Cocconeiopsis sp.1 Navicula sp.6 Cocconeis diminuta Pant.* Navicula sp.9 Cocconeis clandestina A.W.F. Schmidt* Navicula sp.B1

Cocconeis convexa Giffen* Navicula sp.D1 Cocconeis discrepans A.W.F. Schmidt* Navicula sp.D2

Cocconeis euglypta Ehrenb. Nitzschia acuminata (W. Sm.) Grunow

Cocconeis guttata Hust. & Aleem* Nitzschia aequorea Hust.* Cocconeis pediculus Ehrenb. Nitzschia amphibia Grunow

Cocconeis pelta A.W.F. Schmidt* Nitzschia angularis var. affinis (Grunow) Grunow

Cocconeis peltoides Hust.* Nitzschia aurariae Cholnoky* Cocconeis placentula Ehrenb. Nitzschia capitellata Hust. Cocconeis pseudocostata O.E. Romero* Nitzschia coarctata Grunow Nitzschia cf. coarctata Grunow Cocconeis pseudograta Hust.*

Cocconeis scutellum Ehrenb. Nitzschia compressa (J.W. Bailey) Boyer

Cocconeis scutellum var. parva (Grunow) Cleve Nitzschia constricta (Kütz.) Ralfs Cocconeis speciosa W. Greg.

Cocconeis stauroneiformis (Rabenh.) Okuno

Cocconeis cf. stauroneiformis (Rabenh.) Okuno

Cocconeis sp.1 Cocconeis sp.A1 Cocconeis sp.5

Cymatopleura elliptica (Bréb.) W. Sm.

Cymatopleura solea var. apiculata (W. Sm.) Ralfs

Cymbella angusta (W. Greg.) Gusl. Cymbella cymbiformis C. Agardh

Cymbella excisa Kütz.

Cymbella sp.4

Cymbella sp.D1

Denticula subtilis Grunow

Dickieia resistans Witkowski, Lange-Bert. & Metzeltin

Dickieia subinflata (Grunow) D.G. Mann Diploneis bombus (Ehrenb.) Cleve-Euler Diploneis chersonensis (Grunow) Cleve

Diploneis coffaeiformis (A.W.F. Schmidt) Cleve*

Diploneis crabro Ehrenb.

Diploneis fusca (W. Greg.) Cleve Diploneis incurvata (W. Greg.) Cleve

Diploneis litoralis (Donkin) Cleve Diploneis notabilis (Grev.) Cleve

Diploneis notabilis var. tenera Proshk.-Lavr. Diploneis parca (A.W.F. Schmidt) Boyer

Diploneis smithii (Bréb.) Cleve Diploneis stroemii Hust.*

Diploneis suborbicularis (W. Greg.) Cleve Diploneis vacillans (A.W.F. Schmidt) Cleve

Diploneis sp.1

Diploneis sp.2

Encyonopsis microcephala (Grunow) Krammer*

Entomoneis paludosa (W. Sm.) Reimer

Fallacia aequorea (Hust.) D.G. Mann*

Fallacia amphipleroides (Hust.) D.G. Mann*

Fallacia florinae (Moeller) Witkowski*

Fallacia forcipata (Grev.) A. Stickle & D.G. Mann

Fallacia litoricola (Hust.) D.G. Mann* Fallacia oculiformis (Hust.) D.G. Mann*

Fallacia pygmaea (Kütz.) A. Stickle & D.G. Mann

Fallacia subforcipata (Hust.) D.G. Mann

Fallacia tenera (Hust.) D.G. Mann Fallacia sp.7F Nitzschia dissipata (Kütz.) Grunow

Nitzschia frustulum (Kütz.) Grunow

Nitzschia granulata Grunow

Nitzschia grossestriata Hust.* Nitzschia inconspicua Grunow

Nitzschia insignis W. Greg.

Nitzschia lanceolata var. minima Van Heurck

Nitzschia liebetruthii Rabenh. Nitzschia cf. liebetruthii Rabenh. Nitzschia lorenziana Grunow Nitzschia pellucida Grunow

Nitzschia perindistincta Cholnoky*

Nitzschia pusilla (Kütz.) Grunow emend. Lange-Bert.

Nitzschia sigma (Kütz.) W. Sm. Nitzschia spathulata Bréb. Nitzschia vidovichii Grunow

Nitzschia sp.6 Nitzschia sp.8

Oestrupia powellii (Lewis) Heiden* Parlibellus hamulifer (Grunow) E.J. Cox

Parlibellus sp.4D

Petrodictyon gemma (Ehrenb.) D.G. Mann

Petroneis humerosa (Bréb.) A. Stickle & D.G. Mann

Pinnularia cruciformis (Donkin) Cleve

Pinnularia quadratarea (A.W.F. Schmidt) Cleve Pinnularia trevelyana (Donkin) Rabenh.*** Pinnularia viridis (Nitzschiae) Ehrenb.

Pinnularia sp.2D

Plagiotropis elegans (W. Sm.) Grunow Plagiotropis lepidoptera (W. Greg.) Kuntze

Planothidium deperditum (Giffen) Witkowski, Lange-Bert. &

Metzeltin*

Planothidium delicatulum (Kütz.) Round & Bukht.

Planothidium cf. delicatulum (Kütz.) Round & Bukht.

Planothidium cf. delicatulum f.1 (Kütz.) Round & Bukht.

Planothidium dispar (Cleve) Witkowski, Metzeltin & Lange-

Bert

Planothidium lanceolatum (Bréb.) Bukht.

Planothidium quarnerensis (Grunow) Witkowski, Lange-Bert. &

Metzeltin

Planothidium sp.1 Planothidium sp.2 Planothidium sp.2F

Pleurosigma aestuarii (Bréb.) W. Sm. Pleurosigma angulatum (Queckett) W. Sm.

Pleurosigma elongatum W. Sm. Pleurosigma formosum W. Sm.

Fogedia finmarchica (Cleve & Grunow) Witkowski, Metzeltin & Lange-Bert.

Fogedia giffeniana (Foged) Witkowski, Lange-Bert., Metzeltin & Bafana*

Fogedia heterovalvata (Simonsen) Witkowski, Metzeltin & Lange-Bert.*

Fogedia sp.2

Gomphonema angustatum (Kütz.) Rabenh.

Gomphonema sp.1

Gyrosigma fasciola (Ehrenb.) Cleve

Gyrosigma spenceri (Queckett) Griffith & Henfrey

Halamphora acutiuscula (Kütz.) Levkov Halamphora angularis (W. Greg.) Levkov Halamphora coffeaeformis (C. Agardh) Levkov Halamphora tenerrima (Aleem & Hust.) Levkov*

Hantzschia vivax (W. Sm.) Perag.

Haslea subagnita (Proshk.-Lavr.) Makarova & Karajeva

Hippodonta sp.2

Pleurosigma rigidum W. Sm.

Psammodictyon panduriforme var. continua (Grunow) P.J.M. Snoeijs*

Psammodictyon roridum (Giffen) D.G. Mann*

Psammodictyon rudum (Cholnoky) D.G. Mann* Rhoicosphenia abbreviata (C. Agardh) Lange-Bert.

Rhopalodia musculus (Kütz.) O. Müll. Sellaphora pupula (Kütz.) D.G. Mann

Seminavis sp.1

Stauronella indubitabilis Lange-Bert. & Genkal

Staurophora salina (W. Sm.) Mereschk.

Surirella fastuosa (Ehrenb.) Kütz.

Toxonidea insignis Donkin

Trachyneis aspera (Ehrenb.) Cleve

include Gomphonema sp. 1 (Fig. 3: 29), Hippodonta sp. 3 (Fig. 4: 7), Hippodonta sp. 5 (Fig. 2: 15, Fig. 4: 8), Hippodonta sp. 6 (Fig. 2: 16, Fig. 4: 9), Hippodonta sp. 8 (Fig. 4: 10), Amphora sp. 2F (Fig. 3: 25), Astartiella sp. AW B362 1015 (Fig. 2: 10), Navicula sp. D2 (Fig. 3: 11), Navicula sp.146/2 (Fig. 3: 10); Diploneis sp. 1 (Fig. 3: 1, Fig. 4: 20), Diploneis sp. 2 (Fig. 3: 3), Navicula sp. D1 (Fig. 4: 12), Navicula sp. 9 (Fig. 4: 13), and Seminavis sp.1 (Fig. 3: 28, Fig. 4: 25). Some of those species might be new to science, though further data collection is required to confirm or deny this notion.

Classes Coscinodiscophyceae and Fragilariophyceae are represented slightly in the taxocene, at 7.2 and 6.9% of the total species number, respectively. From Coscinodiscophyceae, 5 orders, 9 families, 14 genera, and 22 species were registered, while 6 orders, 6 families, 13 genera, and 20 species were found from Fragilariophyceae. Representatives from class Bacillariophyceae, which belong to 9 orders, 22 families, 51 genera, and 257 species (261 intraspecific taxa) were dominant (85.8%). Order Naviculales was the most abundant in the number of lower taxa, with 9 families, 25 genera, and 122 species observed. A lower richness was observed for the orders Achnanthales (3 families, 6 genera, and 44 species), Bacillariales (1 family, 5 genera, and 34 species), and Thalassiophysales (1 family, 2 genera, and 28 species). The highest species number was observed for the genera Navicula (41 species), followed by Nitzschia (29), Amphora (25), Cocconeis (20), Diploneis (16), Lyrella, Fallacia, and Planothidium (10 species each, respectively) (Appendix 1).

Similarity of species composition in benthic diatom taxocenes from different areas of the Crimean coast

The taxonomic composition of benthic diatoms in the aforementioned sites of the Crimean coast were represented by a total of 543 species, among which 44 species belong to class Coscinodiscophyceae, 42 to Fragilariophyceae, and 457 to Bacillariophyceae (Appendix 1). Species composition between the taxocene of DB and previously investigated areas of the Crimean coast were compared. However, total registered species richness in each sampling area could not be directly matched due to different sampling efforts (number of samles). Therefore, the expected number of species in each of the healthy and polluted comparison areas were evaluated for similar sampling effort (8-9 samples) (Table 3). Notably, a total of 32 samples were analysed for SB, whereas 16 were analysed for BB, and only 8 were analysed for CF.

Recalculation of the observed number of species for each sampling area based on cumulative randomised sequences of species demonstrated that, when considering a similar number of samples (8-9), 126-130 species were observed in SB, while 150-156 species were observed in BB. Thus, due to strong permanent pollution, the species richness in SB or in BB was nearly half of the value registered for each of the pristine areas based on identical sampling effort.

Based on the Bray-Curtis similarity coefficient, the highest species similarity was revealed between the taxocenes of CF and DB (57%) – both of which represent environmentally healthy biotopes. A similarly high value of resemblance coefficient was observed between the taxocenes of heavily polluted SB and BB (Table 4). The lowest similarity was observed between the diatom taxocenes at SB and CF (32.3%) and between SB and DB (36.5%).

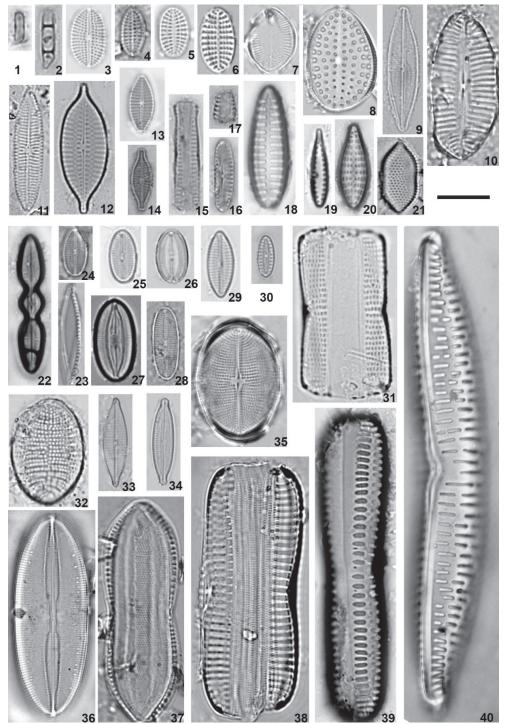


Fig. 2: New for the Black Sea diatom flora species found in Dvuyakornaya Bay (LM): 1 – Achnanthes mercurii; 2 – Plagiogramma tenuissima; 3 – Cocconeiopsis breviata; 4 – Cocconeis discrepans; 5 – Cocconeis peltoides; 6 – Cocconeis pseudocostata; 7 – Cocconeis pseudograta; 8 – Cocconeis guttata; 9 – Astartiella bahusiensis; 10 – Astartiella sp. AW B3621015; 11 – Navicula arenaria; 12 – Fogedia giffeniana; 13 – Fogedia heterovalvata; 14 – Fogedia sp.2; 15 – Hippodonta sp.5; 16 – Hippodonta sp.6; 17 – Opephora gunter-grassii; 18 – Opephora pacifica; 19 – Opephora mutabilis; 20 – Trachysphenia australis; 21 – Psammodictyon panduriforme var. continua; 22 – Caloneis schumanniana var. biconstricta; 23 – Nitzschia aequorea; 24 – Fallacia aequorea; 25 – Fallacia amphipleroides; 26 – Fallacia florinae; 27 – Fallacia oculiformis; 28 – Fallacia litoricola; 29 – Navicula aleksandrae; 30 – Biremis lucens; 31 – Navicula northumbrica; 32 – Cocconeis clandestina; 33 – Navicula gregaria; 34 – Encyonopsis microcephala; 35 – Cocconeis convexa; 36 – Navicula glabriuscula var. elipsoidales; 37 – Psammodictyon roridum; 38 – Biremis ridicula; 39 – Oestrupia powellii; 40 – Navicula petrovii. Scale bar 10 µm.

An analysis of species distribution between studied sites was performed using Venn diagrams (Venn diagrams, 2019) (Fig. 5). Despite their geographical remoteness, the highest number of species unique to each area was found in sites with the lowest degree of pollution –

DB and CF – at which 98 and 96 species were observed, respectively. In contrast, the heavily polluted areas – SB and BB – the number of unique species was nearly two times less (47 and 37 species, respectively). A total of 79 common species were found in pristine sites, while

Table 3. Number of samples, total observed (S_{obs}) and expected (S_{exp}) values of benthic diatom species richness at four investigated coastal sites in Crimea. S_{exp} values were calculated for an equal number of samples (8-9).

| Sampling site | Number of samples | 8 | <u> </u> |
|------------------|-------------------|-----|----------|
| Sampling site | Number of samples | obs | exp exp |
| Dvuyakornaya Bay | 9 | 304 | 304 |
| Cape Fiolent | 8 | 290 | 290 |
| Sevastopol Bay | 32 | 186 | 126–130 |
| Balaklava Bay | 16 | 191 | 150–156 |

32 common species were observed in two polluted areas (SB and BB). Since only 47 species (out of 542) were recorded as common among all studied areas, it can be concluded that technogenic pollution could largely determine the diversity patterns of diatoms in coastal biotopes with similar environmental conditions (Sugie & Suzuki, 2017).

Discussion

The high number of benthic diatom species registered in the DB may due to this area not previously being subjected to anthropogenic disturbance. Levels of pollutants in this bay's soft bottom sediments were either close to the minimum analytical detection limits (for PAHs) or similar to average background levels registered for all offshore bottom areas of the Crimean coast (with the exception of Zn content) (Table 1) (Mitropolsky et al., 2006; Samyshev et al., 2014). Another possible cause of this high Bacillariophyta species richness is the variety of microbiotopes in DB (silty-clayed, rocky, gravel or sandy substrates with macrophyte thickets, and shell debris patches). The high landscape heterogeneity of the sea floor may determine conditions for the development of greater diatom species richness, including the occurrence of rare species, species sensitive to pollution, as well relics of Ponto-Caspian flora (e.g., Navicula petrovii; Witkowski et al., 2014) and invasive species. Notably, the latter could have been brought to the research area from other regions through ballast water or from phytoperiphyton on the hulls of vessels passing nearby DB to the ports of Crimea, Caucasus, and the Sea of Azov. The group of invasive species includes small-cell species, such as: Achnanthidium glyphos, previously known only from a typical habitat in the Indian Ocean (Riaux-Gobin et al., 2010); Fogedia giffeniana, found in the Gulf of Aden (Witkowski et al., 1997); F. heterovalvata, previously noted off the coast of the Baltic Sea and the Caribbean Islands (Witkowski et al., 1997); and Navicula aleksandrae, N. germanopolonica, N. bozenae, and N. viminoides var. cosmomarina (all described from Gdansk Bay, Baltic Sea; Lange-Bertalot et al., 2003). Among the large-cell diatoms, several species also are likely to be invading forms (e.g., Diploneis coffaeiformis, D. stroemii, Mastogloia cuneata, Navicula arenaria, N. besarensis, N. northumbrica, N. cf. fauta, N. cf. lusoria, N. cf. mahoodii, N. cf. opima, Oestrupia powellii, Psammodictyon roridum, P. rudum, and others). Although these species are widely distributed in oceans worldwide, they were recorded for the first time in the Black Sea flora of Bacillariophyta. However, it is possible to assume that several of the aforementioned common small-cell or rare large-cell species are autochthonous forms but were not previously found in the Black Sea due to insufficient research on benthic diatoms. The final conclusion regarding whether the novelty floristic finds of Bacillariophyta (68 species) are invading species can be made after additional taxonomic and molecular/genetic studies. Similar results suggesting high Bacillariophyta species richness (290 species) and a significant number of new floristic finds were also obtained from the ecologically intact area around CF, which is also characterised by a large variety of microbiotopes and very low technogenic pollutant content (heavy metals, chlororganic pesticides, and PAHs) in soft bottom sediments. A total of 68 species and 3 genera were previously recorded as being new to the Black Sea flora within the diatom taxocene of CF, while 3 species and 1 taxonomic combination were registered as new to science, and 4 species were not found in the Black Sea over the last 50 to 100 years of research (Nevrova, 2016). According to the Spearman's rank correlation analysis (Petrov et al., 2005, 2010), diatom species richness showed a significant correlation with most pollution-related variables (Table 1), while factors such as water temperature, salinity, pH, oxygen concentration, and nutrients in the

Table 4. The pairwise similarity (%) of benthic diatoms species composition in biotopes of the South-western and South-eastern coast of Crimea (using Bray-Curtis similarity coefficient).

| | Sevastopol Bay | Cape Fiolent | Balaklava Bay |
|----------------------------|----------------|--------------|---------------|
| Sevastopol Bay (186 sp.) | * | * | * |
| Cape Fiolent (290 sp.) | 32.3 | * | * |
| Balaklava Bay (191 sp.) | 57.8 | 39.1 | * |
| Dvuyakornaya Bay (304 sp.) | 36.5 | 57.1 | 42.6 |

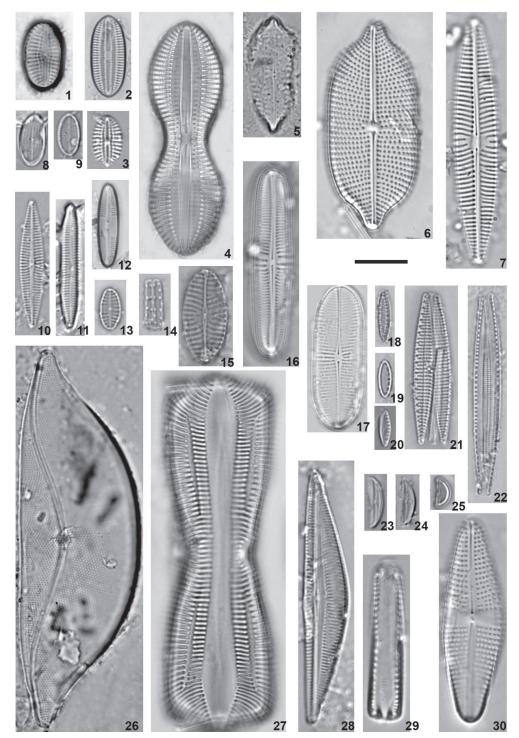


Fig. 3: New for the Black Sea diatom flora species found in Dvuyakornaya Bay (LM): 1 – Diploneis sp.1; 2 – Diploneis parca; 3 – Diploneis sp.2; 4 – Diploneis stroemi; 5 – Navicula ef. opima; 6 – Navicula besarensis; 7 – Navicula erifuga; 8 – Cocconeis sp.5W; 9 – Cocconeis sp.1; 10 – Navicula sp.146/2; 11 – Navicula sp.D2; 12 – Caloneis lancettula; 13 – Planothidium deperditum; 14 – Denticula subtilis; 15 – Navicula ef. lusoria; 16 – Dickieia subinflata; 17 – Dickieia resistans; 18 – Nitzschia aurariae (oblique light); 19 – Nitzschia perindistincta; 20 – Nitzschia inconspicua; 21 – Nitzschia grossestriata; 22 – Nitzschia liebetruthii; 23 – Amphora helenensis; 24 – Amphora exilitata; 25 – Amphora sp. 2F; 26 – Toxonidea insignis; 27 – Pinnularia trevelyana; 28 – Seminavis sp.1; 29 – Gomphonema sp.1; 30 – Achnanthes islandica. Scale bar 10 μm.

near-bottom water layer were only weakly correlated with the species diversity metrics.

Meanwhile, in SB – which has been exposed to intense long-term anthropogenic impact and is distinguished by a homogeneous silty-sandy bottom substrate covering up to 98% of the bay bottom area – a weakened oxygen regime with deeply negative redoxing conditions

and high levels of C_{org} in sediment (Table 1) is characterised by reduced diatom taxocene species richness (186 species). Similarly, in BB, the bottom of which is nearly completely covered with silty deposits with an extremely high level of pollutants (Burgess *et al.*, 2009), the diatom taxocene structure is also characterised by low species richness (191 species) similar to that of SB (Petrov *et*

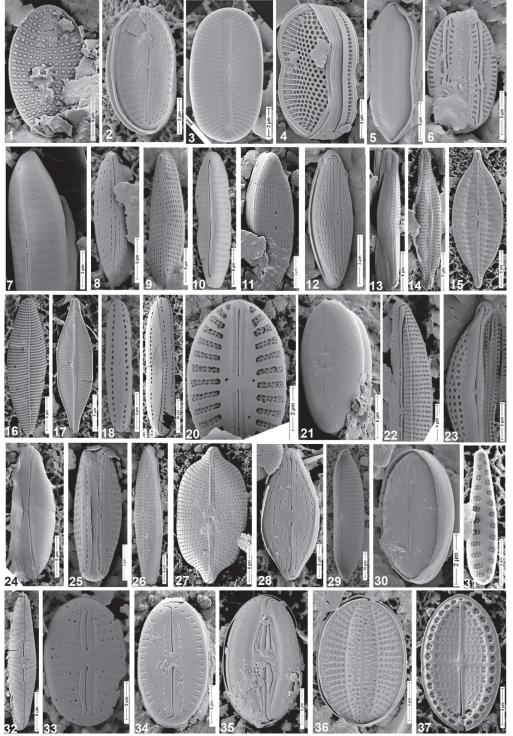


Fig. 4: New for the Black Sea diatom flora species found in Dvuyakornaya Bay (SEM): 1 – Cocconeis guttata; 2 – Cocconeis diminuta; 3 – Cocconeiopsis pullus; 4 – Psammodictyon panduriforme var. continua; 5 – Psammodictyon roridum; 6 – Amphora helenensis; 7 – Hippodonta sp.3; 8 – Hippodonta sp.5; 9 – Hippodonta sp.6; 10 – Hippodonta sp.8; 11 – Navicula germanopolonica; 12 – Navicula sp.D1; 13 – Navicula sp.9; 14 – Fogedia sp.2; 15 – Fogedia giffeniana; 16 – Astartiella bahusiensis; 17 – Astartiella producta; 18 – Biremis lucens; 19 – Biremis ridicula; 20 – Diploneis sp.1; 21 – Diploneis coffaeiformis; 21 – Amphora pusio; 23 – Halamphora tenerrima; 24 – Amphora jostesorum; 25 – Seminavis sp.1; 26 – Navicula parapontica; 27 – Navicula besarensis; 28 – Navicula viminoides var. cosmomarina; 29 – Navicula petrovii; 30 – Amicula specululum; 31 – Opephora minuta; 32 – Mastogloia cuneata; 33 – Fallacia aequorea; 34 – Fallacia florinae; 35 – Fallacia oculiformis; 36 – Cocconeis pelta; 37 – Cocconeis pseudocostata. Scale bar indicated on each micrograph.

al., 2010). Such results are congruent with other studies stating that decreased species richness in diatom assemblages often occurred under reduced heterogeneity of microhabitats and anthropogenically stressed environments

(Heino et al., 2007; Leira et al., 2009; Nevrova, 2014; Stenger-Kovacs et al., 2016).

The differences in diatom taxocene species structure at DB and other compared areas can be due to both exter-

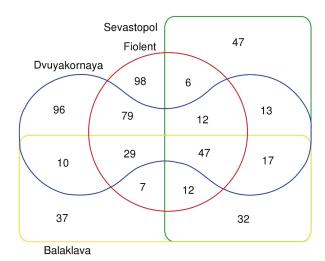


Fig. 5: Comparison of diatom species distribution between studied areas (using a Venn diagram).

nal (environmental) and internal factors that are primarily linked to the phylogenetic relationships of species within the taxonomic tree. Rather low values of species richness and a relatively high ratio of polyspecific branches in a taxonomic tree are often linked to microphytobenthos communities exposed to adverse effects such as anthropogenic pollution (Gottschalk & Kahlert, 2012; Heino et al., 2005, 2007; Petrov et al., 2010; Stenger-Kovacs et al., 2014, 2016). Meanwhile, high species richness and especially taxonomic richness above the species level (i.e., genera and higher) may indicate a considerable taxonomic alignment of the taxocene structure (i.e., proportional representation of taxons at different hierarchical levels of the taxonomic tree). Such features of the architectonics of the hierarchical tree of diatoms typically characterise taxocenes from ecologically healthy biotopes experiencing minimal adverse environmental effects (Leira et al., 2009; Nevrova et al., 2015; Rimet & Bouchez, 2012).

Other possible factors affecting diatom species richness include differences in water turbidity (and the related flow of photosynthetically active radiation (PAR)), oxygen content and redox conditions in the upper layer of bottom sediments, as well as nutrient levels (Kovrigina *et al.*, 2009; 2010, 2016; Mitropolsky *et al.*, 2006). In the present study, only C_{org} content and redox potential (Eh) values in the sediments of compared areas differed significantly (Table 1). Low C_{org} content and highly positive redox potential in the sediments of CF and DB likely influence the survival and occurrence of those low-tolerant diatom species that are absent in SB and BB.

Tests of statistical significance showed that values for nutrients, salinity, and PAR in the near-bottom layers of studied areas did not differ significantly. Results from comparing the levels of abiotic factors led to conclusion that microlandscape heterogeneity as well as the parameters of anthropogenic pollutants and redox conditions in the upper layer of sediments can represent key causes influencing the distinctness of benthic diatom species richness and the structure of taxocenes in biotopes with various environmental conditions.

As a result, in the pristine areas of DB and CF, high

benthic Bacillariophyta species richness was revealed alongside a number of novel taxa to the Black Sea flora. In contrast, high levels of trace metal and organic pollutant accumulation in the bottom sediments of SB and BB exerted a negative influence on the species richness of diatom taxocene structure, though species tolerant to pollution or euribiontic forms prevailed.

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The following information is available for the article:

Appendix 1. Diatom species composition of Dvuyakornaya Bay, Cape Fiolent, Sevastopol Bay and Balaklava Bay (Crimea, the Black Sea) (Excel /on line).