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RESEARCH PAPER

Taxonomic diversity and structure of benthic diatom taxocenes (Bacillariophyta) along the Crimean Coast (the Black Sea)

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ABSTRACT

Diversity and structure of benthic diatom assemblages in 16 Crimean nearshore habitats with varying levels of technogenic bottom area pollution at depths range 0.5–50 m were investigated and accessed using Taxonomic Distinctness indices (TaxDI). In total 793 species and intraspecific taxa, belonging to 736 species, 130 genera, 53 families, 27 orders and 3 classes of Bacillariophyta were registered. The structure of diatom assemblages from polluted sites can be described as having low species richness and a large proportion of mono- and oligospecies branches at the family and order levels. In locations under a moderate anthropogenic impact, the structure was characterised by relatively high species richness and an equal ratio of oligo- and poly-species branches closed at genus level, with the presence of monospecies branches converging at family or order level. Taxocenes in pristine sites were distinguished by the highest species richness and a predominance of poly-species branches closed at different hierarchical levels.

Keywords: hierarchical structure, taxonomic distinctness indices, technogenic pollution

РЕЗЮМЕ

Неврова Е.Л. Таксономическое разнообразие и структура бентосных диатомовых таксоценов (Bacillariophyta) вдоль Крымского побережья (Чёрное море). Исследовано таксономическое разнообразие и структура бентосных диатомовых таксоценов в 16 прибрежных местообитаниях Крыма с разной степенью техногенного загрязнения в диапазоне глубины 0.5–50 м. Для оценки иерархической структуры таксоценов диатомовых исследованных районов использован индекс таксономической отличительности (TaxDI). Всего зарегистрировано 793 вида и внутривидовых таксона, относящихся к 736 видам, 130 родам, 53 семействам, 27 отрядам и 3 классам Bacillariophyta. Структура таксоценов диатомовых в загрязненных районах характеризуется низким видовым богатством и большой долей моно- и олиговидовых ветвей на уровне семейств и порядков. В акваториях с умеренным антропогенным воздействием структура таксоценов имеет относительно высокое видовое богатство и равное соотношение олиго- и поливидовых ветвей, замкнутых на уровне родов, с наличием моновидовых ветвей, конвергирующих на уровне семейств или порядков. Таксоцены в ненарушенных местообитаниях отличаются наибольшим видовым богатством и преобладанием поливидовых ветвей, сходящихся на различных уровнях иерархии.

Ключевые слова: иерархическая структура, индекс таксономической отличительности, техногенное загрязнение

The importance of benthic diatoms to the functioning of coastal ecosystems determines the relevance of study of their biodiversity. It should be preserved so they may be used to assess the state of the marine environment (Blanco et al. 2012, Rimet & Bouchez 2012, Borja et al. 2013, Winter et al. 2013, Stenger-Kovács et al. 2014, Keck et al. 2016). One of the main tasks for Black Sea biodiversity conservation is assessing the structure of the benthic diatom assemblages. The nearshore areas of Crimea are subject to increasing anthropogenic impact, which might cause significant changes to the species composition of benthic diatoms. The intricate structure and high species richness of diatom assemblages in intact water areas can rapidly change due to the elimination of highly sensitive species and their replacement with those species which not susceptible to pollutants. Therefore, a comparative analysis of benthic diatom assemblages in undisturbed coastal habitats and biotopes exposed to anthropogenic impact is an important basis for identifying various aspects of the formation and maintenance of biodiversity under changing environmental conditions (Petrov et al. 2005, 2010, Facca & Sfriso 2007, Heino et al. 2007, Leira et al. 2009, Stenger-Kovács et al. 2014, 2016, Nevrova et al. 2015, Nevrova & Petrov 2019a, b).

Most regional explorations of benthic diatoms have focused on the seasonal dynamics and species composition of the leading species on solid substrates. Soft-bottom diatom assemblages have been studied to a lesser extent, while problems related to diversity assessment remain. Heterogeneous data sets are often represented by simple species lists with no parameters related to the number or biomass of cells. In such cases, the use of traditional indices for measuring biodiversity (Shannon N, Margalef d, Pielou J, etc.) becomes inapplicable when both aspects of diversity are quantified according to the species richness and evenness in the distribution of individuals among species. Applying such indices is also ineffective when the sampling effort or habitat type of Bacillariophyta greatly differs or when compared two taxocenes with the equal species number and similar quantitative characteristics (Warwick & Clarke 1998, 2001, Nevrova et al. 2015). Notably, such taxocenes can include species that are phylogenetically close (belong to the same genus) or distant (belong to different families, orders and classes). In this regard, the taxonomic aspects of diversity can vary greatly, even when the two compared taxocenes have the same species richness. Since relatedness among diatom species and taxonomic systems are still overwhelmingly based on the morphology of frustules and much less on phylogenetic studies, an assemblage of closely related species must be regarded as less diverse than an assemblage of the same number of more distantly related species (e.g., in which the species belong to different taxonomic classes) (Warwick & Clarke 1998, 2001, Leonard et al. 2006, Leira et al. 2009). Based on this premise, a biodiversity measure emphasising the average taxonomic relatedness between species in a community has been developed and applied in biomonitoring (Warwick & Clarke 1998, 2001, Warwick et al. 2002, Arvanitidis et al. 2005). Measures based on the taxonomic structure of assemblages differ from more conventional diversity indices by incorporating the degree to which species are morphologically- and thus evolutionarily-related (according to the Linnean system). These biodiversity assessment indices have proved useful in studies of different groups of organisms in various world regions (Warwick et al. 2002, Ellingsen et al. 2005, Leonard et al. 2006). However, apart from a few recent studies focused on macroinvertebrates (Campbell et al. 2007, Heino et al. 2007, Munari et al. 2009), macroalgae (Mouillot et al. 2005, Chescia et al. 2007) and freshwater diatoms (Leira et al. 2009, Stenger-Kovács et al. 2014, 2016), the measure as a means of assessing anthropogenic effects on marine benthic diatoms is untested. In this context, the work aimed to assess by using TaxDI analysis the current state of diversity and structure of benthic diatom taxocenes off Crimean nearshore habitats under different anthopogenis pressure.

MATERIAL AND METHODS

Study area

Material for this study was sampled at 16 sites off the Crimean coast at depths ranging 0.5–50 m between 1984 and 2016 (Fig. 1). A total of 4 to 37 samples were taken from each site, with two replicates (Nevrova 2022). In total, 190 samples were analysed (Table 1). The sampling and other field studies have been performed in non-restricted and free-accessable areas of the Black Sea and this activity have not been required any permission from the authorities. Prognostic assessment of species richness of benthic diatoms in replicate samples at the regional and polygon scales was performed previously. These assessments determined that any one sample was required to reveal approximately 35 % of the total species richness at the polygon scale (two samples = about 50 %, six samples = about 80 %) At the regional scale, the detection of 80 % of total species richness requires approximately 40 stations, assuming the equiprobability of occurrence of diatom species in samples (Petrov & Nevrova 2013, 2014).

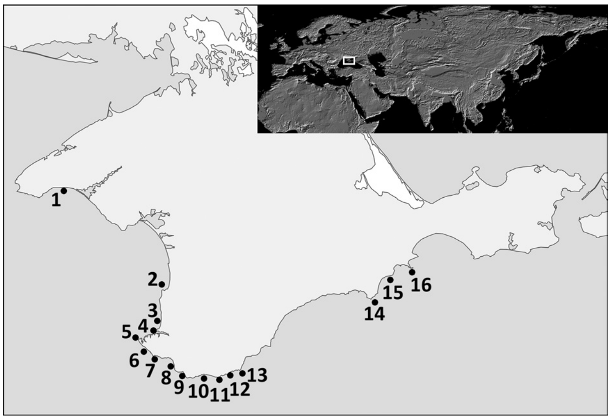

Figure 1 Sampling design along the Crimean coast: 1 – near vil. Marjino; 2 – near r. Belbek; 3 – referent site R3 near Sevastopol; 4, 5, 6 – Karantinnaya Bay, Sevastopol Bay, Inkerman Bay; 7, 8, 9, 10, 11 – Omega Bay, Golubaya Bay, Cape Fiolent, Balaklava Bay, referent site R6 near Balaklava; 12 – Laspi Bay; 13 – Cape Sarych; 14 –near vil. Novyi Svet; 15 – Karadag Nature Reserve; 16 – Dvuyakornaya Bay

Table 1. Sampling efforts and species richness of benthic diatom off Crimean coast

Sampling site	Data	Coordinates	Depth, m	Number of samples	Substrates	Number of sp. and IST
Heavy polluted sites						
Near r. Belbek	05.11.2009	44°39'45"N 33°32'31"E	6 – 19	10	Sandy-silty	243
Karantinnaya Bay	25.08.1994	44°37'05"N 33°30'10"E	0.5 – 32	22	Silty-sandy, rocky	136
Sevastopol Bay	11.07.2001	44°37'19"N 33°31'27"E	4 – 17	31	Silty-sandy	186
Inkerman	06.11.2009	44°36'20"N 33°35'50"E	3 – 10	6	Silty-sandy	116
Balaklava Bay	14.10.2006	44°29'12"N 33°36'53"E	6 – 20	17	Silty-sandy	191
Conventionally healthy sites						
Near vil. Marjino	20.07.2010	45°20'15"N 32°42'12"E	6	4	Sandy	140
Referent site R3	05.11.2009	44°39'01"N 33°31'38"E	12	4	Sandy	119
Omega Bay	28.07.2004	44°35'55"N 33°26'56"E	1.5 – 16	5	Sandy-silty	260
Golubaya Bay	15.08.2009	44°35'03"N 33°22'48"E	1.5 – 6	6	Sandy	124
Cape Fiolent	12.08.2009	44°30'53"N 33°28'18"E	1.5 – 12	16	Sandy	290
Referent site R6	10.11.2009	44°28'25"N 33°37'58"E	12 – 23	4	Sandy	233
Laspi Bay	27.06.1996	44°25'10"N 33°42'27"E	0.5 – 52	37	Silty-sandy	217
Cape Sarych	20.08.2007	44°23'14"N 33°44'17"E	3 – 5	4	Sandy, pebbles	82
Novyi Svet	14.08.2009	44°49'27"N 34°54'25"E	0.5 – 3	4	Sandy	93
Dvuyakornaya Bay	11.08.2008	44°59'28"N 35°22'04"E	2 – 9	12	Sandy	304
Moderately polluted site						
Karadag Nature Reserve	10.08.2010	44°54'53"N 35°13'51"E	5 – 8	8	Sandy, pebbles	300
Total				**190**		**793**

Biological data analysis

Biomaterial from different types of soft-bottom substrates was taken by a meiobenthology sampler (16 cm^2) or Petersen grab-sampler (0.05 m^2). Sample treatment involved preliminary processing in an ultrasonic bath for 20 min, followed by the standard technique using HCl and H_2SO_4 acids with $K_2Cr_2O_7$ addition. For permanent slides for light microscopy (LM), cleaned valves were mounted using Meltmount® or Naphrax® (Nevrova et al. 2015). Sampling treatment for scanning electron microscopy (SEM) involved air-drying Nuclepore Whatman membranes with a drop of the cleaned valves, mounting onto aluminium stubs and coating with gold. Treatment and LM observations were performed at the IBSS RAS (Sevastopol, Russia). LM micrographs were taken using a Nikon Eclipse 600 equipped with a PlanAPO 100× (Institute of Marine Sciences, University of Szczecin, Poland). SEM observations were performed using a Hitachi S4500 (Goethe University, Frankfurt am Main, Germany) and Hitachi SU3500. The slides, stubs and samples are stored in the collection of Dr. Sci. Elena Nevrova (Russia), in the collections of Prof. Dr. Sci. Andrzej Witkowski (Poland), and in the collection of Prof. Dr. Sci. Horst Lange-Bertalot (Germany).

Chemical analysis

Soft sediment samples from most of the studied areas were simultaneously taken for anthropogenic pollutant analysis, which was kindly performed by colleagues from the Institute of Colloid Chemistry and Water Chemistry NASU (Kiev, Ukraine) and Institute of Organic Chemistry NASU (Kiev, Ukraine) using standard techniques (Burgess et al. 2009, 2011, Petrov et al. 2010). Chemical analyses of inorganic and organic pollutants in bottom sediments included metering of 14 parameters: pesticides (sum of DDT and metabolites), total PAHs (sum of 16 isomers), total PCBs (4 congeners) and heavy metal compounds (Ag, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Zn). The content (%) of total organic carbon (TOC) and proportion of silt+clay fractions of the bottom deposites were also measured. The samples for analysis were air-dried, sieved and homogenised in accordance with ISO 11464:2006 standard methods. The metal content (besides Hg) in sediments was determined by graphite (MDL 0.005–0.05 μg × kg^{-1} dry weight) and flame (MDL 2.0–15 μg × kg^{-1}) atomic absorption spectrometry (AAS) following microwave digestion with a concentrated mixture of acids: HNO_3+HCl (3:1). Total Hg in sediments was determined by cold vapour AAS. Organic pollutants (PCBs and pesticides) in grounds were determined by GC/MS (MDL 1.0 μg × kg^{-1}) using a capillary column GC/ECD (MDL 0.05 μg × kg^{-1}), which was followed by Soxhlet extraction with a hexane/acetone (1:1) mixture. The total concentration of PCB homologues – as the sum of tetra-, penta-, hexa-, and heptachlorobiphenyls – was evaluated. The determination of PAHs was performed by HPLC/UV (HP 1050/DAD, MDL 10–20 μg × kg^{-1}) in reversed-phase mode. The sediment grain size ratio of sandy, silty and clay fractions (%) was measured by wet sieving and the gravimetric sedimentation method (Burgess et al. 2009, 2011, Petrov et al. 2010).

Statistical analysis

To conduct the comparative analysis of taxonomical diversity and benthic diatom taxocene structure in Crimean coastal regions, a database for Black Sea Bacillariophyta was created using Microsoft Office Access (Nevrova 2022). It combined available literature sources and own data, whose scope comprises five regions of the Northern part of the Black Sea (NPBS), namely the Caucasian, Crimean, Bulgarian and Romanian neashores as well as the North-Western shelf. The taxonomic database of Black Sea benthic diatoms was primarily based on the review of numerous publications and the own results of benthic surveys conducted in the period of 1984–2016 along the Crimean and Caucasian coasts (Nevrova 2022). Data from the southern part of the Black Sea (coastal region of Turkey) is unavailable. The check-list of Bacillariophyta was prepared according to (Round et al. 1990, Witkowski et al. 2000, 2010, 2014, Levkov 2010, Nevrova et al. 2013).

The most comprehensive list of benthic Bacillariophyta for NPBS (excluding the coast of Turkey) includes 1100 species and intraspecific taxa (IST) and was aggregated into seven hierarchical levels (from IST to Division) (Nevrova 2022). According to taxonomic aggregation, the numerical values of Δ^+ and Λ^+ indices that correspond to the average expected level of hierarchical structure for the NPBS diatom flora were calculated. A quantitative assessment of the hierarchical diversity of diatom taxocene in the studied areas off the Crimean coast was conducted using TaxD indices, where Δ^+ is the index of average taxonomic distinctness (AvTD) and Λ+ is the index of variability (VarTD) (Warwick & Clarke 1998, 2001). AvTD characterizes the vertical evenness of the taxonomic tree along ascending levels of hierarchy. VarTD reflects the horizontal asymmetry of the tree or the different representations of lower taxa in the higher taxa within individual ascending hierarchical branches (Warwick & Clarke, 1998, 2001, Nevrova et al. 2015). To visualise regional differences in the taxonomic structure of the benthic diatom assemblages in relation to the average expected level for NPBS in a subsequent analysis, the TaxDI values were superimposed on the plots, where the X-axis matchs to diatom species number and the Y-axis corresponds to AvTD or VarTD values. The values of the Δ^+ and Λ^+ indices for the diatom taxocenes were defined for each of the studied locations. The points of Δ^+ and Λ^+ indices characterising the hierarchical structures of diatom assemblages in sampling areas are located within the limits of the funnel on the plots, the centre of which corresponds to the average expected value of TaxDI for the NPBS Bacillariophyta flora. The funnel boundaries correspond to the 95 % probability contours of a "cloud" of the mean values of Δ^+ and Λ^+, which were calculated based on 1000-fold random combinations withdrawn from a master list of Black Sea diatom flora (Warwick & Clarke 1998, 2001, Clarke & Gorley 2006). Previous studies have shown that this algorithm allows researchers to reliably assess taxonomic diversity and reveal features of the hierarchical structure of diatom taxocenes under different environmental influence, including technogenic pollution impact (Nevrova 2013a, b, 2014, 2016, Nevrova et al. 2015, Nevrova & Petrov 2019a, b). TaxDI calculations and the comparative analysis of diatom taxocene structure and diversity features between Crimean sites were performed using PRIMERv6 (Clarke & Gorley 2006).

RESULTS

Pollutants in bottom sediments

Results of the chemical analysis of technogenic contaminants in bottom deposites from study sites along the Crimean coast revealed very low pollutant content in areas that were historically less affected by anthropogenic activity, including vil. Marjino, Golubaya Bay, Omega Bay, Cape Aya, Cape Fiolent, Laspi Bay, Cape Sarych, vil. Novyi Svet and Dvuyakornaya Bay (Table 2). The content of technogenic pollutants and trace metals hardly exceeded the average levels for the soft bottom of environmentally intact offshore areas of the Black Sea shelf (numbers in brackets, see Table 2) (Emelyanov et al. 2004, Mitropolsky et al. 2006, Burgess et al. 2009, 2011). The low level of pollution in sediments at all of the aforementioned locations allows us to attribute these waters as having the category of conventionally pristine. Such sites are characterized by an undisturbed structure of diatom taxocenes.

On the contrary, high levels of heavy metals (Cu, Pb, Zn, Ni, Cd, Mn, and Hg) and organic pollutants (PCB, ChOP and PAH) were registered in areas that were exposed to anthropogenic impact. Considerable pollution levels were detected in the coastal zones near river Belbek, Sevastopol Bay, Inkerman, Balaklava and Karantinnaya Bays, which were characterised as heavily anthropogenically disturbed (Burgess et al. 2009, 2011, Nevrova 2013a, b, 2014, Petrov et al. 2005, 2010). In comparison to the aforementioned conventionally pristine biotopes, this high level of accumulated in sediments pollutants (2 to 10 times greater for most elements and up to 50 times higher for Hg and PAHs) can influence the structure of benthic diatom assemblages and cause changes in their taxonomical diversity.

Separately, it is worth discussing the content of pollutants in the Karadag Nature Reserve water area, which was previously considered as reference for seawater purity. Nevertheless, at the end of the 20th century, this location was contaminated due to the constant discharge of wastewater from settlements, the Port of Feodosia and water runoff from the adjacent agricultural area (Emelyanov et al. 2004, Zherko 2004, Mitropolsky et al. 2006, Tikhonova et al. 2016). In the bottom sediments of the Karadag Nature Reserve, the contents of Zn, Ni, Co and Cr in the soft bottom are within the average level, while Pb and As are at the minimum concentration, Mn is below the average level (Tikhonova et al. 2016), and a high concentration of Cd was noted (Silkin et al. 2017). In the soft bottom of the Karadag water area, a constant presence of PCBs, DDT, α-γ-HCH and Heptachlor (average: 151, 9, 7 and 8 ng × g^{-1}, respectively) was detected (Zherko 2004), alongside petroleum hydrocarbons (on average 2.2 mg × g^{-1}) (Tikhonova et al. 2016). The bottom sediments and tissues of hydrobionts from the central part of the Karadag Nature Reserve were most polluted and the DDT content was several times higher than the maximum permissible concentration (Zherko 2004). Thus, despite its previous status as the standard purity site, the water area of Karadag Nature Reserve should now be defined as being between a conventionally healthy site and a moderately polluted one.

As previously confirmed by multivariate statistical analysis, the set of 10 key abiotic parameters, including 3 classes of organic pollutants (PCB, PAH, pesticides) and 7 metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn), achieved the best match for high similarities in the biotic and abiotic matrices (Petrov et al. 2005, 2010). Thus, such a set of abiotic factors can have the greatest combined effect on differences in biotic

Table 2. Mean concentration values of trace metals and 3 main classes of organic pollutants in soft-bottom sediments from studied sites of Crimea

Sites	Environmental variables, dry weight of bottom sediments									
	Metals, μg × g^{-1}							Organic pollutants, ng × g^{-1}		
	Cu (<20)	Pb (<15)	Zn (<80)	Ni (<40)	Cd (<0.5)	Mn (<10)	Hg (<0.04)	PCB (<50)	ChOP (<1)	PAH (<100)
Heavy polluted sites										
Near r. Belbek*	280	780	370	90	0,3	4890	43	78,9	14,2	0
Sevastopol Bay	97.9	96.1	167.2	34.0	0.33	364	1,12	310.5	15.93	2713
Inkerman*	1190	2800	400	320	16,2	5360	60	1014,3	19,8	1550
Karantinnaya Bay	26,2	18,2	26,9	25,1	-	178,3	0,3	155,0	64,2	1,3
Balaklava Bay*	161.4	338.7	232.2	30.8	0,31	333	0.81	121.8	18.80	7054
Conventionally healthy sites										
Referent site R3*	210	230	<20	30	0,1	330	30	22,2	1,9	0
Referent site R6*	10	29	6	9	0	140	0,1	0	0	0
Golubaya Bay*	16,8	121	<1,2	54,8	0,030	49,2	0,02	26,9	0,77	<3
Cape Fiolent*	22,8	21.8	124.3	5,5	0,05	244	0,02	16,3	1,10	<3
Laspi Bay**	7,4	3,7	12,0	1,9	-	6,3	0,042	5,4	2,8	0,1
Novyi Svet*	12,0	68,6	10,6	28,2	0,03	282	0,04	32,0	1,02	<3
Dvuyakornaya Bay*	13,4	45,2	98,4	<1,2	0,03	638	0,02	31,2	1,06	<3
Moderately polluted site										
Karadag Nature Reserve**	No data	Below average	Not exceed average	Not exceed average	High level	Below average	No data	151	9	No data

Notes: PCB – polychlorinated biphenils (sum of 4 congeners), ChOP – Chlorine-organic pesticides (sum of DDTs, DDD and DDE), PAH – polyaromatic hydrocarbons (sum of 16 isomers). Measurements on study areas marked * were kindly provided by colleagues from ICCWC NASU, Kiev. Measurements on the other sites were performed by colleagues from IBSS RAS, Sevastopol (Petrov *et al.,* 2005, 2010). References on heavy metals and organic pollutants on study areas marked **were used: Laspi bay (Medinets et al. 1994), Karadag (Zherko 2004; Tikhonova et al. 2016). Average background level of pollutants in surface sandy/muddy bottom sediments for the coastal zone of Crimea (in brackets) (Emelyanov et al. 2004, Mitropolsky et al. 2006, Polikarpov et al. 1992).

parameters of the diatom taxocenes across the surveyed coastal area. The combined influence of these factors can lead to changes in structure and species diversity features of the benthic diatom assemblages along with the extent of pollution in the most of Black Sea coastal habitats.

Taxonomic composition of Black Sea diatoms

The updated inventory of Black Sea benthic diatoms includes 1100 species and infraspecific taxa (IST) pooled into 953 species, 149 genera, 61 families, 31 orders and 3 classes of Bacillariophyta. The highest diatom species richness ever registered in the Black Sea was recorded off Crimean coast at 80.6 % of the total number of benthic diatom flora. When our results is combined with that of the existing literature, a total of 882 species and IST belonging to 132 genera, 56 families and 29 orders have been observed near the Crimean coast (Nevrova 2022). Only our own data from the investigated sites along the Crimean coast counted 793 species and IST pooled into 736 species, 130 genera, 53 families, 27 orders and 3 classes of Bacillariophyta (Bacillariophyceae: 10 orders; Coscinodiscophyceae: 9 orders; Fragilariophyceae: 8 orders).

Classes Coscinodiscophyceae and Fragilariophyceae in the taxocene were represented poorly (only 7.9 and 8.2 % from the total species number, respectively). From class Coscinodiscophyceae 9 orders, 15 families, 26 genera, 63 species and IST were registered, while 8 orders, 9 families, 26 genera, 65 species and IST were found from Fragilariophyceae. Representatives from class Bacillariophyceae, which belong to 10 orders, 29 families, 78 genera, and 665 species and IST, prevailed in the taxocene (83.9 %).

The most represented families of Bacillariophyta off the Crimean neashores were Naviculaceae Kützing 1844, Bacillariaceae Ehrenberg 1831, Catenulaceae Mereschkowsky 1902, Cocconeidaceae Kützing 1844 (Fig. 2A).

The richest genera of Bacillariophyta along the Crimean coast were *Navicula* Bory 1822, *Nitzschia* Hassall 1845, *Amphora* Ehrenberg 1844, *Cocconeis* Ehrenberg 1837, *Diploneis* Ehrenberg 1894, *Fallacia* A. Stickle et D.G. Mann 1990, *Mastogloia* Thwaites 1856, *Planothidium* Round et Bukhtiyarova 1996, *Lyrella* Karayeva 1978, *Caloneis* Cleve 1894, *Achnanthes* Bory 1822, *Halamphora* (Cleve) Z. Levkov 2009, *Cymbella* C. Agardh 1830, *Thalassiosira* Cleve 1873, *Licmophora* C. Agardh 1827, *Hantzschia* Grunow 1877, *Pinnularia* Ehrenberg 1843, *Fragilaria* Lyngbye 1819 (Fig. 2B). These genera formed the most saturated poly-species branches in benthic diatom hierarchical tree off the Crimean coast.

The most common benthic diatoms off the Crimean coast were *Amphora acuta* W. Gregory, *A. crassa* W. Gregory, *A. graeffeana* Hendey, *A. marina* (W. Smith) Chase, *A. proteus* W. Gregory, *Caloneis liber* (W. Smith) Cleve, *Campylodiscus thuretii* Brébisson, *Cocconeis scutellum* Ehrenberg, *Dimeregramma minor* (W. Gregory) Ralfs, *Diploneis bombus* (Ehrenberg) Cleve-Euler, *D. smithii* (Brébisson) Cleve, *Fallacia forcipata* (Greville) A. Stickle et D.G. Mann, *F. subforcipata* (Hustedt) D.G. Mann, *Grammatophora marina* (Lyngbye) Kützing, *Halamphora coffeaeformis* (C. Agardh) Levkov, *Licmophora abbreviata* C. Agardh *Navicula parapontica* Witkowski, Kulikovskiy, Nevrova et Lange-Bertalot, *Nitzschia acuminata* (W. Smith) Grunow, *N. compressa* (J.W. Bailey) Boyer, *N. sigma* (Kützing) W. Smith, *Paralia sulcata* (Ehrenberg) Cleve, *Pleurosigma elongatum* W. Smith, *Tabularia tabulata* (C. Agardh) P.J.M. Snoeijs, *Thalassionema nitzschioides* (Grunow) Mereschkowsky (Fig. 3: 1–24). Eight novel genera for the Black Sea diatom flora were found: *Amicula* (Witkowski) Witkowski 2000, *Astartiella* Witkowski, Lange-Bert. et Metzeltin 1998, *Chamaepinnularia* Lange-Bert. et Krammer 1996, *Cocconeiopsis* Witkowski, Lange-Bert. et Metzeltin 2000, *Eolimna* Lange-Bert. et W. Schiller 1997, *Lunella* P.J.M. Snoeijs 1996, *Rhoicosigma* Grunow 1867, *Trachysphenia* P. Petit 1877 (Nevrova 2022).

Similarity of benthic diatom taxocene composition along the Crimean coast

Our results were analysed to estimate the diversity features in diatom taxocenes from 16 locations along the Crimean coast. Based on the Bray-Curtis similarity coefficient, the highest resemblance species was revealed between the taxocenes that represent heavily polluted biotopes: Karantinnaya Bay – Sevastopol Bay (64.6) and Balaklava Bay – Sevastopol Bay (57.8). Despite this – and based on the results of chemical analysis (see Table 2) – Laspi Bay belongs to the conventionally clean category. A high similarity was observed between the diatom taxocenes from Laspi Bay – Karantinnaya Bay (64.6) and Laspi Bay – Sevastopol Bay (64.0).

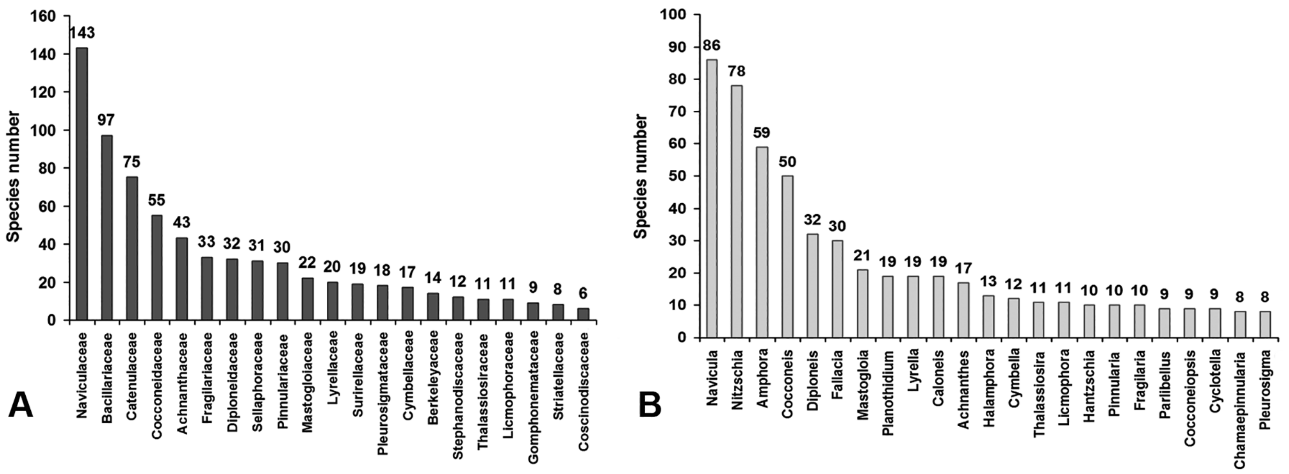

Figure 2 The most representative families (A) and genera (B) of benthic diatoms along the Crimean coast

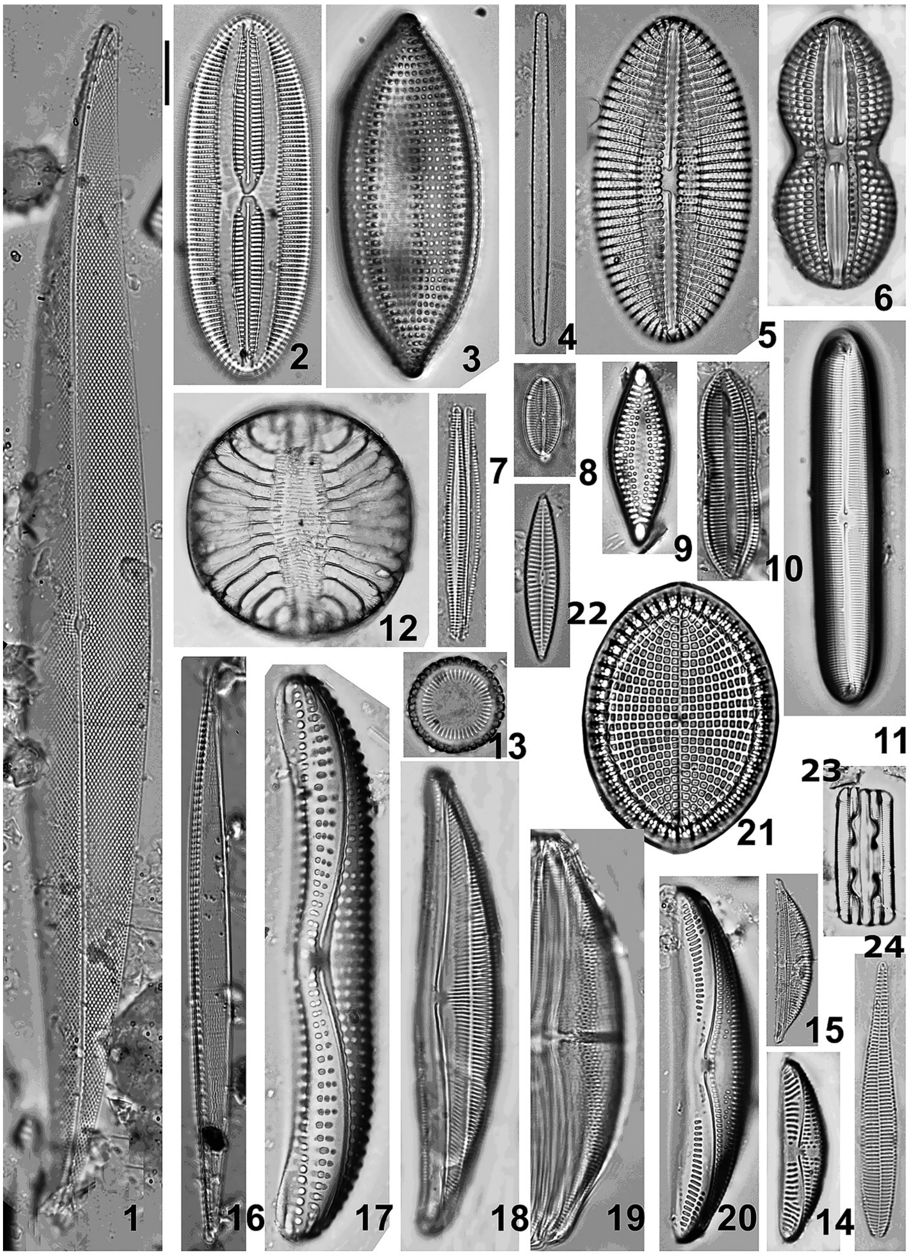

Figure 3 The most common benthic diatoms off the Crimean coast (LM): 1 – *Pleurosigma elongatum* W. Smith, 2 – *Fallacia forcipata* (Greville) A. Stickle et D.G. Mann, 3 – *Nitzschia compressa* (J.W. Bailey) Boyer, 4 – *Thalassionema nitzschioides* (Grunow) Mereschkowsky, 5 – *Diploneis smithii* (Brébisson) Cleve, 6 – *Diploneis bombus* (Ehrenberg) Cleve-Euler, 7 – *Tabularia tabulata* (C. Agardh) P.J.M. Snoeijs, 8 – *Fallacia subforcipata* (Hustedt) D.G. Mann, 9 – *Dimeregramma minor* (W. Gregory) Ralfs, 10 – *Nitzschia acuminata* (W. Smith) Grunow, 11 – *Caloneis liber* (W. Smith) Cleve, 12 – *Campylodiscus thuretii* Brébisson, 13 – *Paralia sulcata* (Ehrenberg) Cleve, 14 – *Amphora marina* (W. Smith) Chase, 15 – *Halamphora coffeaeformis* (C. Agardh) Levkov, 16 – *Nitzschia sigma* (Kützing) W. Smith, 17 – *Amphora crassa* W. Gregory, 18 – *Amphora graeffeana* Hendey, 19 – *Amphora acuta* W. Gregory, 20 – *Amphora proteus* W. Gregory, 21 – *Cocconeis scutellum* Ehrenberg, 22 – *Navicula parapontica* Witkowski, Kulikovskiy, Nevrova et Lange-Bertalot, 23 – *Grammatophora marina* (Lyngbye) Kützing, 24 – *Licmophora abbreviata* C. Agardh. Scale bar 10 mkm

On the other hand, a high level of similarity between the taxocenes of conventionally healthy sites was observed: Dvuyakornaya Bay – Cape Fiolent Fiolent (55.9), Dvuyakornaya Bay – Karadag Nature Reserve (54.3) and Dvuyakornaya Bay – reference site R6 (53.6). Thus, it can be concluded that the traditional assessment of similarity of species composition between diatom assemblages from biotopes with different degrees of anthropogenic pollution does not give unambiguous conclusions. In connection with aforesaid, we tried to assess the dissemblance in taxonomic structure of benthic diatom taxocenes from studied biotopes by using TaxDI.

Assessment of taxonomic diversity features of benthic diatom taxocenes

The index of taxonomic distinctness AvTD (Δ^+) and its variability VarTD (Λ^+) (Warwick & Clarke 2001) were calculated to compare the possible differences in hierarchical diversity features of diatom taxocenes from 16 studied water areas whith significant differences in their level of anthropogenic pollution accumulated in the bottom sediments. The most high values of Δ^+ were revealed for diatom taxocenes from the most polluted sites Inkerman Bay, Balaklava Bay, Sevastopol Bay, Karantinnaya Bay, Belbek (Δ^+ = 84.60; 84.07; 83.62; 83.19, 81.59, respectively), moderately contaminated areas Karadag Nature Reserve (Δ^+ = 82.84) and conventionally healthy location Laspi Bay (Δ^+ = 82.72). The Λ^+ index values for the aforementioned sites are superimposed on the plot much higher than, or close to, the expected TaxDI level (Δ^+ = 82.09, Λ^+ = 316.827) calculated for NPBS Bacillariophyta flora (Fig. 4 A, B). Simultaneously, the lower values of indices Δ^+ corresponding to conventionally intact sites are below the expected level for the Black Sea. There are: Marjino, Novyi Svet, Dvuyakornaya Bay, Omega Bay, referent site R3, referent site R6, Cape Sarych, Golubaya Bay and Cape Fiolent (Δ^+ = 81.28; 79.59; 79.25; 79.21; 79.03; 79.00; 78.52; 78.19; 76.71, respectively) (see Fig. 4A).

Regarding variability, Λ^+ values were either significantly higher than the average expected line for the Black Sea or exceeded the 95 % probability contour for the sites Cape Sarych, Cape Fiolent, Omega Bay, Dvuyakornaya Bay and Sevastopol Bay (Λ^+ = 369.77; 361.07; 348.29; 347.85 and 268.95, respectively) (Fig. 4B). As for VarTD values for the remaining sites, they were within the probable funnel contour and either slightly exceeded or were under the average expected level for the Black Sea.

The effectiveness of both AvTD and VarTD indices for the delineation of distinctions in taxocene structure under various environmental disturbances is the subject of relatively few studies. However, it was assumed that these indices can adequately discriminate ecological alterations along environmental gradients (Salas et al. 2006, Bevilacqua et al. 2009, Prato et al. 2009, Schratzberger et al. 2009, Stenger-Kovács et al. 2014, 2016, Vilmi et al. 2016, Nevrova & Petrov 2019b). Compared to the expected level of TaxDI, a lower degree of vertical hierarchical evenness of the taxocene structure is typical for communities exposed to enduring anthropogenic and technogenic pollution (Ellingsen et al. 2005, Heino et al. 2007, Petrov et al. 2010, Gottschalk & Kahlert 2012, Stenger-Kovács et al. 2014). Conversely, high values of Δ^+ were observed in the substantial vertical evenness of hierarchical structure (Λ^+). In other words, in taxonomic tree, the representation of taxa at different hierarchical levels has a proportional share. High taxonomic variability (Λ^+) used to be observed in pristine and undisturbed sites (Leira et al. 2009, Rimet & Bouchez 2012, Keck et al. 2016, Nevrova & Petrov 2019b, Nevrova 2022).

A taxonomic diversity assessment of benthic diatoms off the Crimean coast based on VarTD showed that the highest Δ^+ index values were revealed for diatom taxocenes from the most polluted sites: Inkerman Bay, Balaklava Bay, Sevastopol Bay, Karantinnaya Bay and Belbek (see Fig. 4B). This occurred despite the distance, differences in hydrological and hydrochemical conditions, and habitat heterogeneity of sea bottoms at the studied sites. Since values of Λ^+ for the aforementioned areas are placed on the plot much higher than the expected TaxDI level for the Black Sea diatom flora, such fact indicates that diatom assemblages at these sites are described by low species richness (see Table 2) and a large share of mono- and oligospecies branches at the family and order levels. This implies that highly pollution-sensitive species cannot withstand the high level of technogenic contaminants accumulated in the bottom sediments, which results in them being eliminated from the taxocene structure.

Taxocenes in the waters of conventionally healthy sites (e.g., Laspi Bay) and moderately polluted sites (e.g., Karadag Nature Reserve) were also characterised by relatively high species richness and an equal ratio of oligo- and poly-species branches closed at the genus level, with the presence of monospecies branches that converged at the family or order levels of hierarchy. The AvTD index values for these sites are slightly greater than the expected average level for the Black Sea flora of Bacillariophyta (see Fig. 4A).

Conversely, the AvTD index values for pristine water areas (i.e., the coast near Marjino, reference site R3, Omega Bay, Golubaya Bay, Cape Fiolent, reference site R6, Cape Sarych, Novyi Svet, Dvuyakornaya Bay) are lower below the expected level for the Black Sea. Indices of VarTD variability for these sites are higher than the expected average level accessed for the Black Sea's diatom flora (see Fig. 4A). Taxonomic trees of diatom taxocene for these healthy sites can be described as having a slight degree of vertical evenness and high variability in the taxonomic distance among the branches, which are primarily formed by polyspecific taxonomic branches closing at the genus level.

The Δ^+ index values for the diatom taxocenes from heavily and moderately polluted sites were significantly higher than the corresponding values for undisturbed biotopes and exceeded the expected average level for the Black Sea diatom flora. Simultaneously, VarTD index values (in comparison with the expected level) were lower for heavily polluted sites and were nearly equal to those of moderately polluted sites. As previously mentioned, the upward trend of AvTD index values with increasing technogenic impact can be related to the gradual disappearance of low-tolerance taxonomically close species from the same genus when oligo- and monospecific branches begin to prevail in the diatom tree architectonics. Previous and current results demonstrate that the TaxDI index should be used as a convenient tool for the quantitative assessment of change in the taxonomical diversity and hierarchy of diatom trees in biotopes affected long-term disturbance by anthropogenic stressors (Nevrova & Petrov 2019b, Nevrova 2022).

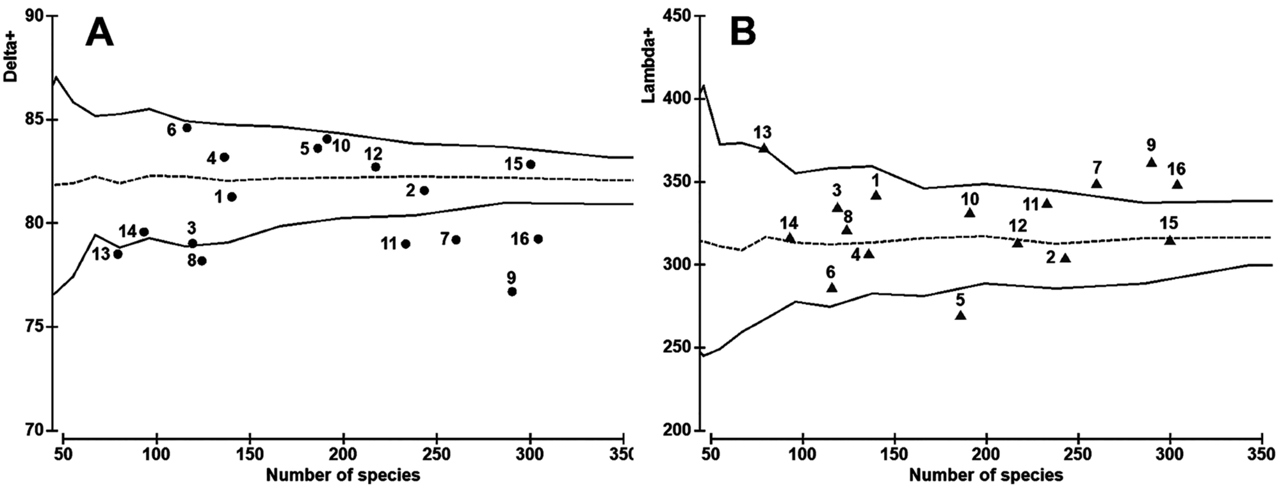

Figure 4 Assessment of taxonomical diversity of benthic diatoms off the Crimean coast, based on AvTD (A) and VarTD (B) (Warwick and Clarke, 1998). Investigated sites along the Crimean coast: 1 – coast near vil. Marjino; 2 – coast near r. Belbek; 3 – referent site R3 near Sevastopol; 4, 5, 6 – Karantinnaya Bay, Sevastopol Bay, Inkerman Bay; 7, 8, 9, 10, 11 – Omega Bay, Golubaya Bay, Cape Fiolent, Balaklava Bay, referent site R6 near Balaklava; 12 – Laspi Bay; 13 – Cape Sarych; 14 – coast near vil. Novyi Svet; 15 – Karadag Nature Reserve; 16 – Dvuyakornaya Bay

As have already been recognised for the North Sea, the decrease in values of Δ^+ and increase in the unevenness of Λ^+ can both indicate environmental degradation, but change in biodiversity are only quantifiable using TaxDI indices (Warwick et al. 2002).

The studied nearshore areas of Crimea affected by varying degrees of anthropogenic disturbance demonstrated sharp distinctions in species richness and diatom taxocene composition. The low species resemblance of diatom species composition between affected and pristine biotopes might be due to the strong effect of pollutants accumulated in sediment. This mainly affects low-resistance diatom species, which have mostly disappeared from heavily polluted sites. The high technogenic load on biotopes led to major changes in the hierarchical patterns of benthic diatom taxocenes. The high species richness of benthic diatoms observed in healthy biotopes can be due to these sites not being subjected to any pollution.

More plausible reason for differences in diatom assemblage structure and species richness can be the heterogeneity of microbiotopes, i.e., the variety of bottom (silty, clay, sandy, volcanic rocky and lime-stone substrates). Many types of soft-bottom sediments and microniches might be favourable for a patchy dispersal over the substrate surface and the successful growth of a large number of diatom species, including those highly sensitive to pollution as well as relics of Ponto-Caspian flora, rare and alien species (Nevrova & Petrov 2019a).

Taxonomic tree of Bacillariophyta for Crimean flora is shaped by branches having three forms of species saturation: mono-, oligo- and poly-species. These elements can be distinguished structurally based on the hierarchical tree of diatom taxocenes. Monospecies branches have only one representative recorded and form a monospecies branch closed into a common node at the level of genus, family or order, while oligospecies branches have two species and poly-species branches have three or more species (Nevrova et al. 2015).

The taxonomic trees of diatom taxocenes from intact water areas are shaped by branches that include various numbers of species. Such branches belong to different hierarchical levels (from genus to order) but are mainly formed by polyspecific taxonomic clusters closing at the common genus level. Their positions on the plot, which correspond to diatom taxocenes from conventionally healthy biotopes, are placed much below the expected level. This arrangement describes the taxocene structure as having medium vertical evenness and high variability and indicates significant differences between the hierarchical structure of the taxocene when compared to the expected level (Nevrova & Petrov 2019b). Notably, in some previous studies (Leonard et al. 2006, Prato et al. 2009, Bevilacqua et al. 2011), the Δ^+ and Λ^+ values most distant from the expected level were also observed in habitats with heavy anthropogenic violation. Simultaneously, in healthy sites subjected to natural influences only, TaxD values were close to the average expected level within a 95 % probability contour.

Additionally, disparities in the taxocene structure of biotopes with different anthropogenic loads could be due to the impact of environmental factors and the internal phylogenetic relationships of species. In intact habitats with favourable conditions, the appearance of many species belonging to the same genus is often observed. In this case, the tree of the taxocene would have a predominance of poly-species branches that are closed at the genus level (Warwick & Clarke 2001, Winter et al. 2013, Vilmi et al. 2016).

Diatom assemblages in heavily and moderately polluted habitats are described by relatively low species richness. The hierarchical structures of their trees are characterised by the dominance of oligospecies branches closed at the genus level and a low quantity of monospecies branches closed at the family and order levels. Such structural features of diatom's taxocene may arise due to a significant degree of pollution and homogeneity of soft substrates; for example, in Sevastopol and Balaklava bays, where nearly the entire bottom area is covered with silty sediments (Petrov et al. 2010, Nevrova 2013a, b, 2014).

Based on the application of TaxDI indices, it was revealed that the strongest changes in their values emerged when a mono- or oligospecific branch was eliminated and closed at a upper taxonomic level (e.g., family or order) (Warwick & Clarke 1998). The degree of diatom resistance to the influence of environmental factors can vary significantly, even between species belonging to the same genus. For example, when such a species or genus disappear (e.g., in the case of a monospecies branch), an entire branch is eliminated from the tree architectonics, which leads to a reduction in diversity of diatom taxocene at the biotope. Notably, such disturbances are most often caused by the impact of strong technogenic pollution or natural stressors (Somerfield et al. 1997, 2009, Warwick & Clarke 2001, Bevilacqua et al. 2011). In heavily polluted bays with homogeneous silty-sandy bottom sediments, the structure of diatom taxocenes is similar and can be described by rather low indicators of species richness, high proportions of mono- and oligospecific branches in the tree structure and a generally moderate degree of vertical hierarchical evenness (Petrov et al. 2010, Nevrova 2013a, b, 2014, 2016, Nevrova & Petrov 2019b).

Since the AvTD and VarTD indices are calculated based on presence/absence data, they do not depend on quantitative data on the diatom abundance and sampling effort, which creates significant advantages when only qualitative analysis data are available (Warwick & Clarke 1998, 2001, Leonard et al. 2006, Bevilacqua et al. 2009, Stenger-Kovács et al. 2014, 2016, Nevrova 2022).

The results obtained from a comparative analysis of the hierarchical structure of diatom taxocenes from various Crimean nearshore habitats allow us to conclude that the formation of the taxonomic tree depends on changes in species richness due to the disappearance (or appearance) of new branches, which are closed at different taxonomic levels of the tree. Species that form new monospecific branches that are closed at a higher level appear – or are found by the researcher – much more rarely in the taxocene structure than closely related species. The reverse process (i.e., the reduction of oligo- or poly-species branches to the status of monospecies) leads to a simplification and impoverishment of taxonomic structure. This can also occur under an adverse long-term impact on the taxocene (e.g., technogenic pollution in Karantinnaya Bay and Inkerman Bay) or due to poor knowledge of the biodiversity of an area. The latter is illustrated by the peculiarities of the diatom taxocenes in less studied (compared to other) biotopes (Cape Sarych, Golubaya Bay, Marjino, Novyi Svet, referent site R3). The emergence (or elimination) of a significant number of new closely related species in the biotope causes much smaller transformations in the hierarchy of taxocene (and TaxDI index values) than the appearance (or reduction) of even a few new species with distant phylogenetic relationships (Somerfield et al. 1997, Warwick & Clarke 2001). Thus, Δ^+ values generally tend to decrease in relation to the average expected line, mainly when closely related species (poly-species) dominate the taxocene.

In summary, when the species richness of a taxocene based on poly-species branches shaped by phylogenetically allied species is higher, while the average value of Δ^+ is lower. Conversely, if the species richness consisted of mono- or oligo species branches with a distant phylogenetic relationship is relatively low, the value of Δ^+ will be higher.

CONCLUSION

The evaluation of TaxDI provides more comprehensive insights into the taxocene structure, which is important to gain a deeper understanding of the biodiversity concept. Differences in the architectonics of the Black Sea's benthic diatom assemblages from biotopes were affected by varying degrees of anthropogenic pollution. This point was revealed and its statistical reliability was assessed. The highest similarity in species richness and taxonomic tree structure occurred between conventionally intact biotopes and between the most heavily polluted water areas. This similarity in diversity features was observed despite the geographical remoteness of these locations, heterogeneity of bottom substrates and differences in hydrological conditions. The low affinity of diatom species composition uncovered by the comparison of affected and intact sites maight be a consequence of heavy pollution impacting taxocene structure when sensitive diatoms disappear.

AvTD values for diatom taxocenes from pristine sites were lower, and their variability was greater than the average level expected for the Black Sea's benthic Bacillariophyta. The trees of diatom taxocenes were mainly formed by poly-species branches with a varying number of species closed at the genus level. The diatom taxocenes structure in heavily polluted sites can be described by higher AvTD values in comparison to the Black Sea expected level. In such hierarchical trees, oligospecies branches were dominant alongside the presence of a lower quantity of monospecies branches that closed at the higher taxonomical levels of family or order.

AvTD and VarTD indices based on a hierarchical structure should be recommended as reliable quantitative tools for diversity assessment under the condition of long-term environmental disturbances to coastal marine habitats. Moreover, when historical or modern data sets are only exist in a qualitative format, applying of TaxD indices may be appropriate. The obtained results for taxonomical distinctness provide a statistically reliable assessment of diatom taxocene structure and could be applied for the conservation of marine flora diversity in the context of modern anthropogenic impacts in the Black Sea.

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