Polish Botanical Journal 55(1): 183–197, 2010

STRUCTURE AND TAXONOMIC DIVERSITY OF BENTHIC DIATOM ASSEMBLAGE IN A POLLUTED MARINE ENVIRONMENT (BALAKLAVA BAY, BLACK SEA)*

ALEXEI PETROV, ELENA NEVROVA, ANNA TERLETSKAYA,
MIKHAIL MILYUKIN & VICTOR DEMCHENKO

Abstract. The study examined changes in the structure and diversity of benthic diatom assemblages from 16 sampling stations covering *ca* 0.3 km^2 of the bottom of industrially polluted Balaklava Bay (Crimea, Black Sea). We analyzed data on diatom abundance and species richness along with measurements of 15 variables: depth, sediment size structure, TOC, metals (Zn, Ni, Cu, Pb, Hg, Cd, Cr, Mn) and organochlorine toxicants (PAHs, pesticides, PCBs). Based on cluster and MDS analyses, two groups of stations were distinguished at 30% similarity level. The first group comprised stations in the inner, most polluted part, and the second corresponded to the outer part of the bay. Diatom assemblages of a certain structure are formed within each group of stations. The species most responsible for similarity/dissimilarity within/between each of the distinguished complexes were determined and are listed. In total, 183 species representing 58 genera were identified. Species richness was highest in the genera *Nitzschia* Hassal (28 species), *Amphora* Ehrenberg (19), *Navicula* Bory (16), *Cocconeis* Ehrenberg (15), *Diploneis* Ehrenberg ex Cleve (11) and *Lyrella* Karayeva (6). Our analyses showed that the combination of depth, percentage of sandy fraction, and the content of TOC, pesticides, PCBs and mercury exerted the most significant impact on diatom assemblage structure and diversity. Changes in benthic diatom taxonomic diversity were evaluated for each station on the basis of average taxonomic distinctness (Δ^+) and variation of the TaxD Index (Λ^+). The taxonomic structure of diatom assemblages in Balaklava Bay is less diverse than the average level expected from the Crimean regional inventory (539 species). The Δ^+ value (80.89) was lowest for the most polluted part of the bay, where species-rich branches of taxonomic tree structure prevailed. The diatom assemblage in the part less impacted had a higher Δ^+ value (82.86), closer to the expected average mode (83.67) for the entire Crimean coast. Overall the results highlight problems with the use of taxonomic diversity measures to detect the impact of adverse environmental conditions based on benthic diatom assemblages.

Key words: benthic diatoms, Black Sea, Crimean coast, diversity, taxonomic distinctness, pollution

Alexei Petrov & Elena Nevrova, Institute of Biology of the Southern Seas, National Academy of Sciences of Ukraine, 2 Nakhimov av., Sevastopol 99011 Ukraine; e-mail: alexpet-14@mail.ru; el_nevrova@mal.ru
Anna Terletskaya, Mikhail Milyukin & Victor Demchenko, Institute of Colloid Chemistry and Water Chemistry National Academy of Sciences of Ukraine, 42 Vernadsky blvd., Kiev 03142, Ukraine; e-mail: annaterlet@ukr.net

INTRODUCTION

Benthic diatoms (Bacillariophyta), the most abundant and ubiquitous group of the microphytobenthos, have the highest population densities and species richness in coastal ecosystems. They are closely associated with certain biotopes directly subjected to environmental influences and sensitive to changes in the levels of various pollutants. These features recommend this group as a reliable indicator of eutrophication and anthropogenic pollution in comprehensive monitoring of aquatic ecosystems (Sladecek 1986; Sathya & Balakrishnan 1988; Watanabe *et al.* 1988, 1990; Descy & Coste 1991; Warwick 1993; Barinova *et al.* 2000, 2006; Gimenez-Casalduero 2001; De la Rey *et al.* 2004; Tavassi *et al.* 2004; Birkett & Gardiner 2005; Nikitina & Shkundina 2009).

Most studies assessing biodiversity in the context of ecological monitoring and bioindication in marine and freshwater ecosystems deal with abun-

---

[*] Dedicated to Dr. Kurt Krammer on the occasion of his 85th birthday

dance and species richness patterns. A variety of univariate numerical diversity indices combining species richness, abundance and evenness have been used to assess the effect of environmental degradation on biota, especially benthic assemblages (see Magurran 2004; Karydis 2009 for review). Traditional diversity measures based on species richness and evenness (e.g., Shannon's H', Pielou J) often have disadvantages in assessment of biodiversity change on large spatial scales and long time scales. In the last decade or so a newer biodiversity measure emphasizing the average taxonomic relatedness between species in a community have been developed and applied for biomonitoring (Warwick & Clarke 1998, 2001).

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 classical Linnean classification system for animals). Here we need to mention that the established taxonomic relatedness among diatom species is based primarily on frustule morphology and much less on genetic affinity. The premise is that 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; Leonard *et al.* 2006; Leira *et al.* 2009).

These biodiversity assessment indices have proved useful in several studies of different groups of organisms in different regions of the world (Warwick *et al.* 2002; Ellingsen *et al.* 2005; Leonard *et al.* 2006; Ceschia *et al.* 2007), but apart from a few studies focused on macroinvertebrate fauna (Heino *et al.* 2005, 2007; Campbell *et al.* 2007) the value of taxonomic distinctness measures as a means of expressing anthropogenic effects on the microphytobenthos is almost untested.

Methods and indices for water quality assessment based on aquatic assemblages are rather widely applied in freshwater ecology (Stoermer 1978; Tilman 1997; Kelly 1998; Dixit *et al.* 1999; Harding *et al.* 2005; Heino *et al.* 2005; Sondergaard *et al.* 2007), but there are very few studies relating to the use of marine benthic diatoms for bioindication of anthropogenically impaired biotopes (Dickman 1998; Izsak *et al.* 2002; Petrov *et al.* 2005, 2008). Most studies concerning Black Sea benthic diatoms deal mainly with taxonomy and floristic analysis, species composition and seasonal dynamics of diatoms (Guslyakov *et al.* 1992; Guslyakov & Nevrova 1998; Ryabushko 2008 for review). Fewer of them address the structure of the diatom assemblages and assess diversity features in relation to the influence of a broad set of environmental factors (Nevrova *et al.* 2003, 2008; Ryabushko *et al.* 2003; Petrov *et al.* 2004, 2005, 2007, 2008; Goldin 2009). Such comparative studies of diatom assemblage structure (especially those considering the different levels of taxonomic resolution) can provide additional information about the influence of environmental changes and technogenic impacts on ecologically relevant measures of diatom diversity in Black Sea coastal regions.

Our study assessed the variation of quantitative characteristics and taxonomic structure (aggregated to higher hierarchical levels) of benthic diatom assemblages resulting from environmental heterogeneity in Balaklava Bay, SW Crimea. We also analyzed differences in taxonomic distinctness indices between diatom assemblages along a gradient of permanent technogenic impacts (by several heavy metals and organochlorine compounds) in the bay bottom area.

MATERIALS AND METHODS

Data on benthic diatoms, including a list of species and their abundances in surface sediment samples together with associated environmental information, and on bottom sediment chemistry, were obtained from the comprehensive ecological surveys carried out in Balaklava Bay (Black Sea, SW Crimea), in October 2006. Sixteen stations representing the bay bottom at 4–27 m depth were investigated (Fig. 1). Duplicate samples taken by tube (surface area 16 cm^2) from the upper (1–4 cm) layer of soft sediments were collected at every station by a diver.

Chemical analyses of inorganic and organic contaminants in sediment samples included measurements of 14 substances: metals (Cu, Zn, Ni, Pb, Cr, Cd, Ag, Mn, Hg), total PCBs, chlorinated pesticides and PAHs. The percentage content of silt+clay fractions and total organic carbon (TOC) of the sediments were also determined.

The sampled sediments were prepared for chemical analysis according to the ISO 11464:2006 standard method. The samples were air-dried, sieved and homogenized. Content of metals (besides Hg) in sediments was determined by graphite (MDL 0.005–0.05 mg/kg dry wt.) and flame (MDL 2.0–15.0 mg/kg dry wt.) atomic absorption spectrometry (AAS) following microwave digestion in Teflon pressure vessels with concentrated HNO_3+HCl (3:1). Total Hg in sediments was determined by cold vapor AAS.

Organic contaminants (pesticides, PCBs) in sediments were determined by GC/MS (MDL 1.0 µg/kg dry wt.) and capillary silica column GC/ECD (MDL 0.05 mkg/kg) following Soxhlet extraction for 16 h with hexane/acetone (1:1). Total concentrations of PCB homologues as tetra-, penta-, hexa-, hepta-chlorobiphenyls were determined. The extracts were cleaned by column chromatography and washing with H_2SO_4 to remove interfering compounds. PAHs were determined by HPLC/UV (HP 1050/DAD, MDL 10–20 mkg/kg dry wt.) in reversed-phase mode. Standard solutions of individual substances or mixtures were used for calibration of equipment. Sediment grain-size composition as sandy, silty and clay fraction percentages was determined by wet sieving and gravimetric sedimentation.

The benthic diatoms of all 16 sediment stations were analyzed. Duplicate diatom samples were examined for abundance, species richness and taxonomic structure.

Only entire intact cells with visible living chloroplasts were counted. All broken cells were rejected from microscope processing and were not used for quantitative and taxonomical analysis. Diatom species were identified to species or variety level (later referred to as species) using the taxonomic classification from Round *et al.* 1990, with additional information (Fourtanier & Kociolek 1999, 2003, 2007; Witkowski *et al.* 2000). The diatom species were counted and expressed per 1 cm^2 of seabed. Species found only on permanent slides (i.e., rare or solitary species) were not included in the quantitative calculation of total density of diatoms. These species, however, were included in the matrix (species density vs stations number) for further quantitative uni- and multivariate statistical analysis. In the matrix the density of such species was indicated as a conventional minimum value of 10 cells · cm^{-2} (Petrov *et al.* 2005).

Sample processing involved preliminary treatment in an ultrasonic bath for 20 min, followed by the standard technique using HCl and H_2SO_4 with the addition of $K_2Cr_2O_7$. Cleaned diatom valves were mounted using Eljashev mountant for light microscopy (Proshkina-Lavrenko 1974). Light microscopy (LM) employed a Carl Zeiss Axiostar microscope equipped with a PlanAPO 100× objective.

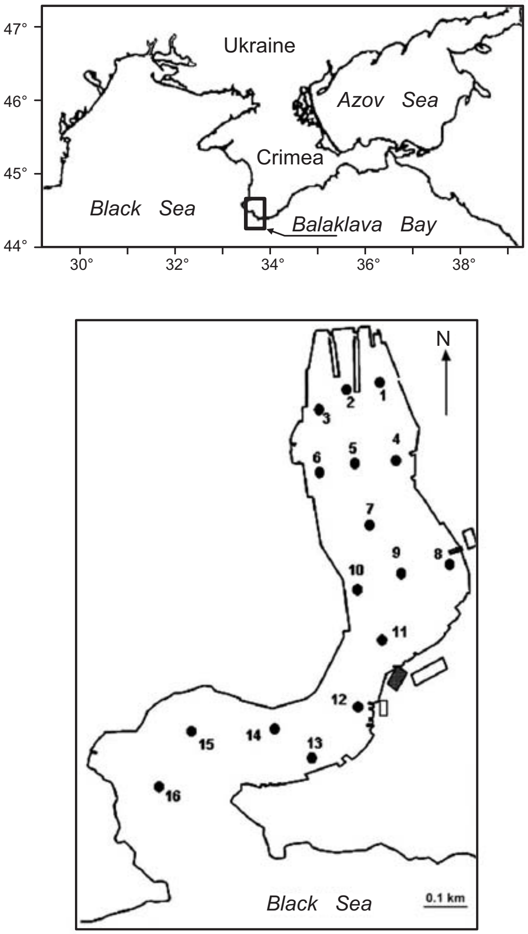

Fig. 1. Schematic map of sampling stations in Balaklava Bay in October 2006 (Black Sea, SW Crimea).

For this research we used the updated Crimean regional inventory (comprising 539 marine benthic diatoms species) based on literature and our own materials (Nevrova & Petrov 2008).

Multivariate statistical analysis was applied to evaluate the spatial distribution of pollutants across the bay and to assess its influence upon the structure and taxonomic diversity of diatom assemblages. Data processing was done with the PRIMER v5 package (Carr 1997; Clarke & Gorley 2001). The environmental gradients along the bay bottom were assessed from the results of PCA analysis. Cluster analysis and nMDS ordination of environmental factors were employed to distinguish the grouping of stations in relation to different levels of anthropopression (Clarke 1993; Carr 1997). The significance of differences between *a priori* separated groups of stations within the bay bottom area was tested with permutation/randomization ANOSIM (analysis of similarity). Similarity between stations was assessed by complete linkage of the Bray-Curtis similarity measure (for double square root-transformed biotic) and Euclidean distance (for non-transformed normalized abiotic) matrices (CLUSTER and MDS routines). The SIMPER routine was applied to reveal the most significant species (Clarke & Warwick 2001). Among such species the indicative ones are mainly responsible for the average similarity within the distinguished diatom assemblages, and discriminating species are mainly responsible for the differences between them corresponding to each of the considered locations.

Spearman rank correlation coefficients (ρ) were used to detect the combination of environmental variables giving the best match for high similarities (low rank) in the biotic (abundance data) and abiotic matrices, that is, to recognize the set of abiotic factors best explaining the spatial differences in benthic diatom assemblages across the surveyed bottom area (BIOENV routine) (Clarke & Warwick 2001).

Taxonomic distinctness indices (TaxDI) were used to estimate the diversity features of the diatom assemblages of the bay (Clarke & Warwick 1998; Warwick & Clarke 1998).

An initial binary presence/absence biotic matrix was constructed, and later the matrix data was aggregated into 7 taxonomic levels (from intraspecific to division). The taxonomic analysis was based on calculation of two taxonomic diversity indices. Average taxonomic distinctness AvTD (Δ^+) reflects the mean path length through the assemblage taxonomic tree (to the phylogenetic common node) connecting every pair of species randomly selected from the list. The AvTD index characterizes the vertical taxonomic evenness along the phylogenetic tree of the given community. Variation in taxonomic distinctness VarTD (Λ^+) describes the variability (variance) of these pairwise path lengths (ωij) between each pair of species i and j, around their mean value Δ^+. The VarTD index characterizes the horizontal unevenness of the taxonomic tree from the conventional averaged level. In other words, VarTD characterizes various representativeness of lower taxa (or successive ratios of lower taxa number to higher ones) for each of the branches composing the whole hierarchical tree (Clarke & Warwick 1999). The combination of AvTD and VarTD in a 2D scatter plot provides a statistically robust summary of the taxonomic relatedness patterns within the diatom assemblages. Calculated values of Δ^+ and Λ^+ are usually located within the simulated 95% probability funnel converging towards increasing species number. The probability funnel (based on 1000-fold random pairwise selections) is created for a range of randomly drawn subsets (m = 20, 30, 40, … 500, etc.) of species (or higher taxonomic categories) from the whole regional inventory, and a plot of the resulting upper and lower limits on a graph of Δ^+ or Λ^+ against m is constructed. The positions of real TaxD index values on such plots corresponded to certain stations, allowing comparison of deviations of the distinctness values (i.e., taxonomic structure) from the expected TaxD level corresponding to the whole Crimean regional inventory. Points located within the probability funnel indicate that the taxonomic diversity of the corresponding sites falls within the expected range revealed from diversity analysis of the entire biogeographical region. Values outside the limits represent a biodiversity measure that departs significantly from what might be expected due to the presence of any pronounced (and long-term) environmental perturbation (Warwick & Clarke 1998).

Table 1. Environmental variables and variation ranges of their values used to characterize Balaklava Bay stations (samples were taken from upper 0–4 cm of bottom sediments).

Environmental variable	Variation range
Depth, m	4.0–23.0
Sandy fraction %	0–60.0
Silt+clay %	3.0–92.2
TOC, %	1.3–6.6
Organic toxicants, mkg · kg^{-1} dry weight	
PAHs	900.0–26000.0
PCBs	2.0–435.0
Pesticides	5.0–93.0
Metals, mkg · kg^{-1} dry weight	
Cu	34.0–350.0
Cd	0.1–0.6
Zn	53.0–600.0
Cr	2.5–67.5
Mn	175.0–470.0
Pb	51.0–1500.0
Hg	0.3–2.0
Ni	9.0–44.0

RESULTS AND DISCUSSION

Measurements of the environmental parameters showed that the depth, grain size of bottom sediment, TOC content and concentrations of toxic compounds vary considerably across the bay bottom (Table 1). There is a pollution gradient of heavy metals and organochlorine compound concentrations.

Principal component analysis revealed a distinct trend along the first three PC axes. PC1 (accounting for 32–36% of total explained variance) is associated with the gradient of heavy metals (Zn, Pb, Cu, Cr, Cd), while PC2 (27–28% of variance) is associated mainly with changes in TOC level and percentage of silty/clay fraction in the sediment. PC3 (12–14% of explained variance) is associated with the distribution of PCBs and pesticides.

Cluster analysis (based on similarity matrix of 15 considered variables) divided the Balaklava Bay bottom into two well-distinguished zones, generally corresponding to the inner (shallower) part and the outer (deeper) part and bay entrance (Fig. 2).

Average values of abiotic variables recorded from the bottom sediments of those parts of the study area are given in Table 2. The content of most of the pollutants (except for Pb, PCBs and PAHs) was higher in the inner part of the bay. The most significant differences were between core stations from inner (station 8) and external (station 16) parts of the bay.

Diatom diversity changes were assessed versus the degree of anthropogenic impact on the basis of detailed data on sediment chemistry and other environmental parameters across Balaklava Bay.

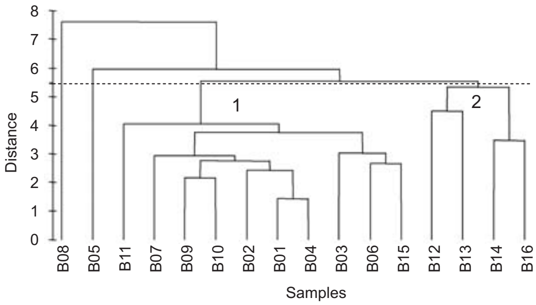

Fig. 2. Dendrogram representing the relative similarity of stations (based on Euclidean distance measure for 15 key environmental variables). Dotted line indicates the integration level (*ca* 5.5) of sampling stations into two spatial groups: 1 – inner part of bay, 2 – outer part.

The updated Crimean inventory at present contains 539 benthic diatom species aggregated into 100 genera, 47 families and 25 orders. Microscopic analysis revealed the presence of 192 taxa belonging to 183 species, 58 genera, 38 families and 21 orders in Balaklava Bay (Table 3). Species richness was highest for genera *Nitzschia* Hassal (28 taxa), *Amphora* Ehrenberg (19), *Navicula* Bory (16), *Cocconeis* Ehrenberg (15), *Diploneis* Ehrenberg ex Cleve (11) and *Lyrella* Karayeva (6). *Paraliales*, *Rhabdonematales*, *Thalassionematales* and *Toxariales* were the most species-poor orders, with only one species recorded in each. The most abundant diatom species of Balaklava Bay are listed in Table 4.

In addition to environmental gradient analysis, spatial grouping of stations based on abun-

Table 2. Mean values of environmental variables in bottom sediment corresponding to two parts of Balaklava Bay. Data for most polluted (station 8) and least polluted (station 16) core stations also indicated.

Location	Variables													
	Zn	Cu	Ni	Pb	Cr	Cd	Ag	Hg	Mn	PCBs	Pesticides	PAHs	TOC	Silt+ clay
Inner part	206.5	146.8	33.1	224.5	34.0	0.3	0.3	0.6	365.1	84.2	15.6	3759.0	2.3	80.9
Outer part	185.5	130.7	16.2	317.6	12.8	0.1	0.3	1.1	225.2	122.7	9.3	10470.0	4.8	36.3
St. 8	600.0	350.0	44.0	1500.0	67.5	0.5	0.4	0.8	325.0	435.0	93.0	2700.0	4.2	72.0
St. 16	53.0	33.8	10.5	51.2	13.8	0.1	0.2	0.4	192.0	79.0	12.0	1200.0	2.8	20.2

Content of metals indicated in mg · kg^{-1} dry weight, organic toxicants in mkg · kg^{-1} dry weight of bottom sediment, TOC and sediment size fraction in percents.

Table 3. Composition of benthic diatom assemblages in Balaklava Bay.

Taxon	Sp.	Taxon	Sp.
THALASSIOSIRALES Gleser & Makarova 1986		RHOICOSPHENIACEAE Chen & Zhu 1983	
THALASSIOSIRACEAE Lebour 1931		*Rhoicosphenia* Grunow 1860	1
Thalassiosira Cleve 1873	7	CYMBELLACEAE Greville 1834	
STEPHANODISCACEAE Glezer & Makarova 1986		*Cymbella* Agardh 1830	2
Cyclotella (Kützing) Brebisson 1838	4	GOMPHONEMATACEAE Kutzing 1845	
Stephanodiscus Ehrenberg 1845	1	*Gomphonema* Ehrenberg 1832	2
MELOSIRALES Crawford 1990		ACHNANTHALES Silva 1963	
MELOSIRACEAE Kützing 1844		ACHNANTHACEAE Kutzing 1845	
Melosira Agardh 1824	1	*Achnanthes* Bory 1822	3
HYALODISCACEAE Crawford 1991		*Planothidium* Round & Bukhtiyarova 1996	2
Hyalodiscus Ehrenberg 1845	2	COCCONEIDACEAE Kutzing 1845	
Podosira Ehrenberg 1840	2	*Cocconeis* Ehrenberg 1837	15
PARALIALES Crawford 1990		NAVICULALES Bessey 1907	
PARALIACEAE Crawford 1988		BERKELEYACEAE Mann 1990	
Paralia Heiberg 1863	1	*Parlibellus* Cox 1988	3
COSCINODISCALES Round & Crawford 1991		DIADESMIDACEAE Mann 1990	
COSCINODISCACEAE Kützing 1845		*Luticola* Mann 1990	1
Coscinodiscus Ehrenberg 1839	3	SELLAPHORACEAE Mereschkowsky 1902	
HEMIDISCACEAE Hendey 1937 em. Simonsen 1975		*Fallacia* Stickle & Mann 1990	3
Actynocyclus Ehrenberg 1837	1	PINNULARIACEAE Mann 1992	
TRICERATIALES Round & Crawford 1990		*Pinnularia* Ehrenberg 1843	2
TRICERATIACEAE (Shütt) Lemmermann 1899		*Caloneis* Cleve 1894	2
Pleurosira (Meneghini) Trevisan 1848	1	DIPLONEIDACEAE Mann 1991	
Triceratium Ehrenberg 1839	1	*Diploneis* Ehrenberg ex Cleve 1894	11
PLAGIOGRAMMACEAE De Toni 1890		NAVICULACEAE Kutzing 1845	
Dimeregramma Ralfs 1861	1	*Navicula* Bory 1822	16
Glyphodesmis Greville 1862	1	*Haslea* Simonsen 1974	1
FRAGILARIALES Silva 1964		*Trachyneis* Cleve 1894	1
FRAGILARIACEAE Greville 1835		*Dickieia* Berkeley ex Kützing 1844 em. Mann 1994	1
Fragilaria Lyngbye 1819	2	*Astartiella* Witkowski, Lange-Bertalot & Metzeltin 1998	1
Synedra Ehrenberg 1830	2	PLEUROSIGMATACEAE Mereschkowsky 1904	
Opephora Petit 1888	1	*Pleurosigma* Smith 1852	2
LICMOPHORALES Round 1991		*Gyrosigma* Hassal 1845	4
LICMOPHORACEAE Kützing 1845		*Rhoicosigma* Grunow 1867	1
*Licmophor*a Agardh 1827	4	PLAGIOTROPIDACEAE Mann 1990	
RHAPHONEIDACEAE Forti 1912		*Plagiotropis* Pfitzer 1871	1
Delphineis Andrews 1977	1	STAURONEIDACEAE Mann 1991	
ARDISSONEALES Round 1991		*Stauronella* Mereschkowsky 1901	1
ARDISSONEACEAE Round 1990		THALASSIOPHYSALES Mann 1991	
Ardissonea De Notaris 1870	3	CATENULACEAE Mereschkowsky 1903	
TOXARIALES Round 1990		*Amphora* Ehrenberg 1844	19
TOXARIACEAE Round 1990		BACILLARIALES Hendey 1939	

Table 3. *Continued.*

Taxon	Sp.	Taxon	Sp.
Toxarium Bailey 1854	1	BACILLARIACEAE Ehrenberg 1831	
THALASSIONEMATALES Round 1990		*Bacillaria* Gmelin 1791	1
THALASSIONEMATACEAE Round 1990		*Hantzschia* Grunow 1877	4
Thalassionema Grunow ex Hustedt 1932	1	*Nitzschia* Hassal 1845	28
RHABDONEMATALES Round & Mann 1990		RHOPALODIALES Mann 1992	
RHABDONEMATACEAE Round & Crawford 1990		RHOPALODIACEAE (Karsten) Topachevsky & Oksiyuk 1962	
Rhabdonema Kützing 1844	1	*Rhopalodia* Müller 1895	2
STRIATELLALES Round 1992		SURIRELLALES Mann 1991	
STRIATELLACEAE Kützing 1846		ENTOMONEIDACEAE Reimer 1976	
Grammatophora Ehrenberg 1840	3	*Entomoneis* Ehrenberg 1845	2
Hyalosira Kützing 1844	1	AURICULACEAE Hendey 1965	
LYRELLALES Mann 1991		*Auricula* Castracane 1873	1
LYRELLACEAE Mann 1991		SURIRELLACEAE Kutzing 1845	
Lyrella Karayeva 1978	6	*Petrodictyon* Mann 1990	1
Petroneis Stickle & Mann 1990	1	*Surirella* Turpin 1828	2
CYMBELLALES Mann 1990		*Campylodiscus* Ehrenberg ex Kützing 1844	3

dance and species richness (biotic matrix) was performed. Such an analysis shows the spatial heterogeneity in diatom assemblage distribution through the bottom area, and it distinguished two well-separated groups of stations at the 30% similarity level (Fig. 3). Similarly to the results of environmental analysis, the first group (stations 1–8 and 10) corresponds to the inner part, and the rest (9 and 11–16) are near the outer part and the mouth of the bay.

Table 4. Leading complex of mass species of benthic diatoms in Balaklava Bay, their maximum and average abundance and occurrence.

Species	Abundance × 10^6 cells · cm^{-2}		Occurrence %
	Average	Max	
Nitzschia compressa (Bailey) Boyer	59.8	180.0	100
Nitzschia sigma (Kützing) W. Smith	30.2	90.0	100
Thalassionema nitzschioides (Grunow) Mereschkowsky	6.0	33.8	100
Cocconeis scutellum Ehrenberg	2.5	11.3	100
Bacillaria paxillifera (O. Müller) Hendey	16.2	124.0	93.8
Nitzschia coarctata Grunow	1.8	45.0	93.8
Diploneis smithii (Brebisson) Cleve	6.4	22.5	93.8
Grammatophora marina (Lyngbye) Kützing	4.9	22.5	93.8
Nitzschia panduriformis Gregory	2.5	16.9	93.8
Synedra tabulata (Agardh) Kützing	6.0	39.4	87.5
Lyrella abrupta (Gregory) Mann	6.4	16.9	87.5
Cymbella angusta (Gregory) Guslyakov	0.4	5.6	81.3
Fallacia forcipata (Greville) Stickle & Mann	3.2	45.0	62.5
Amphora coffeaeformis (Agardh) Kützing	2.8	33.8	62.5
Caloneis liber (W. Smith) Cleve	5.6	33.8	53.0

SIMPER analysis revealed the most significant indicative and discriminating diatom species of each of the assemblages considered, presented in Tables 5 and 6. These results distinguished diatom assemblages of particular structure within each separated part of the bay. In the inner (polluted part), only 2 of 145 top-ranked species, *Nitzschia compressa* (Bailey) Boyer and *Nitzschia sigma* (Kützing) W. Smith, constituted *ca* 82% of total input into the average similarity within the assemblages. In our interpretation these are the most indicative species of the assemblages inhabiting the highly polluted part of the studied bottom area. The diatom assemblage of the less polluted outer part of the bay included 5 top-ranked indicative forms (of the total 136): *Grammatophora oceanica* Ehrenberg, *Bacillaria paxillifera* (O. Müller) Hendey, *Nitzschia compressa* (Bailey) Boyer, *Grammatophora marina* (Lyngbye) Kützing and *Thalassionema nitzschioides* (Grunow) Mereschkowsky. These species together constituted *ca* 50% of the total contribution to average similarity within this assemblage. However, average similarity between stations within the distinguished pollution-related groups was rather low, especially for group 2 (22.3%). This suggests that environmental conditions (pollution level) did not significantly influence the existing differences in the distribution of dominant species within each of pollution-related parts of Balaklava Bay.

On the other hand, comparative analysis of the quantitative development of top-ranked discriminating species in the two parts of the bay revealed high dissimilarity (83.4%) between assemblages. This indicates pronounced differences in key species abundance between the compared assemblages living under different levels of pollution.

The division of all stations into two spatial groups according to levels of environmental factors and information about the set of species corresponding to each group can also be used to find the combination of variables that best matches the high similarity between the biotic and abiotic matrices. It can identify the set of abiotic factors best explaining the spatial differences in benthic diatom assemblage patterns across the surveyed bottom area.

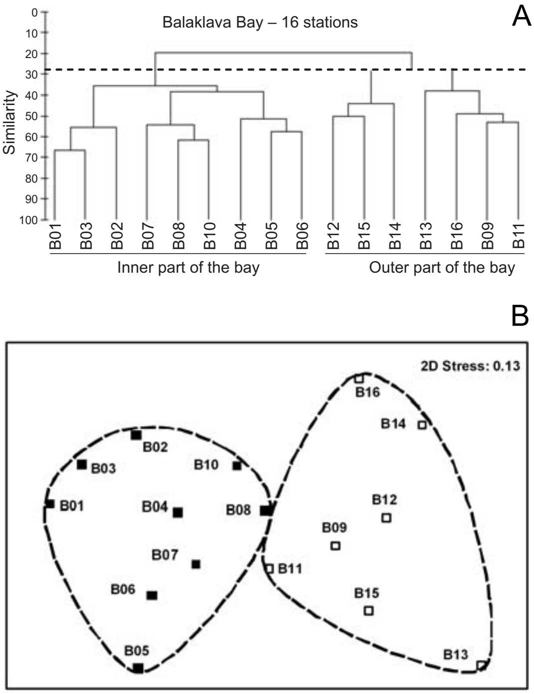

Fig. 3. Results of clustering (A) and nMDS ordination (B) analysis: grouping of stations from Balaklava Bay based on diatom abundance.

Evaluation of the Spearman rank correlation showed that the highest value of the coefficient (ρ_{max} = 0.612) corresponded to a combination of depth, percent of sand fraction, PCBs, TOC, pesticides and Hg. This set of factors gave the maximal matching with the difference in biotic parameters and presumably is the cause of changes in the structure and diversity features of the diatom assemblage along the pollution gradient in the bay.

Earlier work on the distribution of benthic diatoms along environmental gradients in similarly polluted Sevastopol Bay (Black Sea, Crimea) gave a combination of seven key abiotic factors: depth, percentage content of sand fraction, PCBs, Eh, Cd, Mn and Zn (Petrov *et al.* 2005). The first three are the same in the sets of key factors influencing the abundance and diversity of diatoms in the Sevastopol and Balaklava regions. The rest of the key variables for Sevastopol Bay (Cd, Mn, Zn, redox potential Eh) differ from set of key variables detected in the current analysis for Balaklava Bay.

Table 5. Contribution of the most significant indicative species in average similarity within benthic diatom assemblages in two compared locations of Balaklava Bay.

Species in compared locations	N, cells · cm^{-2}	S_i	S_i (%)
1 – inner part (145 sp.) – average similarity 45.7%			
Nitzschia compressa	9136.7	22.1	48.5
Nitzschia sigma	4688.9	15.3	33.5
Other species			18.0
2 – outer part (136 sp.) – average similarity 22.3%			
Grammatophora oceanica	2092.8	4.3	19.3
Bacillaria paxillifer	3381.8	2.6	11.8
Nitzschia compressa	1932.3	1.9	8.8
Grammatophora marina	1049.0	1.8	8.1
Thalassionema nitzschioides	1210.4	1.2	5.4
Other species			46.6

Note: N, cells · cm^{-2} – average abundance of *i-th* species in assemblage, S – similarity function, S_i – absolute and S_i (%) – relative contribution of *i-th* species in average similarity within the diatom taxocenotic complexes.

Dickmann (1998) noted a significant correlation ($P < 0.05$) between high levels of sediment toxicity from metals (Cd, Cr, Cu, Ni, Zn) and decreasing species diversity and deformity of cell morphology of diatoms of the genera *Navicula*, *Achnanthes* and *Fragilaria*.

The next part of this study used AvTD (Δ^+) and VarTD (Λ^+) indices to compare possible deviations in diatom assemblage diversity across the bottom of the bay. We constructed 95% probability 2-D funnel plots for the simulated AvTD and VarTD values, based on the inventory of the Crimean benthic diatom flora, and points for each station were superimposed on the simulation funnel. The AvTD value (81.59) calculated for the whole Balaklava Bay was below the expected average corresponding to the entire Crimean region (83.67). The AvTD value corresponding to the inner part of the bay was lowest (80.89). The phylogenetic structure of the diatom assemblage in permanently polluted Balaklava Bay is shown to be characterized by depressed taxonomic diversity, especially

Table 6. Contribution of the most significant discriminating species in average dissimilarity between benthic diatom assemblages (83.4%) in two compared locations of Balaklava Bay.

Species in compared locations	N, cells · cm^{-2}		D	D_i (%)
	1 – inner part	2 – outer part		
Nitzschia compressa	9136.7	1932.3	15.9	19.1
Nitzschia sigma	4688.9	888.0	8.9	10.7
Bacillaria paxillifer	254.7	3381.9	5.3	6.4
Grammatophora oceanica	67.0	2092.9	4.4	5.3
Synedra tabulata	320.0	968.0	2.3	2.7
Grammatophora marina	70.3	1049.0	2.2	2.6
Thalassionema nitzschioides	132.9	1210.4	2.2	2.6
Coscinodiscus sp. 1	0.0	724.3	1.9	2.3
Other species				48.3%

Note: N, cells · cm^{-2} – average abundance of *i-th* species, D – similarity function, D_i – absolute and D_i (%) – relative contribution of *i-th* species in average dissimilarity (83.4 %) between the diatom complexes.

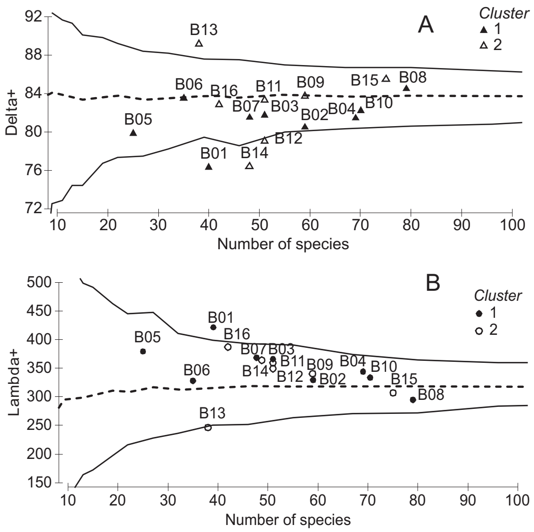

Fig. 4. 95% probability funnel plots for simulated AvTD, Δ^+ (A) and VarTD, Λ^+ (B) values, based on the total list of Crimean benthic diatom flora (502 species). Points indicate the observed Δ^+ and Λ^+ values corresponding to diatom assemblages from each of 16 stations (1st group – stations from inner part, 2nd group – stations from outer part of bay). Dotted line indicates expected average values of TDIs for the whole Crimean region.

at higher hierarchical levels (genus, family). The group of stations corresponding to the outer, less polluted part of the bay has a slightly higher Δ^+ value (82.86), close to the expected average mode from diversity analysis of the Crimean inventory. Figure 4A is a funnel plot with the points indicated for the observed AvTD values corresponding to each of the 16 sampling stations. Most of the points are near or below the expected level but within the lower limit of the probability funnel.

The lower positions of the station points on the AvTD plot (according to expected average mode) is evidence that diatom diversity structure at most of the sampling sites (except stations 13 and 15) is below expectations. Such diversity features are related to decreased taxonomic diversity (in spite of considerable species richness) and with minimal evenness of the whole taxonomic tree due to the rather high ratio of species-rich branches. The most topologically flat (or less vertically even) hierarchical structure of the taxocene at stations 1, 2, 5 and 14 also signify the lowest taxonomic diversity. This result is likely due to elimination of some species-poor and mono-specific branches of the taxonomic tree under permanent environmental disturbances, including anthropogenic pollution (Warwick & Clarke 2001; Warwick *et al.* 2002; Leonard *et al.* 2006; Leira *et al.* 2009).

In the same type of comparative analysis performed with the other taxonomic diversity index (VarTD), only the points of stations 2, 6 and 15 were close to the expected mode of the corresponding funnel ($\Lambda^+ = 308$) (Fig. 4B). These results mean that the aggregation pattern of initial taxa into higher hierarchic levels at these stations is similar to the expected average value of VarTD for the entire Crimean region.

The taxonomic diversity of the rest of the stations (except station 13) was characterized by wide variability of phylogenetic tree topology: the calculated Λ^+ values mapped on the plot are not only above the expected average level but also beyond the upper limit of the 95% probability funnel (see Fig. 4B). This suggests that the diatom assemblages are characterized by uneven phylogenetic structure (i.e., both species-poor and species-rich arms in the taxonomic tree). Taxonomic structure can become asymmetric under the influence of different environmental factors changing considerably across temporal and spatial scales. In such cases the assemblage is usually characterized by pronounced taxonomic heterogeneity, becoming apparent at higher hierarchical levels. Note that the interposition of points on plots Δ^+ and Λ^+ corresponding to the same station usually have a mirror pattern (e.g., see points of stations 1, 5 and 13), where the expected average line can be the axis of symmetry (see Fig. 4A & B).

Results from a similar analysis of taxonomic structure considering higher hierarchical levels (genus, family) are given in Figure 5. The positions of sampling points on the probability funnel plots for three hierarchical levels of diatom diversity in Balaklava Bay indicate a certain shift of points from symmetrical positions around the expected average (dotted line) corresponding to species level. The ellipses on the plots highlight such a shift of a sampling point cloud from the initial rather symmetrical position.

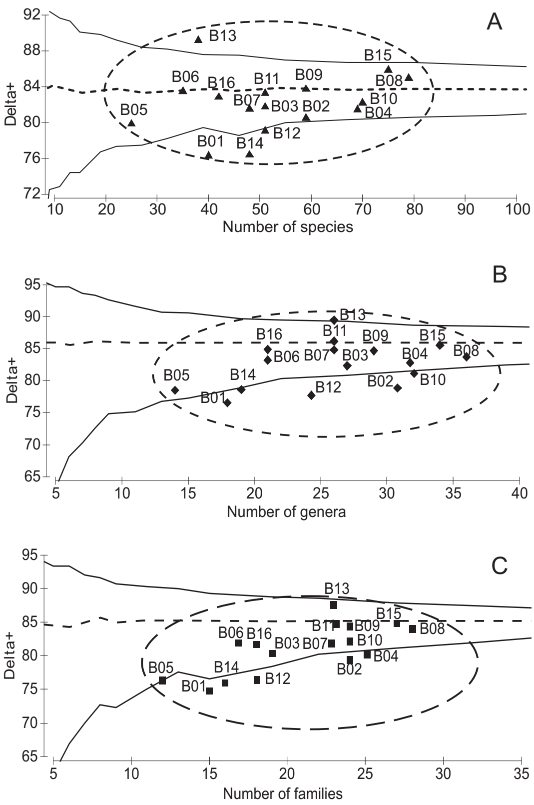

Fig. 5. Simulated AvTaxD (Δ^+) values: probability funnel plots for three hierarchical levels of diatom diversity in Balaklava Bay. A – species level, B – genus level, C – family level. Ellipses indicate shift of sampling points from the initial symmetrical position around the expected average (dotted line), corresponding to species level.

A comparison of these data with the above results for species level provides additional information regarding alteration of the vertical evenness of the diatom assemblage phylogenetic tree, that is, degradation of taxonomic structure under long-term environmental impacts in the bay.

Univariate relationships between key environmental variables distinguished after Spearman coefficient analysis (rank comparison of biotic and environmental matrices), and changes in the AvTD index, are presented in Figure 6. The curve of changes of Δ^+ values along the axes of environmental variables (TOC, sandy fraction) and pollutants (PCBs, pesticides, Hg) is bell-shaped. These newly found relationships show deviation of taxonomic evenness (AvTD index) from the average level, due to the influence of various environmental factors. Taxonomic diversity also increases versus initial levels due to the relative prevalence of species-poor branches in the community structure hierarchy. Such changes could be induced by increasing environmental disturbances in the biotope (Leira *et al.* 2009). However, further increases of environmental disturbances (including pollution) can lead to degradation of community structure, the disappearance of species-poor branches, and their gradual replacement by the species-rich arms in the taxonomic structure of the assemblage. Other authors have also noted that disturbances (natural or anthropogenic) of a biotope tend to eliminate species-poor arms, leaving those that belong to comparatively species-rich taxonomic groups (Clarke & Warwick 1998, 2001). Generally the taxonomic distinctness of degraded locations is usually significantly lower than that of relatively pristine locations for different groups of organisms (e.g., benthic nematodes, coastal fishes, echinoderms) (Warwick *et al.* 2002; Ellingsen *et al.* 2005; Leonard *et al.* 2006; Ceschia *et al.* 2007).

The AvTD and VarTD indices are useful tools for integrated assessment of changes in diversity under environmental degradation (Izsak & Price 2001). They could be important components of monitoring schemes for marine coastal ecosystems, in which evaluation of benthic diatom diversity is considered as a key bioindicator (Leonard *et al.* 2006).

Our study is one of the first attempts in world diatomology to make a comparative assessment of taxonomic diversity features of marine benthic diatom assemblages under different levels of technogenic pollutants. Our results revealed relationships between the values of TaxD indices and levels of key pollutants (heavy metals, PCBs, PAHs) in Black Sea bay sediments. An approach based on comparative evaluation of marine benthic diatom diversity can be recommended for practical monitoring of marine coastal ecosystems subject to permanent anthropogenic impacts, along with other bioindication methods using the different groups of the freshwater microphytobenthos (Schoeman & Haworth 1986; Stevenson & Pan 1999; Harding *et al.* 2005; Heino *et al.* 2005; Karydis 2009).

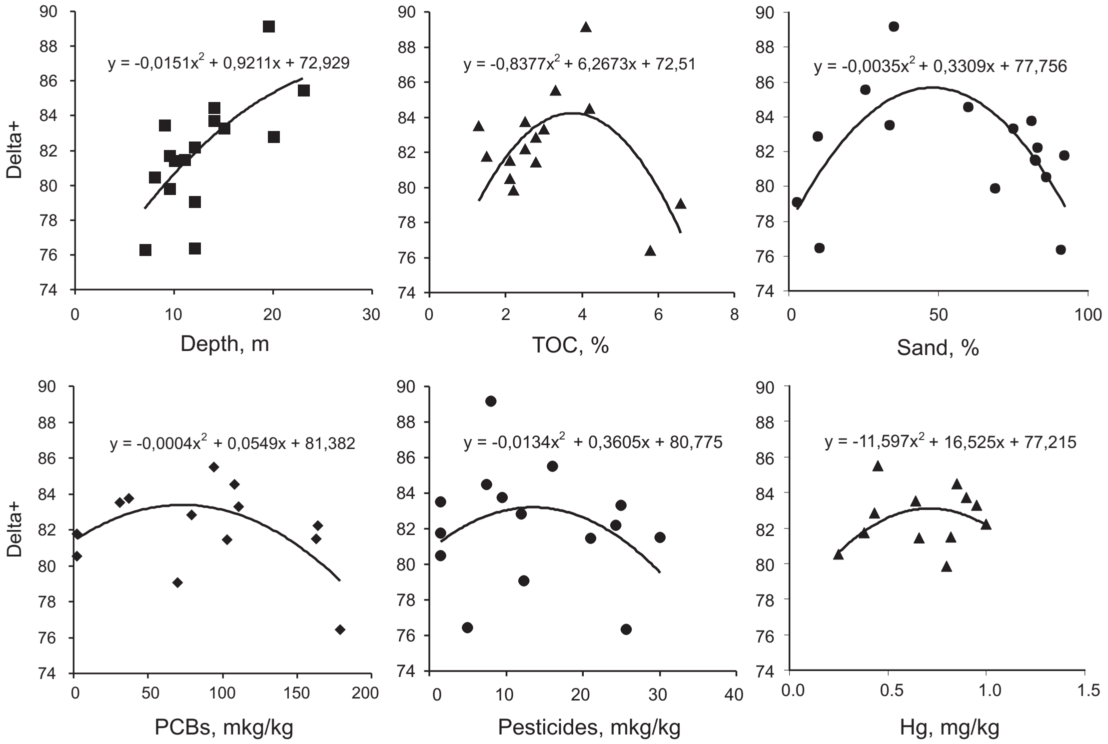

Fig. 6. Relationship between key environmental variables and AvTD index (Δ^+ values) for diatom assemblages from Balaklava Bay stations. A – depth, m; B – TOC, %; C – sand, %; D – PCBs, mkg · kg^{-1}; E – pesticides, mg · kg^{-1}; F – Hg, mg · kg^{-1}.

CONCLUSION

Cluster and MDS algorithms applied for united analyses of 15 abiotic variables and diatom abundance matrices revealed two spatially separated groups of stations in Balaklava Bay: one corresponding to the inner part of the bay and the other near the outer part and the mouth of the bay. The content of the majority of the pollutants, including heavy metals and organochlorine toxicants, was significantly higher in the sediments of the inner part of the bay.

Pronounced differences in the abundance, species richness and taxonomic diversity patterns of benthic diatom assemblages were correlated with the level of anthropogenic impacts in Balaklava Bay. Certain diatom assemblages corresponding to each of the two separated groups of stations were distinguished. The indicative and discriminating species responsible for similarity within each of assemblages and dissimilarity between them were distinguished and are listed.

Evaluation of Spearman rank correlation coefficients ($\rho_{max} = 0.612$) showed that a combination of variables – depth, ratio of sand fraction, content of TOC, pesticides, PCBs and Hg – was best correlated with changes in biotic patterns, suggesting their impact on the structure and diversity features of the diatom assemblages.

Based on assessment of TaxDI, the phylogenetic structure of diatom assemblages in the permanently polluted Balaklava Bay is generally characterized by depressed taxonomic diversity as compared with the expected average level corresponding to the entire Crimean region. The value of the AvTD index was lowest for the inner part of the bay, for which the taxonomic tree of the diatom assemblage is characterized by the relative prevalence of species-rich branches. The structure of assemblages from the less polluted external part of the bay gave higher AvTD values close to the expected average mode corresponding to the Crimean inventory. The results suggest that habitat variability and the gradients of physicochemical factors through the Balaklava Bay alter the abundance and erode the taxonomic structure of the diatom assemblage in conditions of long-term pollution.

Taxonomic distinctness indices are important tools for further development of marine coastal ecosystem monitoring, in which benthic diatom diversity is a key criterion among the bioindication approaches.

ACKNOWLEDGEMENTS. We are grateful to the anonymous reviewers for useful comments and helpful remarks on the manuscript. We thankthe copy editor for improving the English of our manuscript. This research was supported in part by the United States Environmental Protection Agency (Atlantic Ecology Division), through the mediation of the Science and Technology Center in Ukraine, Kiev, and is a contribution to the biodiversity assessment study of STCU Project P-277 (2006–2009).

REFERENCES

PROSHKINA-LAVRENKO A. I. (ed.) 1974. Diatomovye vodorosli SSSR. Izdatel'stvo Nauka, Leningrad.

BARINOVA S. S., MEDVEDEVA L. A. & ANISIMOVA O. V. 2000. Algae as indicators of environmental quality. Institute Nature Conservation Press, Moscow (in Russian with English summary).

BARINOVA S. S., MEDVEDEVA L. A. & ANISIMOVA O. V. 2006. Diversity of algal indicators in the environmental assessment. Pilies Studio, Tel Aviv (in Russian with English summary).

BIRKETT K. & GARDINER S. 2005. The use of epilithic and epiphytic diatoms as indicators of organic pollution in the Cheboygan River, Cheboygan county, Michigan. http://deepblue.lib.umich.edu/handle/2027.42/55043.

CAMPBELL W. B. & NOVELO-GUTIERREZ R. 2007. Reduction in odonate phylogenetic diversity associated with dam impoundment is revealed using taxonomic distinctness. *Fundamental and Applied Limnology* **168**: 83–92.

CARR M. R. 1997. Primer user manual. Plymouth Routines in Multivariate Ecological research. Plymouth Marine Laboratory, Plymouth.

CESCHIA C., FALACE A. & WARWICK R. 2007. Biodiversity evaluation of the macroalgal flora of the Gulf of Trieste (Northern Adriatic Sea) using taxonomic distinctness indices. *Hydrobiologia* **580**: 43–56.

CLARKE K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Austral. J. Ecol.* **18**: 117–143.

CLARKE K. R. & GORLEY R. N. 2001. PRIMER v5: User Manual / Tutorial. PRIMER-E, Plymouth.

CLARKE K. R. & WARWICK R. M. 1998. A taxonomic distinctness index and its statistical properties. *J. Appl. Ecol.* **35**: 523–531.

CLARKE K. R. & WARWICK R. M. 1999. The taxonomic distinctness measure of biodiversity weighting of step lengths between hierarchical levels. *Marine Ecology Progress Series* **184**: 21–29.

CLARKE K. R. & WARWICK R. M. 2001. Change in marine communities: an approach to statistical analysis and interpretation. Ed. 2. PRIMER-E, Plymouth.

DE LA REY P. A., TAYLOR J. C., LAAS A., VAN RENSBURG L. & VOSLOO A. 2004. Determining the possible application value of diatoms as indicators of general water quality: a comparison with SASS 5. *Water SA* **30**(3): 325–332.

DESCY J. P. & COSTE M. 1991. A test of methods for assessing water quality based on diatoms. *Verhandlungen des Internationalen Vereins für Limnologie* **24**: 2112–2116.

DICKMAN M. D. 1998. Benthic marine diatom deformities associated with contaminated sediments in Hong Kong. *Environment International* **24**(7): 749–759.

DIXIT S. S., SMOL J. P., CHARLES D. F., HUGHES R. M., PAULSEN S. G. & COLLINS G. B. 1999. Assessing water quality changes in the lakes of the northeastern United States using sediment diatoms. *Canadian Journal of Fisheries and Aquatic Sciences* **56**: 131–152.

ELLINGSEN K. E., CLARKE K. R., SOMERFIELD P. J. & WARWICK R. M. 2005. Taxonomic distinctness as a measure of diversity applied over a large scale: the benthos of the Norwegian continental shelf. *Journal of Animal Ecology* **74**: 1069–1079.

FOURTANIER E. & KOCIOLEK J. P. 1999. Catalogue of the Diatom Genera. *Diatom Res.* **14**(1): 1–190.

FOURTANIER E. & KOCIOLEK J. P. 2003. Addendum to Catalogue of the Diatom Genera. *Diatom Res.* **18**(2): 245–258.

FOURTANIER E. & KOCIOLEK J. P. 2007. Catalogue of Diatom Names. California Academy of Sciences. http://www.calacademy.org/research/diatoms/names.

GIMENEZ-CASALDUERO F. 2001. Biondicators. Tools for the impact assessment of aquaculture activities on the marine communities. In: *Scientific Report of Universidad de Alicante*, pp. 147–157. Alicante, Spain.

GOLDIN E. B. 2009. Microphytic algae as the bioindicators of environmental situation in the capture places of marine mammals. *Optimization and Protection of Ecosystems* **20**: 105–113 (in Russian with English summary).

GUSLYAKOV N., ZAKORDONEZ O. & GERASIMUK V. 1992. Atlas of diatom algae of benthos of the Black Sea North-West part and adjacent aquatories. Naukova Dumka, Kiev (in Russian with English summary).

GUSLYAKOV N. E. & NEVROVA E. L. 1998. Microphytobenthos. In: Y. P. ZAITSEV & B. G. ALEXANDROV (eds), *Black Sea Biological Diversity. Ukraine*, pp. 41–43 & 199–215. United Nations Publications, New York.

HARDING W. R., ARCHIBALD C. G. M. & TAYLOR J. C. 2005. The relevance of diatom for water quality assessment in South Africa: a position paper. *Water SA* **31**(1): 41–46.

HEINO J., SOININEN J., LAPPALAINEN J. & VIRTANEN R. 2005. The relationship between species richness and taxonomic distinctness in freshwater organisms. *Limnology and Oceanography* **50**(3): 978–986.

HEINO J., MYKRA. H., HAMALAINEN H., AROVIITA J. & MUOTKA T. 2007. Responses of taxonomic distinctness and species diversity indices to anthropogenic impacts and natural environmental gradients in stream macroinvertebrates. *Freshwater Biology* **52**: 1846–1861.

IZSAK C., PRICE A. R. G., HARDY J. T. &. BASSON P. W. 2002. Biodiversity of periphyton (diatoms) and echinoderms around a refinery effluent, and possible associations with stability. *Aquatic Ecosystem Health and Management* **5**(1): 61–70.

IZSAK C. & PRICE A. R. G. 2001. Measuring ß-diversity using a taxonomic similarity index, and its relation to spatial scale. *Marine Ecology Progress Series* **215**: 69–77.

KARYDIS M. 2009. Eutrophication assessment of coastal waters based on indicators: a literature review. *Global NEST Journal* **11**(4): 373–390.

KELLY M. G. 1998. Use of the trophic diatom index to monitor eutrophication in rivers. *Water Research* **32**(1): 236–242.

LEIRA M., CHEN G., DALTON C., IRVINE K. & TAYLOR D. 2009. Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient. *Freshwater Biology* **54**: 1–14.

LEONARD D., CLARKE K., SOMERFIELD P. & WARWICK R. 2006. The application of an indicator based on taxonomic distinctness for UK marine biodiversity assessment. *J. Environm. Managem.* **78**: 52–62.

MAGURRAN A. E. 2004. Measuring Biological Diversity. Blackwell Publishing, Oxford.

NEVROVA E. L. & PETROV A. N. 2008. Taxonomic diversity of benthic diatoms of the Black sea. In: Y. N. TOKAREV, Z. Z. FINENKO & N. V. SHADRIN (eds), *Microalgae of the Black Sea: problems of biodiversity, preservation and biotechnology usage*, pp. 60–84. Ekosi-Gidrophyzika, Sevastopol (in Russian with English summary).

NEVROVA E. L., REVKOV N. K. & PETROV A. N. 2003. Microphytobenthos. In: V. N. EREMEEV & A. V. GAEVSKAYA (eds), *Modern condition of biological diversity in near-shore zone of Crimea* (*the Black Sea sector*), pp. 270–282, 288–302 & 351–362. Ekosi-Gidrophyzika, Sevastopol (in Russian with English summary).

NIKITINA O. A. & SCHKUNDINA F. B. 2009. The isolation of autotrophic benthos indicator species of Sterlitamak city watercourse (Russia). *Algologia* **19**(4): 412–422 (in Russian with English summary).

PETROV A. N. & NEVROVA E. L. 2004. Comparative analysis of taxocene structures of benthic diatoms (Bacillariophyta) in regions with different level of technogenic pollution (the Black Sea, Crimea). *Marine Ecological Journal* **3**(2): 72–83(in Russian with English summary).

PETROV A. N. & NEVROVA E. L. 2007. Database on Black Sea benthic diatoms (Bacillariophyta): its use for a comparative study of diversity pecularities under technogenic pollution impacts. In: *Proceedings of Ocean Biodiversity Informatics: International Conference of Marine Biodiversity Data Management* (*Hamburg, Germany, November 2004*). *IOC Workshop Report, VLIZ Special Publication* **202**(37): 153–165.

PETROV A. N. & NEVROVA E. L. 2008. Assessment of technogenic pollution impact on diversity of benthic diatom taxocene in Balaklava bay (SW Crimea, Black sea). In: N. JASPRICA (ed.), *Abstract book of 20th International Diatom Symposium 2008*, p. 80. Dubrovnik, Croatia.

PETROV A. N., NEVROVA E. L. & MALAKHOVA L. V. 2005. Multivariate analysis of benthic diatoms distribution across the multidimensional space of the environmental factors gradient in Sevastopol Bay (the Black Sea, Crimea). *Marine Ecological Journal* **4**(3): 65–77 (in Russian with English summary).

ROUND F. E., CRAWFORD R. M. & MANN D. G. 1990. The Diatoms. Biology and morphology of the genera. Cambridge University Press, Cambridge.

RYABUSHKO L. I. 2008. Characteristic of microphytobenthos. In: Y. N. TOKAREV, Z. Z. FINENKO & N. V. SHADRIN (eds), *Microalgae of the Black Sea: problems of biodiversity, preservation and biotechnology usage*, pp. 29–50, 130–156 & 157–162. Ekosi-Gidrophyzika, Sevastopol (in Russian with English summary).

RYABUSHKO V. I., ALEEV M. Y., RADCHENKO V. N., RYABUSHKO L. I. & CHUBCHIKOVA I. 2003. Applying of some bioindicators for assessment of impact marine ecosystems. *Ecological safety of coastal and shelf zones and complex research of shelf resources* **2**(7): 144–154 (in Russian with English summary).

SATHYA K. S. & BALAKRISHNAN K. P. 1988. Physiological response of diatom *Nitzschia palea* (Kutz.) W. Sm. to sublethal levels of copper. *Indian Journal of Marine Sciences* **17**(4): 322–325.

SCHOEMANN F. R. & HAWORTH E. Y. 1986. The advantages and disadvantages of employing diatoms as indicators of pollution as opposed to other biological indicators groups. In: M. RICARD (ed.), *Proceedings of the 8th International*

Diatom Symposium: Diatoms as indicators of Pollution, Paris, pp. 75–79. Koeltz Scientific Books, Koenigstein.

SLADECEK V. 1986. Diatoms as indicator of Organic Pollution. *Acta Hydrochimica et Hydrobiologica* **14**(5): 555–556.

SONDERGAARD M. & JEPPESEN E. 2007. Anthropogenic impacts on lake and stream ecosystems, and approaches to restoration. *J. Appl. Ecol.* **44**: 1089–1094.

STEVENSON J & PAN Y. 1999. Assessing environmental conditions in rivers and streams with diatoms. In: E. F. STOERMER & J. P. SMOL (eds), *The diatoms: application for the environmental and earth sciences*, pp. 11–40. Cambridge University Press, Cambridge.

STOERMER E. F. 1978. Phytoplankton assemblages as indicators of water-quality in Laurentian Great Lakes. *Trans. Amer. Microscop. Soc.* **97**: 2–16.

TAVASSI M., BARINOVA S. S., ANISSIMOVA O. V., NEVO E. & WASSER S. P. 2004. Algal indicators of environment in the Nahal Yargan Basin, Central Israel. *International Journal on Algae* **6**(4): 355–382.

TILMAN D. 1997. Distinguishing between the effects of species diversity and species composition. *Oikos* **80**: 185–185.

WARWICK R. M. 1993. Environmental impact studies on marine communities: Pragmatical considerations. *Austral. J. Ecol.* **18**: 63–80.

WARWICK R. M. & CLARKE K. R. 1998 Taxonomic distinctness and environmental assessment. *J. Appl. Ecol.* **35**: 532–543.

WARWICK R. M. & CLARKE K. R. 2001. Practical measures of marine biodiversity based on relatedness of species. *Oceanography and Marine Biology: An Annual Review* **39**: 207–231.

WARWICK R. M., ASHMAN C. M., BROWN A. R., CLARKE K. R., DOWELL B., HART B., LEWIS R. E., SHILLABEER N., SOMERFIELD P. J. & TAPP J. F. 2002. Inter-annual changes in the biodiversity and commuity structure of the macrobenthos in Tees Bay and the Tees estuary, UK, associated with local and regional environmental events. *Marine Ecology Progress Series* **234**: 1–13.

WATANABE T., ASAI K. & HOUKI A. 1988. Numerical water quality monitoring of organic pollution using diatom assemblages. In: F. ROUND (ed.), *Proceedings of the 9th International Diatom Symposium 1986*, pp. 123–141. Koeltz Scientific Books, Koenigstein.

WATANABE T., ASAI K. & HOUKI A. 1990. Numerical simulation of organic pollution in flowing waters. In: P. N. CHEREMISINOFF (ed.), *Encyclopedia Environmental Control Technology. 4. Hazardous Waste: Containment and Treatment*, pp. 252–281. Gulf Publishing Co., Houston, Texas.

WITKOWSKI A., LANGE-BERTALOT H. & METZELTIN D. 2000. Diatom flora of Marine coast 1. *Iconogr. Diatomol.* **7**: 1–925.

Received 13 February 2010