10.2478/v10279-012-0004-8

VERSITA

BOTANICA LITHUANICA
ISSN 2029-932X

2012, 18(1): 27–34

DOMINANT *PSEUDO-NITZSCHIA* (*BACILLARIOPHYTA*) SPECIES IN THE BLACK SEA (UKRAINE)

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Abstract

Terenko L., Terenko G., 2012: Dominant *Pseudo-nitzschia* (*Bacillariophyta*) species in the Black Sea (Ukraine) [Vyraujančios *Pseudo-nitzschia* (*Bacillariophyta*) rūšys Juodojoje jūroje (Ukraina)]. – Bot. Lith., 18(1): 27–34.

Diatoms of the genus *Pseudo-nitzschia* (order *Pennales*) are persistent component of the phytoplankton community in the Black Sea and may reach bloom level during some periods in summer and autumn. Six potentially toxic and non-toxic *Pseudo-nitzschia* species were recorded up to date in the Ukrainian waters. Since 1980s, several mass developments of *Pseudo-nitzschia* spp. have been observed. Morphological analysis revealed *Pseudo-nitzschia delicatissima, P. pseudodelicatissima, P. pungens, P. seriata* as the dominant species in the blooms. Pronounced bloom of *Pseudo-nitzschia* spp. reaching up to 80 % of total phytoplankton abundance and occupying wide area of the Black Sea was observed in June 2001. The summer density peak of *P. delicatissima* and *P. seriata* was observed at 16.0–17.5 °C and salinity 15.2–15.7 ‰ in the coastal waters of Odessa Bay. In December 2005, the density of *Pseudo-nitzschia* spp. in the bottom layers in Odessa region reached $3.0 \cdot 10^6$ cells l^{-1}. In June 2008, a single species *P. pseudodelicatissima* bloomed in the Danube region. The density of the species came up to $12.0 \cdot 10^6$ cells l^{-1}. At the end of May 2009, *P. delicatissima* showed short-term and lower magnitude density peak ($3.1 \cdot 10^6$ cells l^{-1}) in the coastal zone of Odessa Bay. Thus, the *Pseudo-nitzschia* spp. blooms are frequent phenomena in the Black Sea and may occur in any season of a year. These species are indicators of trophic state in marine waters.

Keywords: *Pseudo-nitzschia,* distribution, seasonal dynamics, abundance, the northwestern Black Sea, Odessa Bay, coastal waters of Crimea, the Danube region.

INTRODUCTION

Pseudo-nitzschia H. Peragallo in H. & M. Peragallo are marine planktonic diatoms widespread in the waters of all biogeographical zones and often considered as cosmopolitans (Hasle et al., 1996). Thirty two species of *Pseudo-nitzschia* have been described so far (Ljubešić et al., 2011; Trainer et al., 2012). Ecological and distribution data on *Pseudo-nitzschia* species are quite confusing due to difficulties in different species delineation based on the cell siliceous exoskeleton morphology under light microscopy. Thus, revision of existing species and the description of several new species have been performed by examination of frustules using electron microscopy or molecular data (Casteleyn et al., 2008; Ljubešić et al., 2011).

The particular attention was given to representatives of the genus *Pseudo-nitzschia* as they produce the domoic acid (ASP-toxin), the neurotoxin, which accumulates in the tissues of molluscs, fishes and other filtrating organisms. Such organisms used for nutrition subsequently cause amnesic shellfish poisoning in humans, death of dolphins and fish-eating birds (Bates & Trainer, 2006).

The occurrence of diatoms from the genus *Pseudo-nitzschia* has been noted in the Black Sea since the

middle of the last century under the names of *Nitzschia seriata* Cleve, *N. delicatissima* Cleve, *N. pungens* Grunow ex Cleve (Proshkina-Lavrenko, 1955). *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle was observed for the first time in the plankton of Sevastopol Bay in spring 1949, whereas *P. delicatissima* (Cleve) Heiden – in autumn 1950 (Morozova-Vodianitskaya, 1954). *Pseudo-nitzschia* species are widespread in the Black Sea and quite often grow abundantly causing water blooms in various areas. However, detailed investigations on species diversity, their seasonal changes and spatial distribution of *Pseudo-nitzschia* algae in the Ukrainian part of the Black Sea have not been carried out till now. The data on toxicity of the algae of *Pseudo-nitzschia* genus from the Black Sea are fragmentary and insufficient (Vershinin & Orlova 2008; Ryabushko et al., 2008; Besiktepe et al., 2008).

The aim of this work was to summarize the data related to diversity, population seasonal dynamics and vertical distribution of the dominant *Pseudo-nitzschia* species in the Ukrainian coastal waters of the northwestern Black Sea.

MATERIALS AND METHODS

The study material covers phytoplankton regular monitoring data in the coastal area of Odessa Bay (the northwestern Black Sea). To investigate seasonal changes in *Pseudo-nitzschia* abundance, the samples were collected bi-weekly during 15-year period (1995–2010) (Fig. 1, Table 1). Phytoplankton samples for *Pseudo-nitzschia* spatial and vertical distribution were collected in the Danube region in June 2008 and Odessa region of the Black Sea in December 2005. A total of 754 samples were collected and analysed (Table 1).

Samples for the monitoring of the coastal area were collected using Molchanov's bathometer from the surface (up to 0.5 m) water layer. Unpreserved samples of 1 litre volume were concentrated by reverse filtration through the nucleopore filters (pore diameter 1.5 μm) up to 50–60 ml; these samples may be concentrated repeatedly up to 35–40 ml on necessity. Phytoplankton identification was performed in live material under light microscope "Biolam" (magnification ×600). Algae were counted in the Nageotte chamber (volume 0.05 ml).

Water samples for *Pseudo-nitzschia* spatial and vertical distributions were collected from the surface and bottom layers (5.4–24.0 m) using Molchanov's bathometer and preserved with 40 % neutralized formaldehyde 1:10 solution. The samples were concentrated by sedimentation method and analysed with light microscope as described earlier. Light microscope images of *Pseudo-nitzschia* species were performed with Motic Images 2000 DMWP1-223; scanning electron microscopy was carried out using a LEO-1420 microscope. Frustule samples for microscope analysis were processed after Parsons et al. (1999). The samples were boiled in concentrated HNO_3 for 20 min, and then washed six times with distilled water.

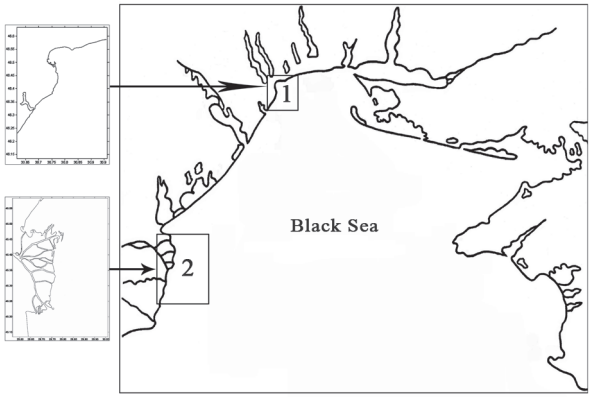

Fig. 1. Location of the study area in the northwestern Black Sea: 1 – Odessa region; 2 – the Danube region

RESULTS AND DISCUSSION

Species diversity. *Pseudo-nitzschia* diatoms have been commonly observed in the Black Sea (Besiktepe et al., 2008). Six species from the genus

Table 1. The number of the investigated phytoplankton samples in different areas of the northwestern Black Sea

Research area	Date	Number of sampling stations	Number of investigated samples
Odessa Bay, coastal area	1995–2010	5–7	710
Odessa region	December 2005	7	14
Danube region	June 2008	15	30

Table 2. *Pseudo-nitzschia* species recorded in the Ukrainian waters of the Black Sea

Species	Northwestern part		Crimean coast	
	1973–1994	1995–2010	1948, 1954	2008
	1	2	3	4
P. calliantha Lundcholm, Moestrup et Hasle		+		+
P. delicatissima (Cleve) Heiden*	+	+	+	+
P. inflatula (Hasle) Hasle				+
P. pseudodelicatissima (Hasle) Hasle*		+		
P. pungens (Grunow ex Cleve) Hasle*	+	+	+	+
P. seriata (Cleve) H. Peragallo*	+	+	+	+

1 – Nesterova, 1998; 2 – original data; 3 – Morozova-Vodianitskaya, 1948, 1954; 4 – Senicheva, 2008; Ryabushko et al., 2008.
* – dominant species, **bold** – potentially toxic species

Pseudo-nitzschia were recorded during this study and after re-examination of the material on the basis of light and electron microscopy (Table 2; Fig. 2). Most usual dominants were three *Pseudo-nitzschia* species – *P. seriata* (Cleve) H. Peragallo, *P. pungens* and *P. delicatissima* – known in the Ukrainian part of the Black Sea since 1950s (Nesterova et al., 2006; Tsarenko et al., 2009). They show the similar yearly development pattern in the area. Species *P. calliantha* Lundcholm, Moestrup et Hasle, *P. pseudodelicatissima* (Hasle) Hasle and *P. inflatula* (Hasle) Hasle have been found recently. *Pseudo-nitzschia calliantha* is rather new species in the Black Sea, which has been recorded in Turkey and the Crimean coastal waters (Bargu et al., 2002; Ryabushko et al., 2008). *P. calliantha* and *P. pseudodelicatissima* have been found for the first time in the northwestern Black Sea also. In spring, *P. calliantha* made up to $1.2 \cdot 10^3$ cells l^{-1} in Sevastopol and Quarantine Bays (Ryabushko et al., 2008), whereas *P. inflatula* – $3.6 \cdot 10^5$ cells l^{-1} in the freshened waters of Sevastopol Bay (Senicheva, 2008).

According to Skov et al. (1999) and Trainer et al. (2012), *P. calliantha*, *P. pseudodelicatissima, P. pungens* and *P. seriata* are known as potentially toxic species able to produce domoic acid, the toxin responsible for amnesic shellfish poisoning. Toxicity of the Black Sea population *P. calliantha* has been confirmed by Bargu et al. (2002), Ryabushko et al. (2008), Besiktepe et al. (2008). Numerous checks on the toxicity of most mass blooming species *P. pseudodelicatissima* and *P. pungens* have appeared to be negative (Vershinin & Orlova, 2008).

Five species known from the Black Sea may be assigned to widespread, cosmopolitan species based on phytogeographical analysis of the genus *Pseudo-nitzschia*. *P. seriata* seems to be arctoborealic species, restricted to cold waters in northern and moderate latitudes (Hasle & Syvertsen, 1997). Hasle (2002) came to the assumption that probably all species producing domoic acid are cosmopolitans.

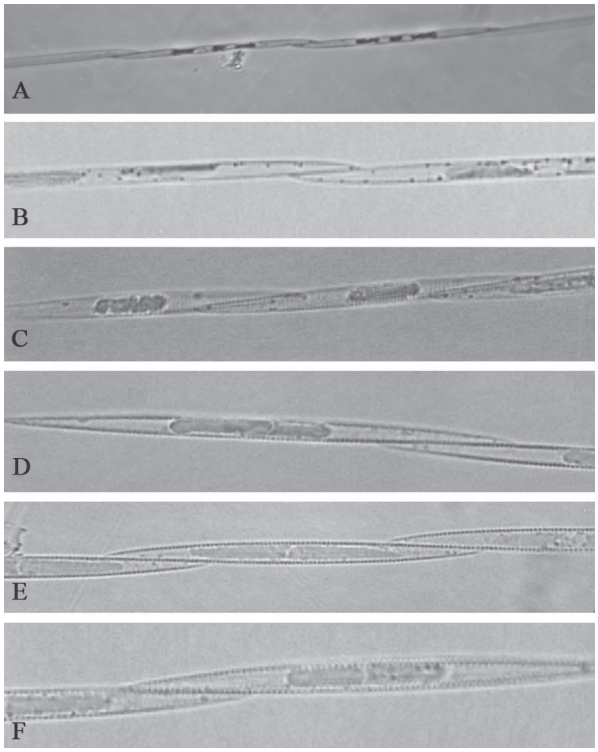

Fig. 2. Light micrographs of *Pseudo-nitzschia* spp. stepped colonies in valve view recorded from the Black Sea: A–B – *P. delicatissima* (June 2001), C–D – *P. pungens* (August 2006), E–F – *P. seriata* (November 2001)

The identification of *Pseudo-nitzschia* diatoms that formed bloom in the Danube region in 2008 was re-examined applying electron microscopy (Fig. 3). The morphology of *P. pseudodelicatissima* is very similar to the morphology of *P. delicatissima,* which is often

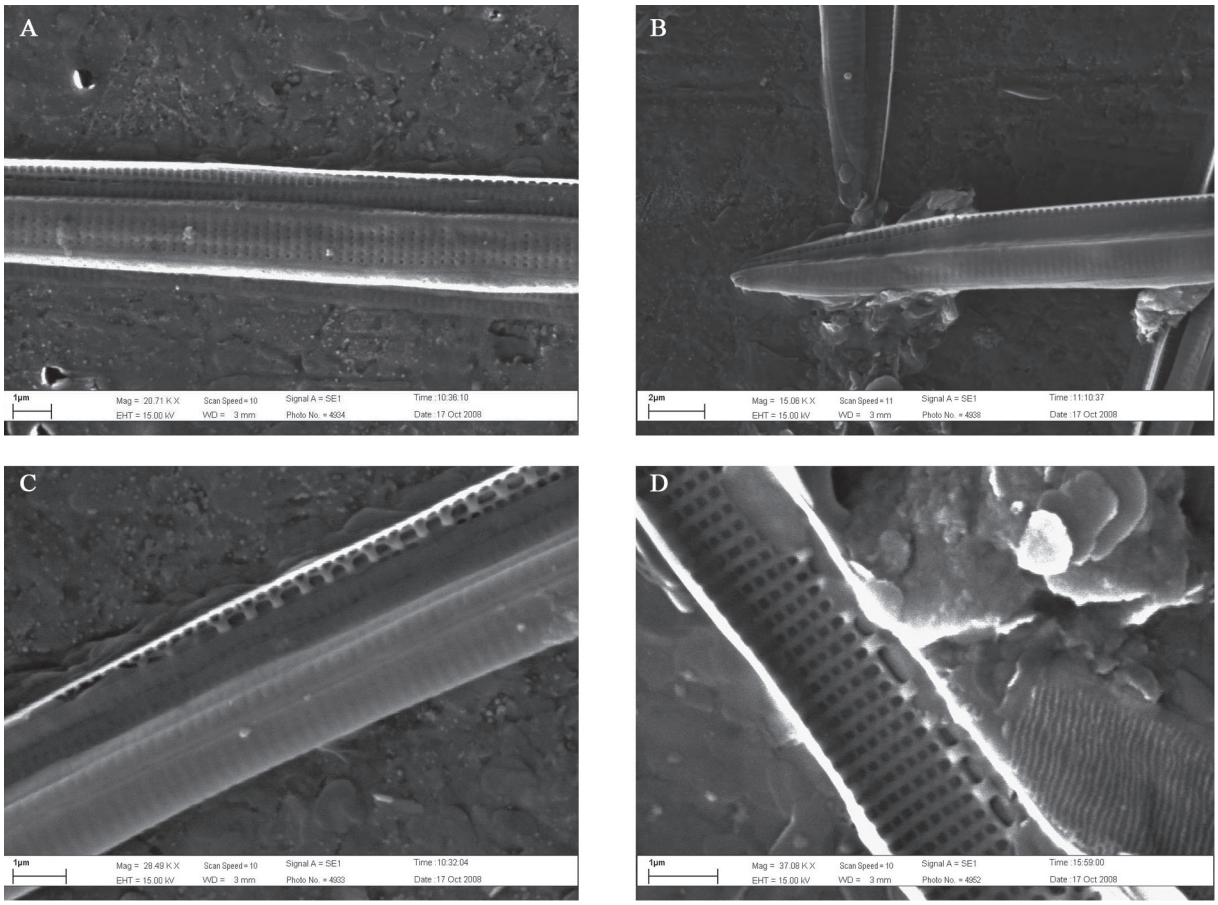

Fig. 3. Scanning electron micrographs of *Pseudo-nitzschia pseudodelicatissima* from the Danube region (June 2008): A, C, D – poroid and striae structure of the central area of valve; B – valve view from the top

blooming species in the Black Sea. Because of the linear shape of the valve and the density of striae, fibulae and poroids as well as the width, *Pseudo-nitzschia* specimens were identified as *P. pseudodelicatissima*.

Seasonal changes. Maximum abundance of *Pseudo-nitzschia* ($12.0 \cdot 10^6$ cells l^{-1}) was observed in the last decade of 2008 at most eutrophicated sites of the Danube region of the Black Sea. This abundance was commensurable with as much as possible registered abundance in 1996 (Fig. 4).

The results of all-year monitoring in the coastal zone of Odessa Bay on *Pseudo-nitzschia* species composition and productivity characteristics as well as the data of more detailed integrated investigations in Odessa and the Danube regions of the Black Sea revealed that *Pseudo-nitzschia* diatoms were permanent constituent of phytoplankton during all vegetation periods, occurring more often from April till October (Table 3). *Pseudo-nitzschia* spp. were found within the wide range of water temperature 5.2–24.0 °C and salinity 6.7–18.0 ‰. Seasonal changes in dominant species of the genus *Pseudo-nitzschia* in the Ukrainian waters of the Black Sea are shown in Table 3. Maximum abundance of most frequent *P. delicatissima* species was noted from May till July in different years when water temperature was 16.8–23.2 °C (Fig. 3). *P. pungens*, a warm-water species, formed density peak in the middle of September at water temperature 21.0 °C, and cold-water *P. seriata* species – at the beginning of December (temperature 8.4 °C).

Usually several *Pseudo-nitzschia* species simultaneously developed in the plankton (Fig. 5). In June, *P. delicatissima* dominated up to 80 % of a total abundance of *Pseudo-nitzschia* population with *P. seriata* being as accompanying species (19 %). In December, *P. pungens* showed clear dominance (79 % of the *Pseudo-nitzschia* abundance) over *P. delicatissima* (21 %) both in superficial and bottom water layers of Odessa Bay.

Table 3. Seasonal changes in dominant species of the genus *Pseudo-nitzschia* in the Ukrainian waters of the Black Sea

Species	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
P. delicatissima				+	+*	+*	+*	+	+	+		
P. pseudodelicatissima						+*						
P. pungens									+	+	+	
P. seriata					+	+	+	+	+	+	+	+*
P. calliantha	+	+		+								
P. inflatula			+	+								

+* – blooming species

Table 4. Abundance ($\times 10^6$ cells l^{-1}) of blooming *Pseudo-nitzschia* in the Ukrainian waters of the Black Sea

Year	Winter	Spring	Summer	Autumn
1996		*P. delicatissima* (16.6)		
2001			*P. delicatissima* (1.5–1.9)	*P. seriata* (0.45)
2005	*Pseudo-nitzschia* spp. (3.0)			
2006			*P. delicatissima* (1.2–1.9)	
2008			*P. pseudodelicatissima* (1.4–12.0)	
2009		*P. delicatissima* (3.1)		

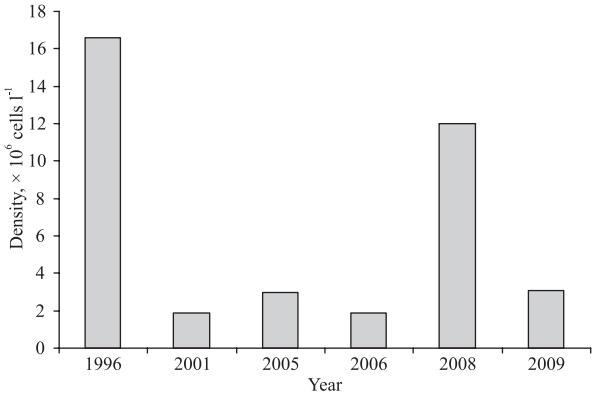

Fig. 4. Interannual variation of maximum abundance of *Pseudo-nitzschia* spp. in the Ukrainian waters of the Black Sea

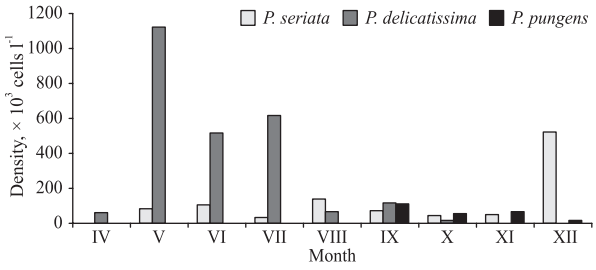

Fig. 5. Seasonal dynamics of *Pseudo-nitzschia* dominant species abundance in the Ukrainian waters of the Black Sea

Non-toxic *Pseudo-nitzschia delicatissima* was the most often bloom-forming species in the Ukrainian waters of the Black Sea. There were four events of *P. delicatissima* fluxes with the highest abundance ($16.6 \cdot 10^6$ cells l^{-1}) observed in spring 1996 (Table 4).

In summer 2001, the phytoplankton in Odessa Bay coastal waters was mainly composed of *Pseudo-nitzschia* diatoms (73.8–83.5 % of total phytoplankton abundance) with a dominance of *P. delicatissima* (Table 4). Water temperature ranged from 16.0 to 17.5 °C, salinity 15.2–15.7 ‰ in the area. *Pseudo-nitzschia* blooms were associated with the development of other diatom taxa (*Skeletonema costatum* (Greville) Cleve, *Cyclotella caspia* Grunow), but in June also were coincident with blooms of mixotrophic *Akashiwo sanguinea* (K. Hirasaka) G. Hansen & Ø. Moestrup, heterotrophic dinoflagellates *Polykrikos schwartzii* Bütschli and *P. kofoidii* Chatton. It should be noted that *Pseudo-nitzschia* spp. bloom in 2001 occupied a wide area of the Black Sea (Bodeanu et al., 2004; Petrova & Velikova, 2004; Vershinin et al., 2004; Terenko & Terenko, 2008). In summer 2006, the bloom of *P. delicatissima* was similar to that registered five years ago.

In May 2009, during short spring peak *Pseudo-nitzschia delicatissima* comprised about 40 % of total phytoplankton abundance and reached $3.1 \cdot 10^6$ cells l^{-1} abundance in the coastal zone of Odessa Bay (water temperature 16.8 °C, salinity 14.5 ‰). The maximum development of this species was observed after bloom of diatom large-cells *Chaetoceros curvisetus* Cleve and *Cerataulina pelagica* (Cleve) Hendey, natural spring bloom of the phytoplankton. Simultaneous

domination of these species occurred after bloom of *S. costatum* ($3.8 \cdot 10^6$ cells l^{-1}). It should be noted that the magnitude of spring blooms of *P. delicatissima* were much higher compared to those in summer. Mass development of *Pseudo-nitzschia* and other diatom species during the spring period in Odessa Bay may be associated with high concentrations of mineral and soluble organic matter coming with the river discharge from Dneprovsko-Bugsky liman.

Among the other *Pseudo-nitzschia* species that formed dense populations in the studied area were *P. delicatissima* and *P. pseudodelicatissima* (Table 4).

Vertical distribution of *Pseudo-nitzschia*. *P. pseudodelicatissima* caused the bloom in the Danube region of the Black Sea at the beginning of June 2008 (Fig. 6). The highest abundance ($10.3–12.0 \cdot 10^6$ cells l^{-1}) was recorded in the surface layer at the stations under direct influence of the Danube waters. The cell number of *Pseudo-nitzschia* varied from $12.0 \cdot 10^6$ cells l^{-1} in the surface to $150 \cdot 10^3$ cells l^{-1} in a bottom layer (average depth 18.0 m), $3.4 \cdot 10^6$ cells l^{-1} on average. So, the cell density was 3.6 times lower in deeper layers. At a distance in this area, the formation of a double layer was registered. Thermo- and halocline separated the Danube waters with a salinity of 2–12 ‰ from the underlying cold water layers of marine origin with a salinity of 16–18 ‰.

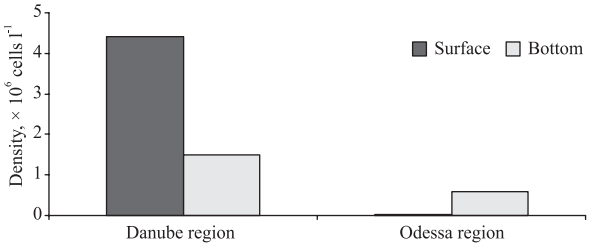

Fig. 6. Average abundance of *Pseudo-nitzschia pseudodelicatissima* in the surface and near bottom layers in the Danube region (June 2008) and Odessa region (December 2005) of the Black Sea

Pseudo-nitzschia spp. winter bloom occurrence and development during the annual cycle remains the least studied phenomena in the Black Sea. In Odessa Bay and adjacent area, *Pseudo-nitzschia* spp. dominated during the first half of December 2005, when the water temperature was 7.0–9.4 °C and salinity 12.0–14.9 ‰. *Pseudo-nitzschia* spp. abundance varied from $6.3 \cdot 10^3$ to $3.0 \cdot 10^6$ cells l^{-1} (average $3.1 \cdot 10^5$ cells l^{-1}). Cell density in the bottom layer (15–24 m) was several times higher compared to the surface layer at most studied stations, reaching a bloom level in one of them (Fig. 6). Deep phytoplankton maxima including species of the genus *Pseudo-nitzschia* in the Black Sea waters have been recorded by Nesterova & Georgieva (1992) and Mikaelyan (1995). Mikaelyan (1995) investigations on winter bloom of *Nitzschia delicatula* (syn. *Pseudo-nitzschia pseudodelicatissima*) in an open part of the sea revealed species accumulation lower the pycnocline, in a depth of 100 m. Pennate diatoms of the genus *Pseudo-nitzschia* are indicators of trophic state of marine waters (Ljubešić et al., 2011). The release of mineral and organic matter from the sediments to the near bottom water layer may promote the intensive development of *Pseudo-nitzschia*. On the other hand, the recorded considerable decrease of biogens in the bottom layer compared to the surface during winter circulation period in the coastal zone of the sea indicates high production processes. Storms during the winter period may pass phytoplankton to the bottom layer and can be a source for a secondary eutrophication in a coastal zone (Garkavaja & Bogatova, 2006). According to Rines et al. (2002), deep populations of *Pseudo-nitzschia* spp. is a recurrent phenomenon perhaps occurring annually in response to hydrographic conditions.

CONCLUSIONS

High abundance of *Pseudo-nitzschia* diatoms reaching to $16.6\ 10^6$ cells l^{-1} in the Ukrainian waters of the Black Sea probably is a result of anthropogenic influence in the coastal areas. Nevertheless, non-toxic *P. delicatissima* was found to be most often blooming species. Four potentially toxic species forming mass developments were found in the area as well. Thus, the initiation of regular monitoring of potentially toxic *Pseudo-nitzschia* species is highly necessary.

ACKNOWLEDGEMENTS

The authors express gratitude to Associate Professor of the University of Copenhagen Dr. Nina Lundholm for electron microscopy micrographs

of *Pseudo-nitzschia* species from the northwestern Black Sea.

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VYRAUJANČIOS *PSEUDO-NITZSCHIA* (*BACILLARIOPHYTA*) RŪŠYS JUODOJOJE JŪROJE (UKRAINA)

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Santrauka

Pseudo-nitzschia genties penatiniai titnagdumbliai yra jūrų aukšto trofiškumo indikatoriai bei būdingas Juodosios jūros planktocenozių komponentas. Ukrainos teritorijos vandenyse randamos šešios toksinės ir netoksinės *Pseudo-nitzschia* genties rūšys. Nuo 1980-ųjų stebėta keletas *Pseudo-nitzschia* genties dumblių „žydėjimų“ Juodojoje jūroje, kurių metu dominavo *P. delicatissima, P. pseudodelicatissima, P. pungens, P. seriata* rūšys. 2001 m. birželį *Pseudo-nitzschia* spp. „žydėjimas“ apėmė didelę Juodosios jūros teritoriją. Šiuo laikotarpiu Odesos įlankos pakrantės vandenyse šios genties dumbliai sudarė 80 % bendros fitoplanktono biomasės, o vyravo *P. delicatissima ir P. seriata* rūšys. Maksimali rūšių biomasė nustatyta, kai vandens temperatūra pasiekė 16,0–17,5 °C, o druskingumas įlankoje buvo 15,2–15,7 ‰. Intensyvus *Pseudo-nitzschia* spp. dumblių vystymasis ($3.0 \cdot 10^6$ ląstelių l^{-1}) taip pat stebėtas Odesos įlankos priedugniniuose sluoksniuose 2005 m. gruodį. 2008 m. birželio mėn. paviršiniame Juodosios jūros prie Dunojaus upės žiočių sluoksnyje intensyviai vystėsi *P. pseudodelicatissima* ($12.0 \cdot 10^6$ ląstelių l^{-1}) rūšis. Trumpalaikis *P. delicatissima* ($3.1 \cdot 10^6$ ląstelių l^{-1}) vystymosi pikas stebėtas 2009 m. gegužę Odesos įlankos paviršiniame vandens sluoksnyje. Taigi, *Pseudo-nitzschia* genties titnagdumbliai gali intensyviai vystytis bet kuriuo metų sezonu, o „žydėjimai“ yra gana dažnas reiškinys Juodojoje jūroje.