OBSERVATIONS ON *PSEUDO-NITZSCHIA* SPECIES IN THE BAY OF KOTOR, SE ADRIATIC SEA

Suncica Bosak [1]*, Lucija Horvat [2], Branka Pestoric [3] and Sladjana Krivokapic [4]
[1] University of Zagreb, Faculty of Science, Division of Biology, Zagreb, Croatia - sbosak@biol.pmf.hr
[2] Rudjer Boskovic Institute, Department of Molecular Biology, Zagreb, Croatia
[3] Institute of Marine Biology, Kotor, Montenegro
[4] University of Montenegro, Faculty of Science, Department of Biology, Podgorica, Montenegro

Abstract
The diatom genus *Pseudo-nitzschia* includes several species capable of producing the neurotoxin domoic acid, the causative agent of Amnesic Shellfish Poisoning (ASP). The scope of this study was to identify species present in the Bay of Kotor, SE Adriatic Sea from the samples collected during the 2008/2009 survey. TEM examination revealed presence of four morphotypes: P. *pseudodelicatissima*, P. cf. *pseudodelicatissima*, P. *calliantha* and P. *subfraudulenta*.
Keywords: Diatoms, Adriatic Sea, Phytoplankton

Introduction
Marine planktonic pennate diatoms of the genus *Pseudo-nitzschia* are widespread and abundant in coastal and oceanic waters all over the world. The genus has received much attention following the discovery that some species are capable of producing the neurotoxin domoic acid, the causative agent of Amnesic Shellfish Poisoning (ASP). There are a few reports about occurrence and ecology of these diatom species from the southern Adriatic coastal waters [1] but none from the Bay of Kotor, a deeply indented bay located in the south eastern coast. This paper represents the first detailed report on findings of Pseudo-nitzschia species in this area.

Material and methods
Sampling was carried out seasonally in 2008/2009 at three stations in the innermost part of the Bay of Kotor. Water samples were collected with Niskin bottles and sub-samples for phytoplankton analysis preserved with neutralized formaldehyde at a final concentration of 1.4%. Phytoplankton composition was identified and enumerated according to Ütermohl. Transmission electron microscope (TEM) observations were made by deposition of acid cleaned material onto firmware metal grids and examination with a Zeiss EM10A microscope.

Results and discussion
The *Pseudo-nitzschia* species found in the Kotor Bay were identified with light microscopy as members of the P. *delicatissima* and P. *seriata* group, based on their valve width (1.5 – 2.8 µm and 4.3 - 6.7 µm, respectively). The cell ends in girdle view were pointed and elongated in the former morphotype therefore it was identified as P. *pseudodelicatissima* species complex. The latter was designated as P. *subfraudulenta* species from the P. *fraudulenta/subfraudulenta* group based on the shape of the valve which was more linear in the mid valve than lanceolate [2]. The cell length range was 85.6 - 195.6 µm. The overlap of cell ends was 1/6-1/4 of cell length. Upon TEM examination, specimens showed 13–17 fibulae and 22–28 striae in 10 µm. The central larger interspace was present and had the width of 5 valve striae. Each stria was biseriate with roundish poroids divided in several sectors separated by smooth space (6 - 7 in 1 µm). The ultrastructural features of the frustules confirmed the species as P. *subfraudulenta* (Hasle) Hasle (Fig. 1. A). Based on the ultrastructural features visible in TEM, P. *pseudodelicatissima*-like specimens were attributed to three distinct morphotypes. Only one morphotype was present in the samples collected in the spring period and together with P. *subfraudulenta* in autumn and winter samples. The cell length range of these cells was 14.3 – 76.8 µm and they overlapped in chains in 1/10 - 1/5 of cell length. Valves had 18 - 22 fibulae and 38 - 40 striae in 10 µm with presence of the central interspace. Each stria was ornamented with square poroids (5 poroids in 1 µm) split into 7 - 10 sectors with central sector present in more than 70% of the poroids. According to these features cells belonging to this morphotype were ascribed to P. *calliantha* Lundholm, Moestrup et Hasle (Fig. 1. B). Second and third morphotype co-occurred in the samples with measured cell length range of 38.9– 144.1 µm and overlap 1/12-1/7 of cell length. The shape of the valve was more linear in LM therefore it was not identified as P. *cuspidata* which has more lanceolate shape of the valves [2]. In TEM specimens of the second morphotype were designated as P. *pseudodelicatissima* (Hasle) Hasle emend. Lundholm, Hasle et Moestrup (Fig. 1. C). Cells had 40 - 44 striae and 22 - 26 fibulae in 10 µm. Striae were uniseriate with 4 - 6 oval to square poroids in 1 µm. In more than 50% of poroids hymen was divided into two large parts. However, in the same samples specimens were present with higher percentage of poroids divided into more sectors (4 - 6). These are not typical features of P. *pseudodelicatissima* and these cells had 40-44 striae and 20-22 striae per 10 µm. Given the possibility that these specimens belong to different species this third morphotype was refered to *P.* cf. *pseudodelicatissima* (Fig.1. D).

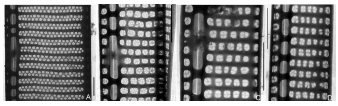

Fig. 1. TEM images: (A) P. subfraudulenta, (B) P. calliantha, (C) P. pseudodelicatissima(D) P. cf. pseudodelicatissima

P. pseudodelicatissima (both second and third morphotype) was found only in the summer with cell concentrations in surface waters of 1.2 x 10^5 cells L^{-1} and it contributed up to 70% of the microphytoplankton. P. *fraudulenta* and potentially toxic P. *calliantha* [4] were present in lower concentrations during the rest of the year with abundances up to 10^4 cells L^{-1} constituting up to 29% and 50% of microphytoplankton, respectively. The cell numbers and environmental parameters for each season are given in Tab 1.

Tab. 1. Seasonal range (min - max) and mean±SD of physical and biological parameters. For all abundance values minimum was 40 cells L^{-1}.

	spring	summer	autumn	winter
Temperature (°C)	14.1 – 15.4 (14.8 ± 0.5)	27.4 – 27.9 (22.3 ± 3.9)	14.6 – 15.9 (17.4 ± 1.6)	12.7 – 14.7 (12.5 ± 0.9)
Salinity	3.2 – 36.0 (26.1 ± 10.7)	28.0 – 36.2 (33.0 ± 2.8)	8.2 – 36.6 (28.6 ± 10.6)	10.1 – 37.0 (31.2 ± 8.5)
Oxygen saturation (%)	67 – 88 (75 ± 7)	104 – 114 (105 ± 4)	85 – 95 (90 ± 3)	61 – 71 (68 ± 2)
Chl *a* (µg L^{-1})	0.2 – 1.1 (0.6 ± 0.3)	0.09 – 1.4 (0.4 ± 0.4)	0.09 – 1.7 (0.7 ± 0.9)	0.3 – 1.9 (0.7 ± 0.4)
Microphytoplankton (cells L^{-1})	1.0×10^3 (0.2×10^3 – 4.8×10^3)	1.8×10^5 (0.1×10^5 – 1.1×10^5)	8.9×10^3 (1.8×10^3 – 1.9×10^4)	1.2×10^4 (2.8×10^3 – 3.8×10^4)
Diatoms (cells L^{-1})	3.9×10^3 (0.6×10^3 – 8.5×10^3)	2.6×10^5 (0.8×10^5 – 9.5×10^5)	1.7×10^4 (1.1×10^4 – 2.2×10^4)	1.2×10^5 (1.8×10^4 – 3.9×10^5)
Pseudo-nitzschia seriata group (cells L^{-1})	n.d.	n.d.	6.8×10^3 (0.9×10^3 – 1.6×10^4)	1.9×10^4 (1.2×10^4 – 5.2×10^4)
Pseudo-nitzschia delicatissima group (cells L^{-1})	1.6×10^3 (1.3×10^3 – 1.8×10^3)	1.2×10^5 (1.6×10^4 – 9.1×10^4)	7.3×10^3 (1.2×10^3 – 1.9×10^4)	4.2×10^3 (7.8×10^2 – 1.3×10^4)

Pseudo-nitschia calliantha, a species for whom the production of domoic acid is well documented [3], represents frequent component of the Kotor Bay phytoplankton assemblages. Blooms of this species are known from Italian coastal waters [1] as well from the middle eastern Adriatic coast [4] and despite the fact that in this investigation it was not recorded in high abundances, it is most likely that blooms of this species occur in these waters. Examination of *Pseudo-nitzschia* species distribution and related environment factors that affect their abundance are therefore essential for predicting potentially harmful algae blooms in the area as it sustains active shellfish farming.

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