Ocean Science Journal, Vol. 43 No. 1, 25-30(2008)

Article

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Potentially Toxic Diatoms *Pseudo-nitzschia fraudulenta* and *P. calliantha* from Russian Waters of East/Japan Sea and Sea of Okhotsk

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Received 14 September 2007; Revised 11 December 2007; Accepted 5 February 2008

Abstract – Potentially toxic diatoms *Pseudo-nitzschia calliantha* and *P. fraudulenta* were found in bottle samples of phytoplankton collected in Amurskii Bay (East/Japan Sea) and in the coastal waters of Sakhalin Island (East/Japan Sea and Sea of Okhotsk) in different seasons during 2002-2006. The mass development of these species occurred in October and November 2002 at water temperatures of 6-16°C and salinities of 28.8-33.5 PSU. The highest concentrations of *P. calliantha* and *P. fraudulenta* were about 2×10^5 cells L^{-1} and 1.5×10^5 cells L^{-1}, respectively. *P. fraudulenta* was found for the first time in the Russian waters of the East/Japan Sea. Morphological descriptions of these species based on observation with light and electron microscopy and information on their ecology are presented. Data on the geographical distribution of these species are supplemented.

Key words – diatoms, *Pseudo-nitzschia calliantha, Pseudo-nitzscha fraudulenta*, morphology

1. Introduction

The marine diatoms of the genus *Pseudo-nitzschia* attract special attention of researchers. Some species of this genus are capable of producing domoic acid known as neurotoxin, which, accumulating in tissues of filter-feeding mollusks, can be transported through a food chain and harmfully affect nervous and digestive systems of man, sea mammalians and birds. The toxic effect is known as "Domoic Acid Poisoning" or "Amnesic Shellfish Poisoning" (Bates *et al*. 1989; Skov *et al.* 1999).

From the beginning of the 1990s until the present time, the incidents of "water bloom" have occurred repeatedly in Peter the Great Bay within East/Japan Sea and in coastal waters of Sakhalin within East/Japan Sea and Sea of Okhotsk. These blooms result from mass development of potentially toxic diatom genus *Pseudo-nitzschia: Pseudo-nitzschia pungens, Pseudo-nitzschia multiseries* and *Pseudo-nitzschia pseudodelicatissima.* However, only a few studies on morphology and ecology of mass species of *Pseudo-nitzschia* from these areas are known (Orlova and Stonik 2001; Stonik *et al.* 2001).

In the autumn of 2002, an outburst of *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia fraudulenta* was recorded in an artificial seawater reservoir within the city of Vladivostok (Amurskii Bay, East/Japan Sea). These species were also found by us when studying bottle samples of phytoplankton collected at the western coast of Sakhalin Island. *P. calliantha* and *P. fraudulenta* are known as potentially toxic species widely spread in the seas of tropical and temperate latitudes of the Northern and Southern hemispheres; quite often the diatoms cause "water blooms" (Takano and Kuroki 1977; Hallegraeff 1994; Hernandez-Becerril 1998; Hasle 2002; Lundholm *et al.* 2003).

No information on these species from the Far Eastern seas of Russia is available except a mention of *P. fraudulenta* in the list of phytoplankton species from the Aniva Bay (Orlova *et al*. 2004). No data on the cell abundances of *Pseudo-nitzschia fraudulenta* in the East/Japan Sea are available.

The present paper describes a case of mass development *P. fraudulenta* and *P. calliantha* in the East/Japan Sea. It was the first discovery of *P. fraudulenta* in Russian waters

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of the East/Japan Sea. Our study with the use of light microscopy (LM) and transmission electron microscopy (TEM) allowed us to give morphological descriptions of *P. fraudulenta* and *P. calliantha*; we present also the ecological characteristics of the species, and expand information on the geographical distribution of these diatoms.

2. Materials and Methods

The study used bathymetric samples of phytoplankton collected in different seasons in 2002-2006 at five stations located in Amurskii Bay within East/Japan Sea and in western coastal waters of Sakhalin Island within East/Japan and Okhotsk Seas (Fig. 1, Table 1). Station 1 was located in a eutrophicated semi-closed man-made seawater basin within the city of Vladivostok. This seawater basin (about 70 000 sq. m) is separated from Amurskii Bay by a rock-fill coffer-dam and has a free water exchange with the bay through a shallow canal (Begun *et al.* 2004). As a rule, weekly series of phytoplankton sampling were carried out in Amurskii Bay at station 1 in August-December of 2002. Phytoplankton samples were collected monthly at western coast of Sakhalin Island at stations 2, 4 and 5 in July 2005, and at station 3 in June 2006.

Samples for quantitative analysis were taken from the surface horizon (0-0.5 m) with the use of a 4-liter Molchanov bathometer. One-liter sample was fixed with Utermöhl's solution and concentrated through sedimentation (Utermöhl 1958) to a volume of 10-15 mL. Algal cells were counted in a 0.05–1 mL Nojott chamber. During high-biomass bloom, a direct cell count was performed in non-concentrated water samples.

Live cells as well as fixed material were examined, photographed and measured using a light microscope (LM) Olympus BX41 (Olympus, Tokyo, Japan). To study the valve structure, material was examined with a JEM-100 S transmission electron microscope (TEM) (JEOL, Tokyo, Japan). Cleaned frustules were treated with 40% H_2SO_4, and then washed with deionized water. For TEM examinations, a drop of rinsed sample was placed on a Formvar-coated grid and air-dried. Terminology follows the works of Anonymous (1975), Ross *et al.* (1979), Mann (1981), and Round *et al.* (1990).

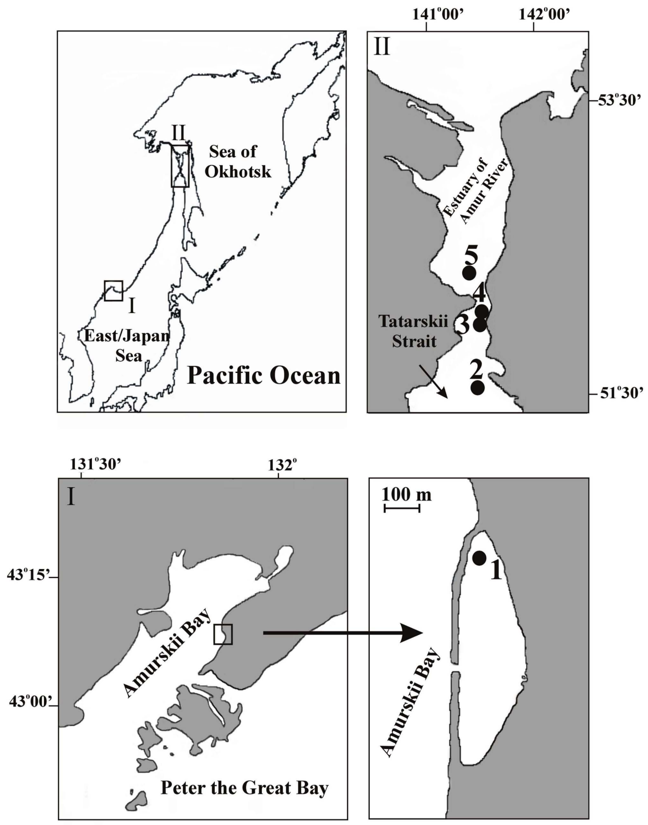

Fig. 1. Location of the sampling stations (1-5). I- Amurskii Bay, East/Japan Sea, II- Western coastal waters of Sakhalin Island, East/Japan Sea and Sea of Okhotsk.

3. Results

Pseudo-nitzschia calliantha Lundholm, Moestrup et Hasle (Figures 2a-d)

· **References**

Lundholm *et al.* (2003), p. 801, fig. 2 A-G.

Table 1. Position of the sampling stations in Russian waters of East/Japan Sea and Sea of Okhotsk

Area	No stations	Sampling date	Latitude N	Longitude E
Amurskii Bay (East/Japan Sea)	1	31 Aug. 2002 –13 Dec. 2002	43°09'	131°54'
Tatarskii Strait (East/Japan Sea)	2	12 Jul. 2005	51°35'	141°25'
	3	10 Jun. 2006	52°02'	141°29'
	4	18 Jul. 2005	52°05'	141°33'
Estuary of the Amur River (Sea of Okhotsk)	5	17 Jul. 2005	52°18'	141°22'

· Description

Overlapping cells form stepped colonies (Fig. 2a). Transapical axis of valves is 1.5-1.8 μm and apical axis is 45-55 μm. Valves are linear in valve view and tapering parts near the tips are very short (Fig. 2b). Eccentric raphe is divided in the middle by a central nodule (Fig. 2c). The number of transapical striae is 31-37 and the number of fibulae is 15-17 in 10 μm. Striae have one row of square to round poroids numbering 4-5 in 1 μm. The hymen of poroids is perforated by 4-10 sectors, resembling a flower pattern. The valve mantle is structured as valve face with 1-2 poroid high (Fig. 2d).

· Remarks

P. calliantha has been recently described as a new species as a result of taxonomical revision of the *Pseudo-nitzschia pseudodelicatissima/cuspidata* complex (Lundholm *et al.* 2003). The structure of the poroid hymen of *P. calliantha*, resembling a flower pattern, is obviously different from the bipartite hymens of *P. pseudodelicatissima* and *P. cuspidata*. The structure of the poroid hymen of *P. calliantha* is also clearly different from *P. caciantha* poroid hymen, comprising four to five sectors (Lundholm *et al.* 2003).

Pseudo-nitzschia fraudulenta (P.T. Cleve) Hasle
(Figures 2e-h)

· Basionym

Nitzschia fraudulenta P.T. Cleve (1897), p. 300, pl. 11.

· References

Hasle (1993), p. 318; Hallegraeff (1994), p. 398, figs. 2 a-k; Hernandez-Becerril (1998), p. 80, figs 8-11.

· Description

Overlapping cells are arranged in colonies (Fig. 2e). Transapical axis of valves is 4-7.5 μm and apical axis is 92-107 μm. Cells are lanceolate in valve view, narrowing towards the pointed ends (Fig. 2f, g). Eccentric raphe is divided in the middle by a central nodule. The number of striae is 19-24 and the number of fibulae is 16-24 in 10 μm. The striae have 2 rows (at the cell ends) or 2-3 rows (in the middle part of valve) of round large poroids numbering 5-8 in 1 μm (Fig. 2h, i). The hymen of poroids is perforated by 4-8 sectors with an irregular unperforated part in the middle (Fig. 2j).

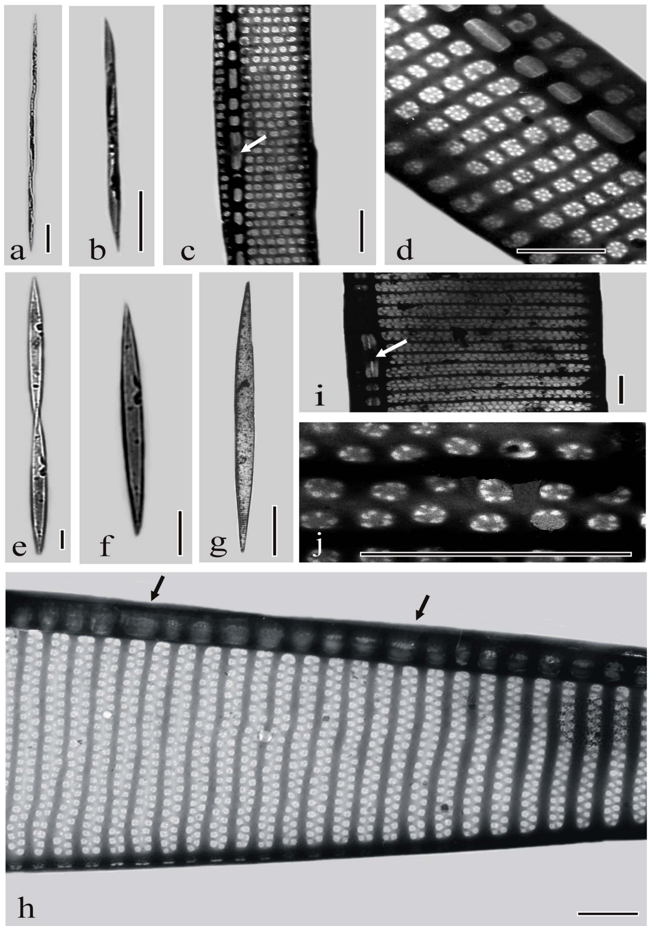

Fig. 2. (a)-(d) *Pseudo-nitzschia calliantha*, (a) chain of three cells, (b) whole valve, (c) middle part of valve showing central nodule (arrow); (d) – part of valve showing mantle and valve striae structure; (e)-(j) *Pseudo-nitzschia fraudulenta*, (e) chain of cells showing cell overlap 1/6-1/7 of total cell length, (f) whole valve, (g) part of valve showing striae and fibulae, (h) part of valve showing unequal number of striae and fibulae (arrows), (i) middle part of valve showing central nodule (arrow), (j) structure of valve striae. (a, b, e, f) LM, (c, d, g-j) TEM. Scale bar is equal to 10 μm (a, b, e-g) and 1 μm (c, d, i, j, h).

· Remarks

The species was found for the first time in Russian waters of the East/Japan Sea. Generally, the morphological description of the frustule of *P. fraudulenta* from Amurskii Bay and from the coastal waters of Sakhalin agrees well with the descriptions presented by other authors (Hasle 1965; Hallegraeff 1994; Hasle *et al*. 1996). A majority of descriptions of *P. fraudulenta* presented in the literature indicates that the number of the fibulae coincides approximately with number of transapical striae (Hallegraeff 1994; Hernandez-Becerril 1998; Hasle *et al*. 1996). However, according to our data, the numbers of transapical striae and fibulae in *P. fraudulenta* specimens varied significantly. So, we investigated with the use of TEM fifteen specimens from the samples collected in the period of outbreak of the species. In four cells, the number of striae (22-24) did not coincide with the number of fibulae (16-20) (Fig. 2h). In other specimens, the numbers of striae (19-24) and fibulae (18-24) were nearly the same. Similar data on discrepancy of the numbers of striae and fibulae in *P. fraudulenta* specimens from the coastal waters of the Northern Atlantic are presented by Hasle (1965). Probably, the marked differences in the numbers of transapical striae and fibulae result from morphological variability inherent in the local populations of this species from various areas of the world ocean.

In phytoplankton of the Amurskii Bay, *P. calliantha* was sampled from August till November at water temperatures of 3-24°C and salinities of 23-33.5 PSU. The species *P. fraudulenta* was recorded in phytoplankton from September till December at water temperatures of 1-16°C and salinities of 28.3-33.5 PSU. For the period of our study, cell counts of diatoms *P. calliantha* and *P. fraudulenta* varied within the range from 9×10^3 to 2.1×10^5 cells L^{-1} and from 0.2×10^3 to 1.5×10^5 cells L^{-1}, respectively. Mass development of the genus *Pseudo-nitzschia* occurred in October - November at water temperatures of 6-16°C and salinities of 28.8-33.5 PSU on the background of low abundace of phytoplankton (0.5×10^6 cells L^{-1} -1.2×10^6 cells L^{-1}). In the period, two peaks of cell densities of *Pseudo-nitzschia* spp. (Fig. 3) were registered. The first relatively low peak of density (1.2×10^5 cells L^{-1} for *P. calliantha* and 1×10^5 cells L^{-1} for *P. fraudulenta*) was observed in the first half of October at water temperatures of 14-16°C and salinities of 28.8-31 PSU. *P. calliantha* and *P. fraudulenta* contributed, respectively, about 10% and 20% of total cell counts of phytoplankton. In this season, the diatom *Skeletonema costatum* predominated quantitatively with 67-70% contribution to the total cell counts of the phytoplankton community. This diatom species is a typical component of autumn phytoplankton in Amurskii Bay and an eutrophic level indicator (Stonik and Orlova 2002). The second maximum in cell numbers of *P. calliantha* (up to 2.1×10^5 cells L^{-1}) and *P. fraudulenta* (up to 1.5×10^5 cells L^{-1}) was recorded in the beginning of November with rapid decline of water temperature to 6°C and a slight increase of salinity to 33.5 PSU. In this period, *P. calliantha* and *P. fraudulenta* contributed, respectively, 38% and 27% of the total cell densities of phytoplankton. Besides *Pseudo-nitzschia* species, other typical diatom species of Amurskii Bay for autumn season also developed in considerable amounts: *Thalassionema nitzschioides, S. costatum,* and *Chaetoceros curvisetus*.

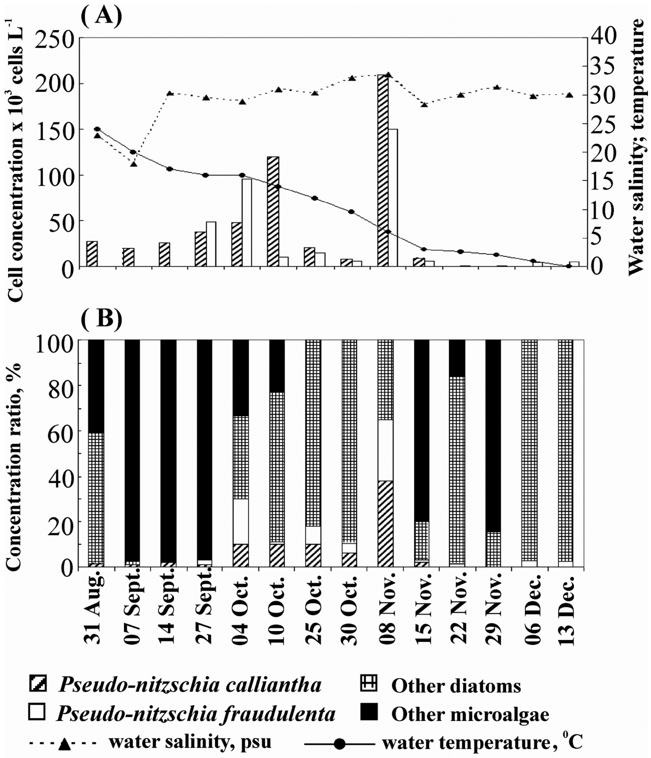

Fig. 3. Dynamics of the population densities (cells L^{-1}) of *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia fraudulenta* in relation to salinity and temperature variations (A), and the ratio of the population densities of *Pseudo-nitzschia calliantha*, *Pseudo-nitzschia fraudulenta* and other groups of phytoplankton (B) at station 1 during August-December 2002.

In the coastal waters around Sakhalin Island, *P. calliantha* was found at Stations 2, 3, and 5 between June and July. Population density of the species varied from 0.3×10^3 cells L^{-1} to 8×10^4 cells L^{-1}. The maximum density of P. *calliantha* was registered at Station 5 in Estuary of the Amur River in July with water temperature of 14.1°C and salinity of 28.8 PSU. *P. fraudulenta* was recorded at Stations 3, 4, and 5 in June – July. In this period, total density of phytoplankton varied within the range from 0.4×10^3 to 4×10^4 cells L^{-1}. The highest density of this species was registered in Tatarskii Strait at Station 4 in July with water temperature of 9.5°C and salinity 13.7‰.

4. Discussion

The data obtained in the study show that *P. calliantha* and *P. fraudulenta* are predominant in the fall season in Peter the Great Bay, with cell abundances exceeding 10^5 cells l^{-1} during this period and comprising 30-40% of the total phytoplankton abundance. The mass development of these species was recorded by us in October - November at water temperatures of 6-16°C; that agrees well with the data available on vegetation of *P. fraudulenta* in waters of the Northern Atlantic at water temperatures of 9-14.4°C (Hasle, 1965) and vegetation of *P. calliantha* at the coast of Canada at water temperatures of 10-18°C (Martin *et al*. 1993).

Unfortunately, plankton samples at the western coast of Sakhalin were taken only in summer. In this connection, the data obtained in our study on the ecology of *P. calliantha* and *P. fraudulenta* in this area shall be considered only as preliminary. No doubt, an expansion of the sampling period in these areas and the use of electron microscopy will significantly extend our knowledge on species composition and distribution of diatoms belonging to the genus at the western coast of Sakhalin Island.

The species considered in the present work are widespread both in the equatorial area, and in neritic and oceanic waters of temperate latitudes of the Northern and Southern hemispheres (Hasle 2002; Lundholm *et al*. 2003). Only limited data on distribution of *P. calliantha* in the Northern Pacific are available and include records at the coasts of Chile, Australia and Vietnam (Lundholm *et al*. 2003). In our research, the species was found in Amurskii Bay, in the northern part of the East/Japan Sea (Station 2 and 3), and also in the Sea of Okhotsk (Station 5). Thus, the northernmost record of *P. calliantha* within the Northern Pacific is limited to the Estuary of the Amur River at about 52°18 N, 141°22 E.

The species *P. fraudulenta* has been found in the Northern Pacific at the western coast of the USA, the coasts of Mexico, Chile, Australia, New Zealand, South Korea, and Japan (Cho *et al*. 2002; Hasle 2002). These data can be supplemented by our findings of *P. fraudulenta* in Amurskii Bay, in the northern part of the East/Japan Sea (Station 3 and 4), and also in the Sea of Okhotsk (Station 5). As Hasle (2002) noted, the northernmost limit of distribution of *P. fraudulenta* within the Pacific Ocean runs at the western coast of the USA at about 48°30 N. Our data allow us to move this northernmost limit of distribution northwards to the area of the Estuary of Amur River within the Sea of Okhotsk (52°18 N, 141°22 E). Thus, our evidence reveals a wider range of *P. calliantha* and *P. fraudulenta* distribution in waters of the Northern Pacific in comparison with the earlier literature data and confirms the assumption of Hasle (2002) on cosmopolitan distribution of these species.

P. calliantha and *P. fraudulenta* are known as potentially toxic species capable of producing domoic acid (Martin *et al*. 1990; Rhodes *et al*. 1998; Lundholm *et al*. 2003). Concentration of domoic acid in the cells of these species during the period of their mass development has not yet been examined. However, it has been found that the abundance of these potentially toxic species may exceed the maximum safe limit (10^3-10^5 cells L^{-1}) in the autumn season when, in countries with a well-developed mariculture, the harvest of edible marine mollusks is frozen (Andersen 1998). In this connection, we find it necessary to perform plankton monitoring in order to control the appearance and spread of potentially toxic diatoms as well as to conduct shellfish monitoring throughout Amurskii Bay, where commercial aquaculture farms and recreational zones are situated.

Acknowledgements

This research was supported by the grant RFBR 06-04-48649 and by the Far Eastern Branch of the Russian Academy of Sciences (FEB RAS), through grants 06-I-II-16-057, 06-I-II-11-034, and 06-III-A06-167 to T.Yu. Orlova, and by grant 04-04-49747 and the Russian Science Support Foundation to I.V. Stonik.

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