Annual cycle of *Pseudo-nitzschia* species in Outer Oslofjorden, Norway

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Abstract

The annual cycle of the diatom genus *Pseudo-nitzschia* was examined in Outer Oslofjorden, Norway. Vertical net tows (2 5- 0 m depth) and seawater samples (1m depth) were collected monthly for one year (June 2009 -June 2010). The diversity of *Pseudo-nitzschia* species were recorded from live and preserved material with light microscopy and from acid cleaned samples viewed in scanning (SEM) and transmission electron microscopy (TEM). *Pseudo-nitzschia* species were present in every sample collected during the year. Nine species of the genus were identified, of which eight are potentially toxic: *Pseudo-nitzschia delicatissima, P. pseudodelicatissima, P. calliantha, P.* cf. *cuspidata, P. pungens, P. multiseries, P. fraudulenta* and *P. seriata.* Two species, *Pseudo-nitzschia calliantha* and *P. delicatissima*, were the most frequently observed and present in 9 out of 11 samples. The highest concentration of *Pseudo-nitzschia* spp. was recorded in January 2010 (1, 61 x 10^6 cells L^{-1}).

Introduction

Species of the genus *Pseudo-nitzschia* H. Peragallo (H. & M. Peragallo, 1900) are widely distributed and present in all biogeographic zones (Hasle 2002; Casteleyn *et al.* 2008). Earlier investigations from Norwegian waters have shown a considerable variation in the species composition of the genus *Pseudo-nitzschia*, both geographically and seasonally (Hasle *et al.* 1996). The genus contains more than 30 species and several of them are found in Norwegian waters (e.g. *P. delicatissima, P. fraudulenta, P. granii, P. heimii, P. pungens, P. multiseries, P. calliantha, P. seriata, P. obtusa, P. americana)*. Twelve species of *Pseudo-nitzschia* have been documented to produce domoic acid (DA) a neurotoxin that causes amnesic shellfish poisoning (Moestrup 2005; Moschandreou *et al* 2010). The ability to produce DA varies among species, thus an exact identification and knowledge about their geographical and seasonal occurrence at the species level is important. Monitoring of microalgae (including toxic species like *Pseudo-nitzschia*) is based on light microscopy; nevertheless a precise identification of *Pseudo-nitzschia* at the species level requires verification by electron microscopy and/or molecular biological tools.

The genus *Pseudo-nitzschia* is a common component of the phytoplankton in Norwegian waters. The aim of the present study is to examine the composition and abundance through the annual cycle of *Pseudo-nitzschia* in Outer Oslofjorden in the Northern Skagerrak.

Materials and methods

Samples were collected monthly during one year period from June 2009 to June 2010, at station OF2 (59.186668N, 10.691667E) in Northern Skagerrak, Norway. Seawater samples collected at 1 m depth were preserved in Lugol's solution (1% of final concentration) and cell counts were made according to the protocol of Uthermöhl (1958). Vertical net-tows (20 µm mesh size) from 25 - 0 m depth were preserved with formaldehyde (2% final concentration) and with Lugol's

solution (1% final concentration). In order to remove organic material the formaldehyde preserved net samples were acid cleaned (Throndsen *et al.* 2007). The cleaned frustules were mounted on stubs and grids and viewed in a Hitachi FEG S-4800 SEM and Philips CM-1000 TEM.

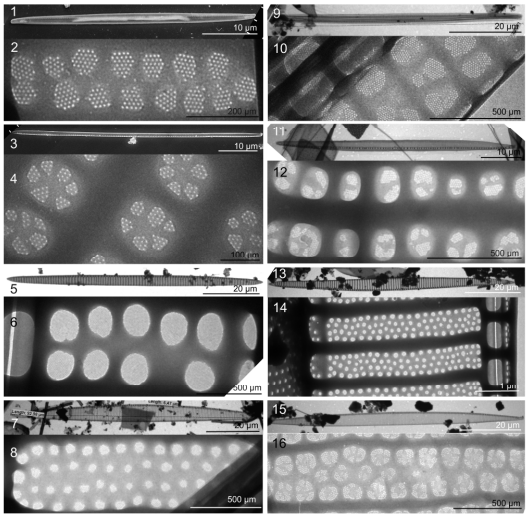

Figure 1. SEM and TEM micrographs of eight potentially toxic *Pseudo-nitzschia* species; *delicatissima* – group: (1, 2) *P. delicatissima*; (3, 4) *P. calliantha*; (9, 10) *P. pseudodelicatissima*; (11, 12) *P.* cf. *cuspidata.; seriata* – group: (5, 6) *P. pungens*; (7, 8) *P. seriata;* (13, 14) *P. multiseries*; (15, 16) *P. fraudulenta.*

Results

Occurrence and cell counts

Pseudo-nitzschia species were present in all samples with densities ranging from 500 cells L^{-1} in June 2010 to 1.61 million cells L^{-1} in January 2010 (Table 1). In January 2010, the high cell density was mainly related to a bloom of *P. delicatissima* and *P. calliantha*. The *delicatissima*-group (species with valve width less than ca. 3 µm) dominated all samples except the April and May samples of 2010.

Species diversity and identification

Pseudo-nitzscia species identification was based on morphometric characteristics (Hasle *et al.*1996, Lundholm *et al.* 2003). A total of ten species of the genus *Pseudo-nitzschia* were found, nine of them were identified: *P. calliantha, P. delicatissima, P. pseudodelicatissima, P.* cf. *cuspidata*, *P. fradulenta, P. seriata, P. pungens, P. multiseries* and *P. americana*. An unidentified *Pseudo-nitzschia* species resembles *P. pseudodelicatissima* in all morphometric characteristics but differs in poroid structure. *Pseudo-nitzschia calliantha* and *P. deli-catissima* were the most frequently observed and present in 9 out of the 11 examined samples. The species composition changed through the year and the number of species present in one sample varied from one in June 2010 to seven in June and October 2009 (Table 1).

Table 1. Abundance and monthly occurrence of *Pseudo-nitzschia* spp. during June 2009- June 2010 in Outer Oslofjord, Norway.

***Pseudo-nitzschia* species**	**22.06 2009**	**05.08 2009**	**22.09 2009**	**20.10 2009**	**17.11 2009**	**09.12 2009**	**21.01 2010**	**11.03 2010**	**13.04 2010**	**11.05 2010**	**22.06 2010**
***Pseudo-nitzschia* spp. cells L^{-1}**	**99100**	**15000**	**217500**	**91500**	**150100**	**11400**	**1615900**	**141200**	**1400**	**800**	**500**
***delicatissima*- group**											
P. calliantha	x	x	x	x	x	x	x	x		x	
P. delicatissima	x			x	x	x	x	x	x	x	x
P. pseudodelicatissima	x			x							
*P.*cf. *cuspidata*					x	x					
Pseudo-nitzschia sp.	x			x							
***seriata*- group**											
P. fraudulenta	x	x		x						x	
P. seriata	x	x						x	x	x	
P. pungens	x		x	x	x	x				x	
P. multiseries				x	x						
P. americana						x	x				

Discussion

Occurrence and cell density of *Pseudo-nitzschia* spp. varied through the seasons, with a more or less gradual increase from June 2009 until January 2010 (bloom) followed by a subsequent decrease. The *delicatissima*- group dominated the samples and the most common *Pseudo-nitzschia* species were *P. calliantha* and *P. delicatissima* (the only representative of the genus in June 2010). Eight potentially toxic *Pseudo-nitzschia* species were detected in the present study: *Pseudo-nitzschia pungens*, *P. multiseries*, *P. fraudulenta, P. seriata*, *P. delicatissima*, *P. pseudodelicatissima, P. calliantha* and *P.* cf. *cuspidata* (Fig. 1). Seven of the nine identified *Pseudo-nitzschia* species have earlier been encountered regularly in Norway; the exceptions are P. *cuspidata* and *P. pseudodelicatissima* that are recent additions to the Skagerrak phytoplankton flora.

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