



Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Blooms of the potentially toxic diatom *Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle in coastal waters of the northern Adriatic Sea (Croatia)

Daniela Marić^{a,*}, Zrinka Ljubešić^b, Jelena Godrijan^a, Damir Viličić^b, Ivana Ujević^c, Robert Precali^a

^a Center for Marine Research, Ruđer Bošković Institute, G. Paliaga 5, 52210 Rovinj, Croatia

^b University of Zagreb, Faculty of Science, Department of Botany, Rooseveltov trg 6, 10000 Zagreb, Croatia

^c Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovića 63, 21000 Split, Croatia

ARTICLE INFO

Article history:

Received 16 June 2010

Accepted 10 January 2011

Available online xxx

Keywords:

Pseudo-nitzschia sp.

phytoplankton

Pseudo-nitzschia calliantha

domoic acid (DA)

Adriatic sea

ABSTRACT

The genus *Pseudo-nitzschia* contains more than 30 species, though most of them are discernible only on the basis of ultrastructural or genetic differences. We present a taxonomic study of *Pseudo-nitzschia calliantha* Lundholm, Hasle & Moestrup in the coastal waters of the northern Adriatic Sea together with domoic acid (DA) measurements on natural and breeding populations of shellfish. Sampling carried out monthly at 13 stations during 2007 showed *Pseudo-nitzschia* species to be present with high abundances during the entire year, forming blooms in coastal waters (reaching abundances of up to 1.2×10^6 cells L⁻¹) during the late summer/early autumn, when they contributed up to 70% of the whole phytoplankton community. Ultrastructural examination provided evidence that the observed blooms were formed by the potentially toxic species *P. calliantha*. After the *P. calliantha* bloom, DA was detected at 3 stations along the north-eastern Adriatic coast with concentrations ranging from 0.11 to 1.32 µg g⁻¹ in shellfish tissue. The distribution patterns of the species were analyzed statistically in order to explain the relationships between environmental variables and population dynamics. We here report on a mass appearance of the potentially toxic *P. calliantha* in the coastal waters of the northern Adriatic.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Diatoms of the genus *Pseudo-nitzschia* are common constituents of marine phytoplankton worldwide and especially in temperate seas (Hasle, 2002). The genus *Pseudo-nitzschia* (Bacillariophyta) comprises more than 30 species of which several, including *P. calliantha*, are reported to be domoic acid (DA) producers (Lundholm et al., 2003). Diatoms are important primary producers at the base of the food web in the northern Adriatic. Several *Pseudo-nitzschia* species are thereby introducing DA to higher trophic levels which make it even more important to monitor and control the genus *Pseudo-nitzschia* with species level resolution. The genus *Pseudo-nitzschia* is often subdivided into two light microscope-discernible groups. Cells wider than 3 µm are combined into the so called *P. seriata* group, while cells less than 3 µm in width are gathered in the so called *P. delicatissima* group. Most species within the latter group are only determinable after ultrastructural examination. The *P. delicatissima* group comprises more than 12 potentially toxic species producing DA, the cause of amnesic shellfish poisoning (Jeffery et al., 2004).

Pseudo-nitzschia calliantha Lundholm, Hasle & Moestrup, was described during the revision of two morphologically similar species: *P. pseudodelicatissima* and *P. cuspidata* (Hasle) emend. Lundholm, Hasle & Moestrup. The revision was based on valve dimensions, fine structure of the hymen poroids, cingulum morphology and molecular data (Lundholm et al., 2003). Investigations on *P. calliantha* distribution and ecological preferences have generated considerable scientific interest since it is probably a cosmopolitan species associated with the production of DA (Lundholm et al., 1997, 2003). In the Adriatic Sea *P. calliantha* was discovered for the first time in samples from two sites in the northern and middle Adriatic (Lundholm et al., 2003). The species was also reported from the southern part along the western Adriatic coast and from one site in the eastern-middle Adriatic where it was reported together with population dynamics in relation to physical and chemical variables (Burić et al., 2008).

Different species of the genus *Pseudo-nitzschia* are present in phytoplankton assemblages of the Adriatic Sea throughout the entire year and are often found to be dominant in phytoplankton samples (Viličić et al., 2007, 2008, 2009). However, the actual species composition and species succession is, due to the limitations of light microscopical determination, still unknown and requires further examination.

* Corresponding author.

E-mail address: daniela.maric@cim.irb.hr (D. Marić).

During high abundances of toxin producing *Pseudo-nitzschia* species, DA can be detected in seawater and furthermore accumulates through the food chain (Bates et al., 1998; Bates, 2000; Bates and Trainer, 2006). Although it is known that *Pseudo-nitzschia* species are an important constituent of the phytoplankton community in the Adriatic Sea, so far no associations between DA production and *Pseudo-nitzschia* sp. abundances have been shown.

In many countries worldwide, mariculture areas are monitored for the occurrence of toxic plankton species and for the accumulation of corresponding toxins (Ferreira et al., 2007). Mariculture of plankton feeding animals such as shellfish is affected and such areas have to be closed after the detection *Pseudo-nitzschia* blooms. This not only raises questions about their distribution but also raises concerns about the increasing threat of amnesic shellfish poisoning (ASP) events (Hasle, 2002). The first reported case of *Pseudo-nitzschia* toxicity occurred in 1987 in Canada after the consumption of DA contaminated mussels *Mytilus edulis* (Bates et al., 1998). The causative organism was found to be *P. multiseriata* which was blooming in the area at the time and declined shortly before the toxicity event. The DA was found in mussel tissue and in the phytoplankton samples. After the 1987 ASP event, the Canadian government enacted a 20 µg DA g⁻¹ mussel flesh action limit which, when exceeded, would authorize the closure of shellfish farms (Waldichuk, 1989). This action limit has also been adopted by the European Union (Anonymous, 2002). Besides the ASP events there were several cases of domoic acid poisoning (DAP) which involved birds and marine mammals. Several different *Pseudo-nitzschia* species were found to be responsible for those episodes e.g. *P. australis* with a maximum concentration 4 × 10⁴ cells L⁻¹ in plankton samples during the DAP.

DA in the Adriatic Sea was recorded in 2000 for the first time on the Emilia-Romagna coast (Italy) (Ciminiello et al., 2005). Since then DA seems to have appeared on several occasions, reaching e.g. up to 6.55 µg g⁻¹ in January 2006 in the Krka estuary, eastern-middle Adriatic (Ujević et al., 2010).

As *Pseudo-nitzschia* species are monitored only on a genus level due to the limitations of light microscopy in species determination, there are insufficient data on the spatio-temporal distribution of different species from this, including in the Adriatic Sea. Therefore, no conclusions on the ecological preferences of the different species of this genus could be drawn to date. So far the prediction of mass occurrences of these toxic diatoms has not been confirmed and is generally attributed to the lack of knowledge about the ecology of *Pseudo-nitzschia* species (Sahraoui et al., 2009). Nevertheless, more and more observations of different species belonging to the genus *Pseudo-nitzschia* are reported around the world, thus showing most species to be globally distributed. Therefore, reports on the occurrences of *Pseudo-nitzschia* species are of particular and general public interest.

The main objectives of this study were (i) the determination of the potentially toxic *Pseudo-nitzschia* sp. and their abundance in the phytoplankton guilds of the northern Adriatic Sea, (ii) to describe bloom dynamics and taxonomy of the potentially toxic *P. calliantha* in relation to DA accumulation and/or retention in natural and breeding shellfish populations in the northern Adriatic Sea, (iii) to determine the temporal and spatial distribution of the genus *Pseudo-nitzschia* in the northern Adriatic and (iv) to study chemical and physical factors influencing the distribution patterns of the potentially toxic *P. calliantha*.

1.1. Study area

The northern Adriatic is a shallow basin with an average depth of 35 m. The trophic state, the vertical structure of the water column, the salinity distribution and the local circulation patterns

are influenced by fresh water inputs of rivers from the Italian coast and by the highly saline and oligotrophic waters from the southern part of the Adriatic basin (Marini et al., 2010). The Po river outflow and meteorological forcing are the main factors influencing the hydrography of the water column and subsequently the phytoplankton annual dynamics in the northern Adriatic (Bernardi Aubry et al., 2004; Viličić et al., 2009). These hydrographical features combine to make the system quite heterogeneous with across-shelf and longitudinal trophic gradients resulting in complex inhomogeneous distributions of phytoplankton composition, abundance and biomass (Polimene et al., 2006). Diatoms are the dominant phytoplankton group during the majority of the year and in most areas of the basin, while flagellates dominate mainly in areas under the direct influence of river inflows (Bernardi Aubry et al., 2004; Viličić et al., 2009).

2. Material and methods

2.1. Microscopy analysis

A total of 541 plankton samples were analyzed during the study period. Water samples were collected with 5 L Niskin bottles at the surface, 5 m, 10 m, 20 m, 30 m and 2 m above bottom. Samples were collected monthly at 13 stations in the northern Adriatic during 2007 (Fig. 1).

Phytoplankton samples were preserved in 2% (final concentration) neutralized formaldehyde (Kemika, Ltd. 36% solution) and analyzed within one month after sampling. Sub-samples of 50 mL were allowed to sediment for 24 h and cells were counted on an inverted Zeiss Axiovert 200 phase contrast microscope following the Utermöhl method (Utermöhl, 1958). Total phytoplankton abundances include microphytoplankton (cell-size 20–200 µm) and nanophytoplankton (cell-size 2–20 µm) (Sieburth et al., 1978).

A more extensive microscopic analysis for *Pseudo-nitzschia* species composition was directed towards the north-eastern

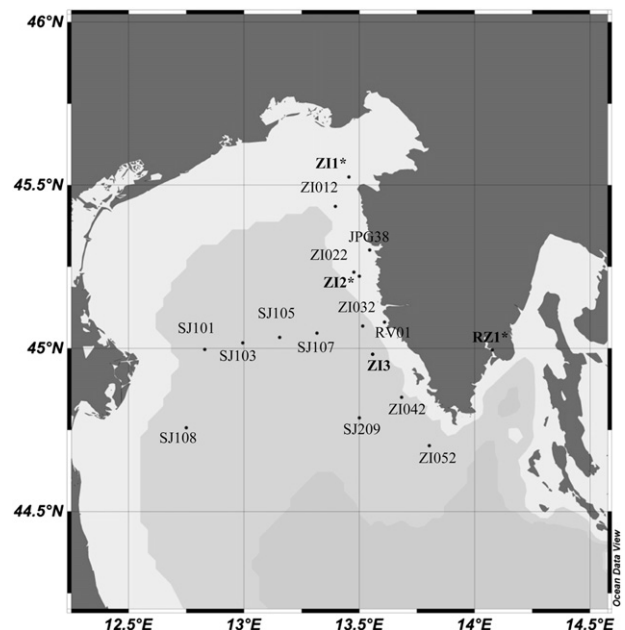


Fig. 1. Map of the northern Adriatic with the Gulf of Trieste and Venice Bay. Depicted are the sampling stations examined in this work. Localization of the 6 sampling stations (JPG38, ZI012, ZI022, SJ107, ZI042, SJ209) where *P. calliantha* was identified as the dominant diatom species during the autumn bloom. Asterisks indicate the stations where DA was found (ZI11*, ZI2*, ZI3, RZ1*).

transect along the Istrian coastline. Additional net phytoplankton samples (53 μm mesh) were collected during the *Pseudo-nitzschia* blooms in September–October at these sampling stations and used for detailed taxonomic analysis. For transmission electron microscopy *Pseudo-nitzschia* frustules were first acid cleaned (HNO_3 and H_2SO_4) and then rinsed with distilled water. A drop of clean net material was placed on formvar-coated grids, air dried and examined with a FEI Morgagni 268 D transmission electron microscope. For morphometry and analysis of the valves the procedures from Lundholm et al. (2003) and Caroppo et al. (2005) were followed.

2.2. Domoic acid analysis

To find possible toxin accumulations in shellfish tissue, DA was measured weekly/biweekly during 2007 at 6 shellfish farms and 3 natural habitats (Z11, Z12, Z13) (DA positive stations are marked with * in Fig. 1) along the north-eastern coastline on natural population of *Flexopecten proteus* (Z11), *Pecten jacobaeus* (Z12) and *Ostrea edulis* (Z13), and *Mytilus galloprovincialis* from an area with intense farming (RZ1). *F. proteus* (Z11), *P. jacobaeus* (Z12) and *O. edulis* (Z13) dwell on bottom habitats deeper than 25 m.

Qualitative and quantitative determination of ASP toxins, DA and *epi*-DA followed the protocol proposed by Quilliam et al. (1995). For representative samples about 100 g of soft tissue were used. Homogenized samples (approximately 4 g) were extracted with 16 mL $\text{MeOH}/\text{H}_2\text{O}$ (1:1) at 10000 rpm for 5 min. After 40 min centrifugation at 4000 rpm, 5 mL of the supernatant was filtered through a 0.45 μm membrane filter (HVHP, Millipore). Subsequent clean up by strong anion exchange (SAX) solid phase extraction (SPE) was necessary to avoid tryptophan interference. Since DA can decompose when frozen, prepared SAX-cleaned extracts had to be analyzed immediately.

The HPLC system consisted of a Varian ProSTAR 230 Solvent Delivery Module, 310 UV/Vis Detector, 335 Photodiode Array Detector and a 410 Autosampler. A standard calibration curve was performed by using an externally certified DA calibration solution for domoic acid (National Research Council of Canada, Halifax, NS, Canada) prepared in seven different concentrations (0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 25.0 $\mu\text{g mL}^{-1}$) and measured in triplicates by HPLC.

The column used was a Pinnacle II C18, 250 \times 4.6 mm (Restek), with a C18 Guard Cartridge (20 \times 4 mm) and the temperature was 40 $^\circ\text{C}$. Domoic acid was detected by UV absorption at a wavelength of 242 nm which is the absorption maximum of DA. The mobile phase consisted of 100 mL acetonitrile, 0.2 mL trifluoroacetic acid and up to 1000 mL deionized water. Domoic acid has a retention time of around 12.8 min.

2.3. Environmental analysis

Temperature and salinity were measured using a CTD probe (SBE25, Sea-Bird Electronics, Inc., Bellevue, Washington, USA). Dissolved oxygen concentration was determined following the Winkler titration method (Parsons et al., 1984) while the saturation percentage of dissolved oxygen in each water sample was calculated following the Benson and Krause equation (UNESCO, 1986). Sub-samples for the determination of nitrate (NO_3^-), phosphate (PO_4^{3-}) and siliceous acid ($\text{Si}(\text{OH})_4$) were measured using a Shimadzu UV-Mini 1240 spectrophotometer (Ivančić and Degobbi, 1984; Parsons et al., 1984). Chlorophyll was measured by standard oceanographic methodologies (Parsons et al., 1984).

2.4. Statistical analysis

Statistical analysis and data representation was produced using the software Systat 12, Golden software Surfer 8.00, PRIMER 5 and Statistica 6.0. The dataset was transformed logarithmically ($\log(x+1)$) to obtain a normal distribution. A standard Spearman rank was used to elaborate direct correlations between all parameters. For principal component analysis (PCA) of physical and chemical variables and *Pseudo-nitzschia* abundance the statistical software package PRIMER 5 was used (Clarke and Warwick, 2001).

3. Results

3.1. The genus *Pseudo-nitzschia* in the northern Adriatic

Cells of the genus *Pseudo-nitzschia* were found in 336 of 541 samples collected during 2007 at all stations in the northern

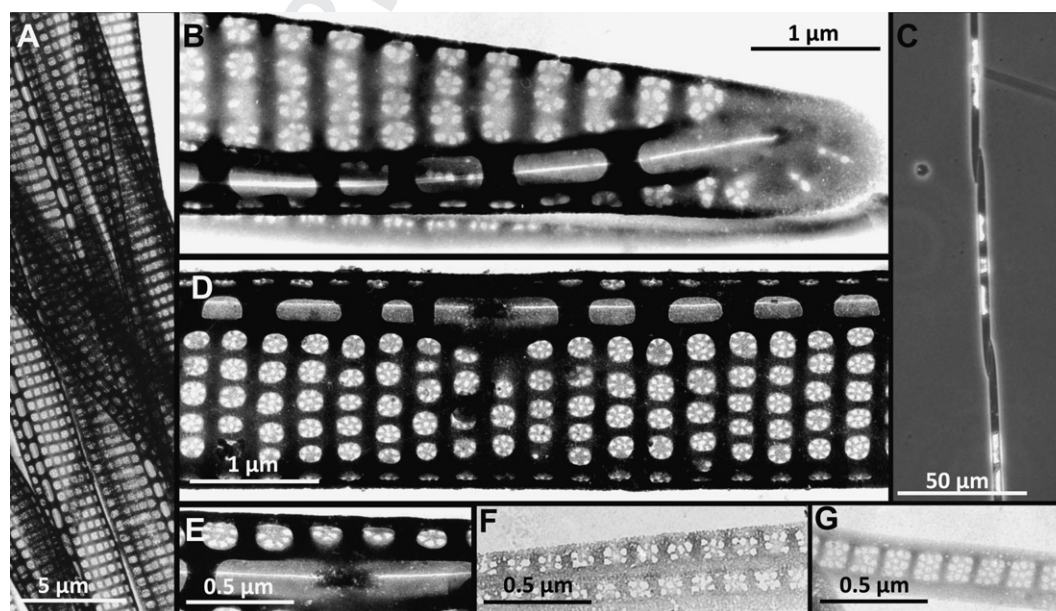


Fig. 2. Transmission electron micrographs (TEM) illustrate the ultrastructural details leading to the determination *P. calliantha*; (A) TEM of several frustules, (B) parts of the valve showing the tip, (C) light micrograph, (D, E) middle and central part of the mantle and stria structure with the central nodule and (F, G) valvocopula and cingular bands.

Table 1 Abiotic factors: Ranges of abiotic factors measured and Spearman correlations between the abiotic factors given and the abundances of the *Pseudo-nitzschia* sp.

Complete dataset	Temper.	Salinity	Nitrate	Nitrite	Ammonia	TIN	Silica	Phosphate	Oxygen	pH
	[°C]		[μM]	[μM]	[μM]	[μM]	[μM]	[μM]	[μM]	
Minimum	9	22.31	0.58	0.01	0.01	0.66	0.01	n.d.	2.04	7.96
Maximum	28.16	38.37	19.51	3.72	3.43	20.58	28.29	0.56	10.05	8.54
Arithmetic Mean	16.792	37.67	1.922	0.308	0.265	2.464	3.367	0.037	5.158	7.773
Standard Deviation	4.709	1.038	1.825	0.45	0.427	2.095	3.672	0.066	1.417	1.865
Spearman correlations with <i>Pseudo-nitzschia</i> sp. abundances	0.2	-0.292	0.035	-0.051	-0.078	-0.007	-0.031	0.117	-0.113	0.175

Significant correlations ($P < 0.05$) are highlighted in bold font.

Adriatic. In particular, the genus was continuously present throughout 2007 with maximum values (10^6 cells L^{-1}) in the period from August to October (Table 2). In order to characterize the late summer/autumn bloom, further examinations were performed on net phytoplankton samples.

3.2. Taxonomic study of *Pseudo-nitzschia calliantha* in the northern Adriatic

After taxonomic study of *Pseudo-nitzschia calliantha* using electron microscopical examinations and detailed taxonomic measurements, the summer/autumn bloom was attributed to the recently described *P. calliantha*. The colonies were linear in shape in valve view with a short overlap in girdle view (Fig. 2C). The overlapping part of the tips and valves was very short (11.7–13.5 μm) and the eccentric raphe was divided in the middle by the central space (Fig. 2E). The apical axis ranged from 59 to 112 μm in length with pointed frustule ends, while the transapical axis of valves varied from 0.7 to 1.7 μm. Fibulae were regularly spaced with 17–27 in 10 μm (Table 1). The central part of the valve has a central nodule and a larger central interspace. The number of striae was 33–45 in 10 μm and they were composed of a single row of large round poroids with a central unperforated area surrounded by 4–10 finely perforated sectors (Fig. 2). Valvo-copulae were 2–3 poroids wide and 3–4 poroids high (Fig. 2F, G). The range of ultrastructural characteristics measured in the present work, in comparison with literature data, is listed in Table 3. For those values highlighted in bold, our results exceed the thus far reported ranges for the species *P. calliantha*: minimum width was 0.7 μm (as opposed to the thus far 1.3 μm), the maximum number of fibulae in 10 μm was 27 (as opposed to 26) and the maximum number of striae in 10 μm was 45 (as opposed to 40).

Table 2 Ranges of the measured morphological characteristics of *P. calliantha* with the corresponding environmental factors and abundances given.

	Sampling station and sampling date	ZI012	SJ107	SJ209	ZI042	ZI022	SJ107	JPG38
		21.9.07.	24.9.07.	02.10.07.	21.10.07.	15.10.07.	17.10.07.	29.10.07.
Abundance and dynamics	min (cells L^{-1})	227200	164595	100320	74480	230040	395028	164595
	max (cells L^{-1})	303880	285298	241406	106400	606258	1207030	200806
	avg (cells L^{-1})	276427	232078	175959	88768	378484	842178	73634
	st. dev.	42726,04	62546,27	62079,47	14434,00	40481,18	426620,21	116314,00
Morphology	Cell width in μm (sd)	0,9 (0,2)	1,37 (0,3)	1,3 (0,2)	1,04 (0,3)	1,43 (0,2)	1,63(0,3)	1,4 (0,1)
	Fibulae in 10 μm (sd)	21,77 (2,5)	22,12 (1,5)	20,69 (3,2)	22,14 (3,3)	19,66 (1,2)	20,66 (2,5)	21,5 (2,1)
	Striae in 10 μm (sd)	37,55 (2,8)	37,87 (2,6)	38,46 (2,8)	39,85 (4,6)	37,33 (3,2)	35 (2,1)	40 (2,8)
	Araeolae in 10 μm (sd)	43 (7,2)	51,87 (6,6)	53,69 (7,4)	57,14 (3,5)	72,66 (23,1)	51,83 (6,8)	58,5 (0,7)
Ecology	Temperature (°C)	21,27	22,12	21,03	20,31	19,56	19,73	16,97
	Salinity	37,52	37,41	37,79	37,82	37,70	37,13	37,57
	Phosphate (μM)	0,01	0,01	0,00	0,00	0,01	0,01	0,03
	Nitrate (μM)	1,27	1,21	1,86	2,11	2,13	2,06	2,83
	Nitrite (μM)	0,2	0,04	0,03	0,12	0,14	0,09	0,52
	Ammonium (μM)	0,06	0,00	0,06	0,15	0,12	0,12	0,19
	TIN (μM)	1,3	1,25	1,95	2,38	2,38	2,27	3,54
	Silicate (μM)	0,17	0,11	0,48	1,99	1,83	0,32	2,94
	Chl (μM)	0,33	0,35	0,26	0,43	0,60	1,08	1,1

3.3. Domoic acid (DA) measurements on natural and cultured populations of shellfish

A total number of 342 shellfish samples were analyzed for DA during 2007 at 6 shellfish farms and 3 natural stations. Domoic acid was detected in bottom dwelling mussels during the autumn period on 4 occasions. DA was detected in low concentrations, ranging from 0.13 μg g^{-1} to 0.45 μg g^{-1} . DA was detected in *Flexopecten proteus* at station ZI1 and *Pecten jacobaeus* at ZI2 (Fig. 3). The period with the highest abundance of *P. calliantha* (October) preceded the appearance of DA in this area. The maximum DA concentration (1.32 μg DA g^{-1}) was recorded on the 19th of November at the intensive farming station RZ1 in the tissue of *M. galloprovincialis* (Fig. 3) which was growing throughout the entire water column, not just on the bottom.

3.4. Distribution patterns and the bloom of the potentially toxic species *P. calliantha*

The spatial distribution of *Pseudo-nitzschia* species appears patchy (Figs. 4 and 5). The highest abundances ($>5 \times 10^5$ cells L^{-1}) on the longitudinal transect in October 2007 were at the surface and at 10 m depth (Fig. 4). Along the Istrian coast maximum abundances were also noted beneath the surface and at 10 m depth, with a maximum of 6×10^5 cells L^{-1} at approx. 45°N at station ZI032. The *P. calliantha* bloom started on August 21st 2007 and lasted until November 2007.

3.5. Environmental parameters

Ranges of environmental parameters with basic statistics (minimum, maximum, arithmetic mean and standard deviation)

Table 3Morphological ranges so far reported for *P. calliantha*. Values from this report exceeding reported ranges to date are highlighted in bold font. n.r. = not reported.

<i>P. calliantha</i>	Length μm		Width μm		Fibulae in 10 μm		Striae in 10 μm		Poroids in 1 μm		R./p.	References
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max		
	60	63	1.3	1.4	19	20	39	40	6	6	n.r.	Bargu and Lundholm 2002
	44	66	1.7	1.7	18	19	37	39	5	5	1	Lundholm et al., 2003
	41	68	1.7	1.7	17	20	36	39	5	5	1	Lundholm et al., 2003
	41	63	1.5	1.7	20	21	36	38	4	6	n.r.	Lundholm et al., 2003
	47	72	1.5	1.8	19	21	36	38	5	5	n.r.	Lundholm et al., 2003
	62	98	1.5	1.8	5	19	34	38	5	5	n.r.	Lundholm et al., 2003
	69	69	1.4	1.6	18	22	36	38	4	5	n.r.	Lundholm et al., 2003
	90	90	1.5	1.7	18	22	34	38	5	5	n.r.	Lundholm et al., 2003
	67	77	1.5	1.8	20	22	38	39	5	6	n.r.	Lundholm et al., 2003
	n.r.	n.r.	1.4	1.4	16	20	36	36	4	5	1	Lundholm et al., 2003
	66	77	1.4	1.8	16	18	34	34	5	5	1	Lundholm et al., 2003
	83	87	1.4	1.8	16	21	34	36	5	5	1	Lundholm et al., 2003
	n.r.	n.r.	1.5	1.7	15	19	34	39	4	5	1	Lundholm et al., 2003
	n.r.	n.r.	1.4	1.4	17	20	34	36	5	6	1	Lundholm et al., 2003
	80	110	1.5	2.2	18	20	n.r.	36	n.r.	n.r.	1	Caroppo et al., 2005
	n.r.	n.r.	1.7	2.4	18	24	32	39	4	6	n.r.	Amato et al., 2007
	n.r.	n.r.	1.7	2.6	17	25	30	40	4	6	n.r.	Amato et al., 2007
	62	100	1.5	2	16	20	37	39	6	10	1	Spatharis et al., 2007
	41	123	1.1	2.4	15	26	30	40	4	5	1	Quijano-Scheggia et al., 2008
	40	87	1.5	1.8	15	20	32	38	5	6	1	Orlova et al., 2008
	63	94	1.5	1.8	14	18	35	39	4	6	n.r.	Orlova et al., 2008
	64	98	1.5	2.1	17	19	39	39	4	5	n.r.	Buric et al. 2008
	59	112	0.7	1.7	17	27	33	45	4	10	n.r.	this work
Mean	56.8	81.8	1.5	1.8	16.6	21.0	34.8	38.4	4.6	5.8		mean

are shown in Table 2. The water temperature ranged from 9 °C (winter) to 28.16 °C (summer). The warming of the water began in May, reaching temperatures around 17 °C, and peaking in July–August when a sharp thermocline was established. In November thermal homogeneity throughout the water column was recorded. The lowest salinity during 2007 was recorded at stations SJ101 (22.31) and the highest values were recorded in December at a number of stations, with an annual maximum of 38.37 (mean values were 37.47). The maximum nitrate concentration was detected in the surface layer in August at station SJ101 (19.51 μM) and the lowest at the same station in July (0.58 μM) with a mean value of 1.92 μM . The minimum value of nitrite was in summer at SJ101 (0.01 μM) and the maximum value was measured on the same station in October with 3.72 μM (mean value 0.308 μM).

Ammonia ranged from not detectable (n.d.) to 3.43 μM in September at SJ108 while the mean value was 0.26 μM . Total inorganic nitrogen ranged from 0.66 to 20.58 μM (SJ101, 0m, August) with a mean value of 2.46 μM .

$\text{Si}(\text{OH})_4$ concentration varied widely throughout the year. Minimum values were measured in October (0.01 μM) in the surface layer and the highest values were also measured in October in the bottom layer, where a concentration of 28.29 μM was recorded. The mean value of $\text{Si}(\text{OH})_4$ during the entire sampling period was 3.36 μM . Phosphate levels remained relatively invariable and low through the sampling period with the maximum value (0.56 μM) recorded in the bottom layer during August and October (SJ108 and SJ105) while the mean value was 0.04 μM . The highest level of chlorophyll *a* was found in October during the diatom

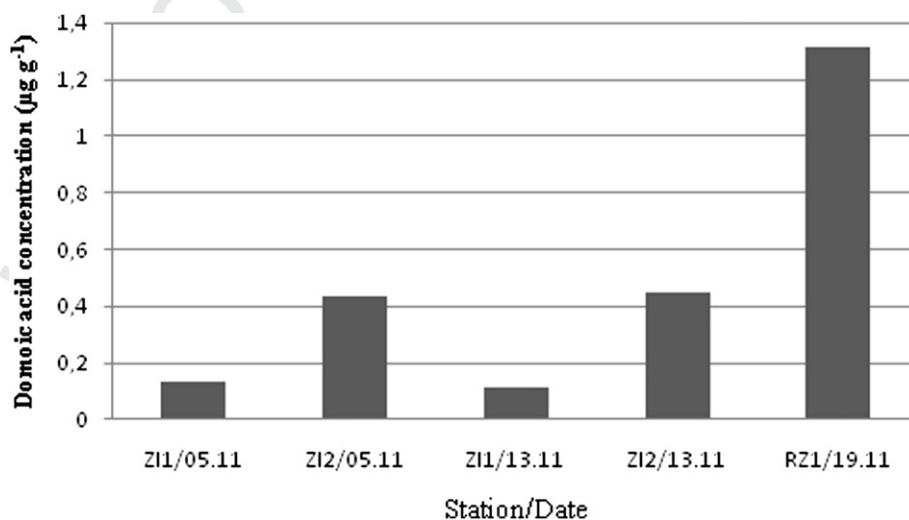


Fig. 3. Domoic acid concentration in natural shellfish samples of *Flelopekten proteus* (Z11) and *Pecten jacobaeus* (Z12) and a farmed *Mytilus galloprovincialis* (RZ1) from the northern Adriatic in 2007.

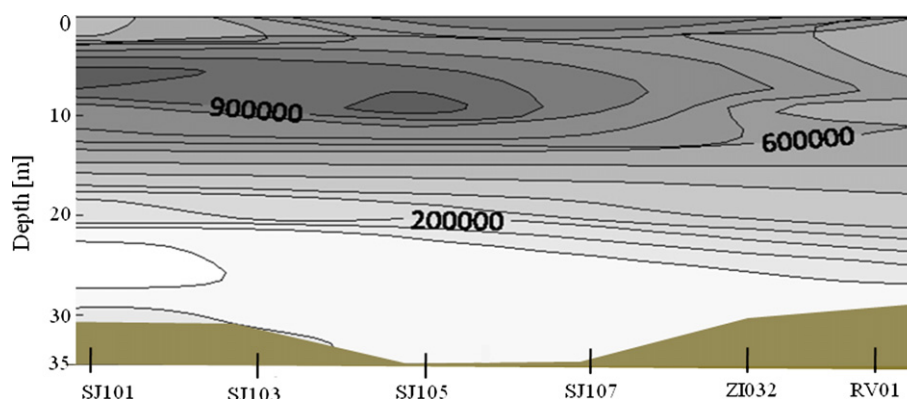


Fig. 4. Longitudinal transect across the northern Adriatic. Spatial distribution of *Pseudo-nitzschia* sp. in October 2007 along the stations SJ101, SJ103, SJ105, SJ107, ZI032 and RV01.

bloom at 20 m depth with $1.24 \mu\text{g L}^{-1}$ at station SJ107, while the mean values were considerably lower ($0.46 \mu\text{g L}^{-1}$). The lowest value was observed in July with $0.092 \mu\text{g L}^{-1}$ at the same station. The average Si:DIN ratio was 1.05, with the lowest values in autumn (0.32), and higher average values in spring.

3.6. Environmental parameters accompanying the *P. calliantha* bloom

The temperature during the *Pseudo-nitzschia calliantha* bloom ranged from 16.97°C to 22.12°C (Table 1); the lowest salinity was recorded at SJ107 with 37.13 to a maximum of 37.82 at station ZI042. Phosphate values were low, ranging from not detectable to $0.03 \mu\text{M}$. Silicate ranged from $0.11 \mu\text{M}$ to $2.94 \mu\text{M}$ while the chlorophyll measurements were maximal in accordance with maximal cell abundance, with chlorophyll *a* reaching a value of $1.08 \mu\text{M}$. More details regarding nutrient and chlorophyll *a* concentrations with the corresponding morphological measurements of *P. calliantha* cells are shown in Table 1. Even though a relatively large range of nitrate species, silica and chlorophyll *a* concentrations were recorded throughout our samples, the corresponding morphological ranges of *P. calliantha* showed no significant correlations with abiotic parameters.

3.7. Statistical results

The ranges of abiotic parameters throughout the entire dataset and correlations are given in Table 2. *Pseudo-nitzschia* abundances are significantly ($p < 0.05$) correlated with the concentrations of total inorganic nitrogen and silica as well as with temperature and salinity.

The PCA generated 5 principal components; the first three explained 96.3% of the variance of the original data set. The first

principal component (PC1) accounted for 54.4% of the total variance within the data set and primarily related to silicate and temperature. The second principal component (PC2) expressed 33.1% of the variance and related to nitrates, while the third principal component (PC3) was significantly related to temperature and nitrites and explained 8.8% of the variance. On the scatter plot, two groups could be distinguished: summer and autumn group, which were related to higher temperatures and higher *Pseudo-nitzschia* sp. abundance and the winter and spring group preferring lower temperatures and higher silica concentrations (Fig. 6).

4. Discussion

Ultrastructural examinations revealed that the *Pseudo-nitzschia* bloom, lasting from August to October along the eastern coast of the northern Adriatic Sea, comprised of the potentially toxic species *P. calliantha*. The morphological ranges of *P. calliantha* valves were similar amongst the different sampling sites. Our measurements exceeded the thus far reported ranges for *P. calliantha* (Table 3) (Lundholm et al., 2003; Caroppo et al., 2005; Amato et al., 2007; Spatharis et al., 2007; Quijano-Scheggia et al., 2008). Namely, the minimum cell width was $0.7 \mu\text{m}$ (as opposed to the thus far reported $1.3 \mu\text{m}$), the maximum number of fibulae in $10 \mu\text{m}$ was 27 (as opposed to 25) and the maximum number of striae in $10 \mu\text{m}$ was 45 (as opposed to 40). During the bloom it was found that *P. calliantha* was strongly dominant, contributing up to 70% of the total phytoplankton abundance.

Pseudo-nitzschia calliantha was already found to be producing DA toxin in several areas worldwide, as well as in cultures (Martin et al., 1990; Lundholm et al., 1997, 2003; Besiktepe et al., 2008) even though several cultures from, for example, Vietnam and Italy

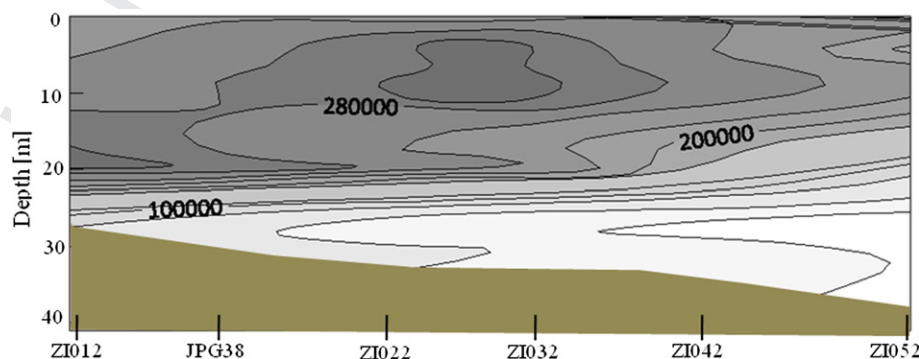


Fig. 5. Transect along the north-eastern Adriatic (Istrian) coast: Spatial distribution of the *Pseudo-nitzschia* sp. in October 2007 along the stations ZI012, JPC38, ZI022, ZI032, ZI042 and ZI052. The maximal abundance was found at station ZI032.

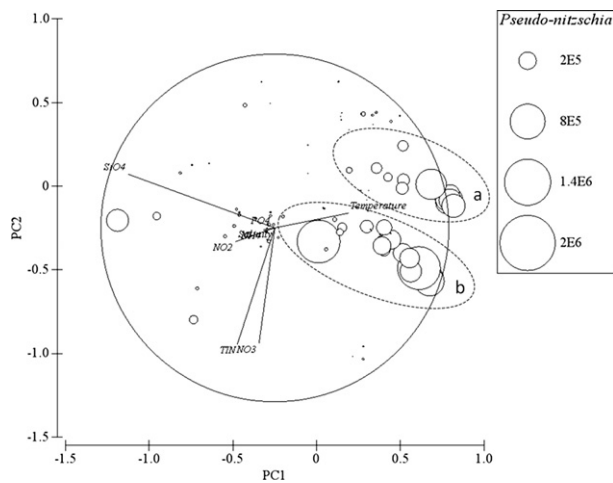


Fig. 6. Scatterplot of the first two principal components, following PCA analysis of physico-chemical parameters, revealing two distinguishable groups representing the winter/spring (a) and summer/autumn (b) mass abundances of *Pseudo-nitzschia* species.

(Venice) were found not to produce the toxin (Lundholm et al., 2003; Besiktepe et al., 2008). The first record of DA in the Adriatic Sea reported DA in shellfish tissue (*M. galloprovincialis*) along the Emilia-Romagna coast in 2000, as described by Ciminiello et al. (2005). Samples at that time were analyzed by HILIC/MS with subsequent work by Ciminiello et al. (2005) recording the presence of DA as a new toxin that entered the shellfish toxin profile ($2.5 \mu\text{g g}^{-1}$). Such results clearly demonstrate that there are DA-producing species in the Adriatic Sea. Although concentrations of DA in the analyzed samples were far below the regulatory limit, careful monitoring of this toxin in shellfish was strongly recommended (Ciminiello et al., 2005).

During the observed *Pseudo-nitzschia calliantha* bloom in autumn 2007 in the northern Adriatic Sea, DA in natural shellfish populations was also detected. Low concentrations of DA were measured in natural benthic mussel populations of *F. proteus* and *P. jacobaeus*. These low DA concentrations can probably be explained by the observation that the maximal abundance of *P. calliantha* was recorded at 10 m depth in the water column and not in the bottom layer where the shellfish live. Results here differed from the survey made by Honsell et al. (2006) on *Pseudo-nitzschia* sp. in the Gulf of Trieste in 2005. In their results the most abundant species were *P. calliantha* ($5.4 \times 10^3 \text{ cells L}^{-1}$) and *P. delicatissima* ($2.0 \times 10^3 \text{ cells L}^{-1}$). However, DA concentrations in both natural phytoplankton net samples and sea water samples were under the detection limit of both Biosense ASP ELISA (10 pg mL^{-1}) and HILIC-MS (3 ng mL^{-1}) in MRM positive ion mode transition at $m/z 312 > 266$. Those observations could be explained by low *Pseudo-nitzschia* sp. concentrations ($10^3 \text{ cells L}^{-1}$) or by the presence of not toxic strains. Data presented here, however, show that a bloom event of *P. calliantha* in the northern Adriatic preceded elevated DA concentrations in shellfish tissue.

Besiktepe et al. (2008) found DA-producing *Pseudo-nitzschia calliantha* strains in the phytoplankton community of the Black Sea. They calculated the toxin content per cell between non-detectable and up to $1.3 \text{ pg DA cell}^{-1}$ with the HPLC-FMOC method. Experiments on batch cultures show that DA levels were variable over time within the cells. The maximum DA values they measured were in the early exponential phase and during the late exponential and stationary phase of their cultures. This differed from the experiments conducted by Thessen and his group (2009) which compared DA production in *P. multiseriis*, *P. fraudulenta* and

P. calliantha. From their results it is evident that *P. calliantha* is a weak DA producer. DA production in batch cultures was observed only when the cultures were in the stationary phase, once cell division has stopped, and the cultures were limited by P or Si with sufficient N and light (Thessen et al., 2009). Hence, this could partly explain the relatively low DA concentrations found in the present study. When the bottom dwelling shellfish that were examined came into contact with the *P. calliantha* bloom, this population was probably already in the late stationary phase and limited by nutrients. This is shown by Spearman correlations (e.g. negative correlation with silica) and the very low phosphorous concentrations measured. Perhaps this could explain the low DA concentration in shellfish tissue, while such high cell abundances were measured in water samples from the northern Adriatic Sea. For further analysis, and better understanding of this topic, we propose further experiments with *P. calliantha* cultures from the northern Adriatic, with an aim to quantify the DA produced per cell and under conditions reflecting the northern Adriatic in late autumn.

Pseudo-nitzschia calliantha has been observed in geographically widespread locations which indicate a fairly cosmopolitan distribution (Lundholm et al., 2003). Routinely performed light microscopical observations showed that species of the genus *Pseudo-nitzschia* were present in the northern Adriatic throughout the entire year (Viličić et al., 2009). In general, the abundances attributed to the genus *Pseudo-nitzschia* followed the total phytoplankton trends with highest cell densities during early spring or in summer and autumn. The spatial and temporal distribution of *Pseudo-nitzschia* sp. throughout the entire year, in a wide range of ecological conditions, is probably explainable by a considerable diversity within the genus. In light microscopy (LM), *P. manii*, *P. calliantha*, *P. caciiantha*, *P. delicatissima* and *P. cuspidata* are indistinguishable, while in electron microscopy the species differ in poroid density and arrangement, and in the number of sectors on the poroid hymens (Lundholm et al., 2003; Amato and Montresor, 2008). Differences in cell width are very small and hence difficult to be estimated by LM. Moreover, one species name may actually represent a number of cryptic species, a phenomenon which has been recently described in detail for *P. delicatissima* (Amato et al., 2007). Thus far, very little is known about the relationship among particular species and the environmental conditions which lead to their growth, even though some researchers showed significant correlations between *Pseudo-nitzschia* species and abiotic factors (Trainer et al., 2002; Kaczmarek et al., 2007).

The present study showed a significant correlation between the abundances of species attributed to the genus *Pseudo-nitzschia* (as a group) and total inorganic nitrogen, silica, temperature and salinity. The positive correlation with temperature corresponds with the observation of higher abundances, or even blooms, in autumn when water temperatures are still elevated as was also found for *P. calliantha* abundance in the north-western Mediterranean (Quijano-Scheggia et al., 2008). Negative correlations with total inorganic nitrogen and silica are likely to be an effect of the high abundances during our sampling which led to a depletion of the aforementioned nutrients and salts in the water column. Our observations contrast with the conclusions drawn for the southern Adriatic by Caroppo et al. in 2005, where the bloom of *P. calliantha* occurred in winter months and was negatively correlated with water temperature and positively correlated with nutrient availability. *Pseudo-nitzschia* species exhibit a wide range of physiological tolerances demonstrating their ability to dominate a variety of marine habitats (Bates et al., 1998). A significant negative correlation between *Pseudo-nitzschia* species abundance and phosphate concentration in the north-western Adriatic coastal waters was previously reported (Penna et al., 2006). In contrast, a significant correlation between the *Pseudo-nitzschia* sp. abundance and

phosphate concentration was not found in this study, which agrees with the findings for the Catalan coastal waters (Quijano-Scheggia et al., 2008). Given this variety of different correlations found in different places and years it seems that the measured abiotic factors alone are not sufficient to predict the ecological circumstances leading to *Pseudo-nitzschia* sp. mass occurrences. We would also expect a considerably increased significance of correlations between abundances and abiotic factors if abundances were reported on a species specific level.

Marchetti et al. (2004) performed their measurements in the Juan de Fuca eddy. This peculiar natural environment provided a unique environment to study the influence of nutrients on *Pseudo-nitzschia* abundances and DA accumulation in the field. They suggested 3 conditions which could result in elevated DA concentrations: low Si:N ratios might allow *Pseudo-nitzschia* to out-compete other diatoms if Si is utilized to low or limiting concentrations; the N supply, with respect to Si and P, must be high to ensure that *Pseudo-nitzschia* does not experience N-limitation and thus is able to produce DA; the third condition is a long residence time of toxin producing stains (Marchetti et al., 2004). It may be assumed that low Si:N ratios in the northern Adriatic Sea favour the growth of *Pseudo-nitzschia* species over other diatoms due to lower silica requirements, as had already been indicated before (Sommer, 1994; Marchetti et al., 2004). The lowest values of Si:N ratio coincided with *Pseudo-nitzschia* species blooms in the present and other studies in the Adriatic (Penna et al., 2006; Burić et al., 2008).

Day-length can affect growth rates, cell yield and toxin production and influence which species of *Pseudo-nitzschia* becomes dominant during the bloom. This is in accordance with the autumn bloom in the northern Adriatic (Fehling et al., 2005). We may assume that the lower irradiance, shorter photoperiod and the suitable Si:N ratio coincided with and stimulated the observed *P. calliantha* bloom and DA production. In attempts to forecast harmful algal blooms in the future, it is necessary go beyond traditional environmental parameters typically measured in monitoring programmes and further examine the physiology of the phytoplankton community (Marchetti et al., 2004).

5. Conclusions

For the first time measurable values of DA in natural mussel populations were found co-occurring with a *P. calliantha* bloom in the Adriatic Sea. The measured DA concentrations were low, probably due to the fact that the mussels were sampled from the sea floor while highest cell concentrations were measured at around 10 m depth. We can speculate that the accumulated DA in clams originates from sinking or dying cells filtered by the mussels or by the remnants of the bloom after sinking. We therefore suggest that more studies and experiments should be conducted on cell cultures of *P. calliantha* to gain further insight into the toxin production and/or its possible distribution in the northern Adriatic Sea. With up to 1.2×10^6 cells L^{-1} of the toxin producing species *P. calliantha* near the Croatian coast, our findings certainly demand closer and species specific monitoring in the area with parallel toxin measurements.

Acknowledgements

The research was financially supported by the Ministry of Science, Education and Sports of the Republic of Croatia (Croatian National Monitoring Programme – Jadran, and projects 098-0982705-2731, 119-1191189-1228 and 001-0010501-0848). We are thankful to Martin Pfannkuchen for valuable discussions and support in computational analysis. We thank our college Dr. Daniel Lyons for English language improvement. The authors are grateful

to the crew of the RV “Vila Velebita” with special thanks to A. Bakota. We thank three anonymous referees for their suggestions which enabled us to improve the manuscript.

References

- Amato, A., Kooistra, W.H.C.F., Levaldi Ghiron, J.H., Mann, D.G., Pröschold, T., Montresor, M., 2007. Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist* 158, 193–207.
- Amato, A., Montresor, M., 2008. Morphology, phylogeny, and sexual cycle of *Pseudo-nitzschia mannii* sp. nov. (Bacillariophyceae): a pseudo-cryptic species within the *P. pseudodelicatissima* complex. *Phycologia* 47, 487–497.
- Anonymous, 2002. Regulation (EC) No 853/2004 of the European Parliament and of the Council of April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Communities Legislation L220, 22–86.
- Bates, S.S., Garrison, D.L., Horner, R.A., 1998. Bloom Dynamics and Physiology of Domoic Acid Producing *Pseudo-nitzschia* Species. Springer-Verlag, Heidelberg, 267–292 pp.
- Bates, S.S., 2000. Domoic-acid-producing diatoms: another genus added! *Journal of Phycology* 36, 978–983.
- Bates, S.S., Trainer, V., 2006. The ecology of harmful diatoms. In: Granéli, E., Turner, J.T. (Eds.), *Ecology of Harmful Algae*. Springer-Verlag, Berlin, pp. 81–88.
- Bernardi Aubry, F., Berton, A., Bastianini, M., Socal, G., Aciri, F., 2004. Phytoplankton succession in a coastal area of the NW Adriatic, over a 10-year sampling period (1990–1999). *Continental Shelf Research* 24, 97–115.
- Besiktepe, S., Ryabushko, L., Ediger, D., Yilmaz, D., Zengin, A., Ryabushko, V., Lee, R., 2008. Domoic acid production by *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle (bacillariophyta) isolated from the Black Sea. *Harmful Algae* 7, 438–442.
- Burić, Z., Viličić, D., Mihalić, K.C., Carić, M., Kralj, K., Ljubesić, N., 2008. *Pseudo-nitzschia* blooms in the Zrmanja River estuary (Eastern Adriatic Sea). *Diatom Research* 23, 51–63.
- Caroppo, C., Congestri, R., Bracchini, L., Albertano, P., 2005. On the presence of *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle and *Pseudo-nitzschia delicatissima* (Cleve) Heiden in the Southern Adriatic Sea (Mediterranean Sea, Italy). *Journal of Plankton Research* 27, 763–774.
- Ciminiello, P., Dell’Aversano, C., Fattorusso, E., Forino, M., Magno, G.S., Tartaglione, L., Quilliam, M.A., Tubaro, A., Poletti, R., 2005. Hydrophilic interaction liquid chromatography/mass spectrometry for determination of domoic acid in Adriatic shellfish. *Rapid Communications in Mass Spectrometry* 19, 2030–2038.
- Clarke, K.R., Warwick, R.M., 2001. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*. Plymouth Marine Laboratory, Plymouth, 172 pp.
- Fehling, J., Davidson, K., Bates, S.S., 2005. Growth dynamics of non-toxic *Pseudo-nitzschia delicatissima* and toxic *P. seriata* (Bacillariophyceae) under simulated spring and summer photoperiods. *Harmful Algae* 4, 763–769.
- Ferreira, J.G., Vale, C., Soares, C.V., Salas, F., Stacey, P.E., Bricker, S.B., Silva, M.C., Marques, J.C., 2007. Monitoring of coastal and transitional waters under the E.U. water framework directive. *Environmental Monitoring and Assessment* 135, 195–216.
- Hasle, G.R., 2002. Are most of the domoic acid-producing species of the diatom genus *Pseudo-nitzschia* cosmopolites? *Harmful Algae* 1, 137–146.
- Honsell, G., Dell’Aversano, C., Vuerich, F., Sosa, S.S., Tartaglione, L., Tubaro, A., 2006. *Pseudo-nitzschia* and ASP in the northern Adriatic sea. In: 12th International Conference on Harmful Algae, Copenhagen, Denmark, pp. 193.
- Ivančić, I., Degobis, D., 1984. An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. *Water Research* 18, 1143–1147.
- Jeffery, B., Barlow, T., Moizer, K., Paul, S., Boyle, C., 2004. Amnesic shellfish poison. *Food and Chemical Toxicology* 42, 545–557.
- Kaczmarek, I., Martin, J.L., Ehrman, J.M., LeGresley, M.M., 2007. *Pseudo-nitzschia* species population dynamics in the Quoddy Region, Bay of Fundy. *Harmful Algae* 6, 861–874.
- Lundholm, N., Skov, J., Pocklington, R., Moestrup, Ø., 1997. Studies on the marine planktonic diatom *Pseudo-nitzschia*. 2. Autecology of *P. pseudodelicatissima* based on isolates from Danish coastal waters. *Phycologia* 36, 381–388.
- Lundholm, N., Moestrup, Ø., Hasle, G.R., Hoef-Emden, K., 2003. A study of the *Pseudo-nitzschia pseudodelicatissima/cuspidata* complex (Bacillariophyceae): what is *P. pseudodelicatissima*? *Journal of Phycology* 39, 797–813.
- Marchetti, A., Trainer, V.L., Harrison, P.J., 2004. Environmental conditions and phytoplankton dynamics associated with *Pseudo-nitzschia* abundance and domoic acid in the Juan de Fuca eddy. *Marine Ecology Progress Series* 281, 1–12.
- Marini, M., Grilli, F., Guarnieri, A., Jones, B., Klajic, Z., Pinardi, N., Sanxhaku, M., 2010. Is the southeastern Adriatic Sea coastal strip an eutrophic area? *Estuarine, Coastal and Shelf Science* 88, 395–406.
- Martin, J.L., Haya, K., Burrige, L.E., Wildish, D.J., 1990. *Nitzschia pseudodelicatissima* – a source of domoic acid in the Bay of Fundy, eastern Canada. *Marine Ecology Progress Series* 67, 177–182.
- Parsons, T.R., Maita, Y., Lalli, C.M., 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, Toronto.
- Penna, A., Ingarao, C., Ercolessi, M., Rocchi, M., Penna, N., 2006. Potentially harmful microalgal distribution in an area of the NW Adriatic coastline: sampling procedure and correlations with environmental factors. *Estuarine, Coastal and Shelf Science* 70, 307–316.

- 1021 Polimene, L., Pinardi, N., Zavatarelli, M., Colella, S., 2006. The Adriatic Sea ecosystem
1022 seasonal cycle: validation of a three-dimensional numerical model. *Journal of*
1023 *Geophysical Research* 111, 3–19.
- 1024 Quijano-Scheggia, S., Garcés, E., Sampedro, N., Van Lenning, K., Flo, E., Andree, K.,
1025 Fortuño, J.M., Camp, J., 2008. Identification and characterisation of the dominant
1026 *Pseudo-nitzschia* species (Bacillariophyceae) along the NE Spanish coast
1027 (Catalonia, NW Mediterranean). *Scientia Marina* 72, 343–359.
- 1028 Quilliam, M.A., Xie, M., Hardstaff, W.R., 1995. A rapid extraction and cleanup
1029 procedure for the liquid chromatographic determination of domoic acid in
1030 unsalted seafood. *J AOAC Int* 78, 543–554.
- 1031 Sahraoui, I., Hlaili, A.S., Mabrouk, H.H., Léger, C., Bates, S.S., 2009. Blooms of the
1032 diatom genus *pseudo-nitzschia* H. peragallo in Bizerte lagoon (Tunisia, SW
1033 Mediterranean). *Diatom Research* 24, 175–190.
- 1034 Sieburth, J.M., Smetacek, V., Lenz, J., 1978. Pelagic ecosystem structure: heterotrophic
1035 compartments of the plankton and their relationship to plankton size –
1036 fractions. *Limnology and Oceanography* 23, 1256–1263.
- 1037 Sommer, U., 1994. Are marine diatoms favoured by high Si:N ratios? *Marine Ecology*
1038 *Progress Series* 115, 309.
- 1039 Spatharis, S., Danielidis, D.B., Tsirtsis, G., 2007. Recurrent *Pseudo-nitzschia calliantha*
1040 (Bacillariophyceae) and *Alexandrium insuetum* (Dinophyceae) winter blooms
1041 induced by agricultural runoff. *Harmful Algae* 6, 811–822.
- 1042 Thessen, A.E., Bowers, H.A., Stoecker, D.K., 2009. Intra- and interspecies differences
1043 in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources.
1044 *Harmful Algae* 8, 792–810.
- 1045 Trainer, V.L., Hickey, B.M., Horner, R.A., 2002. Biological and physical dynamics of
1046 domoic acid production off the Washington coast. *Limnology and Oceanography*
1047 47, 1438–1446.
- 1048 Ujević, I., Ninčević-Gladan, Z., Roje, R., Skejić, S., Arapov, J., Marasović, I., 2010.
1049 Domoic acid—a new toxin in the Croatian Adriatic shellfish toxin profile.
1050 *Molecules* 15, 6835–6849.
- 1051 UNESCO, 1986. Progress on Oceanographic Tables and Standards 1983–1986: Work
1052 and Recommendations of the UNESCO/SCOR/ICES/IAPSO Joint Panel. UNESCO
1053 50, Paris.
- 1054 Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton Meth-
1055 odik. *Mitteilung Internationale Vereinigung Fuer Theoretische Unde Amge-
1056 wandte Limnologie* 9, 1–38.
- 1057 Viličić, D., Bosak, S., Burić, Z., Caput-Mihalić, K., 2007. Phytoplankton seasonality and
1058 composition along the coastal NE Adriatic Sea during the extremely low Po
1059 River discharge in 2006. *Acta Botanica Croatica* 66, 101–115.
- 1060 Viličić, D., Terzić, S., Ahel, M., Burić, Z., Jasprica, N., Carić, M., Mihalić, K.C., Olujić, G.,
1061 2008. Phytoplankton abundance and pigment biomarkers in the oligotrophic,
1062 eastern Adriatic estuary. *Environmental Monitoring and Assessment* 142,
1063 199–218.
- 1064 Viličić, D., Djakovac, T., Burić, Z., Bosak, S., 2009. Composition and annual cycle of
1065 phytoplankton assemblages in the northeastern Adriatic Sea. *Botanica Marina*
1066 52, 291–305.
- 1067 Waldichuk, M., 1989. Amnesic shellfish poison. *Marine Pollution Bulletin* 20,
1068 359–360.