Long-Term Variations in Species Composition of Bloom-Forming Toxic *Pseudo-nitzschia* Diatoms in the North-Western Sea of Japan during 1992–2015

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**Abstract:** This review aimed to summarize original and published data on the bloom events caused by toxic diatoms of the genus *Pseudo-nitzschia* and to assess long-term variations in the composition of bloom-forming species in the northwestern Sea of Japan during 1992–2015. This information is crucial to understanding the potential threat of toxic blooms and their effects. A change in species composition was observed within the genus *Pseudo-nitzschia*: it was dominated by *Pseudo-nitzschia multiseries* during 1992–1993, 2002, and a shift towards the dominance of *Pseudo-nitzschia multistriata* and *Pseudo-nitzschia calliantha* occurred in 2005–2015. We assume that the increased sea surface temperature recorded from Amursky Bay in the period of 1998–2009 compared to the data of 1980–1989 might be one of the causative factors for the shift observed. The absence of highly toxic *Pseudo-nitzschia multiseries* among the bloom-forming species is considered as one of the possible explanations for the lack of damage from the blooms of *Pseudo-nitzschia* over the past 15 years in the study area.

**Keywords:** *Pseudo-nitzschia*; bloom; domoic acid; long-term variations; northwestern Sea of Japan

1. Introduction

*Pseudo-nitzschia* H. Peragallo (1900) is a worldwide distributed genus of pennate diatoms. Species of this genus are widely known as potential domoic acid (DA) producers and also as an important component of phytoplankton communities [1]. Long-term series of observations on changes in dominant diatoms of the genus *Pseudo-nitzschia* are crucial for understanding the potential threat of toxic blooms and their effects. In recent years, extensive datasets of long-term variations in the density of phytoplankton, including species belonging to the genus *Pseudo-nitzschia* from different areas, were studied to document the temporal variations in phytoplankton communities associated with environmental factors and climate change events [2–6]. Nevertheless, long-term population dynamics of bloom-forming *Pseudo-nitzschia* species are rarely reported for the Northwest Pacific [7].

In terms of species diversity, Russian waters of the northwestern Sea of Japan are among the most productive areas in the Northwest Pacific [8], which is explained by its position, being in the mixing zone of the floras of arctic/boreal and tropical phytogeographic regions [9]. Harmful algal bloom events were permanently recorded from the Russian coastal waters of the Sea of Japan near the areas of aquaculture farms and popular coastal tourist sites since 1992 [10]. Nevertheless, in the study area, no economic or social impacts connected with toxigenic *Pseudo-nitzschia* species were observed.

Diatoms of the genus *Pseudo-nitzschia* are a dominant group of toxic plankton microalgae in the northwestern Sea of Japan, constituting up to 75–98% of the total phytoplankton density in bloom seasons [11,12]. Extensive datasets on species and genetic diversity, certain bloom events, and domoic acid concentration in cultures of the dominant *Pseudo-nitzschia* species isolated from the northwestern Sea of Japan were accumulated over the past
third decades [11–15]. However, no studies on long-term changes in the composition of *Pseudo-nitzschia* species that cause blooms in this body of water were carried out to date.

The purpose of the present work was to summarize original data and those available in literature concerning bloom events caused by toxic diatoms of the genus *Pseudo-nitzschia* and to assess long-term variations in the composition of bloom-forming species near the city of Vladivostok, Sea of Japan, during the period from 1992 to 2015.

2. Study Area and Sampling

The work was based on phytoplankton samples collected during the period from June 1992 to December 2015 in Peter the Great Bay, northwestern Sea of Japan. Peter the Great Bay is divided by the Muraviev-Amursky Peninsula and a group of islands into two smaller bodies of water: the Amursky and Ussuriisky bays (Figure 1). The most complete dataset on species composition and density of species belonging to the genus *Pseudo-nitzschia* in the study area was obtained for the coastal waters off the city of Vladivostok in Amursky and Ussuriisky bays [11,12,15,16]. In these bays, seven sampling stations were set (Figure 1, Table 1). The longest series of observations (the summer–autumn seasons from 1992 to 2005 and year-round samplings from 2007 to 2015) were made at monitoring station 1 in the northeastern part of Amursky Bay (Figure 1, Table 1). In Amursky Bay, phytoplankton samples were generally collected twice a month. In Ussuriisky Bay, phytoplankton was sampled once a month from the surface horizon in the autumn–winter periods of 2012, 2013, and 2015. The oceanographic characteristics of the sampling area were given elsewhere [17,18].

![Figure 1. Study area and sampling stations 1–7. Station 1 is a permanent monitoring station (indicated by asterisk).](image-url)
Table 1. Time, location, and number of samples of the sampling stations 1–7 in Amursky and Ussuriisky bays, northwestern Sea of Japan. See Figure 1 for the location of samples.

| Area          | Station | Dates                          | No. of Samples | Latitude N | Longitude E |
|---------------|---------|--------------------------------|----------------|------------|-------------|
| Amursky Bay   | 1       | 20 April 1993–17 December 1993| 14             | 43°12'     | 131°54'     |
|               | 1       | 4 June 1997–5 December 1997   | 21             | 43°12'     | 131°54'     |
|               | 1       | 6 June 2002–11 September 2002 | 4              | 43°12'     | 131°54'     |
|               | 1       | 14 June 2005–30 December 2005| 31             | 43°12'     | 131°54'     |
|               | 1       | 26 January 2007–29 December 2015| 205         | 43°12'     | 131°54'     |
|               | 2       | 25 June 1992–25 October 1992  | 9              | 43°10'     | 131°52'     |
|               | 3       | 31 August 2002–20 November 2005| 39            | 43°11'     | 131°54'     |
|               | 4       | 10 May 2001–20 October 2002   | 11             | 43°01'     | 131°47'     |
| Ussuriisky Bay| 5       | 13 November 2012–19 December 2012| 2               | 43°04'     | 131°57'     |
|               | 5       | 11 November 2013–23 December 2013| 2            | 43°04'     | 131°57'     |
|               | 5       | 15 October 2015–5 November 2015| 2              | 43°04'     | 131°57'     |
|               | 6       | 11 November 2013–23 December 2013| 2            | 43°05'     | 131°58'     |
|               | 6       | 15 October 2015–5 November 2015| 2              | 43°05'     | 131°58'     |
|               | 7       | 13 November 2012–19 December 2012| 2              | 43°11'     | 132°06'     |
|               | 7       | 11 November 2013–23 December 2013| 2            | 43°11'     | 132°06'     |
|               | 7       | 15 October 2015–5 November 2015| 2              | 43°11'     | 132°06'     |

The sampling and the cell counting techniques are described in detail in the works of Stonik et al. [12,16]. Light microscopic (LM) and transmission electron microscopic (TEM) studies were used for counting and identification to species level [11,12, 14–16]. Based on the long-term datasets, we assumed a concentration of >1 × 10^5 cells L^-1 to be a provisional limit (a limit level), above which any increase in the density of *Pseudo-nitzschia* spp. would be considered as a fact of bloom [15].

3. Statistical Analysis

Spearman’s within-year correlation analysis was used during the period of blooms of *Pseudo-nitzschia multistriata/calliantha* in the autumn of 2005 to study the relationship of species density with water temperature and salinity and concentration of ammonium nitrogen in water [19]. Data on all above environmental factors necessary for such an analysis were only available for the autumn 2005. The relationship of species density with water temperature was studied based on year-round sampling at station 1 during 2007–2015 using Spearman’s correlation analysis of the whole data set. The data were statistically processed with Statistica 7 software (StatSoft Inc., Tulsa, OK, USA).

The similarity in species composition and abundance among samples collected during 1993–2015 (station 1) was analyzed by calculating the Bray–Curtis similarity coefficient [20] based on relative abundance data for different species.

For graphical representation of the data set from the 1993–2015 sampling period (station 1), non-metric multidimensional scaling (NMDS) ordination was performed [21], which resulted in five groups. Based on samples scores from NMDS, a k-means cluster analysis was performed with Statistica v. 7.0 (StatSoft Inc., Tulsa, OK, USA). The significance of the resulting groups was tested using the one-way analysis of similarities (ANOSIM). NMDS and ANOSIM analyses were carried out using the software package PRIMER 6.1.12 (Primer-E Ltd., Plymouth, UK).
4. Results
4.1. The Bloom Events

The dominant bloom-forming *Pseudo-nitzschia* spp. and their maximum concentrations during bloom events are shown in Figure 2 and Table 2. The species that caused bloom events in Peter the Great Bay were identified earlier using the morphological studies and a phylogenetic analysis of ribosomal genes [11,13–16,22]. An analysis of long-term data on *Pseudo-nitzschia* bloom events in Amursky Bay showed that *Pseudo-nitzschia multiseries* was the dominant species in these areas in the early 1990s. Thus, the most intense bloom event of *Pseudo-nitzschia multiseries* was recorded in June 1992 at station 2 at a temperature of 15.3–16.1 °C after heavy rains. The maximum density in the bloom spot reached $1.1–3.5 \times 10^6$ cells L$^{-1}$ (79–98% of the total phytoplankton density) [13]. In June 1993, a bloom of *Pseudo-nitzschia multiseries* ($1–1.2 \times 10^6$ cells L$^{-1}$) occurred again in the northeastern Amursky Bay at station 1 at a temperature of 14.2–15.8 °C [16]. Subsequently, this species was recorded once more (at a highest concentration of $1.4 \times 10^6$ cells L$^{-1}$) in September 2002 at a temperature of 12.5–14 °C [12]. After 2002, *Pseudo-nitzschia multiseries* was not found in the study area. *Pseudo-nitzschia pungens* was found as a permanent component of phytoplankton throughout the study period, with maximum concentration ($5.9 \times 10^5$ cells L$^{-1}$) in autumn 1997 at station 1.

![Figure 2. Dominant species in the period *Pseudo-nitzschia* bloom events in Amursky Bay in 1992–2015. The bold horizontal line corresponds to a provisional limit for the bloom events.](image)

Most of the bloom events observed in Amursky and Ussuriisky bays from 1997 to 2015 were caused by *Pseudo-nitzschia calliantha* (Table 2). In Amursky Bay, the maximum concentration of this species ($2.7 \times 10^6$ cells L$^{-1}$) was registered in November 1997 at a water temperature of 5–6 °C and a salinity of 34.5‰. Among other species that caused blooms since 2002, *Pseudo-nitzschia multistriata* and *Pseudo-nitzschia fraudulenta* were also identified. The most intense bloom recorded after 2002 was caused by *Pseudo-nitzschia multistriata* (up to $0.9 \times 10^6$ cells L$^{-1}$ or 67% of the total *Pseudo-nitzschia* density) and *Pseudo-nitzschia calliantha* ($1.2 \times 10^5$ cells L$^{-1}$ or 9%) in October 2005 at station 1 at a water temperature of 6–12 °C and a salinity of 31–33.5‰. After 2005, *Pseudo-nitzschia* density exceeded the limit level of $1 \times 10^5$ cells · L$^{-1}$ in three cases: $1.7 \times 10^5$ cells L$^{-1}$ in...
September 2007, dominated by *Pseudo-nitzschia calliantha*; $2.5 \times 10^5$ cells · L$^{-1}$ in September 2012, dominated by *Pseudo-nitzschia multiseries*; and $1.1 \times 10^5$ cells L$^{-1}$ in November 2012, dominated by *Pseudo-nitzschia calliantha* (Figure 2).

**Table 2.** *Pseudo-nitzschia* bloom events and sea surface temperature (SST) and salinity (SSS) in the northwestern Sea of Japan during 1992–2015.

| Species                  | Station | Dates                | Maximum Concentration (Cells L$^{-1}$) | Conditions                                           | Data from |
|--------------------------|---------|----------------------|----------------------------------------|-----------------------------------------------------|-----------|
| *Pseudo-nitzschia multiseries* | 2       | June 1992            | $1.1-3.5 \times 10^7$                  | After heavy rains at SST of 15.3–16.1 °C             | [13]      |
| *Pseudo-nitzschia multiseries* | 1       | June 1993            | $1-1.2 \times 10^6$                    | SST of 14.2–15.8 °C                                  | [16]      |
| *Pseudo-nitzschia multiseries* | 1       | September 2002       | $1.4 \times 10^6$                     | SST of 12.5–14 °C                                   | [12]      |
| *Pseudo-nitzschia calliantha* | 1       | November 1997        | $2.7 \times 10^6$; $9 \times 10^5$    | SST of 5–6 °C and SSS of 34.5                        | [16]      |
| *Pseudo-nitzschia fraudulenta* *Pseudo-nitzschia pungens* | 3       | October–November 2002 | $2.1 \times 10^6$; $1.5 \times 10^5$ | Water temperature of 6–16 °C and SSS of 28.8–33.5   | [22]      |
| *Pseudo-nitzschia multiseries* *Pseudo-nitzschia calliantha* | 1       | October 2005         | $0.9 \times 10^6$; $1.2 \times 10^5$  | SST of 6–12 °C and SSS of 31–33.5                    | [19]      |
| *Pseudo-nitzschia calliantha* | 1       | September 2007       | $1.7 \times 10^5$                     | SST of 20 °C                                        | [15]      |
| *Pseudo-nitzschia multiseries* | 1       | September 2012       | $2.5 \times 10^5$                     | SST of 17.8 °C and SSS of 28.                       | [15]      |
| *Pseudo-nitzschia calliantha* | 1       | November 2012        | $1.1 \times 10^5$                     | SST of 6.8 °C                                       | [15]      |
| *Pseudo-nitzschia calliantha* | 5       | November 2012        | $1.4 \times 10^6$                     | SST of 6 °C                                          | Unpublished data |
| *Pseudo-nitzschia calliantha* | 6       | November 2012        | $1.1 \times 10^6$                     | SST of 6.5 °C                                       | Unpublished data |
| *Pseudo-nitzschia calliantha* | 7       | November 2012        | $1.2 \times 10^6$                     | SST of 6 °C                                          | Unpublished data |
| *Pseudo-nitzschia calliantha* | 7       | November 2013        | $1.9 \times 10^5$                     | SST of 5 °C                                          | Unpublished data |
| *Pseudo-nitzschia calliantha* | 6       | October 2015         | $3.5 \times 10^5$                     | SST of 10.4 °C                                      | Unpublished data |
| *Pseudo-nitzschia multiseries* | 7       | October 2015         | $1.7 \times 10^5$                     | SST of 14.3 °C                                      | Unpublished data |

There were five cases of blooms caused by *Pseudo-nitzschia calliantha* in November 2012, 2013, and October 2015, and one bloom of *Pseudo-nitzschia multiseries* in October 2015 in Ussuriisky Bay (Table 2). The maximum cell density of *Pseudo-nitzschia calliantha* ($1.4 \times 10^6$ cells L$^{-1}$) was recorded in November 2012 from station 5; the maximum concentration cell density of *Pseudo-nitzschia multiseries* ($1.7 \times 10^5$ cells L$^{-1}$) was recorded in October 2015 from station 7.
The data obtained showed that the highest concentration values of *Pseudo-nitzschia* in Amursky and Ussuriisky bays in 2005–2015 were determined by blooms of *Pseudo-nitzschia multistriata* and *Pseudo-nitzschia calliantha*, whose maximum density reached $0.9 \times 10^6$ cells L$^{-1}$ and $1.4 \times 10^6$ cells L$^{-1}$, respectively [15,19].

Thus, in northwestern Sea of Japan, there was a shift in the dominant *Pseudo-nitzschia* species since 2002: blooms caused by *Pseudo-nitzschia multiseries* were no longer observed, and *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia multistriata* became the causative organisms of *Pseudo-nitzschia* bloom events (Table 2, Figure 2).

The period 2007–2015, when the materials were taken year-round, was undoubtedly better studied in comparison with other periods of the study when some bloom events possibly could not be recorded by us due to the insufficient duration of the collections. However, from 2007 to 2015, we observed no bloom events caused by *Pseudo-nitzschia multiseries*, while most of the bloom events were caused by *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia multistriata*. These observations confirm the conclusion of the current study of long-term changes in the species composition of bloom-forming *Pseudo-nitzschia*.

4.2. Long-Term Variations in Species Composition of Bloom-Forming *Pseudo-nitzschia*

Phytoplankton samples from the permanent monitoring station 1 were analyzed to study long-term variations in species composition of bloom-forming *Pseudo-nitzschia*. The NMDS ordination plot graphically represents the variation in the species composition of *Pseudo-nitzschia* during blooms events in 1993–2015 (station 1). Five clusters (A–E) based on the dominant species were separated (Figures 3 and 4). The optimal number of clusters resulted from a k-means cluster analysis was five; these clusters corresponded to groups resulted by NMDS analysis. The Euclidian distances between clusters ranged of 49.6 to 62.8. The resulting clusters also were supported by the one-way ANOSIM test (Global $R = 0.997$, number of permutations: 999, number of permuted $R$ statistics greater than or equal to Global $R$: 0; for the results of the pair-wise test, see Table 3). The overlay of the MSD plot with the cluster dendrogram similarity lines indicated the respective maximum boundary values for discrimination of clusters. The maximum relative similarity of all clusters was about 0% (Bray–Curtis similarity), while the similarity between groups of clusters B, C, D, and cluster E was 3.8%. The maximum similarity was found between clusters B and C (16.4%).

![Figure 3. Cont.](image)
Thus, the analysis supports the conclusion that there was a change in bloom-forming species composition within the genus *Pseudo-nitzschia*; it was dominated by *Pseudo-nitzschia multiseries* during the early 1990s and 2002, and a shift towards the dominance of *Pseudo-nitzschia multistriata* and *Pseudo-nitzschia calliantha* occurred in 2005–2015.

**Figure 3.** Non-metric multidimensional scaling (NMDS) ordination analysis of cell density data of bloom-forming *Pseudo-nitzschia* species for different years of collection (n = 275). Five clusters (A–E) are separated. The cluster A formed by *Pseudo-nitzschia multiseries* is clearly distinctive (arrow) from clusters B–E formed by other bloom-forming species (a). Plot (b) shows detailed structure of clusters B–E.

**Figure 4.** Non-metric multidimensional scaling (NMDS) ordination analysis of cell density (cells L⁻¹) showing five clusters (A–E). The cell densities of *Pseudo-nitzchia multiseries* (red circles), *Pseudo-nitzchia pungens* (orange circles), *Pseudo-nitzchia calliantha* (blue circles), *Pseudo-nitzchia multistriata* (green circles), and other *Pseudo-nitzchia* species (grey circles) are given for clusters.
Table 3. Results of one-way ANOSIM pair-wise test to compare the similarity between clusters resulted by non-metric multidimensional scaling (NMDS) and k-means analyses. The number permutations was 999 and the number of permuted statistics greater than or equal to R-statistics was zero for all cluster combinations.

| Cluster | R-Statistics | Significance Level |
|---------|--------------|--------------------|
| A, B    | 1            | 0.1%               |
| A, C    | 1            | 0.1%               |
| A, D    | 1            | 0.1%               |
| A, E    | 1            | 0.1%               |
| B, E    | 0.999        | 0.1%               |
| B, C    | 0.976        | 0.1%               |
| B, D    | 1            | 0.1%               |
| E, C    | 0.997        | 0.1%               |
| E, D    | 1            | 0.1%               |
| C, D    | 1            | 0.1%               |

Cluster A formed by the \textit{Pseudo-nitzschia multiseries}-dominated collections of 1993 and 2002 was distinctive from all others (Figure 3a). It should be noted that blooms of this species with a cell density of more than $1 \times 10^6$ cells L$^{-1}$ were observed in 1993 and 2002 only (Figures 3a and 4).

Clusters B (collections of 1997, 2005, 2007–2015) and C (collections of 1997, 2005, 2007–2010, 2012, 2013, 2015) combined samples dominated by \textit{Pseudo-nitzschia pungens} and \textit{Pseudo-nitzschia calliantha} (Figures 3b and 4). The maximum concentration of \textit{Pseudo-nitzschia pungens} ($5.9 \times 10^5$ cells L$^{-1}$) was recorded in autumn 1997. In the following years, cell density of \textit{Pseudo-nitzschia pungens} did not exceed $6 \times 10^4$ cells L$^{-1}$ and accounted for 60%–100% of total cell density of \textit{Pseudo-nitzchia}. The most intensive bloom events of \textit{Pseudo-nitzschia callianta} were observed in autumn of 1997 (up to $2.7 \times 10^6$ cells L$^{-1}$). In the following years, this species was less abundant and reached up to $1 \times 10^5$ cells L$^{-1}$ in autumn of 2007 and 2012.

Cluster D was formed by \textit{Pseudo-nitzschia multistriata}-dominated collections of 2005, 2010, 2012, and 2015 (Figures 3b and 4). The most intensive bloom of \textit{P. multistriata} was registered in autumn of 2005 with a maximum density of about $1 \times 10^6$ cells L$^{-1}$. Another bloom event caused by \textit{Pseudo-nitzschia multistriata} was recorded in autumn of 2012 (up to $2.5 \times 10^5$ cells L$^{-1}$).

The last cluster (E) combined collections of 1997, 2007–2011, and 2014 dominated by other species of \textit{Pseudo-nitzchia} (\textit{Pseudo-nitzschia delicatissima}, and \textit{Pseudo-nitzchia} sp.), with their densities of up to a few thousand cells L$^{-1}$ (Figures 3b and 4).

Thus, the analysis supports the conclusion that there was a change in bloom-forming species composition within the genus \textit{Pseudo-nitzchia}; it was dominated by \textit{Pseudo-nitzschia multiseries} during the early 1990s and 2002, and a shift towards the dominance of \textit{Pseudo-nitzschia multistriata} and \textit{Pseudo-nitzschia calliantha} occurred in 2005–2015.

4.3. A Shift towards the Dominance of \textit{Pseudo-nitzschia multistriata} and \textit{Pseudo-nitzchia calliantha} and Long-Term Variations in Water Temperature

A decrease in the density of \textit{Pseudo-nitzschia multiseries} in different years in the past and in the first ten years of the current century was observed in different areas of the world ocean, including the northeastern coast of Canada [23], the coastal waters of Denmark [2,24,25], and California [26]. In the Northwest Pacific, \textit{Pseudo-nitzschia multiseries} reached the highest concentration (1.1–3.5 $\times$ 10$^7$ cells L$^{-1}$) in the coastal waters of Russia and Korea [13,27] in the 1990s. DA accumulated to extremely high concentrations (30.8–33.5 mg kg$^{-1}$) in bivalves, \textit{Mytilus edulis}, from the waters of Korea during blooms of this species [27]. After 2002, no bloom events of this species were recorded from the area of our study.

Blooms caused by \textit{Pseudo-nitzschia multiseries} in western Scottish Waters are known to frequently take place at low water temperatures during the cold season, while blooms of the
Pseudo-nitzschia pseudodelicatissima complex (including Pseudo-nitzschia calliantha), Pseudo-
nitzschia australis, and Pseudo-nitzschia pungens are associated mainly with the warm sea-
son [28]. The experiments with laboratory-reared cultures showed that Pseudo-nitzschia mul-
tiseries grows faster at lower temperatures (5 °C and 0 °C) [29–31] than does Pseudo-nitzschia 
pungens, which stops the cell division at 10 °C [31]. For this reason, some researchers [2] 
asumed an effect of seawater warming on reduction in the density of Pseudo-nitzschia mul-
tiseries and a competitive balance between this species and other Pseudo-nitzschia species.

As noted in the work of Lundholm et al. [2], the sharp decrease in Pseudo-nitzschia multiseries 
density may represent a combined effect of increased nutrient levels and water 
temperature. We did not find Pseudo-nitzschia multiseries in Amursky and Ussuriisky bays 
from 2005 to 2015, while blooms of Pseudo-nitzschia multistriata and Pseudo-nitzschia calliantha 
were observed. During the period of the most intense bloom caused by Pseudo-nitzschia multistriata 
and Pseudo-nitzschia calliantha in the autumn of 2005 in Amursky Bay, correlations of their density and the total density of Pseudo-nitzschia spp. with salinity, ammonium 
nitrogen concentration, and water temperature were studied [19]. Thus, Spearman’s corre-
lation analysis revealed a negative relationship between total density of Pseudo-nitzschia and salinity and ammonium nitrogen concentration (Table 4). A weak positive relationship 
between the density of bloom-forming Pseudo-nitzschia species and the water temperature 
was supported (Table 4). In particular, a weak correlation was found between the water 
temperature and the density of Pseudo-nitzschia multistriata (Spearman’s correlation coefficient 
$r = 0.432, p \leq 0.05$) and a weak correlation between the water temperature and the total density of Pseudo-nitzschia spp. ($r = 0.428, p \leq 0.05$) [19]. Furthermore, a quite strong 
negative correlation between the density and the concentration of ammonium nitrogen in 
water was recorded for Pseudo-nitzschia calliantha ($r = −0.691, p = 0.0005$), which supports 
the data reported by a number of authors that the increase in Pseudo-nitzschia abundance 
seems to be a response to eutrophication [2,32–34].

Table 4. Spearman’s coefficient of correlation between the cell density of bloom-forming Pseudo-
nitzschia species and environmental factors based on data from phytoplankton sampling (n = 21) in 
Amursky Bay in autumn 2005. Statistically significant results ($p \leq 0.05$) are marked in bold (data 
from Stonik et al. [19]).

| Factor          | Pseudo-nitzschia spp. | Pseudo-nitzschia multistriata | Pseudo-nitzschia calliantha |
|-----------------|-----------------------|-------------------------------|-----------------------------|
| NH$_4$          | $-0.521$              | $-0.487$                      | $-0.691$                    |
| Salinity        | $-0.445$              | $-0.441$                      | $-0.232$                    |
| Water temperature | $0.428$              | $0.432$                       | $0.204$                     |

Spearman’s correlation analysis of the whole data set was also performed in the 
current study based on data from year-round phytoplankton sampling from 2007 to 2015 
(Table 5). The densities of Pseudo-nitzschia calliantha, Pseudo-nitzschia pungens, Pseudo-nitzschia 
multistriata, and the total density of Pseudo-nitzschia spp. were positively correlated with 
water temperature ($p < 0.05$).

Table 5. Spearman’s coefficient of correlation (r) between the cell density of bloom-forming Pseudo-
nitzschia species and water temperature based on data from year-round phytoplankton sampling 
(n = 205) in Amursky Bay (station 1) during 2007–2015. Statistically significant results ($p < 0.05$) 
are presented.

| Species           | r        |
|-------------------|----------|
| Pseudo-nitzschia calliantha | 0.278   |
| Pseudo-nitzschia pungens      | 0.188   |
| Pseudo-nitzschia multistriata  | 0.154   |
| Pseudo-nitzschia spp.         | 0.464   |
Although the statistical positive correlation does not directly indicate a causal relation, the correlation implies that water temperature might be one of the causative factors for the increased cell concentrations of *Pseudo-nitzschia multistriata* and *Pseudo-nitzchia calliantha* in Amursky Bay after 2002.

An earlier year-round study of phytoplankton investigated the dynamics of the *Pseudo-nitzschia* spp. density in Amursky Bay during 2007–2013 and revealed high cell densities in autumn, which can be considered as a season of *Pseudo-nitzschia* bloom events [15]. Zuenko and Rachkov [18] analyzed long-term climatic variations in water temperature in Amursky Bay over a more than 30 year period (1980—2013). During this period, waters of the bay showed a warming trend. Thus, a comparison of the average monthly sea surface temperatures (SST) in the period from 1980–1989 to 1998–2009 indicates a temperature increase in all months except March in Amursky Bay, with the most pronounced warming recorded in autumn (Figure 5), i.e., during the season of *Pseudo-nitzschia* spp. bloom in the northwestern Sea of Japan [15]. Thus, events of blooms caused by *Pseudo-nitzschia multistriata* and *Pseudo-nitzschia calliantha* were registered in Amursky Bay in autumn of 2005–2009, at a 0.9–1.4 °C increase in water temperature in comparison with the period of 1980–1989 (Table 6).

![Figure 5. Difference of mean monthly SST between 1980–1989 and 1998–2009 in Amursky Bay. Reproduced with permission from Federal Research Institute of Fisheries and Oceanography, Pacific Branch (TINRO) from (Climatic Changes of Temperature, Salinity and Nutrients in the Amur Bay of the Japan Sea. Zuenko Yu. I.; Rachkov V.I. Izvestiya TINRO 2015, 183, 186–199).](image)

**Table 6.** *Pseudo-nitzschia* bloom events in Amursky Bay during 2005–2009 and the difference of mean monthly SST in Amursky Bay between 1980–1989 and 1998–2009, adapted from [18].

| Species and Temperature Differences                      | Months and Years of Bloom Events |
|---------------------------------------------------------|----------------------------------|
| *Pseudo-nitzschia multistriata*                         | nd                               |
| *Pseudo-nitzschia calliantha*                           | 2007                             |
| Difference of mean monthly SST                          | 0.9 °C                           |
|                                                        | 1.4 °C                           |

nd, not detected.

The climate of the Russian Far East is monsoon and is characterized by sharp variations in hydrometeorological processes during winter and summer monsoons. As regards the warm season of the year, when *Pseudo-nitzschia* bloom events occur, it should be noted that
the initial summer monsoon phase shows a tendency to weaken due to the weakening of the Okhotsk High. This process is caused by an increase in the atmosphere’s thermal insulation properties (the so-called “greenhouse effect”). The inter-decadal fluctuations in the strength of the Okhotsk High are observed against the background of global trends, presumably due to the Arctic oscillation. The intensity of the summer monsoon in the final phase is determined by the distribution of atmospheric pressure in the North Pacific (Hawaiian) High pressure system, which also tends to weaken and is subject to inter-decadal fluctuations [18,35].

The relationships of the observed water temperature variations in Amursky Bay with large-scale climate changes were analyzed [18]. A conclusion was made that the water warming in Amursky Bay is induced mainly by the weakening summer monsoon, which determines weather conditions in Primorsky Krai, and the cross-shelf water exchange [18]. Based on these data, we assume that one of the factors responsible for the increase in the density of *Pseudo-nitzchia multistriata* and *Pseudo-nitzchia calliantha* off the Primorsky Krai coast may be the increased SST recorded from Amursky Bay in the period of 1998–2009 compared to the data of 1980–1989. This increase in water temperature is induced mainly by the long-term climatic changes such as, in particular, the weakening of the summer monsoon, which, in turn, is related to the long-term downward trend of the Okhotsk High index in 1990–2015 [18,35].

5. DA Content of *Pseudo-nitzschia* Cultures

Data on the DA content of *Pseudo-nitzschia* cultures from Russian waters of the Sea of Japan and coastal waters of Japan and Korea accumulated to date indicate that *P. multiseries* is the only highly toxic species in the Northwest Pacific (Table 7). Thus, according to an HPLC-fluorescence analysis, *Pseudo-nitzchia multiseries* isolated and cultured from Japanese and Russian waters had a high DA content with maximum concentrations of 5390 ng mL\(^{-1}\) and 317 ng mL\(^{-1}\) DA or 140 and 20.8 picogram cell\(^{-1}\), respectively [11,36]. The presence of DA in the cultures of *Pseudo-nitzchia multistriata* and *Pseudo-nitzchia calliantha* was confirmed for the laboratory-reared cultures isolated from the northwestern Sea of Japan [15]. The use of an Amnesic Shellfish Poisoning (ASP) direct Competative enzyme-linked assay (cELISA) kit (Biosence laboratories AS, Norway, Association of Official Agricultural Chemists (AOAC) official method 2006.02) showed that *Pseudo-nitzchia multistriata* and *Pseudo-nitzchia calliantha* strains isolated during their bloom events from the northwestern Sea of Japan produced relatively low levels of DA (up to 0.57 and 0.44 picogram cell\(^{-1}\), respectively). A low level of cellular DA (0.0004 picogram cell\(^{-1}\)) was also found in the strains of *Pseudo-nitzchia pungens var. pungens* from the northwestern Sea of Japan. Thus, in recent years, numerous *Pseudo-nitzschia* bloom events in the area of our study were not accompanied by significant accumulation of DA in the diatom.

Table 7. Maximum concentrations of domoic acid (DA) produced in laboratory-reared *Pseudo-nitzschia* cultures isolated from the northwestern Pacific Ocean during the period of their bloom.

| Species | Maximum DA Concentration (Picogram DA Cell\(^{-1}\)) |
|---------|-----------------------------------------------------|
| *Pseudo-nitzchia multiseries* | 20.8 (Peter the Great Bay, Russia\(^a\)) 1.03–2.4 (Jinhae and Chinhae bays, South Korea\(^b\)) 1.15–140 (Okkiray Bay, Japan\(^c\)) 5.7 (Ofunato Bay, Japan\(^d\)) |
| *Pseudo-nitzchia pungens var. pungens* | 0.0004\(^e\) |
| *Pseudo-nitzchia calliantha* | 0.44\(^e\) |
| *Pseudo-nitzchia multistriata* | 0.57\(^e\) |

\(^{a}[11];\(^{b}[27,31];\(^{c}[36];\(^{d}[37];\(^{e}[15].

6. Conclusions

We consider the relatively low concentration of DA in the clones of *Pseudo-nitzchia multistriata* and *Pseudo-nitzchia calliantha* as an important factor to explain that no serious toxic events (poisoning of humans and marine animals and significant accumulation of
DA in shellfish) were observed in the area of our study to date, despite these species being regularly recorded from the Russian waters of the northwestern Sea of Japan at concentrations corresponding to a bloom event (>10^5 cells L^-1). Thus, the absence of *Pseudo-nitzschia multiseries*, the only highly toxic species registered in Peter the Great Bay among the dominant species, may be one of the possible causes of the absence of significant damage from algal blooms over the past 15 years.

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