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THE EFFECT OF TEMPERATURE ON THE GROWTH OF TWO SPECIES OF *PSEUDO-NITZSCHIA* H. PERAGALLO (BACILLARIOPHYTA) IN LABORATORY CULTURES ISOLATED FROM THE SEA OF JAPAN

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Diatoms of the genus *Pseudo-nitzschia* H. Peragallo, 1900, known as producers of the neurotoxic domoic acid, regularly cause algal blooms in the Russian Far Eastern seas. Temperature is an important factor affecting diatom blooms; however, its effect on the growth of this group of microalgae from the Sea of Japan has not been sufficiently studied. In this regard, growth characteristics of two diatom species were investigated in laboratory culture within the temperature range of +5 to +20 °C. The test involved direct counting in a Nageotte chamber. Cell density, growth rates, and generation time were evaluated. As found, the maximum average density of *P. fraudulenta* reached 2.2×10^5 cells·L^{-1} on the 16th day of the experiment at +18 °C. For this species, at +18 °C, the growth rate (0.11–0.16 div.·day^{-1}) remained relatively high, and the generation time (4.4–6.7 days) was relatively low for most of the test. The maximum mean density of *P. hasleana*, 5×10^5 cells·L^{-1}, was recorded on the 16th day of the experiment at +17 °C. For this species, high growth rate (0.2–0.92 div.·day^{-1}) and low generation time (0.8–3.6 days) were recorded at +17 °C from the 2nd to the 10th day of the test. The average densities of *P. fraudulenta* and *P. hasleana* were statistically significantly higher at +17 °C and +18 °C, respectively, than at the other temperatures studied (Tukey's test, $p < 0.05$). As recorded, when *P. fraudulenta* clones were grown at +10, +16, and +18 °C, and when *P. hasleana* clones were cultured at +14, +17, and +20 °C, their cells remained viable and continued to divide. When the cultivation temperature for *P. fraudulenta* and *P. hasleana* was lowered to +5 and +7 °C, respectively, division slowed down dramatically, and cell density was statistically significantly lower than at higher temperatures (Tukey's test, $p < 0.05$). Ranges of tolerant temperature during *P. fraudulenta* and *P. hasleana* cultivation were found to be within +10…+18 °C and +14…+17 °C, respectively. The revealed lower temperature tolerance limits for the two species during cultivation (+10 and +14 °C) corresponded to water temperatures under which *P. fraudulenta* and *P. hasleana* blooms were observed in the natural environment (+6…+16 and +10…+16 °C, respectively). The study demonstrated the broad adaptive potential of the investigated species to temperature changes.

Keywords: *Pseudo-nitzschia fraudulenta*, *Pseudo-nitzschia hasleana*, laboratory cultivation, temperature, Sea of Japan

Diatoms of the genus *Pseudo-nitzschia* H. Peragallo, 1900 are a dominant taxon of toxic planktonic microalgae in the northwestern Sea of Japan: those account for 75–98% of the total phytoplankton density during bloom seasons [Orlova et al., 2008]. They are known as potential producers of neurotoxic domoic acid [Bates et al., 2018; Liu et al., 2021; Zhou et al., 2024] and belong to one of the most

numerous groups of toxic phytoplankton regularly causing algal blooms in the Far Eastern seas of Russia [Stonik, 2021; Stonik, Orlova, 2018; Stonik et al., 2011, 2019]. Temperature plays a decisive role in the metabolic processes of Bacillariophyta and other microalgae, affects photosynthesis and nutrient absorption, and mediates enzymatic processes in cells [Claquin et al., 2008; Davison et al., 1991; Klochkova, Lelekov, 2022; Kuzmin, 2025; Raven, Geider, 1988]. Thus, temperature is a significant factor of the occurrence and development of diatom blooms [Fu et al., 2012; Ryabushko et al., 2008]. Two species, *Pseudo-nitzschia fraudulenta* (Cleve) Hasle, 1993 and *Pseudo-nitzschia hasleana* Lundholm, 2012, are regularly recorded in Peter the Great Bay (the Sea of Japan) as an important component of phytoplankton involved in the formation of water blooms [Stonik, Orlova, 2018; Stonik, Zinov, 2023; Stonik et al., 2008]. However, environmental factors, primarily temperature, driving the abundance and physiological state of *Pseudo-nitzschia* spp. from the Sea of Japan have not been sufficiently studied.

In this regard, the aim of the work is to investigate under experimental conditions the effect of temperature on the dynamics of density, division rate, and generation time in laboratory cultures of *Pseudo-nitzschia fraudulenta* and *P. hasleana*: diatoms isolated from waters of the Russian sector of the Sea of Japan.

MATERIAL AND METHODS

The object of the study is unialgal cultures of diatoms of the genus *Pseudo-nitzschia* isolated from Peter the Great Bay, Sea of Japan. *P. fraudulenta* culture (clone MBRU-PF-16) was isolated from the Amur Bay (N43.2°, E131.91°) in November 2016 at water temperature of +5.2 °C. *P. hasleana* culture (clone MBRU-PH-18) was isolated from the Patrokl Bay of the Ussuri Bay (N43.07°, E131.96°) in November 2018 at water temperature of +6.8 °C. The cultures are maintained in the "Marine Biobank" core facility at NSCMB FEB RAS [2024].

The cultures were grown on f/2 nutrient medium [Guillard, Ryther, 1962]. Before the experiment, both clones were pre-adapted to studied temperatures for four days. Cultures at the exponential growth stage were used as an inoculum. For the test, the cultures were transferred to Erlenmeyer flasks (250 mL) with a culture suspension volume of 200 mL; the light flux intensity was of 3,500 lux, and photoperiod was 12 h : 12 h (light : darkness). *P. fraudulenta* culture was grown at +5, +10, +16, and +18 °C; *P. hasleana* culture, at +7, +14, +17, and +20 °C (in three flasks at each value). Binder KBW 400 climate chambers (Germany) were used to create the required temperature conditions. Illumination in the chambers was provided by daylight fluorescent lamps. The intensity of the luminous flux was measured with a UNI-T mini UT383 00-00007443 light meter (UNI-T, China). The experiments were performed in triplicate.

The cell density was estimated by direct counting in a 0.05-mL Nageotte counting chamber under an Olympus BX41 light microscope (Japan) at 20× magnification. Cells were counted every two days. Samples for quantitative analysis were taken in triplicate 2–3 h after the end of the dark period, and the suspension was thoroughly mixed and fixed with Utermöhl's solution [Fedorov, 1979].

The specific growth rate of the culture was calculated based on cell concentration data by the formula [Zaika, 1972]:

$$\mu = \frac{\ln X_1 - \ln X_0}{T_1 - T_0},$$

where X_1 and X_0 are concentration values corresponding to the time of growth T_1 and T_0.

The cell density doubling time (generation time) was estimated by the formula [Jones et al., 1963]:

$$g = \frac{\ln 2}{\mu} = \frac{0.693}{\mu},$$

where g is the generation time;

μ is the specific growth rate.

A test for a reliable relationship between temperature, age of the culture, and cell density was performed using a two-factor analysis of variance. Data on cell density, growth rate, and generation time depending on temperature were subjected to a one-factor analysis of variance. For multiple comparisons of means, Tukey test was applied. All calculations were performed in Statistica 7.0 [StatSoft, 2025].

RESULTS

As revealed using a two-factor analysis of variance, both factors considered in the experiment, temperature and age of culture, and their interaction were statistically significant ($p < 0.001$) and affected *Pseudo-nitzschia* spp. cell density (Table 1).

Table 1. Results of a two-factor analysis of variance on the effect of *Pseudo-nitzschia fraudulenta* and *P. hasleana* cultivation conditions on cell density

Factor	*F*		*p*
	Pseudo-nitzschia fraudulenta	*Pseudo-nitzschia hasleana*	
	942.3	12,764.37	0.00
Temperature	373.7	477.33	0.00
Age of the culture	42.8	1,238.92	0.00

Note: all values of the correlation coefficients, the Fisher's criterion (*F*) and probability (*p*), were significant.

The dynamics of *P. fraudulenta* and *P. hasleana* density under cultivation conditions at different temperatures is shown in Fig. 1.

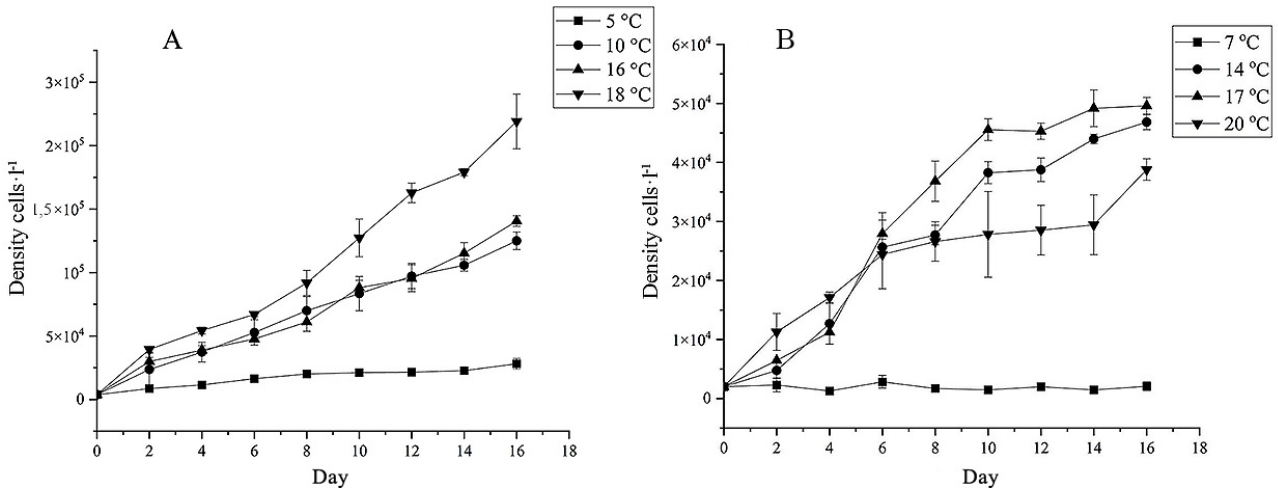

Fig. 1. Dynamics of cell densities of *Pseudo-nitzschia fraudulenta* cells (A) and *P. hasleana* cells (B) at different temperature (the means and standard deviations are given)

Pseudo-nitzschia fraudulenta. The initial cell density in the experiment was 3.7×10^3 cells·L^{-1}. Throughout the test, there was an increase in total cell density in a range of temperatures +10 to +18 °C (Fig. 1A). The maximum mean density (2.2×10^5 cells·L^{-1}) was recorded on the 16th day of the experiment at +18 °C, and the minimum one (8.6×10^3 cells·L^{-1}) was registered on the 2nd day at +5 °C. When the cultivation temperature was reduced to +5 °C, the mean density was 2.8×10^4 cells·L^{-1} on the day 16; it is approximately an order of magnitude lower than at the end of the test at +18 °C. The mean cell density at +18 °C was statistically significantly higher (Tukey test, $p < 0.05$) than at other temperature values throughout the experiment. From the 4th day of cultivation and until the end of the test, there were no statistically significant differences between the mean density values at +10 and +16 °C (Fig. 1A, Table 2).

Table 2. Statistically significant differences between *Pseudo-nitzschia fraudulenta* cell abundance curves at different temperature based on the Tukey matrix at $p < 0.05$

Day	Temperature, °C			
	+5	+10	+16	+18
2	+10, +16, +18	+5, +16, +18	+5, +10, +18	+5, +10, +16
4	+10, +16, +18	+5, +18	+5, +18	+5, +10, +16
6	+10, +16, +18	+5, +18	+5, +18	+5, +10, +16
8	+10, +16, +18	+5, +18	+5, +18	+5, +10, +16
10	+10, +16, +18	+5, +18	+5, +18	+5, +10, +16
12	+10, +16, +18	+5, +18	+5, +18	+5, +10, +16
14	+10, +16, +18	+5, +18	+5, +18	+5, +10, +16
16	+10, +16, +18	+5, +18	+5, +18	+5, +10, +16

The mean growth rates during cultivation at +5 °C (0.1–0.18 div.·day^{-1}) and +10 °C (0.14–0.23 div.·day^{-1}) were higher than at other temperatures from day 2 to days 6–8. From the 8th to the 14th day, the growth rates dropped reaching a minimum (0.01 div.·day^{-1}) against the backdrop of the maximum generation time (40.5 days) on days 10–12 at +5 °C (Table 3).

Table 3. Growth rate (div.·day^{-1}) and generation time (days) during *Pseudo-nitzschia fraudulenta* cultivation at different temperature

Days	+5 °C		+10 °C		+16 °C		+18 °C	
	μ	g	μ	g	μ	g	μ	g
2–4	0.14 ± 0.063	5.5 ± 1.902	0.23 ± 0.067	3.3 ± 0.631	0.13 ± 0.011	5.3 ± 0.234	0.16 ± 0.016	4.4 ± 0.267
4–6	0.18 ± 0.051	4.1 ± 1.070	0.17 ± 0.056	4.2 ± 1.187	0.1 ± 0.024	6.9 ± 1.464	0.11 ± 0.015	6.7 ± 1.039
6–8	0.1 ± 0.051	9.2 ± 5.016	0.14 ± 0.056	6.4 ± 4.450	0.12 ± 0.024	6.1 ± 2.258	0.16 ± 0.015	4.7 ± 1.520
8–10	0.03 ± 0.051	35.1 ± 17.094	0.09 ± 0.056	11.4 ± 5.253	0.11 ± 0.024	6.4 ± 1.340	0.16 ± 0.015	4.5 ± 1.548
10–12	0.01 ± 0.004^{b,c}	40.5 ± 2.428$^{a–c}$	0.08 ± 0.031	10.5 ± 5.477^{a}	0.11 ± 0.020^{b}	6.4 ± 1.240^{b}	0.12 ± 0.037^{c}	5.9 ± 2.141^{c}
12–14	0.03 ± 0.004^{b,c}	31.5 ± 12.416	0.04 ± 0.031	16.6 ± 2.056	0.1 ± 0.020^{b}	7.8 ± 2.554	0.05 ± 0.037^{c}	15.8 ± 5.026
14–16	0.1 ± 0.004^{b,c}	11 ± 5.004	0.08 ± 0.031	8.7 ± 2.199	0.1 ± 0.020^{b}	7.4 ± 2.112	0.1 ± 0.037^{c}	7.7 ± 2.535

Note: the values of growth rates and generation time are provided in the columns μ and g, respectively, with confidence intervals. Footnotes to the means mark results that are statistically significantly different (Tukey's test, $p < 0.05$) under the following cultivation conditions: +5 and +10 °C (a); +5 and +16 °C (b); +5 and +18 °C (c).

Cultivation at +16 and +18 °C revealed relatively high growth rates (0.11–0.16 div.·day^{-1}) and low values of generation time (4.4–6.9 days) during most of the experiment, from the 2nd to the 12th day (Table 3). On days 12–14 at +16…+18 °C, a decrease in the growth rate and a rise in the generation time were recorded compared to the values in the previous periods of cultivation (from day 2 to day 12) (Table 3).

A slight increase in the growth rate at the end of the test, on the 14–16th days, at +5, +10, and +18 °C was noticeably lower than the values in the first half of the experiment (Table 3). On days 10–14 of cultivation, statistically significant differences in the mean growth rates (Tukey test, $p < 0.05$) at +5 and +16 °C, as well as at +5 and +18 °C, were registered. The growth rates at +5 °C were noticeably lower during most of the test than at a higher temperature (Table 3), and this determined the low cell density in the experiment at +5 °C (Fig. 1A).

Pseudo-nitzschia hasleana. The initial cell density in the test was 2 × 10^3 cells·L^{-1}. Throughout the experiment on *P. hasleana* culturing, we revealed a rise in the total cell density in the temperature range +14 to +20 °C (Fig. 1B).

The maximum mean density (5 × 10^5 cells·L^{-1}) was recorded on day 16 at +17 °C, and the minimum mean density (1.3 × 10^3 cells·L^{-1}) was noted on day 4 at +7 °C. At cultivation temperature of +7 °C, on the 6th day of the experiment, the cell density increased to 2.8 × 10^3 cells·L^{-1}, and then it dropped; only on the 12th day, it rose to 2 × 10^3 cells·L^{-1} and remained at this level until the end of the test (Fig. 1B). The differences in the mean density values at all studied temperature values (+7, +14, +17, and +20 °C) were statistically significant (Tukey test, $p < 0.05$) only on days 10–12 of the experiment (Table 4).

Table 4. Statistically significant differences between *Pseudo-nitzschia hasleana* cell abundance curves at different temperature based on the Tukey matrix at $p < 0.05$

Day	Temperature, °C			
	+7	+14	+17	+20
2	+17, +20	+20	+7, +20	+7, +14, +17
4	+14, +17, +20	+7	+7	+7
6	+14, +17, +20	+7	+7, +20	+7, +17
8	+14, +17, +20	+7, +17	+7, +14, +20	+7, +17
10	+14, +17, +20	+7, +17, +20	+7, +14, +20	+7, +14, +17
12	+14, +17, +20	+7, +17, +20	+7, +14, +20	+7, +14, +17
14	+14, +17, +20	+7, +20	+7, +20	+7, +14, +17
16	+14, +17, +20	+7	+7, +20	+7, +17

The mean cell density at +17 °C was noticeably higher (Tukey test, $p < 0.05$) than that for all other temperature values on the 8–12th days of the test (Table 4). On days 14–16, no statistically significant differences were found between the mean density at +14 and +17 °C (Fig. 1B, Table 4).

When *P. hasleana* was cultured at +14, +17, and +20 °C, high growth rates were recorded during the first half of the experiment, and at 7 °C, during the second half (Table 5).

Thus, at a temperature of +14 °C, the highest growth rates (0.73–0.98 div.·day^{-1}) were registered from the 2nd to the 6th day. From days 6–8 to 8–10, the values decreased from 0.54 to 0.32 div.·day^{-1}. From the 10th to the 12th day and until the end of the experiment, the growth rate at this temperature

was relatively low, with a minimum (0.01 div.·day^{-1}) on the 10–12th day (Table 5). High growth rates (0.2–0.92 div.·day^{-1}) were noted at +17 °C from day 2 to day 10, and at +20 °C, from day 2 to day 8 (0.2–0.47 div.·day^{-1}). From the 10th day until the end of the test, the growth rates at +14, +17, and +20 °C dropped dramatically compared to the values in the first part of the experiment (Table 5). The exceptions were relatively high growth rate (0.19 div.·day^{-1}) and long generation time (5.1 days) on days 14–16th at +20 °C.

Table 5. Growth rate (div.·day^{-1}) and generation time (days) during *Pseudo-nitzschia hasleana* cultivation at different temperature

Days	+7 °C		+14 °C		+17 °C		+20 °C	
	μ	g	μ	g	μ	g	μ	g
2–4	0.08 ± 0.003$^{a–c}$	8.7 ± 0.347$^{a–c}$	0.98 ± 0.075^{a,d,e}	0.7 ± 0.054^{a}	0.54 ± 0.164^{b,d,e}	1.4 ± 0.506^{b}	0.47 ± 0.130^{c,e}	1.6 ± 0.447^{c}
4–6	0.05 ± 0.023$^{a–c}$	15.5 ± 5.613$^{a–c}$	0.73 ± 0.240^{a}	1.0 ± 0.283^{a}	0.92 ± 0.159^{b}	0.8 ± 0.138^{b}	0.20 ± 0.097^{c}	4.0 ± 1.876^{c}
6–8	0.07 ± 0.001^{b,c}	9.8 ± 0.200$^{b–e}$	0.54 ± 0.031$^{a,d–e}$	6.4 ± 2.368$^{d.e}$	0.28 ± 0.033^{b,d}	2.5 ± 0.323^{b,d}	0.22 ± 0.050^{c,e}	3.3 ± 0.888^{c,e}
8–10	0.16 ± 0.001^{b,c}	4.8 ± 2.086	0.32 ± 0.031^{d,e}	2.1 ± 0.089^{e}	0.20 ± 0.033^{b,d}	3.6 ± 1.167	0.08 ± 0.010^{c,e}	11.4 ± 5.949^{e}
10–12	0.15 ± 0.049$^{a–c}$	4.9 ± 1.796^{a}	0.01 ± 0.008^{a}	7.2 ± 1.190^{a}	0.02 ± 0.016^{b}	18.3 ± 3.928	0.06 ± 0.017^{c}	12.1 ± 3.136
12–14	0.25 ± 0.049$^{a–c}$	2.9 ± 0.464$^{b.c}$	0.14 ± 0.008^{a}	5.3 ± 1.942	0.07 ± 0.016^{b}	12.1 ± 5.488^{b}	0.06 ± 0.017^{c}	11.0 ± 1.865^{c}
14–16	0.31 ± 0.049^{a,b}	2.3 ± 0.464^{b,c}	0.04 ± 0.017^{a}	9.7 ± 1.942	0.01 ± 0.020^{b}	8.1 ± 5.488^{b}	0.19 ± 0.123	5.1 ± 1.865^{c}

Note: the values of growth rates and generation time are provided in the columns μ and g, respectively, with confidence intervals. Footnotes to the means mark results that are statistically significantly different (Tukey's test, $p < 0.05$) under the following cultivation conditions: +7 and +14 °C (a); +7 and +17 °C (b); +7 and +20 °C (c); +14 and +17 °C (d); +14 and +20 °C (e).

Cultivation at +7 °C showed as follow: from the 2nd to the 8th day, the growth rates remained relatively low (0.05–0.08 div.·day^{-1}), and the generation time was high (8.7–15.5 days). Only on days 8–10, the growth rates began to rise (Table 5). Throughout most of the test at +7 °C, the growth rates were statistically significantly lower (Tukey test, $p < 0.05$) than at a higher temperature, and this mediated the low density at the minimum temperature investigated (Fig. 1B).

DISCUSSION

Our data show that *P. fraudulenta* and *P. hasleana* are capable of remaining in a viable state throughout the experiment and actively divide within the temperature ranges of +10…+18 °C and +14…+20 °C, respectively. The mean cell density of *P. fraudulenta* at +18 °C was statistically significantly higher than at other values (Tukey test, $p < 0.05$) throughout the test (Fig. 1A, Table 2). The mean cell density of *P. hasleana* at +17 °C was noticeably higher (Tukey test, $p < 0.05$) than that at other temperatures on days 8–12 (Fig. 1B, Table 4). At a temperature tolerant for *P. fraudulenta* and *P. hasleana* cultivation, in most cases, the growth rates at the end of the experiment (from the 10–12th to 14–16th days) were lower, and the generation time was higher than during the previous part of the test, despite small peaks on the 14–16th day at +18 °C for *P. fraudulenta* and at +20 °C for *P. hasleana* (Tables 3, 5).

This indicated a slowdown in cell growth towards the end of the experiment. It can be assumed as follows: on days 14–16, due to the release of nutrients resulting from the decomposition of dying cells and the effect of bacterial exometabolites, which can promote the growth of microalgae, small secondary peaks in density and growth rate were recorded. Due to the low growth rates and longer generation time, the mean cell density of *P. fraudulenta* and *P. hasleana* when cultured at +5 and +7 °C, respectively, was significantly lower than that at a higher temperature (Tukey test, $p < 0.05$) (Fig. 1A and B).

So, the ranges of tolerant temperatures for *P. fraudulenta* and *P. hasleana* cultivation were +10 to +18 °C and +14 to +20 °C, respectively. The optimal temperatures for the development of these species in culture were +18 and +17 °C, respectively. Since the clones we studied were isolated from the natural environment at relatively low water temperatures (about +5…+7 °C), *P. fraudulenta* and *P. hasleana* appear to be adapted to surviving in a wide range of temperatures. This is consistent with the available literature data on the widespread distribution of these species in plankton at high, low, and temperate latitudes [Bates et al., 2018].

Literature data support our findings on the development of the investigated species in a wide range of temperature for cultivation. According to previous studies, for *P. fraudulenta* isolates from different areas of the World Ocean, the optimal ranges of water temperatures for growth were +10…+15 °C [Ayache et al., 2021; Fehling et al., 2006; Gai et al., 2018; Thessen et al., 2009] and +18.5…+26.5 °C [Delegrange et al., 2018]. Other authors reported a temperature of (20.8 ± 0.8) °C for a *P. fraudulenta* clone from coastal waters of France [Claquin et al., 2008]. In the North Atlantic, *P. fraudulenta* vegetation began at a water temperature of +9…+14.4 °C [Hasle, 1965]. Importantly, this did not differ much from our data on the species blooms in Peter the Great Bay (Sea of Japan) within the temperature range of +6…+16 °C [Stonik et al., 2008]. As for *P. hasleana*, according to literature data, water temperature favorable for its development off the coast of Australia exceeded +16 °C [Ajani et al., 2013]. Water blooms caused by *Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle, 2003 and *P. hasleana* in Peter the Great Bay were previously registered by us at water temperature of +10…+16 °C [Stonik, Zinov, 2023]. Thus, lower limits of temperature conditions for culturing two species we revealed (+10 and +14 °C) are consistent with the parameters of water temperature in the natural environment (Peter the Great Bay, Sea of Japan) at which blooms of *P. fraudulenta* (+6…+16 °C) and *P. hasleana* (+10…+16 °C) were observed.

The occurrence of high adaptive capabilities of *Pseudo-nitzschia* species in relation to temperature, along with high genetic variability in natural populations of this genus [Evans et al., 2005], suggest that *P. fraudulenta* and *P. hasleana* may gain advantages for growth in a relatively wide range of water temperatures, +10…+18 °C and +14…+20 °C, respectively, in Russian waters of the Sea of Japan. The obtained data can be used to predict blooms of the analyzed species in the study area.

Conclusions. When *Pseudo-nitzschia fraudulenta* clones were grown at temperatures of +10, +16, and +18 °C, and *Pseudo-nitzschia hasleana* clones were grown at +14, +17, and +20 °C, cells remained in a viable state and continued to divide. When the cultivation temperature of *P. fraudulenta* and *P. hasleana* was lowered to +5 and +7 °C, respectively, division slowed down dramatically.

The ranges of tolerable temperatures during *P. fraudulenta* and *P. hasleana* cultivation were +10 to +18 °C and +14 to +20 °C, respectively. The revealed lower limits of the range of temperatures for cultivation of these two species (+10 and +14 °C) are consistent with the parameters of water temperature in the natural environment, Peter the Great Bay (Sea of Japan), under which blooms of *P. fraudulenta* (+6…+16 °C) and *P. hasleana* (+10…+16 °C) were observed.

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REFERENCES

1. Zaika V. E. *Specific Production of Aquatic Invertebrates.* Kyiv : Naukova dumka, 1972, 147 p. (in Russ.). https://repository.marine-research.ru/handle/299011/1127
2. Klochkova V. S., Lelekov A. S. Study of the temperature effect on the specific growth rate of *Arthrospira platensis* culture. *Trudy Karadagskoi nauchnoi stantsii imeni T. I. Vyazemskogo – prirodnogo zapovednika RAN*, 2022, no. 1 (21), pp. 40–50. (in Russ.). https://doi.org/10.21072/eco.2022.21.05
3. Kuzmin D. V. *Razrabotka platformy po polucheniyu biologicheski aktivnykh soedinenii iz fotosinteziruyushchikh mikroorganizmov* : avtoref. dis. … d-ra biol. nauk : 1.5.6 / Vserossiiskii nauchno-issledovatel'skii institut sel'skokhozyaistvennoi biotekhnologii. Dolgoprudny, 2025, 47 p. (in Russ.)
4. *Marine Biobank. Resource Collection. Core Shared Research Facility* / National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences : [site]. Vladivostok, 2024. (in Russ.). URL: https://marbank.dvo.ru/index.php/ru/ [accessed: 25.11.2024].
5. Ryabushko L. I., Besiktepe S., Ediger D., Yilmaz D., Zenginer A., Ryabushko V. I., Lee R. I. Toxic diatom of *Pseudo-nitzschIa calliantha* Lundholm, Moestrup et Hasle from the Black Sea: Morphology, taxonomy, ecology. *Morskoj ekologicheskij zhurnal*, 2008, vol. 7, no. 3, pp. 51–60. (in Russ.). https://repository.marine-research.ru/handle/299011/973
6. Stonik I. V., Orlova T. Y. Domoic acid–producing diatoms of the genus *Pseudo-nitzschia* H. Peragallo, 1900 (Bacillariophyta) from the North Pacific. *Biologiya morya*, 2018, vol. 44, no. 5, pp. 299–306. (in Russ.). https://doi.org/10.1134/S0134347518050017
7. Fedorov V. D. *O metodakh izucheniya fitoplanktona i ego aktivnosti* : [uchebnoe posobie]. Moscow : Izd-vo Mosk. un-ta, 1979, 167 p. (in Russ.)
8. Ajani P., Murray S., Hallegraef G., Brett S., Armand L. First reports of *Pseudo-nitzschia micropora* and *P. hasleana* (Bacillariaceae) from the Southern Hemisphere: Morphological, molecular and toxicological characterization. *Phycological Research*, 2013, vol. 61, iss. 3, pp. 237–248. https://doi.org/10.1111/pre.12020
9. Ayache N., Lundholm N., Gai F., Hervé F., Amzil Z., Caruana A. Impacts of ocean acidification on growth and toxin content of the marine diatoms *Pseudo-nitzschia australis* and *P. fraudulenta*. *Marine Environmental Research*, 2021, vol. 169, art. no. 105380 (9 p.). https://doi.org/10.1016/j.marenvres.2021.105380
10. Bates S., Hubbard A., Lundholm N., Montresor M. *Pseudo-nitzschia*, *Nitzschia*, and domoic acid: New research since 2011. *Harmful Algae*, 2018, vol. 79, pp. 3–43. https://doi.org/10.1016/j.hal.2018.06.001
11. Claquin P., Probert I., Lefebvre S., Véron B. Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae. *Aquatic Microbial*

Ecology, 2008, vol. 51, no. 1, pp. 1–11. https://doi.org/10.3354/ame01187

12. Davison I. R., Greene R. M., Podolak E. J. Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Marine Biology*, 1991, vol. 110, iss. 3, pp. 449–454. https://doi.org/10.1007/bf01344363
13. Delegrange A., Lefebvre A., Gohin F., Courcot L., Vincent D. *Pseudo-nitzschia* sp. diversity and seasonality in the southern North Sea, domoic acid levels and associated phytoplankton communities. *Estuarine, Coastal and Shelf Science*, 2018, vol. 214, pp. 194–206. https://doi.org/10.1016/j.ecss.2018.09.030
14. Evans K. M., Kuhn S. F., Hayes P. K. High levels of genetic diversity and low levels of genetic differentiation in North Sea *Pseudo-nitzschia pungens* (Bacillariophyceae) populations. *Journal of Phycology*, 2005, vol. 41, iss. 3, pp. 506–514. https://doi.org/10.1111/j.1529-8817.2005.00084.x
15. Fehling J., Davidson K., Bolch C., Tett P. Seasonality of *Pseudo-nitzschia* spp. (Bacillariophyceae) in western Scottish waters. *Marine Ecology Progress Series*, 2006, vol. 323, pp. 91–105. https://doi.org/10.3354/meps323091
16. Fu F. X., Tatters A. O., Hutchins D. A. Global change and the future of harmful algal blooms in the ocean. *Marine Ecology Progress Series*, 2012, vol. 470, pp. 207–233. https://doi.org/10.3354/meps10047
17. Gai F. F., Hedemand C. K., Louw D. C., Grobler K., Krock B., Moestrup Ø., Lundholm N. Morphological, molecular and toxigenic characteristics of Namibian *Pseudo-nitzschia* species – including *Pseudo-nitzschia bucculenta* sp. nov. *Harmful Algae*, 2018, vol. 76, pp. 80–95. https://doi.org/10.1016/j.hal.2018.05.003
18. Guillard R. R. L., Ryther J. H. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Canadian Journal of Microbiology*, 1962, vol. 8, no. 2, pp. 229–239. https://doi.org/10.1139/m62-029
19. Hasle G. R. *Nitzschia* and *Fragilariopsis* species studied in the light and electron microscopes. II. The group *Pseudo-nitzschia*. *Skrifter utgitt av det Norske Videnskaps-Akademi i Oslo. Matematisk-Naturvitenskapelig Klasse Ny Serie*, 1965, vol. 18, pp. 1–45.
20. Jones R. F., Speen H. L., Kury W. Studies on the growth of the red alga *Porphyridium cruentum*. *Physiologia Plantarum*, 1963, vol. 16, iss. 3, pp. 636–643. https://doi.org/10.1111/j.1399-3054.1963.tb08342.x
21. Liu C., Ji Y., Zhang L., Qiu J., Wang Z., Liu L., Zhuang Y., Chen T., Li Y., Niu B., Li A. Spatial distribution and source of biotoxins in phytoplankton from the South China Sea, China. *Journal of Hazardous Materials*, 2021, vol. 418, art. no. 126285 (10 p.). https://doi.org/10.1016/j.jhazmat.2021.126285
22. Orlova T. Yu., Stonik I. V., Aizdaicher N. A., Bates S. S., Léger C., Fehling J. Toxicity, morphology and distribution of *Pseudonitzschia calliantha*, *P. multistriata* and *P. multiseries* (Bacillariophyta) from the northwestern Sea of Japan. *Botanica Marina*, 2008, vol. 51, no. 4, pp. 297–306. https://doi.org/10.1515/bot.2008.035
23. Raven J. A., Geider R. J. Temperature and algal growth. *New Phytologist*, 1988, vol. 110, no. 4, pp. 441–461. https://doi.org/10.1111/j.1469-8137.1988.tb00282.x
24. *StatSoft* : [site]. Moscow, 2025. URL: https://statsoftai.ru/ [accessed: 25.01.2025].
25. Stonik I. V. Long-term variations in species composition of bloom-forming toxic

Pseudo-nitzschia diatoms in the northwestern Sea of Japan during 1992–2015. *Journal of Marine Science and Engineering*, 2021, vol. 9, iss. 6, art. no. 568 (13 p.). https://doi.org/10.3390/jmse9060568

26. Stonik I. V., Orlova T. Y., Chikalovets I. V., Aizdaicher N. A., Aleksanin A. I., Kachur V. A., Morozova T. V. *Pseudo-nitzschia* species (Bacillariophyceae) and the domoic acid concentration in *Pseudo-nitzschia* cultures and bivalves from the northwestern Sea of Japan, Russia. *Nova Hedwigia*, 2019, Bd 108, Heft 1–2, S. 73–93. https://doi.org/10.1127/nova_hedwigia/2018/0502
27. Stonik I. V., Orlova T. Yu., Begun A. A. Potentially toxic diatoms *Pseudo-nitzschia fraudulenta* and *P. calliantha* from Russian waters of East/Japan Sea and Sea of Okhotsk. *Ocean Science Journal*, 2008, vol. 43, iss. 1, pp. 25–30. https://doi.org/10.1007/BF03022428
28. Stonik I. V., Orlova T. Yu., Lundholm N. Diversity of *Pseudo-nitzschia* H. Peragallo from the western North Pacific. *Diatom Research*, 2011, vol. 26, iss. 1, pp. 121–134. https://doi.org/10.1080/0269249x.2011.573706
29. Stonik I. V., Zinov A. A. Changes in the composition of bloom-forming toxic *Pseudo-nitzschia* diatoms in surface waters in Ussuri Bay, northwestern Sea of Japan, during the autumn seasons of 2017–2022. *Journal of Marine Science and Engineering*, 2023, vol. 11, iss. 5, art. no. 1024 (14 p.). https://doi.org/10.3390/jmse11051024
30. Thessen A. E., Bowers H. A., Stoecker D. K. Intra- and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. *Harmful Algae*, 2009, vol. 8, iss. 5, pp. 792–810. https://doi.org/10.1016/j.hal.2009.01.003
31. Zhou C.-Y., Pan C.-G., Peng F.-J., Zhu R.-J., Hu J.-J., Yu K. Simultaneous determination of trace marine lipophilic and hydrophilic phycotoxins in various environmental and biota matrices. *Marine Pollution Bulletin*, 2024, vol. 203, art. no. 116444 (8 p.). https://doi.org/10.1016/j.marpolbul.2024.116444

ВОЗДЕЙСТВИЕ ТЕМПЕРАТУРЫ НА РОСТ ДВУХ ВИДОВ *PSEUDO-NITZSCHIA* H. PERAGALLO (BACILLARIOPHYTA) В ЛАБОРАТОРНЫХ КУЛЬТУРАХ, ИЗОЛИРОВАННЫХ ИЗ ЯПОНСКОГО МОРЯ

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Диатомовые водоросли рода *Pseudo-nitzschia* H. Peragallo, 1900, продуцирующие нейротоксичную домоевую кислоту, нередко интенсивно размножаются в дальневосточных морях России, что вызывает цветения воды. Температура известна как важный фактор, влияющий на развитие диатомей, однако его воздействие на рост этой группы микроводорослей из Японского моря исследовано недостаточно. Изучены особенности роста в лабораторной культуре двух видов диатомей — *Pseudo-nitzschia fraudulenta* (Cleve) Hasle, 1993 и *Pseudo-nitzschia hasleana* Lundholm, 2012 — в диапазоне температуры от +5 до +20 °C. Методом прямого подсчёта в камере Нажотта оценены плотность клеток, темпы роста и время генерации. Установлено, что максимальная средняя плотность клеток *P. fraudulenta* достигала $2{,}2 \times 10^5$ кл.·л$^{-1}$ на 16-е сутки опыта

при +18 °C. Для этого вида при +18 °C темпы роста (0,11–0,16 дел.·сут$^{-1}$) оставались относительно высокими, а время генерации (4,4–6,7 сут) — относительно низким в течение большей части эксперимента. Максимальная средняя плотность клеток *P. hasleana*, 5×10^5 кл.·л$^{-1}$, отмечена на 16-е сутки опыта при +17 °C. Для этого вида высокие темпы роста (0,2–0,92 дел.·сут$^{-1}$) и низкое время генерации (0,8–3,6 сут) зарегистрированы при +17 °C с 2-х по 10-е сутки эксперимента. Средняя плотность клеток *P. fraudulenta* при +18 °C оказалась статистически достоверно выше, чем при других изученных значениях температуры (тест Тьюки, $p < 0,05$) на протяжении всего опыта. Средняя плотность клеток *P. hasleana* при +17 °C была статистически значимо выше (тест Тьюки, $p < 0,05$) таковой при других температурах на 8–12-е сутки эксперимента. Установлено, что при выращивании клонов *P. fraudulenta* при +10, +16 и +18 °C и клонов *P. hasleana* при +14, +17 и +20 °C клетки оставались в жизнеспособном состоянии и продолжали делиться. При понижении температуры культивирования *P. fraudulenta* и *P. hasleana* до +5 и +7 °C соответственно деление резко замедлялось, а плотность клеток была статистически значимо ниже, чем при более высокой температуре (тест Тьюки, $p < 0,05$). Установлены диапазоны толерантной температуры при выращивании диатомей — от +10 до +18 °C для *P. fraudulenta* и от +14 до +17 °C для *P. hasleana*. Выявленные нижние границы температурных условий для культивирования двух видов (+10 и +14 °C) согласуются с параметрами температуры воды в природной среде, при которых отмечены цветения *P. fraudulenta* (+6…+16 °C) и *P. hasleana* (+10…+16 °C). Показаны широкие адаптивные возможности изученных видов по отношению к температуре.

Ключевые слова: *Pseudo-nitzschia fraudulenta*, *Pseudo-nitzschia hasleana*, лабораторное культивирование, температура, Японское море