1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2023, vol. 8, no. 3, pp. 47–61
https://marine-biology.ru

UDC 582.261.1-152.4

INTERACTIONS OF THE DIATOM ALGAE *PSEUDO-NITZSCHIA HASLEANA* AND *THALASSIOSIRA PSEUDONANA* IN THE MIXED CULTURE

© 2023 **Zh. V. Markina[1] and A. Yu. Popik[2]**

[1]A. V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS, Vladivostok, Russian Federation
[2]Institute of Automation and Control Processes, FEB RAS, Vladivostok, Russian Federation
E-mail: *zhannav@mail.ru*

Received by the Editor 16.05.2022; after reviewing 08.06.2022;
accepted for publication 04.08.2023; published online 21.09.2023.

Representatives of the genus *Pseudo-nitzschia* (Bacillariophyta) cause blooms in different areas of the World Ocean. Therefore, it is necessary to know their ecological features, including the way those interact with other species of unicellular algae. Moreover, for rapid identification of these algae in the environment, a certain technique is needed. Thus, we assessed the dynamics of cell abundance for *Pseudo-nitzschia hasleana* and *Thalassiosira pseudonana* in mono- and mixed cultures by their direct counting in a Nageotte chamber. Temperature curves of chlorophyll *a* fluorescence obtained by laser-induced fluorescence in a temperature chamber were also analyzed. The experiments lasted for 14 days. As shown, *P. hasleana* had different effect on *T. pseudonana* depending on initial abundance of *T. pseudonana*. At initial concentration of 0.8×10^4 cells·mL^{-1}, a pronounced stimulation of the growth of this diatom occurred. At initial concentrations of 1.6×10^4 and 3.2×10^4 cells·mL^{-1}, *T. pseudonana* growth was inhibited. In the mixed culture, *T. pseudonana* remained at the stationary growth phase, while in a monoculture, the population entered the dying phase by the 14th day of the experiment. *T. pseudonana* had an inhibitory effect on *P. hasleana* growth. The experiment with *P. hasleana* and *T. pseudonana* co-cultivation showed as follows: chlorophyll *a* fluorescence of the mixture is more affected by the microalga with much higher concentration. The fluorescent signal of two separately cultivated monocultures can potentially be used to search for these cultures in a mixture.

Keywords: *Pseudo-nitzschia hasleana*, *Thalassiosira pseudonana*, allelopathy, chlorophyll *a* fluorescence, microalgae identification

Natural phytoplankton communities are affected by many environmental factors. Those can cause blooms or, conversely, prevent them [Lima-Mendez et al., 2015]. As shown, the dominance of *Pseudo-nitzschia* spp. toxic complex is associated with a decrease in N : Si ratio in the presence of sewage effluents. *Pseudo-nitzschia australis* Frenguelli, 1939 is capable of urea osmotrophy and active growth on it, which is the reason for blooms of this species [Burkholder et al., 2008]. The effect of biotic factors, in particular, microalgae interaction with each other, remains a less studied problem [Long et al., 2018]. Evaluation of the growth of mixed microalgae cultures in a laboratory experiment is one of the ways to analyze biotic interactions. Specifically, population–population relationships are investigated; boundaries of the stability of coexisting species are determined; and the conditions for their dominance and elimination are assesses [Mikheev et al., 2018]. However, there is still no standardized methodology for studying the effects of algal populations on each other, as in toxicological research [Long et al., 2018].

Pseudo-nitzschia representatives are ubiquitous in the waters of the World Ocean [Huang et al., 2009; Sobrinho et al., 2017; Trainer et al., 2012; Yasakova, 2013]. Those are of interest to researchers not only because of their periodic blooms, but also because of the presence of domoic acid which is toxic to warm-blooded animals [Trainer et al., 2012]. *Pseudo-nitzschia* abundance in a monospecific bloom can reach 1×10^6 cells·mL^{-1} [Louw et al., 2017], and the bloom can last for two months [Bates et al., 1989]. At the same time, *Pseudo-nitzschia* spp. can account for 99% of the total phytoplankton [Lundholm et al., 2005].

In the phytoplankton community, together with *Pseudo-nitzschia* spp., representatives of another genus of diatoms, *Thalassiosira*, are regularly recorded [Balzano et al., 2017; Orlova et al., 2009]. These genera were shown to have the same iron requirement [Cohen et al., 2017]. *Thalassiosira pseudonana* is involved in the phytoplankton succession cycle and is of great ecological importance as a species affecting the formation of phytoplankton blooms [Ianora et al., 2011]. The interest in this microalga is, among other things, due to cases of salmon death during its mass reproduction [Mardones, 2020]. Species of this genus are often found in the waters of temperate and polar seas [Harris et al., 1995].

As a rule, the mutual effect of cultures is assessed on allelopathically aggressive species, and to a lesser extent, on coexisting ones [Phatarpekar et al., 2000]. We have shown earlier that fluorescent characteristics of *Pseudo-nitzschia* can be used to identify it in water [Popik et al., 2022]. However, due to the mutual effect of algae during co-cultivation, the question arises whether the joint growth of different species can also affect chlorophyll *a* fluorescence in microalgae, making it difficult to identify them in the natural environment. Therefore, the aim of this work is to study growth and temperature curves of chlorophyll *a* fluorescence in diatoms *Pseudo-nitzschia hasleana* and *Thalassiosira pseudonana* in the mixed culture.

MATERIAL AND METHODS

The objects of study were strains of unicellular algae cultures, *Pseudo-nitzschia hasleana* Lundholm, 2012 MBRU_PH18 and *Thalassiosira pseudonana* Hasle & Heimdal, 1970 MBRU_TSP-02 (Bacillariophyta). The algae were grown on medium f [Guillard, Ryther, 1962] prepared on the basis of filtered and sterilized seawater with a salinity of 32‰, in 250-mL Erlenmeyer flasks with 100 mL of a culture medium, at a temperature of +18 °C, an illumination intensity of 70 μmol·m^{-2}·s^{-1}, and a light–dark period of 14 h : 10 h (light : dark). Cultures at the exponential growth stage were used as inoculum. Initial cell concentrations were 0.1×10^4 cells·mL^{-1} for *P. hasleana* and 0.8×10^4, 1.6×10^4, and 3.2×10^4 cells·mL^{-1} for *T. pseudonana*. Biovolume ratios for *P. hasleana* : *T. pseudonana* cells were 1 : 1, 1 : 2, and 1 : 4. The biovolume of *P. hasleana* was 210 μm^3, and that of *T. pseudonana* was 26.5 μm^3. Algae biovolumes were calculated by the formulas from [Hillebrand et al., 1999].

The experiments were conducted in two stages. At the first one, the dynamics of the microalgae abundance in monocultures at various initial concentrations was studied, while at the second one, the microalgae growth in the mixed culture of *P. hasleana* and *T. pseudonana* was investigated. The experiments lasted for 14 days. Sampling for cell counting was carried out on the 3rd, 7th, 10th, and 14th day. Cell abundance was established in a Nageotte chamber. Fluorescence spectra of microalgae, as well as temperature curves of chlorophyll *a* fluorescence intensity and chlorophyll *a* fluorescence wavelength, were determined according to the methods described earlier [Popik et al., 2022; Voznesenskiy et al., 2019]. The experiments were carried out in triplicate. The data were statistically processed in MS Excel. The graphs show the mean values and standard deviations.

RESULTS

***Pseudo-nitzschia hasleana* and *Thalassiosira pseudonana* growth in monocultures.** For 3 days, the concentration of *P. hasleana* cells remained low, and by the 7th day, it increased to 1×10^4 cells·mL^{-1} (Fig. 1). From the 10th to 14th day, cell abundance rose from 3.8×10^4 to 32.4×10^4 cells·mL^{-1}.

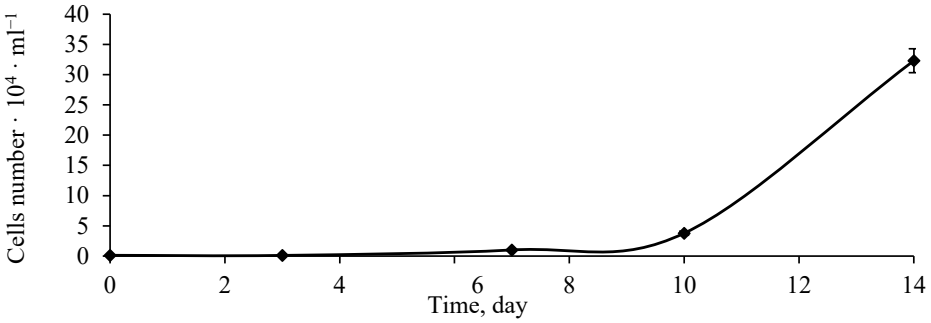

Fig. 1. Growth curve of *Pseudo-nitzschia hasleana* in the monoculture

By the 3rd day of the experiment, the abundance of *T. pseudonana* cells did not differ significantly at all initial cell concentrations (Fig. 2). On the 10th day, the maximum value was registered. On the last days, cell abundance in cultures decreased.

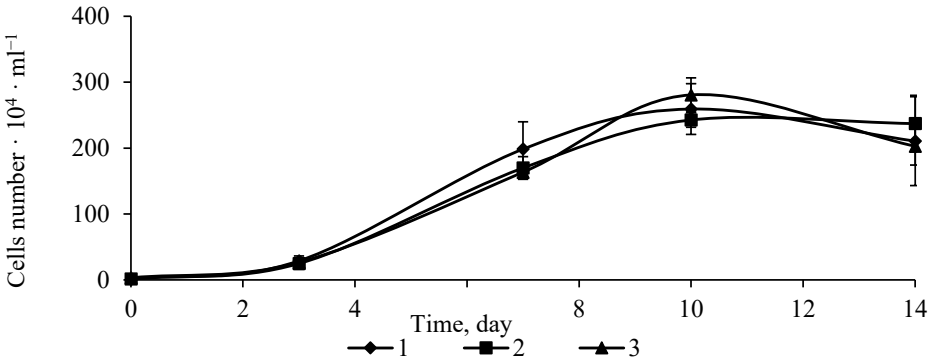

Fig. 2. Growth curve of *Thalassiosira pseudonana* in the monoculture. Initial concentration of cells, cells·mL^{-1}: 1, 0.8×10^4; 2, 1.6×10^4; 3, 3.2×10^4

***Pseudo-nitzschia hasleana* and *Thalassiosira pseudonana* growth in mixed cultures.** The abundance of *P. hasleana* cells increased after the 3rd day of the experiment (Fig. 3). At initial concentration of *T. pseudonana* cells of 0.8×10^4 cells·mL^{-1}, *P. hasleana* entered the stationary growth phase on the 7th day; at higher initial *T. pseudonana* concentrations, the abundance of *P. hasleana* cells rose even on the last day.

The abundance of *T. pseudonana* cells in the mixture increased from the beginning of the experiment at all its initial concentrations (Fig. 4). At 3.2×10^4 cells·mL^{-1}, the alga growth was inhibited after the 7th day: cell abundance was 137×10^4 cells·mL^{-1}, while in the monoculture, the value was 203×10^4 cells·mL^{-1}.

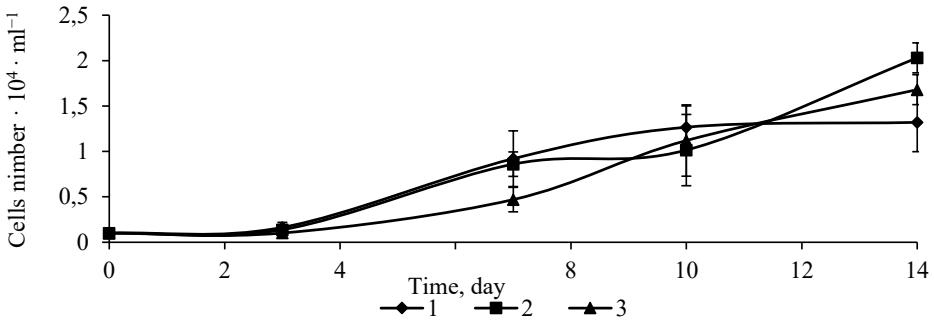

Fig. 3. Growth curve of *Pseudo-nitzschia hasleana* in the mixed culture with *Thalassiosira pseudonana*. Initial concentration of *T. pseudonana* cells, cells·mL^{-1}: 1, 0.8×10^4; 2, 1.6×10^4; 3, 3.2×10^4

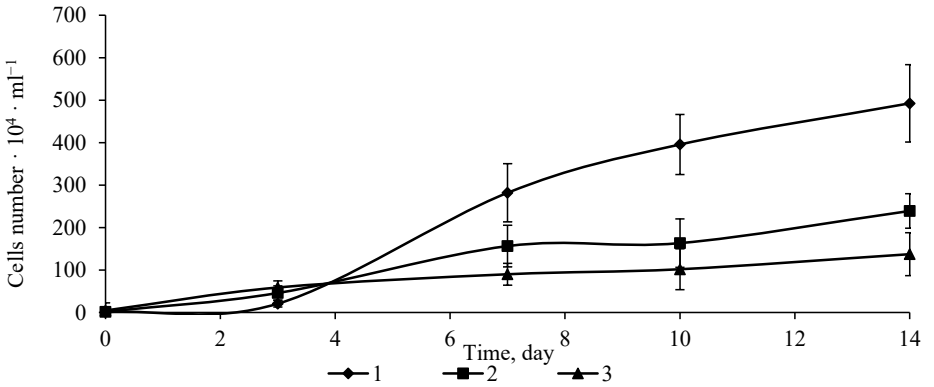

Fig. 4. Growth curve of *Thalassiosira pseudonana* in the mixed culture with *Pseudo-nitzschia hasleana*. Initial concentration of *T. pseudonana* cells, cells·mL^{-1}: 1, 0.8×10^4; 2, 1.6×10^4; 3, 3.2×10^4

Fluorescence of *P. hasleana* cells during the first week correlates with their concentration. As *Pseudo-nitzschia* sp. cultures grow, cell size decreases [Lelong et al., 2012; Trainer et al., 2012]. Therefore, the amount of chlorophyll *per* cell drops, which results in decreased fluorescence. This effect should be observed with longer cultivation, but even in our experiment, a drop in the intensity of chlorophyll *a* fluorescence was recorded on the 14th day compared to that on the 7th day (Figs 5, 6).

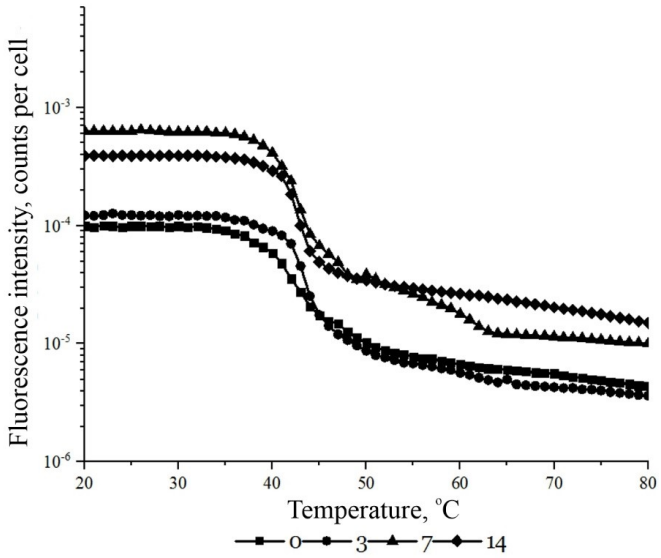

Fig. 5. Fluorescence temperature curves of *Pseudo-nitzschia hasleana* cells during cultivation for two weeks: 0, the beginning of the experiment; 3, the 3rd day; 7, the 7th day; 14, the last day

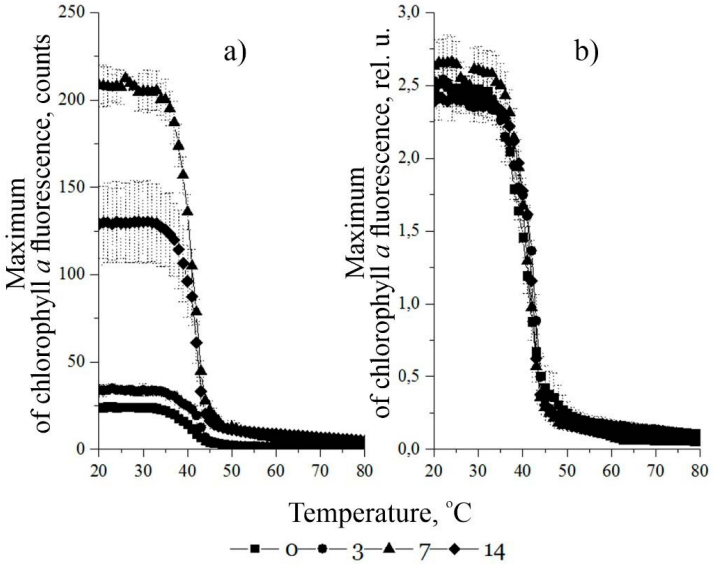

Fig. 6. Temperature curves of *Pseudo-nitzschia hasleana* chlorophyll *a* fluorescence: a, absolute values; b, normalized to mean intensity. The time of the experiment: 0, the beginning; 3, the 3rd day; 7, the 7th day; 14, the last day

The shape of temperature curves of chlorophyll *a* fluorescence for the microalga *P. hasleana* (Fig. 6) was analyzed by us earlier [Popik et al., 2022]. Temperature curves of chlorophyll *a* fluorescence wavelength for *P. hasleana* monoculture, which were obtained during the experiment, showed as follows. Within the range of +20…+40 °C, the maximum intensity of chlorophyll *a* fluorescence occurred at a wavelength of 682.5 nm (Fig. 7).

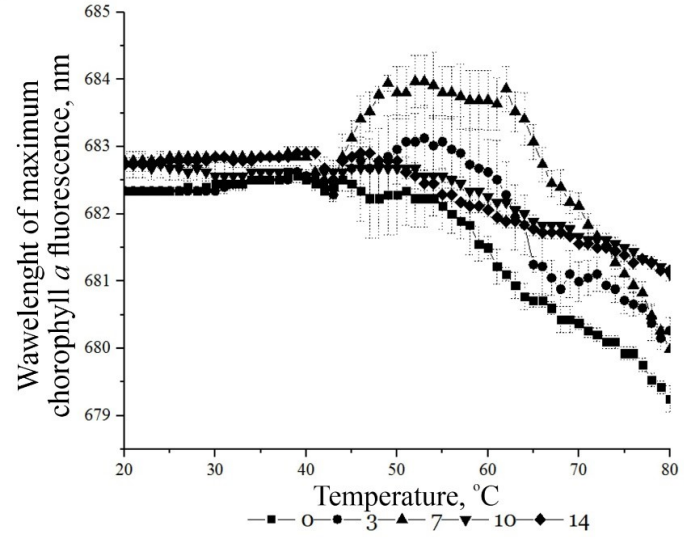

Fig. 7. Changes in the wavelength of chlorophyll *a* fluorescence maximum for *Pseudo-nitzschia hasleana* monocultures. The time of the experiment: 0, the beginning; 3, the 3rd day; 7, the 7th day; 10, the 10th day; 14, the last day

T. pseudonana monoculture, sown at a concentration of 0.8×10^4 cells·mL^{-1}, reaches growth limits (the stationary phase) within 10 days. Then, the culture begins to die off, which may manifest itself in a decrease in the intensity of fluorescence of individual cells (Fig. 8).

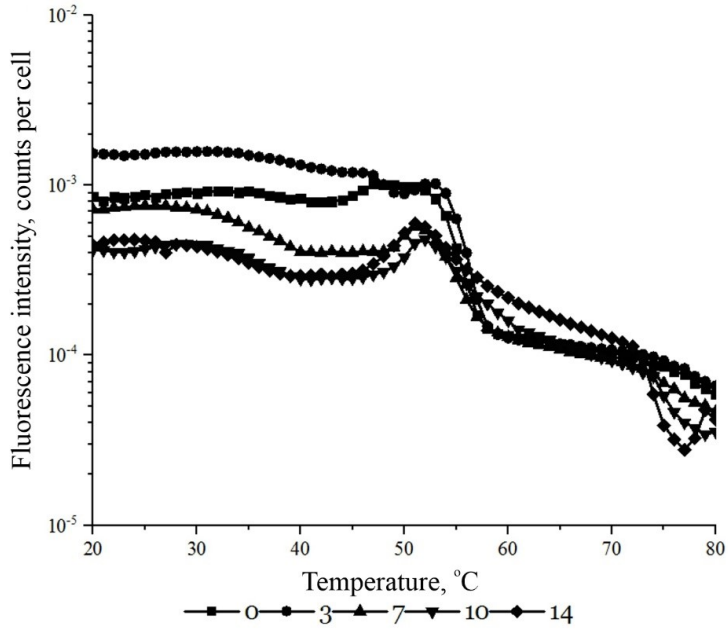

Fig. 8. Fluorescence temperature curves of *Thalassiosira pseudonana* cells during cultivation for two weeks. The time of the experiment: 0, the beginning; 3, the 3rd day; 7, the 7th day; 10, the 10th day; 14, the last day

It can be concluded that the microalga in laboratory culture is in approximately the same state as microalgae during real bloom. In this case, the normalized fluorescence temperature curve (hereinafter NFTC) of the culture changes (Fig. 9), and the differences in its form correspond to three stages: NFTC at low concentrations (the 0th day), NFTC of a growing culture (the 3rd day), and NFTC of a "stagnating" culture (the 7th–14th days) with high concentration (Fig. 10). If we do not take into account a rise in chlorophyll *a* fluorescence observed for *T. pseudonana* on the 3rd day of the experiment, we can conclude that there is an inverse correlation between an increase in the culture concentration and chlorophyll *a* fluorescence. A rise in chlorophyll *a* fluorescence after reseeding may be caused by the corresponding stress of the culture.

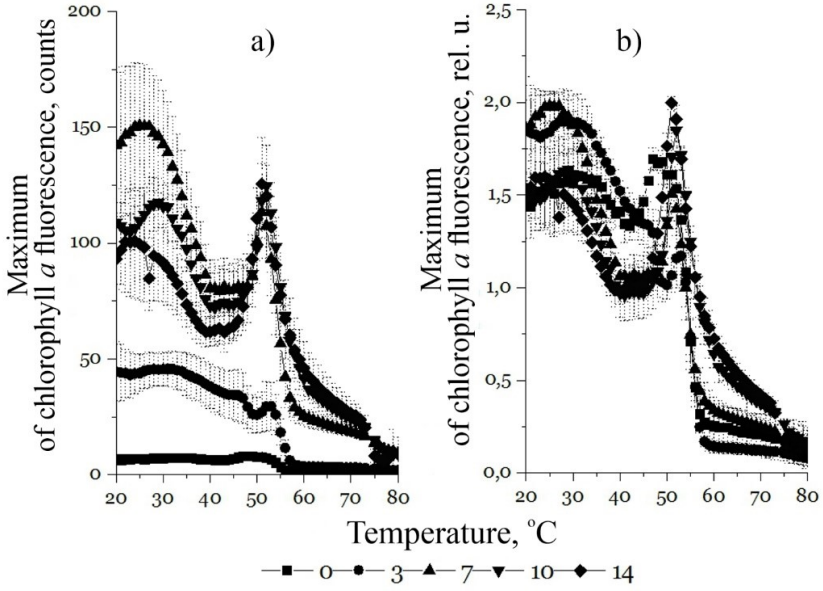

Fig. 9. Fluorescence temperature curves of chlorophyll *a* for the culture of the microalga *Thalassiosira pseudonana*: a, absolute values; b, normalized to mean intensity. The time of the experiment: 0, the beginning; 3, the 3rd day; 7, the 7th day; 14, the last day

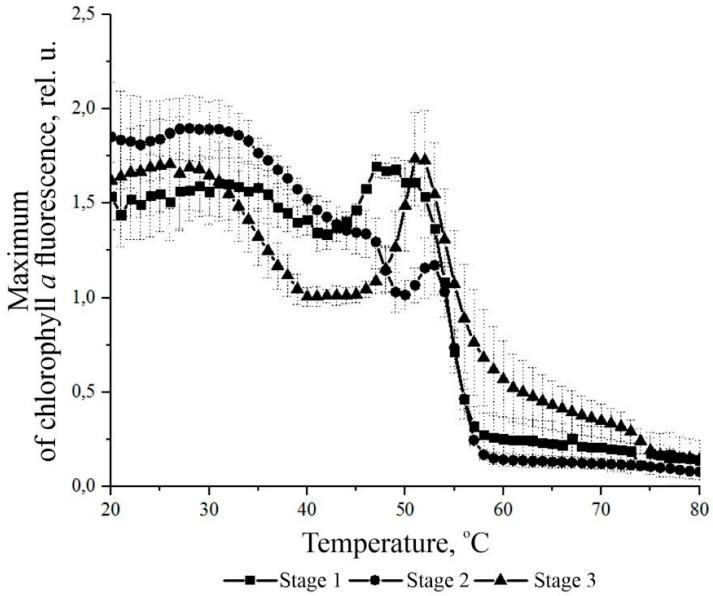

Fig. 10. Fluorescence temperature curves of chlorophyll *a* for the culture of the microalga *Thalassiosira pseudonana* corresponding to different stages of development. Stage 1 is the initial one, during which there is no significant growth; stage 2 corresponds to rapid, exponential growth; stage 3 is the stage of "stagnation"

For *T. pseudonana*, temperature curves of fluorescence for all three stages have certain similarities. These are stable high chlorophyll *a* fluorescence in the range of +20…+32 °C, the presence of a local maximum of its fluorescence at +50…+53 °C, and stabilization of chlorophyll *a* fluorescence at a low level at temperatures above +60 °C. At the same time, the initial stage is characterized by higher value of chlorophyll *a* fluorescence at a local maximum, than that for normal temperatures. For the growth stage, the local maximum of chlorophyll *a* fluorescence is significantly lower in terms of its intensity than fluorescence at initial stages. The local maximum of the intensity of chlorophyll *a* fluorescence at the "stagnation" stage is comparable to the intensity at +20 °C.

At all stages of cultivation, temperature curves of chlorophyll *a* fluorescence for the monoculture remain relatively stable (Fig. 11); at +20…+45 °C, the wavelength of the maximum for chlorophyll *a* fluorescence is 685.5 nm. The difference in the wavelength of the maximum on the 1st day from that on other days may be due to the adaptation of the monoculture during its reseeding. Also, temperature curves of chlorophyll *a* fluorescence wavelength are stable for all days of cultivation within the range of +45…+52 °C. In this range, there is a sharp drop in the wavelength of the maximum for chlorophyll *a* fluorescence from 685.5 to 680.5 nm. Then, a slight increase in the wavelength is observed for 2–3 min (dT = 2…3 °C), which is followed by its slow monotonic decrease. At the same time, within the range of +55…+80 °C, temperature curves of chlorophyll *a* fluorescence wavelength for the culture at various stages of cultivation begin to differ from each other. This may be due to the different composition of pigment–protein complexes for a culture going through all stages of its growth.

Since *Pseudo-nitzschia* are capable of forming red tides [Trainer et al., 2012] and often co-evolve with other diatoms, studying fluorescent characteristics of mixtures of *Pseudo-nitzschia* and other microalgae is of particular interest for their further use in environmental monitoring. Due to different growth rates, on the 7th day of the experiment, microalgae in *P. hasleana* : *T. pseudonana* mixture had the concentrations of 1 : 30. With such a ratio, the effect of *P. hasleana* culture fluorescence in the mixture becomes insignificant: the main contributor to the fluorescent signal is *T. pseudonana*. Since fluorescent characteristics are planned to be used for environmental monitoring, there are no prospects in the investigation of mixtures, in which the fluorescent signal of chlorophyll *a* for *P. hasleana* cannot be measured.

Therefore, it was decided not to determine chlorophyll *a* fluorescence for mixtures during further cultivation. For the mixtures studied, in temperature curves of chlorophyll *a* fluorescence wavelength, the microalga *T. pseudonana* predominated (Fig. 12). The form of NFTC of the mixtures is highly correlated with the form of NFTC of *T. pseudonana*, as can be seen when comparing NFTC of a mixture and NFTC obtained as the sum of NFTC of the monocultures.

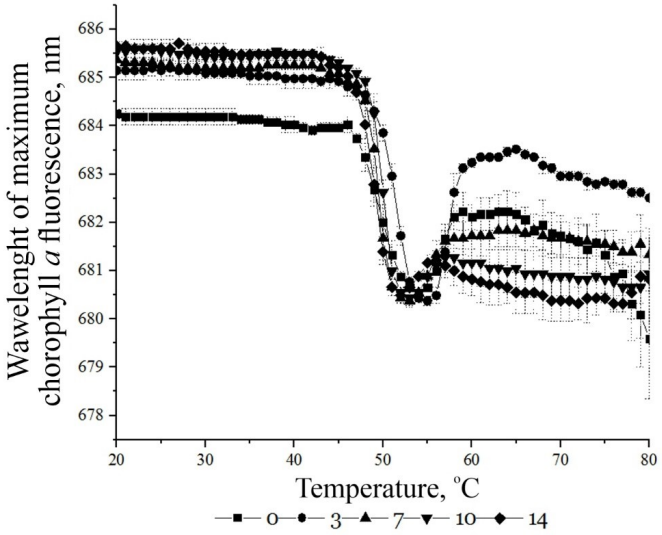

Fig. 11. Changes in chlorophyll *a* fluorescence maximum for monocultures of the microalga *Thalassiosira pseudonana*. The time of the experiment: 0, the beginning; 3, the 3rd day; 7, the 7th day; 10, the 10th day; 14, the last day

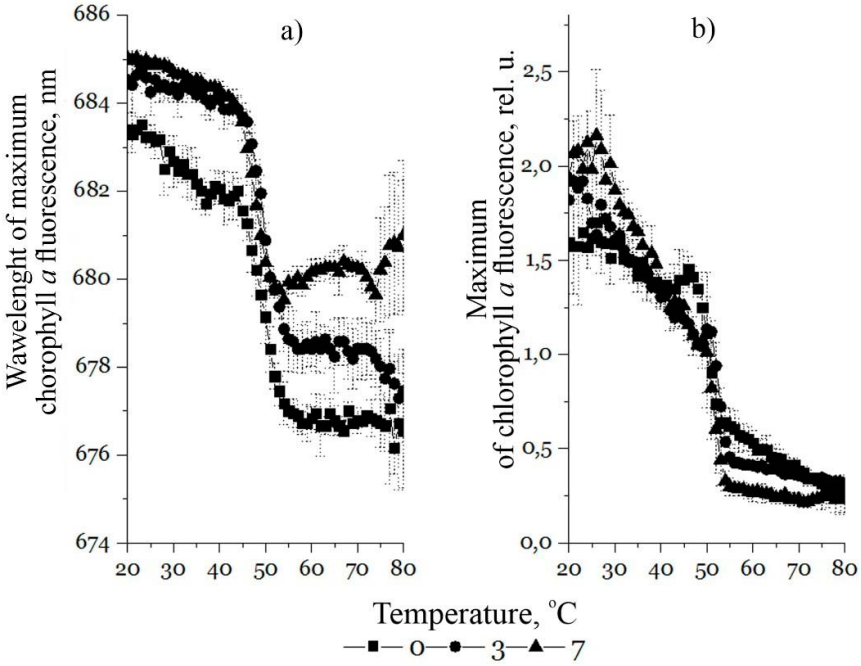

Fig. 12. Fluorescent characteristics of the mixed culture of *Pseudo-nitzschia hasleana* and *Thalassiosira pseudonana*: a, temperature curves of chlorophyll *a* fluorescence wavelength of the mixture; b, normalized fluorescence temperature curves of the mixture. The time of the experiment: 0, the beginning; 3, the 3rd day; 7, the 7th day

Fig. 13 provides a comparison of NFTC of mixtures and summed monocultures. The summation of NFTC was carried out based on the proportional ratio of cells in the culture mixture. When summing NFTC for the 0th day, we used NFTC of *T. pseudonana* at the initial stage. When summing NFTC for the 3rd and 7th days, we used NFTC of *T. pseudonana* at the exponential growth stage.

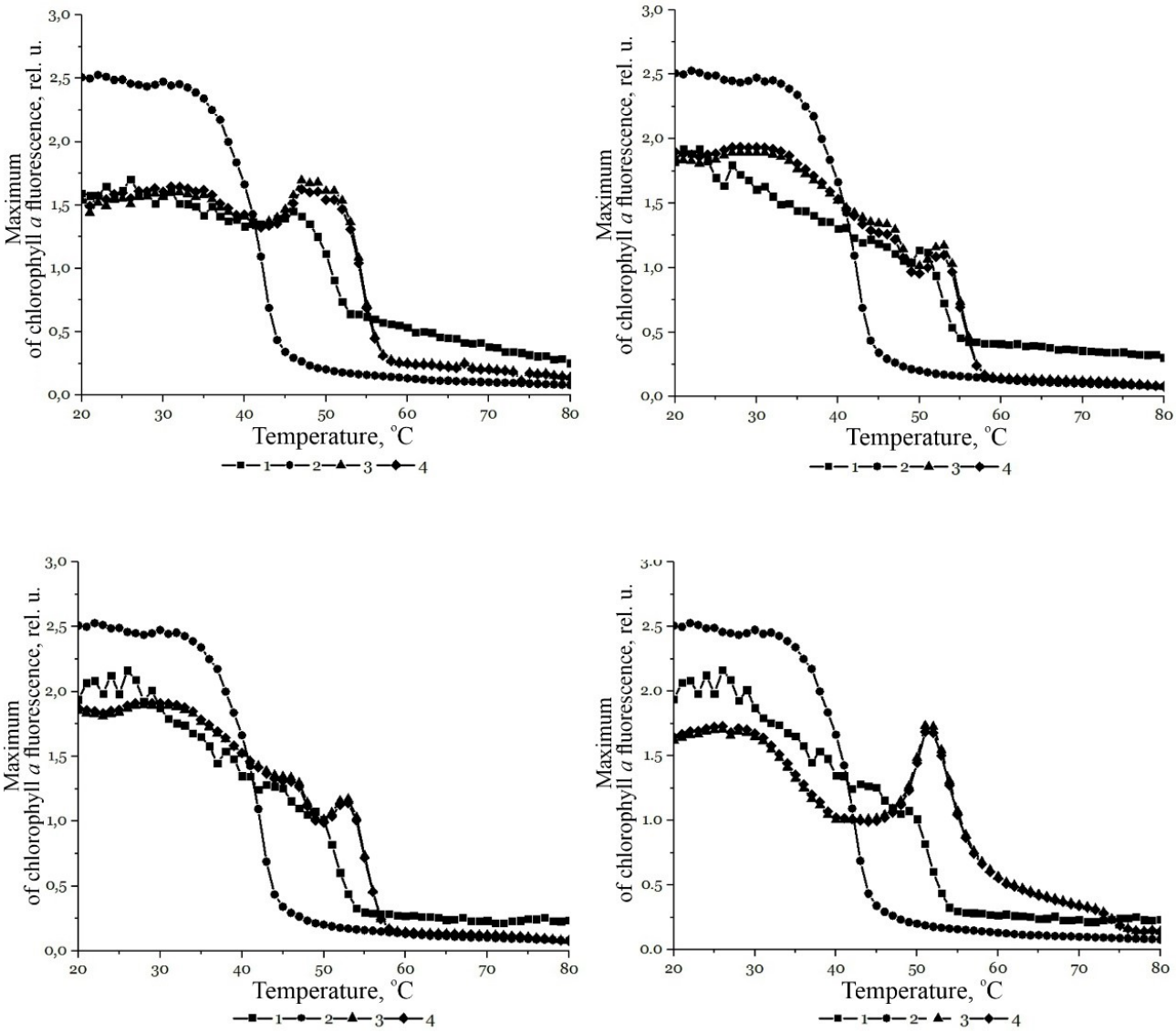

Fig. 13. Fluorescence temperature curves of the mixed culture of *Pseudo-nitzschia hasleana* and *Thalassiosira pseudonana* compared to fluorescence temperature curves of the corresponding monocultures and their mathematical sum: a, normalized fluorescence temperature curves (NFTC) of *Thalassiosira pseudonana* at the initial stage; b, *Thalassiosira pseudonana* NFTC at the beginning of the growth stage; c, *Thalassiosira pseudonana* NFTC at the end of the growth stage; d, *Thalassiosira pseudonana* NFTC at the "stagnation" stage; 1, measured characteristic of the mixture; 2, characteristics of *Pseudo-nitzschia hasleana* monoculture; 3, characteristics of *Thalassiosira pseudonana* monoculture; 4, characteristics of monoculture mixture

DISCUSSION

Exploitative competition (competition for a limiting resource) is one of the biotic factors that determine the structure of the phytoplankton community. Under such conditions, an organism with less consumption may be more successful than other ones in a given community and become a new dominant. Another strategy is interference competition. Specifically, an organism inhibits the growth of other ones directly or indirectly *via* the secretion of chemicals, cell–cell interactions, and so on. In eutrophic water areas, interference competition comes to the fore [Zhao et al., 2018]. Artificial media for microalgae cultivation are nutrient-rich; apparently, when *P. hasleana* and *T. pseudonana* are co-cultivated, interference competition occurs rather than exploitative one.

When algae were co-cultivated, no alterations in cell size and morphology were noted in any of the species. Interestingly, in the study of the effect of the macrophyte *Pyropia haitanensis* (T. J. Chang & B. F. Zheng) N. Kikuchi & M. Miyata, 2011 on *Pseudo-nitzschia multiseries* (Hasle) Hasle, 1995 and *Pseudo-nitzschia pungens* (Grunow ex Cleve) G. R. Hasle, 1993, valve curvature and chloroplast condensation were registered [Patil et al., 2020]. Also, in experiments on dinoflagellates of the genus *Alexandrium* Halim, 1960, when co-cultivated with other microalgae, *inter alia* diatoms, dinoflagellates negatively affected cell abundance and morphology of target species. Moreover, their physiological state changed under the effect of metabolites released by dinoflagellates: there were inhibition of photosystem II, an increase in the content of reactive oxygen species in cells, changes in lipid composition, membrane damage, and cell immobilization and sedimentation [Long et al., 2018; Tan et al., 2019; Zheng et al., 2016].

The growth of *T. pseudonana* with initial concentrations of 1.6×10^4 and 3.2×10^4 cells·mL^{-1} was suppressed when *P. hasleana* began to grow more intensively. At the same time, the stimulation of growth was recorded for *T. pseudonana* at the lowest initial concentration, 0.8×10^4 cells·mL^{-1}. As shown earlier, the initial concentration of monoculture cells in the mixture affects the response of the microalga to metabolites of another species. Specifically, when *Skeletonema costatum* (Greville) Cleve, 1873 was cultivated on *Heterosigma akashiwo* (Y. Hada) Y. Hada ex Y. Hara & M. Chihara, 1987 filtrates, *S. costatum* growth was inhibited at low cell concentration and was not affected at high one [Yamasaki et al., 2009]. The same was observed when cultivating *Phaeodactylum tricornutum* Bohlin, 1898 with *Prorocentrum donghaiense* D. Lu, 2001 [Cai et al., 2014]. Small algal species are thought to be more susceptible to allelopathic substances than large ones [Felpeto et al., 2019; Prasetiya et al., 2016]. At the same time, small species gain a competitive advantage due to their rapid growth [Mikheev et al., 2018]. In general, whether a toxic or toxin-sensitive species has an advantage depends on which species becomes a dominant in the environment [Hulot, Huisman, 2004]. In the experiment with *P. hasleana* and *T. pseudonana*, both species in mixed cultures had a mainly inhibitory effect on each other – with the exception of *T. pseudonana* at the lowest initial cell concentration in the medium. Apparently, interactions between algae depend on their species; so, it is now difficult to see a universal pattern of microalgae interaction. To date, the most studied toxic algae in terms of their effects on other species are *Alexandrium* dinoflagellates [Long et al., 2018; Zheng et al., 2016].

As shown in the experiments aimed at *Pseudo-nitzschia multiseries* and *Bacillaria* sp. co-cultivation, in *Bacillaria* sp., the abundance decreased by 50–70% [Sobrinho et al., 2017]. Inhibition of *T. pseudonana* growth in a mixed culture with *P. hasleana* was observed on the 3rd day of the experiment. Cell abundance of *Rhodomonas salina* (Wislouch) D. R. A. Hill & R. Wetherbee, 1989, *Chattonella marina* (Subrahmanyan) Hara & Chihara, 1982, and *Akashiwo sanguinea* (K. Hirasaka) Gert Hansen

& Moestrup, 2000, both due to lysis and growth inhibition, decreased when co-cultivated with *P. pungens*. At the same time, in *Prorocentrum minimum* (Pavillard) J. Schiller, 1933 and *Phaeocystis globosa* Scherffel, 1899, cell abundance in a mixed culture with *P. pungens* remained the same as in a monoculture [Xu et al., 2015].

Domoic acid has no toxic effect on microalgae [Lundholm et al., 2005; Poulin et al., 2018]. In this regard, it can be assumed that the inhibition of *T. pseudonana* growth results from the release of other substances. Diatoms are known to produce large amounts of polyunsaturated aldehydes [Pichierri et al., 2017], which trigger a cascade of reactions causing microalgal cell death *via* apoptosis [Ianora et al., 2011].

According to the theory of the paradox of the plankton, the great diversity of planktonic species in an ecosystem with limited resources is possible only if their cell concentrations are balanced with the availability of light and nutrients [Hutchinson, 1961]. To date, allelopathy is considered a key component in competition between microalgae [Ternon et al., 2018]. It can be assumed as follows: in natural communities, the interaction of *Thalassiosira* and *Pseudo-nitzschia* species is one of the limiters of their reproduction at a high nutrient content. Previously, on the example of *S. costatum* and *H. akashiwo*, it was shown that the interaction between these species is one of the factors of the monospecific bloom formation [Yamasaki et al., 2007]. An increase in the abundance of some species in the phytoplankton community can reduce the pressure of grazers on other species of this community. Thus, in the South China Sea, if *S. costatum* abundance increases, zooplankton pressure on *P. pungens* decreases; importantly, it is the second key factor, after temperature, for this species [Huang et al., 2009].

Conclusion. *Pseudo-nitzschia hasleana* and *Thalassiosira pseudonana* affected each other in a mixed culture. The effect of *P. hasleana* on *T. pseudonana* depended on the initial concentration of *T. pseudonana* cells. Specifically, at 0.8×10^4 cells·mL^{-1}, a pronounced stimulation of its growth occurred. At initial concentrations of 1.6×10^4 and 3.2×10^4 cells·mL^{-1}, inhibition of *T. pseudonana* growth was registered, and the effect increased with a rise in its initial concentration. However, in the mixed culture, *T. pseudonana* was at the stationary growth phase, while in the monoculture, the population began to die off. *T. pseudonana* had an inhibitory effect on *P. hasleana* growth, and *P. hasleana* abundance in the mixed culture was 16 times lower by the end of the experiment, than that in the monoculture. The experiment with co-cultivation of *P. hasleana* and *T. pseudonana* showed that chlorophyll *a* fluorescence in the mixture is more affected by the microalga with significantly higher concentration. There was no change in the curves of individual cultures in the mixtures.

The work was carried out with the financial support of the Russian Science Foundation grant No. 21-74-30004.

Acknowledgement. Cultures of the microalgae *Pseudo-nitzschia hasleana* MBRU_PH18 and *Thalassiosira pseudonana* MBRU_TSP-02 (Bacillariophyta) were provided by the Marine Biobank resource collection of the National Scientific Center of Marine Biology, FEB RAS (http://marbank.dvo.ru).

REFERENCES

1. Balzano S., Percopo I., Siano R., Gourvil P., Chanoine M., Marie D., Vaulot D., Sarno D. Morphological and genetic diversity of Beaufort Sea diatoms with high contributions from the *Chaetoceros neogracilis* species complex. *Journal of Phycology*, 2017, vol. 53, iss. 1, pp. 161–187. https://doi.org/10.1111/jpy.12489
2. Bates S. S., Bird C. J., de Freitas A. S. W., Foxall R., Gilgan M., Hanic L. A., Johnson G. R., McCulloch A. W., Odense P., Pocklington R.,

Quilliam M. A., Sim P. G., Smith J. C., Subba Rao D. V., Todd E. C. D., Walter J. A., Wrigh J. L. C. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 1989, vol. 46, no. 7, pp. 1203–1215. https://doi.org/10.1139/f89-156

3. Burkholder J. A. M., Glibert P. M., Skelton H. M. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae*, 2008, vol. 8, iss. 1, pp. 77–93. https://doi.org/10.1016/j.hal.2008.08.010
4. Cai Z., Zhu H., Duan S. Allelopathic interactions between the red-tide causative dinoflagellate *Prorocentrum donghaiense* and the diatom *Phaeodactylum tricornutum*. *Oceanologia*, 2014, vol. 56, iss. 3, pp. 639–650. https://doi.org/10.5697/oc.56-3.639
5. Cohen N. R., Ellis K. A., Lampe R. H., McNair H., Twining B. S., Maldonado M. T., Brzezinski M. A., Kuzminov F. I., Thamatrakoln K., Till C. P., Bruland K. W., Sunda W. G., Bargu S., Marchetti A. Diatom transcriptional and physiological responses to changes in iron bioavailability across ocean provinces. *Frontiers in Marine Science*, 2017, vol. 4, art. no. 360 (20 p.). https://doi.org/10.3389/fmars.2017.00360
6. 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
7. Harris A. S. D., Medlin L. K., Lewis J., Jones K. J. *Thalassiosira* species (Bacillariophyceae) from a Scottish sea-loch. *European Journal of Phycology*, 1995, vol. 30, iss. 2, pp. 117–131. https://doi.org/10.1080/09670269500650881
8. Hillebrand H., Dürselen C. D., Kirschtel D., Pollingher U., Zohary T. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, 1999, vol. 35, iss. 2, pp. 403–424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x
9. Huang C., Lin X., Lin J., Du H., Dong Q. Population dynamics of *Pseudo-nitzschia* pungens in Zhelin Bay, China. *Journal of the Marine Biological Association of the United Kingdom*, 2009, vol. 89, iss. 4, pp. 663–668. https://doi.org/10.1017/S0025315408002919
10. Hulot F., Huisman J. Allelopathic interactions between phytoplankton species: The roles of heterotrophic bacteria and mixing intensity. *Limnology and Oceanography*, 2004, vol. 49, iss. 4, pt 2, pp. 1424–1434. https://doi.org/10.4319/lo.2004.49.4_part_2.1424
11. Hutchinson G. E. The paradox of the plankton. *The American Naturalist*, 1961, vol. 95, no. 882, pp. 137–145. https://doi.org/10.1086/282171
12. Felpeto A. B., Śliwińska-Wilczewska S., Klin M., Konarzewska Z., Vasconcelos V. Temperature-dependent impacts of allelopathy on growth, pigment, and lipid content between a subpolar strain of S*ynechocystis* sp. CCBA MA-01 and coexisting microalgae. *Hydrobiologia*, 2019, vol. 835, iss. 1, pp. 117–128. https://doi.org/10.1007/s10750-019-3933-8
13. Ianora A., Bentley M. G., Caldwell G. S., Casotti R., Cembella A. D., Engström-Öst J., Halsband C., Sonnenschein E., Legrand C., Llewellyn C. A., Paldavičienė A., Pilkaityte R., Pohnert G., Razinkovas A., Romano G., Tillmann U., Vaiciute D. The relevance of marine chemical ecology to plankton and ecosystem function: An emerging field. *Marine Drugs*, 2011, vol. 9, iss. 9, pp. 1625–1648. https://doi.org/10.3390/md9091625
14. Lelong A., Hégaret H., Soudant P., Bates S. S. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: Revisiting previous paradigms. *Phycologia*, 2012, vol. 51, iss. 2, pp. 168–216. https://doi.org/10.2216/11-37.1
15. Lima-Mendez G., Faust K., Henry N., Decelle J., Colin S., Carcillo F., Chaffron S., Ignacio-Espinosa J. C., Roux S., Vincent F., Bittner L., Darzi Y., Wang J., Audic S., Berline L., Bontempi G., Cabello A. M., Coppola L., Cornejo-Castillo F. M., D'Ovidio F., de Meester L., Ferrera I., Garet-Delmas M.-J., Guidi L., Lara E., Pesant S., Royo-Llonch M., Alazar G., Sánchez P., Sebastian M., Souffreau C., Dimier C., Picheral M., Searson S., Kandels-Lewis S., Tara

Oceans Coordinators, Gorsky G., Not F., Ogata H., Speich S., Stemmann L., Weissenbach J., Wincker P., Acinas S. G., Sunagawa S., Bork P., Sullivan M. B., Karsenti E., Bowler C., de Vargas C., Raes J. Determinants of community structure in the global plankton interactome. *Science*, 2015, vol. 348, no. 6237, art. no. 1262073 (10 p.). https://doi.org/10.1126/science.1262073

16. Long M., Tallec K., Soudant P., Le Grand F., Donval A., Lambert C., Sarthou G., Jolley D. F., Hégaret H. Allelochemicals from *Alexandrium minutum* induce rapid inhibition of metabolism and modify the membranes from *Chaetoceros muelleri*. *Algal Research*, 2018, vol. 35, pp. 508–518. https://doi.org/10.1016/j.algal.2018.09.023
17. Louw D. C., Doucette G. J., Voges E. Annual patterns, distribution and long-term trends of *Pseudo-nitzschia* species in the northern Benguela upwelling system. *Journal of Plankton Research*, 2017, vol. 39, iss. 1, pp. 35–47. https://doi.org/10.1093/plankt/fbw079
18. Lundholm N., Hansen P. J., Kotaki Y. Lack of allelopathic effects of the domoic acid-producing marine diatom *Pseudo-nitzschia multiseries*. *Marine Ecology Progress Series*, 2005, vol. 288, pp. 21–33. https://doi.org/10.3354/meps288021
19. Mardones J. I. Screening of Chilean fish-killing microalgae using a gill cell-based assay. *Latin American Journal of Aquatic Research*, 2020, vol. 48, iss. 2, pp. 329–335. https://dx.doi.org/10.3856/vol48-issue2-fulltext-2400
20. Mikheev M. A., Ipatova V. I., Spirkina N. E. Biotic interactions between two species of microalgae in mixed culture. *Moscow University Biological Sciences Bulletin*, 2018, vol. 73, iss. 2, pp. 63–68. https://doi.org/10.3103/S0096392518020062
21. Orlova T. Yu., Stonik I. V., Shevchenko O. G. Flora of planktonic microalgae of Amursky Bay, Sea of Japan. *Russian Journal of Marine Biology*, 2009, vol. 35, iss. 1, pp. 60–78. https://doi.org/10.1134/S106307400901009X
22. Patil V., Abate R., Wu W., Zhang J., Lin H., Chen C., Liang J., Sun L., Li X., Li Y., Gao Y. Allelopathic inhibitory effect of the macroalga *Pyropia haitanensis* (Rhodophyta) on harmful bloom-forming *Pseudo-nitzschia* species. *Marine Pollution Bulletin*, 2020, vol. 161, pt A, art. no. 111752 (12 p.). https://doi.org/10.1016/j.marpolbul.2020.111752
23. Phatarpekar P. V., Sreepada R. A., Pednekar C., Achuthankutty C. T. A comparative study on growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with monocultures. *Aquaculture*, 2000, vol. 181, iss. 1–2, pp. 141–155. https://doi.org/10.1016/S0044-8486(99)00227-6
24. Pichierri S., Accoroni S., Pezzolesi L., Guerrini F., Romagnoli T., Pistocchi R., Totti C. Allelopathic effects of diatom filtrates on the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*. *Marine Environmental Research*, 2017, vol. 131, pp. 116–122. https://doi.org/10.1016/j.marenvres.2017.09.016
25. Popik A., Gamayunov E., Voznesenskiy S., Markina Zh., Orlova T. The study of fluorescence features of microalgae from the genus *Pseudo-nitzschia* and the possibility of their detection in water. *Algal Research*, 2022, vol. 64, art. no. 102662 (10 p.). https://doi.org/10.1016/j.algal.2022.102662
26. Poulin R. X., Poulson-Ellestad K. L., Roy J. S., Kubanek J. Variable allelopathy among phytoplankton reflected in red tide metabolome. *Harmful Algae*, 2018, vol. 71, pp. 50–56. https://doi.org/10.1016/j.hal.2017.12.002
27. Prasetiya F. S., Safitri I., Widowati I., Cognie B., Decottignies P., Gastineau R., Morançais M., Windarto E., Tremblay R., Mouget J. L. Does allelopathy affect co-culturing *Haslea ostrearia* with other microalgae relevant to aquaculture? *Journal of Applied Phycology*, 2016, vol. 28, iss. 4, pp. 2241–2254. https://doi.org/10.1007/s10811-015-0779-y
28. Sobrinho B. F., De Camargo L. M., Sandrini-Neto L., Kleemann C. R., da Costa Machado E., Mafra L. L. Growth, toxin production and allelopathic effects of *Pseudo-nitzschia multiseries* under iron-enriched conditions. *Marine Drugs*, 2017, vol. 15, iss. 10, art. no. 331 (16 p.). https://doi.org/10.3390/md15100331
29. Tan K., Huang Z., Ji R., Qiu Y., Wang Z., Liu J. A review of allelopathy on microalgae.

Microbiology, 2019, vol. 165, iss. 6, pp. 587–592. https://doi.org/10.1099/mic.0.000776

30. Ternon E., Pavaux A. S., Marro S., Thomas O. P., Lemée R. Allelopathic interactions between the benthic toxic dinoflagellate *Ostreopsis* cf. *ovata* and a co-occurring diatom. *Harmful Algae*, 2018, vol. 75, pp. 35–44. https://doi.org/10.1016/j.hal.2018.04.003
31. Trainer V. L., Bates S. S., Lundholm N., Thessen A. E., Cochlan W. P., Adams N. G., Trick C. G. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae*, 2012, vol. 14, pp. 271–300. https://doi.org/10.1016/j.hal.2011.10.025
32. Voznesenskiy S. S., Gamayunov E. L., Popik A. Yu., Markina Zh. V., Orlova T. Yu. Temperature dependence of the parameters of laser-induced fluorescence and species composition of phytoplankton: The theory and the experiments. *Algal Research*, 2019, vol. 44, art. no. 101719 (11 p.). https://doi.org/10.1016/j.algal.2019.101719
33. Yamasaki Y., Shikata T., Nukata A., Ichiki S., Nagasoe S., Matsubara T., Shimasaki Y., Nakao M., Yamaguchi K., Oshima Y., Oda T., Ito T., Jenkinson I. R., Asakawa M., Honjo T. Extracellular polysaccharide-protein complexes of a harmful alga mediate the allelopathic control it exerts within the phytoplankton community. *The ISME Journal*, 2009, vol. 3, iss. 7, pp. 808–817. https://doi.org/10.1038/ismej.2009.24
34. Yamasaki Y., Nagasoe S., Matsubara T., Shikata T., Shimasaki Y., Oshima Y., Honjo T. Allelopathic interactions between the bacillariophyte *Skeletonema costatum* and the raphidophyte *Heterosigma akashiwo*. *Marine Ecology Progress Series*, 2007, vol. 339, pp. 83–92. https://doi.org/10.3354/meps339083
35. Yasakova O. N. The seasonal dynamics of potentially toxic and harmful phytoplankton species in Novorossiysk Bay (Black Sea). *Russian Journal of Marine Biology*, 2013, vol. 39, iss. 2, pp. 107–115. https://doi.org/10.1134/S1063074013020090
36. Xu N., Tang Y. Z., Qin J., Duan S., Gobler C. J. Ability of the marine diatoms *Pseudo-nitzschia multiseries* and *P. pungens* to inhibit the growth of co-occurring phytoplankton *via* allelopathy. *Aquatic Microbial Ecology*, 2015, vol. 74, no. 1, pp. 29–41. https://doi.org/10.3354/ame01724
37. Zhao M., Chen X., Ma N., Zhang Q., Qu D., Li M. Overvalued allelopathy and overlooked effects of humic acid-like substances on *Microcystis aeruginosa* and *Scenedesmus obliquus* competition. *Harmful Algae*, 2018, vol. 78, pp. 18–26. https://doi.org/10.1016/j.hal.2018.07.003
38. Zheng J.-W., Li D.-W., Lu Y., Chen J., Liang J.-J., Zhang L., Yang W.-D., Liu J.-S., Lu S.-H., Li H.-Y. Molecular exploration of algal interaction between the diatom *Phaeodactylum tricornutum* and the dinoflagellate *Alexandrium tamarense*. *Algal Research*, 2016, vol. 17, pp. 132–141. https://doi.org/10.1016/j.algal.2016.04.019

ВЗАИМОДЕЙСТВИЕ ДИАТОМОВЫХ ВОДОРОСЛЕЙ *PSEUDO-NITZSCHIA HASLEANA* И *THALASSIOSIRA PSEUDONANA* В СМЕШАННОЙ КУЛЬТУРЕ

Ж. В. Маркина[1], А. Ю. Попик[2]

[1]Национальный научный центр морской биологии имени А. В. Жирмунского ДВО РАН, Владивосток, Российская Федерация

[2]Институт автоматики и процессов управления ДВО РАН, Владивосток, Российская Федерация

E-mail: *zhannav@mail.ru*

Представители рода *Pseudo-nitzschia* (Bacillariophyta) вызывают цветения в разных районах Мирового океана, поэтому важно знать экологические особенности этих видов, в том числе то, как они взаимодействуют с другими видами одноклеточных водорослей. Кроме того,

необходима методика быстрой идентификации данных водорослей в среде. В связи с этим нами оценена динамика численности клеток *Pseudo-nitzschia hasleana* и *Thalassiosira pseudonana* в моно- и смешанных культурах путём их прямого подсчёта в камере Нажотта. Также проанализированы температурные кривые флуоресценции хлорофилла *a*, полученные методом лазерно-индуцированной флуоресценции в температурной камере. Опыты проводили в течение 14 суток. Показано, что *P. hasleana* оказывала различное действие на *T. pseudonana* в зависимости от начальной численности *T. pseudonana*. При начальной концентрации $0{,}8 \times 10^4$ кл.·мл$^{-1}$ происходила выраженная стимуляция роста этой диатомовой водоросли. При начальных концентрациях $1{,}6 \times 10^4$ и $3{,}2 \times 10^4$ кл.·мл$^{-1}$ отмечено ингибирование её роста. В смешанной культуре *T. pseudonana* оставалась в стационарной фазе роста, тогда как в монокультуре популяция входила в фазу отмирания к 14-м суткам опыта. *T. pseudonana* ингибировала рост *P. hasleana*. Эксперимент с совместным культивированием *P. hasleana* и *T. pseudonana* показал, что на флуоресценцию хлорофилла *a* смеси оказывает большее воздействие та микроводоросль, концентрация которой значительно выше. Флуоресцентный сигнал двух культивируемых отдельно монокультур потенциально может быть использован для их поиска в смеси.

Ключевые слова: *Pseudo-nitzschia hasleana*, *Thalassiosira pseudonana*, аллелопатия, флуоресценция хлорофилла *a*, идентификация микроводорослей