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THE EFFECT OF NITROGEN AND PHOSPHORUS ON THE ACCUMULATION OF EXTRACELLULAR MARENNINE-LIKE PIGMENT IN THE CULTURE OF *HASLEA KARADAGENSIS* (BACILLARIOPHYTA)

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Currently, five diatom species from the genus *Haslea* are known to produce marennine pigments which slightly differ in their physicochemical characteristics. The available data on these pigments primarily relate to *H. ostrearia*, but given the biodiversity noted, it is important to analyze the pigments in other representatives of the genus, specifically *H. karadagensis*, endemic to the Black Sea. The aim of this study was to investigate the effect of nitrogen and phosphorus on the accumulation of marennine-like pigments in *H. karadagensis* cultures. Literature data on the effects of these essential biogenic elements on pigment accumulation in the cultures are conflicting. For the clones analyzed, the absence of a significant correlation was established between the specific productivity in relation to the release of extracellular marennine and levels of nitrogen and phosphorus in a medium. Growth rates and dynamics of extracellular marennine accumulation in *H. karadagensis* cultures are determined.

Keywords: diatoms, *Haslea karadagensis*, marennine, biogenic elements, pigment accumulation, specific productivity, cell abundance

Marennine is a water-soluble pigment produced by some diatoms from the genus *Haslea*. Pigments of the marennine group are blue, blue-green, or grey-green in color, depending on the producer species, and have antibacterial, antiviral, antiproliferative [Bergé et al., 1999; Carbonnelle et al., 1999; Gastineau et al., 2012b, c], and antioxidant activity [Pouvreau et al., 2008]. Also, they exhibit allelopathy towards bacteria, some microalgae, and invertebrates [Pouvreau et al., 2007; Prasetiya et al., 2016, 2020].

Despite practical interest in using marennine in oyster aquaculture on the Atlantic coast of France [Gastineau et al., 2012c, 2018] and more than a century of its study, the functional purpose, molecular structure, and diversity of pigments of this group remain unclear. It is believed that the pigment is capable of screening excess photosynthetically active radiation, thus acting as a photoprotector [Schubert et al., 1995]. Marennine is known to have 2 forms, differing in chemical composition and spectral characteristics [Pouvreau et al., 2006]. The intracellular form of this pigment is accumulated in the apical parts of cells. The extracellular form of marennine released from a cell into the environment has slightly different physicochemical properties and a lower molecular weight. It has long been believed that the only species capable of producing marennine

is *Haslea ostrearia* (Gaillon) Simonsen. The description in 2012 of a second marennine producer, *H. karadagensis* Davidovich, Gastineau & Mouget [Gastineau et al., 2012a], recorded in the coastal Black Sea area, initiated the study of the marennine diversity [Gastineau et al., 2014].

According to D. Neuville and P. Daste [1972; 1978], the release of marennine by *H. ostrearia* is driven by unfavorable environmental conditions, especially nitrate starvation in the presence of light. T. Lebeau [Lebeau et al., 2000] shares this opinion, claiming that insufficient nitrogen stresses cells thus causing elevated pigment synthesis. R. Nghiem Xuan [Nghiem Xuan et al., 2021] confirms that it is the limitation of silicon and nitrogen, rather than deficiency, that is responsible for a high concentration of extracellular marennine revealed in *H. ostrearia* cultures. Some authors explain a gain in the content of extracellular marennine in a culture by cell lysis when a significant cell density and deficiency of nutrients are reached [Nghiem Xuan et al., 2020], ignoring the fact of the existence of two different pigment forms. Other authors take a different view, claiming that the key factor of the marennine release is the amount and spectral composition of light [Mouget et al., 1999, 2004, 2005; Prasetiya et al., 2016]. A rise in the marennine production was recorded with a decrease in the cell size of *H. ostrearia* throughout its life cycle [Pedron et al., 2023]. Currently, five species capable of producing marennine-like pigments are known. In addition to above-mentioned, those include *H. provincialis* Gastineau, Hansen & Mouget [Gastineau et al., 2016], *H. nusantara* Mouget, Gastineau & Syakti [Prasetiya et al., 2019], and *H. silbo* Gastineau, Hansen & Mouget [Gastineau et al., 2021]. Marennine-like pigments (hereinafter marennine) exhibit some differences in their physicochemical characteristics [Gastineau et al., 2014; Pouvreau et al., 2006]. The diversity of pigments determined the aim of the present work: to reveal the effect of nitrogen and phosphorus concentrations on the efficiency of the marennine production and changes in the cell density in a culture of the diatom *Haslea karadagensis* which had not been previously studied in this regard.

MATERIAL AND METHODS

In an experiment with the addition of biogenic elements, three clones of the marine diatom *H. karadagensis* were used: 23.1113-KS, 23.1129-KE, and 23.1227-KC. Those are descendants of the parental pairs 22.1128-KC + 23.1129-KU, 22.1129-KA + 22.1129-KB, and 22.1228-KE + 22.1128-KH, respectively, from the Collection of Diatoms of the World Ocean (Karadag Scientific Station, IBSS, https://ibss-ras.ru/about-ibss/structure-ibss/tsentry-kollektivnogo-polzovaniya/collection-of-diatoms-of-world-ocean/). Clones 23.1129-KE and 23.1113-KS were characterized by higher concentration of extracellular marennine and cell density, while 23.1227-KC was less productive. On average, cell sizes for clones 23.1129-KE, 23.1113-KS, and 23.1227-KC were (15.3 ± 0.28), (48.5 ± 0.32), and (56.1 ± 0.28) μm, respectively.

Pure cultures were maintained under non-axenic conditions in 50-mL glass Erlenmeyer flasks. A modified artificial ESAW medium (Enriched Seawater, Artificial Water) [Polyakova et al., 2018] was used as a medium, with a salinity level of 20‰ taken as standard cultivation conditions. Cultures were grown at a temperature of +20 °C at two levels of illuminance, 3.3 and 7.2 klx, with the aid of LED lamps LLED-05-T5-FITO-14W-W [ATL Business (Shenzhen) Co., Ltd., China]. The photoperiod was 14 h / 10 h (light / dark). Illuminance was measured with a lux meter Yu-116 (USSR). To the flasks, 0.5 mL of inoculum with a cell density of 100 cells·mL^{-1} was added.

In six clones of *H. karadagensis*, for 20 days, the concentration of pigment released into the medium was measured every 2 days. The cell concentration was estimated in a Fuchs–Rosenthal chamber. The rate of cell division for the first 5 days was determined by a change in the cell density according to the exponential growth model [Wood et al., 2005]. The rate of the marennine accumulation in cultures for the same clones was calculated in a similar manner.

During the study, 24 different nitrogen concentration options and 29 phosphorus concentration options in 154 combinations were tested. For two clones, 12 combinations involving nitrogen and phosphorus were tested at a higher illuminance (7.2 klx).

The main biogenic elements (nitrogen and phosphorus) were preliminarily excluded from the composition of the completely artificial ESAW medium; then, their required amount was added in the form of $NaNO_3$ and NaH_2PO_4 compounds. Concentrations in the medium were within ranges of 0.136–43.2 mg·L^{-1} for nitrogen and 0.014–4.481 mg·L^{-1} for phosphorus. The modified ESAW medium served as a control.

The duration of the culture growth in the experiments was 10 days, during which they were in the exponential growth phase, not reaching the stationary one. In this case, the release of extracellular marennine by cells into the culture medium was intravital as evidenced by the absence of dead cells (empty frustules). In a 10-day period, extracellular marennine was accumulated in the cultures in a concentration sufficient for spectrophotometric analysis.

Non-specific absorption was extremely low, and this allowed the use of unfiltered medium containing dissolved extracellular marennine for spectral analysis. Optical density was measured in a cuvette with an optical path length of 2 cm. Absorption spectra were recorded with a PromEcoLab PE-5400UF spectrophotometer (Shanghai Mapada Instruments Co., Ltd., China) and supplied Scan54 software.

To calculate the concentration of extracellular marennine in *H. karadagensis* according to the Bouguer–Lambert–Beer law, we used the only currently known values of the molar extinction coefficient [ε677 = 120,000 L·mol^{-1}·cm^{-1} for a wavelength of 677 nm] and the molar mass [M = (9,893 ± 1) Da] obtained for extracellular marennine in *H. ostrearia* [Pouvreau et al., 2006]. The wavelength of 677 nm corresponds to the maximum optical density in the red region of the spectrum for extracellular forms of marennine of both species. The cell concentration in the cultures was calculated on the 10th day of the experiment.

The mean values of extracellular marennine content, cell density, and specific cell productivity in terms of extracellular marennine (hereinafter specific productivity) are given in the text and figures taking into account the error of the mean.

RESULTS AND DISCUSSION

Under our standard culture conditions, exponential growth of *H. karadagensis* cells was recorded for at least 5–6 days (Fig. 1A). This is consistent with the data of authors who reported, at different nutrient concentrations, a late exponential phase on the 8th day of cultivation [Pedron et al., 2023]. Subsequently, growth was declining, and cells formed clusters making it impossible to accurately count them in the following days.

During the first week, the marennine concentration in the culture also increased exponentially, and during the following month, linearly (Fig. 1B). The graph of the accumulation rate of extracellular marennine shows as follows: 5–8 days are required to obtain a pigment concentration sufficient for spectrophotometric analysis. During this period, the *H. karadagensis* culture is in the exponential growth

phase, cells do not die, and only the extracellular form of the pigment is accumulated in the medium. By the end of the month, clonal variability in terms of both cell density and extracellular marennine concentration became evident.

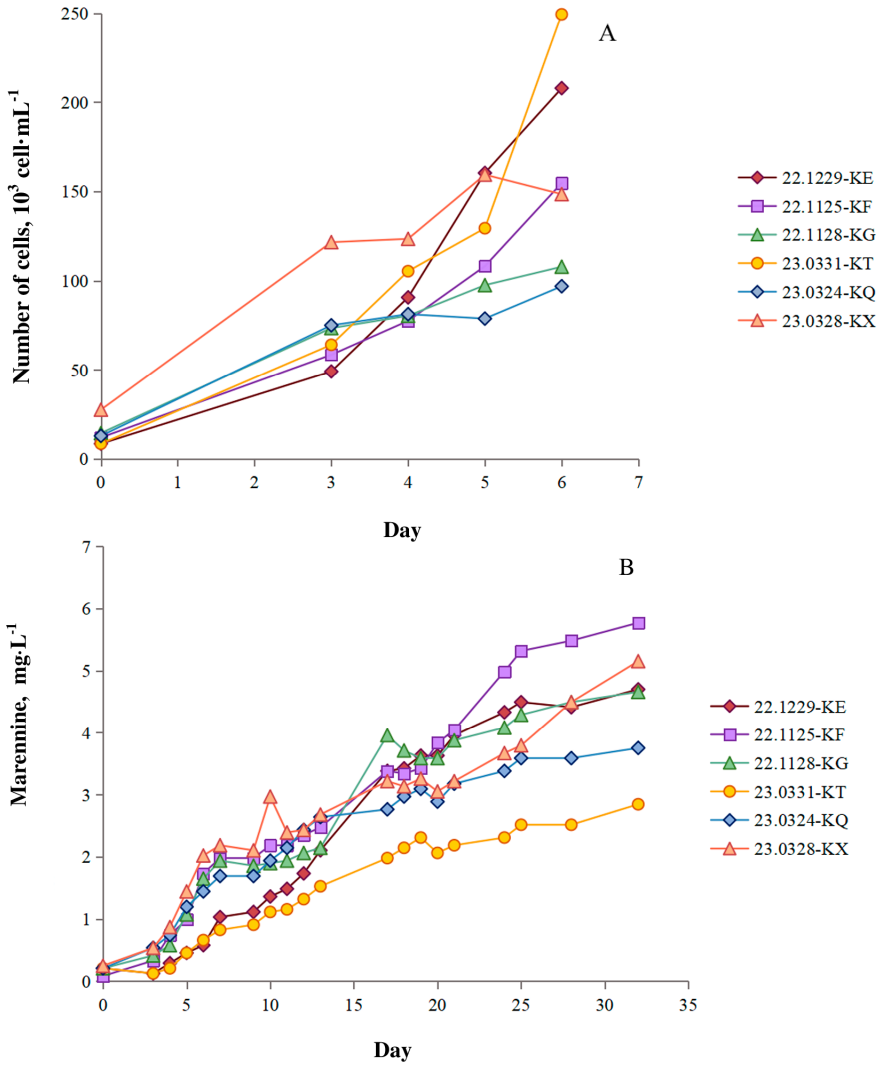

Fig. 1. The cell density growth (A) and extracellular marennine accumulation (B) in *Haslea karadagensis* cultures under standard cultivation conditions. The legend indicates the names of the clones used

With a gain in nitrogen and phosphorus content, we recorded a drop in the concentration of extracellular marennine accumulated in the medium. This dependence was approximated by an exponential function with a high correlation (Fig. 2A, B). Furthermore, the cell density in the cultures significantly decreased with an increase in nitrogen concentration (Fig. 2C), while a rise in phosphorus concentration in the medium within the studied range did not result in a decline in the cell density (Fig. 2D). Our data are quite consistent with the results of other authors for *H. ostrearia* cultures [Lebeau et al., 2000; Neuville, Daste, 1972, 1978; Nghiem Xuan et al., 2021].

In the cultures of the more productive clones, 23.1129-KE and 23.1113-KS, a mean of 1.8 mg·L^{-1} of marennine was accumulated at a nitrogen concentration of 0.177–43.200 mg·L^{-1}, phosphorus concentration of 0.018–4.784 mg·L^{-1}, and illuminance of 3.3 klx. The mean cell density and specific productivity were 77,197 cells·mL^{-1} and 30 pg·cells^{-1}, respectively.

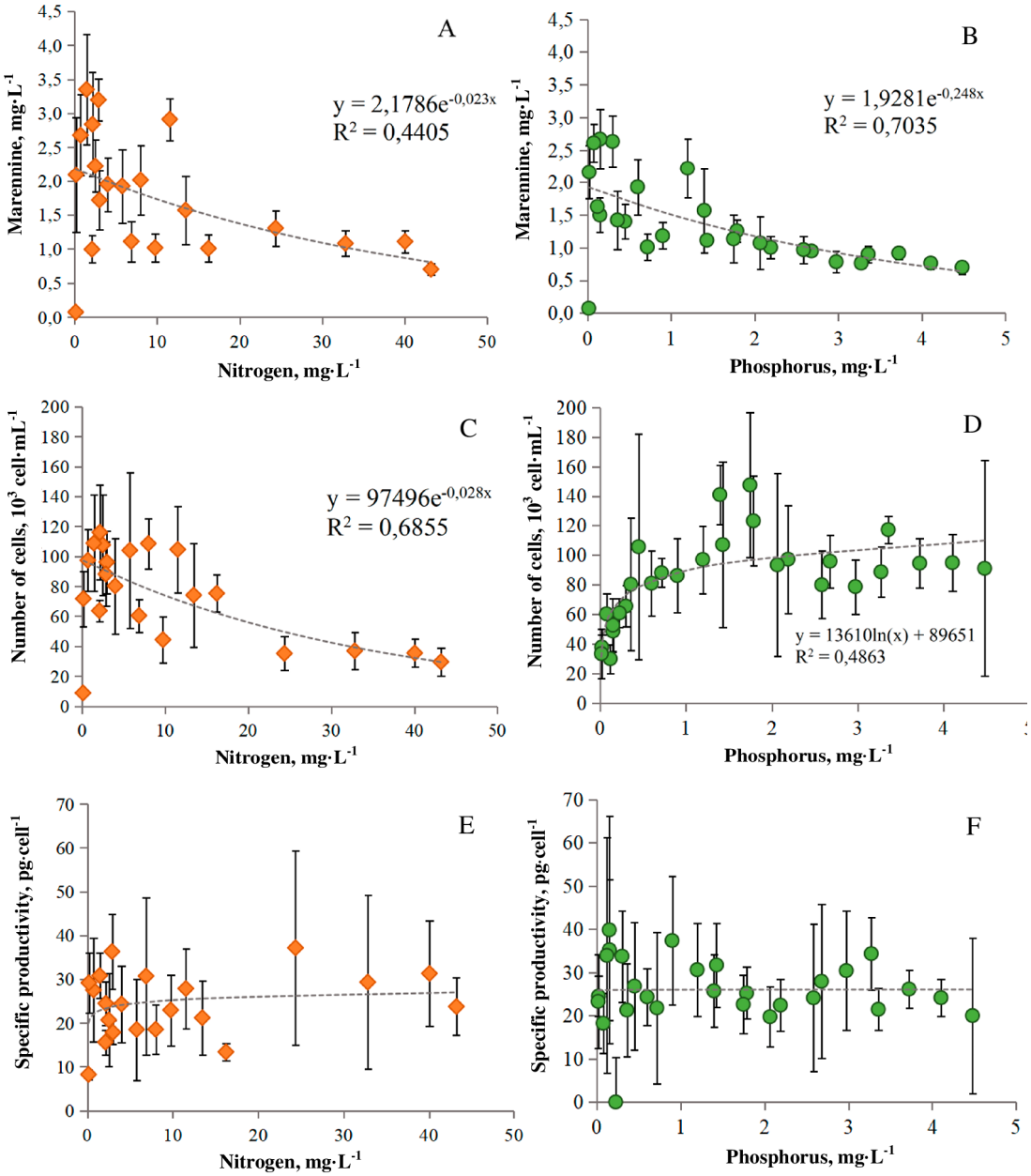

Fig. 2. The mean values of extracellular marennine content (A, B), cell density (C, D), and specific extracellular marennine productivity (E, F) in *Haslea karadagensis* culture for different concentration of nitrogen and phosphorus in the medium at the illuminance level of 3.3 klx. When approximating the data, concentrations of biogenic elements close to zero were excluded (0.136 μg·L^{-1} for nitrogen and 0.014 μg·L^{-1} for phosphorus)

The less productive clone, 23.1227-KC, was characterized by the mean marennine concentration of 1.25 mg·L^{-1}. In the experiments with this clone, the maximum nitrogen content (128.5 mg·L^{-1}) was tested; however, it did not lead to a significant shift in the marennine concentration. No cell death due to nitrogen poisoning was revealed.

For the illuminance level of 7.2 klx, the mean value of extracellular marennine concentration was 3.14 mg·L^{-1}. The cell density was 58,177 cells·mL^{-1}, and the specific productivity was 60 pg·cells^{-1}, *i. e.*, twice as high as at lower illuminance.

At close-to-zero concentrations of biogenic elements, 0.136 mg·L^{-1} of nitrogen and 0.014 mg·L^{-1} of phosphorus, the marennine production was minimal and averaged 0.51 mg·L^{-1}. However, even a slight increase in their content, nitrogen to 0.177 mg·L^{-1} and phosphorus to 0.018 mg·L^{-1}, led to the same concentration of cells and marennine in the medium as with a higher supply of biogenic elements (Fig. 3).

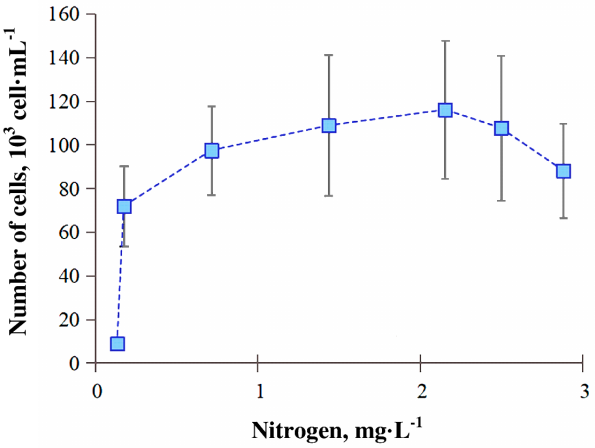

Fig. 3. The cell density in the *Haslea karadagensis* culture after 10 days of cultivation under low nitrogen concentration

According to the data obtained, the specific cell productivity of the *H. karadagensis* culture for marennine (its extracellular form) did not depend on the concentration of the main biogenic elements in a fairly wide range, except for minimal values, and averaged about 30 pg·cells^{-1} (Fig. 2E, F). As assumed, the key factor limiting pigment production is illuminance, since marennine is believed to perform a photoprotective function by shielding chloroplasts from ultraviolet radiation [Schubert et al., 1995]. As noted above, the spectral composition of light is also important: the marennine production significantly increased when *H. ostrearia* cultures were illuminated with blue light compared to illumination with the control white light [Mouget et al., 2005].

The density of diatom cells, according to some authors, is determined chiefly by the availability of silicon [Nghiem Xuan et al., 2021]. The second crucial element affecting the cell density growth is phosphorus [Turpin et al., 1999]. In the ESAW medium we used, the silicon concentration was 3 mg·L^{-1}, and it was quite sufficient for the consumption by cells during 10 days of cultivation.

The marennine accumulation in a medium was shown to depend on its composition [Nghiem Xuan et al., 2020]. It was also noted as follows: if the medium contains both organic and inorganic sources of nitrogen and phosphorus, the marennine production is low. Importantly, the same as in our experiments, an increase in nitrogen content to certain values initially favored the marennine synthesis, and subsequently inhibited the cell density growth and the pigment accumulation in the medium. The composition of the ASW medium (Artificial Seawater) is most similar to that of ESAW. According to the data provided [Nghiem Xuan et al., 2020], a medium developed on the basis of the ES1/3 medium turned out to be optimal for the marennine accumulation. Moreover, in the marennine synthesis, the role of both the composition and the concentration of biogenic elements was significant.

In addition to their general theoretical value, the results obtained have practical significance for biotechnology, in particular for the aquaculture of filter-feeding molluscs, such as oysters. Marennine not only improves their organoleptic properties and increases their commercial value,

but, being a biologically active substance, has a preventive effect: it protects against diseases [Gastineau et al., 2012c, 2018]. Currently, biotechnology for the marennine production involving cultures of *Haslea* representatives is not used; oyster farmers rely on natural and poorly predictable outbreaks of algae development in ponds where these molluscs are kept. Notably, under natural conditions, it is impossible to achieve such a high concentration of marennine as in laboratory cultures. Thus, in a field experiment, when water supplied to oyster ponds was enriched with nitrogen, phosphorus, and silicon, the maximum observed content of extracellular marennine was 2.7 mg·L^{-1} [Turpin et al., 1999]. In a non-laboratory experiment, the marennine concentration reached 3.4 mg·L^{-1} [Turpin et al., 2001]. In *H. ostrearia* culture in a photobioreactor, the marennine content was an order of magnitude higher: 20–30 mg·L^{-1} [Rossignol et al., 2000].

Another noteworthy factor is clonal variability. In our study, three *H. karadagensis* clones were used, and two of them exhibited higher productivity in terms of extracellular marennine synthesis. A similar pattern of variation was recorded in experiments with *H. ostearia* [Mouget et al., 2005; Pedron et al., 2023]. As found, the clones differed in cell size, and this indicates they were at different stages of the life cycle. The highest productivity in terms of pigment accumulation was demonstrated by medium-sized cells. The exact reasons for the variability are unknown, and this may become a topic for new research.

Conclusions. For the marennine-producing diatom *Haslea karadagensis*, we revealed no significant correlation between nitrogen and phosphorus concentration in a medium and the specific productivity in terms of extracellular marennine synthesis. Relatively low content of these biogenic elements, 0.177 mg·L^{-1} of nitrogen and 0.018 mg·L^{-1} of phosphorus, was sufficient for optimal growth of *H. karadagensis* cultures and accumulation of extracellular pigment in the medium. An increase in their concentration negatively affected the cell density growth and accumulation of extracellular marennine in the culture.

The key drivers of the productivity of marennine accumulation were illuminance and, likely, clonal variability. An increase in illuminance from 3.3 to 7.2 klx stimulated a nearly twofold boost in both extracellular pigment concentration and specific productivity. The effect of intraclonal differences requires further study.

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REFERENCES

1. Polyakova S. L., Davidovich O. I., Podunay Yu. A., Davidovich N. A. Modification of the ESAW culture medium used for cultivation of marine diatoms. *Marine Biological Journal*, 2018, vol. 3, no. 2, pp. 73–80. (in Russ.). https://doi.org/10.21072/mbj.2018.03.2.06
2. Bergé J.-P., Bourgougnon N., Alban S., Pojer F., Billaudel S., Chermann J.-C., Robert J.-M., Franz G. Antiviral and anticoagulant activities of a water-soluble fraction of the marine diatom *Haslea ostrearia*. *Planta Medica*, 1999, vol. 65, iss. 7, pp. 604–609. https://doi.org/10.1055/s-1999-14032
3. Carbonnelle D., Pondaven P., Morançais M., Massé G., Bosch S., Jacquot C., Briand G., Robert J.-M., Roussakis C. Antitumor and antiproliferative effects of an aqueous extract from the marine diatom *Haslea ostrearia* (Gaillon) Simonsen against solid tumors: Lung carcinoma (NSCLC-N6), kidney carcinoma (E39) and melanoma (M96) cell lines. *Anticancer Research*, 1999, vol. 19, iss. 1A, pp. 621–624.

4. Gastineau R., Davidovich N. A., Bardeau J.-F., Caruso A., Leignel V., Hardivillier Y., Jacquette B., Davidovich O. I., Rincé Y., Gaudin P., Cox E. J., Mouget J.-L. *Haslea karadagensis* (Bacillariophyta): A second blue diatom, recorded from the Black Sea and producing a novel blue pigment. *European Journal of Phycology*, 2012a, vol. 47, iss. 4, pp. 469–479. https://doi.org/10.1080/09670262.2012.741713
5. Gastineau R., Davidovich N., Hansen G., Rines J., Wulff A., Kaczmarska I., Ehrman J., Hermann D., Maumus F., Hardivillier Y., Leignel V., Jacquette B., Méléder V., Hallegraeff G., Yallop M., Perkins R., Cadoret J.-P., Saint-Jean B., Carrier G., Mouget J.-L. *Haslea ostrearia*-like diatoms: Biodiversity out of the blue. In: *Advances in Botanical Research*. Vol. 71. *Sea Plants* / N. Bourgougnon (Ed.). UK : Academic Press, 2014, pp. 441–465. https://doi.org/10.1016/B978-0-12-408062-1.00015-9
6. Gastineau R., Hansen G., Davidovich N. A., Davidovich O. I., Bardeau J.-F., Kaczmarska I., Ehrman J. M., Leignel V., Hardivillier Y., Jacquette B., Poulin M., Morançais M., Fleurence J., Mouget J.-L. A new blue-pigmented hasleoid diatom, *Haslea provincialis*, from the Mediterranean Sea. *European Journal of Phycology*, 2016, vol. 51, iss. 2, pp. 156–170. https://doi.org/10.1080/09670262.2015.1110861
7. Gastineau R., Hansen G., Poulin M., Lemieux C., Turmel M., Bardeau J.-F., Leignel V., Hardivillier Y., Morançais M., Fleurence J., Gaudin P., Méléder V., Cox E. J., Davidovich N. A., Davidovich O. I., Witkowski A., Kaczmarska I., Ehrman J. M., Onís E. S., Quintana A. M., Mucko M., Mordret S., Sarno D., Jacquette B., Falaise C., Séveno J., Lindquist N. L., Kemp P. S. Jr., Eker-Develi E., Konucu M., Mouget J.-L. *Haslea silbo*, a novel cosmopolitan species of blue diatoms. *Biology*, 2021, vol. 10, iss. 4, art. no. 328 (27 p.). https://doi.org/10.3390/biology10040328
8. Gastineau R., Hardivillier Y., Leignel V., Tekaya N., Morançais M., Fleurence J., Davidovich N. A., Jacquette B., Gaudin P., Hellio C., Bourgougnon N., Mouget J.-L. Greening effect on oysters and biological activities of the blue pigments produced by the diatom *Haslea karadagensis* (Naviculaceae). *Aquaculture*, 2012b, vol. 368–369, pp. 61–67. https://doi.org/10.1016/j.aquaculture.2012.09.016
9. Gastineau R., Pouvreau J.-B., Hellio C., Morançais M., Fleurence J., Gaudin P., Bourgougnon N., Mouget J.-L. Biological activities of purified marennine, the blue pigment produced by the diatom *Haslea ostrearia* and responsible for the greening of oysters. *Journal of Agricultural and Food Chemistry*, 2012c, vol. 60, iss. 14, pp. 3599–3605. https://doi.org/10.1021/jf205004x
10. Gastineau R., Prasetiya F. S., Falaise C., Cognie B., Decottignies P., Morançais M., Méléder V., Davidovich N. A., Turcotte F., Tremblay R., Pasetto P., Dittmer J., Bardeau J.-F., Pouvreau J.-B., Mouget J.-L. Marennine-like pigments: Blue diatom or green oyster cult? In: *Blue Biotechnology: Production and Use of Marine Molecules* : in 2 vols / S. La Barre, S. S. Bates (Eds). Weinheim : Wiley-VCH Verlag GmbH & Co. KGaA, 2018, vol. 2, pp. 529–551. https://doi.org/10.1002/9783527801718.ch16
11. Lebeau T., Gaudin P., Junter G.-A., Mignot L., Robert J.-M. Continuous marennin production by agar-entrapped *Haslea ostrearia* using a tubular photobioreactor with internal illumination. *Applied Microbiology and Biotechnology*, 2000, vol. 54, iss. 5, pp. 634–640. https://doi.org/10.1007/s002530000380
12. Mouget J.-L., Rosa P., Tremblin G. Acclimation of *Haslea ostrearia* to light of different spectral qualities – confirmation of 'chromatic adaptation' in diatoms. *Journal of Photochemistry and Photobiology B: Biology*, 2004, vol. 75, iss. 1–2, pp. 1–11. https://doi.org/10.1016/j.jphotobiol.2004.04.002
13. Mouget J.-L., Rosa P., Vachoux C., Tremblin G. Enhancement of marennine production by blue light in the diatom *Haslea ostrearia*. *Journal of Applied Phycology*, 2005, vol. 17, iss. 5, pp. 437–445. https://doi.org/10.1007/s10811-005-0561-7
14. Mouget J.-L., Tremblin G., Morant-Marceau A., Morançais M., Robert J.-M. Long-term photoacclimation of *Haslea ostrearia* (Bacillariophyta): Effect of irradiance on growth rates, pigment

content and photosynthesis. *European Journal of Phycology*, 1999, vol. 34, iss. 2, pp. 109–115. https://doi.org/10.1080/09670269910001736162

15. Neuville D., Daste P. H. Production du pigment bleu par la diatomée *Navicula ostrearia* (Gaillon) Bory, maintenue en culture uni-algue sur milieu synthétique carencé en azote. *Comptes rendus de l'Académie des Sciences. Sér. D Sciences Naturelles*, 1972, vol. 274, pp. 2030–2033.
16. Neuville D., Daste P. H. Recherches sur le déterminisme de la production de marennine par la diatomée marine *Navicula ostrearia* (Gaillon) Bory en culture *in vitro*. *Revue générale de botanique*, 1978, vol. 85, pp. 255–303.
17. Nghiem Xuan R., Mouget J.-L., Turpin V., Jaouen P., Pruvost J. Optimization of the growth and marennine production by the diatom *Haslea ostrearia* in photobioreactor. *Algal Research*, 2021, vol. 55, art. no. 102251 (17 p.). https://doi.org/10.1016/j.algal.2021.102251
18. Nghiem Xuan R., Safitri I., Mouget J.-L., Pruvost J., Turpin V., Jaouen P. Design of an artificial culture medium to optimize *Haslea ostrearia* biomass and marennine production. *Algal Research*, 2020, vol. 45, art. no. 101653 (11 p.). https://doi.org/10.1016/j.algal.2019.101653
19. Pedron E., Gargouch N., Mouget J.-L., Tremblay R., Deschênes J.-S., Massé A., Gonçalves O. Hybrid photobioreactor operation for the intensified production of *Haslea ostrearia* and marennine in function of strain variability. *Algal Research*, 2023, vol. 75, art. no. 103285 (9 p.). https://doi.org/10.1016/j.algal.2023.103285
20. Pouvreau J.-B., Housson E., Le Tallec L., Morançais M., Rincé Y., Fleurence J., Pondaven P. Growth inhibition of several marine diatom species induced by the shading effect and allelopathic activity of marennine, a blue-green polyphenolic pigment of the diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen. *Journal of Experimental Marine Biology and Ecology*, 2007, vol. 352, iss. 1, pp. 212–225. https://doi.org/10.1016/j.jembe.2007.07.011
21. Pouvreau J.-B., Morançais M., Fleury F., Rosa F., Thion L., Cahingt B., Zal F., Fleurence J., Pondaven P. Preliminary characterization of the blue-green pigment "marennine" from the marine tychopelagic diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen. *Journal of Applied Phycology*, 2006, vol. 18, iss. 6, pp. 757–767. https://doi.org/10.1007/s10811-006-9087-x
22. Pouvreau J.-B., Morançais M., Taran F., Rosa P., Dufossé L., Guérard F., Pin S., Fleurence J., Pondaven P. Antioxidant and free radical scavenging properties of marennine, a blue-green polyphenolic pigment from the diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen responsible for the natural greening of cultured oysters. *Journal of Agricultural and Food Chemistry*, 2008, vol. 56, iss. 15, pp. 6278–6286. https://doi.org/10.1021/jf073187n
23. Prasetiya F. S., Decottignies P., Tremblay R., Mouget J.-L., Sunarto S., Iskandar I., Dhahiyat Y., Cognie B. Not only greening: The effects of marennine produced by *Haslea ostrearia* on physiological traits of three bivalve species. *Aquaculture Reports*, 2020, vol. 18, art. no. 100546 (8 p.). https://doi.org/10.1016/j.aqrep.2020.100546
24. Prasetiya F. S., Gastineau R., Poulin M., Lemieux C., Turmel M., Syakti A. D., Hardivillier Y., Widowati I., Risjani Y., Iskandar I., Subroto T., Falaise C., Arsad S., Safitri I., Mouget J.-L., Leignel V. *Haslea nusantara* (Bacillariophyceae), a new blue diatom from the Java Sea, Indonesia: Morphology, biometry and molecular characterization. *Plant Ecology and Evolution*, 2019, iss. 152, no. 2, pp. 188–202. https://doi.org/10.5091/plecevo.2019.1623
25. 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
26. Rossignol N., Jaouen P., Robert J.-M., Quéméneur F. Production of exocellular pigment by the marine diatom *Haslea ostrearia* Simonsen in a photobioreactor equipped with immersed ultrafiltration membranes. *Bioresource Technology*, 2000, vol. 73, iss. 2,

pp. 197–200. https://doi.org/10.1016/S0960-8524(99)00171-6
27. Schubert H., Tremblin G., Robert J.-M., Sagert S., Rincé Y. *In-vivo* fluorescence measurement of photosynthesis of *Haslea ostrearia* Simonsen in relation to marennine content. *Diatom Research*, 1995, vol. 10, iss. 2, pp. 341–349. https://doi.org/10.1080/0269249X.1995.9705354
28. Turpin V., Robert J.-M., Goulletquer P. Limiting nutrients of oyster pond seawaters in the Marennes-Oléron region for *Haslea ostrearia*: Applications to the mass production of the diatom in mesocosm experiments. *Aquatic Living Resources*, 1999, vol. 12, iss. 5, pp. 335–342. https://doi.org/10.1016/S0990-7440(99)00114-X
29. Turpin V., Robert J.-M., Goulletquer P., Massé G., Rosa P. Oyster greening by outdoor mass culture of the diatom *Haslea ostrearia* Simonsen in enriched seawater. *Aquaculture Research*, 2001, vol. 32, iss. 10, pp. 801–809. https://doi.org/10.1046/j.1365-2109.2001.00615.x
30. Wood A. M., Everroad R. C., Wingard L. M. Measuring growth rates in microalgal cultures. In: *Algal Culturing Techniques* / R. A. Andersen (Ed.). New York : Elsevier Academic Press, 2005, pp. 269–286. https://doi.org/10.1016/B978-012088426-1/50019-6

ВЛИЯНИЕ АЗОТА И ФОСФОРА НА НАКОПЛЕНИЕ ВНЕКЛЕТОЧНОГО МАРЕННИН-ПОДОБНОГО ПИГМЕНТА В КУЛЬТУРЕ *HASLEA KARADAGENSIS* (BACILLARIOPHYTA)

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В настоящее время известно пять видов диатомовых водорослей из рода *Haslea*, которые способны продуцировать пигменты маренниры, слегка различающиеся по своим физико-химическим характеристикам. Имеющиеся сведения относятся главным образом к маренниру *H. ostrearia*. Это делает актуальным изучение маренниры-подобных пигментов у других представителей рода, в частности у *H. karadagensis* — эндемика Чёрного моря. Цель исследования заключалась в анализе влияния азота и фосфора на накопление внеклеточного маренниры-подобного пигмента в клоновых культурах *H. karadagensis*. Следует отметить противоречивость литературных данных о влиянии этих основных биогенных элементов на накопление маренниры в культурах. Для изученных клонов *H. karadagensis* установлено отсутствие значимой корреляции между удельной продуктивностью клеток в отношении выделения внеклеточного маренниры-подобного пигмента и уровнями азота и фосфора в среде. Определены темпы роста численности клеток и динамика накопления внеклеточного пигмента в культурах *H. karadagensis*.

Ключевые слова: диатомовые водоросли, *Haslea karadagensis*, маренниры, биогенные элементы, накопление пигмента, удельная продуктивность, численность клеток