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Research Article

INFLUENCE OF SALINITY ON THE GROWTH OF *NODULARIA SPUMIGENA* MERTENS

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Abstract

The plankton material was sampled in August 1997 from the Gulf of Gdansk. The Cyanobacterium *Nodularia spumigena* strain (NSG 0897) was isolated from the samples. The effect of salinity on the growth of *Nodularia spumigena* was studied in the laboratory. Salinity had a significant effect on the number of cells, optical density, concentration of chlorophyll *a*, dry mass and growth rate. The *Nodularia spumigena* strain grew well in salinities 4-16 PSU. A salinity of 8 PSU is the best for the growth of this strain.

INTRODUCTION

Nodularia spumigena Mertens belongs to the filamentous cyanobacteria (blue-green algae), which are able to fix dissolved molecular nitrogen and occasionally produce hepatotoxin. Blooms of this species, occurring in the late summer have been observed for several years as a regular phenomenon in the Baltic Sea. Such blooms, in which *Nodularia spumigena* plays an important role have been noticed since the beginning of the nineteenth century in the Baltic waters. They occur in the coastal zone as well as in the open sea (Kononen 1992). The Bothnian Bay is the only exception where the blooms are very rare because of a lower temperature and different hydrographic conditions (Kharu *et*

al. 1994). In the North Sea and in Ellesmere (the brackish lake) blooms of this species have been noticed (Nehring 1993). *Nodularia spumigena* occurs also in Australian waters. It blooms very often in numerous estuaries and bays along the southern and western coasts of Australia and New Zealand. Many of them are mentioned as toxic (Blackburn 1996). However, the first reference to a toxic strain of *Nodularia spumigena* comes from Australia (Carmichael 1994).

Single blooms of *Nodularia spumigena* and mixed blooms in which this species plays a codominant role are very often toxic (Blackburn *et al.* 1996, Seller 1997). The toxicity is caused by nodularin – the five amino-acid, one of the hepatotoxins. They damage the liver and kill animals by causing blood to pool in the liver. It damages liver cells, detains blood in the liver and within a few hours causes death as a result of a shock and an insufficiency of the circulatory system (Carmichael 1994). The distemper of the liver function and also the appearance of cancer tumours can be caused by even lower concentrations of this toxin. The toxin is present in cells of toxic strains (it doesn't occur in non-toxic strains) and it gets into the water (or medium) when the cells are broken (Lehtimäki *et al.* 1994).

There have been many investigations on defining the biological and environmental parameters responsible for *Nodularia spumigena* blooms which are a serious threat to natural waters. The blossoming of water can not be explained only by the biological features of the species but this phenomenon is induced by many environmental factors which play an important limiting and controlling role (Kononen 1992). Among the main controlling factors are the trophic parameters *e.g.* phosphorus and nitrogen supply and the N:P ratio. The limiting factors are: temperature (for the Baltic strains of this species the temperature optimum ranges between 20-25 °C), light (there are different optima given by many authors depending on the individual features of the treated strain, ranging from 40-80 $\mu\text{mol m}^{-2}\text{s}^{-1}$) as well as salinity. There are many authors who consider salinity to be a limiting factor causing blooms and having influence on the distribution of this species (Kononen 1992, Lehtimäki *et al.* 1994).

Therefore the aim of this paper is to define the optimum of salinity when the growth and biological production of *Nodularia spumigena* are the most effective.

MATERIAL AND METHODS

The strain of *Nodularia spumigena* used for investigation is stored with the culture collection of the Laboratory of Marine Plant Ecology of the University of Gdańsk (number collection NSG 0897). The plankton material was sampled

from the Gulf of Gdansk at a station situated close to the promenade boulevard in Gdynia (Fig. 1). The sample was taken in August 1997 during an earlier stage of the bloom when the temperature of water was 18 °C, salinity - 7,4 PSU and weather was sunny and windless.

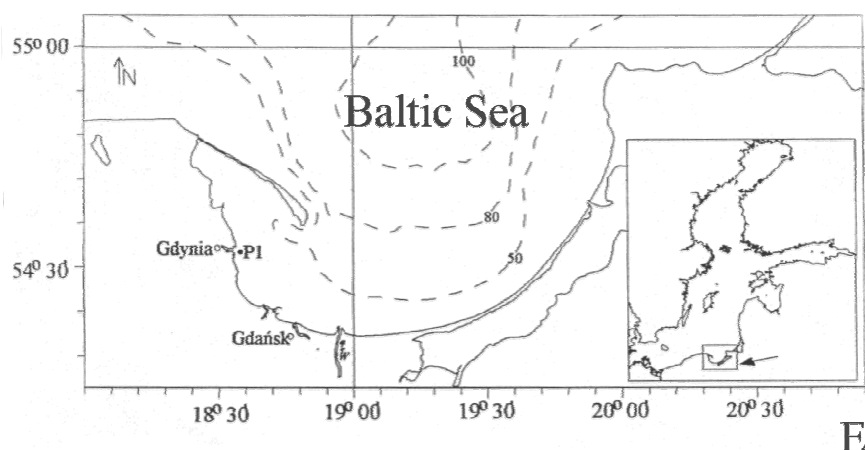


Fig. 1. Location of the sampling station (P₁).

Isolation

Single filaments of *Nodularia spumigena* have been isolated from the plankton material under the binocular microscope using the micropipette for the spiral filaments and the preparatory needle for the non-spiral ones. The isolated filaments were out on Petri dishes with mineral substratum (medium MN of. Waterbury and Stanier) condensed with 0,5 % agar- agar. The material was placed in the culture chamber and a temperature of 20 °C , incubation of 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and photoperiod of 16 h of light and 8 h of dark were used. Microscopic examinations were performed every 3-4 days. The symptoms of visible growth have been observed after about 3 weeks of examinations. The colonies free of other algae were placed along with a small piece of agar, with a sterile scalpel onto new dishes and incubated for two weeks under the same conditions. When the growth of *Nodularia* was distinctly visible, the purity of colonies was examined using an inverted microscope. Because there were some troubles with the transfer from agar to the liquid medium, the semi-liquid medium was used during 6 weeks. Such a semi-liquid medium contains only 0,1 % of agar with a little amount of a liquid medium added every three days. After this period the strain started to grow in a normally liquid medium. The strain

isolated in the described way is stored in nonaxenic cultures in mineral media: MN and 0,5 ASN III .

Growth testing

Sea water with a salinity of 37 PSU was diluted using deionised water for getting the following salinities 4, 8, 12, 16, 24, 30, 35 PSU. Every salinity was completed by mineral components of medium BG 11 (after Stanier 1971). The 0 PSU was made using only deionized water.

The experiment was carried out at a temperature of 20 °C, which is contained in the range of the thermal optimum conditions for Baltic strains (Sivonen *et al.* 1989, Lehtimäki *et al.* 1994). The light intensity of 70 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and photoperiod of 16 h of light and 8 h of dark were chosen because these parameters are optimal for Baltic strains (Lehtimäki *et al.* 1994) and are very similar to light conditions during the blooms. Cool white florescent tubes of the Sylwania 40W type were used. Light intensity was measured with the LI Cor indicator, model LI-189s.

The acclimatization of cultures to the chosen salinity was done in the experiment conditions in a period of two weeks. Erlenmeyer flasks with a volume of 250 ml, containing 100 ml of the liquid media were used. For every combination 3 repetitions were done. The injective volume of inoculum contained approximately $7 \cdot 10^3$ cells in 1 ml. The experiment lasted 15 days. The growth was examined every 5 days.

The material was filtered out by the Whatman GF/C glass fiber, then dried to a constant weight at a temperature of 60 °C for 24 h and weighed with an accuracy of 0,1 mg. 50 ml of the liquid material was filtered to get a dry mass. The optical density of cultures was measured using the spectrophotometer UV-VIS 1202 with a wavelength of 750 nm and a one- cm glass cuvette. The extraction of chlorophyll was done according to Strickland and Parson methods (Strickland, Parson 1972).

The concentration of chlorophyll *a* has been calculated after Jeffrey and Humphrey's equation (Jeffrey, Humphrey 1975). Membrane filters (Millipore HAWG 047 00) were used for counting the cells after Holmes' method (Holmes 1954). The growth rate per day was calculated as

$$k' = \ln (N_2/N_1) / (t_2 - t_1)$$

where N_2 is the number of cells at time 2 (t_2) and N_1 is the number of cells at time 1 (t_1). The growth rate was calculated over days 5-10, which corresponded to the logarithmic-phase stage of growth (Blackburn *et al.* 1996).

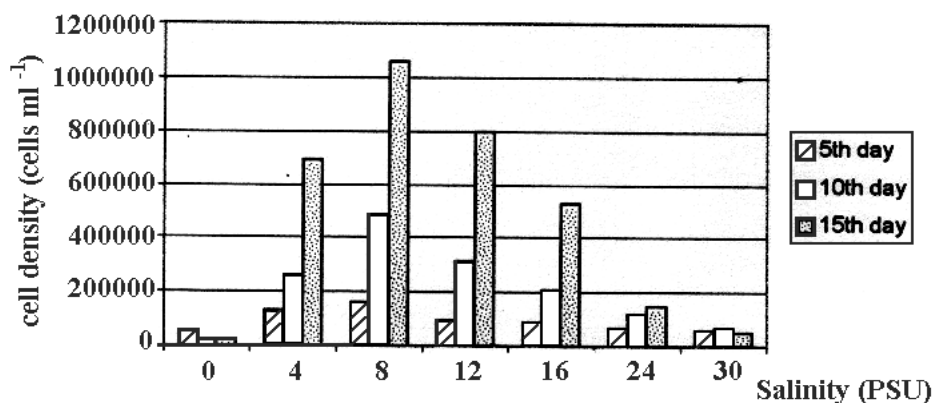


Fig. 2. The effect of salinity (PSU) on cell density of the *Nodularia spumigena* strain from the Baltic Sea.

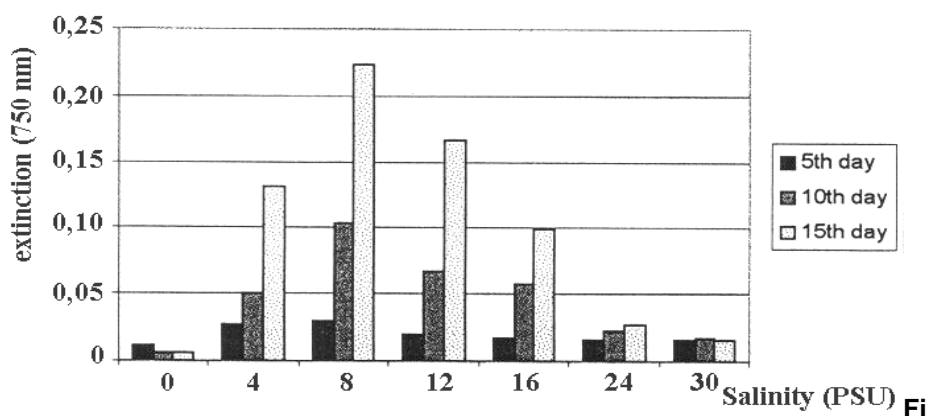


Fig. 3. The effect of salinity (PSU) on optical density (extinction) of *Nodularia spumigena* strain from Baltic Sea.

RESULTS AND DISCUSSION

A salinity of 8 PSU is the best for the growth of the strain of *Nodularia spumigena* isolated from the Gulf of Gdansk under the conditions of light and temperature in which the experiment had been carried out. This feature is typical of all investigated parameters: the number of cells (Fig. 2), optical density (Fig. 3), the concentration of chlorophyll *a* (Fig. 4) and dry mass (Fig. 5). A sufficient growth was observed in a salinity of 4 PSU, 12 PSU and 16 PSU as well.

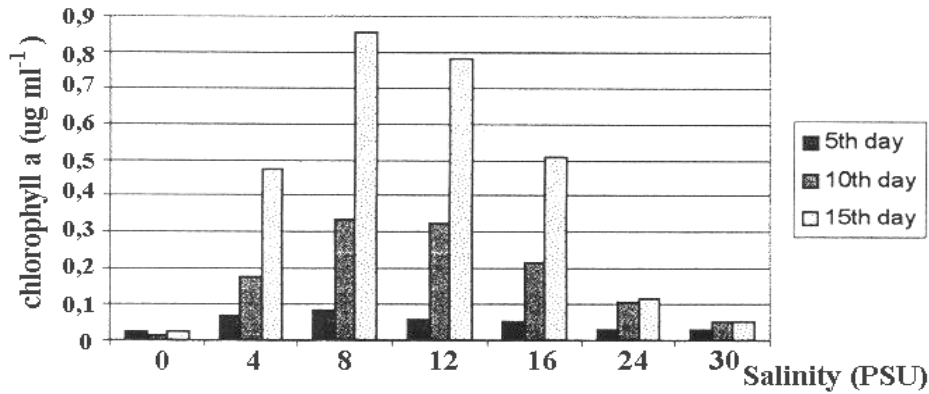


Fig. 4. The effect of salinity (PSU) on concentration of chlorophyll a of *Nodularia spumigena* strain from the Baltic Sea.

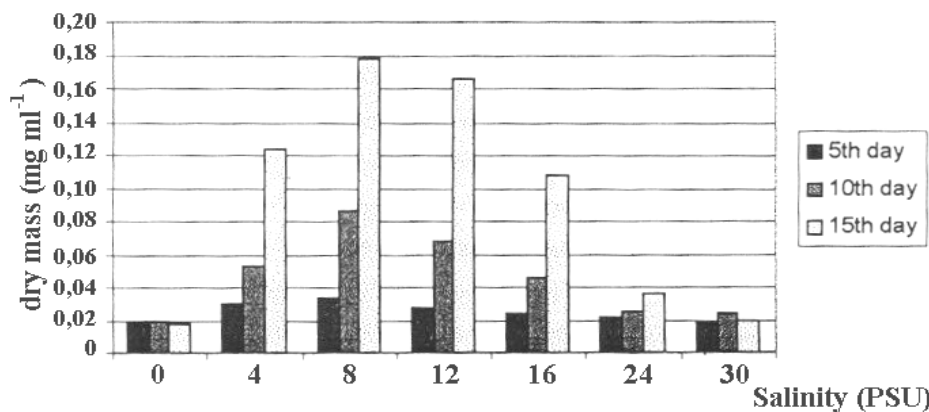


Fig. 5. The effect of salinity (PSU) on dry mass of *Nodularia spumigena* strain from the Baltic Sea.

A significantly worse growth was observed in a salinity of 24 PSU and very weak - in 30 PSU. A growth of 35 PSU was not noticeable, living cells were not noticed on the 5th day of cultivation. On the contrary, in the fresh water medium the investigated strain grew well on the first few days. Although the number of cells on the 5th day of cultivation was higher than in inoculum, on the following days its number decreased. On the 15th day of the observation the filaments had very few living cells. Changes in the appearance of filaments were observed in high salinity as well. They were unnaturally short, strongly bended and tangled. It is especially characteristic of the culture of 30 PSU, where such a deformation makes it difficult to count the cells.

The growth rate like the other investigated parameters is also the best in a salinity of 8 PSU and is 0.247 d^{-1} . In a 12 PSU salinity the growth rate was high as well – 0.227 d^{-1} . A somewhat lower value of this rate was noticed in a salinity of 4 PSU and 16 PSU - 0.131 d^{-1} and 0.177 d^{-1} respectively. The lowest rate was observed for a high salinity, e.g. 0.106 d^{-1} for 24 PSU and 0.01 d^{-1} for 30 PSU.

The obtained results show that the strain isolated from the Gulf of Gdańsk grows well in a range of salinity between 4 and 12 PSU. It confirms the ecological adaptation of this species to natural conditions. Blooms of *Nodularia spumigena* occur very frequently in the southern and central part of the Baltic Sea, where salinity is similar to that mentioned above. However, in the Gulf of Finland where salinity is below 4 PSU and in the Danish Straits with a salinity of 13-17 PSU this species occurs very rarely (Lehtimäki *et al.* 1994, Kahru *et al.* 1994). The result obtained by us corresponds to the data published by Lehtimäki *et al.* (1994) for two Baltic strains of *Nodularia spumigena*. They grow very well in a salinity of 5-11 PSU although the growth rate was significantly lower (0.16 d^{-1}) than in the case of our examined strain. The medium composition could probably explain this difference. Lehtimäki *et al.* (1994) did not use nitrogen in the medium. A very interesting data was obtained for the strains from Australian waters. They grow well in a wide range of salinity from 0 to 35 PSU (Blackburn *et al.* 1996). Under freshwater conditions the results of cell division and the growth rate were somewhat lower than in saline water. It means that the Baltic strains are less tolerant to salinity than those from Australian waters.

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