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SCELETONEMA COSTATUM (GREV.) CLEVE CULTURE IN ARTIFICIAL SEA WATER

1. INTRODUCTION

Diatom cultures in artificial sea water have been the object of interest of many researchers [1, 10, 21, 25, 26]. Laboratory experiments with cultures of marine algae enable the tracing of all the life cycles of the organisms. They afford studies of growth and cell divisions, also supplying additional information for the needs of modern taxonomy [15].

Sceletonema costatum is an important neritic organism [3] and considered an indicator of inshore phytoplankton [5]. As eurythermic and euryhaline organism it occurs in both tropical and subpolar waters [18], in marine ecosystems and brackish rivers [27].

The species is commonly used in experimental cultures [3]. It has a rapid rate of reproduction, two or three times a day, on average [22, 23, 29, 30, 35], but quantitative fluctuations have been observed in parallel cultures maintained in similar conditions. These fluctuations did not always depend on the initial number of cells in the cultures [5].

The nutrients which are most important for the growth of *S. costatum* and other diatoms are nitrogen, phosphorus and silicon. A decrease of these three nutrients was observed in the marine environment during the mass flowering of diatoms [2]. Silicon was found to limit the growth of natural populations of such diatoms as *S. costatum* [35] and other species [4, 11]. In cultures where the quantities of nitrogen and phosphorus, but not silicon, were increased, an inhibition of diatom growth was observed, the former were subsequently replaced by green microflagellates [7]. It is possible that the quantitative nitrogen to phosphorus ratio in the natural environment may stimulate or inhibit the growth of the most valuable plant plankton such as diatoms and green algae [34].

Typical marine diatoms perish or develop poorly when the salinity

droops to below 15‰. After adding sucrose or sodium chloride to the medium the diatom continued cell division at salinities of 5‰ [12].

The purpose of the present study was to investigate the effect of changes in the amounts of inoculum, nutrients and salinity on the growth of the Baltic diatom *Sceletonema costatum* cultured in artificial sea water. The ingredients of the culture medium which was prepared according to the methods of Guillard [10] and other authors [21, 25] are also shown.

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2. CULTURE METHOD

2.1. Preparation of the ASPM medium

The ASPM medium consists of artificial sea water with a salinity of about 35‰, and several other solutions with separately prepared nutrients, buffor and vitamins (Tab.).

The basic ASPM solution (solution I, Tab.) was prepared without concentration by dissolving the ingrediants in 10 dcm³ double-distilled water. In the case of cultures in large containers $NaHCO_3$ was added to the sterile medium. The carbonate solution was separately sterilized by filtering. Since the basic solution was diluted to a lower salinity, boronic acid was aded from a separate mixture before autoclaving the prepared medium. The boronic mixture contained 0,6 g H_3BO_3 in 100 cm³. H_2O; 4 cm³ of this was added for every 1 dcm³ of the medium. Boron is needed by many marine and freshwater diatoms [10].

Macroelements (solution II, Tab.) were added to the medium from 1000 fold concentrated stock solutions. Sodium metasilicate was dissolved after heating the solution and the addition of some drops of HCl prevented its precipitation after adding it to the medium. One to 10 cm³ of solution of each macroelement was added to 1 dcm³ of the medium. Nitrates were added prior to autoclaving. Phosphates and metasilicates were autoclaved separately and added to a sterile medium.

Trace elements (solutions III, Tab.) were prepared in two stages.

Composition of 1 dcm³ of artificial sea water ASPM [10]
Skład 1 dcm³ sztucznej wody morskiej ASPM [10]

I. ASPM basic solution Rozwtór podstawowy ASPM	1. NaCl	26,73 g
	2. $MgCl_2 \times 6H_2O$	4,82 „
	3. $MgSO_4 \times 7H_2O$	6,40 „
	4. $CaCl_2 \times 6H_2O$	2,28 „
	5. KCl	0,72 „
	6. $NaHCO_3$	0,19 „
	7. KBr	0,07 „
	8. H_3BO_3	0,06 „
II. Macroelements Makroelementy	9. $NaNO_3$	10 - 100 mg
	10. K_2HPO_4	1 - 10 „
	11. $Na_2SiO_3 \times 5H_2O$	7 - 70 „
III. Trace metals Mikroelementy	12. FeEDTA	10,00 mg
	13. $ZnSO_4 \times 6H_2O$	0,22 „
	14. $MnCl_2 \times 4H_2O$	81,0 µg
	15. $Na_2MoO_4 \times 2H_2O$	13,0 „
	16. $CoCl_2 \times 6H_2O$	2,3 „
	17. $CuCl_2 \times 2H_2O$	1,1 „
	18. KJ	0,66 „
	19. $SrCl_2 \times 6H_2O$	0,40 mg
	20. LiCl	0,08 „
	21. $AlCl_3$	10,0 µg
	22. RbCl	2,8 „
IV. Buffer Bufor	23. TRIS pH=7,2	100 - 500 mg
V. Vitamins Witaminy	24. B_1	0,2 mg
	25. B_6	1 µg
	26. B_{12}	1 µg

First 100 cm³ solutions were prepared from the following salts: $ZnSO_4 \times 6H_2O$ — 2,2 g, $MnCl_2 \times 4HO$ — 810 mg, $Na_2MoO_4 \times 2H_2O$ — 130 mg, $CoCl_2 \times 6H_2O$ — 23 mg, $CuCl_2 \times 2H_2O$ — 11 mg, KJ — 6,6 mg. Each of the Zn, Mn, Mo, Co and Cu salts was dissolved in 100 cm³ distilled water which was previously made acidic with pH=4,5. Iodine solution was alkalized with NaOH to pH 8—9. The remaining trace metals — Sr, Al, Rb, Li are stable in neutral solutions and a single stock solution may be prepared [10]. 100 cm³ of such solution contained 4 g $SrCl_2 \times 6H_2O$, 100 mg $AlCl_3$, 28 mg RbCl and 800 mg LiCl. In the second stage one working stock solution was prepared containing all trace metals. 10 g FeEDTA was boiled for 10 minutes in 0,8 dcm³ of distilled water made acidic with HCl. After cooling, 10 cm³ of each stock solution of the microelements were added and diluted to 1 dcm³ with distilled water, pH=2. 1 cm³ to 1 dcm³ ASPM was added before autoclaving.

Buffer (solution IV, Tab.) was prepared by dissolving 50 g TRIS (2-amino-2-hydroxymethylo-1,3-propandiol) in 100 cm³ of distilled water, mixing and adding 29,3 cm³ HCl until pH=7,2 was obtained.

1—5 cm^3 per 1 dcm^3 ASPM was added to the medium before autoclaving.

Vitamins (solutions V, Tab.) were added to the sterile medium directly from purchased ampoules. The following doses were instilled with micropipetts: vit. B_1 — 0,2 mg dcm^{-3} ASPM, vit. B_{12} — 1 µg dcm^{-3} ASPM, vit. B_6 — 1µg dcm^{-3} ASPM.

2.2. Culture materials

Diatoms for cultures were collected from the Bay of Gdańsk in April 1978. A polyethylene barrel containing unfiltered sea water was brought to The Institute of Ecology in Dziekanów Leśny and placed outside in the shade. The source of plankton for cultures was the sediment from the bottom of the barrel containing resting cells of diatoms. Several species of marine diatoms, among them *Sceletonema costatum*, were obtained from this sediment six months after — in september 1978. After inoculation in the ASPM medium cells multipled quickly and after five to ten days they reached a high number. In mixed cultures containing several species, *S. costatum* quickly became dominant, replacing other species of diatoms of the genera Chaetoceros, *Thalassiosira* and constituing more than 95% of the populations.

Pure one-species diatom cultures were obtained by the isolation, under a microscope, of single cells or colonies from the initial mixed cultures. A Pasteur pipette was used for the isolation of cells. Diatom cells were rinsed in several droops of the medium containing antibiotics, after which they were transferred into a sterile medium. The ASPM solution containing antibiotics was prepared by dissolving 20 mg of penicillin G and 10 mg streptomycin in 100 cm^3 ASPM.

2.3. Experimental conditions

The cultures were maintained for a period of two weeks. Cells were transfered to a new medium in the first week during the logarithmic phase of growth, when the rate of cell division was no less than 0,3 in 24 hours. This value was calculated from the question:

$$K = \ln \frac{N}{No} \left(\frac{1}{t \ln 2} \right)$$

where $\ln 2 = 0{,}6931$, No — initial number of cells, N — final cell number, t — time in days [4].

Measurements were made with Burker's type chamber for counting blood cells. Inoculum contained the number of cells necessary to obtain

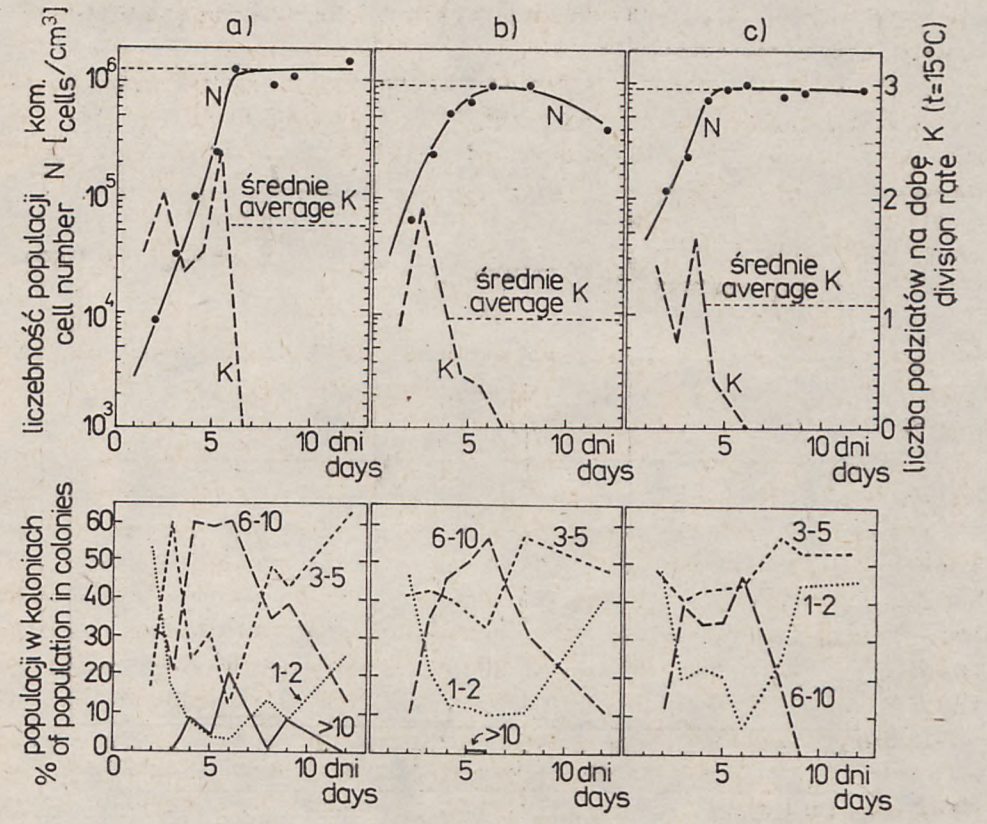

Fig. 1. Influence of the size of inoculum on the growth of *S. costatum*

Rys. 1. Wpływ wielkości inokulum na wzrost hodowli *S. costatum*

a concentration of 10^3—10^4 cells cm^{-3} of the new medium. Usually 0,1—1 cm^3 of the old culture was enough to obtain such an amount of cells. The cultures were maintained in 125 cm^3 flasks at a continuous light of 3000 lux (two 8W neon bulbs at a distance of 30 cm) and a constant temperature of 10°C (283°K or 288°K). Salinity was 20‰, the dose of macroelements was enlarged twice.

The growth of about 40 cultures was followed during this research; the size of inoculum, amounts of nutrients and salinity were changed in succesive experiments.

In experiment No 1 (Fig. 1) the effect of the size of inoculum on the growth of *S. costatum* was investigated. Three cultures of the clone S-1 were used. In culture a) (Fig. 1) the inoculum was 3×10^3 cells cm^{-3} of the new medium, in the culture b) it was 3×10^4 cells cm^{-3} and in culture c) — $4{,}5\times10^4$ cells cm^{-3}. Cultures were kept at 15°C.

In the second experiment different amounts of nutrients were used in the medium (Figs 2, 3, 4), 26 cultures were used. Figure 2 shows three cultures of clone S-1. In culture a) (Fig. 2) a single dose of

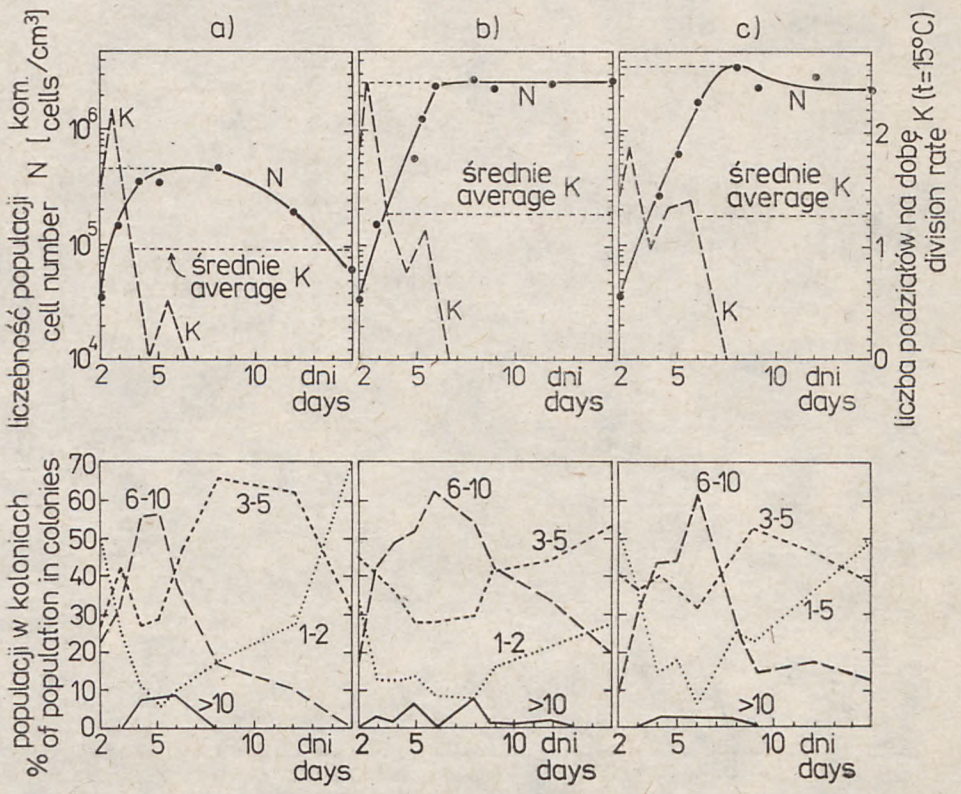

Fig. 2. Growth of *S. costatum* in different concentrations of macroelements (N, P, Si)

Rys. 2. Wpływ dawki makroelementów (N, P, Si) na wzrost *S. costatum*

macroelements was added, in culture b) (Fig. 2) a five fold greater amount of nitrogen, phosphorus and silicon was used, and culture c) (Fig. 2) contained ten times higher the amount of nutrients than in culture a). In these three cultures nutrients were taken from one stock solution. A single dose of macroelements contained 10 mg $NaNO_3$ dmc^{-3} ASPM, 1 mg K_2HPO_4 dcm^{-3} ASPM, 7 mg $Na_2SiO_3 \times 5H_2O$ dcm^{-3} ASPM. Cultures were kept at 15°C. Culture a) (Fig. 3) contains a single dose of macroelements and a five times greater dose of trace metals. A single dose of microelements equalled 1 cm^3 of working stock solution of microelements per dcm^3 ASPM. In cultures b) (Fig. 3) five times greater amounts of macro- and microelements were used. In cultures c) (Fig. 3) the medium contained a ten-fold dose of mackroelements and a single dose of microelements. Cultures d) contained ten-fold doses of macro- and microelements. Cultures a) shown in Fig. 4 had single doses of all nutrients. Cultures b) (Fig. 4) had ten-fold doses of nitrogen and phosphorus and single doses of silicon and microelements. Cultures c) (Fig. 4) had ten-fold doses of nitrogen, phosphorus and

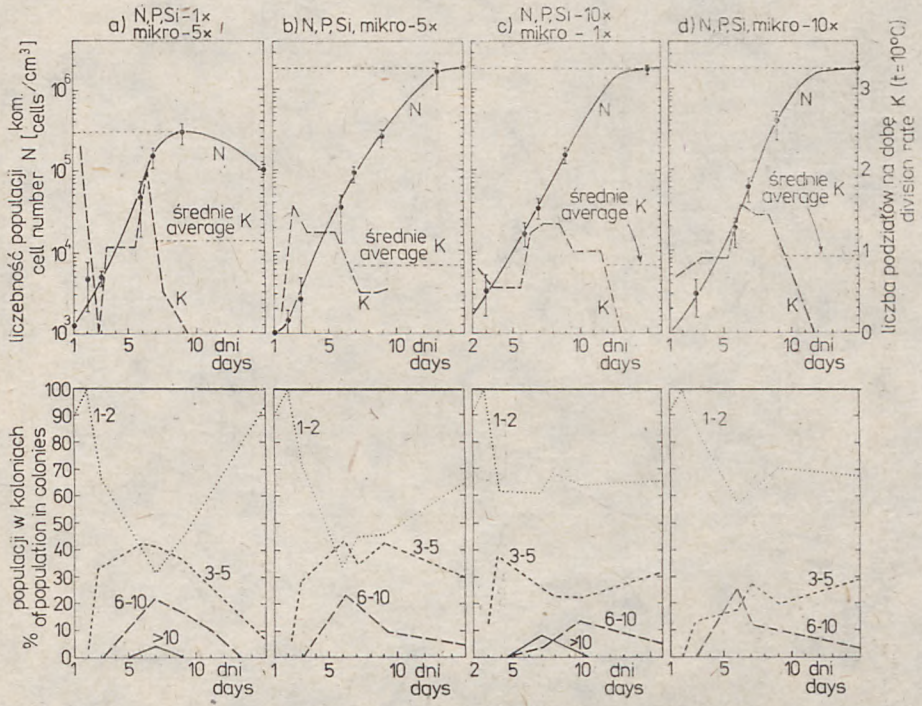

Fig. 3. Growth of *S. costatum* in different concentrations of nutrients (N, P, Si, trace metals)

Rys. 3. Wpływ dawki biogenów (N, P, Si, mikroelementów) na wzrost *S. costatum*

microelements and a single dose of silicon. Cultures d) (Fig. 4) contained ten-fold doses of all nutrients. Cultures shown in Figs 3 and 4 were maintained at +10°C. Growth curves are for three cultures each. In both cases (Figs 3, 4) clone S-1 was used. Nutrients were added from separate solutions.

The effect of salinity on the growth of *S. costatum* was investigated in the third experiment (Figs 5 and 6). Cultures a) were grown in media with a salinity of 10‰, cultures b) — a salinity of 20‰, and cultures c) in media with a salinity of 30‰. Fig. 5 shows clone S-1 and Fig. 6 clone S-2. Growth curves are for single cultures mentained at 15°C.

3. RESULTS

Results of the effect of the size of inoculum on the growth of *S. costatum* (Fig. 1) lead to the conclusion that optimal inoculum should not exceed 10^4 cells cm^{-3} of the new medium. The smaller inoculum, the

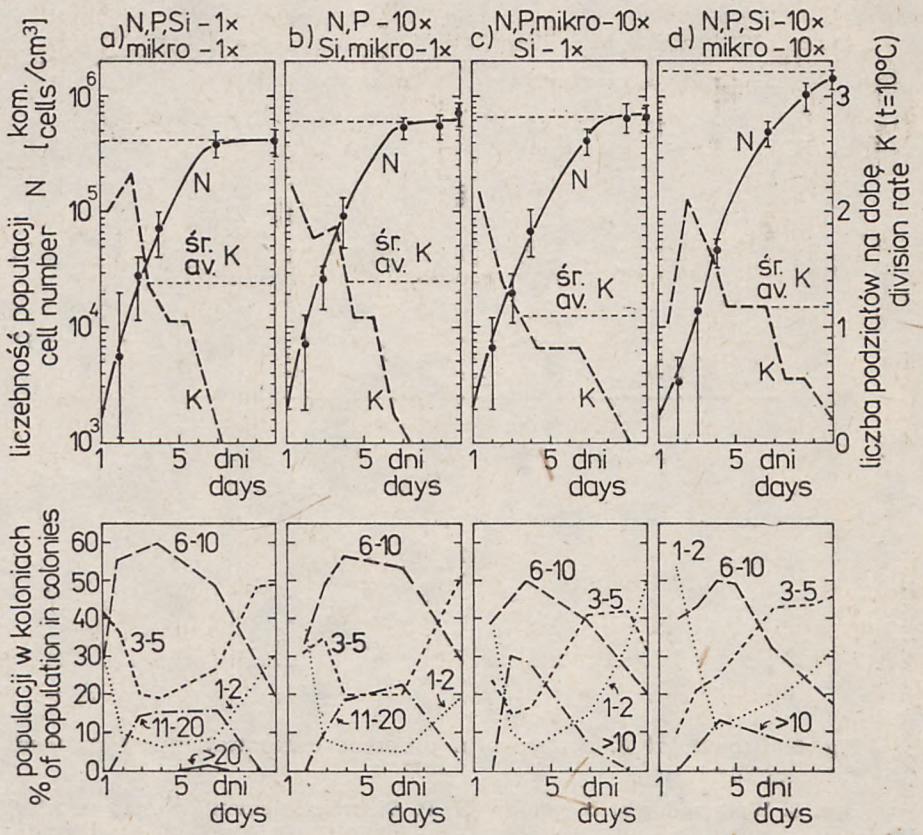

Fig. 4. Growth of *S. costatum* in different concentrations of nutrients
Rys. 4. Wpływ dawki biogenów na wzrost *S. costatum*

longer the logarithmic phase of growth, which is the phase of full physiological activity of diatoms. Culture a) (Fig. 1) showed the longest phase of growth (as in the Figs. 3 and 4). Diatoms had the highest rate of growth and formed the highest number of multicellular chains. Inoculum was lowest (about 10^3 cells cm^{-3} of the new medium).

In the ASPM medium the macroelements — nitrogen, phosphorus, and silicon are indispensible for the growth of diatoms (Figs. 2, 3, 4). An increase of the amounts of nitrogen and phosphorus alone without increasing the silicon dose, did not cause any apparent increase in the number of cells (Fig. 4a, b, c, d). When five and ten times greater doses of macroelements were added, the maximum quantities of cells were similar in both cases (Fig. 2b, c, Fig. 3 b, d). This shows that is sufficient to add a five-fold amount of macroelements to increase the maximum number of cells. The addition of greater doses did not affect the apparent quantity of cells (Fig. 2 b, c, Fig. 3 b, d). Even when a ten-fold dose of macroelements is added, a single dose of microelements

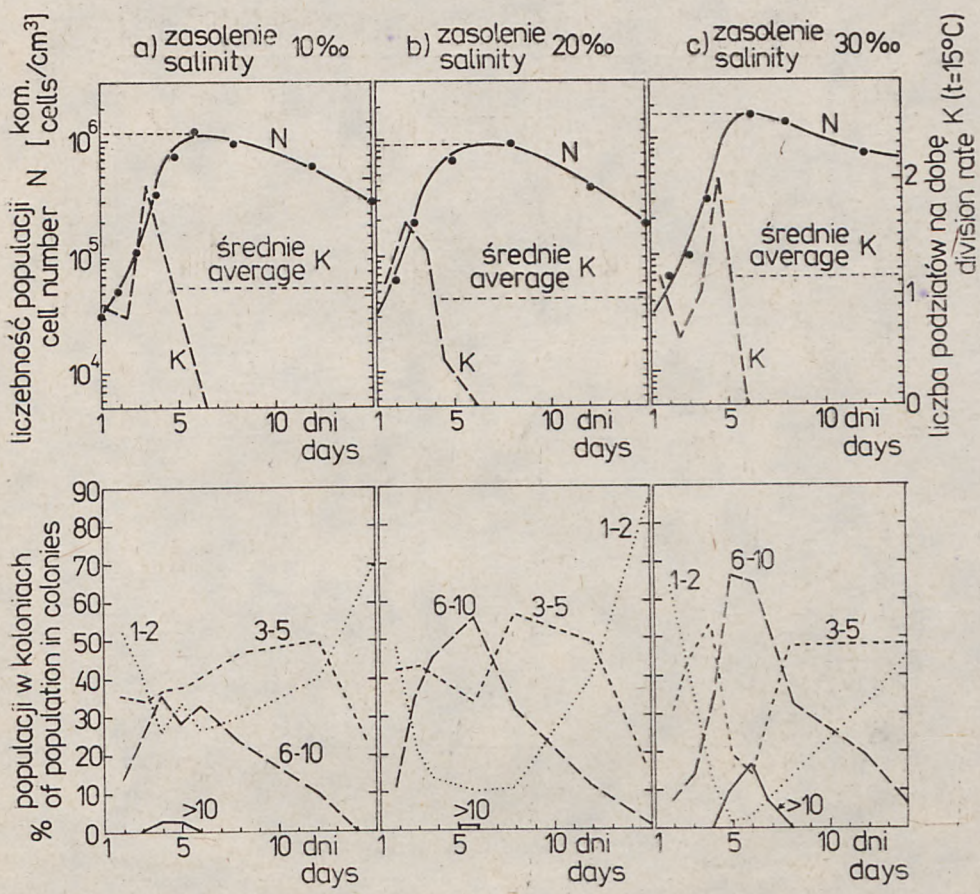

Fig. 5. Growth of *S. costatum* in different salinities

Rys. 5. Wzrost *S. costatum* w pożywkach o różnym zasoleniu

can be used, and they will not limit the growth of diatoms in the ASPM medium (Fig. 3 c, d, Fig. 4 b, c). A large amount of nutrients in the medium increases the lenght of the log phase of growth, as well as the stationary phase (Fig. 2 c, Fig. 3 b, c, d, Fig. 4 d). Multicellular chains lasted for the longest time in cultures with an increased amount of macroelements (Fig. 2 b, c, Fig. 3 b, c, d, Fig. 4 d). Multicellular chains occurred for the shortest time in cultures with single nutrient doses (Fig. 2 a, Fig. 3 a, Fig. 4a). On ageing, these cultures contained the highest number of short chains. The phase of senescence began earlier in these cultures than in others. The results suggest that for the active growth of *S. costatum* in cultures over a period of about eight days, The ASPM medium should contain a five-fold amount of macroelements (that is 50 mg $NaNO_3$ dcm^{-3} ASPM, 5 mg K_2HPO_4 dcm^{-3} ASPM, 35 mg $Na_2SiO_3 \times 5H_2O$ dcm^{-3} ASPM). In the case of an organism with such a fast growth rate, a smaller amount of nutrients would make it necess-

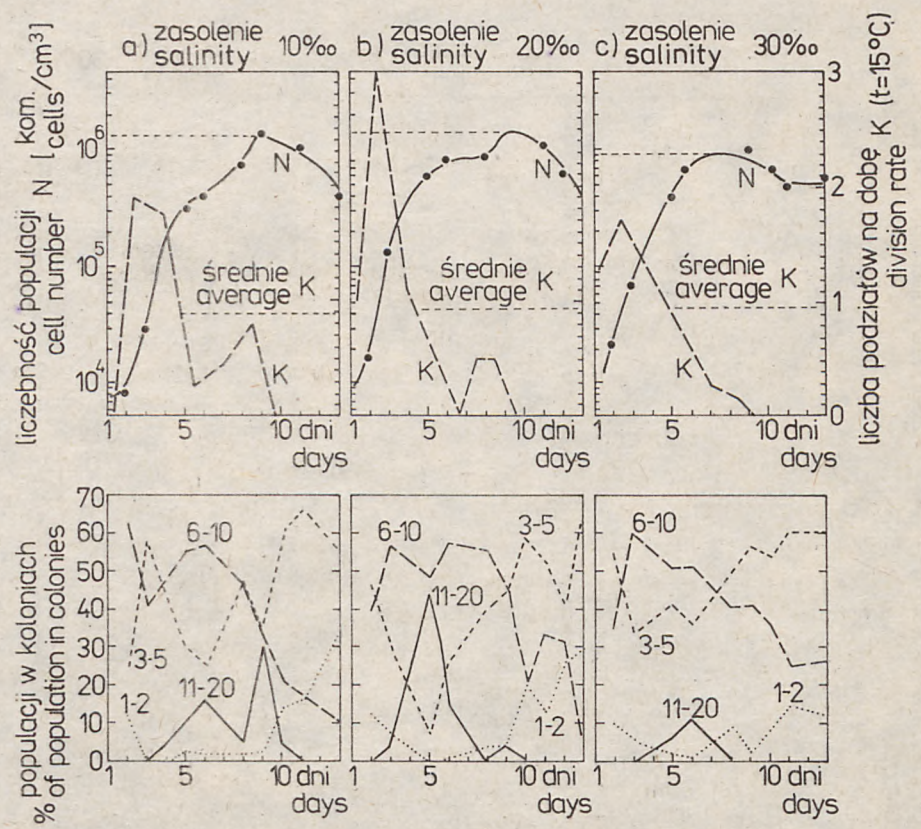

Fig. 6. Growth of *S. costatum* in different salinities

Rys. 6. Wzrost hodowli *S. costatum* w pożywkach o różnym zasoleniu

ary to transfer the culture into a fresh medium every four or five days.

Investigations on the effects of salinity on the growth rates of *S. costatum* showed them to be similar at salinities ranging between 10‰ and 30‰ (Figs. 5, 6).

Analysis of figures 1 to 6 shows, that the highest number of long chains occur during the period of active growth and at the beginning of the stationary phase. There is a certain rellationship between the value of "K", wchich denotes growth rate expressed by the number of cell divisions in 24 hours, and the number of chains consisting of more than ten cells. If the growth rate of the cultures reaches two or more divisions in 24 hours, after one or two days from this moment there is a formation of the longest chains (of more that ten cells) which constitute more than 10‰ of the whole population (Figs. 1 a, Figs. 2 a, b, Figs. 4 a, b, c, d, Fig. 5 c, Fig. 6 a, b).

4. DISCUSSION

The problem of the effect of the initial size of a population in a culture on the logarithmic growth has only been treated marginally in the literature on the methods of diatom cultures. Guillard [10] who gave a complete discription of the methods of culturing diatoms and other algae gave only approximate sizes of inoculum in cm^3. Another author [5] called attention to the quantitative fluctuations of *S. costatum* culture irrespective of the size of inoculum. However, she noted the lowest increase of cells in cultures with the least size of inoculum.

It should be added that the division of diatoms is inverse in proportion to the size of the cells. It has been calculated [32] that large diatoms more than 100—200 µm in diameter, have the slowest rates of division. At the most they divide once in 24 hours. Medium sized cells divided once in nine hours. Small diatoms, 10 µm in diameter (as *S. costatum*) had one cell division every five hours [32]. These are the highest cell divisions. Most frequently, diatoms divide slower — depending on the temperature, light and nutrient conditions [22, 23, 29, 30, 35].

This was also the case in the present study, where the number of cell divisions for various diatom species, appropriate amounts of inoculum should be chosen for each species. Inoculum may be small if we have to deal with a very small species or with a preauxosporal form. For large diatoms the inoculum may be bigger, as there is a slow increase in their cell numbers. Thus the length of the logarithmic phase of growth during which the species retains full physiological activity depends on the size of inoculum and the rate of cell division.

The results of the present studies showed that nitrates, phosphates and silicates are the most important nutrients for the growth of *S. costatum* cultured in the ASPM medium. This was found by authors who studied this [7, 29, 35] and other species of planktonic diatoms [2, 4, 10, 11].

Compared to the ASPM medium described by Guillard [10], the medium used here contains ten times smaller amounts of trace metals (except of Fe, Tab.). In spite of this, a single dose of microelements is sufficient when there is a ten-fold increase in the amount of macroelements (Tab., Figs 3 d and 4 c).

Sceletonema costatum isolated from the South Baltic Sea where the salinity is about 7—8‰ grew equally well in media with salinities of 10, 20 and 30‰. The fact that the species in abundant in the Baltic Sea contradicts the opinion of Droop [6] who suggested that *S. costatum* does not develop in waters with salinities below 10‰. According to him this species develop in water with a sodium content ranging between 3 000 mg Na and 12 000 mg Na dcm^{-1} medium (which corresponds a sal-

inity of 10—40‰). It may be that Atlantic diatoms in the Baltic Sea are well adapted to lower salinities.

The use of resting cells from old stagnant surface water to obtain diatom cultures is a new method and has not been tried by authors of studies known to me.

Fig. 7. *S. costatum* in culture (Phot. by author)

Rys. 7. *S. costatum* w hodowli (Fot. autora)

Any authors obtained planktonic diatoms from bottom sediments [8, 19, 24, 36, 37]. French and Hargraves [9] described a numerous literature which was depending of resting spores formation, survival and germination. Zgurovskaya [36] found, that some of freshwater diatom species were able to survival in bottom sediments as a vegetative forms. Umebayashi [31] preserved five diatom species for a period of 9—34 months in dark and low temperature condition. This according to a method used by myself. I preserved many diatoms from the North Sea, the shelf waters of northern France, and the vicinity of King George Island in the Antarctic in the same way. The following forms were obtained from the North Sea in October 1979: *Thalassiosira hyalina* (Grun) Cleve, *Eucampia zoodiacus* Ehr., *Sceletonema costatum*, *Chaetoceros spp*, *Thalassiosira spp*. From the shelf waters of France I received *S. costatum*, *Thalassiosira spp*, *Coscinodiscus oculus*, *Biddulphia sp.*, *Chaetoceros spp*. I was able to isolate a few species from water samples collected in Antarctic: two species of the genus *Thalassiosira*, *Chaeto-*

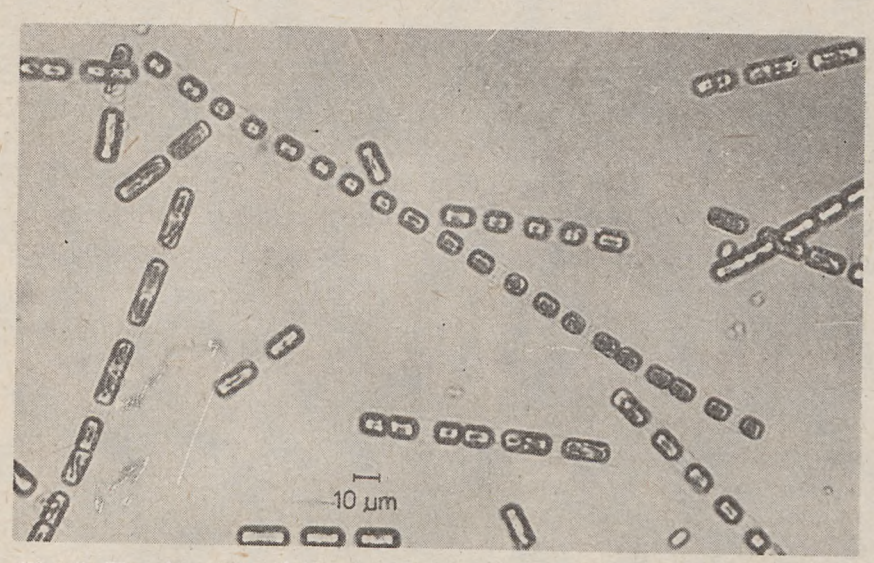

Fig. 8. The forming of the auxospore of *S. costatum* (Phot. by author)
Rys. 8. Tworzenie auksospory u *S. costatum* (Fot. autora)

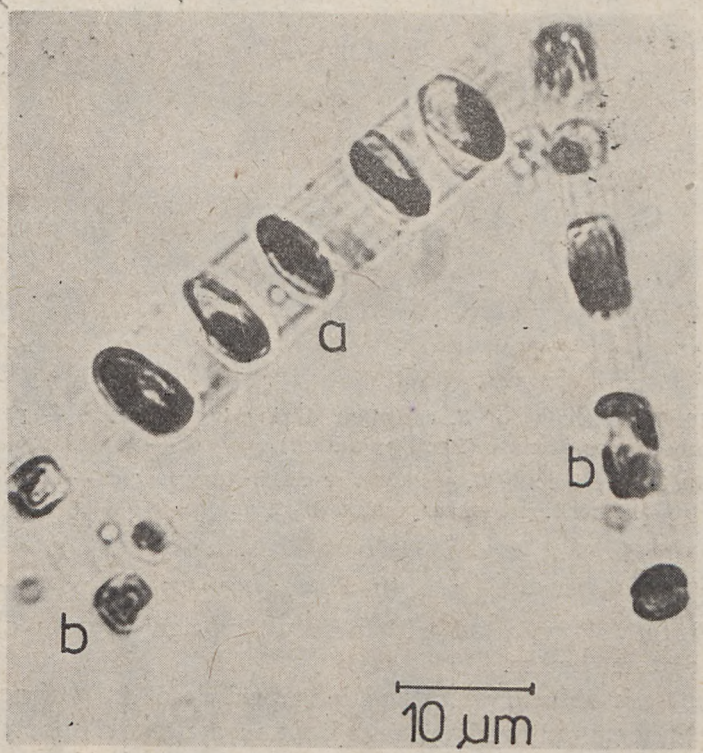

Fig. 9. Chains of *S. costatum*; *a* — postauxosporal cells, *b* — preauxosporal cells (Phot. by author)

Rys. 9. Łańcuszki *S. costatum*; *a* — komórki postauksosporalne, *b* — komórki preauksosporalne (Fot. autora)

ceros socialis Lauder and *Fragilariopsis sp.* Containers with water samples from European seas were examined seven-ten days after sampling. Thanks to this vegetative forms of diatoms were still present. Water from Antarctic did not contain vegetative forms. The barrel was kept more than a month in a ship's cold room at 10°C, without ligth and aeration. No nutrients were added. Despite this, after introducing a few cm^3 of the ASPM medium a growth of diatoms and protozoans was ap-

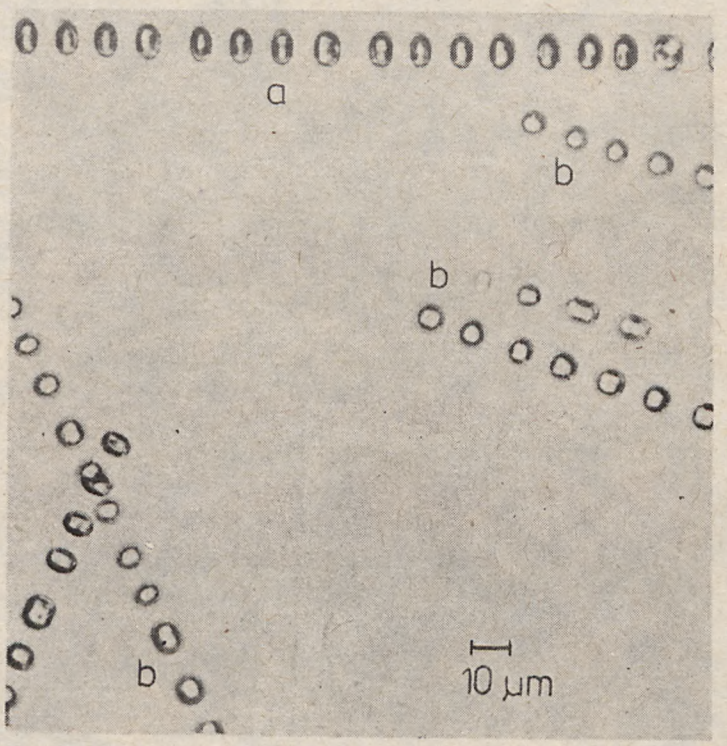

Fig. 10. Long chains of *S. costatum* during log phase; *a* — postauxosporal cells, *b* — preauxosporal cells (Phot. by author)

Rys. 10. Długie kolonie *S. costatum* w czasie fazy log; *a* — komórki postauksosporalne, *b* — komórki preauksosporalne (Fot. autora)

parent after a few days of incubation in the light. Two month later the procedure was repeated with a resultant new growth of the same Antarctic diatom species.

Diatoms are able to withstand long periods of in darkness. Heterotrophy of diatoms cultured without light in a mineral medium containing glucose or galactose has been investigated [33]. The species grew without light for a year and divided slowly, once every few days.

The described method of diatom isolation by picking up cells by means of pipettes and washing them in a medium containing antibiotics

is in common use [14, 26]. All single species clones were obtained in this way. Diatom isolation without dipping them in a solution with antibiotics did not yeld satisfactory results. Even if diatoms did grow, this growth was inhibited by bacteria which sometimes caused the death of new cultures. Longer cultured species, after passing through the period of adaptation to the artificial medium (about one — four months) in which they divided slowly were able to divide twice as fast. The present of bacteria in this case did not inhibit diatom growth.

Not many authors have paid attention to the structure of chains in diatom cultures, as has been shown in the present paper (Figs 1—6). Smayda and Boleyn [29] made observations on the sinking rates of *S. costatum* depending on the number of cells in the chain and found that the longest chains had the lowest sinking rates.

The following conclusion can be drawn from the present discussion: in favourable conditions *S. costatum* forms multicellular chains which float the longest in the water (Fig. 10). Research on species in natural conditions should include investigations on the colony structure. Conclusions as to the stage of growth of a given diatom population can be drawn of multicellular forms.

5. CONCLUSIONS

1. The size of inoculum affects the length of the logarithmic growth phase in diatom cultures. Optimal inoculum for *S. costatum* in the ASPM medium should contain no more than 10^3—10^4 cells cm^{-3} of new medium.

2. In the ASPM medium, the nutrients which give the maximal numbers of diatom cells are the macroelements nitrogen, phosphorus, and silicon. The amount of nutrients suggested here should contain 50 mg $NaNO_3$ dcm^{-3} ASPM, 5 mg K_2HPO_4 dcm^{-3} ASPM, 35 mg $Na_2SiO_3 \times 5H_2O$ dcm^{-3} ASPM and 1 cm^3 of trace metals solution per 1 dcm^3 of the medium. An addition of a greater amount of nutrients did not result in an increase in the population of *S. costatum*.

3. *S. costatum* divides in the same way in media with salinities of 10‰, 20‰ and 30‰. This confirms that the species is euryhaline.

4. At a temperature of 10°C and a continuous light of 3 000 lux the cells of the species investigated divided twice in 24 hours.

5. In favourable conditions *S. costatum* multiplies fast and forms long chains consisting of 20—30 cells. In growth limiting conditions, the colonies break into one or two cell fragments.

6. *S. costatum* probably forms resting cells. The diatom investigated during this study was obtained from detritus sedimented from unfiltered old sea water.

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HODOWLA *SCELETONEMA COSTATUM* (GREV.) CLEVE W SZTUCZNEJ WODZIE MORSKIEJ

Streszczenie

Sceletonema costatum (Grev.) Cleve jest centryczną okrzemką charakterystyczną dla przybrzeżnych wód morskich [3, 5, 16, 27].

Gatunek ten izolowano z osadu starej bałtyckiej wody morskiej. Kilka cm^3 detritusu przeniesiono do sztucznej wody morskiej ASPM (tab.) [10], w ten sposób otrzymano hodowlę wielogatunkową. W celu otrzymania jednogatunkowych szczepów izolowano pojedyncze kolonie *S. costatum*.

Przedmiotem badań były wielkość inoculum (rys. 1), zawartość biogenów (N, P, Si, mikroelementów), (rys. 2, 3, 4) i zasolenie (rys. 5, 6). Stwierdzono, że wielkość inoculum może wpływać na długość trwania fazy logarytmicznego wzrostu (rys. 1). Optymalne inoculum nie powinno przekraczać liczby 10^3—10^4 kom. cm^{-3} nowej pożywki. Najważniejszymi biogenami w pożywce ASPM są ortofosforan, azotan i metakrzemian (tab.). Wykazano, że optymalne koncentracje tych biogenów powinny wynosić 50 mg $NaNO_3 \times dcm^{-3}$, 5 mg $K_2HPO_4 \times dcm^{-3}$ i 35 mg $Na_2SiO_3 \times 5H_2O \times dcm^{-3}$ ASPM (rys. 3b) i 1 cm^3 roztworu mikroelementów na 1 dcm^3 ASPM (tab., rys. 3). Wyższe koncentracje tych biogenów nie powodowały wyraźnego wzrostu liczby komórek (rys. 3 c, d, rys. 4 d). *S. costatum* rozmnaża się tak samo przy zasoleniu 10, 20 i 30‰ (rys. 5, 6).

Omawiany gatunek może się rozmnażać szybko, ponad dwa razy na dobę w 10°C i przy stałym oświetleniu ok. 3 000 lux. Podczas fazy log okrzemka tworzy wielokomórkowe łańcuchy (ok. 10—30-komórkowe). Podczas fazy stacjonarnej i starzenia kolonie rozpadają się na krótkie odcinki (1—2-komórkowe).

Sceletonema costatum prawdopodobnie tworzy komórki spoczynkowe. To może tłumaczyć utrzymanie się przy życiu komórek tego gatunku przez pół roku w osadzie starej wody morskiej, stojącej w cieniu, bez napowietrzania. Po wprowadzeniu osadu do pożywki otrzymano wzrost wielogatunkowej hodowli okrzemek po kilku dniach.

Opisano przygotowanie pożywki ASPM opartej na metodzie Guillarda [10] i innych autorów [21, 25].

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