

Culture Collection of Baltic Algae (CCBA) and characteristic of some strains by factorial experiment approach

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With 7 figures and 9 tables in the text

Abstract: Culture Collection of Baltic Algae (CCBA), founded in the 1980s, has a university status. It puts emphasis on Baltic cyanobacteria, green algae and diatoms. Now it contains about forty original algal strains isolated from the coastal waters of the Southern Baltic. Curator of CCBA is Dr. ADAM LATAŁA, e-mail address: oceal@univ.gda.pl. The complete list of original Baltic strains and strains acquired from other collections, which are maintained in CCBA, will appear on the web site: <http://www.ocean.univ.gda.pl/~ccba/>.

At present there is no significant algal culture collection in Poland. Therefore, an enrichment of CCBA with new algal strains isolated from different inland waters in Poland is planned. Special attention would be paid to algal species threatened by extinction, rare, endemic, toxic, harmful or typical of our region.

The growth of Baltic strains has been characterized by factorial experiment approach. The effects of salinity, light and temperature, as well as their interaction have been determined. The brackishwater character showed: *Chlorella vulgaris*, *Oocystis submarina*, *O. parva*, *Stichococcus bacillaris*, *Cyclotella meneghiniana*, *Skeletonema costatum*, and *Nodularia spumigena*, cyanobacterium *Phormidium amphibium* was the marine strain and green algae from the genus *Scenedesmus* and *Monoraphidium* were freshwater strains. The growth of the above strains was strongly affected by light and temperature interaction. This effect was especially distinct in diatoms. However, the influence on the light alone was not so well-marked.

For six strains: *Monoraphidium convolutum*, *Amphora coffeaeformis*, *Entomoneis punctulata*, *Haslea spicula*, *Nitzschia thermaloides* and the second strain of *Skeletonema costatum* only influence of salinity was investigated. It occurred that green alga was a typically freshwater species, *Skeletonema costatum* showed marine character, however the remaining strains were brackishwater.

Introduction

As a result of anthropogenic action the aquatic and terrestrial flora and fauna suffer serious quantitative and qualitative changes. Negative human intervention in natural environment is manifested, among others, by threat of extinction of many

species. Therefore, the investigations on biodiversity of biological communities are still increasing. They concern mainly bacteria and fungi because of their medical and industrial uses. Although microalgae, being the main primary producers of organic matter, play an important role in water ecosystems, the studies on these organisms and their commercial exploitation are rather scarce. However, it should be underlined that the amounts of biomass produced in one year by algae and higher plants inhabiting terrestrial ecosystems are comparable (ANDERSEN 1996). Algae and cyanobacteria form an extremely diverse group of organisms. Unfortunately, they are still insufficiently recognized. Microscopic dimensions, poor species concepts, necessity of cultivation as well as molecular and ultrastructural investigations are a few reasons for this lack of knowledge. The collections of algal cultures are very important in microbiology and phycology since they are a source of reliable material used in environmental studies, education or biotechnology. Being specific repositories for living organisms, culture collections of algae and cyanobacteria have promoted phycological research since many decades. Furthermore, the changes in environment reduced the diversity of natural algal flora and therefore, there is an absolute requirement for the *ex situ* conservation of microalgal cultures in European countries.

The Culture Collection of Baltic Algae (CCBA) is housed at the Institute of Oceanography and has a university status. The main objective of the CCBA is to isolate, maintain and supply microalgal strains for use in research, teaching and biotechnology (LATAŁA & SUROSZ 1998, 1999, LATAŁA et al. 2005). At present there is no significant algal culture collection in Poland. Therefore, it is planned to enrich CCBA with new algal strains isolated from Baltic and different inland waters in Poland. Special attention would be paid to algal species threatened by extinction, rare, endemic, toxic, harmful or typical of our region. It will extend the knowledge of algal biodiversity and help to preserve the microbial and biological resources of the region. The above task would be realized basing on Centre of Excellence for Baltic Development, Education and Research (BALTDER) in co-operation with the outstanding European collections of algae.

The objective of this paper is a short presentation of the CCBA at the Institute of Oceanography, University of Gdańsk and an autecological characteristic of some CCBA strains by factorial experiment approach. The investigations were carried out on microalgal strains which are characteristic since they are currently present in the coastal waters of the Gulf of Gdańsk.

Material and methods

The CCBA cultures are unialgal. They are free from fungal contamination. Microscopic observations did not show bacterial contamination in the media. Since the cultures are maintained in sterile conditions, it could be assumed that any bacteria present in the cultures may be bounded to algal cell wall.

The strains are maintained in liquid media or on agar slants (STEIN 1979). The majority of strains grow in liquid f/2 medium (GUILLARD 1975, MCLACHLAN 1979) prepared on the base of natural Baltic water, salinity 7–8 PSU. Cyanobacteria are cultivated in BG-11 medium (STANIER et al. 1971). The cultures are kept at 18°C, intensity of photosynthetic

active radiation (PAR) of about 5–10 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and photoperiod L:D 16:8. The source of light are fluorescent lamps, Sylvania cool-white 40 W. The serial transfers of agar and liquid cultures into the fresh media vary from three weeks to two months.

Algae and cyanobacteria maintained in CCBA were isolated from the natural samples of Baltic water collected at the stations situated along the coast of the Gulf of Gdańsk. For isolation purposes, dilution and single cell isolation methods were used. Unialgal cultures were established using well-known methods like washing with microcapillary pipette, streak and spray plating (HOSHAW & ROSOWSKI 1979). All strains were isolated and identified by A. LATAŁA. The identification of some taxonomically difficult strains was verified by the specialists: cyanobacteria and green algae – FATIMA SANTOS and FRANTIŠEK HINDÁK, diatoms BOŽENA BOGACZEWCZ-ADAMCZAK and ALEKSANDRA ZGRUNDO.

The effects of PAR, temperature and salinity on the growth of nine strains have been determined by factorial experiment approach. The experiments were performed on well growing collection species, which are essential elements of microalgae assemblages in the coastal waters of the Gulf of Gdańsk. The following strains were examined: green algae *Chlorella vulgaris* BEIJERINCK – BA-2 (LA-1/685); *Scenedesmus armatus* var. *subalternans* G. M. SMITH – BA-6 (LA-6/685); *Oocystis submarina* LAGERHEIM – BA-1 (AL-2/682); *Monoraphidium contortum* (THURET) KOMÁRKOVÁ-LEGNEROVÁ – BA-5 (AL-5/587); *Stichococcus bacillaris* NÄGELI – BA-9 (AL-9/682); diatoms *Cyclotella meneghiniana* KÜTZING – BA-10 (AL-10/586); *Skeletonema costatum* (GREVILLE) CLEVE – BA-11 (LA-11/493); cyanobacteria *Phormidium amphibium* (AG. ex. GOM.) ANAGNOSTIDIS et KOMÁREK – BA-13 (AL-13/994); *Nodularia spumigena* MERT. in JÜRGENS – BA-15 (AL-15/999).

In four green algae species: *Scenedesmus acutus* MEYEN – BA-8 (AL-8/487); *Scenedesmus acuminatus* (LAGERHEIM) CHODAT – BA-7 (LA-7/586); *Oocystis parva* W. et G. S. WEST – BA-3 (AL-3/487) and *Monoraphidium griffithii* (BERKELEY) KOMÁRKOVÁ-LEGNEROVÁ – BA-4 (AL-4/586) the influence of light, temperature and salinity on their growth was only determined. For one strain of green alga *Monoraphidium convolutum* (CORDA) KOMÁRKOVÁ-LEGNEROVÁ BA-17 and five diatoms *Amphora coffeaeformis* (C. A. AG.) KÜTZING – BA-16, *Entomoneis punctulata* W. SMITH BA-39, *Haslea spicula* (HICKIE) LANGE-BERTALOT – BA-28, *Nitzschia thermaloides* HUSTEDT BA-14 and *Skeletonema costatum* (GREVILLE) CLEVE BA-98 influence of salinity was only investigated.

The batch cultures (LEE & SHEN 2004) were carried out in 150 cm^3 of f/2 or BG-11 medium in 300 cm^3 glass Erlenmeyer flasks. The incubation vessels were kept in thermostats equipped with fluorescent lighting. The strains were incubated under a 16:8 h L:D cycle. The source of light were fluorescent lamps (Sylvania cool-white 40 W) and, to achieve higher light intensity, combined with halogen lamps (Sylvania 100 W). Light intensity was adjusted with neutral filters. The intensity of photosynthetic active radiation (PAR) was measured using a quantum-meter Li Cor (LI-189) with a cosine collector. The required salinities of the media were obtained by dilution of Atlantic water (37 PSU) with tap water. Then the water was filtered through GF/C Whatman glass filters and kept in darkness in plastic containers. The population density was measured after two-weeks' incubation. The number of cells or filament units were determined microscopically in Fuchs-Rosenthal and Bürker chambers. The effects of PAR (from 10–40 to 160–200 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), temperature (from 8–15 to 25–38°C) and salinity (0–35 PSU) on the growth of strains have been examined.

In factorial experiment method the values of the independent variables occur at the same intervals. Moreover, replicate number is always the same, in our investigations all experimental variants were run in triplicate. It enables calculations by creating polynomials that are mutually orthogonal. The method makes possible to determine the influence of the investigated factors on algal growth by calculation of regression equation. The fitting of the polynomial by the method ξ' (ksi prim) (SNEDECOR & COCHRAN 1967, OKTABÁ 1986) was simplified by the use of tables of orthogonal polynomials (FISHER & YATES 1963). The calculations were made by original computer programme FACT using the results of cell number (per 1 cm^3 of the medium) after 14th day of cultivation. The results have been presented graphically as response surface form using calculated regression equation and computer programme SURFER. Two-way analysis of variance ANOVA was

used to assess the main effects and their interaction (BRZEZIŃSKI & STACHOWSKI 1981, OKTABA 1986).

Results

Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk was created in the 1980s. Curator: Dr. ADAM LATAŁA, e-mail address: oceal@univ.gda.pl. Now CCBA holdings are mainly Baltic cyanobacteria, green algae and diatoms, although there are representatives of the other algal groups, like flagellates. They are mainly used by national institutions for scientific (LATAŁA 1991, LATAŁA et al. 1991, LATAŁA & SUROSZ 1998, 1999, LATAŁA & MISIEWICZ 2000) and educational purposes.

CCBA has been gradually developed and now it contains about forty original Baltic algal strains. Future plans include purification of existing strains to axenic state and collaboration with other collections and taxonomic experts to verify the taxonomy of new isolates. Moreover, the algal test organisms recommended by the International Organization for Standardization (ISO) or the European Committee for Standardization (CEN) are cultured and supplied for research and analysis of water quality by biological methods.

The complete list of original Baltic strains identified to species level and their microscopic images and strains acquired from other collections, which are maintained in CCBA, is being prepared and it will appear in 2006 on the web site, at Institute of Oceanography homepage <http://www.ocean.univ.gda.pl/~ccba/>. Besides the actual strains numbers, some strains were also marked with the numbers previously used in articles, which are placed in parentheses.

The effect of such main factors as salinity, temperature and irradiance on the growth of some strains isolated from the Baltic Sea has been characterized by factorial experiment approach. Some selected data to characterize their properties are presented below.

3.1 Green algae

Chlorella vulgaris BEIJERINCK – BA-2 (LA-1/685), unicellular.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 7–8 PSU (Fig. 7a), good growth between 0 and 24 PSU, no growth at 35 PSU.

Temperature: optimum temperature 26–28°C (Fig. 1), growth from 14°C, no growth at 5–10°C.

Light: optimum PAR intensity 160–180 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At 120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ better growth in photoperiod than at continuous illumination.

The results of variance analysis (Tab. 1) show that about 57 % of total sum of squares is accounted for by light, while the influence of temperature and interaction of main factors reached lower values, 30 % and 13 % respectively.

Scenedesmus armatus var. *subalternans* G.M. SMITH – BA-6 (LA-6/685), mainly 2- and 4-celled coenobia.

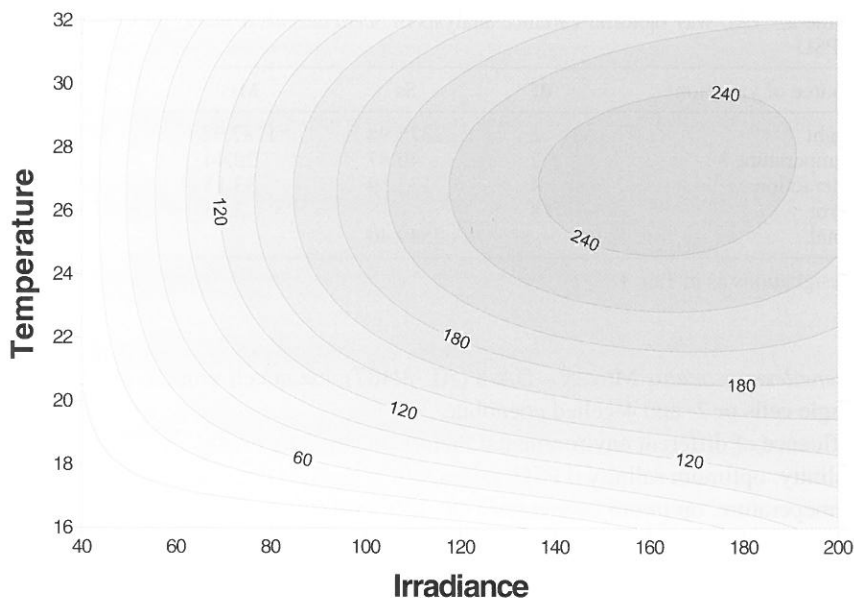


Fig. 1. Population density of *Chlorella vulgaris* [N. 100000 per ml] at different temperature [°C] and irradiance (PAR) [$\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$] and at 8 PSU.

Influence of different environmental factors on strain's growth:

Salinity: the best growth at 0 PSU, 15 PSU is upper growth limit, no growth at 20 PSU (Fig. 7i).

Temperature: optimum temperature 24–26°C, very weak growth at 5°C, better from 10°C.

Light: optimum PAR intensity 160–200 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At 120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ better growth in photoperiod than at continuous illumination.

The results of variance analysis (Tab. 2) show that above 93% of total sum of squares is accounted for by light sum of squares, while the influence of temperature and interaction of the main factors, although could be accepted at 0.05 significance level, reached very low values, 1.6% and 5.2% respectively. Thus, light is the most important factor contributing to the observed variation in this green alga growth.

Table 1. Two-way factorial variance analysis of *Chlorella vulgaris* growing at 8 PSU

Source of variation	df	Ss	Mss	F values
Light	2	122890.13	61445.06	239.05*
Temperature	2	64740.07	32370.04	125.94*
Interaction	4	28166.88	7041.72	27.40*
Error	18		257.04	
Total		215797.08		

df – degrees of freedom, Ss – Sum of squares, Mss – Mean square, F – Fisher F-test statistic, * – significance at 0.05

Table 2. Two-way factorial variance analysis of *Scenedesmus armatus* growing at 0 PSU

Source of variation	df	Ss	Mss	F values
Light	2	2375.94	1187.97	389.06*
Temperature	2	40.87	20.44	6.69*
Interaction	4	132.59	33.15	10.86*
Error	18		3.05	
Total		2549.40		

Designations as in Tab. 1.

Scenedesmus acutus MEYEN – BA-8 (AL-8/487), mean cell volume 50–75 μm^3 , single cells or 2- and 4-celled coenobia.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 0 PSU, no growth at 8 PSU (Fig. 7h).

Temperature: optimum temperature 18–22°C, weak growth at 5°C, no growth at 38°C.

Light: optimum PAR intensity 120–160 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At 120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ better growth in photoperiod than at continuous illumination.

Scenedesmus acuminatus (LAGERHEIM) CHODAT – BA-7 (LA-7/586), mean cell volume about 230 μm^3 , single cells or 2- and 4-celled coenobia.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 0 PSU, good growth at 2.5 PSU, no growth at 15 PSU (Fig. 7g).

Temperature: optimum temperature 34°C, very good growth at 22–30°C, good growth at 10°C and 38°C, no growth at 5°C.

Light: optimum PAR 80 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At 120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ better growth in photoperiod than at continuous illumination.

Oocystis submarina LAGERHEIM – BA-1 (AL-2/682), mean cell volume 270 μm^3 , single cells or coenobia consisted of some cells surrounded by the wall of the mother cell.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 11.5–15 PSU, weaker growth in the range of 0–35 PSU (Fig. 7f).

Temperature: optimum temperature 23–24°C (Fig. 2), very weak growth at 5°C, no growth at 38°C.

Light: optimum PAR intensity 120–140 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At 120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ better growth at continuous illumination than in photoperiod.

Above 33% of total sum of squares is accounted for by interaction of main factors sum of squares (Tab. 3). 34% and 32% are accounted for light and temperature, respectively.

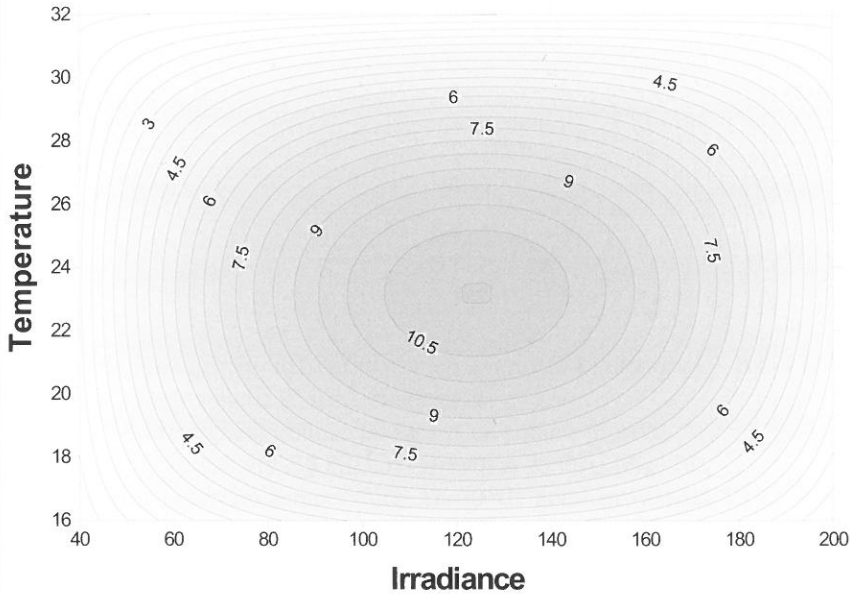


Fig. 2. Population density of *Oocystis submarina* [N. 100000 per ml] at different temperature [°C] and irradiance (PAR) [$\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$] and at 0 PSU.

Oocystis parva W. et G.S. WEST – BA-3 (AL-3/487), mean cell volume $100 \mu\text{m}^3$, single cells, sometimes 2- or 3-celled coenobia.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 7,5 PSU, very weak growth at 0 PSU, no growth at 30 PSU (Fig. 7e).

Temperature: optimum temperature 18°C , weak growth at 5 and 34°C , no growth at 38°C .

Light: optimum PAR intensity $80 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At $120 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ better growth in photoperiod than at continuous illumination.

Monoraphidium contortum (THURET) KOMÁRKOVÁ-LEGNEROVÁ – BA-5 (AL-5/587), mean cell volume $35 \mu\text{m}^3$, single cells.

Table 3. Two-way factorial variance analysis of *Oocystis submarina* growing at 0 PSU

Source of variation	df	Ss	Mss	F values
Light	2	97.39	48.70	110.55*
Temperature	2	92.73	46.37	105.27*
Interaction	4	94.76	23.69	53.78*
Error	18		0.44	
Total		284.88		

Designations as in Tab. 1.

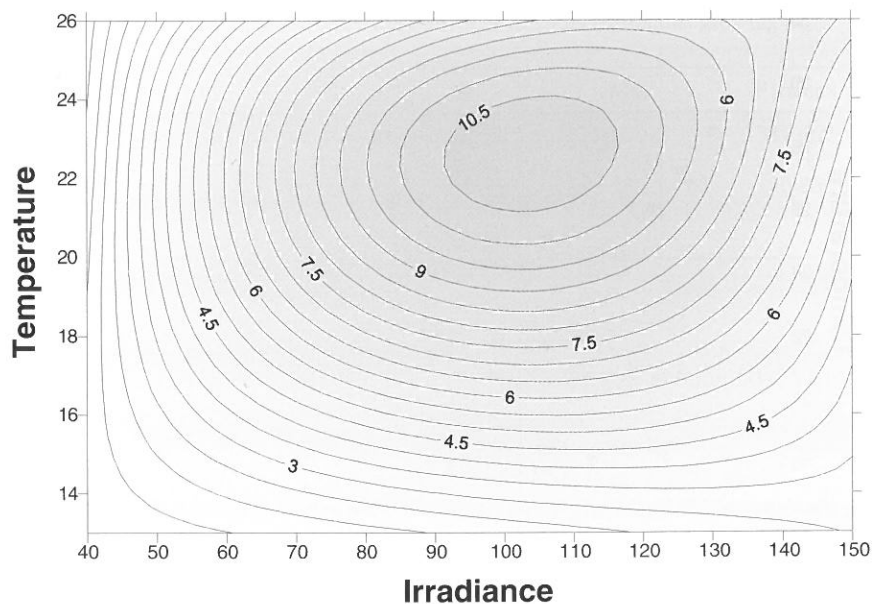


Fig. 3. Population density of *Monoraphidium contortum* [N. 1000000 per ml] at different temperature [°C] and irradiance (PAR) [$\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$] and at 0 PSU.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 0 PSU, good growth at 11.5 PSU, very weak growth at 15 PSU (Fig. 7b).

Temperature: optimum temperature 22–23°C (Fig. 3), good growth at 14–24°C, no growth at 34–38°C.

Light: optimum PAR intensity 100–110 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, photoperiod does not affect the growth rate.

41% of total sum of squares is accounted for by light sum of squares (Tab. 4). 33% and 26% were accounted for interaction of the main factors and temperature, respectively.

Table 4 Two-way factorial variance analysis of *Monoraphidium contortum* growing at 0 PSU

Source of variation	df	Ss	Mss	F values
Light	3	92.02	30.67	93.41*
Temperature	2	147.08	73.54	223.93*
Interaction	6	118.62	19.77	60.20*
Error	24		0.33	
Total		357.73		

Designations as in Tab. 1.

Table 5. Two-way factorial variance analysis of *Stichococcus bacillaris* growing at 24 PSU

Source of variation	df	Ss	Mss	F values
Light	2	22.12	11.06	305.12*
Temperature	2	83.21	41.60	1147.98*
Interaction	4	63.92	15.98	440.96*
Error	18		0.04	
Total		169.25		

Designations as in Tab. 1.

Monoraphidium griffithii (BERKELEY) KOMÁRKOVÁ-LEGNEROVÁ – BA-4 (AL-4/586), mean cell volume 95 μm^3 , single cells.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 2.5 PSU, no growth at 25 PSU (Fig. 7d).

Temperature: optimum temperature 22–30°C, good growth at 18°C, weak growth at 10°C, no growth at 5 and 38°C.

Light: optimum PAR intensity 150–250 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Stichococcus bacillaris NÄGELI – BA-9 (AL-9/682), mean cell volume 38–43 μm^3 , single cells, exceptionally 2- or 3-celled forms.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 7.35 PSU, good growth in the range of 0–25 PSU, very weak growth at 30–35 PSU (Fig. 7j).

Temperature: Optimum temperature 23–25°C (Fig. 4), weak growth at 5–10°C, no growth at 34–38°C.

Light: optimum PAR intensity 120–140 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At 120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ better growth in photoperiod than at continuous illumination.

The influence of temperature on growth was considerably higher, which was reflected in the results of variance analysis (Tab. 5). About 49% of total sum of squares is accounted for by temperature sum of squares. 37% and 13% were accounted for interaction of the main factors and light, respectively.

Monoraphidium convolutum (CORDA) KOMÁRKOVÁ-LEGNEROVÁ BA-17 was the only one strain of green algae that only salinity was investigated. The best growth was observed in fresh water (Fig. 7c). In the range 8–16 PSU growth was weaker and no growth was observed at highest salinities.

Diatoms

In CCBA diatoms are represented, among other, by the following strains:

Cyclotella meneghiniana KÜTZING – BA-10 (AL-10/586), cell volume 2000–7600 μm^3 , single cells.

Influence of different environmental factors on strain's growth:

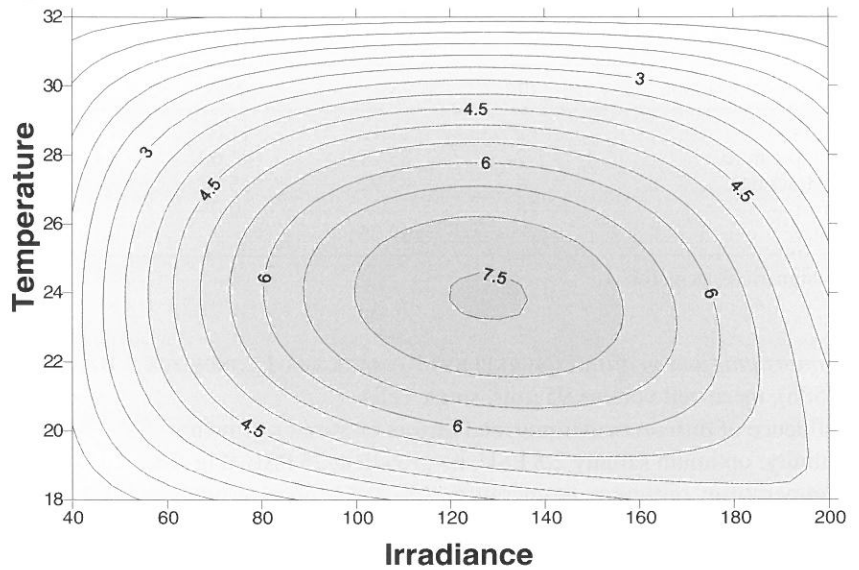


Fig. 4. Population density of *Stichococcus bacillaris* [N. 1000000 per ml] at different temperature [°C] and irradiance (PAR) [$\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$] and at 24 PSU.

Salinity: optimum salinity 0–16 PSU, weaker growth to 24 PSU, no growth at 32 PSU (Fig. 7l).

Temperature: optimum temperature 26–30°C, good growth 18–34°C, no growth at 38°C

Light: optimum PAR 80–120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

The results of variance analysis (Tab. 6) show that about 55 % of total sum of squares is accounted for by interaction of main factors, while the influence of temperature and light reached 32 % and 13.5 %, respectively.

Skeletonema costatum (GREVILLE) CLEVE – BA-11 (LA-11/493), short chains of cells.

Influence of different environmental factors on strain's growth:

Salinity: good growth in the range of salinity 8–24 PSU, no growth at 0 and 32 PSU (Fig. 7p).

Table 6. Two-way factorial variance analysis of *Cyclotella meneghiniana* growing at 8 PSU

Source of variation	df	Ss	Mss	F values
Light	2	43458801664	21729400832	11.40*
Temperature	3	102744064000	34248022016	17.97*
Interaction	6	176134668290	29355778048	15.40*
Error	24		1905862016	
Total		322337538050		

Designations as in Tab. 1.

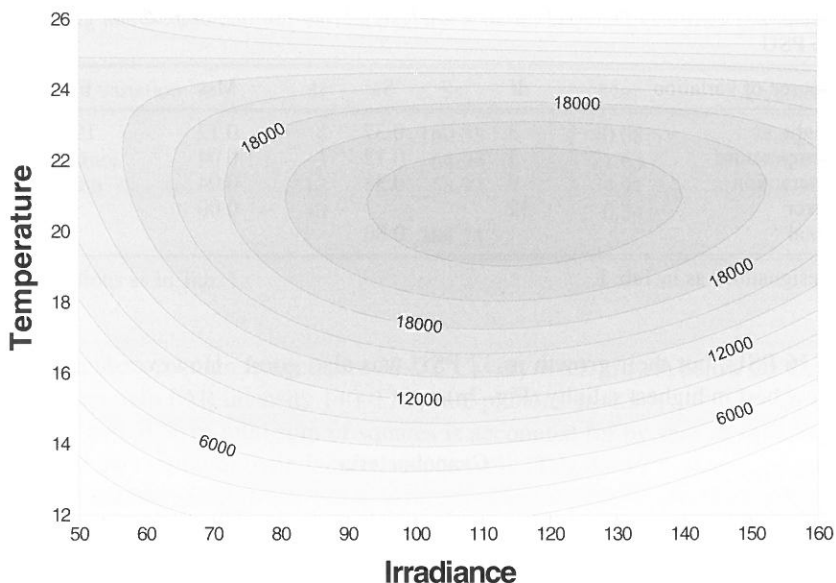


Fig. 5. Population density of *Skeletonema costatum* – BA-11 [N. 100 per ml] at different temperature [°C] and irradiance (PAR) [$\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$] and at 24 PSU.

Temperature: optimum temperature 20–22°C (Fig. 5), good growth at 8–26°C.

Light: optimum PAR intensity 90–120 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Only 3% of total sum of squares is accounted for by light sum of squares (Tab. 7). 57% and 39% were accounted for temperature and interaction of the main factors, respectively.

For another five strains of diatoms *Amphora coffeaeformis* (C.A.AG.) KÜTZING – BA-16, *Haslea spicula* (HICKIE) LANGE-BERTALOT – BA-28, *Entomoneis punctulata* W. SMITH – BA-39, *Nitzschia thermaloides* HUSTEDT – BA-14 and *Skeletonema costatum* (GREVILLE) CLEVE – BA-98 only salinity influence on their growth was investigated. In all strains, no growth was observed in fresh water. The best growth of four strains *A. coffeaeformis* (Fig. 7k), *H. spicula* (Fig. 7n.), *E. punctulata* (Fig. 7m) and *N. thermaloides* (Fig. 7o) was observed in range

Table 7. Two-way factorial variance analysis of *Skeletonema costatum* (BA-11) growing at 24 PSU

Source of variation	df	Ss	Mss	F values
Light	2	89581864	44790932	19.46*
Temperature	3	1219380224	406460064	176.55*
Interaction	6	835960256	139326704	60.52*
Error	24		2302175.75	
Total		2144922368		

Designations as in Tab. 1.

Table 8. Two-way factorial variance analysis of *Phormidium amphibium* growing at 26 PSU

Source of variation	df	Ss	Mss	F values
Light	3	0.37	0.12	1912.59*
Temperature	3	0.12	0.04	602.73*
Interaction	9	0.38	0.04	665.34*
Error	32		0.00	
Total		0.86		

Designations as in Tab. 1.

6–16 PSU, but their growth in 32 PSU was also good. However *S. costatum* grew best in highest salinity (Fig. 7p).

Cyanobacteria

In our collection cyanobacteria are the smallest group. They are represented by the following strains:

Phormidium amphibium (AG. ex. GOM.) ANAGNOSTIDIS et KOMÁREK – BA-13 (AL-13/994), thin filaments of different length.

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 26–32 PSU, no growth at 0 PSU, very weak growth at 2 PSU (Fig. 7s).

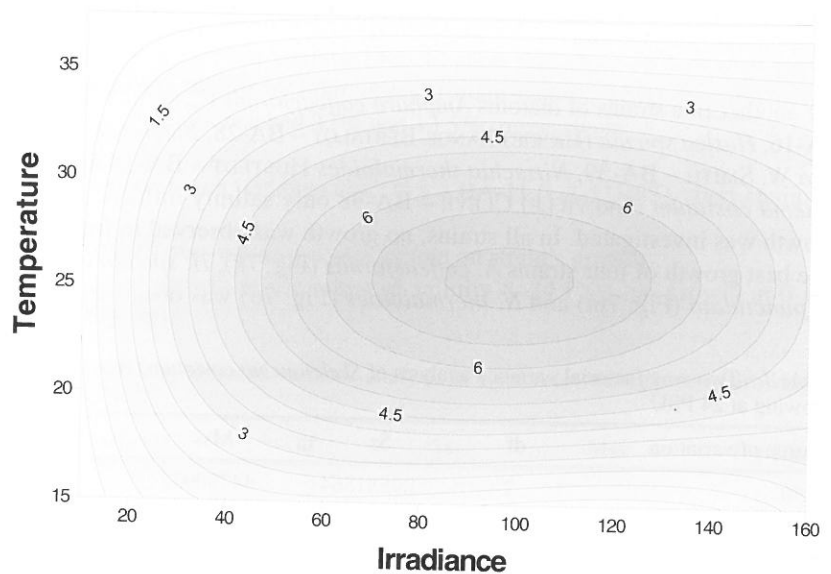


Fig. 6. Population density of *Nodularia spumigena* [N. 1000000 per ml] at different temperature [°C] and irradiance (PAR) [$\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$] and at 16 PSU.

Table 9. Two-way factorial variance analysis of *Nodularia spumigena* growing at 16 PSU

Source of variation	df	Ss	Mss	F values
Light	4	160.33	40.08	74.42*
Temperature	3	64.94	21.65	40.19*
Interaction	12	58.97	4.91	9.12*
Error	40		0.54	
Total		284.24		

Designations as in Tab. 1.

Temperature: optimum temperature 30–32°C, very weak growth below 20°C.

Light: optimum PAR intensity 140–170 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

34% and 48% of total sum of squares is accounted for by sum of squares of light and interaction of main factors, respectively (Tab. 8). 17% were accounted for temperature.

Nodularia spumigena MERT. in JÜRGENS – BA-15 (AL-15/999), trichome width 7.4 to 9.4 μm , mean distance between heterocytes 50 μm .

Influence of different environmental factors on strain's growth:

Salinity: optimum salinity 8–16 PSU, relatively good growth from 0 PSU to 32 PSU (Fig. 7r).

Temperature: optimum temperature 25–26°C (Fig. 6), weak growth at 7.5°C, growth stops almost completely at 37.5°C

Light: optimum PAR intensity 90–110 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

About 56% of total sum of squares is accounted for by temperature sum of squares, while 23% and 20% were accounted for light and interaction of the main factors, respectively (Tab. 9).

Discussion

It is planned to enrich CCBA with new strains isolated from Baltic waters as well as from different freshwater bodies. Efforts must be done to isolate and maintain various strains originating from different parts of the country. Thus, CCBA will help to investigate biodiversity of aquatic environment in Poland and through isolation, collection and maintenance of new strains it could contribute to protect microalgae species threatened by extinction. The other important task of CCBA is to determine autecology of the algal strains. A close examination of the effect of such factors as salinity, light or temperature on the growth of strains isolated from different environments will increase possibilities to use algae as bioindicators of water environment.

The chlorococcal green algae are not common in marine environment but they can dominate the phytoplankton of enclosed estuaries or lagoons, especially in summer and fall (LEVINTON 2001). In the Southern Baltic green algae are the per-

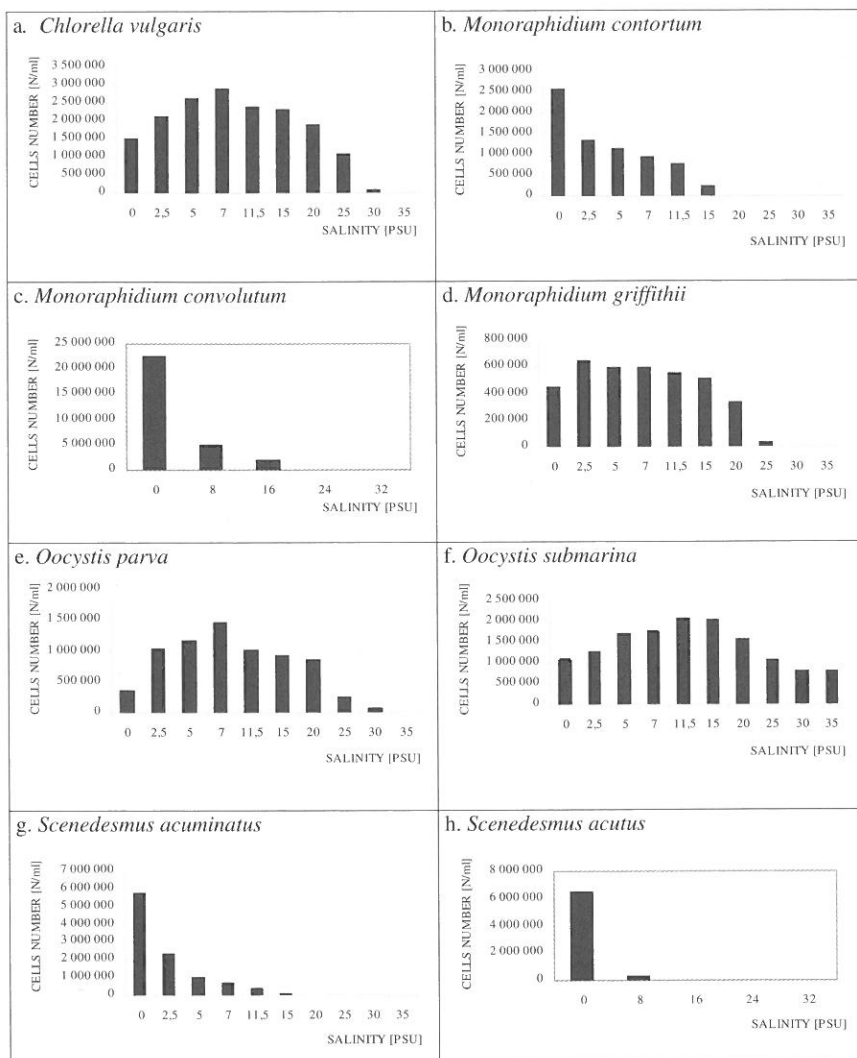


Fig. 7. Effect of salinity on population density of: green algae (a–j), diatoms (k–p) and blue-green algae (r–s).

manent component of phytoplankton, especially in late spring and summer (PLIŃSKI 1995). However, they never reach high density and do not create blooms. Many of them are freshwater species tolerant of low salinity (7–8 PSU). However, typical brackish water or even marine species could also be found. The optimum salinity values for the growth of planktic green algae indicate that a lot of the Baltic strains examined preferred fresh waters. At higher salinities (a dozen or so PSU) their growth was totally arrested. The species from the genus *Scenedesmus* and *Monoraphidium* were freshwater strains. It corroborates the

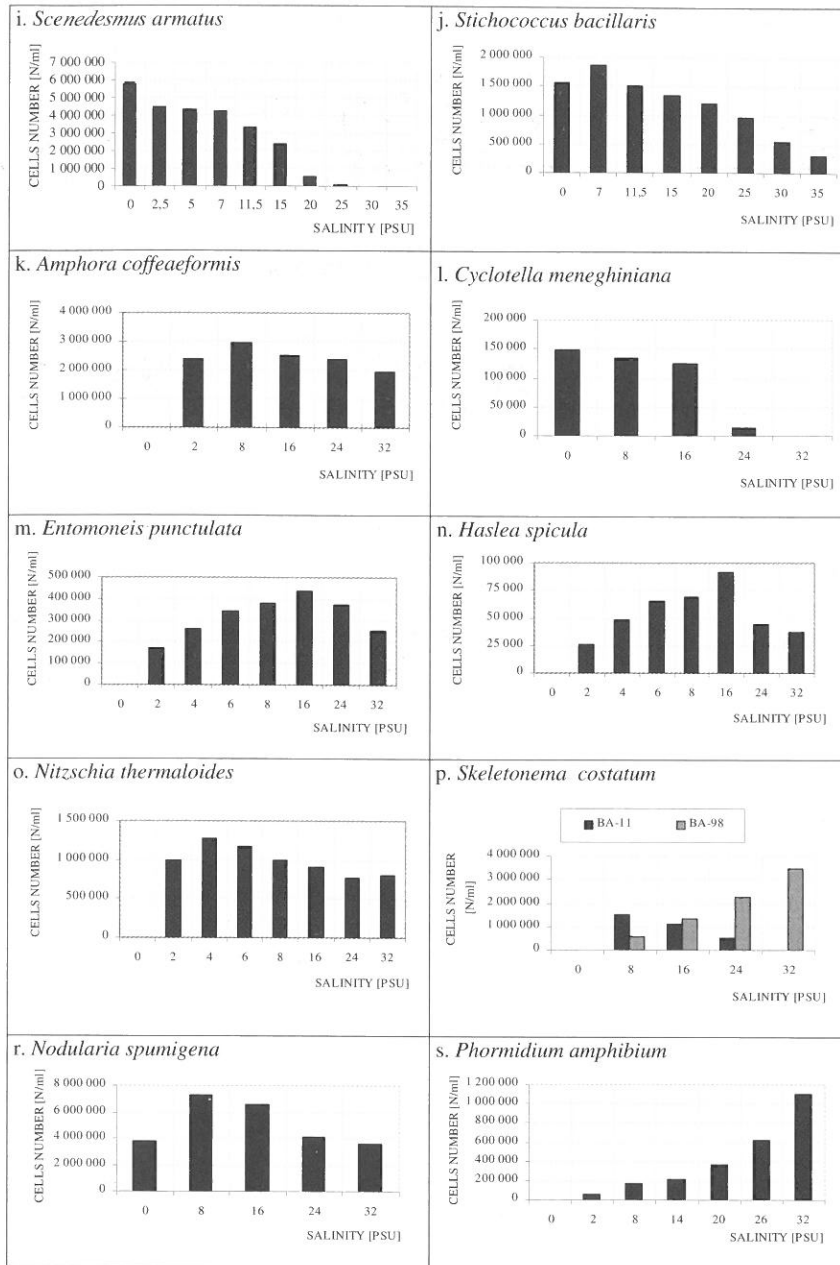


Fig. 7. (Continued)

statement that in the Southern Baltic the majority of planktic green algae are freshwater species (LATAŁA 1991). *C. vulgaris*, *O. parva* and *S. bacillaris* displayed brackishwater character. Their growth optimum was noted at 7–8 PSU, which is typical salinity of the Gulf of Gdańsk. In *O. submarina* growth optimum was observed even at higher salinity range, 11.5–15 PSU, and it was the only one species tolerant of high salinity (35 PSU).

The diatoms are the most species-rich group of algae and play an essential role in global primary production in marine environment and biogeochemical cycles. In a temperate-boreal zone, usually in the early spring, phytoplankton dominated by diatom species is characterized by a dramatic increase in density (LEVINTON 2001). In the Southern Baltic diatoms are the main element of spring and autumn phytoplankton blooms (PLIŃSKI 1995). However, because of difficulties in cultivation and long-term maintenance, diatoms are not numerous represented in algal culture collections. The examined strains of diatoms are brackishwater species. The best growth of *C. meneghiniana* was found in fresh or low salinity waters. Higher salinity, typical of marine environment, strongly reduces the growth of the above species. In the Baltic Sea *C. meneghiniana* is very frequently noted only in waters, where salinity does not exceed ~ 10 PSU (SNOEIJIS 1993). The diatom, *S. costatum*, occurs in many seas and open oceanic waters. It is of cosmopolitan nature (DRING 1998). In spring it is also found all over the Baltic Sea (SNOEIJIS 1993). Its ecophysiological demands are highly differentiated and genetically fixed but morphological characters are stable (GALLAGHER 1982). Both Baltic strains of this species do not grow in fresh water. One of them, *S. costatum* BA-11, does not tolerate salinity above 30 PSU. However, its good growth in the broad salinity range, 8–24 PSU, points at its typically brackishwater character. The second strain, *S. costatum* BA-98, opposite to the first one grew best at the highest salinity what indicates its marine character. Considering the recent results of researches describing morphological and genetical diversity of *Skeletonema* species we can assume that these strains are representatives of two different species, but this needs further investigations.

In marine environment filamentous cyanobacteria occur abundantly in coastal waters of limited circulation as well as in brackish water (LEVINTON 2001). In the Southern Baltic cyanobacteria are numerous in summer when they usually create intense blooms (KONONEN 1992, SELNER 1997). *A. flos-aquae* is the most common species but it was impossible to maintain it at laboratory conditions. Filamentous Baltic cyanobacteria are very tolerant of salinity changes. *N. spumigena* forms wide-spread, often toxic blooms in nearshore waters and saline lakes all over the world (LEHTIMÄKI 2000, MAZUR et al. 2003). Different strains of this species show the best growth at salinity of a dozen or so PSU. At optimum light and temperature conditions the Baltic strain of *N. spumigena*, isolated from the Gulf of Gdańsk, also reaches maximum population density at 8–16 PSU (Fig. 7r). Relatively good growth of *N. spumigena* was also noted in fresh water, similarly as in the case of two other Baltic strains investigated by MOISANDER (2002), whereas the other Baltic strains did not grow at salinity lower than 3 PSU (LEHTIMÄKI et al. 1997).

Temperature showed a significant influence on the growth of all strains. The only exception was *S. armatus*. Its growth was strongly dependent on light intensity whereas the effect of temperature was slight but statistically significant. It was observed that in majority of the species studied the optimum temperature range was 20–30°C. This range is typical of phytoplankton species originating from the boreal zone (FELFÖLDY 1961). In the majority of investigated strains optimum growth irradiance was in the range 80–150 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and it is in agreement with the literature data (CHAN 1978). In most species examined a very strong effect of light and temperature interaction on growth was noted. It was especially profound in diatoms. Moreover, in these algae the growth was relatively weakly influenced by light intensity.

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