

Effects of salinity,
temperature and light
on the growth and
morphology of green
planktonic algae*

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Growth of algae

Morphology of algae

Salinity

Temperature

Light

Green algae

Chlorella vulgaris

Scenedesmus armatus

S. acuminatus

S. acutus

Monoraphidium contortum

M. griffithii

Oocystis submarina

O. parva

Stichococcus bacillaris

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Abstract

The influence of the main environmental factors such as salinity, temperature and light intensity on the growth and cell volume of 9 planktonic green algae isolated from the Gulf of Gdańsk was studied. The species were cultivated in the salinity range of 0-35 psu, temp. 5-38°C and light intensity (PAR) 20-380 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. The species examined differed widely in their optimum growth conditions - 4 were brackish-water species, and 5 fresh-water species. With respect to temperature requirements, groups of three species each belonged to high-temperature, mesothermophilous and low-temperature strains. Most species required relatively high light intensities, because in 6 of them growth was saturated at 120 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

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1. Introduction

Planktonic algae are the principal producers of organic matter in aquatic ecosystems. The presence and growth intensity of the individual species and phytoplankton groups are determined by their optimum ecological requirements as to light, temperature, nutrients or salinity (Mikulski, 1982; Collier *et al.*, 1978). Identification of the basic environmental factors influencing algal growth and distribution is possible to a certain extent through *in situ* experiments. However, exact data can be obtained only under controlled laboratory conditions (Fogg and Thake, 1987).

The ecology of planktonic algae from the Gulf of Gdańsk is interesting because of the non-specific features of the physico-chemical environment. The relatively low salinity (7–8 psu) and the current inflow of large amounts of fresh waters rich in suspended matter, pollutants, and biogenic substances accelerating the eutrophication process are the main factors affecting the changes in phytoplankton species composition. The phytoplankton from the Gulf of Gdańsk consists of marine, brackish-water and freshwater species. One of the important groups are the green algae, which are especially abundant in late spring and summer (Pliński *et al.*, 1985).

In the present experiments, the influence of the main environmental factors, such as salinity, light and temperature, on the growth and morphology of some unicellular green algae isolated from the Gulf of Gdańsk was studied.

2. Material and methods

The following 9 species of green algae were examined:

1. *Chlorella vulgaris* LA-1/685,
2. *Scenedesmus armatus* var. *subalternans* LA-6/685,
3. *Scenedesmus acuminatus* LA-7/586,
4. *Scenedesmus acutus* LA-8/487,
5. *Monoraphidium contortum* LA-5/587,
6. *Monoraphidium griffithii* LA-4/586,
7. *Oocystis submarina* LA-2/682,
8. *Oocystis parva* LA-3/487,
9. *Stichococcus bacillaris* LA-9/682.

Most of these species are typical of the Gulf of Gdańsk, *i.e.* in some periods of the growing season they are found at all the stations situated in different parts of the bay (Pliński *et al.*, 1985). Pure algal cultures were isolated from natural plankton communities and belong to the Laboratory of Marine Plant Ecology Collection, University of Gdańsk (Latała, 1989).

Batch cultures of algae were grown in 300 cm³ Erlenmeyer flasks, each containing 150 cm³ of medium. The medium was prepared from Atlantic water (35 psu) or water taken at the GN station (18°48'E, 54°32'N) situated in the central part of the Gulf of Gdańsk. As applied in the F/2 medium (Guillard, 1975) the water was enriched with nutrients and microelements (without metasilicate and vitamins). The batch cultures were stirred vigorously once a day. The inoculum was taken from cultures grown at 18 ± 1°C, 27 μE·m⁻²·s⁻¹ and 7.35 psu. The experimental cultures, each in 5 replicates, were not adapted to new temperature, light and salinity conditions.

The following combinations of salinity, temperature and light intensity were used:

- Salinity – 0, 2.5, 5, 7.35, 11.5, 15, 20, 25, 30 and 35 psu at 18 ± 1°C and 27 μE·m⁻²·s⁻¹ (PAR – photosynthetically available radiation; spectral range 400-700 nm). Different salinities were prepared from Atlantic water (35 psu) diluted with distilled water. The source of fresh water was tap water. The cultures were illuminated with 6 fluorescent tubes (POLAMP LF, 25 W) in a 16:8 L:D cycle (16 h light : 8 h dark).
- Temperature – 5, 10, 14, 18, 22, 26, 30, 34 and 38 ± 1°C at 7.35 psu and 27 μE·m⁻²·s⁻¹ (PAR). The medium was prepared from water from the Gulf of Gdańsk (7.35 psu). The light source consisted of 6 fluorescent tubes (POLAMP LF, 25 W). The algae were incubated in a 16:8 L:D cycle.
- Light intensity (PAR) – 20, 30, 45, 80, 120, 150, 270 and 380 μE·m⁻²·s⁻¹ at 18 ± 1°C and 7.35 psu. The cultures were illuminated with high-pressure sodium lamps and the light intensity was controlled with neutral filters. A 16:8 L:D cycle was used. The medium was prepared from water from the Gulf of Gdańsk (7.35 psu).

The light intensity (PAR) measurements were carried out using an FF-01 phytophotometer (quanta meter with a spectral range of 400–700 nm) produced in Poland. The cell number was determined by counting them in a Bürker chamber. In order to determine the cell morphology the mean cell size of 50 cells in the exponential growth phase was calculated. The cell volume was estimated according to Edler (1979).

Regression analysis was performed and the correlation coefficients between the cell volume or maximum cell number and different light, temperature or salinity conditions were calculated. To evaluate the statistical significance of regression equations and correlation coefficients, Student's t-test and analysis of variance were done.

3. Results

Wide ranges of salinity, temperature and light intensity were applied to obtain detailed information on individual species' requirements.

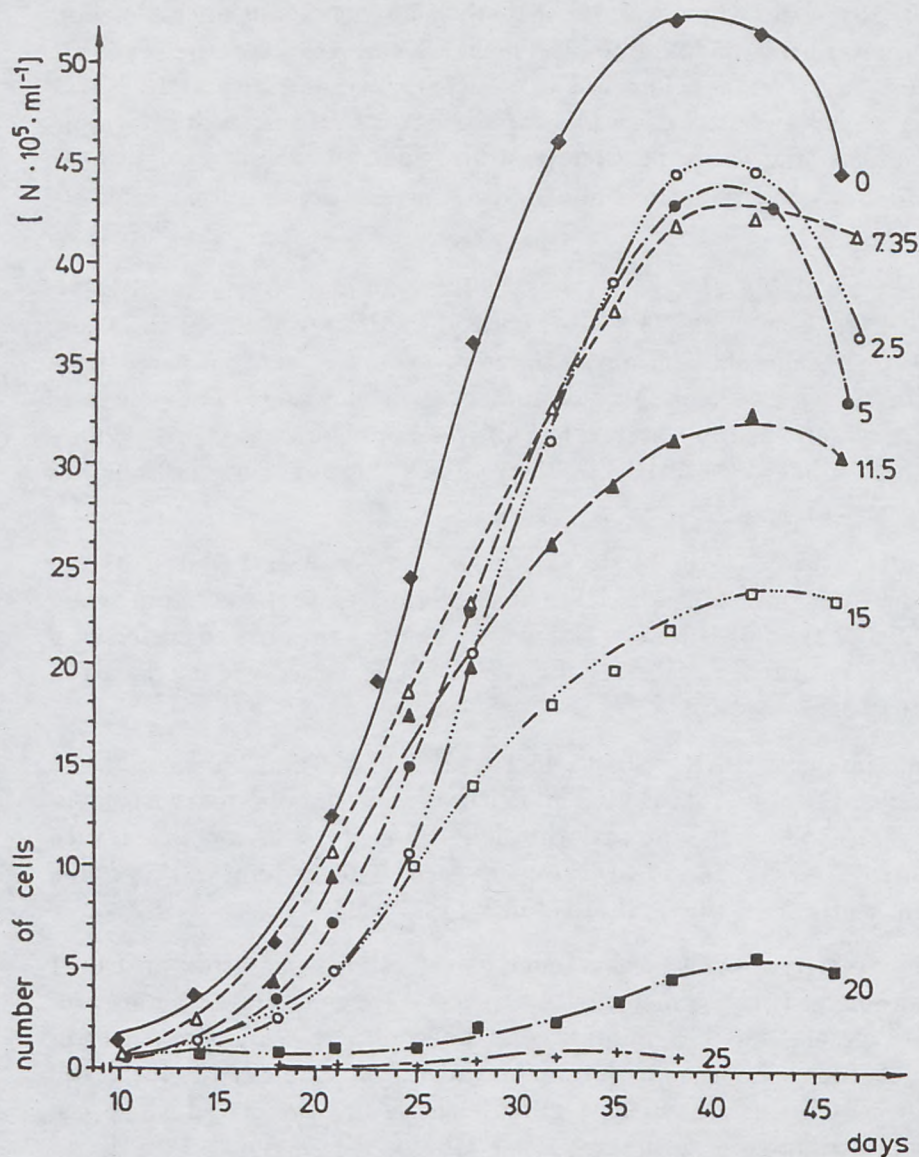


Fig. 1. *Scenedesmus armatus* growth as a function of salinity (psu). Salinity values are denoted on each curve

Five of the species investigated grow best in fresh water (2, 3, 4, 5) or at a very low salinity of 2.5 psu (6). They also exhibit low resistance to higher

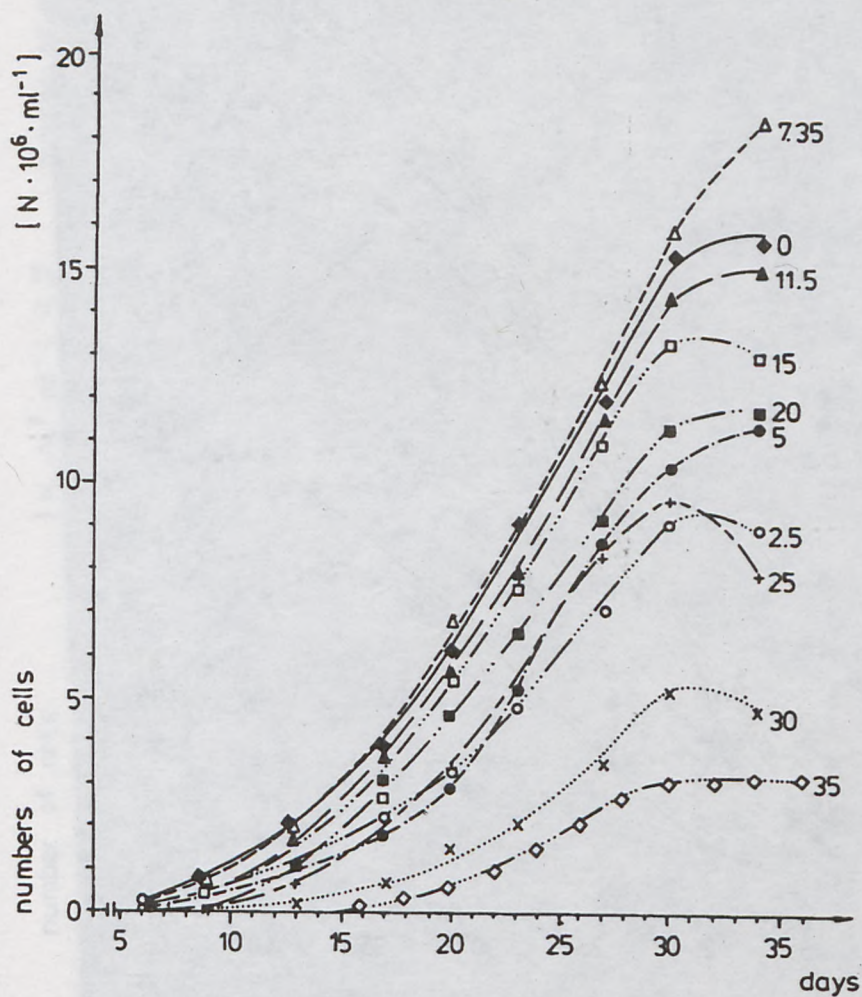


Fig. 2. *Stichococcus bacillaris* growth as a function of salinity (psu). Salinity values are denoted on each curve

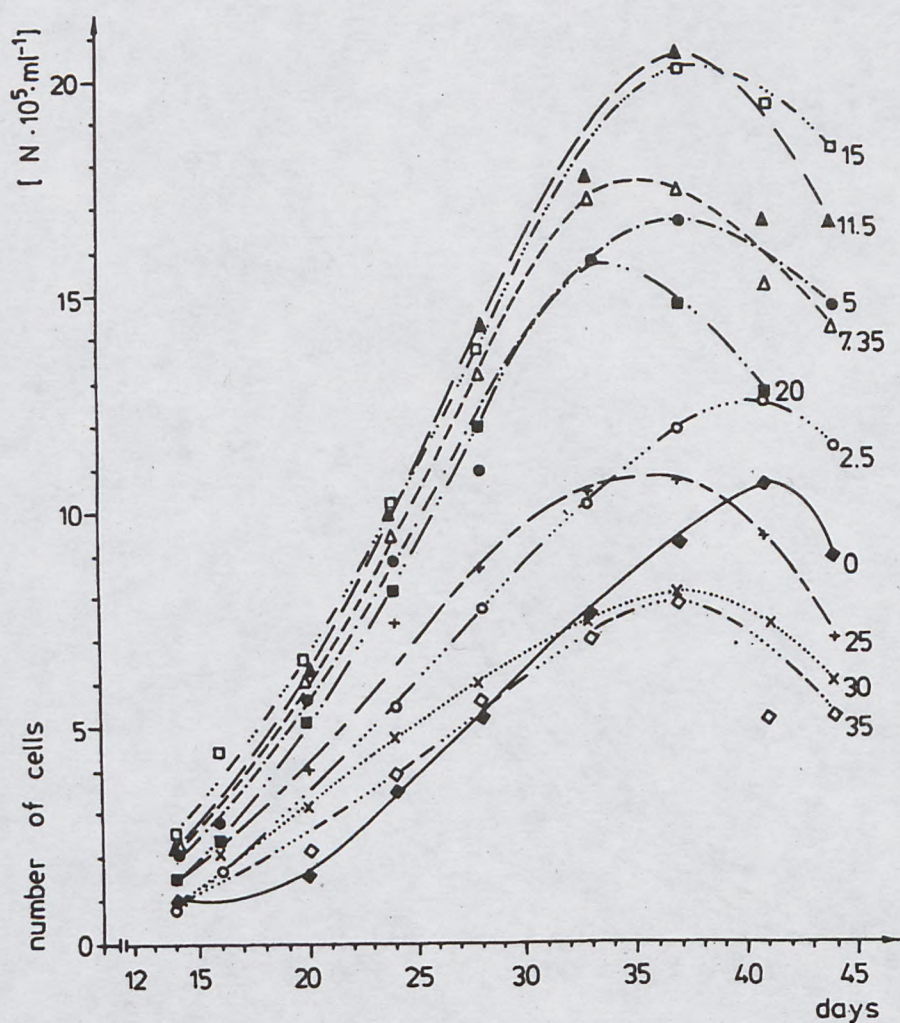


Fig. 3. *Oocystis submarina* growth as a function of salinity (psu). Salinity values are denoted on each curve

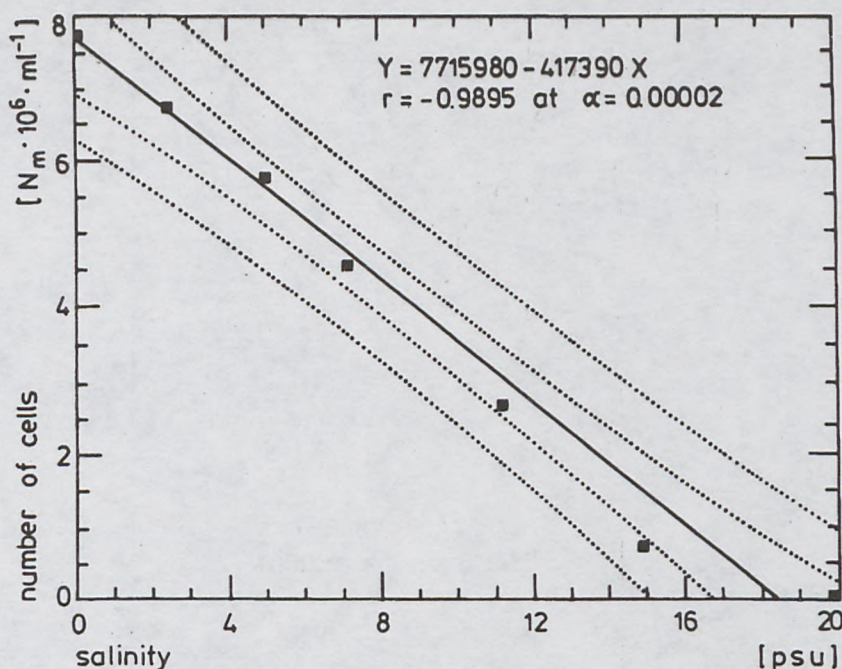


Fig. 4. Regression between salinity and maximum cell number (N_m) of *Scenedesmus acutus*. Dotted lines represent confidence and prediction limits (0.95)

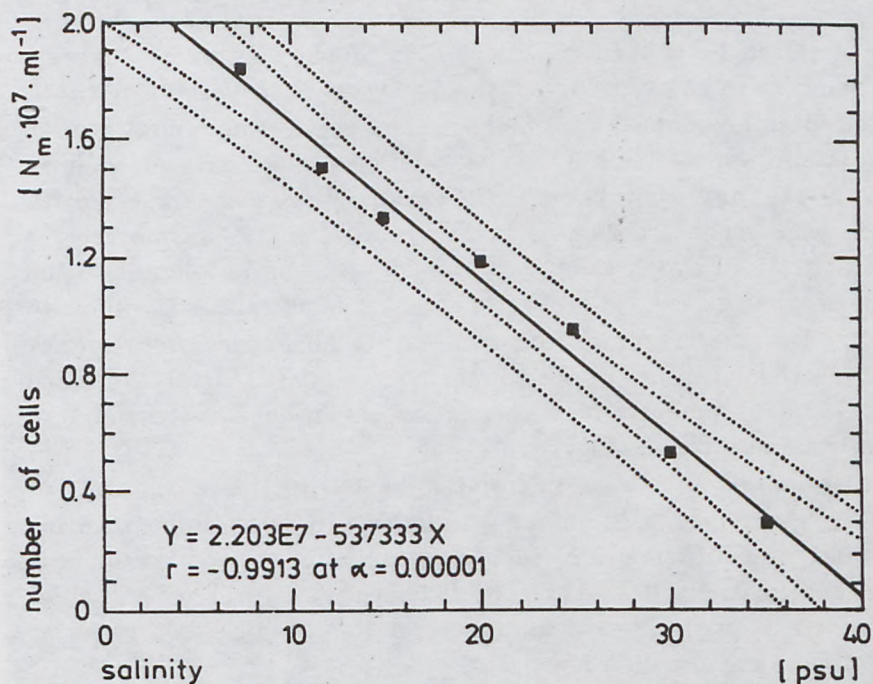


Fig. 5. Regression between salinity and maximum cell number (N_m) of *Stichococcus bacillaris*. Dotted lines represent confidence and prediction limits (0.95)

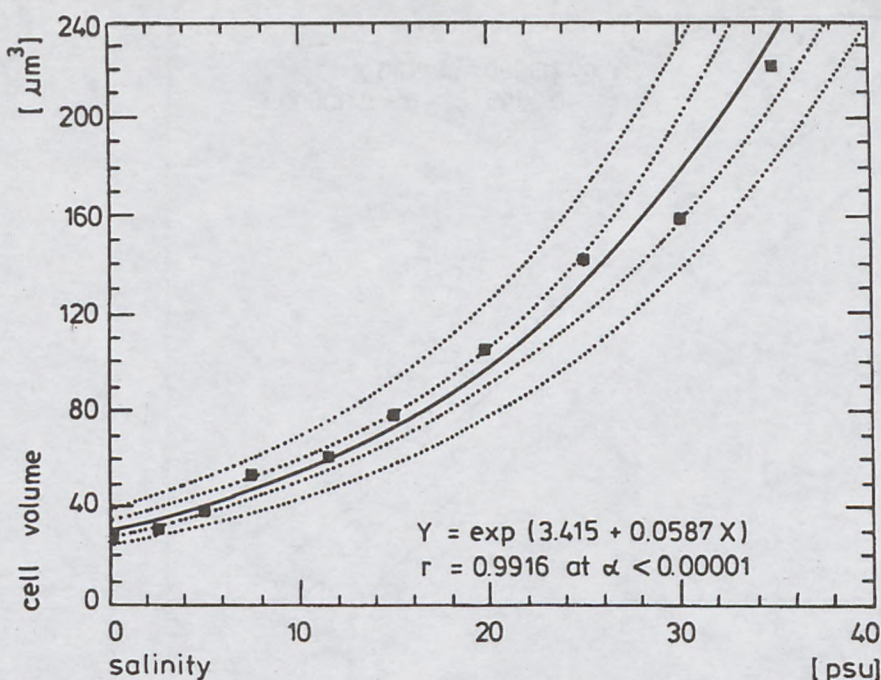


Fig. 6. Regression between salinity and cell volume in *Stichococcus bacillaris*. Dotted lines represent confidence and prediction limits (0.95)

salinity since a decrease in their growth at 15 or 20 psu was noted. Figure 1 illustrates the growth curves in one of these species, *Scenedesmus armatus*. Three of the species examined (1, 8 and 9) show the most abundant growth at 7.35 psu (typical of the Gulf of Gdańsk) and also tolerate a wide salinity range of 0–30 psu, and even 35 psu (*Stichococcus bacillaris*). The growth curves of *S. bacillaris* are presented in Figure 2. *Oocystis submarina* (7) grows best at relatively high salinities, 11.5–15 psu, and is tolerant to the whole range of salinity used, i.e. 0–35 psu (Fig. 3). In all species the increase in salinity reduces the maximum cell number. In all *Scenedesmus* species the correlation coefficients were greater than $r = -0.984$ (Fig. 4). In three species (1, 8 and 9) similarly high correlation coefficients in the range of 7.35–35 psu were also found (Fig. 5).

Different salinities have a considerable influence on the morphology of green algae. In the species examined an increase in cell volume with increasing salinity was observed. *Stichococcus bacillaris* showed the highest correlation coefficient, $r = 0.9915$ (Fig. 6), between the cell volume and different salinities (0–35 psu). The calculated correlation coefficients for species 1, 2, 3 and 4 were greater than $r = 0.974$.

The effect of temperature on algal growth varies. Five species (1, 2, 3, 6, 9) grow very well at high temperatures, 26–30°C, and in

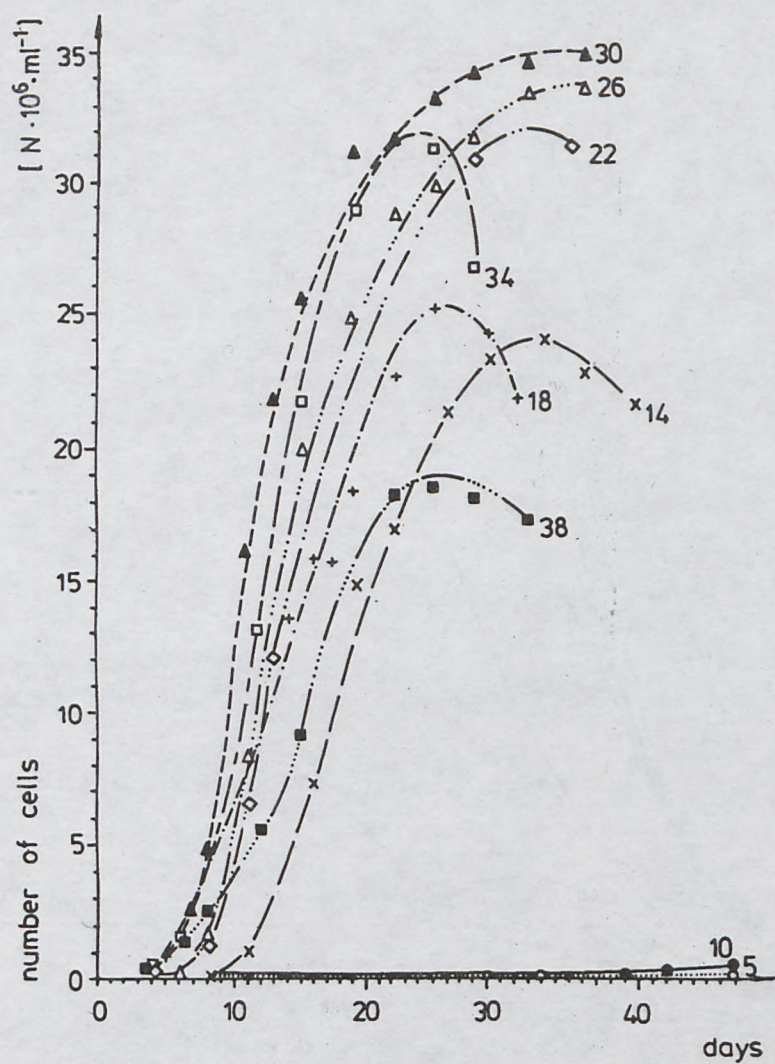


Fig. 7. *Chlorella vulgaris* growth as a function of temperature ($^{\circ}C$). Temperature values are denoted on each curve

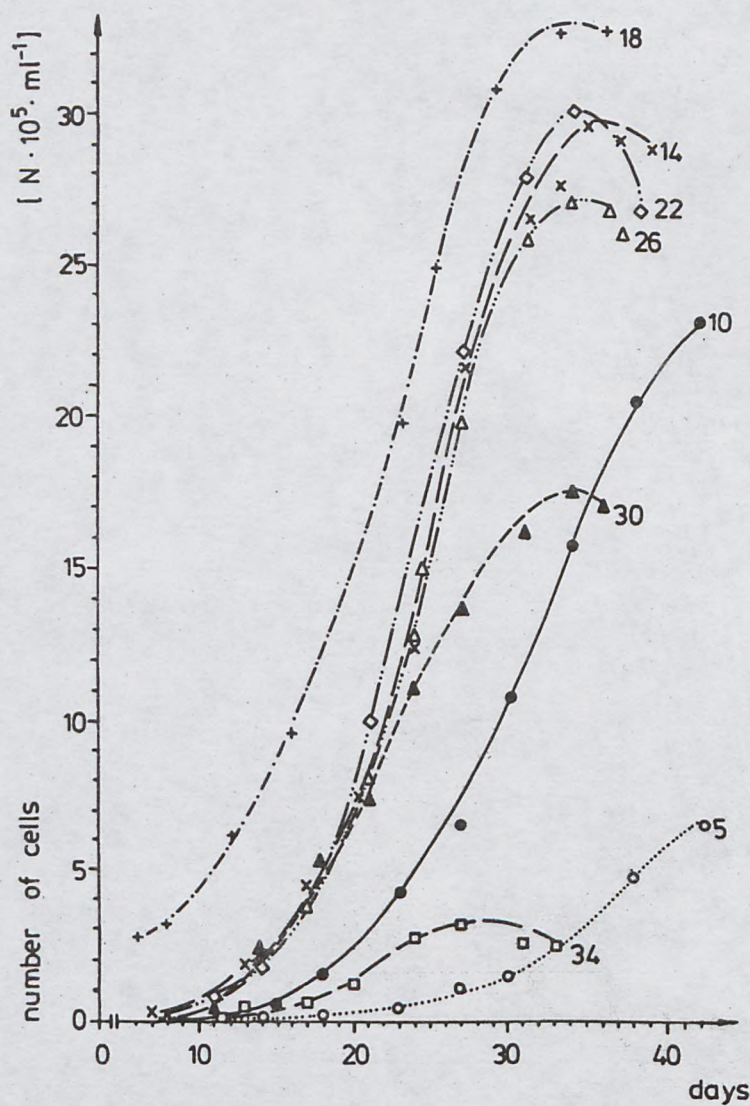


Fig. 8. *Oocystis submarina* growth as a function of temperature ($^{\circ}\text{C}$). Temperature values are denoted on each curve

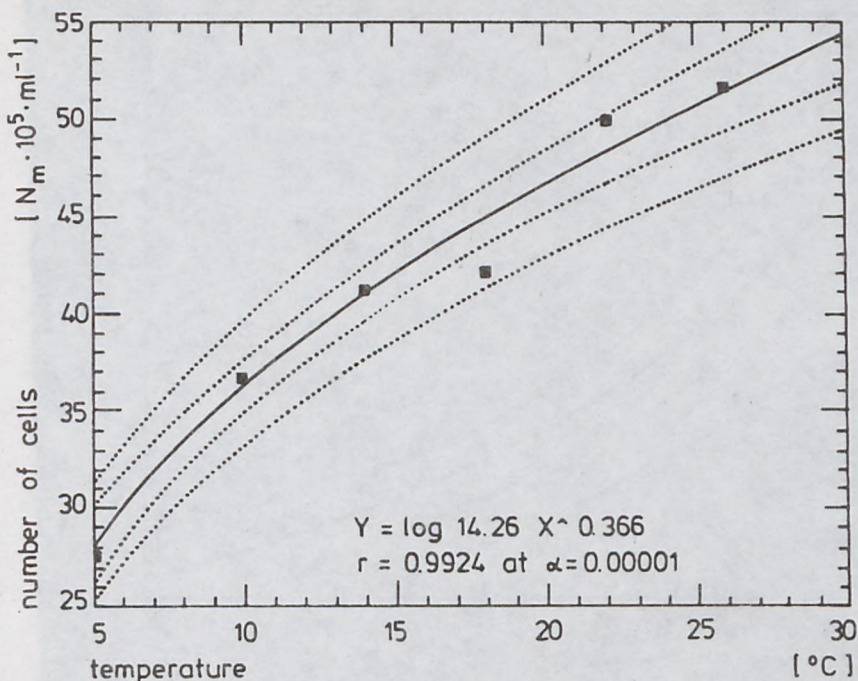


Fig. 9. Regression between temperature and maximum cell number (N_m) of *Scenedesmus armatus*. Dotted lines represent confidence and prediction limits (0.95)

Scenedesmus acuminatus the most luxuriant growth was observed even at 34°C. Typical growth curves for *Chlorella vulgaris* cultivated at different temperatures are illustrated in Figure 7. These species grow poorly or not at all at 5–10°C. The remaining species reveal optimum growth at 18°C, and grow also at the lowest temperature used (5°C), but do not tolerate the highest temperature (38°C). Such a situation is illustrated, for example, in Figure 8 for *Oocystis submarina*. *Scenedesmus armatus* (Fig. 9) showed the highest correlation between temperature and maximum cell number in the 5–30°C range.

The influence of temperature on algal morphology varied widely and a high correlation between temperature and cell volume in the 10–30°C range was noted only for *Scenedesmus armatus* (Fig. 10).

These algae require high light intensities. *Monoraphidium griffithii* grows best at 150–270 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, whereas 5 species (1, 2, 4, 5, 9) grow best at a high PAR intensity (120 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and in a 16:8 L:D cycle, *i.e.* the minimal daily quantum illumination causing growth saturation is equal to 7 $\text{E} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$. Figure 11 illustrates the growth curves at different light intensities for one of these species, *Scenedesmus armatus*. The above-mentioned species also grow at the highest radiation intensity, 380 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, but this growth is rather poor. Two species (3 and 8)

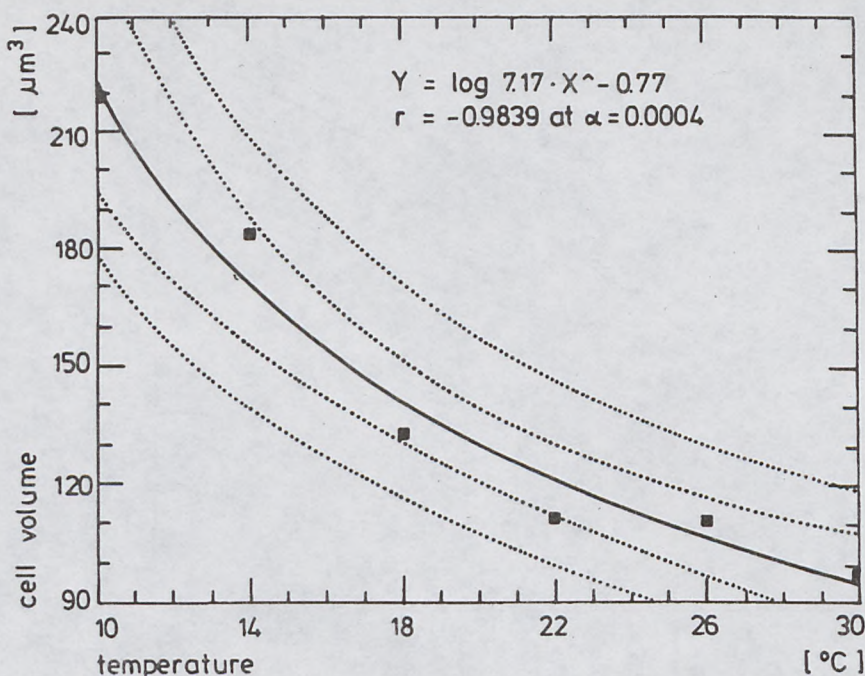


Fig. 10. Regression between temperature and cell volume in *Scenedesmus armatus*. Dotted lines represent confidence and prediction limits (0.95)

grow best at a lower light intensity, $80 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and only *Oocystis submarina* (Fig. 12) grows very well at $45 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In the case of the last species a high correlation between the PAR in the $45\text{--}380 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ range and the maximum number of cells was obtained (Fig. 13).

Different light intensities did not affect the cell morphology of 4 species (3, 4, 5, 6), whereas multidirectional changes in cell size with increasing light intensity were observed in the other species.

4. Discussion

Salts dissolved in water influence living organisms by the chemical properties of ions present in the solution and by the osmotic effect (Guillard, 1962). Salinity fluctuations also affect gas solubility, especially that of CO_2 , as well as water density and viscosity (Chlebowicz, 1988). The optimum salinity for the growth of many planktonic algae was determined (Braarud, 1961; Guillard and Myklestad, 1970). According to Proskina-Lawrenko (1963), the optimum salinity for a given species is the same as in its natural habitat. The results of the present studies indicate that only 3 species (1, 8, 9) exhibited the best growth in a salinity typical of the Gulf of Gdańsk, whereas the majority of the algae examined preferred fresh waters. This

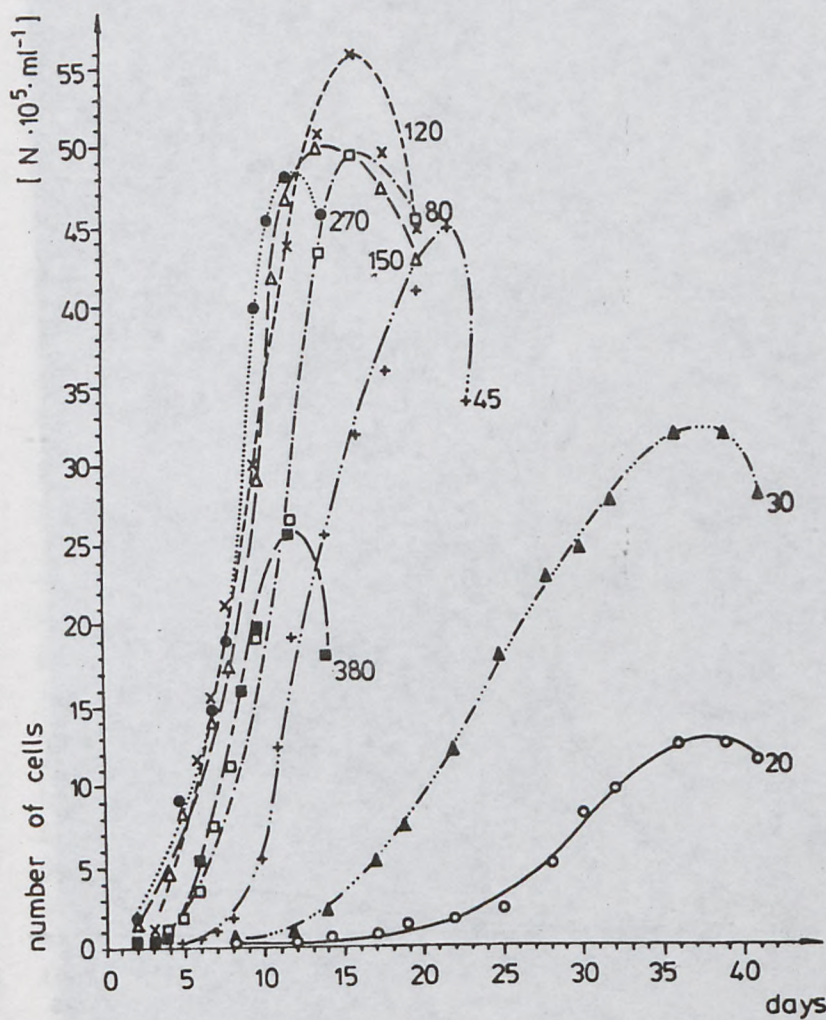


Fig. 11. *Scenedesmus armatus* growth as a function of light intensity PAR ($\mu E \cdot m^{-2} \cdot s^{-1}$). PAR values are denoted on each curve

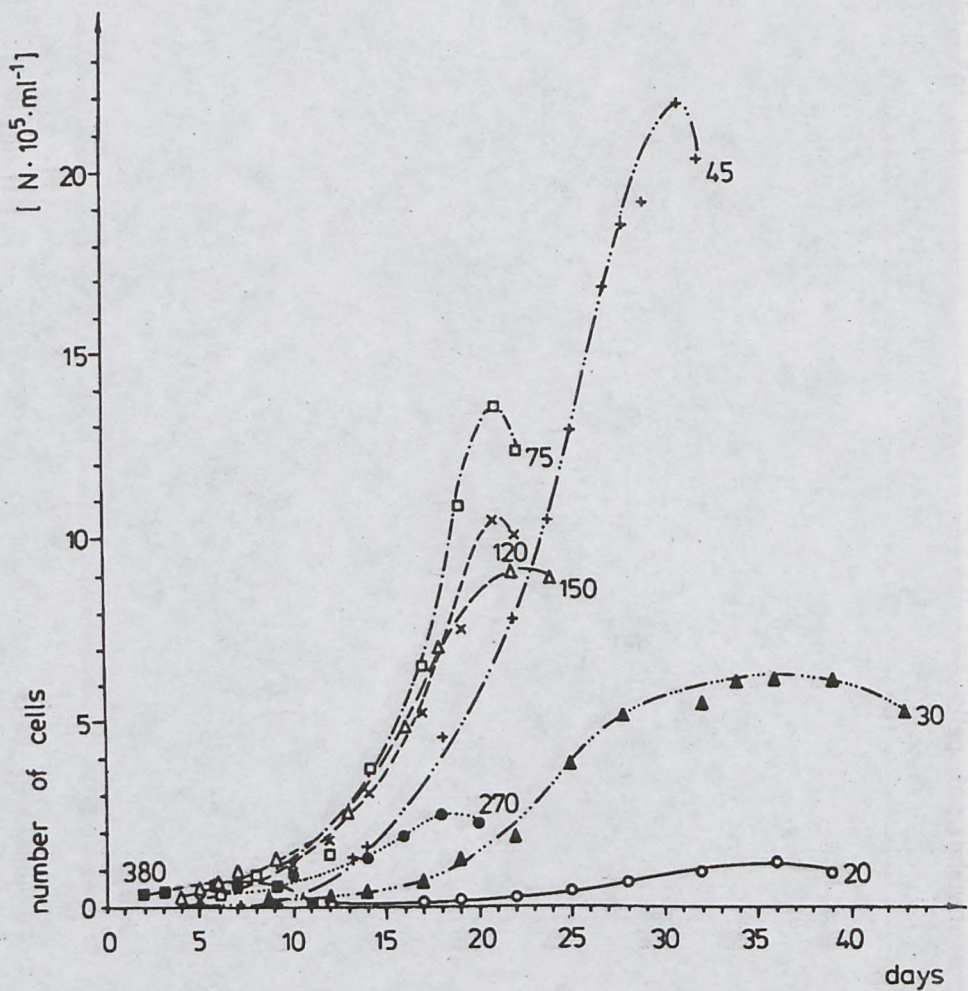


Fig. 12. *Oocystis submarina* growth as a function of light intensity PAR ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). PAR values are denoted on each curve

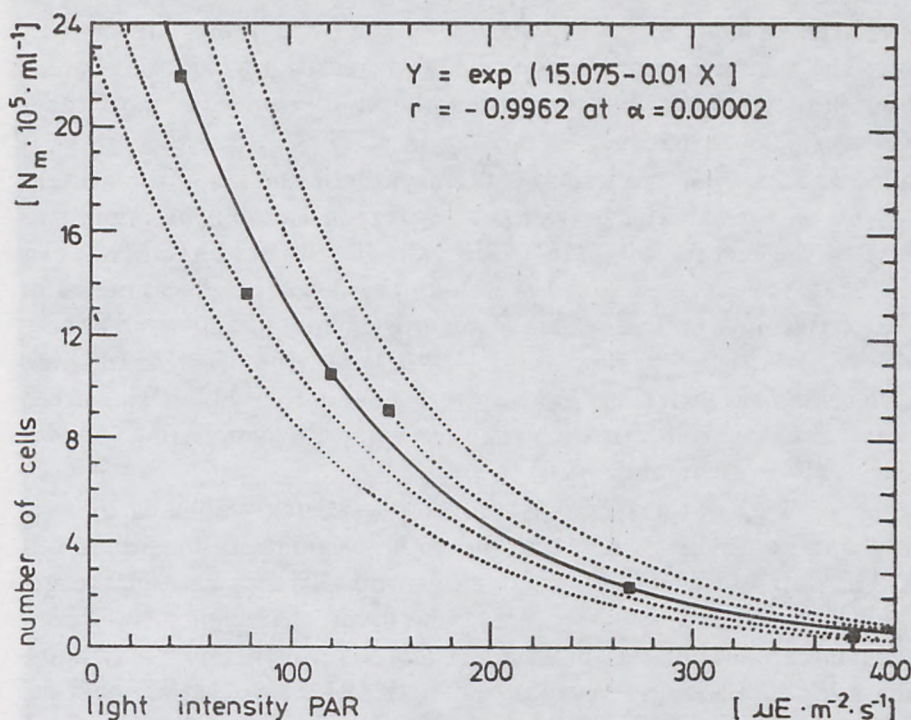


Fig. 13. Regression between light intensity PAR ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and maximum cell number (N_m) in *Oocystis submarina*. Dotted lines represent confidence and prediction limits (0.95)

fact confirms the typical freshwater nature of most planktonic green algae (Starmach, 1963).

Chlorella species, very common in fresh waters, show great adaptability to various environmental conditions. The brackish nature of the *Chlorella* strain from the Gulf of Gdańsk confirms the data on similar strains which grow equally well in fresh and in sea water (George, 1957). *Stichococcus* species also inhabit various environments, such as soil, glacier surfaces or fresh water (Starmach, 1972). According to George (1957), the *Stichococcus bacillaris* strain 379/5 isolated from the marine environment is of a euryhaline nature. However, the latest studies (Hayward, 1974) indicate that it grows best at a very low salinity of 0.2 psu, and although this species does tolerate salinities up to 100 psu, cell numbers are observed to decrease with increasing salinity. The strain isolated from the Gulf of Gdańsk has a typical brackish-water character, exhibiting the best growth at 7.35 psu; the highest salinity used (35 psu) strongly reduced its growth (Fig. 2).

In the brackish-water species investigated in the present study, an increase in salinity results in a gradual decrease in the maximum cell number and biomass per 1 cm^3 of medium. However, in the case of marine spe-

cies the restrictive effect of salinity was revealed only at values higher than 7.35 psu. The regression equations and high correlation coefficients point to a close relationship between the cell number and salinity. In most cases these relationships are linear.

The metabolism rate and thus the photosynthesis and respiration intensities depend on the salt concentration in water. In halophilous organisms a decrease in the metabolism rate at low salinities was observed (Lobban *et al.*, 1985). It is connected with the well-known dwarfism phenomenon of marine organisms present in brackish water ecosystems (Remane and Schlieper, 1971; Medwecka-Kornaś and Kornaś, 1972). On the other hand, high sodium chloride concentrations have a greater inhibitory influence on cell division and daughter cell formation than on biomass accumulation (Soeder and Stengel, 1974; Richmond, 1986).

The morphology of the species examined was strongly affected by salinity. In all species a salinity increase resulted in a significant increase in cell volume. The data obtained here are in agreement with Soeder and Stengel (1974) and Setter and Greenway (1979), who found that higher NaCl concentrations mainly inhibit cell division in *Chlorella* and thereby delay autospore liberation. Thus, as a consequence of active photosynthetic processes, larger cells could be formed. Such relationships are especially pronounced in green algae, because *e.g.* dinoflagellates do not reveal changes in cell morphology within the range of tolerated salinity (White, 1978).

Temperature is undoubtedly one of the most important factors controlling the growth of many algal species. In most *Chlorococcales* the optimum growth temperature is in the range of 20–30°C (Felföldy, 1961; Jankowski, 1964). Komárek and Ružička (1969) divided the strains of chlorococcal algae into several groups according to their temperature optima. In accordance with this division, *Chlorella vulgaris* (1) *Scenedesmus armatus* (2) and *Scenedesmus acuminatus* (3) are high-temperature strains with temperature optima between 30 and 38°C, *Monoraphidium contortum* (5), *M. griffithii* (6) and *Stichococcus bacillaris* (9) are mesothermophilic strains with temperature optima of 20–30°C, whereas *Scenedesmus acutus* (4), *Oocystis submarina* (7) and *O. parva* (8) are low-temperature strains with temperature optima below 20°C. To compare this classification with the results obtained here, it could be said that the mesothermophilous species do not grow at $\leq 10^\circ\text{C}$ and less, whereas the low-temperature species tolerate 5°C, but do not grow at 38°C.

Payer *et al.* (1980) have studied the effect of temperature on the growth of 34 algal strains, mainly of the *Scenedesmus*, *Chlorella* and *Monoraphidium* genera. The optimal temperature obtained were in most cases higher by a few degrees than in the present study. However, it should be stressed

that the species examined by Payer *et al.* (1980) and those studied here were different. According to Komárek and Ružička (1969), *Scenedesmus quadricauda* tolerates 14–36°C, with an optimum temperature of 30–32°C. The main difference between the above-mentioned species and the three *Scenedesmus* species tested in this study lies in the fact that the latter grew at a relatively low temperature of 5–10°C, but their growth was often very poor.

Temperature has a relatively large influence on organism morphology. In most species with an extensive geographical range, smaller forms are observed in warm environments (Margalef, 1955). Because an increase in temperature results in an increase in metabolic rate, this relationship is connected with the biological principle that the smaller the organism, the more active the metabolism; in other words, the metabolic rate per volume or weight unit decreases as the organisms increases in size (Sournia, 1982). The algae also show a decrease in cell volume with a temperature increase (Jørgensen, 1968). Komárek and Ružička (1969) demonstrated the strong influence of temperature on *Scenedesmus quadricauda* cells and coenobia. An increase in temperature up to 30°C resulted in a decrease in the mean cell size. In the 40–74.5°C temperature range Kullberg (1977) found a linear relationship between decreasing temperature and mean cell size in the thermophilous blue-green algae *Synechococcus lividus*; the correlation coefficient was $r = 0.977$.

The algae, especially the planktonic ones, were able to adapt themselves rapidly and actively to different light conditions. When light limits the higher cell division rate with increasing light intensity, the biomass increases up to light saturation of algal growth. Light intensities saturating the growth of different algal species are generally in the range of 40–100 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Chan, 1978; Durbin, 1974; Paasche, 1968). In the present study similar results were obtained, *i.e.* 45–120 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. High light intensities inhibit algal growth and the literature data on the algae of various systematic groups are in the range of 120–600 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Holt and Smayda, 1974; Sorokin and Krauss, 1958; Durbin, 1974; Chan, 1978; Jitts *et al.*, 1964). Sorokin and Krauss (1958) found that in green algae these values were between 600 and 1200 ftc (80–260 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). According to Komárek and Ružička (1969), 0.19 $\text{cal} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$ PAR (660 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) is lethal for *Scenedesmus quadricauda* after a few days of cultivation. Most of the species studied tolerated the highest light intensity used (380 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), but their growth was not so pronounced as at lower light intensities. In most species the inhibitory influence of light intensity was demonstrated even at 150 or 270 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Data on the effects of different light intensities on algal morphology are relatively scarce. The earlier investigations indicate that a gradual increase in light intensity results in an increase in cell size (Morimura, 1959). Komárek and Ružička (1969) found that the increase in *Scenedesmus quadricauda* cell size with increasing light intensity was more pronounced at 23°C than at 30°C. Sorokin *et al.* (1972) demonstrated that a gradual increase in light intensity not only stimulates an increase in *Stichococcus* cell length, but simultaneously affects chloroplast morphology: its shape changes from lammellar at low to spiral at higher light intensities. The 5 species examined also showed a tendency to increase in cell size with increasing light intensity.

The green algae investigated in the present study differ in their optimum growth requirements. These differences could partly explain the variability in phytoplankton distribution and periodicity of its occurrence in the Gulf of Gdańsk.

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