

EXPERIMENTAL  
ARTICLES

## Intrapopulation Heterogeneity of the Fluorescence Parameters of the Marine Plankton Alga *Thalassiosira weissflogii* at Various Nitrogen Levels

E. N. Voronova<sup>a</sup>, L. V. Il'ash<sup>a</sup>, S. I. Pogosyan<sup>a,1</sup>, A. Yu. Ulanova<sup>a</sup>, D. N. Matorin<sup>a</sup>,  
Man-gi Cho<sup>b</sup>, and A. B. Rubin<sup>a</sup>

<sup>a</sup>Biology Faculty, Moscow State University

<sup>b</sup>Dongseo University, the Republic of Korea

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**Abstract**—Fluorescence of the marine alga *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle with open ( $F_o$ ) and closed ( $F_m$ ) reaction centers of photosystem 2 (PS 2) and its relative variable fluorescence ( $F_v/F_m$ ) were measured at various levels of inorganic nitrogen. A significant heterogeneity of the population in terms of these parameters was revealed. Some cells within the population were more sensitive to nitrogen deficiency, and their photosynthetic apparatus was disrupted to a greater extent. The cells within a population also differed in terms of their ability to recover after incubation at low nitrogen levels. Enhancement of nitrogen deficiency resulted in an increase in the variability of the  $F_o$  and  $F_v/F_m$  values of the cells. Fluorescence variability decreased at a less pronounced deficiency. Fluorescence variability should be taken into consideration in the studies concerning responses of algae to changes in nutrient contents.

*Key words:* *Thalassiosira weissflogii*, nitrogen deficiency, microfluorimetry.

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Illumination intensity and concentrations of biogenic elements are the main factors determining the photosynthetic activity of phytoplankton. Nitrogen levels are of paramount importance for marine phytoplankton. In marine water, both illumination intensity values and inorganic nitrogen concentrations are highly variable, irrespective of the time scale involved. After periods characterized by high contents of biogenic elements in the environment due to such hydrological factors as upwelling and vertical mixing, or advection, nitrogen concentrations decrease (eventually to analytical zero values), owing to nitrogen consumption by phytoplankton. Accordingly, the nitrogen-supplied growth phase is followed by a phase characterized by growth and photosynthesis limitation caused by nitrogen deficiency. A prolonged lack of nitrogen supply may result in nitrogen starvation [1]. No unified method of determination and delimitation of stages of starvation and nutrient depletion of algae have been suggested up to now. We regard starvation as an extreme case of limitation of cell activity by a lack of an indispensable nutrient. It is characterized by depletion of the intracellular stock of the nutrient, a zero growth rate of the population, and an incipient decrease in cell numbers in the population.

A decrease in the nitrogen supply results in decreased photosynthetic activity of the algae, affecting both its light and dark stages. Nitrogen limitation decreases their capacity to take up light energy and the photosynthesis efficiency, which primarily manifests itself in a decrease in relative variable fluorescence,  $F_v/F_m$  [2–4].

The decrease in photosynthetic activity under inorganic nitrogen deficiency is reversible. Once the necessary nutrient is made available to algae, an increase in the efficiency of photosynthetic reactions occurs [1, 3–6]. In the first place, the quantum yield is raised to the normal level; i.e., priority is given to an increase in  $F_v/F_m$  [6].

The dynamics of algal fluorescence parameters during a decrease in the nitrogen level and after eliminating the nitrogen deficiency is relatively well understood at the population and phytoplankton community level. Nevertheless, almost no studies have been conducted up to now on the responses of individual cells in a population to various nitrogen levels. The variability of relative variable fluorescence of individual algal cells was assayed in a small number of populations in algal cultures [7–9] and under environmental conditions [10, 11]. In studies with natural phytoplankton, emphasis was placed on the interpopulation and not the intrapopulation differences in algal photosynthetic activity. These early studies already revealed cell heterogeneity

<sup>1</sup> Corresponding author; e-mail: pogosyan@biophys.msu.ru

within populations in terms of  $F_o$  and  $F_v/F_m$ , as well as the differences in heterogeneity degree among populations within a community [7, 8, 10, 11]. Differences in the photosynthetic properties of individual cells within a population suggest a functional heterogeneity of cells within a population. This is a prerequisite for the population's capacity for adaptation and survival under changeable environmental conditions. Therefore, research on population heterogeneity is essential for understanding the dynamics of phytoplankton and its productivity and for elucidating the main factors responsible for them.

In this work, the intrapopulation variability of fluorescence parameters in the population of the marine diatomic alga *Thalassiosira weissflogii* was assayed at various inorganic nitrogen levels: (i) during nitrogen-supplied growth; (ii) during nitrogen limitation, and (iii) during the recovery period after nitrogen starvation.

## MATERIALS AND METHODS

This study was conducted with algologically pure cultures of the marine planktonic alga *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle (*Bacillariophyceae*).

**Cultivating.** Culture media were prepared on the basis of artificial sea water with 30‰ salinity, pasteurized three times prior to the addition of nutrients. The cultures were grown in 1-l flasks with 0.5 l of the medium on a shaker at 20°C under illumination intensity of 3  $\mu\text{E}/(\text{m}^2 \text{ s})$  in the range of physiologically active radiation (PAR) generated by OSRAM L58W25 daylight luminescence lamps. For *T. weissflogii*, this illumination intensity corresponds to the light-limited part of the *P/E* curve [5]. The light period duration was 14 h per day.

**Experimental protocol.** The studies were carried out on the cultures with severe nitrogen deficiency, i.e., nitrogen-starved algae. To obtain starved cultures, the algae were inoculated on a medium supplemented with all the substances present in the *f/2* medium [12] except nitrates. Nitrogen (as sodium nitrate) was added at a concentration of 0.176 mM. This concentration is contained in the *f/10* medium [12]. The algae were cultivated on the medium with a low nitrogen content until the  $F_v/F_m$  ratio decreased to a value of  $\leq 0.2$ . Low  $F_v/F_m$  values point to a severe nitrogen deficiency [1, 2]. The population growth rate was zero or the cell number in the population started to decrease.

Nitrogen-starved algae were inoculated on the *f/10* medium and grown in an enrichment culture for 22 days. The inoculum titer was 40000 cells/ml. Cell numbers and fluorescence parameters (both in cell suspensions and individual cells) were determined during the cultivation. Three experiments were carried out according to this protocol. The cultures with the maximum possible nitrogen supply were obtained by subculturing

the algae on the *f/2* medium with 0.88 mM nitrogen three times with 1 week intervals.

**Tested parameters.** Cell numbers in algal populations were determined by direct counting in a Goryaev chamber under a light microscope. The volume of algal cells was estimated using the geometrical similarity method by measuring their size. The relative growth rate was calculated as follows:  $\mu = 1/T (\ln N_{t+1} - \ln N_t)$  days<sup>-1</sup>, where  $N_t$  is the cell number at time point  $t$ ,  $N_{t+1}$  is the cell number at time point  $t + 1$ , and  $T$  (days) is the interval between observations.

Chlorophyll fluorescence parameters in algal suspensions were measured in the algae that were dark-adapted for 15 min, using the pulse fluorimeter designed at the Biophysics Department of the Faculty of Biology, Moscow State University. Fluorescence values obtained with open reaction centers ( $F_o$ ) were estimated by exciting the system with weak 5  $\mu\text{s}$  test pulses at an average quantum density of 1  $\mu\text{E}/(\text{m}^2 \text{ s})$  that were generated by blue light-emitting diodes (450–470 nm). To enhance the precision of the measurements, they were done 100 times at 80-ms intervals; the mean values were determined and used in further calculations. To measure fluorescence with closed reaction centers ( $F_m$ ), algal suspensions were illuminated for 300 ms with test pulses and reaction center-saturating light with a quantum density of 6000  $\mu\text{E}/(\text{m}^2 \text{ s})$  from halogen lamps with blue SZS-22 filters. During the photosynthesis-saturating flash, 100 measurements of fluorescence intensity were carried out; the mean value of  $F_m$  was used in subsequent calculations. Chlorophyll fluorescence excited by test light pulses was monitored using a red KS-18 filter transmitting the light with  $\lambda > 680$  nm. Using the  $F_m$  and  $F_o$  values obtained, we calculated relative variable fluorescence ( $F_v/F_m = (F_m - F_o)/F_m$ ). This dimensionless value provides an estimate of the potential quantum efficiency of PS 2 [13].

The similar parameters were measured in individual algal cells ( $F_o^i$ ,  $F_m^i$ ,  $F_v^i/F_m^i$ ) with a microfluorimeter designed at the Biophysics Department of the Faculty of Biology, Moscow State University. It consists of a LYUMAM I-3 fluorescence microscope (LOMO, Russia) with a FMEL-1A fluorimetric adaptor that is equipped with pulse light sources, a computer-based light source-controlling system and automatic signal monitoring and accumulation device. Individual cell fluorescence was excited with an L400CWO12K, T4 Round red light-emitting diode (Ledtronics, United States) with an irradiation wavelength of 612 nm. This enabled decreasing the background light intensity from the camera with the sample. However, using a red light-emitting diode in this set-up necessitated the application of a filter for farther red light (KS-19) that is permeable for light with wavelengths over 700 nm. This prevented the registration of near-red range fluorescence. The quantum flow density of exciting light was 1.6 and 12000  $\mu\text{E}/(\text{m}^2 \text{ s})$  while measuring  $F_o^i$  and  $F_m^i$ ,

respectively [7]. In order to decrease the measurement errors,  $F_o^i$  and  $F_m^i$  were measured up to 100 times, and the average values were calculated. The measurement time for the average values of  $F_o^i$  and  $F_m^i$  was 9 and 0.3 s, respectively. The sample included 40–70 cells for each measurement. All fluorescence measurements were performed after incubating the algae in the dark for 15 min.

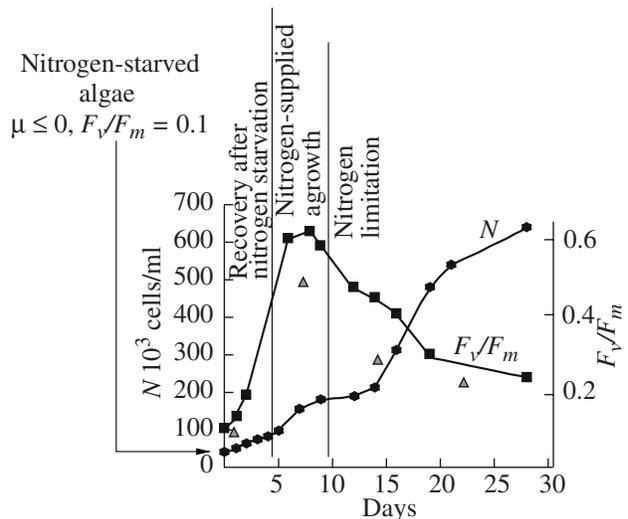
## RESULTS

After inoculating a nitrogen-starved *T. weissflogii* culture in the *f/10* medium, the cell number continued to increase for 25 days (Fig. 1). The population growth rate was the highest during the first 7 days, whereupon it decreased. The relative variable fluorescence yield values of the starving culture were low. After the addition of nitrogen,  $F_v/F_m$  increased from day 1 to day 8 of cultivation, followed by a drop in the photosynthetic activity (Fig. 1).

In algal enrichment cultures, the concentration of biogenic elements in the medium decreases during growth and nutrient consumption until it reaches the threshold level at which nutrient utilization is impossible. Nitrogen-limited algae are characterized by rapid consumption of added nitrates, resulting in the accumulation of a significant intracellular nitrogen pool [14]. Calculations based on the maximum values of inorganic nitrogen consumption by *T. weissflogii* presented in the literature [14] reveal that nitrate nitrogen added at a concentration of 0.176 mM was already consumed on the 2nd or 3rd day of cultivation. Further biomass accumulation proceeds at the expense of intracellular nitrogen pools. Their depletion results in an increasing nitrogen deficiency in the cells. Accordingly, the degree of nitrogen limitation in enrichment cultures of algae increases with an increase in their biomass. The nitrogen limitation degree is maximal during the stationary growth phase and at the onset of a decrease in cell number when rate of the cell death equals or exceeds the rate of population growth.

The calculations taking into account the nitrogen cell quota in *T. weissflogii* [14], the quantity of nitrogen added to the medium, and the dynamics of algal cell numbers reveal that on the 7th day of cultivation, the cell quota exceeds the cell nitrogen content. Hence, the nitrogen-starved culture inoculated on a nitrate-containing medium consecutively underwent the following stages: (i) a high degree of nitrogen deficiency (nitrogen starvation), a zero growth rate, and low  $F_v/F_m$  values (0.10–0.11); (ii) the recovery after nitrogen starvation and an increase in growth rate and  $F_v/F_m$ ; (iii) nitrogen-supplied growth and maximum  $F_v/F_m$  values; and (iv) nitrogen limitation and a decrease in  $F_v/F_m$  and growth rate (however,  $\mu > 0$ ).

The average cell volume in the population varied depending on the nitrogen level. In a nitrogen-starved



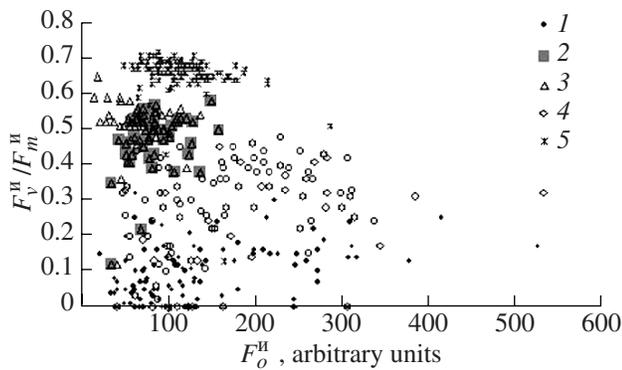
**Fig. 1.** Dynamics of cell numbers ( $N$ ), relative variable fluorescence values in cell suspensions ( $F_v/F_m$ ), and average relative variable fluorescence values of individual cell ( $F_v^i/F_m^i$ ) in the alga *Thalassiosira weissflogii*.

and a nitrogen-provided culture, it was  $865 \pm 283 \mu\text{m}^3$  and  $1293 \pm 375 \mu\text{m}^3$ , respectively. The average cell volume was 1140 to  $1026 \mu\text{m}^3$  (the difference is statistically insignificant) in the populations with different nitrogen levels. The coefficient of variation did not exceed 40%.

**Variable fluorescence of individual *T. weissflogii* cells.** Populations with various levels of nitrogen supply are characterized by a high variability of the fluorescence parameters of individual cells including the fluorescence values with open reaction centers ( $F_o^i$ ) and the relative variable fluorescence values ( $F_v^i/F_m^i$ ) (Fig. 2,3, and 4 and Table).

The highest cell variability in terms of  $F_o^i$  ( $CV = 68\%$ ) and  $F_v^i/F_m^i$  ( $CV = 74\%$ ) was revealed in a nitrogen-starved population. The  $F_v^i/F_m^i$  values did not exceed 0.3 (Figs. 2 and 4), while the percentage of cells with  $F_v^i/F_m^i$  values between 0.2 and 0.3 was only 6% of the whole cell number in the population. The  $F_v^i/F_m^i$  values were zero in approximately 10% of the cells.

The average  $F_o^i$  values decreased, and the average  $F_v^i/F_m^i$  increased in the populations supplied with nitrogen and recovering after nitrogen starvation. The fluorescence parameters of individual cells in the population were less variable than in starving algae (Table). In the populations recovering after nitrogen starvation and supplied with nitrogen, the  $F_v^i/F_m^i$  values over 0.50 were detected in 33% and over 50% of the cells, respec-



**Fig. 2.** Fluorescence yield with open reaction centers ( $F_o^i$ )

and relative variable fluorescence values ( $F_v^i/F_m^i$ ) in *T. weissflogii* cells in a nitrogen-starved culture (1); a culture recovering after nitrogen starvation (2); a nitrogen-supplied culture (3); a nitrogen-limited culture (4); and a culture supplied with a maximum nitrogen amount (5). The data are based on the results of three experiments on the *f/10* medium (1–4) and two experiments on the *f/2* medium (5).

tively. We detected no cells with  $F_v^i/F_m^i$  values below 0.10 or with completely inhibited photosynthetic activity. The  $F_v^i/F_m^i$  values exceeded 0.60 in 94% of the cells in the population with the maximum possible nitrogen supply which was obtained by subculturing the algae on the *f/2* medium three times with 1-week intervals.

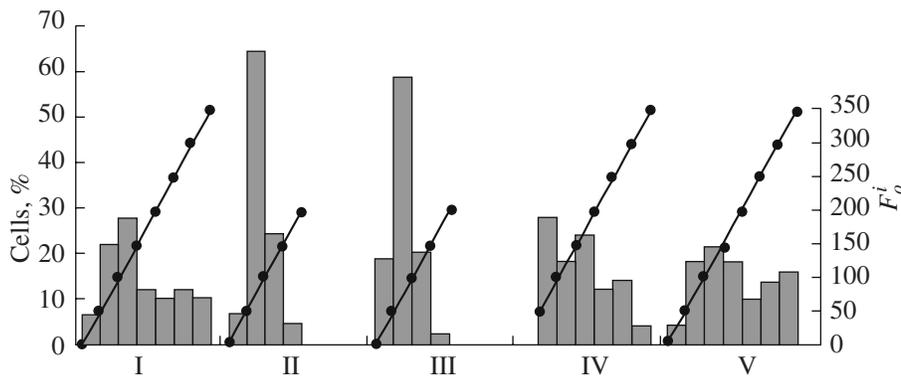
The average  $F_o^i$  values were higher and the  $F_v^i/F_m^i$  values were lower in nitrogen-limited populations than in nitrogen-supplied cultures. The variability of individual cell fluorescence parameters increased within

the population. Cells with  $F_v^i/F_m^i < 0.10$  were detected. An increase in the degree of nitrogen deficiency associated with culture aging resulted in an increase in the percentage of the cells with  $F_v^i/F_m^i < 0.10$ , and the cells incapable of photosynthesis were detected ( $F_v^i/F_m^i = 0$ ). Cells with  $F_v^i/F_m^i > 0.50$  were not present in the population.

The average  $F_o^i/F_m^i$  values in the population were usually below the  $F_v^i/F_m^i$  values determined in the cell suspensions of all populations, regardless of their nitrogen level (Figs. 1 and 5).

## DISCUSSION

The dynamics of biogenic elements in enrichment cultures of algae is concordant with that in natural waters provided that biogenic elements are introduced into the ecosystem only once, their pool is not replenished for a long time, and the efflux of algal cells from the ecosystem is insignificant. Semi-closed lagoons with a low rate of water renovation are examples of the ecosystems with this pattern of biogenic elements [15]. A long-term lack of nutrient supply may result in biogenic starvation of the algae [6]. Presently, there is no unified approach to determining and delimiting the starvation and limitation stages of algal development with respect to various nutrients. Some researchers consider a lack of a nutrient in the environment a sufficient condition to conclude that the algae are at the starvation stage, whereas nutrient limitation corresponds to growth at a low nutrient concentration [1]. Alternatively, starving algae are construed as cells with a zero growth rate and a high ratio between the cell carbon and nitrogen (or phosphorus) levels [4]. We regard starva-



**Fig. 3.** Cell distribution in terms of  $F_o^i$  in *T. weissflogii* populations at various nitrogen levels: in nitrogen-starved cultures (I), in cultures recovering after nitrogen starvation (II), in nitrogen-supplied cultures (III), and in nitrogen-limited cultures on day 12 (IV) and day 22 (V) of cultivation. The data are based on the results of three experiments on the *f/10* medium. Each distribution pattern was plotted based on  $F_o^i$  measurements in at least 50 cells (see Fig. 2). Lines with filled circles,  $F_o^i$  values.

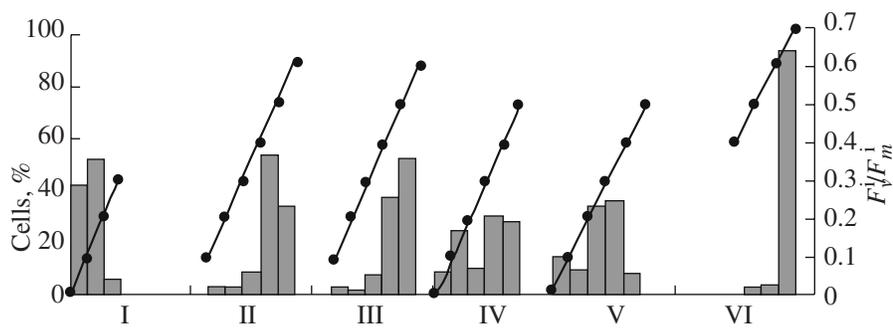
Average population values of fluorescence with open reaction centers ( $F_o^i$ ) and the relative variable fluorescence ( $F_v^i/F_m^i$ ) of individual cells in *T. weissflogii* cultures at various nitrogen levels. CV, coefficient of variation. The data are based on the results of five experiments

Medium	Nitrogen supply		$(F_o^i)$ , arbitrary units	$(F_v^i)/(F_m^i)$
	Starving culture	Average	138	0.10
		Standard deviation	93	0.08
		CV, %	68	74
<i>f/10</i> medium	Recovery after nitrogen starvation	Average	86	0.47
		Standard deviation	29	0.08
		CV, %	33	18
	Nitrogen-supplied culture	Average	76	0.49
		Standard deviation	31	0.09
		CV, %	40	18
	Nitrogen limitation	Average	175	0.28
		Standard deviation	90	0.13
		CV, %	52	47
<i>f/2</i> medium	Culture supplied with a maximum possible nitrogen amount	Average	117	0.65
		Standard deviation	37	0.08
		CV, %	31	13

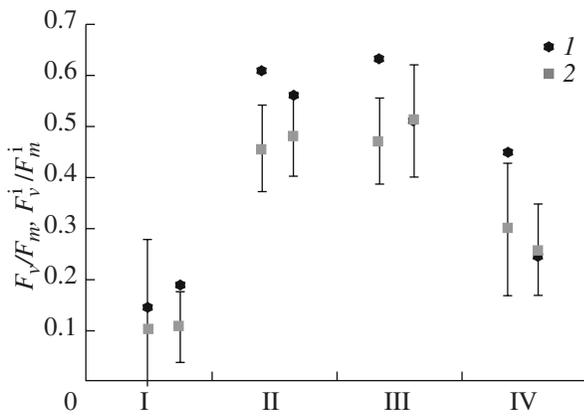
tion as the extreme degree of limiting cell processes by a lack of an indispensable nutrient, when the cell pool of this nutrient is depleted, the growth rate is zero, or the cell number in the population starts decreasing. Despite the different opinions, it is generally accepted that, during both limitation and starvation with respect to a nutrient, the algae involved are in a state of physi-

ological stress caused by a lack of an indispensable nutrient [1–4, 6, 16].

Maximum relative variable fluorescence values in a nitrogen-starved *T. weissflogii* cell suspension did not exceed 0.11, and the average  $F_v^i/F_m^i$  value for the population was 0.10. In a similar fashion, low  $F_v^i/F_m^i$  values



**Fig. 4.** Cell distribution in terms of  $F_v^i/F_m^i$  in *T. weissflogii* populations at various nitrogen levels: in nitrogen-starved cultures (I), in cultures recovering after nitrogen starvation (II), in nitrogen-supplied cultures (III), and in nitrogen-limited cultures on day 12 (IV) and day 22 (V) of cultivation. The data are based on the results of three experiments on the *f/10* medium and two experiments on the *f/2* medium. Each distribution pattern was plotted based on  $F_v^i/F_m^i$  measurements in at least 50 cells (see Fig. 2). Lines with filled circles,  $F_v^i/F_m^i$  values.



**Fig. 5.** Relative variable fluorescence values in a *T. weissflogii* cell suspension ( $F_v/F_m$ , 1) and average relative variable individual cell fluorescence values  $F_v^i/F_m^i$ , 2) in populations grown at various nitrogen levels: in nitrogen-starved cultures (I), in cultures recovering after nitrogen starvation (II), in nitrogen-supplied cultures (III), and in nitrogen-limited cultures (IV). The confidence intervals for the average  $F_v^i/F_m^i$  values corresponds to the standard deviations. The data are based on the results of three experiments on the *f/10* medium.

(0.20) were revealed in a nitrogen-starved culture of the green alga *Dunaliella tertiolecta* [6]. Low  $F_v/F_m$  values are consistent with the general pattern of disruptions in the photosynthetic apparatus under nitrogen limitation, which is caused by suppression of protein and pigment synthesis at the translation level [13]. For example, the  $F_v/F_m$  decrease is due to a number of factors including (i) inhibition of electron transfer at the level of the primary quinone acceptor *Qa* [17]; (ii) disrupted transfer of the excitation energy from the light-harvesting complex to reaction centers [2]; (iii) an increase in the antenna relative size, mostly due to a decreased content of chlorophyll bound to the photosystem reaction centers, while the chlorophyll of light-harvesting complexes remains more stable [17]; (iv) a decrease in the PS 2 reaction center number per cell; and (v) accumulation of inactive reaction centers [2,3,18], owing to a decrease in the repair rate of proteins *D*<sub>1</sub> and CP47 [13].

A nitrogen-starved *T. weissflogii* population was characterized by a high individual cell variability in terms of  $F_v^i/F_m^i$  values ( $CV = 74\%$ ) and fluorescence with open reaction centers ( $CV = 68\%$ ). Taking into account a significant genotypic cell heterogeneity in planktonic algal populations [19–21] and the fact that the intrapopulation variability in cell size, growth rate [19], and toxicant resistance [22] are associated with the differences in cell genotypes, we suggest that the high intrapopulation variability of the fluorescence parameter is due to the genetic heterogeneity of a population. Spontaneous mutagenesis is one of the factors

possibly involved in maintaining the genetic heterogeneity of the populations [21].

The fluorescence level with open reaction centers is regarded as an indirect index of the concentration of algal photosynthetic pigment. For example, a relationship between  $F_o$  and chlorophyll *a* concentration was revealed [23]. Accordingly, a high variability of  $F_o^i$  values in a nitrogen-starved population may testify to significant differences in the content of photosynthetic pigment between individual cells. These differences cannot be due to cell size variability only, because the cell size is considerably less variable. The coefficient of variation of the cell volume in a nitrogen-starved population was only 32%.

Cells with high fluorescence values with open reaction centers ( $F_o^i > 200$  arbitrary units) occurred only in nitrogen-starved and nitrogen-limited *T. weissflogii* populations (Fig. 3). The average  $F_o^i$  values for populations were also higher in the cultures under nitrogen deficiency (table). The high  $F_o^i$  values are probably due to decoupling antennas from the PS 2 reaction centers [2].

About 10% of the cells in a nitrogen-starved *T. flogii* population were characterized by a zero PS 2 quantum yield. The  $F_o^i$  values of these cells varied from 39 to 133 arbitrary units. Apparently, these were unviable, moribund cells. Photosynthetic pigment degradation and photosynthetic activity inhibition are characteristic of the second stage of algal cell death that follows cell membrane disruption. Thereupon, nuclear DNA fragmentation and cell lysis occur [24]. According to [24], the photosynthetic activity of unviable cells is only 10% of that of viable cells. Considering these data and the fact that a nitrogen-starved *T. weissflogii* population demonstrates the maximum  $F_v^i/F_m^i$  values of 0.30, we

suggest that cells with  $F_v^i/F_m^i \leq 0.03$  are also unviable. Therefore, unviable cells accounted for 20% of the nitrogen-starved *T. weissflogii* population. A high death rate under nitrogen limitation was revealed in the diatoms *T. weissflogii* [25] and *Ditylum brightwellii* [26] and in freshwater cyanobacteria [27]. However, despite the high death rate, the population includes actively dividing cells. This was demonstrated for the diatom *Chaetoceros calcitrans* [24] and the green alga *Dunaliella tertiolecta* [6]. This population heterogeneity in terms of the population's physiological state (ranging from a progressive loss of viability to an actively dividing state) can be due to the heterogeneity of its genotype [28].

The presence of unviable cells in the nitrogen-starved *T. weissflogii* population appears to be the reason for its relatively low growth rate ( $\mu = 0.23 \text{ day}^{-1}$ ) during the first day after inoculation on a nitrogen-containing medium. Since unviable cells may account for 20% of the *T. weissflogii* population under nitrogen star-

vation, the expected growth rate of the viable part of the population is 0.95–1.01 day<sup>-1</sup>. This is consistent with the values reported in the literature for nitrogen-supplied *T. weissflogii* cultures [14].

A nitrogen-starved *T. flogii* population does not contain cells with  $F_v^i/F_m^i$  values over 0.30. This  $F_v^i/F_m^i$  distribution pattern differs from that of a *T. weissflogii* population illuminated with high-intensity light. The average  $F_v^i/F_m^i$  values decreased from 0.58 to 0.15 upon illuminating a nitrogen-supplied *T. weissflogii* culture with a light intensity of 4000  $\mu\text{E}/\text{m}^2 \text{ s}$ ; however, the population still contained the cells with  $F_v^i/F_m^i$  values over 0.50 [8].

The variability of individual cell fluorescence parameters was lower in nitrogen-limited *T. weissflogii* cells whose nitrogen deficiency was less severe than that of starved cells. The average  $F_v^i/F_m^i$  values were higher than in starved cells. The percentage of unviable cells (with  $F_v^i/F_m^i \leq 0.03$ ) increased with an increase in the degree of nitrogen deficiency. It was 4 to 14% of the cell number in the population, which was below the percentage in a starved culture.

Once nitrogen is made available to the algae sustaining nitrogen deficiency (for nitrogen-starved and -limited algae), their photosynthetic apparatus is restored and the growth rate increases. The time required for complete restoration of the photosynthetic apparatus and the onset of the population's growth varies in a species-specific fashion, depending on the initial degree of nitrogen deficiency, the redox state of the available inorganic nitrogen, and the illumination intensity [3–6]. The duration of the period required for incorporation of inorganic nitrogen into the macromolecules is also species-specific and varies from 3 to 20 h [29]. These limits correspond to the time required for monitoring changes in the state of the photosynthetic apparatus and algal metabolic rates [3–6, 30]. Relative variable fluorescence is one of the first parameters to increase [4–6, 30]. Restoring the operation of the photosynthetic apparatus and increasing the cell's metabolic rate result in an increase in the growth rate of algal populations [4, 6, 30].

The average  $F_m^i$  values in *T. weissflogii* populations recovering after nitrogen deficiency were lower, and the average  $F_v^i/F_m^i$  values were higher than those in nitrogen-deficient algae. Individual cell fluorescence parameters were less variable than in nitrogen-starved and -limited algae (table). In a similar fashion, a decrease in  $F_v^i/F_m^i$  variability occurred in natural phytoplankton populations that recovered after incubating in an iron-limited medium [11]. Presumably, the lesser  $F_v^i/F_m^i$  variability of the cells in nutrient-supplied populations

can be explained as follows. The maximum possible relative variable fluorescence value is an evolutionarily conserved parameter that does not exceed 0.70 irrespective of the algal species and is determined by the specific features of the photosynthesis process [13]. The algae that could not photosynthesize under optimum conditions with the maximum efficiency were eliminated by natural selection. Therefore, populations lack cells with the  $F_v^i/F_m^i$  values genetically fixed at submaximal levels.

The  $F_v/F_m^i$  values exceeded 0.50 in 33% and over 50% of the cells recovering after nitrogen starvation and supplied with nitrogen, respectively. We detected no unviable cells in these populations. The  $F_v^i/F_m^i$  values exceeded 0.60 in 94% of the cells of the populations were grown on the *f/2* medium and supplied with maximum possible nitrogen amounts. The high  $F_v^i/F_m^i$  values in the algae grown on the comparatively nitrogen-rich *f/2* medium suggest that the algae did not completely recover after nitrogen starvation if lower nitrogen amounts were added (the *f/10* medium).

The average  $F_v^i/F_m^i$  values were somewhat lower than the  $F_v/F_m$  values in cell suspensions in all tested populations, regardless of their nitrogen supply. The difference may be due to the different light filters employed for monitoring fluorescence in cell suspensions and under the microscope (see Materials and Methods). Microfluorimetric detection of quanta with wavelengths over 700 nm is likely to reduce the  $F_v^i/F_m^i$  values in contrast to the  $F_v/F_m$  values. This may be caused by a larger contribution of PS 1 chlorophyll to the fluorescence data obtained. In addition, the difference between the  $F_v^i/F_m^i$  and the  $F_v/F_m$  values may be due to the peculiarities of the microscopic measurement techniques. Locating a cell in the field and focusing on it requires illuminating the sample. The  $F_m^i$  data obtained fail to reach the maximum per flash values, owing to a lack of complete darkness. Analogously, the  $F_v^i/F_m^i$  values measured in individual cells with a microscope equipped with a PAM-fluorimeter were below the respective  $F_v/F_m$  values [11].

In summary, the population of the alga *T. weissflogii* is characterized by a variability of individual cell fluorescence parameters (including both fluorescence with open reaction centers and relative variable fluorescence) depending on the nitrogen amounts available to the cells involved. In nitrogen-limited and nitrogen-starved populations, part of the cells are more sensitive to the nutrient deficiency, and the disruptions in the operation of their photosynthetic machinery are more serious. Cells within a population also differ in terms of their capacity to recover after a period of nutrient limi-

tation. Once nitrogen is made available for the algae, some of their cells require less time for their recovery than the rest of the population. Cell variability in terms of  $F_o^i$  and  $F_v^i/F_m^i$  increases with an increased degree of nitrogen deficiency; it decreases if the deficiency becomes less serious. Hence, the population heterogeneity is more pronounced under stress. This fact should be taken into account in studies on the response of algae to various factors (nutrient supply, light conditions, presence of toxic substances, etc.) that are conducted with continuous cultures. Under stress, cells with lower photosynthetic activity and, therefore, a lower growth rate (lower than the flow rate) are washed out. The population becomes increasingly homogeneous. The resulting population's response, therefore, is at variance with its real behavior.

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