Editorial Manager(tm) for Hydrobiologia
Manuscript Draft

Manuscript Number: HYDR3230R2

Title: A dynamic model of darkness tolerance for phytoplankton: model description

Article Type: Primary research paper

Section/Category:

Keywords: Phytoplankton; darkness tolerance; dynamic model; cell quota; catastrophic death; lag phase

Corresponding Author: Eiichi Furusato,

Corresponding Author's Institution: Saitama University

First Author: Eiichi Furusato

Order of Authors: Eiichi Furusato; Takashi Asaeda, Dr.

Manuscript Region of Origin:

Abstract: To analyze various effects of prolonged darkness on phytoplankton population dynamics, we developed a dynamic model of darkness tolerance for phytoplankton and investigated its characteristics. To construct the basic concepts of the model, we categorized various changes in abundance of phytoplankton during prolonged darkness and after reillumination into several patterns, and then considered the physiological processes producing these patterns. The nature of darkness tolerance was considered to incorporate previously experienced light conditions, including darkness, as a physiological activity, and members of the same phytoplankton species exhibit different dynamics even in identical light conditions due to such career effects. Taking this into consideration, we developed a cell quota model in relation to darkness tolerance. State variables

for abundance were indicated by cell numbers and physiological condition by three intracellular carbon pools with different physiological functions.
Using our model, we analyzed the various changes in abundance of phytoplankton in relation to exposure to prolonged darkness. Various responses in terms of phytoplankton abundance to prolonged darkness and after reillumination were successfully reproduced by the model that simply assumed that deterioration of physiological mechanics, such as photosynthetic functions, was due to a prolonged dark condition. Based on the results of calculation and assumptions for the model, we discuss the limitations, problems, and effectiveness of the model.

Response to Reviewers: Attached file include the response to comments.

Eiichi Furusato
Department of Environmental Science & Technology, Saitama University, 255 Shimo-okubo, Sakura-ku, Saitama-shi, Saitama 338-0825, Japan
E-mail:furusato@ah8.mopera.ne.jp
Telephone & Facsimile: +81-48-858 3123

August 24, 2008.

Dr. Koen Martens
Editor-in-Chief, *Hydrobiologia*

Re: Submission of revised manuscript (HYDR3230)

Dear Dr. Martens,

Thank you very much for your letter of June 24, 2008, with regard to our manuscript, "A dynamic model of darkness tolerance for phytoplankton: model description" (HYDR3230), and sending the comments from the reviewers.

I am attaching our revised manuscript, together with responses to the reviewers' comments.

We hope that the manuscript has been improved satisfactorily for publication in *Hydrobiologia*.

Thanking you,
Yours truly,

EIICHI FURUSATO
Corresponding Author

HYDR 3230: A dynamic model of darkness tolerance for phytoplankton: model description

Incorporation of reviewers' comments in the revised manuscript

Table 1 Response to general comments

Reviewers' comment	Response of authors
General comments of Reviewer #2	Thank you for your interest in our research and fruitful comments. We agree with your comments. We have modified the manuscript substantially, as described below. [Balanced presentation of advantages and novelties] According to the comment, we have corrected 1st. paragraph of "Discussion" for balanced presentation.

Table 2 Responses to specific comments of Reviewer #2

No.		Reviewers' comments	Response of authors
1	Page 4 line 5	"Succession" or "success"?	What we meant was that we are referring "succession" ~~stand the sentence~~ as the ecological concept. However, if this is not suitable, we should modify it.
2	P4 L 9	Replacement	We modified the sentence according to the comment.
3	P5 L 19	Replacement	We modified the sentence according to the comment.
4	P 6 L 13	Repeat	We modified the sentence according to the comment.
5	P 7 L 7-8	Synchronizing the tense	The tenses have been synchronized.
6	P 10 L 22	"model of Geider et al. (1996)"	We modified the sentence according to the comment.
7	P 13 L 16-17	Eq. 2	We modified the sentence according to the comment.
8	P 30 L 2	Add a ".".	We modified the sentence according to the comment.
9	P 32 L 17	Eulerian approach	We modified the sentence according to the comment.

We wish to thank you for your guidance and comments, which were invaluable in improving the manuscript.

A dynamic model of darkness tolerance for phytoplankton: model description

Eiichi FURUSATO*, Takashi ASAEDA

Department of Environmental Science & Technology, Saitama University, 255 Shimo-okubo, Sakura-ku, Saitama-shi, Saitama 338-0825, Japan

Key words: Phytoplankton, darkness tolerance, dynamic model, cell quota, catastrophic death, lag phase

* Corresponding author. Tel.: +81 48 858 3123; fax: +81 48 858 3123. E-mail address: furusato@ah8.mopera.ne.jp (E. Furusato).

This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to *Hydrobiologia*.

Abstract

To analyze various effects of prolonged darkness on phytoplankton population dynamics, we developed a dynamic model of darkness tolerance for phytoplankton and investigated its characteristics. To construct the basic concepts of the model, we categorized various changes in abundance of phytoplankton during prolonged darkness and after reillumination into several patterns, and then considered the physiological processes producing these patterns. The nature of darkness tolerance was considered to incorporate previously experienced light conditions, including darkness, as a physiological activity, and members of the same phytoplankton species exhibit different dynamics even in identical light conditions due to such career effects. Taking this into consideration, we developed a cell quota model in relation to darkness tolerance. State variables for abundance were indicated by cell numbers and physiological condition by three intracellular carbon pools with different physiological functions.

Using our model, we analyzed the various changes in abundance of phytoplankton in relation to exposure to prolonged darkness. Various responses in terms of phytoplankton abundance to prolonged darkness and after reillumination were successfully reproduced by the model that simply assumed that deterioration of physiological mechanics, such as photosynthetic functions, was due to a prolonged dark condition. Based on the results of calculation and assumptions for the model, we discuss the limitations, problems, and effectiveness of the model.

Introduction

Prolonged darkness is one of the important ecological factors relating not only to the dynamics of a phytoplankton population and community but to also the management of water quality and aquatic ecosystems. The avoidance of loss is necessary for survival of populations and for competitive advantage. The general loss processes of phytoplankton are hydraulic wash out, sedimentation, grazing, and infection (Reynolds, 1984, 1997). Death due to prolonged darkness is also one of the loss factors (Reynolds, 1997). Recently, programmed cell death (PCD) in phytoplankton induced by prolonged darkness has been reported (Segovia et al., 2003; Franklin & Berges, 2004).

The vertical distribution of phytoplankton is often highly heterogeneous (Moll & Stoermer, 1982; Reynolds, 1984; Klausmeier & Litchman, 2001). The prominent vertical aggregations of phytoplankton in oligotrophic and mesotrophic water bodies are deep chlorophyll maxima (DCM) due to limitation of nutrients, which are often supplied from below (Moll & Stoermer, 1982; Klausmeier & Lichtman, 2001). DCM has been observed in lakes (Tilzer et al., 1977; Abbott et al., 1984; Coon et al., 1987; Gervais, 1997) and in the oceans (Takahashi et al., 1989; Moore et al., 1998; Jochem, 1999). These deep layers are often below the level of 1% surface light intensity in oligotrophic lakes (Kiefer et al., 1972; Vincent, 1978; Moll & Stoermer, 1982) and oceans (Antia, 1976; Murphy & Cowles, 1997; Peters, 1996). In ice-covered polar seas and lakes, light is absent during the polar night, yet the presence of various phytoplankton such as diatoms (Wright, 1964; Bunt & Lee, 1972; Palmisano & Sullivan, 1982), Cryptophyceans (Morgan & Kalff, 1975), Chrysophyceans (Rodhe, 1955), cyanobacterias, and Chlorophyceans (McKnight et al., 2000) beneath the ice have been

reported. Furthermore, thick ice and snow cover prevent light penetration. Thus, these phytoplanktons survive in darkness. In addition to such natural conditions, artificial circulation systems (Pastorok et al., 1982) installed in eutrophic reservoirs for prevention of cyanobacterial bloom entrain surface phytoplankton into deep aphotic layers by density currents (Visser et al., 1996; Furusato et al., 2004). The succession of a phytoplankton community will depend on the relationship between hydraulic conditions and the ecophysiological characteristics of phytoplankton. Furusato et al. (2004) suggested that *Microcystis aeruginosa*, a typical bloom-forming and harmful cyanobacterium, has low darkness tolerance. Conversely, Wu et al. (2008) reported that *M. aeruginosa* has endurance and the ability to adapt to the stress of darkness. Clarification of the characteristics of the darkness tolerance of undesirable cyanobacteria can provide important information for water quality management. Furthermore, darkness tolerance is an important factor in facilitating stock culture maintenance (Antia & Cheng, 1970).

From the ecological viewpoint, we need to pay attention to the effects of prolonged darkness on population dynamics of the autotrophic vegetative cells of phytoplankton. Among the several mechanisms known for the survival of phytoplankton in dark condition are formation of resting stages, heterotrophic nutrition, and reduced respiratory activity (Dehning & Tilzer, 1989). In addition to the formation of resting spores by diatoms (Sicko-Goad et al., 1989), akinetes by cyanobacteria (Li et al., 1997), and cysts by dinoflagellates (Rengefors & Anderson, 1998), altered energy acquisition modes such as heterotrophy (White & Shiro, 1975) and phagotrophy (Bird & Kalff, 1987) are active strategies of adaption to darkness. However, Gibson & Smith (1982) suggested that heterotrophic growth of phytoplankton is impossible *in situ* because the rate of organic

material uptake becomes a limiting factor. Besides, the formation of resting stages and availability of heterotrophic nutrition are not always shown. In such conditions, the darkness tolerance of autotrophic vegetative cells affects the dynamics of the phytoplankton community and competition. In this paper, we use the term "darkness tolerance" to mean the tolerance of an autotrophic vegetative cell to prolonged darkness without formation of resting stages.

The time scale of darkness investigated in this study was from a few days to several weeks. The effects of variations in light intensity including darkness in periods of less than several hours on phytoplankton have been extensively studied as photoacclimation or photoadaptation (Woods & Onken, 1982; Falkowski et al., 1985; Lande & Lewis, 1989; Takahashi et al., 1989; Geider et al, 1996, 1998; Cianelli et al., 2004). However, periods of darkness longer than a few hours sometimes occur *in situ* in natural (Smayda & Mitchell-Innes, 1974; Morgan & Kalff, 1975; Vincent, 1978; McKnight et al., 2000) and artificial conditions (Furusato et al., 2004). Some studies suggest the distinct differences in darkness tolerance among various phytoplankton were confirmable after a few days or weeks of darkness (Peters, 1996; Peters & Thomas, 1996; Furusato et al., 2004). Thus, this study covered periods of darkness from a few days to several weeks.

Conflicting reports of changes in abundance of phytoplankton not only during prolonged darkness but also after reillumination have been reported. Generally, specific mortality rates under certain external conditions like at constant temperature appear to be constant. However, the rate of decrease in cell numbers during prolonged darkness is not constant. Phytoplankton cell numbers do not always decrease immediately after the transition to darkness (Handa, 1969; Selvin et al., 1988; WRDPC, 1989; Gervais, 1997; Franklin & Berges, 2004). Short-term increases in abundance during periods of

darkness (Handa, 1969; Griffiths, 1973; White & Shiro, 1975; Peters, 1996; Peters & Thomas, 1996; Gervais, 1997; Berges & Falkowski, 1998; Jochem, 1999; Furusato et al., 2004) and retention of abundance during certain periods of darkness have also been reported (Hellebust & Terborgh, 1967; Griffiths, 1973; Selvin et al., 1988: Dehning & Tilzer, 1989; Gervais, 1997; Berges & Falkowski, 1998; Jochem, 1999; Furusato et al., 2004). Further, logarithmic growth does not always start immediately after reillumination (Peters, 1996; Murphy & Cowles, 1997; Jochem, 1999; Furusato et al., 2004), but often starts after a lag phase (Griffiths, 1973; Dehning & Tilzer, 1989; WRDPC, 1989; Peters, 1996; Peters & Thomas, 1996; Gervais, 1997; Jochem, 1999; Franklin & Berges, 2004; Furusato et al., 2004). In addition, several studies reported that growth did not occur after reillumination (Antia, 1976; WRDPC, 1989; Gervais, 1997; Jochem, 1999; Franklin & Berges, 2004). These conflicting patterns suggest the variety of darkness tolerances of phytoplankton. However, little is known about the characteristics of tolerance in response to prolonged darkness.

As far as we know, no mathematical model for darkness tolerance has been reported to date. Many of the studies cited above have reported on the vegetative reproduction of phytoplankton cells in an autotrophic metabolic state under several dark conditions. However, they were confined to laboratory experiments and field observations.

Under such circumstances, we propose a dynamic model of autotrophic vegetative phytoplankton cells, which enables the estimation of the effects of darkness over several days on phytoplankton population dynamics. Because this model would be a first attempt to analyze phytoplankton dynamics in relation to prolonged dark conditions on a time scale of a few days to several weeks, we focused only on the effects of light, including prolonged darkness. Thus, the effects of other factors such as temperature and

nutrients on physiological processes were not considered in the present version of the darkness-tolerance model. Our present model assumes nutrient-saturated conditions and a constant temperature. The extension of the model will be discussed in the Discussion section.

Materials and Methods

Categorization of darkness tolerance

We proposed a novel categorization of the changes in abundance of phytoplankton, as shown in Fig. 1, and used it as the basis for a darkness-tolerance model. Various changes in abundance of phytoplankton, not only during prolonged darkness but also after reillumination have been reported (Table 1), but very few attempts have been made to classify the diversity of phytoplankton dynamics from a comprehensive viewpoint. Here, we suggest a new categorization. The tolerance patterns under prolonged darkness were divided into three types: D1, D2, and D3. Type D1 is typified by an increase in abundance immediately after the transition to darkness, and then cell numbers decrease or remain constant. D2 is characterized by preservation of the initial abundance of cells for a certain period after the transition to darkness. D3, which seems to be the most general pattern, is characterized by a rapid decrease immediately after the transition to darkness. Changes in cell numbers during regrowth after reillumination were also divided into three types: L1, L2, and L3. In type L1, regrowth at a rate similar to that before the transition to darkness starts immediately after reillumination. Type L2 is characterized by the presence of a lag phase in which the growth rate is low. Finally, there is type L3, in which regrowth does not occur after reillumination, and the population ultimately disappears. Type L3 is an

Fig. 1

Table 1

interesting case where the light history affects phytoplankton dynamics.

We assumed that each darkness-tolerance type has several phases of simple physiological conditions, which are shown in Fig. 1. Types D1, D2, and D3 comprise basically a tolerant phase and a catastrophic phase. In the tolerant phase, cell numbers do not decrease rapidly and physiological states do not deteriorate due light deficiency. The tolerant phase includes short-term cell division. We assumed the catastrophic phase to be the condition in which cell numbers decrease rapidly. Like the prolonged darkness types, the combination of a lag phase and a logarithmic regrowth phase characterizes types L1, L2, and L3 after reillumination. We assumed that the lag phase is a state that does not represent the growth rate expected from the ambient light intensity, even after reillumination.

Model description

In the model, state variables of abundance and physiological state were separated. These were cell number and three cellular carbon contents, respectively. We assumed that a history of having experienced darkness periods in the past accumulates in the intracellular physiological conditions, that physiological phases were due to these conditions, and that these phases determine the changes in abundance without direct response to external light conditions. Hence, to analyze darkness tolerance, it is necessary to represent the effects of darkness on the integrity and degradation of the physiological state and define the rates of increase and decrease in abundance corresponding to intracellular physiological conditions. Fig. 2 shows the relationship among these components, cell numbers, and several important processes in the model. List of definitions of variables and parameters is shown in Table 2.

Fig. 2

Table 2

Changes in cell number were assumed to consist of cell division and death, given by Eq. (1).

$$\frac{dN}{dt} = \left(G_{div} - M\right) \cdot N \tag{1}$$

where N is the number of cells, G_{div} is the rate of cell division , and M is the mortality rate. Because the cell number is not decreased by respiration metabolism itself, a term for respiration is not included. To focus on darkness tolerance, grazing by zooplankton was ignored in this study.

The cell division rate is related to intracellular carbon concentration using the Droop formula (Droop, 1973).

$$G_{div} = G_{div.m} \cdot \left(\frac{E - E_{\min}}{E}\right) \tag{2}$$

where $G_{div.m}$ is the maximum cell division rate, E is the intracellular carbon concentration of the biosynthetic apparatus, and E_{min} is the minimum value of E for cell division. Generally, the Droop model has been used to represent the effects of the cell quota on growth rate (Droop, 1973). Although the Droop model was often applied to nutrients, such as nitrogen and phosphorus (Janse et al., 1992; Cerco et al., 2004; Mooij et al., 2007) and vitamins (Droop, 1973), we used the cell carbon concentration as the cell quota in the Droop model. There have been several studies dealing with the relationship between the cell carbon concentration and growth rate under not only CO_2-limited conditions (Goldman & Graham, 1981) but also CO_2-replete conditions (Falkowski et al., 1985; Thompson et al., 1991). Klausmeier et al. (2004) used a multiple resources version, including the cell carbon concentration, of Droop's model

for phytoplankton growth, with equations for cell number. Rhee (1978) reported that cell protein, RNA, and free amino acids were all related to the growth rate by a saturation function. Because the relationship between the quantity of E and the organic nitrogen concentration was implicitly assumed to be linear function in our model, these chemical components correspond to E. Thus, we postulated the dependence of the cell division rate on E.

We modeled the discontinuous increase in the mortality rate under prolonged darkness as Eq. (3).

$$M = \begin{vmatrix} M_0 & \text{if:} & E & \geq E_{th.c} \\ M_c & & & < E_{th.c} \end{vmatrix} \quad (3)$$

where $E_{th.c}$ is the threshold value of E for the catastrophic phase, M_0 is the mortality rate in the tolerant phase, meaning the physiological integrity state, and M_c is the mortality rate in the catastrophic phase. The transition from tolerant to catastrophic phases due to deterioration of the metabolic state was represented as changes in the mortality rate according to $E_{th.c}$. Morgan & Kalff (1975) reported that the death rate of *Cryptomonas erosa* was shown to increase according to a decrease in cell volume. Dehning & Tilzer (1989) reported that the mortality rate increased when the intracellular carbon content decreased to a certain point. Thus, we assumed that the mortality rate changes according to $E_{th.c}$.

The intracellular carbon concentration, which regulates the rate of increase and decrease of abundance, of the three functional groups was represented by the allocations of photosynthates to each carbon pool and the respiratory loss.

Functional groups of intracellular carbon pools

Table 3

The total cellular organic carbon consists of the sum of three components, as shown in Eq. (4) (Table 3). We used the functional groups of cellular carbon adopted in the model of Geider et al. (1996).

$$IC = E + L + R \tag{4}$$

where IC is the total intracellular carbon content, E is the biosynthetic apparatus, L is the light-harvesting apparatus, and R is the stored energy reserve.

Separate cellular carbon pools represent characteristics of physiological functions relating to darkness tolerance. Carbon, a component of most cellular matter, exists in various chemical forms and has diverse physiological functions. Griffiths (1973) reported the importance of enzymes and proteins that carry out the photosynthetic function to the ability of diatoms to survive in darkness. The results of their experiments strongly suggested that the reduction in total protein following prolonged culture in the dark correlates well with the observed changes in photosynthetic capacity. Carbohydrates as an energy reservoir decrease when short-term cell division occurs after the transition to darkness (Handa, 1969). Besides, the Chla concentration varies with fluctuations in light intensity as photoadaptation occurs. Thus, we used the above division of cellular carbon.

State equations of cellular carbon

The three intracellular carbon pools were assumed to vary in quantity according to the proportion of photosynthates and the inherent respiration rate of each component. Eqs. (5), (6), and (7) show the state equations of each component.

$$\frac{dE}{dt} = \left(P^E \cdot \rho_E - r_0 \right) \cdot E \quad (5)$$

$$\frac{dL}{dt} = P^E \cdot \rho_L \cdot E - r_0 \cdot L \quad (6)$$

$$\frac{dR}{dt} = P^E \cdot \rho_R \cdot E - r \cdot R \quad (7)$$

where P^E is the E-specific rate of photosynthesis, ρ_E is the proportion of photosynthates allocated to the synthesis of E, r_0 is the maintenance metabolic rate, r is the respiratory consumption rate of reserve material, ρ_L is the proportion of photosynthates allocated to synthesis of L, and ρ_R is the proportion of photosynthates allocated to synthesis of R.

Different respiration rates were used to describe the variations in each component according to its properties. The respiration rate is the sum of the maintenance metabolic rate independent of growth and the increase in the growth rate, being equivalent to biosynthesis costs, as will be more fully described later. Reserve carbohydrates are consumed for biomass synthesis during short-term cell division after transfer to darkness, although other cellular components, such as protein and Chla, do not decrease (Handa, 1969; Foy & Smith, 1980). Thus, we used a distinct respiration rate for R.

Photosynthetic rate

Asymptotic exponential equation type models (Geider et al., 1996) were used to describe the *PI* curve, and deterioration of the photosynthetic ability in the lag phase was represented as a change in the maximum photosynthetic rate according to $E_{th.lag}$. The photosynthetic rate was represented as Eq. (8).

$$P^E = P^E{}_m \left[1 - \exp\left(\frac{-\sigma \cdot I \cdot L}{P^E{}_m \cdot E} \right) \right] \quad \text{if: } E < E_{th.lag} \qquad P^E{}_m \equiv P^E{}_{m.lag} \tag{8}$$

where $E_{th.lag}$ is the threshold value of E for the lag phase, $P^E{}_m$ is the maximum E-specific rate of photosynthesis, $P^E{}_{m.lag}$ is the $P^E{}_m$ in the lag phase, σ is the functional cross-section of L, I is the irradiance, and σ corresponds to the initial slope of the PI curve. The maximum photosynthetic rate is changed from $P^E{}_m$ to $P^E{}_{m.lag}$ when E is less than the threshold value of E for the lag phase ($E_{th.lag}$). Generally, temperature and nutrients affect the photosynthetic rate. However, because the main aim of our investigation was the development of a model focusing on light conditions, including prolonged darkness, only light intensity was considered as a limiting factor for photosynthesis.

The lag phase is an important factor for representing various types of darkness tolerances in reillumination. The lag phase after prolonged dark conditions is characterized by a reduction in the cell division rate compared to that of sound cells. The effect of accumulations of the history of past light conditions on the cell division rate can be expressed by Eq. (2). In addition to this effect, a decline in the maximum photosynthetic rate (Dehning & Tilzer, 1989), degradation in the dark reaction of photosynthesis (Griffiths, 1973; Franklin & Berges, 2004), and declining maximum quantum yield (Dehning & Tilzer, 1989; Franklin & Berges, 2004) have been reported. Thus, these decreases in photosynthetic potential and the reduction in the photosynthetic rate itself have to be included in a darkness tolerance model. Griffiths (1973) and Dehning & Tilzer (1989) stated that the lag phase after reillumination includes periods of repair and reconstruction of the photosynthetic function, which is damaged by prolonged darkness. Griffiths suggested that the deterioration of photosynthetic capacity is largely

due to a decrease in the enzymatic components of the photosynthetic machinery. Thus, if a physiologically faithful model is to be developed, the amount of photosynthetic and biosynthetic apparatus has to correlate with the maximum photosynthetic rate (P^E_m). However, little is known about the relationship between photosynthesis and biosynthesis and the maximum photosynthetic rate (P^E_m). Therefore, we assumed that the maximum photosynthetic rate under saturated light intensity in the lag phase changes from P^E_m to the rate decreased by darkness deterioration ($P^E_{m.lag}$).

Respiration rate

The respiration rate was expressed as a linear function of G_{div} (Eq. (9)).

$$r = r_0 + k_r \cdot G_{div} \tag{9}$$

where r_0 is the maintenance metabolic rate, and k_r is a coefficient of the effect of the cell division rate on the respiratory rate. r_0 is the respiration rate under the conditions in which growth does not occur and represents the basic maintenance metabolism necessary to sustain physiological activity. k_r is the cost of synthesis of new cell components (Geider & Osborne, 1989). The respiration rate has important effects on the survival of phytoplankton in prolonged darkness (Dehning & Tilzer, 1989; Peters, 1996; Peters & Thomas, 1996). Generally, the respiration rate equals only 10% of the photosynthetic rate (Parsons et al., 1984) under a nutrient-replete condition. However, the respiration rate actually changes by multiples of 10 or more according to the growth rate, even in the same species and at the same temperature (Geider & Osborne, 1989). The reason is that assimilated carbohydrate catabolism enables growth by synthesis of various cellular materials such as proteins and enzymes. Thus, the respiration rate should be a function of the rate of conversion of *R* to *E* and *L*. However, our present model assuming simple

physiological processes does not include this metabolism. Thus, we represent the respiration rate as a function of growth. Obviously, cell division is one of the phases of the cell cycle, consisting of protein synthesis, replication of DNA, spindle formation, and chromosome alignment. Thus, this process is not expensive. However, in our model, the cell division rate are treated like the growth rate because we use cell number as a variable state of abundance. Thus, we modeled respiration processes relating to cell division.

Allocation rate of photosynthates

The proportions of photosynthates directed to synthesis of each cellular carbon component were specified as follows (Geider et al., 1996):

$$\rho_E = \kappa_E \tag{10}$$

$$\rho_L = \kappa_L \cdot \frac{P^E \cdot E}{\sigma \cdot I \cdot L} \tag{11}$$

$$\rho_R = \kappa_L \cdot \left(1 - \frac{P^E \cdot E}{\sigma \cdot I \cdot L}\right) \tag{12}$$

where κ_E is the constant proportion of photosynthates directed to synthesis of the biosynthetic apparatus, and κ_L is the maximum proportion of photosynthates directed to synthesis of the light-harvesting component. Conservation of mass requires that $\kappa_{E+}\kappa_L =$ 1.0.

We used Eqs. (10), (11), and (12) regardless of the dark condition. $P^E \cdot E$ and $\sigma \cdot I \cdot L$ in the above equations are equivalent to carbon fixation and light harvesting, respectively. Thus, the rates of allocation to the storage pool (R) and photosynthetic apparatus (L)

depend on the balance of dark and light reactions. Geider et al. (1996) used 0.6 as κ_E and 0.4 as κ_L for two distinct phytoplanktons, and we followed this. Although little is known about the effects of darkness on the allocation of photosynthates, taking the results for *Phaeodactylum tricornutum* (Griffiths, 1973) into consideration, we assumed that prolonged darkness did not affect the allocation of photosynthates to some intracellular components.

Parameterization

The parameters used for analysis (Table 4, 5) were based on the calculated values from experimental results and the theory of allometry as described below. Experimental results using *Scenedesmus acuminatus* (Dehning & Tilzer, 1989), *Cryptomonas phaseolus* (Gervais, 1997), and *Phormidium* sp. PC type (WRDPC, 1989) were selected as examples of phytoplankton dynamics in a prolonged dark condition. *Microcystis aeruginosa* (Furusato et al., 2004) was selected as an example of several darkness periods and after reillumination.

Table 4

Table 5

Because not only cell number data but also carbon and protein cell concentrations have been measured for *S. acuminatus*, the parameter values for this alga were calculated using experimental data. Cell number data were used for definition of $G_{div.m}$, M_0, and M_c. Cell carbon concentration data were used for estimation of r_0, which has important effects on dynamics in darkness. $E_{th.c}$, which sets transition timing from the tolerant phase to the catastrophic phase, was defined by experimental protein values at the end of the tolerant phase. Transformation from protein to carbon was obtained using a general ratio of carbon to protein (0.5 [pg C pg^{-1} protein]) (Geider et al., 1996). Although lipid, as the main component of thylakoids in *E*, cannot be neglected in the estimate of $E_{th.c}$, values transformed from protein were used in this analysis.

Because the experimental results for *Phormidium* sp. and *C. phaseolus* did not include a carbon cell quota, only parameters for variation in cell number, such as $G_{div.m}$, M_0, and M_c, were defined by experimental data. Values of $E_{th.c}$, E_{min}, and r_0 were estimated by fitting them to cell number changes.

The parameters of *Microcystis aeruginosa* used to model dynamics of phytoplankton in darkness and reillumination were defined as described below. The value of $G_{div.m}$ was estimated based on the allometric equation ($G_{div.m}$ = 1.142 ($S\ V^{-1}$) $^{0.375}$) (Reynolds, 1997): cell volume (*M. aeruginosa*), 101 [μm^3 $cell^{-1}$] (Imamura & Yasuno, 1981); corresponding diameter, 3.5 [μm]; and corresponding to surface/volume ratio (S / V), 0.86 [μm^{-1}]. In the laboratory culture, colonies have not been formed, and the single cell volume was used. M_0 and M_c were calculated by changes in abundance of experiments. The value of P^E_m corresponding to $G_{div.m}$ through κE ($G_{div.m} = \kappa E \cdot P^E_m$) was used. The value of P^E_m was assumed to be half of $P^E_{m.lag}$. Each threshold value for E, such as E_{min}, $E_{th.c}$, and $E_{th.lag}$, and r_0 was estimated by fitting it to cell number changes. σ was calculated from Reynolds (1997) ($\sigma = 0.257(m\ S\ V^{-1})^{0.236}$), where m = 3.5 [μm] and $S\ V^{-1}$ = 0.86 [μm^{-1}]. The value of κ_r from the literature was used (Geider & Osborne, 1989).

Initial conditions

Tables 4 and 5 show the initial conditions defined as below described.

Scenedesmus acuminatus

The initial cell number was defined based on the experimental value. The initial cellular carbon concentration was calculated from the following assumptions based on laboratory experiments (Dehning & Tilzer, 1989). Total cellular carbon (IC = 21.35 [pg C $cell^{-1}$]) was calculated from their experimental value of the dry weight of cells (49.6

[pg D.W. cell^{-1}]) using the general carbon/D.W. ratio (0.43 [g C g^{-1} D.W.] (Strickland, 1965)) of Chlorophyceae. The initial value of E was calculated from the experimental value (Dehning & Tilzer, 1989) of protein (26.4 [pg protein cell^{-1}]) using the above carbon/protein ratio (0.5 [g C g^{-1} protein]). These values correspond closely to κ_E (0.6). The initial value of L was calculated from the experimental values of Chla (0.72 [pg Chla cell^{-1}]) (Dehning & Tilzer, 1989) using the general Chla/carbon ratio (0.2 [g Chla g^{-1} C]) of the light harvesting apparatus (Geider et al., 1996). R is the remainder of the cellular carbon components.

Phormidium sp.

The initial cell number was defined based on the experimental value (WRDPC, 1989). In defining the cellular carbon concentration, the cellular volume (22.11 [μm^3 cell^{-1}]) of *P. tennue* (Imamura & Yasuno, 1981) was used because the original experiments focused on 2-methylisoborneol production in a eutrophied reservoir in Japan. The initial value of the total cellular carbon concentration (IC) (3.63 [pg C cell^{-1}]) was calculated by the D.W. and cell volume transformation equation (D.W. = 0.47 $V^{0.99}$) (Reynolds, 1984), and the general carbon/D.W. ratio in cyanobacteria (0.36 [g C g-1 D.W.] (Strickland, 1965). E was calculated using κE (0.6). The initial value of L was calculated by an empirical equation of the relationship between the cell volume (V) and cell Chla of cyanobacteria (log Chla = 1.00 log$V^{-2.261}$) (Reynolds 1984) and by using the general Chla/carbon ratio (0.2 [g Chla g^{-1} C]) of the light harvesting apparatus (Geider et al., 1996). R is the remainder of the cellular carbon components of this phytoplankton.

Cryptomonas phaseolus

The initial cell number was defined based on an experimental value (Gervais, 1997).

For definition of cellular carbon concentrations, the cell volume (250 [μm^3 $cell^{-1}$]) of *C. erosa* was used (Imamura & Yasuno, 1981). Like *P. tennue*, the initial values of *E*, *L*, and *R* were estimated from cell volume.

Microcystis aeruginosa

The definitions of the initial conditions of *M. aeruginosa* were similar to those of *Phormidium* sp.

Results

From the experimental results, including the changes in abundance under prolonged darkness over several days and after the transition to reillumination, representative examples of each darkness-tolerance type (Dehning & Tilzer, 1973; WRDPC, 1989; Gervais, 1997; Furusato et al. 2004) were selected for simulations. In all these experiments, different inorganic mediums were used and evidence of resting stages, such as cyst and akinetes formation, was not observed. Thus, these experimental results can be used to analyze the effects of prolonged darkness on the changes in abundance of autotrophic vegetative cells of phytoplankton. Discrete approximations in time to the differential equations were solved with a fourth order Runge-Kutta method. Time steps were adjusted by trial and error to be small enough that doubling the time steps did not significantly change the simulation results. This resulted in a time step of 1 hour for the model.

Sensitivity analysis

A sensitivity analysis was performed to investigate the effects of the model parameters

on the results by changing them $\pm 50\%$. The index below was used to determine sensitivity (Jørgensen & Bendoricchio, 2001).

$$S = \left| \frac{\Delta C / C}{\Delta P / P} \right| \tag{13}$$

where C is the logarithm of the number of cells, and P is the parameter value. The logarithm was used because we observed extreme variations in cell numbers. If the S value was equal to 10, C varied by 500% because P values were changed by 50%.

Analyses were performed separately for darkness and reillumination because it was envisioned that the sensitivity to the parameters values differed in both prolonged darkness and reillumination. Table 6 shows the results of sensitivity analysis. As might be expected, the effects of photosynthetic parameters are limited to reillumination. In prolonged darkness, we found that the model was the most sensitive to M_c, and the S values of r_0 and $E_{th.c}$ were the next largest. On the other hand, parameters relating to photosynthesis and cell division tended to be highly sensitive during reillumination. In spite of the use of cell number as state variable under consideration, the S value of the parameters, which directly regulates the changes in cell number, such as $G_{div.m}$, was not the largest. That P^E_m and κ_E had a larger S value than $G_{div.m}$ is a reflection of importance of the cell carbon quota in the darkness-tolerance model.

Table 6

Simulation results

Prolonged darkness

Figs. 3 and 4 show the calculated results of the three types of darkness tolerance (D1, D2, and D3). The parameter values shown in Table 4 were used. *Scenedesnmus acuminatus* (Chlorophyeae) represents a typical type D2 pattern (Dehning & Tilzer,

Fig. 3

1989). In this experiment, dark incubation was done for about 50 days in two water temperature conditions to investigate the effects of prolonged darkness on the physiology, as well as cell morphology and changes in cell number. The cell number after reillumination was not determined. Because various cellular organic materials were analyzed in this study, values converted from the protein concentration were used for simulation as observed *E*. Parameter values were defined by calculation and conversion from observed data such as cell numbers and *E* (Table 4). Our model successfully represented the results of the experiment, which are a decrease in the cellular carbon concentration for the maintenance of metabolism at a uniform rate through the experimental periods and a rapid decrease in cell numbers after certain darkness periods

The next calibration was conducted for dark incubation data of *Cryptomonas phaseolus* (Cryptophyceae) (Gervais, 1997) as a representative example of type D3 (Fig. 4A). *Cryptomonas* produces deep chlorophyll maxima in mesotrophic or eutrophic lakes (Gervais, 1997). Changes in abundance during 10 days of darkness were studied by culture experiments.

Fig. 4

We defined several parameters, such as the respiration rate and threshold values related to *E*, by calibration because this study did not directly analyze intracellular organic carbon. The cell number started to decrease immediately after the transition to darkness. It decreased particularly rapidly after 6 days of darkness. Assuming that the tolerant phase shifted to the catastrophic phase at 6 days of darkness, we aimed to represent the phenomenon by changes in the mortality rate. As shown in Fig. 4A, the calculation results showed the tendency of *C. phaseolus* to correspond to type D3.

Fig. 4B shows the results of a simulation using cyanobacteria (*Phormidium* sp. PC

type) (WRDPC, 1989) as an example of type D1. This species dominates in eutrophicated reservoirs and causes a musty odor. To study the effects of artificial circulation on prevention of growth of this cyanobacterium, it was maintained in darkness in the laboratory for 20 days. The cell number had increased by approximately 10% 3 days after the transition to darkness, but it decreased rapidly after that. The threshold values of the intracellular carbon concentration were determined by tuning because intracellular materials were not measured in this study. The results in Fig. 4 show that the model was able to successfully reproduce the dynamics of this species.

Reillumination after several periods of prolonged darkness

We analyzed the characteristics of darkness tolerance after reillumination by using laboratory experiment results for *Microcystis aeruginosa* (Furusato et al., 2004) (Fig. 5) as a typical cyanobacterium causing water-bloom in eutrophied water bodies. Artificial circulation using a bubble plume, also referred to as destratification, is a general measure for prevention of cyanobacterial bloom in eutrophic fresh water (Hawkins & Griffiths, 1993). Furusato et al. (2004) suggested that the entrainment of phytoplankton to an aphotic layer was one of the mechanisms of the measure. To evaluate the effects of darkness on the dynamics of this phytoplankton in artificial circulation, changes in cell number during 0, 5, 10, 15, and 20 days of darkness and after reillumination were obtained in the experiment. The temperature was 25℃.

Fig. 5

This experiment demonstrated that types L1 and L2 can occur even in the same species depending on the length of the period of darkness. In prolonged darkness, three phases, consisting of a gradual increase, gradual decrease, and rapid decrease in cell numbers, occurred and each lasted 5 days. This is consistent with type D1. After reillumination, growth resumed (type L1), and cell numbers reached the same maximum regardless of

the period of darkness. However, more than 10 days of darkness resulted in the occurrence of a lag phase (type L2).

Calculation was done continuously from darkness to reillumination by using the same parameters, which were based on allometry (Reynolds, 1984, 1997) and tuning (Table 5). Saturation in abundance in the stationary phase after reillumination, corresponding to carrying capacity, was represented by the saturation density function.

Table 6

By using the same parameter values, our model was able to reproduce the various changes in cell number of each different darkness period. Fig. 5 shows a comparison between calculation results and observations. Some deviations remain because of simple assumptions about metabolic mechanics in our model. For example, there was a remarkable decrease in cell number over 10 days of darkness, a decrease after the stationary phase in the control and 10 days darkness, and gradual growth after reillumination in the cases of 15 and 20 days darkness. However, it is considered that this model can represent the behaviors of types L1 and L2 occurring after reillumination periods, in addition to the changes in abundance of type D1 in 20 days of darkness.

Discussion

The model provides a conceptual framework for describing and modeling darkness tolerance. It summarizes and incorporates all present knowledge into a system, which helps to understand phytoplankton on the population-scale. Although the model depends on the simple assumption that the quantitative conditions of phytoplankton cells (intracellular carbon concentrations of some functional pools) represent qualitative

factors (integrity of metabolism), the calculation results replicated various changes in abundance relating to darkness tolerance. However, the model remains hypothetical, not only because many remaining factors need to be verified but also because other assumptions may produce equally good simulations. Thus, more research measuring the internal carbon concentrations by laboratory methods is needed. However, in some situations, simplified (semi-theoretical) models are easier to apply to an actual system than a full-fledged model that uses a lot of unrecognizable parameters. Furthermore, as described below, simple assumptions used in this study (Fig. 6) may well represent the intrinsic mechanism of various darkness-tolerance types.

Fig. 6

Expression mechanism of darkness tolerance

Prolonged darkness

The physiological activity immediately after the transition to darkness causes various patterns of change in abundance. It has been reported that the degrees of physiological senescence in the pre-culture (Antia & Cheng, 1970) and the growth condition before the transition to darkness (Griffiths, 1973; Gervais, 1997) affect the dynamics after the transition. This agrees with the simple assumptions of our model. The relationship between E and E_{min} or $E_{th.c}$ before the transition to darkness determines which type of darkness tolerance occurs. If E is greater than E_{min} immediately after the transition to darkness, a short-term increase in cell number (type D1) due to cell division can occur in our model. Furthermore, circadian rhythm and synchronized cell division are considered to be two of the causes of this type of cell division. In the converse condition, cell division cannot occur (type D2). When $E_{th.c}$ is greater than E, type D3 behavior occurred.

After a certain period from the transition to darkness, these two parameters, E_{min} and

$E_{th.c}$, also determine the dynamics. For type D1, the decrease in E continues not only by respiration but also by biomass synthesis for cell division. When E becomes lower than E_{min}, cell division is terminated in our model. Some laboratory experiments showed a rapid decrease in organic matter corresponding to short-term cell division after the transition to darkness (Handa, 1969; Peters, 1996), and cell division in prolonged darkness was confined to the immediate aftermath of the transition to darkness (Griffiths, 1973; Peters, 1996; Gervais, 1997; Berges & Falkowski, 1998; Jochem, 1999; Furusato et al., 2004). These observations also agree with the mechanics of our model. For type D1 as well as for D2, when E fell below E_{thrM}, cell numbers rapidly decreased as the catastrophic phase. Because the decrease of E is determined by R_0, these mechanics of darkness tolerance indicate the importance of R_0.

After reillumination

The physiological condition or integrity immediately before the transition to reillumination determines the dynamics after reillumination. If E is greater than E_{min}, cell division starts immediately after the transition to reillumination (type L1). In the converse condition, a lag phase occurs until E exceeds E_{min} due to photosynthesis (type L2). Further, if E is lower than $E_{th.lag}$, the lag phase is prolonged because the increase in E itself is delayed by the low photosynthetic rate ($P^E_{m.lag}$). The lag phase is terminated and shifts to the logarithmic regrowth phase when the E increased by the low photosynthetic rate in the lag phase reaches E_{min}. Incidentally, phytoplankton sometimes totally disappears due to a continuation of the decrease in cell number without regrowth after reillumination (type L3). When E cannot increase because of a degraded photosynthetic rate that is lower than the respiration rate and a slow cell division rate that is lower than the mortality rate in

1 the catastrophic phase, no further regrowth after reillumination occurs, even in light
2 conditions. These mechanics of the model agree with the effects of the length of darkness
3 (Peters, 1996; Peters & Thomas, 1996; Furusato et al., 2004; Franklin & Berges, 2004),
4 light intensity (Morgan & Kalff, 1975; Gervais, 1997) and light-dark cycle (Hellebust &
5 Terborgh, 1967) before the transition to darkness on the growth after reillumination. Thus,
6 our model based on simple physiological assumptions is able to analyze the various
7 responses of phytoplankton to not only prolonged darkness but also after reillumination.
8
9 ***Physiological accuracy of model***
10 The reliability and accuracy of the model depend on the assumptions for model
11 formulation and the parameter values, defining the physiological conditions of each
12 phase for darkness tolerance. Generally, the Droop model is applied to limiting nutrients.
13 In our model, the cellular carbon concentration of biosynthetic machinery (E) was used
14 for Droop model (Eq. (2)). Droop (1983) stated that carbon is not suitable for use in the
15 Droop model. The reason is that the yield of carbon to cellular weight remains
16 unchanged compared to other nutrients such as nitrogen and phosphorus. However, our
17 model uses the carbon concentration per cell, which varies according to the
18 physiological state. Thus, Eq. (2) is consistent with Droop's conditions. Whether
19 inorganic carbon is a limiting factor for primary production or not is the debatable
20 question. Inorganic carbon is decreased by photosynthesis itself, so some studies
21 reported a carbon limitation in ocean (Goldman & Graham, 1981; Riebesell et al., 1993)
22 and fresh water (Talling, 1976; Shapiro 1990; Maberly, 1996). On the other hand, a
23 number of CO_2-concentrating mechanisms, which compensate for low affinity of
24 ribulose 1,5-bisphosphate carboxylase-oxygenase as one of the causes of carbon

limitation, have been found among the different groups of phytoplankton (Badger & Price, 1992; Giordano et al., 2005). However, these focused on the dynamics of inorganic carbon in the euphotic layer. Because the present model is geared toward the phytoplankton dynamics in not only euphotic zones but also the aphotic layer, the assumption of carbon limitation cannot always be applied. Thus, representation of the dynamics of the inorganic carbon concentration in water and parameterization by considering carbon acquisition mechanisms depending on species will be needed for a more accurate model.

Parameter values calculated from experiments and theoretical values based on allometry were used. However, in addition to M_c and $E_{th.c}$ determining the characteristics of the catastrophic phase in darkness, $P^E_{m.lag}$ and $E_{th.lag}$, which determine the characteristics of the lag phase after reillumination, had to be defined by fitting them to cell number changes. These include highly sensitive parameters in sensitivity analysis. Because our model depends on the simple assumption that the physiological condition of cells is represented by the cellular carbon contents, these parameters are important.

If the catastrophic phase is the result of a loss of integrity (Dehning & Tilzer, 1989; Franklin & Berges, 2004) or homeostasis of cells (Furusato et al., 2004), the inherent parameters of each phytoplankton cannot be defined and the validity of the model will be reduced. However, taking into consideration recent findings on PCD in phytoplankton (Segovia et al., 2003; Franklin & Berges, 2004), we are able to determine the parameters relating to the catastrophic phase. PCD, one of the methods of resistance to infection that occurs in cells of higher plants and animals, results in apoptotic and paraptotic morphotypes. One cause of PCD is environmental stresses. Recently, it has been reported that prolonged darkness caused rapid PCD in phytoplankton (Berges &

Falkowski, 1998; Franklin & Berges, 2004). Franklin & Berges (2004) stated that cell death as PCD triggered by energy limitation is probably a significant process in the structuring of phytoplankton populations. If the catastrophic phase is caused by PCD, each phytoplankton possibly has its own inherent expression condition. Although what triggers PCD is unknown, if the expression mechanisms of PCD are revealed in the future, the catastrophic phase can be analyzed by relating it to the regulation rules of our model.

Parameter values such as $E_{th.lag}$ and E_{min} reflect intrinsic species characteristics of the lag phase as a darkness-tolerance property. Peters (1996) and Peters & Thomas (1996) reported that the lag phase is used to repair the photosynthetic function that had deteriorated in prolonged darkness. Thus, a species has an inherent physiological machinery of the lag phase, although more physiological research is needed.

Darkness periods experienced by phytoplankton in situ

In periods of darkness longer than the time scale we assumed, distinct physiological functions have to be included in the model. The spatial distribution of phytoplankton is determined by hydraulic phenomena. In addition, the penetration of light into aquatic ecosystems is affected by absorption and scattering processes that are affected by the water quality (Kirk, 1994). Thus, the light conditions experienced by phytoplankton vary according to the physical and chemical conditions of water bodies. Deep-living phytoplankton in the aphotic layer below the thermocline will be entrained to a surface euphotic zone by vertical mixing due to not only winter cooling but also strong winds. Vincent (1978) reported that the length of residence of an aphotic phytoplankton population in Lake Tahoe might exceed 12 months because of the absence of such vertical mixing. Sea-ice and pelagic phytoplankton in polar regions must survive long periods of winter darkness (Smayda & Mitchell-Innes, 1974). Density currents caused

by artificial circulation in eutrophic reservoirs will entrain surface phytoplankton into deep aphotic layers in periods from a few days to several weeks (Furusato et al., 2004). Although our model focused on the time scale of a few days to several weeks, different processes will occur during longer periods of darkness. Diatoms living in polar regions and having a strong tolerance for darkness often become resting cells without morphological differences from vegetative cells by reducing their cellular metabolism (Peters, 1996; Peters & Thomas, 1996). Thus, physiological processes, which have to be included in a darkness-tolerance model, depend on the length of prolonged darkness. To analyze this phenomenon, further studies are needed.

Extension of the model

The effects of darkness tolerance on the competition among populations depend on the inherent characteristics of each water body. In order to estimate this, a comprehensive phytoplankton model (e.g., Janse et al., 1992; Mooij et al., 2007) that includes various factors (physical, geochemical, and ecological processes) is effective. However, to extend the model, not only improvement in the accuracy of the values of physiological parameters but also consideration of the problems outlined below are needed. These are other factors relating to darkness tolerance in addition to light, such as water temperature, nutrients, the effects of externally caused death, such as those due to predation and infection on the death processes, and the limitations of the Eulerian model for analyzing darkness tolerance.

Temperature

Water temperature is important factor in phytoplankton ecophysiology from the viewpoint of not only governing the rate of almost all metabolic processes but also

darkness tolerance. There are many studies that have pointed out the effects of temperature on the strength of darkness tolerance (e.g., Smayda & Mitchell-Innes, 1974) as temperature affects all biochemical reactions. This is because respiration plays an important role in preservation of stored energy for survival. Wu et al. (2008) suggested that the difference in the response of *Microcystis aeruginosa* to darkness in their study compared to those of Furusato et al. (2004) depends on water temperature. Furthermore, the vertical profile of light intensity is closely tied to water temperature. Thus, the transitions from light to dark and inverse conditions often accompany changes in temperature. To extend the model relating to water temperature to analyze phytoplankton dynamics *in situ*, the function of temperature in photosynthesis and respiration will have to be included. All the rates and time constants should be temperature-dependent in an enhanced, temperature-aware model.

Nitrogen

Chemical factors, particularly nitrogen, have to be related to the darkness-tolerance model. In addition to physical factors besides the length of periods of darkness, such as water temperature (Griffiths, 1973; Antia, 1976; Dehning & Tilzer, 1989), the effects of chemical conditions such as medium composition, pH, and salinity (Antia & Cheng, 1970) on darkness tolerance have been suggested by some experimental laboratory studies. In particular, nitrogen plays important role in not only general physiological processes but also the characteristics of darkness tolerance. Under N-limitation, synthesis of amino acids, protein, and various cellular organelles becomes suppressed. On the other hand, the carbohydrate concentration will increase simultaneously. Thus, N-limitation leads to a reduction in the allocation rate for E and L components. Furthermore, it also decreases the maximum photosynthetic rate (Geider et al., 1998). In

addition to the above processes, some studies suggest the importance of nitrogen in darkness tolerance. The induction of specific proteases in the catastrophic phase (Berges & Falkowski, 1998) and the decrease in cellular protein and photosynthetic enzymes in the lag phase (Griffiths, 1973; Dehning & Tilzer, 1989) have been observed. Additionally, if a phytoplankton includes sufficient carbohydrate reserves in its cell, it will be able to take up nitrogen even in the aphotic layer. Our present model assumes a N-replete condition. However, if we consider various environmental conditions *in situ*, particularly nitrogen, extensions of the model described below will be necessary. One is the separation of nitrogen uptake and growth. Another is representation of the effects of nitrogen quota on both allocation rate to synthesis of the three-carbon components and subsequent photosynthetic ability. Like the photoacclimation model (Geider et al., 1998), the cellular carbon-nitrogen ratio plays an important role in various physiological processes.

Pathogenic infection

External causes of death, such as pathogenic infection, have to be considered in analyzing darkness tolerance. Although our study dealt with internal mortality, the death of phytoplankton *in situ* is affected by external factors such as predation by zooplankton. Pathogenic infection of physiologically deteriorated cells is another external cause of death (Reynolds, 1984). Jochem (1999) reported that cell death increased more during prolonged dark incubation with bacteria than without bacteria. Generally, the death process tends to be modeled simply in dynamic phytoplankton models. However, a mathematical term for infection will be needed because of the importance of the death process in darkness tolerance. Pathogenic infection depends on the rate of contact between the host cells and pathogens (Proctor & Fuhrman, 1991; Beltrami & Carroll,

1994). The burst size of pathogens, particularly viruses or phages, is also one of the factors affecting the infection process (Brown et al., 2006). Several infection models dealing with the interaction between virus and phytoplankton have been proposed (Beltrami & Carroll, 1994; Chattopadhyay & Arino, 1999; Singh et al., 2004).

Limitations of Eulerian model

By adding state variables relating to intracellular carbon components, a comprehensive ecosystem model including physical, chemical, and biological processes will allow analysis of phytoplankton dynamics relating to darkness tolerance in various natural conditions. Most of the differences in the underwater environment in which phytoplankton exists from the terrestrial ecosystem is that water movements define the distribution and transport of phytoplankton. In particular, hydraulic conditions determine prolonged dark conditions experienced by phytoplankton. If mixed depth exceeds euphotic depth, phytoplankton experiences darkness for a certain period. Conversely, in stable stratified water bodies having shallower mixed depth, phytoplankton suffers from prolonged darkness after settling out, except for motile phytoplankton such as cyanobacteria and dinoflagellate. Thus, the mixing process is important to analyze darkness tolerance. Such biological-physical interactions have been modeled in 2 fundamentally different ways, that is to say, the Eulerian model and the Lagrangian model. Eulerian formulations treat phytoplankton populations in terms of their bulk properties, and the averaged photosynthetic response in computed at fixed grid points in the water column. Alternatively, Lagrangian formulations compute phytoplankton growth along the trajectories of individual phytoplankton cells. Traditionally, the Eulerian models are used for analyzing physicochemical and biological processes in comprehensive ecosystem models. Many of the processes that determine the growth and

survival of individual phytoplankton cells are non-linear. The Eulerian continuum method will generally be less accurate than Lagrangian methods because it will basically average non-linear equations (Woods & Onken, 1982). However, the reduced computation requirement and the easier evaluation of aggregated/population properties are advantages of the Eulerian approach.

The Lagrangian approach to simulating phytoplankton dynamics minimizes the errors arising from this non-linearity by simulating the dynamics of many individual phytoplankton cells. Furthermore, it is not too computing-intensive to be used in an ecosystem model (Ross & Sharples, 2007). Some studies have analyzed the photoadaptive response and turbulence-induced vertical displacement (Woods & Onken, 1982; Lizon et al., 1998; Cianelli et al., 2004) and the dynamics of motile phytoplankton (Broekhuizen, 1999) by Lagrangian model. In particular, considering that the characteristics of darkness tolerance are the result of the accumulation of the effects of the history of past experiences of darkness, the Lagrangian model, dealing with individual phytoplankton cells as particles in fluid, is suitable to analyze darkness tolerance.

Conclusion

This paper proposes a dynamic model of autotrophic vegetative cells of phytoplankton. This model enables estimation of the effects of darkness over several days on phytoplankton population dynamics. In the construction of the basic concepts of the model, we categorized the results of many experiments dealing with darkness into several types of changes in abundance, and we assumed several physiological phases consisting of these various types. Then, assuming that these phases correspond to the physiological

integrity, we developed a model in which degrees of health were connected with quantitative conditions of intracellular functional components. Descriptions of both sound and deteriorated physiological states relating to darkness tolerance were formulated by the traditional framework of phytoplankton population dynamics. In the sensitivity analysis, we found that the parameters relating to regulation of carbon cell quota, particularly E, tended to be more sensitive to changes in abundance than the parameters that directly regulate cell number, such as $G_{div.m}$. Taking these tendencies into consideration, a simulation was done using several experimental laboratory data in which prolonged darkness caused various changes in abundance. The model successfully represented the various changes. Furthermore, we discussed the limits and extensibility of the model. Although in this paper we described the characteristics of responses to only light conditions as a submodel, we expect further extension to a whole biogeochemical model that includes various physical, chemical, and biological factors such as temperature, nutrients, and pathogens. This will make it possible to analyze competition corresponding to the strength of darkness tolerance of each phytoplankton. In addition, the simple physiological assumptions in the model are expected to be useful in problem-finding studies on darkness tolerance.

Acknowledgements

We are extremely grateful for thoughtful comments of two anonymous reviewers.

References

Abbott, M.R., K.L. Denman, T.M. Powell, P.J. Richerson, R.C. Richards & C.R. Goldman, 1984. Mixing and the dynamics of the deep chlorophyll maximum in Lake Tahoe. Limnology and Oceanography 29: 862-878.

Antia, N.J., 1976. Effects of temperature on the darkness survival of marine microplanktonic algae. Microbial Ecology 3: 41-54.

Antia, N.J. & J.Y. Cheng, 1970. The survival of axenic cultures of marine planktonic algae from prolonged exposure to darkness at 20 C. Phycologia 9: 179-183.

Badger, M.R. & G.D. Price, 1992. The CO_2 concentrating mechanism in cyanobacteria and microalgae. Physiologia Plantarum 84: 606-615.

Beltrami, E. & T.O. Carroll, 1994. Modeling the role of viral disease in recurrent phytoplankton blooms. Journal of Mathematical Biology 32: 857–863.

Berges, J.A. & P.G. Falkowski, 1998. Physiological stress and cell death in marine phytoplankton: Induction of proteases in response to nitrogen or light limitation. Limnology and Oceanography 43: 129-135.

Bird, D.F. & J. Kalff, 1987. Algal phagotrophy: Regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae). Limnology and Oceanography 32: 277-284.

Broekhuizen, N., 1999. Simulating motile algae using a mixed Eulerian-Lagrangian approach: does motility promote dinoflagellate persistence or coexistence with diatoms?. Journal of Plankton Research 21: 1191-1216.

Brown, C.M., J.E. Lawrence & D.A. Campbell, 2006. Are phytoplankton population density maxima predictable through analysis of host and viral genomic DNA content?. Journal of

Marine Biological Association of the United Kingdom 86: 491-498.

Bunt, J.S. & C.C. Lee, 1972. Data on the composition and dark survival of four sea-ice microalgae. Limnology and Oceanography 17: 458-461.

Cerco, C.F., M.R. Noel & D.H. Tillman, 2004. A practical application of Droop nutrient kinetics (WR 1883). Water Research 38: 4446-4454.

Chattopadhyay, J. &. O. Arino, 1999. A predator-prey model with disease in the prey. Nonlinear Analysis 36: 747-766.

Cianelli, D., M.R. D'alcala, V. Saggiomo & E. Zambianchi, 2004. Coupling mixing and photophysiological response of Antarctic plankton: a Lagrangian approach. Antarctic Science 16: 133-142.

Coon, T. G., M. Lopez, P. J. Richerson, T. M. Powell & C. R. Goldman, 1987. Summer dynamics of the deep chlorophyll maximum in Lake Tahoe. Journal of Plankton Research 9: 327–344.

Dehning, I. & M.M. Tilzer, 1989. Survival of *Scenedesnmus acuminatus* (Chlorophyceae) in darkness. Journal of Phycology 25: 509-515.

Droop, M.R., 1973. Some thoughts on nutrient limitation in algae. Journal of Phycology 9: 264-272.

Droop, M.R., 1983. 25 years of algal growth kinetics; a personal view. Botanica Marina 26: 99-112.

Falkowski, P.G., Z. Dubinsky & K. Wyman, 1985. Growth-irradiance relationships in phytoplankton. Limnology and Oceanography 30: 311-321.

Foy, R.H. & R.V. Smith, 1980. The role of carbohydrate accumulation in the growth of planktonic *Oscillatoria* species. British Phycological Journal 15: 139-150.

Franklin, D.J. & J.A. Berges, 2004. Mortality in cultures of the dinoflagellate *Amphidinium carterae* during culture senescence and darkness. Proceedings of the Royal Society of

London, Series B: Biological Science 271: 2099-2107.

Furusato, E., T. Asaeda & J. Manatunge, 2004. Tolerance for prolonged darkness of three phytoplankton species, *Microcystis aeruginosa* (Cyanophyceae), *Scenedesmus quadricauda* (Chlorophyceae), and *Melosira ambigua* (Bacillariophyceae). Hydrobiologia 527: 153-162.

Geider, R.J., H.L. MacIntyre & T.M. Kana, 1996. A dynamic model of photoadaptation in phytoplankton. Limnology and Oceanography 41: 1-15.

Geider, R.J., H.L. MacIntyre & T.M. Kana, 1998. A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. Limnology and Oceanography 43: 679-694.

Geider, R.J. & B.A. Osborne, 1989. Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth. New Phytologist 112: 327-341.

Gervais, F., 1997. Light-dependent growth, dark survival, and glucose uptake by cryptophytes isolated from a freshwater chemocline. Journal of Phycology 33: 18-25.

Gibson, C.E. & R.V. Smith, 1982. Freshwater phytoplankton. In: Carr, N.G. & B.A. Whitton (Eds), The Biology of Cyanobacteria. Blackwell Scientific Publications, Oxford: 463-489.

Giordano, M., J. Beardall & J.A. Raven, 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annual Review of Plant Biology 56: 99-131.

Goldman, J.C. & S.J. Graham, 1981. Inorganic carbon limitation and chemical composition of two freshwater green microalgae. Applied and Environmental Microbiology 41: 60-70.

Griffiths, D.J., 1973. Factors affecting the photosynthetic capacity of laboratory cultures of the diatom *Phaeodactylum tricornutum*. Marine Biology 21: 91-97.

Handa, N., 1969. Carbohydrate metabolism in the marine diatom *Skeletonema costatum*. Marine

Biology 4: 208-214.

Hawkins, P. R. & D. J. Griffiths, 1993. Artificial destratification of a small tropical reservoir: effects upon the phytoplankton. Hydrobiologia 254: 169-181.

Hellebust, J.A. & J. Terborgh, 1967. Effects of environmental conditions on the rate of photosynthesis and some photosynthetic enzymes in *Dunaliella tertiolecta* Butcher. Limnology and Oceanography 12: 559-567.

Imamura, N. & M. Yasuno, 1981. Changes in standing crop of phytoplankton at Takahamairi Bay in Lake Kasumigaura. Research Report from the National Institute for Environmental Studies No. 22: 123-148. (in Japanese)

Janse, J.H., T. Aldenberg & P.R.G. Kramer, 1992. A mathematical model of the phosphorus cycle in Lake Loosdrecht and simulation of additional measures. Hydrobiologia 233: 119-136.

Jochem, F.J., 1999. Dark survival strategies in marine phytoplankton assessed by cytometric measurement of metabolic activity with fluorescein diacetate. Marine Biology 135: 721-728.

Jørgensen, S.E. & G. Bendoricchio, 2001. Fundamental of Ecological Modelling (3rd Edition). Developments in Environmental Modelling 21. Elsevier, Amsterdam.

Kiefer, D.A., O. Holm-Hansen, C.R. Goldman, R. Richards & T. Berman, 1972. Phytoplankton in Lake Thaoe: Deep-living populations. Limnology and Oceanography 5: 418-422.

Kirk, J.T.O, 1994. Light and photosynthesis in aquatic ecosystems, 2nd edition. Cambridge University Press. Cambridge.

Klausmeier, C.A. & E. Litchman, 2001. Algal games: The vertical distribution of phytoplankton in poorly mixed water columns. Limnology and Oceanography 46: 1998-2007.

Klausmeier, C.A., E. Litchman, T. Daufresne & S.A. Levin, 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429: 171-174.

Lande, R. & M.R. Lewis, 1989. Models of photoadaptation and photosynthesis by algal cells in a turbulent mixed layer. Deep-Sea Research 36: 1161-1175.

Li R., M. Watanabe & M. Watanabe, 1997. Akinete formation in planktonic *Anabaena* spp. (cyanobacteria) by treatment with low temperature. Journal of Phycology 33:576-584.

Lizon, F., L. Seuront & Y. Lagadeuc, 1998. Photoadaptation and primary production study in tidally mixed coastal waters using a Lagrangian model. Marine Ecology Progress Series 169: 43-54.

Marberly, S.C., 1996. Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. Freshwater Biology 35: 579-598.

McKnight, D.M., B.L. Howes, C.D. Taylor & D.D. Goehringer, 2000. Phytoplankton dynamics in a stable stratified Antarctic lake during winter darkness. Journal of Phycology 36:852-861.

Moll, R.A. & E.F. Stoermer, 1982. A hypothsis relating trophic status and subsurface chlorophyll maxima in lakes. Archiv fur Hydrobiologie 94: 425-440.

Mooij, W.M., J.H. Janse, L.N. De Senerpont Domis, S. Huisman & B.W. Ibelings, 2007. Predicting the effect of climate change on temperate shallow lakes with the ecosystem model PCLake. Hydrobiologia 584: 443-454.

Moore, L.R., G. Rocap & S.W. Chisholm, 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecoypes. Nature 393: 464-467.

Morgan, K. & J. Kalff, 1975. The winter dark survival of an algal flagellate- *Cryptomonas erosa* (Skuja). Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie 19: 2734-2740.

Murphy, A.M. & T.J. Cowles, 1997. Effects of darkness on multi-excitation in vivo fluorescence and survival in a marine diatom. Limnology and Oceanography 42: 1444-1453.

Palmisano, A.C. & C.W. Sullivan, 1982. Physiology of sea ice diatoms. II. Dark survival of

three polar diatoms. Canadian Journal of Microbiology 29: 157-160.

Parsons, T.R., M. Takahashi & B. Hargrave, 1984. Biological Oceanographic Processes (3rd Edition). Pergamon Press, New York.

Pastorok, R.A., M.W. Lerenzen & T.C. Ginn, 1982. Environmental aspect of artificial aeration and oxygenation of reservoirs: A review of theory, techniques, and experiences. NTIS Technical Report.

Peters, E., 1996. Prolonged darkness and diatom mortality: II. Marine temperate species. Journal of Experimental Marine Biology and Ecology 207: 43-58.

Peters, E. & D.N. Thomas, 1996. Prolonged darkness and diatom mortality I: Marine Antarctic species. Journal of Experimental Marine Biology and Ecology 207: 25-41.

Proctor, L.M. & J.A. Fuhrman, 1991. Roles of viral infection in organic particle flux. Marine Ecology Progress Series 69: 133-142.

Rengefors, K. & D.M. Anderson, 1998. Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates. Journal of Phycology 34: 568-577.

Reynolds, C.S., 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge.

Reynolds, C.S., 1997. Vegetation Processes in the Pelagic: A Model for Ecosystem Theory. Excellence in Ecology, 9. Ecology Institute, Oldendorf/Luhe, Germany.

Rhee, G-Y., 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. Limnology and Oceanography 23: 10-25.

Riebesell, U., D.A. Wolf-Gladrow & V. Smetacek, 1993. Carbon dioxide limitation of marine phytoplankton growth rate. Nature 361: 249-251.

Rodhe, W., 1955. Can plankton production proceed during winter darkness in subarctic lakes?. Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie 12: 117-122.

Ross, O.N. & J. Sharples, 2007. Phytoplankton motility and the competition for nutrients in the thermocline. Marine Ecology Progress Series 347: 21-38.

Segovia, M., L. Haramaty, J.A. Berges & P.G. Falkowski, 2003. Cell death in the unicellular chlorophyte *Dunaliella tertiolecta*. A hypothesis on the evolution of apoptosis in higher plants and metazoans. Plant Physiology 132: 99-105.

Selvin, R., B. Reguera, I. Bravo & C.M. Yentsch, 1988. Use of fluorescein diacetate (FDA) as a single-cell probe of metabolic activity in Dinoflagellate cultures. Biological Oceanography 6: 505-511.

Shapiro, J., 1990. Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie 24: 38-54.

Sicko-Goad, L., E.F. Stoermer & J.P. Kociolek, 1989. Diatom resting cell rejuvenation and formation: time course, species records and distribution. Journal of Plankton Research 11: 375-389.

Singh, B.K., J. Chattopadhyay & S. Sinha, 2004. The role of virus infection in a simple phytoplankton zooplankton system. Journal of Theoretical Biology 231: 153-166.

Smayda, T.J. & B. Mitchell-Innes, 1974. Dark survival of autotrophic, planktonic marine diatoms. Marine Biology 25, 195-202.

Strickland, J.D.H., 1965. Production of organic matter in the primary stages of the marine food chain. In Riley, J.P. & G. Skirrow (Eds.), Chemical Oceanography. Academic Press, New York: 477-610.

Takahashi, M., S. Ichimura, M. Kishino & N. Okami, 1989. Shade and chromatic adaptation of phytoplankton photosynthesis in a thermally stratified sea. Marine Biology 100: 401-409.

Talling, J.F., 1976. The depletion of carbon dioxide from Lake water by phytoplankton. The Journal of Ecology 64: 79-121.

Thompson, P.A., P.J. Harrison & J.S. Parslow, 1991. Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. Journal of Phycology 27: 351-360.

Tilzer, M.M., H.W. Paerl & C.R. Goldman, 1977. Sustained viability of aphotic phytoplankton in Lake Tahoe (California-Nevada). Limnology and Oceanography 22: 84-91.

Vincent, W.F., 1978. Survival of aphotic phytoplankton in Lake Tahoe throughout prolonged stratification. Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie 20: 401-406.

Visser, M.P., W.B. Ibelings, B. Van der Veer, J. Koedood & R.L. Mur, 1996. Artificial mixing prevents nuisance blooms of the cyanobacterium *Microcystis* in Lake Nieuwe Meer, the Netherlands. Freshwater Biology 36: 435-450.

Water Resource Development Public Corporation (WRDPC), 1989. *Phormidium* laboratory culture experiment technical report. (in Japanese)

White, A.W. & M. Shiro, 1975. Heterotrophic growth of filamentous blue-green alga *Plectonema boryanum*. Archives of Microbiology 102: 123-127.

Woods, J.D. & R. Onken, 1982. Diurnal variation and primary production in the ocean – preliminary results of a Lagrangian ensemble model. Journal of Plankton Research 4: 735-756.

Wright, R.T., 1964. Dynamics of a phytoplankton community in an ice-covered lake. Limnology and Oceanography 9: 163-178.

Wu, Z, L. Song & R. Li, 2008. Different tolerances and responses to low temperature and darkness between waterbloom forming cyanobacterium *Microcystis* and a green alga *Scenedesmus* Hydrobiologia 596: 47-55.

Legend to figures

Figure 1. Diagrammatic representation of various changes in phytoplankton abundance during prolonged darkness and reillumination.

Figure 2. Diagrammatic representation of regulation of cell division rate and physiological state in dynamic model of darkness tolerance. Boxes with thick line: state variables, boxes with thin line: parameters, solid arrows: positive effects, dashed arrows: negative effects.

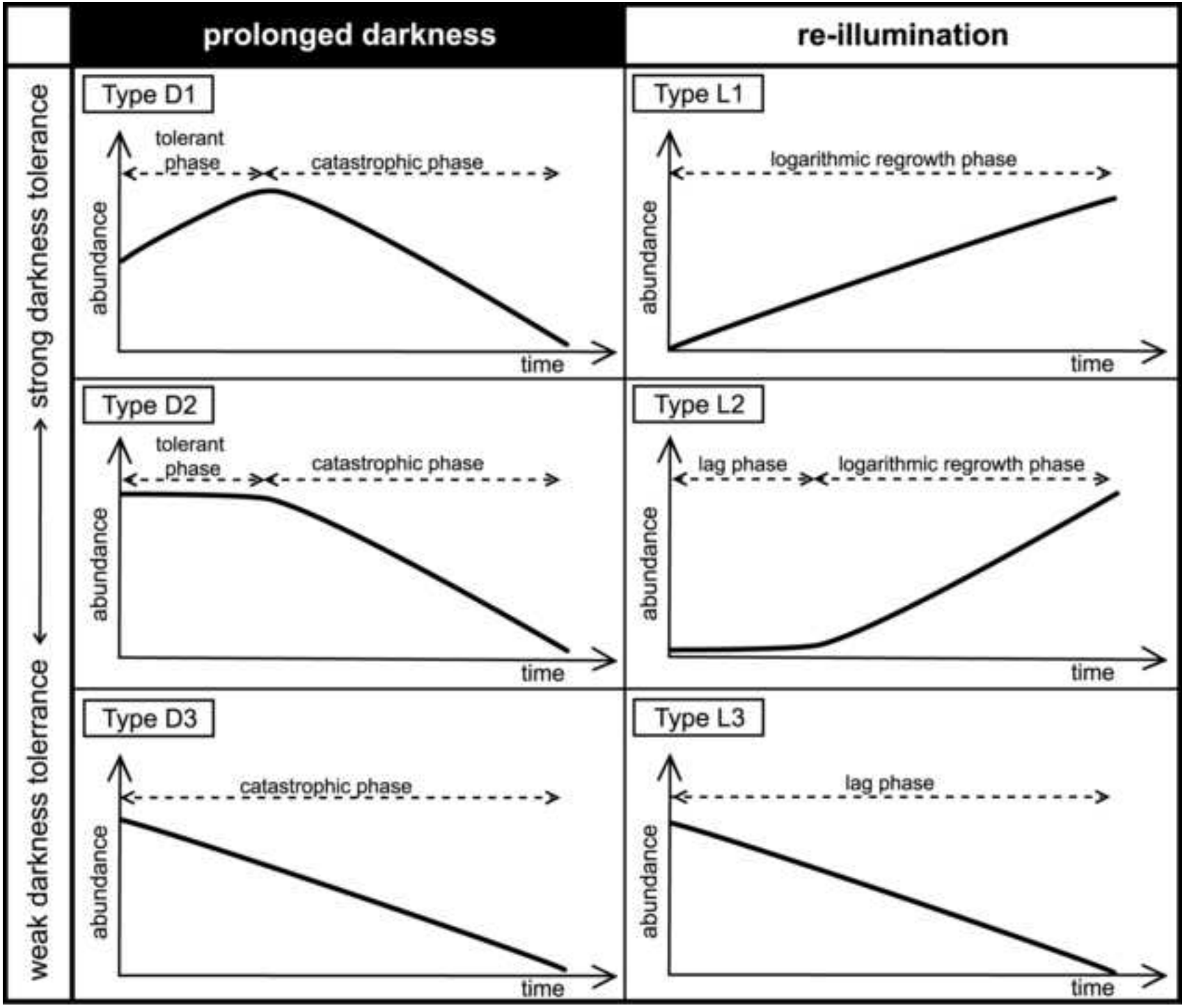
Figure 3. Comparison of model simulations and observations of *Scenedesmus acuminatus* during dark incubation (observation data of Dehning & Tilzer, 1989). A: Simulations and observations of changes in cell number, and B: cellular carbon concentration of biosynthetic apparatus. Each figure contains values simulated at 22°C (solid lines) and at 7°C (dashed line) and values observed at 22°C (black circles) and at 7°C (white circles).

Fig. 4. Comparison of model simulations (solid lines) and observations (black circles) of changes in abundance during dark incubation. A: *Cryptomonas phaseolus* (Gervais, 1997) as a representative of type D3. B: *Phormirium* sp. PC type (WRDPC, 1989) as a representative of type D1.

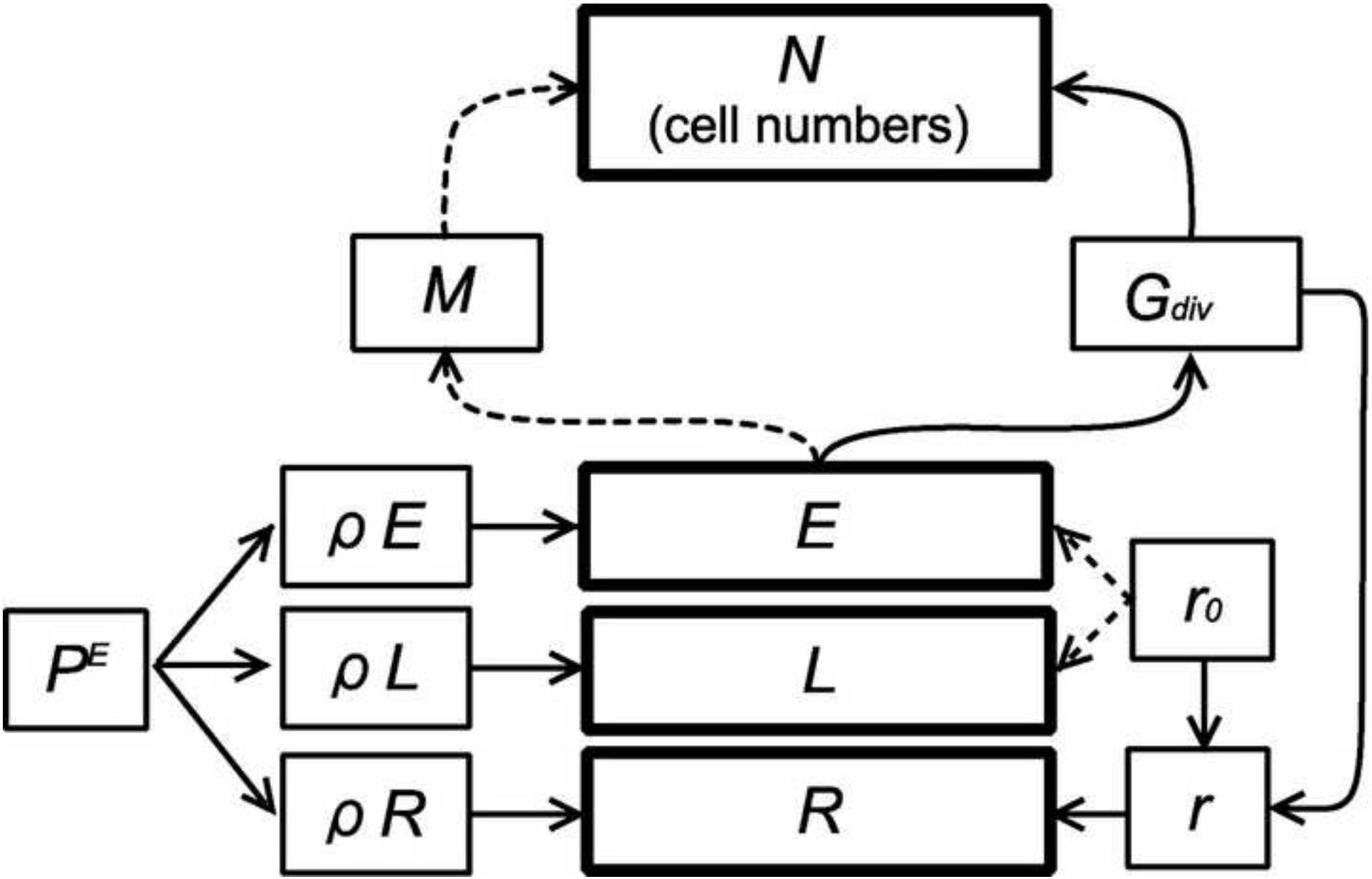
Figure 5. Comparison of model simulations (solid lines) and observations (white circles) of changes in cell numbers for *Microcystis aeruginosa* in a dark and reilluminated batch culture (data from Furusato et al., 2004). A: control L-D cycle, B: 5 days of darkness, C: 10 days of darkness, D: 15 days of darkness, E: 20 days of darkness. Shaded area represents dark incubation.

Figure 6. Relationship between cellular carbon concentration and physiological state in darkness-tolerance model.

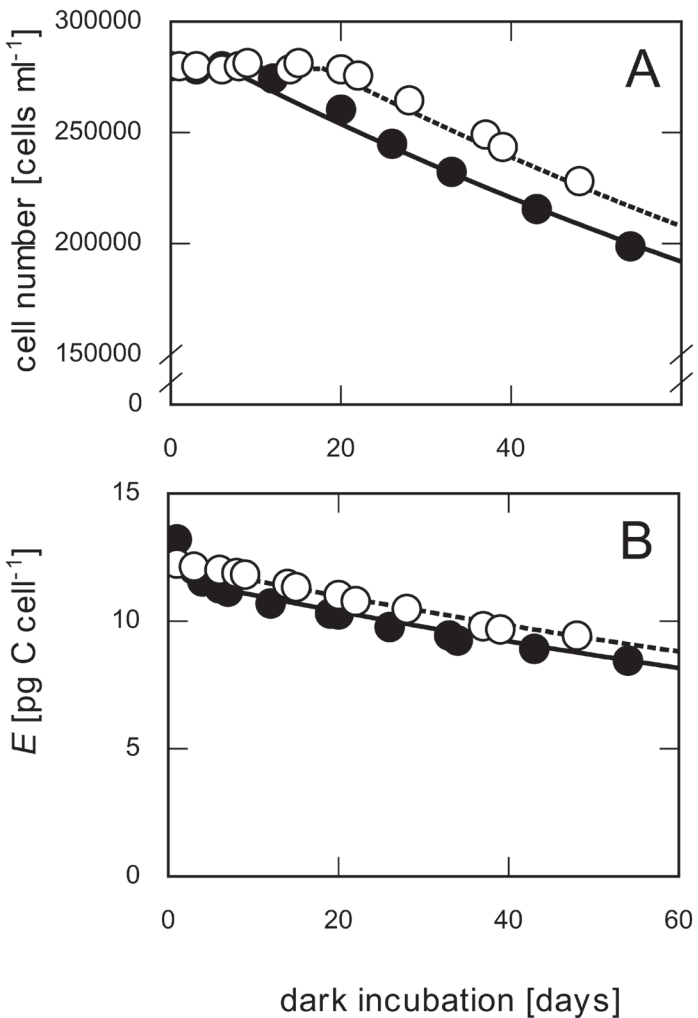
line figure (fig. 1)
Click here to download high resolution image



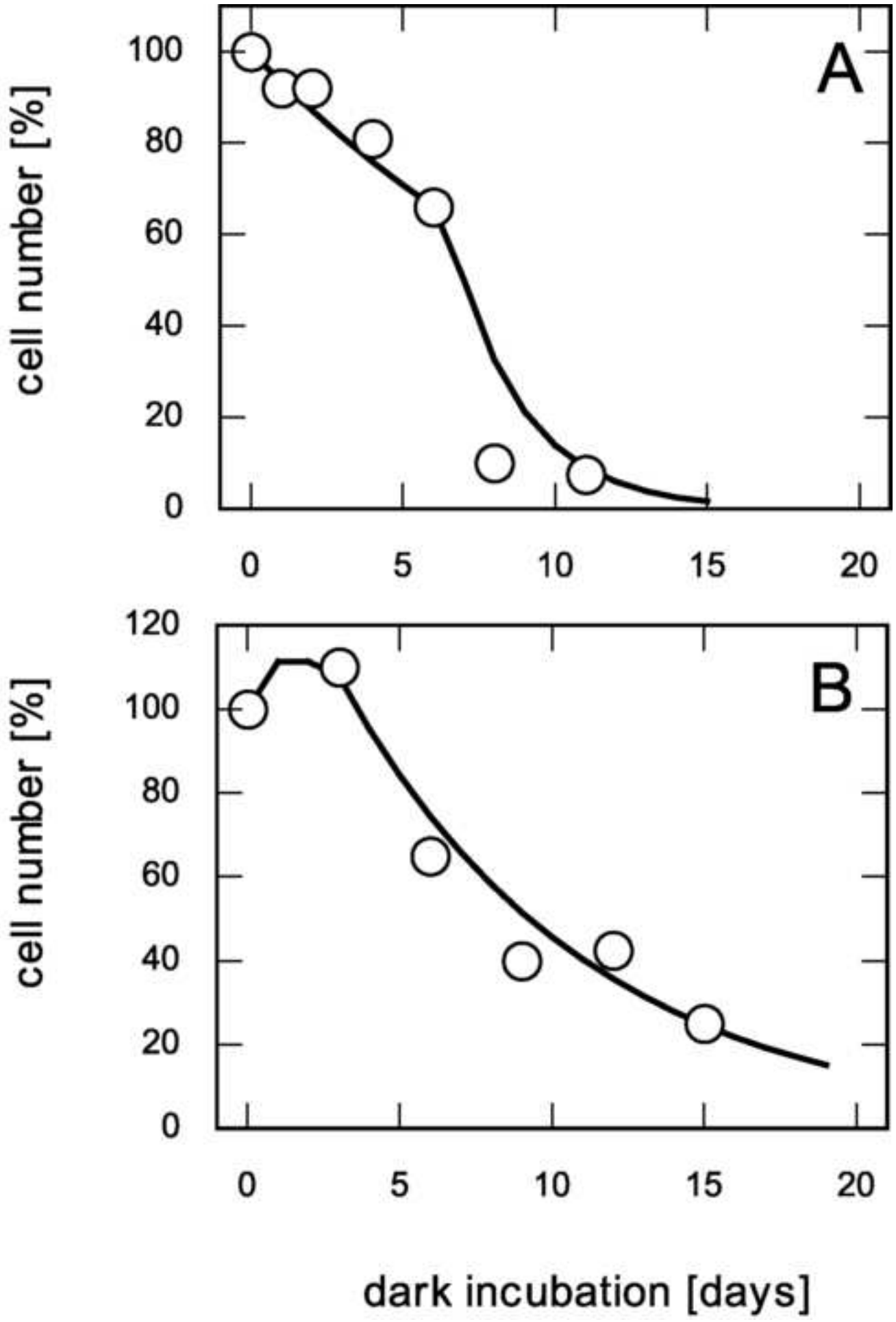
line figure (fig. 2)
Click here to download high resolution image



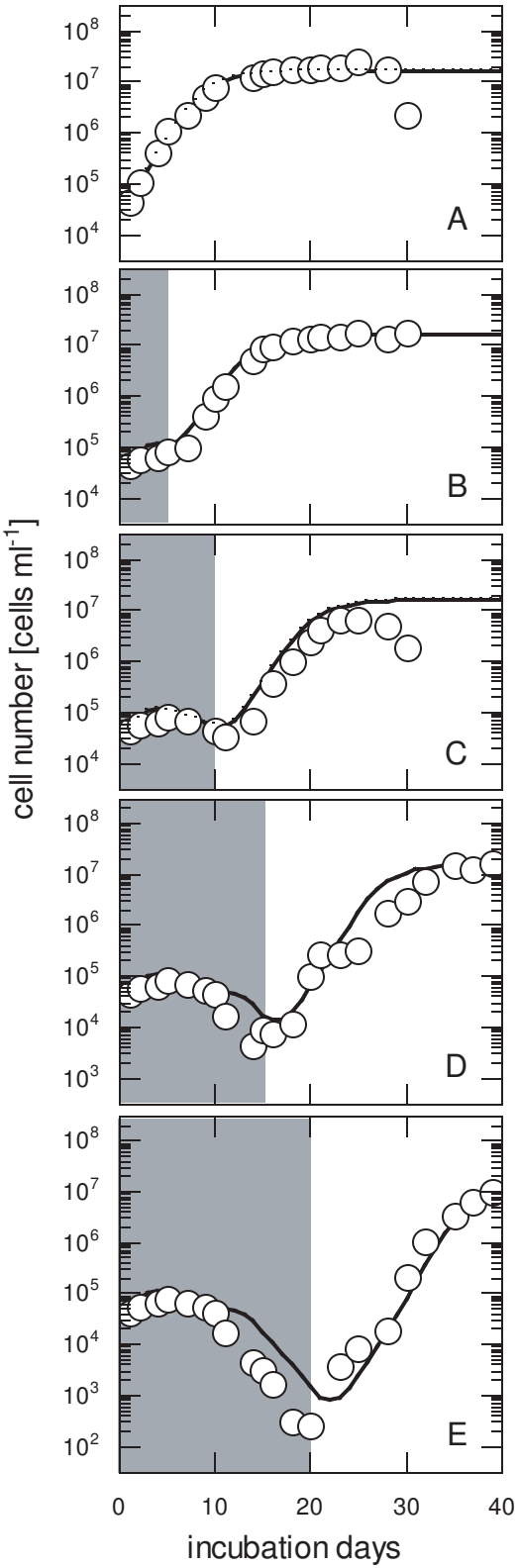
line figure (fig. 3)
Click here to download line figure: fig3.eps



line figure (fig. 4)
Click here to download high resolution image



line figure (fig. 5)
Click here to download line figure: fig5.eps



line figure (fig. 6)
Click here to download line figure: fig6.eps

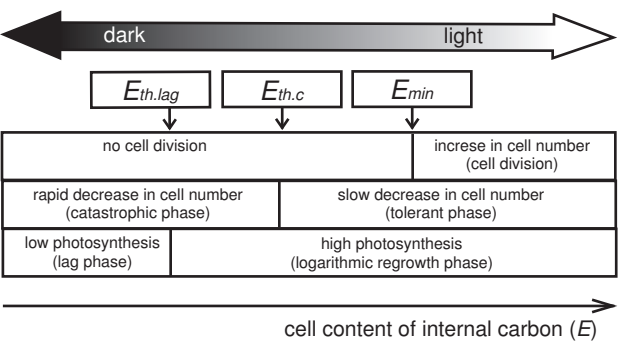


table
Click here to download table: Tables.doc

Tables

Table 1 Types of darkness tolerance of various phytoplankton[a]

Type	CLASS	Species[b]	Reference
D1	CYANOPHYCEAE	*Microcystis aeruginosa* (10)	Furusato et al. (2004)
		Plectonema boryanum (5)	White & Shiro (1975)
	CHLOROPHYCEAE	*Dunaliella tertiolecta* (6)	Berges & Falkowski (1998)
		Scenedesmus quadricauda (>20)	Furusato et al. (2004)
	BACILLARIOPHYCEAE	*Ditylum brightwellii* (>60)	Peters (1996)
		Phaeodactylum tricornutum (>7)	Griffiths (1973)
		Proboscia inermis (>74)	Peters & Thomas (1996)
		Rhizosolenia setigera (20)	Peters (1996)
		Skeletonema costatum (>10)	Handa (1969)
		Thalassiosira antarctica (>74)	Peters (1996)
		Thalassiosira tumida (>74)	
		Thalassiosira punctigera (>60)	
		Thalassiosira wessflogii (>13)	Berges & Falkowski (1998)
	CRYPTOPHYCEAE	*Cryptomonas rostratiformis* (2～4)	Gervais (1997)
		Cryptomonas cf. *ovata* (2)	
	HAPTOPHYCEAE	*Chrysochromulina hirta* (10)	Jochem (1999)
		Pavlova lutheri (11)	
		Prymnesium parvum (9)	
D2	CHLOROPHYCEAE	*Brachiomonas submarina* (10)	Jochem (1999)
		Dunaliella tertiolecta (>7)	Hellebust & Terborgh (1967)
		Scenedesmus acuminatus (10～15)	Dehning & Tilzer 1989)
	BACILLARIOPHYCEAE	*Bacteriastrum* sp. (11)	Jochem (1999)
		Melosira ambigua (>20)	Furusato et al. (2004)
		Phaeodactylum tricornutum (>3)	Griffiths (1973)
		Thalassiosira weissflogii (>63)	Murphy & Cowles (1997)
	CRYPTOPHYCEAE	*Cryptomonas* cf. *ovata* (2)	Gervais (1997)
		Cryptomonas phaseolus (2)	
	DINOPHYCEAE	*Protogonyaulax affinis* (>6)	Selvin et al. (1988)
D3	CYANOPHYCEAE	*Phormidium* sp. (PCtype)	WRDPC [c] (1989)
		Phormidium sp. (PEtype)	
	CRYPTOPHYCEAE	*Chroomonas* sp.	Gervais (1997)
		Cryptomonas phaseolus,	
		Cryptomonas undulata	
	DINOPHYCEAE	*Amphidinium carterae*	Franklin & Berges (2004)
		Gymnodinium catenatum	Selvin et al. (1988)
L1	CHLOROPHYCEAE	*Scenedesmus quadricauda*	Furusato et al. (2004)
	BACILLARIOPHYCEAE	*Bacteriastrum* sp.	Jochem (1999)
		Ditylum brightwellii	Peters (1996)
		Melosira ambigua	Furusato et al. (2004)
		Thalassiosira weissflogii	Murphy & Cowles (1997)
	HAPTOPHYCEAE	*Chrysochromulina hirta*	Jochem (1999)
L2	CYANOPHYCEAE	*Microcystis aeruginosa*	Furusato et al. (2004)
		Phormidium sp. (PCtype)	WRDPC [c] (1989)
		Phormidium sp. (PEtype)	
	CHLOROPHYCEAE	*Brachiomonas submarina*	Jochem (1999)
		Scenedesmus acuminatus	Dehning & Tilzer (1989)

Table 1 Types of darkness tolerance of various phytoplankton[a] (continued)

Type	CLASS	Species[b]	Reference
L2	BACILLARIOPHYCEAE	*Ditylum brightwellii*	Peters (1996)
		Phaeodactylum tricornutum	Griffiths (1973)
		Proboscia inermis	Peters & Thomas (1996)
		Rhizosolenia setigera	Peters (1996)
		Thalassiosira antarctica	Peters & Thomas (1996)
		Thalassiosira punctigera	Peters (1996)
		Thalassiosira tumida	Peters & Thomas (1996)
	CRYPTOPHYCEAE	*Cryptomonas phaseolus*	Gervais (1997)
		Cryptomonas undulata	
	DINOPHYCEAE	*Amphidinium carterae*	Franklin & Berges (2004)
L3	CYANOPHYCEAE	*Phormidium* sp. (PC type)	WRDPC [c] (1989)
	HAPTOPHYCEAE	*Pavlova lutheri*	Jochem (1999)
		Prymnesium parvum	
	BACILLARIOPHYCEAE	*Rhizosolenia setigera*	Peters (1996)
	CRYPTOPHYCEAE	*Cryptomonas phaseolus*	Gervais (1997)
		Cryptomonas undulata	
	DINOPHYCEAE	*Amphidinium carterae*	Franklin & Berges (2004)

[a] Data presented were selected from experiments in which changes in abundance were confirmed. The results of experiments in which only the possibility of regrowth after reillumination was studied (Antia & Cheng 1970; Smayda & Mitchell-Innes 1974; Antia 1976) were excluded.
[b] Type D1 and D2 values in parentheses mean periods of tolerance phase[d].
[c] Water Resource Development Public Corporation (Japan)

Table 2 List of definitions of variables and parameters

Variable	Definition	Typical units
E	Cellular carbon content of biosynthetic machinery	pg C cell^{-1}
E_{min}	Minimum E Content	pg C cell^{-1}
$E_{th.c}$	Threshold value of E to catastrophic phase	pg C cell^{-1}
$E_{th.lag}$	Threshold value of E to lag phase	pg C cell^{-1}
G_{div}	Cell division rate	day^{-1}
$G_{div.m}$	Maximum cell division rate	day^{-1}
IC	Cellular carbon content	pg C cell^{-1}
I	Irradiation	mol photons m^2 day^{-1}
k_r	Coefficient of respiratory rate on growth rate	-
L	Cellular carbon content of photosynthetic apparatus	pg C cell^{-1}
N	Cell number	cells ml^{-1}
M	Mortality rate	d^{-1}
M_0	Mortality rate at tolerant phase	d^{-1}
M_c	Mortality rate at catastrophic phase	d^{-1}
P^E	E specific rate of photosynthesis	d^{-1}
P^E_m	Maximum E specific rate of photosynthesis	d^{-1}
$P^E_{m.lag}$	Maximum E specific rate of photosynthesis at lag phase	d^{-1}
R	Cellular carbon content of storage pool	pg C cell^{-1}
r	Respiratory consumption of reserve material rate	d^{-1}
r_0	Maintenance metabolic rate	d^{-1}
κ_E	Proportion of biosynthate allocated to synthesis of E	-
κ_L	Maximum proportion of biosynthate allocated to synthesis of L	-
ρ_E	Proportion of photosynthetate allocated to synthesis of E	-
ρ_L	Proportion of photosynthetate allocated to synthesis of L	-
ρ_R	Proportion of photosynthetate allocated to synthesis of R	-
σ	Functional cross-section of L	m^2 mol^{-1} photons

Table 3 Three intracellular components of darkness-tolerance model

Component	Symbol	Property	Response to light intensity
Biosynthetic apparatus	*E*	Machinery of biosynthesis and cell replication, including Calvin cycle enzymes	Independent of light intensity
Light-harvesting apparatus	*L*	Chloroplasts, photosynthetic pigment-protein complexes, and supporting membranes	Increases in low irradiance (photoadaptation)
Energy storage reserves	*R*	Polysaccharides and lipids serving as energy storage reserves	Increases in high irradiance

Table 4 Parameters and initial conditions used to model dynamics of phytoplankton in prolonged darkness [a] (see Materials and Methods in the text)

Parameter or initial condition	Units	*S. acuminatus*		*P.* sp (PC type)	*C. phaseo-lus*
		22℃	7℃ [b]		
Parameters					
$G_{div.m}$	d^{-1}	-	-	1.14	-
M_0	d^{-1}	0.0	0.00025	-	0.069
M_c	d^{-1}	0.007	0.007	0.123	0.43
$E_{th.c}$	pg C $cell^{-1}$	11.29	11.03	1.3	14
E_{min}	pg C $cell^{-1}$	-	-	1.7	-
r_0	d^{-1}	0.0060	0.0055	0.15	0.1
Initial conditions					
N	cells ml^{-1}	28,000		164,000	10,000
IC	pg C $cell^{-1}$	21.35		3.63	44.98
E	pg C $cell^{-1}$	13.2		2.18	26.64
L	pg C $cell^{-1}$	3.6		0.61	9.7
R	pg C $cell^{-1}$	4.55		0.84	8.64

[a] The maximum cell division rate ($G_{div.m}$) and other related parameters of *S. acuminatus* and *C. phaseolus*, in which cell division in darkness was not confirmed in each experiment, are not defined.

[b] Similar initial conditions were used for case: 22℃ and 7℃.

Table 5 Parameters and initial conditions of *Microcystis aeruginosa* used to model dynamics of phytoplankton in darkness and reillumination (see Materials and Methods in the text)

Parameter or initial condition	Units	Values
Parameter		
$G_{div.m}$	d^{-1}	1.04
M_0	d^{-1}	0.125
M_c	d^{-1}	0.49
P^E_m	d^{-1}	1.6
$P^E_{m\_lag}$	d^{-1}	0.8
E_{min}	pg C $cell^{-1}$	2.3
$E_{th.c}$	pg C $cell^{-1}$	0.8
$E_{th.lag}$	pg C $cell^{-1}$	0.2
σ	m^2 mol^{-1} photons	0.39
r_0	d^{-1}	0.125
k_r	-	0.1
Initial condition		
N	cells ml^{-1}	164,000
IC	pg C $cell^{-1}$	16.34
E	pg C $cell^{-1}$	9.8
L	pg C $cell^{-1}$	2.78
R	pg C $cell^{-1}$	3.76

Table 6 Results of sensitivity analysis

Parameter	S		
	Dark conditions	Reillumi-nation	Average
$G_{div.m}$	0.000	0.213	0.107
M_0	0.001	0.001	0.001
M_c	1.452	0.026	0.739
P^E_m	0.000	0.528	0.264
$P^E_{m.lag}$	0.000	10.613	5.306
E_{min}	0.099	0.157	0.128
$E_{th.c}$	0.106	0.004	0.055
$E_{th.lag}$	0.000	0.031	0.015
σ	0.000	0.567	0.283
r_0	0.145	1.693	0.919
k_R	0.000	0.002	0.001
κE	0.000	6.402	3.201