様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科研費 KAKENHI

科学研究費助成事業　　研究成果報告書

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機関番号：１２４０１
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研究課題名（和文）藍藻の強光ストレスに対する反応の解明と強光阻害を用いたアオコ対策法の開発

研究課題名（英文）Study the response of Cyanobacteria to strong light stress and development of cyanobacterial control measures using light inhibition.

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：短時間の強光を用いることで藍藻の生育を抑制することができる。強光によって、それらの生理と成長行動が変化する。藍藻の酸化ストレスは光強度の増加とともに増加する。しかし、反応は種異的である。長時間の極端光（850~900 PAR)では、細胞は強光照射に適応している。ストレスが解消されると、細胞は積極的に回復する。暗黒暴露(30日)は藍藻を完全に変色させるが、迅速に回復する。単独の極端光は藍藻の生育を有望に抑制できないことが理解できる。水生植物のアレロパシーは異なる光条件を結合することで有効に藍藻の生長を抑制できる。この研究の結果、強光とアレロパシーを組み合わせることで藍藻を抑制することができる。

研究成果の学術的意義や社会的意義
水システムでの藍藻の生長は大きな問題である。飲用水問題、中毒、嫌な匂いと審美価値の喪失により、藍藻は社会に影響を与える。しかも、Egeria densaは侵襲的な大型植物である。地球温暖化により、日本を含めてE. densaの伝播が増加する。現在の研究結果により、侵入種の生産利用は生物学的に藍藻の生長を抑制できることが分かった。この方法は藍藻の物理と化学制御と比較して、より安全で費用効果がある。極端な光照射による細胞硬化と藍藻の積極的な曝露後の回復は新たな発見である。生理パラメータと形態パラメータとの関係が得られる。これらの関係は藍藻の生育やストレスのシミュレーションや予測に重要である。

研究成果の概要（英文）：Cyanobacteria growth can be suppressed using extreme light exposure for a shorter duration. Due to the extreme light, their physiology and growth behaviors were altered. Oxidative stress of P. ambiguum and M. aeruginosa increased with the light intensity. The responses were species-specific. Under long-duration extreme light (850 - 900 PAR), M. aeruginosa cells were adapted to stress. When the stress is removed, cells recovered rapidly. Darkness exposure (30 d) completely discolored M. aeruginosa and P. galeata, but were recovered aggressively. It is understood that extreme light alone cannot continously suppress cyanobacterial growth. The Egeria densa allelopathy combined with light conditions can effectively suppress the M. aeruginosa. The outcome of this research is that cyanobacteria can be suppressed by combining high light exposure and allelopathy.

研究分野：応用生態工学

キーワード：アオコ発生　藍藻制御　極端な光　酸化ストレス　水生植物のアレロパシー　生理と形態パラメータ　生物の相互作用　ストレス回復

１．研究開始当初の背景

Cyanobacterial growth in water bodies is a widely discussed environmental topic in many parts of the world due to the excessive environmental problems they cause. Among these problems, water toxification, bad odors, clogging of water supplies, and loss of esthetic values are significant. The health hazards caused by cyanotoxins are extensive—temporary sickness to organ damage or even death can result when cyanotoxin-contaminated water is consumed. Various measures have been proposed or are currently practiced for cyanobacteria control, and these can be mainly categorized as chemical, physical, and biological methods. Biological, chemical, and physical factors collectively determine the occurrence and distribution of cyanobacteria in the environment. Physical factors, such as temperature and light, influence the growth and distribution of cyanobacteria. Previous research have confirmed the unfavorable light conditions bring oxidative stress to photosynthetic species, including cyanobacteria. The photosynthetic species produce reactive oxygen species (ROS) as a byproduct of the photosynthesis process, which is harmful when accumulated in cells. Therefore, a balance between ROS generation and antioxidant activities is required to maintain cell homeostasis. Unfavorable conditions, including excess light, can disturb this balance, leading to oxidative stress. Therefore, focusing on extreme light (unfavorable) exposure to control cyanobacteria is important to strengthen cyanobacteria control further.

２．研究の目的

The cyanobacterial control measures like air bubbling and mixing can suppress the growth of cyanobacteria by pushing them to depths in which the light intensity is not enough for the growth. This can be achieved in water bodies with high water columns. Also, the cost of operation of circulatory systems is high due to the consumption of a large amount of energy. Further, for shallow water bodies, those methods cannot apply. The ROS-induced inhibition can be promoted by subjecting cyanobacteria to high light; it will be more convenient, cost-effective, and can be applied to shallow water bodies.
The suppression of cyanobacteria using highlight is required extensive research on oxidative stress, antioxidant responses, suppression, and recovery of cyanobacteria since the current research is not sufficient. The present research was conducted to extensive knowledge on stress responses of cyanobacteria and to suggest a sustainable method to suppress cyanobacterial growth.

３．研究の方法

(1) Cyanobacterial species *Pseudanabaena galeata* and *Microcystis aeruginosa* were exposed to different light intensities (0, 10, 30, 50, 100, 300, and 600 μmol m^{-2} s^{-1}) for 2 and 8 days, under controlled conditions. The growth, pigmentation, oxidative stress, and antioxidant responses were quantified. The fitting of the data with different growth models was checked.

(2) *Phormidium ambiguum* and *M. aeruginosa* were exposed to diurnal light-intensity variation to investigate their favorable and stressed phases during a single day. The photosynthetically active radiation (PAR) started at 0 μmol m^{-2} s^{-1} (06:00 h), increased by ~25 μmol μmol m^{-2} s^{-1} or ~50 μmol m^{-2} s^{-1} every 30 min, peaking at 300 μmol m^{-2} s^{-1} or 600 μmol m^{-2} s^{-1} (12:00 h), and then decreased to 0 μmol m^{-2} s^{-1} (by 18:00 h). The growth, oxidative stress, and antioxidant responses were quantified.

(3) *M. aeruginosa* was exposed to extreme light (850-900 μmol m^{-2} s^{-1}) for 8 days, and the changes in response to the light exposure were observed. Post-exposure recovery performances were accessed by allowing Cyanobacteria to recover under optimum conditions for 8 days. The growth, pigmentation, oxidative stress, and antioxidant responses were quantified.

(4) *M. aeruginosa* was exposed to different flow velocities (0, 0.4, 0.8 and 1.2 m s^{-1}) under different light intensities (0, 50, 300, 700, 1200 μmol m^{-2} s^{-1}). A new flow simulation system was designed. Cyanobacterial stress responses were quantified.

(5) The effect that the coexistence of *E. densa* on *M. aeruginosa* was tested under different photosynthetically active radiation (PAR) intensities. The growth, chlorophyll-a, oxidative stress, and antioxidant activities were quantified after 7 days of coexistence with *E. densa*. A

control experiment without *E. densa* was conducted for *M. aeruginosa* under the same conditions to distinguish the effect of the PAR intensity from the allelopathic effect.

4．研究成果

(1) The OD730, chlorophyll content, oxidative stress and antioxidant responses of *P. galeata* and *M. aeruginosa* were negatively affected by PAR intensity >30 and < 50 μmol m^{-2} s^{-1}. The 30 – 50 μmol m^{-2} s^{-1} PAR range can be considered as the favorable range for both species. *M. aeruginosa* has a higher tolerance for extreme light conditions compared with *P. galeata*. The growth responses of the two species were fit with simulated results of three growth models **(Figure 1)**.

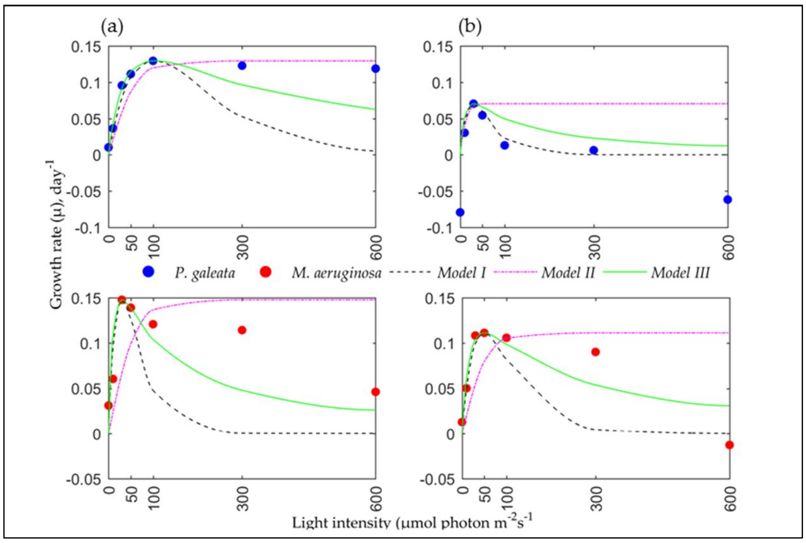

Figure 1. Growth rates of *Pseudanabaena galeata* and *Microcystis aeruginosa* as a function of light intensity at different time intervals (a) 2 days and (d) 8 days. The observed data are fitted with Model proposed by Steele (Model I), Platt and Jassby (Model II) and Peeters and Eilers (Model III).

(2) *P. ambiguum* and *M. aeruginosa* are highly responsive to the diurnally varying PAR intensity. H_2O_2 and antioxidant levels increased with the gradually increasing light intensities (300 or 600 μmol m^{-2} s^{-1} PAR) and reduced light intensity. However, the response curves were species-specific **(Figure 2)**.

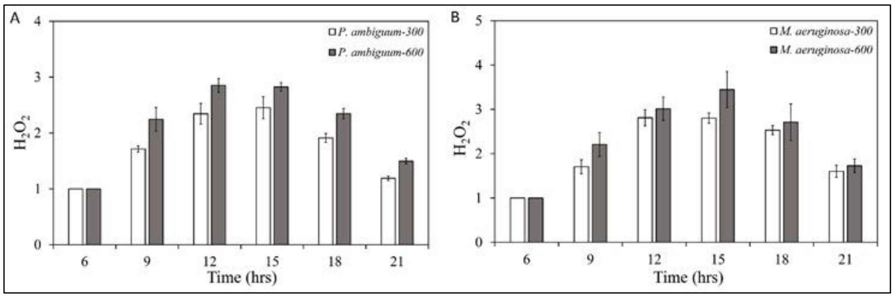

Figure 2. Diurnal variations in the H_2O_2 contents of *Phormidium ambiguum* and *Microcystis aeruginosa*. The numbers 300 and 600 represent the maximum photosynthetically active radiation (PAR) intensities for two different treatment conditions, where the maximum PAR intensity was reached at 12:00 h. The error bars represent the standard deviations.

(3) *M. aeruginosa* can adjust its physiology and growth to continue exposing extreme light (850–900 μmol m^{-2} s^{-1} PAR), to surpasses the stress period. The post-stress recovery capacity was aggressive and was positively correlated to the exposure duration. These findings suggest that exposure to light stress causes the hardening of *M. aeruginosa* (**Figure 3**). This finding highlights the research requirement on the post-stress recovery capacity of *M. aeruginosa* and highlights stress exposure suppression of cyanobacteria should be carefully conducted.

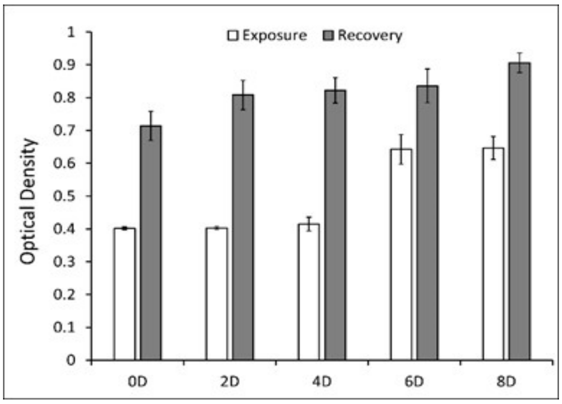

Figure 3. Optical density (OD730) of *Microcystis aeruginosa* under extreme light exposure and the OD730 of the same samples after 8 days recovery. 'Exposure' represents the diurnal variation of OD730 under high light exposure and the 'Recovery' represents the OD730 of the same samples after 8 days of recovery. 0D, 2D, 3D, 6D, and 8D represent the days.

(4) Under the combined effect of flow velocity and high light exposure, *M. aeruginosa* showed mixed responses and found that the velocity can be beneficial to escape from high light conditions **(Figure 4)**.

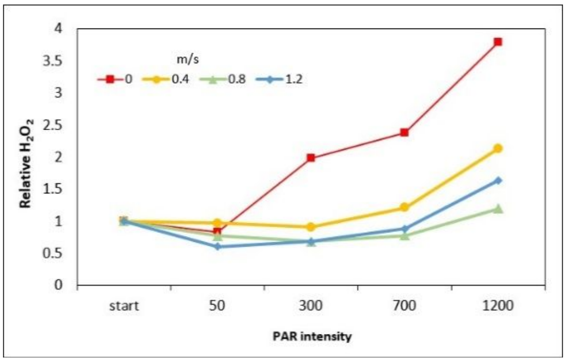

Figure 4. The relative change of cellular H_2O_2 content of *Microcystis aeruginosa* under different light intensities and flow velocities.

(5) The allelopathy of *E. densa* on *M. aeruginosa* is significantly influenced by the PAR intensity. PAR intensities lower or higher than the favorable range (50–100 μmol m^{-2} s^{-1}) further suppressed *M. aeruginosa* under coexistence **(Figure 5)**. Research has revealed that the allelopathic effect of *E. densa* can be utilized to biological suppression of *M. aeruginosa* under various light conditions. Interestingly combine with moderately high light is enough for such control. Therefore, we recommend combining biological control measures and light-induced stress to suppress cyanobacteria rather than extreme light exposure.

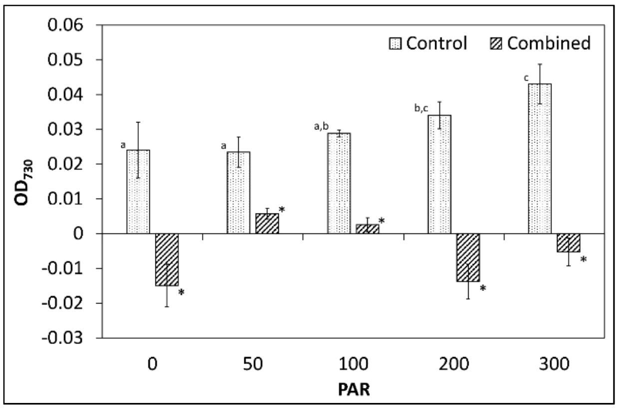

Figure 5. Change of the optical density (OD730) from starting OD730 of *Microcystis aeruginosa*. The exposure period is 7 days. X-axis is PAR in μmol m^{-2} s^{-1}. "Control" and "Combined" represent the *M. aeruginosa* only and *Egeria densa*-*M. aeruginosa* coexistence conditions, respectively.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　2件）

１．著者名 Mudalige Don Hiranya Jayasanka Senavirathna, Guligena Muhetaer, Hongyu Yan, Bahaguri Aihemaiti, Takeshi Fujino	４．巻 232
２．論文標題 Stress and Recovery Responses of Microcystis aeruginosa Exposed to Extreme Light for Different Durations	５．発行年 2021年
３．雑誌名 Water Air and Soil Pollution	６．最初と最後の頁 229
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s11270-021-05175-3	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Mudalige Don Hiranya Jayasanka Senavirathna, Guligena Muhetaer, Keerthi Sri Senarathna Atapaththu, Takeshi Fujino	４．巻 232
２．論文標題 Egeria densa Allelopathy on Microcystis aeruginosa Under Different Light Intensities and Preliminary Insight into Inter-Parameter Relationships	５．発行年 2021年
３．雑誌名 Water Air and Soil Pollution	６．最初と最後の頁 135
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s11270-021-05088-1	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Mudalige Don Hiranya Jayasanka Senavirathna, Guligena Muhetaer, Liu Zhaozhi, Takeshi Fujino	４．巻 36
２．論文標題 Allelopathic influence of low concentration Microcystis aeruginosa on Egeria densa under different light intensities	５．発行年 2020年
３．雑誌名 Chemistry and Ecology	６．最初と最後の頁 1-19
掲載論文のDOI（デジタルオブジェクト識別子） 10.1080/02757540.2020.1798939	査読の有無 有
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１．著者名 Guligena Muhetaer, Mudalige Don Hiranya Jayasanka Senavirathna, Takeshi Fujino	４．巻 8
２．論文標題 Oxidative Stress and Antioxidant Responses of Phormidium ambiguum and Microcystis aeruginosa Under Diurnally Varying Light Conditions	５．発行年 2020年
３．雑誌名 Microorganisms	６．最初と最後の頁 890
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/microorganisms8060890	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

1．著者名 Guligena Muhetaer, Takashi Asaeda, Senavirathna M.D.H. Jayasanka, Mahendra B. Baniya, Helayaye D.L. Abeynayaka, M. Harun Rashid, HongYu Yan	4．巻 12(2), 407
2．論文標題 Effects of Light Intensity and Exposure Period on the Growth and Stress Responses of Two Cyanobacteria Species: Pseudanabaena galeata and Microcystis aeruginosa	5．発行年 2020年
3．雑誌名 Water	6．最初と最後の頁 N/A
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/w12020407	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

1．発表者名 Guligena Muhetaer, Takashi Asaeda, Helayaye Damitha Lakmali Abeynayaka, Senavirathna MDH Jayasanka
2．発表標題 Effects of different light intensity on the growth and stress responses of Paeudanabaena galeata (cyanobacteria)
3．学会等名 International Symposium on Ecohydraulics, 2018（国際学会）
4．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

－

6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関			
スリランカ	Department of Limnology and Water Tec.	Faculty of Fisheries and Marine Sc.	University of Ruhuna	