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## RESEARCH ARTICLE

# Blue light irradiation increases the relative abundance of the diatom *Nitzschia palea* in co-culture with cyanobacterium *Microcystis aeruginosa*

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## Abstract

Lake eutrophication is associated with cyanobacterial blooms. The pennate diatom *Nitzschia palea* (*N. palea*) inhibits the growth of the cyanobacterium *Microcystis aeruginosa* (*M. aeruginosa*); therefore, increasing the relative abundance of *N. palea* may contribute to the inhibition of *Microcystis* blooms. Several studies have demonstrated that blue light irradiation promotes diatom growth and inhibits cyanobacterial growth. In this study, we evaluated the effects of blue light irradiation on *N. palea* and *M. aeruginosa* abundance. Monocultures and co-cultures of *N. palea* and *M. aeruginosa* were exposed to blue light and fluorescent light at 32  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The relative abundance of *N. palea* under fluorescent light decreased gradually, whereas the abundance under blue light was relatively higher (approximately 74% and 98% under fluorescent light and blue light, respectively, at the end of the experiment). The inhibition efficiency of blue light on the growth rate of *M. aeruginosa* was related to the light intensity. The optimal light intensity was considered 20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  based on the inhibition efficiency of 100%. Blue light irradiation can be used to increase the abundance of *N. palea* to control *Microcystis* blooms.

## Practitioner Points

- The effects of blue light irradiation on *N. palea* abundance was discussed.
- Monocultures and co-cultures of *N. palea* and *M. aeruginosa* were exposed to blue light and to fluorescent light.
- The relative abundance of *N. palea* increased upon irradiation with blue light in co-culture with *M. aeruginosa*.

## KEYWORDS

algae, competition, light-emitting diode, water blooms

## INTRODUCTION

Lake eutrophication is one of the most serious environmental problems worldwide (Bhagowati & Ahamad, 2019) and is associated with the occurrence of water blooms (O'Neil et al., 2012). The increase in atmospheric CO<sub>2</sub> gas concentration promotes the growth of the cyanobacterium *Microcystis aeruginosa* (Ma et al., 2019). Consequently, the occurrence of water blooms is expected to increase worldwide due to global warming. The negative impacts of water blooms on various organisms are well known. For example, phytoplankton diversity decreases during cyanobacterial blooms (Niu et al., 2011; Toporowska & Pawlik-Skowrońska, 2014) as it is inversely related to cyanobacterial density (Bockwoldt et al., 2017).

*Nitzschia palea* is a widely distributed pennate diatom (Kim Tiam et al., 2018), which is detected with *M. aeruginosa* from lake water samples in several environments (Haroon et al., 2020; Romo & Miracle, 1994); we confirmed that it coexists with *Microcystis* spp. in reservoirs. Diatoms are much higher food quality for zooplankton than cyanobacteria (Brett & Muller-Navarra, 1997; Gulati & Demott, 1997; Fujibayashi et al., 2021). Furthermore, *N. palea* can destroy *Microcystis* colonies (Hao et al., 2021). Therefore, artificially promoting the growth of *N. palea* may represent an effective strategy for inhibiting *Microcystis* blooms.

Optimal growth conditions vary among algae. Paerl (2014) reported that the optimal temperature for cyanobacterial growth is relatively higher than that for eukaryotic plankton, such as diatoms and green algae. For example, the growth rate of *M. aeruginosa* increases significantly with increasing temperature at 15–30°C (Coles & Jones, 2000), whereas the pennate diatom *Synedra* sp. attains the highest growth rate at 25°C (Li et al., 2017). The maximum cell yield of *N. palea* decreases at 30°C (Watanabe et al., 2020). However, it is difficult to control environmental factors such as temperature to increase the abundance of *N. palea*.

The wavelength of light affects the physiological characteristics of algae. Irradiation with blue light has been shown to promote diatom growth (Holdsworth, 1985; Li et al., 2020; Shikata et al., 2009), whereas it adversely affects the growth of various cyanobacteria (Wyman & Fay, 1986), including *M. aeruginosa* (Khan et al., 2016; Tan et al., 2020), *Pseudanabaena mucicola* (Khatoon et al., 2018), *Synechocystis* PCC6803 (Bland & Angenent, 2016), *Cyanobacterium aponinum* (Meng et al., 2018), and *Spirulina platensis* (Chen et al., 2010; Wang et al., 2007). Luimstra et al. (2018, 2019) reported that growth is considerably reduced in the phycobilisome-containing cyanobacterium *Synechocystis*

sp. upon absorbing blue light owing to phycobilisomes. Since the introduction of blue light irradiation in lakes is relatively easy, it may be used to increase the abundance of *N. palea*.

In this study, we focused on the effects of blue light irradiation on *N. palea* abundance. Blue light irradiation may be an effective measure against water bloom in small lakes where installing light-emitting diodes (LEDs) is easier due to the small surface area. Monocultures and co-cultures of *N. palea* and *M. aeruginosa* were exposed to blue light and to fluorescent light as a control.

## MATERIAL AND METHODS

### Algae cultivation

*M. aeruginosa* NIES-102 was obtained from the National Institute for Environmental Studies (NIES), Ibaraki, Japan. *N. palea* was isolated from a water bloom at Fujinohira dam, Saga Prefecture, Japan, in 2015 (Watanabe et al., 2019). *N. palea* was identified via electron microscopy. Both species were cultivated in a WC medium (Guillard & Lorenzen, 1972) containing 11 mg Si L<sup>-1</sup> at pH 8.0, as described by Amano et al. (2012), which was used in all experiments. Cultures were incubated at 25°C and exposed to 32 μmol photons m<sup>-2</sup> s<sup>-1</sup> and a light–dark cycle of 12:12 h, and shaken once daily.

### Effects of blue light irradiation on monocultures of *M. aeruginosa* and *N. palea*

*M. aeruginosa* and *N. palea* were cultured individually in 150 mL medium in a 300 mL Erlenmeyer flask, with an initial cell density of 4000 cells mL<sup>-1</sup> for each species. The cultures were irradiated using a 3 W blue LED (Kashinoki Sogyo Co. Ltd., Tokyo, Japan) and fluorescent light (control) at 32 μmol photons m<sup>-2</sup> s<sup>-1</sup>, which has not been specifically assessed in previous studies (Tan et al., 2020; Watanabe et al., 2019; Wyman & Fay, 1986). The light–dark cycle was 12:12 h. This experiment was performed in triplicate at 25°C until the stationary growth phase was attained. Sampling was performed every 2 or 3 days to count cell density. At each sampling time, 1 mL of culture was collected twice from each flask. *N. palea* was separated from the flask bottom via vigorous manual shaking and pipetting and collected using a sterilized glass pipette. *M. aeruginosa* was collected without vigorous shaking.

## Effects of blue light irradiation on co-cultures of *M. aeruginosa* and *N. palea*

*M. aeruginosa* and *N. palea* were cultured together in 150 mL medium in a 300 mL Erlenmeyer flask. The initial cell density was 4000 cells mL<sup>-1</sup> for each species. The co-culture was irradiated using a blue LED or fluorescent light in triplicate under the same conditions as described in the monoculture experiment. The sampling was performed as mentioned above.

### Analysis of growth rate

Cell density was evaluated every 2 or 3 days using optical plastic plankton counters (Matsunami Glass Industry, Osaka, Japan) and a microscope (BH2-RFCA; Olympus, Tokyo, Japan). The specific growth rate in each flask was determined based on the cell density during the exponential growth phase using equation 1, where  $\mu$  represents the specific growth rate (day<sup>-1</sup>), and  $C_1$  and  $C_2$  represent the cell density (cells mL<sup>-1</sup>) of *M. aeruginosa* and *N. palea* at the culture times of  $t_1$  and  $t_2$  (day), respectively.

$$\mu = 1/(t_2 - t_1) \ln C_2/C_1 \quad (1)$$

The growth rate was determined from the average of growth rates in three flasks of the same treatment system.

The relative abundance of *N. palea* under fluorescent light and blue light was evaluated using equation (2) based on the cell number as described by Chellappa et al. (2009).

Relative abundance (%) = cell density of *N. palea* (cells mL<sup>-1</sup>)/cell density of *N. palea* + *M. aeruginosa* (cells mL<sup>-1</sup>) (2).

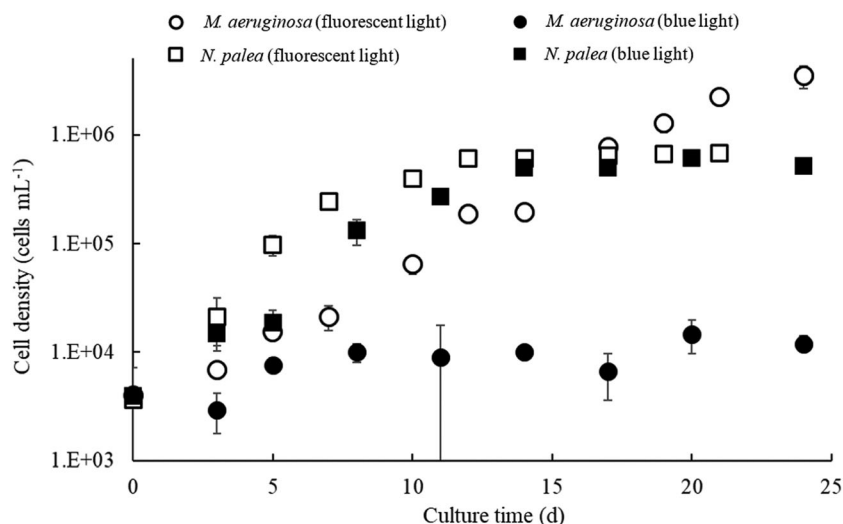
### Statistical analysis

A Student's *t* test was performed to compare the differences in the specific growth rate of the monoculture between fluorescent light and blue light. The effects of light quality, presence of *M. aeruginosa*, and their interaction effects on the growth rate of *N. palea* in co-culture were analyzed via two-way analysis of variance (ANOVA).

## RESULTS

### Effects of blue light irradiation on monocultures of *M. aeruginosa* and *N. palea*

*M. aeruginosa* showed growth under fluorescent light until the end of the experiment and the growth rate was 0.33 day<sup>-1</sup>, whereas it showed minimal growth under blue light. Exponential growth under blue light was observed up to day 8 (Figure 1) and the growth rate was 0.11 day<sup>-1</sup>, which was significantly lower than that obtained under fluorescent light ( $p < 0.01$ ). *N. palea* showed growth under both light conditions. The specific growth rate for *N. palea* under fluorescent light (0.62 day<sup>-1</sup>) was higher than that under blue light (0.36 day<sup>-1</sup>) ( $p < 0.01$ ). No significant difference was observed in the maximum cell yield of *N. palea* between fluorescent light and blue light ( $p > 0.05$ ).



**FIGURE 1** Change in cell density of monocultures of *Microcystis aeruginosa* and *Nitzschia palea* cultured under fluorescent light or blue light irradiation. Error bars represent standard deviation of triplicate

## Effects of blue light irradiation on co-cultures of *M. aeruginosa* and *N. palea*

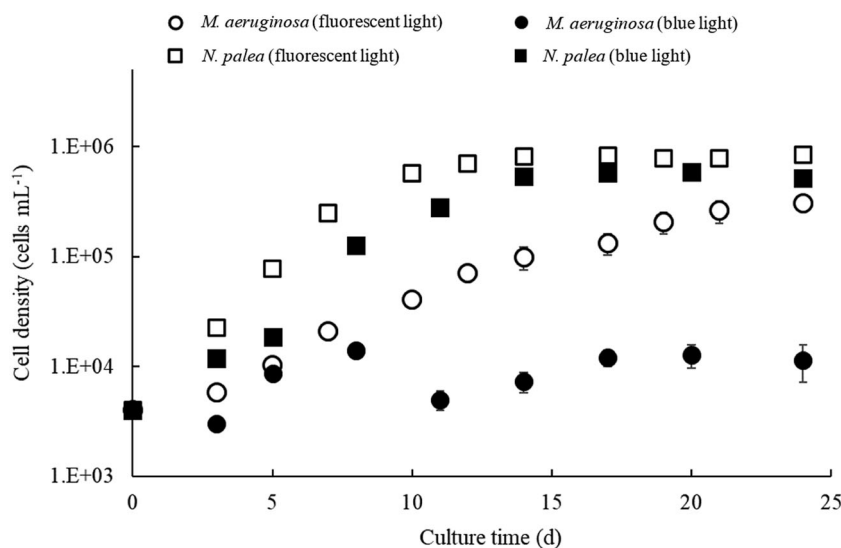
The growth of *M. aeruginosa* in co-culture with *N. palea* was similar to that of the monoculture (Figure 2); the growth rate was  $0.26 \text{ day}^{-1}$  and  $0.30 \text{ day}^{-1}$  under fluorescent light and blue light, respectively. *N. palea* reached the stationary growth phase by day 14 under both light conditions, and the growth rate was  $0.60 \text{ day}^{-1}$  and  $0.36 \text{ day}^{-1}$  under fluorescent light and blue light, respectively. Two-way ANOVA showed that the presence of *M. aeruginosa* and light conditions had no significant interaction with the specific growth rate of *N. palea*. The relative abundance of *N. palea* under fluorescent light gradually decreased (Figure 3). *N. palea* had a relatively higher abundance under blue light, and there was a

significant difference between the two light conditions on day 14 ( $p < 0.01$ ).

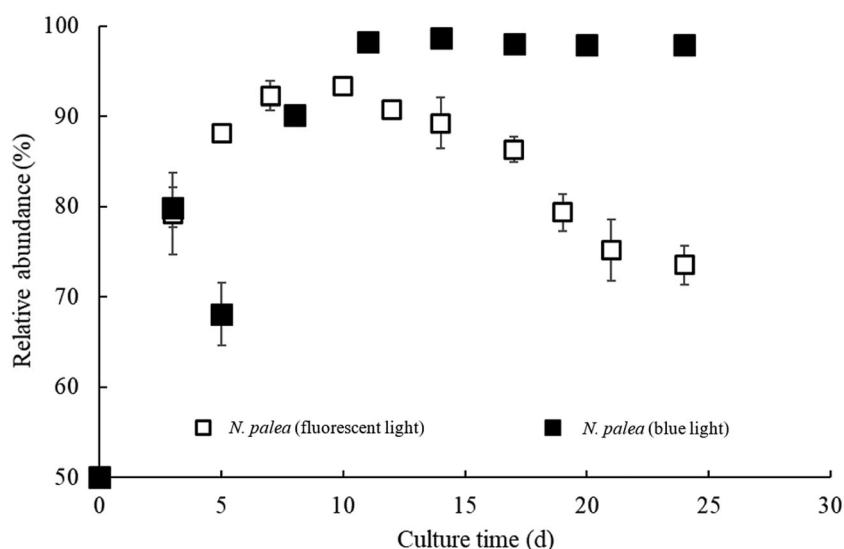
## DISCUSSION

Blue light irradiation has been shown to promote diatom growth and reduce the growth of cyanobacteria such as *M. aeruginosa*. Therefore, it was hypothesized that blue light irradiation can reduce the occurrence of *Microcystis* blooms. The relative abundance of *N. palea* increased upon irradiation with blue light in co-culture with *M. aeruginosa* in this study.

The growth curves of *M. aeruginosa* were significantly different under fluorescent and blue light conditions. The specific growth rate of *M. aeruginosa* under fluorescent



**FIGURE 2** Change in cell density of *Microcystis aeruginosa* and *Nitzschia palea* in co-culture under fluorescent light or blue light irradiation. Error bars represent standard deviation of triplicate



**FIGURE 3** The relative abundance of *Nitzschia palea* in co-culture with *Microcystis aeruginosa*. Error bars represent standard deviation of triplicate

light ( $0.33 \text{ day}^{-1}$ ) was similar to that reported by Ohkubo et al. (1991) obtained from the same strain cultivated under similar conditions ( $0.32 \text{ day}^{-1}$ ). However, the growth rate obtained using blue light in this study ( $0.11 \text{ day}^{-1}$ ) was different from that reported in a previous study ( $0.00 \text{ day}^{-1}$ ) (Watanabe et al., 2019). This difference may be attributed to light intensity because the intensity used in the previous study was  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Therefore, the relationship between light intensity and inhibition effects was compared to that reported in other studies. The extent of the inhibitory effect of blue light on the growth of *M. aeruginosa* differed among the studies (Figure S1). However, the culture conditions varied among the studies (Table S1). For instance, Wyman and Fay (1986) and Tan et al. (2020) used BG-11 medium at different concentrations, suggesting that the available nutrient concentrations must have differed between the studies. The growth rate follows the Droop equation, which is a model affected by the intracellular content, and the nutrient uptake rate is calculated using the Michaelis–Menten equation, which is affected by the nutrient concentration in the medium (Ducobu et al., 1998; Mikawa et al., 2016). Since the half-saturation constant for nutrient uptake is usually smaller than the concentration of the medium, it is considered that the growth rate does not differ depending on the type of medium unless the nutrient is depleted. Li et al. (2014) demonstrated that there is no significant difference in the growth rate of *M. aeruginosa* even when a medium with different nutrient concentrations is used.

Temperature is known to affect algal growth. It has been reported that the growth rate of *M. aeruginosa* increases with increasing temperature (Imai et al., 2009; You et al., 2018). Li et al. (2014) demonstrated that the growth rate of *M. aeruginosa* is higher at  $25^\circ\text{C}$  than that at  $20^\circ\text{C}$ . Therefore, the growth rate reported by Wyman and Fay (1986) is expected to increase further when cultured at  $25^\circ\text{C}$  (as in this experiment). The growth rate at  $20\text{--}45 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  was obtained at the same temperature. Therefore, the difference in the growth rates at  $20\text{--}45 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  was related to factors other than temperature.

The light–dark cycle used in the studies was not the same. Zevenboom and Mur (1984) reported that the growth rate of *M. aeruginosa* is approximately the same in the 12:12 and 24:0 h light–dark cycles. Furthermore, the difference in growth rate related to different light–dark cycles is smaller under the light intensity, which is insufficient to cause saturation with respect to the growth rate (Zevenboom & Mur, 1984). Blue light intensities of 10, 20, and  $32 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  are not considered sufficient to cause saturation (Figure S1). If the relationship between the light–dark cycle and the growth rate is

similar to that of fluorescent light, then the difference in the growth rate under blue light at different light–dark cycles is presumed to be small.

The growth rate varies depending on the strain. Despite cultivation under the same experimental conditions including BG-11 medium at  $25^\circ\text{C}$  under fluorescent light at  $45 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , different growth rates of *M. aeruginosa* were reported by Li et al. (2014) ( $0.60 \text{ day}^{-1}$ ) and Tan et al. (2020) ( $0.32 \text{ day}^{-1}$ ). However, the growth rates of strains used in this study and that reported by Watanabe et al. (2019) were the same. The growth rate under blue light was  $0 \text{ day}^{-1}$  at  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Watanabe et al., 2019), whereas that under  $32 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  was  $0.11 \text{ day}^{-1}$ . Overall, the observed difference in the growth rate of *M. aeruginosa* was determined by the light intensity. The growth rate tends to be minimum when the blue light intensity is approximately  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Figure S1).

The specific growth rate for *N. palea* under fluorescent light ( $0.62 \text{ day}^{-1}$ ) was higher than that under blue light ( $0.36 \text{ day}^{-1}$ ). The specific growth rate under blue light ( $0.23 \text{ day}^{-1}$ ) has been reported to be higher than that under fluorescent light ( $0.21 \text{ day}^{-1}$ ) (Watanabe et al., 2019). The difference in the specific growth rate at each light condition between the previous and current studies is likely due to the difference in light intensity. The increase in the specific growth rate of *N. palea* was not higher under blue light than that under fluorescent light (Figure S1). Diatoms contain fucoxanthin, which is a natural pigment (Wang et al., 2018). The adsorption of fucoxanthins is optimal in the range of 480–560 nm, although some light absorption occurs in the range of 420–470 nm, which represents the blue wavelength (Papagiannakis et al., 2005). However, the growth rate of *N. palea* did not increase considerably under blue light at  $32 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Similarly, Mouget et al. (2004) reported that the growth rate of diatom *Haslea ostrearia* at  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  was significantly higher under blue light than under white light, whereas there was no significant difference in the growth rates between both light conditions at  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Fucoxanthin promotes *N. palea* growth under blue light; however, it may be slow to respond to increased light intensity. Considering the competition with *M. aeruginosa*, the best light intensity was  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . When fluorescent light was replaced with blue light at  $32 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , the growth rate of *M. aeruginosa* reduced by 67% ( $0.33$  to  $0.11 \text{ day}^{-1}$ ), whereas that of *N. palea* reduced by only 42%. Since the reduction in growth rate of *N. palea* via blue light irradiation at both 20 and  $32 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (0% and 42%, respectively) was smaller than that of *M. aeruginosa*



(100% and 67%, respectively), blue light irradiation was considered successful in inhibiting the growth of *M. aeruginosa*, indicating that *M. aeruginosa* was in relatively disadvantageous conditions. We demonstrated that *N. palea* was dominant in the co-culture upon irradiation with blue light at  $32 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

Irradiation with blue light may increase the relative abundance of *N. palea* in small lakes where LEDs are relatively easy to install, thereby inhibiting *Microcystis* blooms. However, because the intensity of blue light is much lower than the intensity of sunlight, *Microcystis* blooms can increase and subsequently decrease the effects of the increasing relative abundance of *N. palea*. Blue light irradiation may be effective for treating water bodies in which sunlight is difficult to reach. Irradiation may be effective in the middle and bottom layers of the water body, or where sunlight is artificially shielded. For example, the use of black shade balls can suppress evaporation of the reservoir by blocking sunlight; in addition, it can prevent algal blooms (Haghighi et al., 2018). Irradiation in such an environment may provide a competitive advantage to *N. palea*. It has been reported that diatoms, such as *Nitzschia* spp., can bloom and cause problems like discoloration of water (Mitrovic et al., 2008). Irradiation with blue LED while monitoring diatom growth is required to control diatom blooms. Irradiation during the night may be also effective. It is necessary to assess the impact of irradiation on the ecosystem because an artificial light at night (ALAN) is recognized as a source of light pollution (Falchi et al., 2016; Holker et al., 2010). However, Grubisic et al. (2017) reported that the proportion of diatoms increases when irradiation is performed using white LEDs into the flumes for 3 weeks as an ALAN. Therefore, irradiation with blue light instead of white light is expected to increase the abundance of *N. palea*.

## CONCLUSIONS

The relative abundance of *N. palea* increased upon irradiation with blue light in co-culture with *M. aeruginosa*. Therefore, blue light can be used to increase the relative abundance of *N. palea* to control *Microcystis* blooms.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## AUTHOR CONTRIBUTIONS

Shunsuke Watanabe: performed the experiments, writing draft; data curation. Naoki Matsunami and Ikki Okuma: Performed the experiments. Podiapien Tannen Naythen: helped isolate *Nitzschia*; proofread the manuscript. Megumu Fujibayashi and Takahiro Kuba: Review and editing. Aimin Hao and Yasushi Iseri supervised the experiments. All authors read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (Watanabe, S.), upon reasonable request.

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