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# Growth of *Gracilaria* sp. in Monoculture and Polyculture System with Milkfish (*Chanos chanos* Forsk) in Traditional Ponds, Brebes Regency, Central Java

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**Abstract**

*Gracilaria* sp. has a high economic value, which can be cultivated in monoculture or polyculture with other cultivars, such as milkfish (*Chanos chanos* Forsk). Polyculture of *Gracilaria* sp. with milkfish can increase the growth of *Gracilaria* sp. because the metabolic products of milkfish are helpful as a source of nutrients for *Gracilaria* sp. This study aimed to (1) examine the effect of *Gracilaria* sp. cultivation in monoculture and polyculture with milkfish on the growth of *Gracilaria* sp. and (2) the nutrient content of the water. This study used an experimental method with two treatments and three replications using six (6) earth ponds: *Gracilaria* sp. monoculture and polyculture with milkfish. Growth and nutrient content (nitrate and phosphate) data were analyzed using a t-test. The results of the study showed that the growth of *Gracilaria* sp. polyculture with milkfish provided absolute growth of 3615.89 ± 330.84 g; Relative Growth Rate (RGR) 180.79 ± 16.54 %; and Specific Growth Rate (SGR) 2.29 ± 0.13 %/day were significantly higher, compared with the growth of *Gracilaria* sp. monoculture. Monoculture *Gracilaria* sp showed absolute growth of 1912.71 ± 386.94 g; RGR 95.64 ± 19.35 %; and SGR 1.48 ± 0.22 %.day$^{-1}$. Nutrient content in polyculture pond waters revealed nitrate 1.08 ± 0.09 mg.L$^{-1}$ and phosphate 0.18 ± 0.04 mg.L$^{-1}$ were significantly higher compared to nutrient content in monoculture pond waters which were nitrate 0.71 ± 0.22 mg.L$^{-1}$ and 0.07 ± 0.04 mg.L$^{-1}$. Polyculture Gracilaria and seaweed provide higher production due to the enrichment from nutrient content.

**Keywords:** Gracilaria, milkfish, monoculture, nitrate, phosphate, polyculture

## INTRODUCTION

*Gracilaria* sp. has an advantage; its high tolerance to environmental conditions makes it easier to cultivate. One of the *Gracilaria* sp. production centers in Central Java is located in Brebes Regency, with production reaching 40,247 tons (DKP Brebes, 2014). *Gracilaria* sp. production in the district is carried out in monoculture and polyculture ponds. Multiple species with different feeding habits can be grown together in a polyculture system, which minimizes land use, produces products of greater diversity, and increases economic profitability.

*Gracilaria* sp. polyculture system is widely practiced with milkfish (*Chanos chanos* Forsk). The growth of *Gracilaria* sp. cultivated in polyculture with milkfish is better than in monoculture (Suryani *et al.,* 2022). That is thought to be because, in polyculture systems, the supply of nutrients for *Gracilaria* sp. growth is more than in monoculture systems. Nutrients come from milkfish's metabolic waste (Widyastuti & Setiadi 2021). *Gracilaria* sp. proliferates along with the abundance of nutrient supply. Moreover, milkfish, microscopic plant-eating animals, can eat moss and plankton in the pond that are considered as pest for Gracilaria sp (Sukmawantara *et al.,* 2021).

In addition to nutrients, *Gracilaria* sp. requires sunlight penetration for its growth. In general, *Gracilaria* sp. culture in Brebes Regency uses the broadcast method and can only be applied to ponds with water transparency that can penetrate to the pond's bottom. In some areas, the transparency of the water is low, so the sunlight can only effective in a limited depth (around 30 cm from the surface). That condition is indicated by the decrease in *Gracilaria* sp. production by 0.5 dry tons.ha$^{-1}$ in Brebes Regency (BPS Brebes 2021). A net line method method of *Gracilaria* sp. culture in


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the ponds is asolution when the pond's water transparancy does not reach the pond bottom. The net line method uses a net raft to plant floating seaweed. A 3x3 m net was tightened in square bamboo, then every corner was thighted with big bamboo and plugged into the pond bottom. Therefore, the study on the net line method for *Gracilaria* sp. monoculture and polyculture with milkfish (*Chanos chanos* Forsk) is needed. This study aimed to examine the effect of netline *Gracilaria* sp. monoculture and polyculture with milkfish (*Chanos chanos* Forsk) on Gracilaria sp.'s growth and the nutrient content in the ponds water used.

## MATERIALS AND METHODS

This study was implemented in Randusanga Wetan Village, Brebes District, Brebes Regency, Central Java. The test materials used were *Gracilari*a sp. from Tangerang City, Banten, and milkfish (average size: weight 15 g and length 10 cm) from Brebes Regency, Central Java. Research equipmwnts include Water Quality Checker (Thermo Scientific™ Orion™ Star A Series) for monitoring the dissolved oxygen in the water, and water salinity, pH meter (EZDO PH-5011) for monitoring pH; Secchi disk for monitoring the water transparancy; spectrophotometer (DR3900 Visible Spectrophotometer) for nutrient analyses; and electric balance (CAMRY).

The research method used was the experimental method. The research design used two treatments with three replications. The treatments used in this study are as follows: M: Monoculture of *Gracilaria* sp.; P: Polyculture of *Gracilaria* sp. with milkfish (*Chanos chanos* Forsk); The research design showing the location of the treatment ponds can be seen in Figure 1.

*Gracilaria* sp. was cultivated on 3 m² net lines installed in three monoculture ponds and three polyculture ponds. *Gracilaria* sp. was stocked at a density of 2 $kg.m^{-2}$, while milkfish stocking density was 1 $fish.m^{-2}$. The stocking of the species was done in the morning. *Gracilaria* sp. was monitored weekly by cleaning the mud attached to *Gracilaria* sp.

Data collected included growth data (absolute growth, relative growth rate/RGR, specific growth rate/SGR), nutrient content (nitrate and phosphate), and water quality during 45 days of cultivation. A sampling of *Gracilaria* sp. weight and analysis of nutrient content (nitrate and phosphate) in the cultivation media were conducted every two weeks. Nitrate, phosphate, and ammonia levels were analyzed using a DR3900 Visible Spectrophotometer. Nitrate levels were determined in the range of 0.3 - 30.0 $mg.L^{-1}$ using the cadmium reduction method at a wavelength of 500 nm. Determination of phosphate levels was carried out by ascorbic acid in the range of 0.02 - 3.00 $mg.L^{-1}$ and a wavelength of 700 - 880 nm. Determination of ammonia levels using the salicylate method with levels of 0.01 - 0.50 $mg.L^{-1}$ at a wavelength of 655 nm. The following methods were described by the National Standardization Agency of Indonesia: 06-6989.30-2005, 6989.99-2011 and 06-6989.9-2004 (SNI 2004). The cultivation media's water quality was checked daily, including dissolved oxygen using a D.O. meter, temperature and salinity using a Water Quality Checker, pH using a pH meter, and brightness using a Secchi disk. Ammonia analysis is done every two weeks.

Absolute growth (W) was calculated based on the difference between the final weight of *Gracilaria* sp. at the end of the study (Wt) and the initial weight of *Gracilaria* sp. at the beginning of the study (Wo) using the method of Effendy (1997), The relative growth rate is following formula of Effendy (1997). The specific growth rate is calculated using formula of Effendie, 1997. SGR is Specific Growth Rate ($\%.day^{-1}$) t is period of culture (days)

*Gracilaria* sp. growth and nutrient content (nitrate and phosphate) data were analyzed using an Independent Sample T-Test with a = 0.05. Before the t-test, the data were first tested for normality and homogeneity. Decision-making for the t-test was done by looking at the probability number, which accepts Ho if P>0.05 and rejects Ho if P<0.05. The data analysis was carried out using SPSS software version 25.

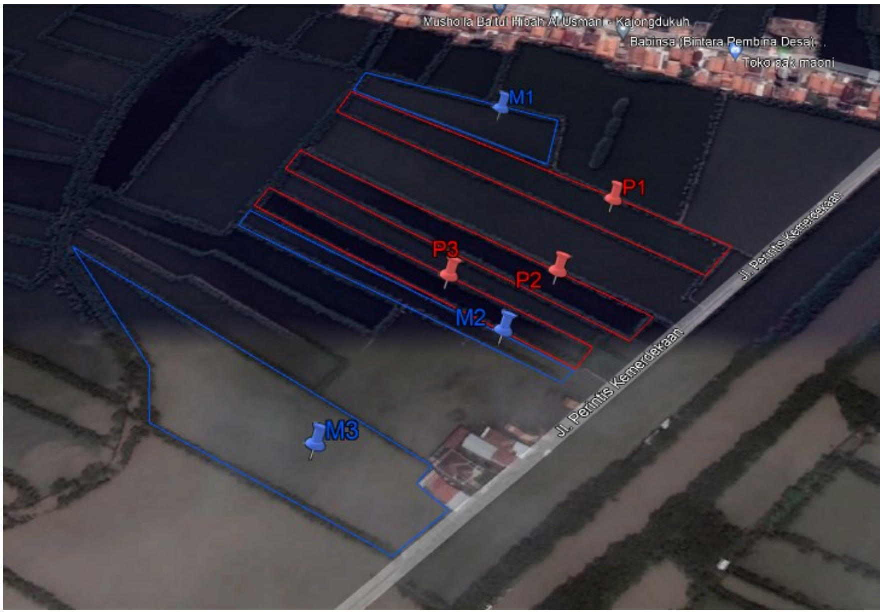


**Figure 1.** Research Design: M1) Monoculture 1; P1) Polyculture 1; P2) Polyculture 2; P3) Polyculture 3; M2) Monoculture 2; and M3) Monoculture 3.

## RESULT AND DISCUSSION

The cultivation of *Gracilaria* sp. monoculture and polyculture with milkfish (*Chanos chanos* Forsk) has a significant effect (P < 0.05) on the growth of *Gracilaria* sp., which includes absolute growth (Figure 2), relative growth rate (Figure 3), and specific growth rate (Figure 4). The growth results in the study showed that the average value of absolute growth of the monoculture treatment was 1912.71 g, while the polyculture treatment was 3615.89 g. The average RGR value of the monoculture and polyculture cultivation system treatments were 95.64% and 180.79%, respectively. Furthermore, the average SGR values of monoculture and polyculture cultivation were 1.48%.day$^{-1}$ and 2.29%.day$^{-1}$ respectively. The average value of absolute growth, RGR, and SGR showed that the growth of *Glaciaria* sp. cultivated with a polyculture system with milkfish (*Chanos chanos* Forsk) was higher than in monocultures. This is because, in the polyculture system, the supply of nutrients for the growth of *Gracilaria* sp. is higher than in the monoculture system. The behavior of milkfish as microscopic plant eaters can help remove moss attached to *Glacilaria* sp., thus accelerating the absorption of nutrients by *Gracilaria* sp. Nutrients, especially nitrates and phosphates, are the main limiting factors for algae growth in aquatic ecosystems (Shakouri & Balouch 2020).

In addition to the advantages of the polyculture system, applying the net line method in *Gracilaria* sp. cultivation also resulted in better growth of *Gracilaria* sp. compared to those cultivated using the broadcast method. *Gracilaria* sp. cultivation in polyculture with milkfish using the net line method resulted in an RGR of 180.79 %. The RGR value is higher than the RGR of *Gracilaria* sp. polyculture with milkfish using the broadcast method, which only produces an RGR of 72.36 % (Suryani *et al.,* 2022). The increased growth of *Gracilaria* sp. is due to the penetration of sunlight obtained by *Gracilaria* sp. being more optimal so that the photosynthesis process can work better. The condition of the study ponds with depth and turbidity is too high, which impacts the lack of photosynthesis process *Gracilaria* sp. cultivated with the broadcast method because the penetration of sunlight cannot reach the bottom of the pond. Applying the net line method in the cultivation of *Gracilaria* sp., which is polyculture with milkfish, is considered feasible because it produces a decent SGR, which reaches 2.29 %.day$^{-1}$. Seaweed is feasible to cultivate if it grows by up to 2 %.day$^{-1}$ (Ariyati, *et al.,* 2016; Hendri *et al*. 2018; Wijayanto *et al*. 2020).

The*Gracilaria* sp. monoculture and polyculture with milkfish (*Chanos chanos* Forsk) has a significant effect (P < 0.05) on the nitrate content in pond waters (Figure 5). The results showed that the nitrate content of monoculture ponds was 0.71 ± 0.22 mg.$L^{-1}$, while polyculture ponds amounted to 1.08 ± 0.09 mg.$L^{-1}$. Nitrate content in the pond waters of the polyculture system is higher than in the pond waters of the monoculture system. This is because, in the polyculture system, milkfish (Chanos chanos Forsk) are cultivated with *Gracilaria* sp. Milkfish produce metabolic waste that can be converted into nitrate through nitrification. The nitrate content in this study pond is classified in the range suitable for the cultivation of *Gracilaria* sp. The range of nitrate values suitable for seaweed growth is 0.1 - 3.5 mg.$L^{-1}$ (Nadlir *et al*. 2019). Nitrate content in pond waters is critical because it is needed to support the metabolic process, growth, and survival of *Gracilaria* sp (Agustang, Mulyani & Indrawati 2019). Nitrate is the primary material in the photosynthesis process of *Glacilara* sp. (Lachmann *et al*. 2019). Seaweed's biomass increases with water nitrate levels (Wang *et al*. 2023).

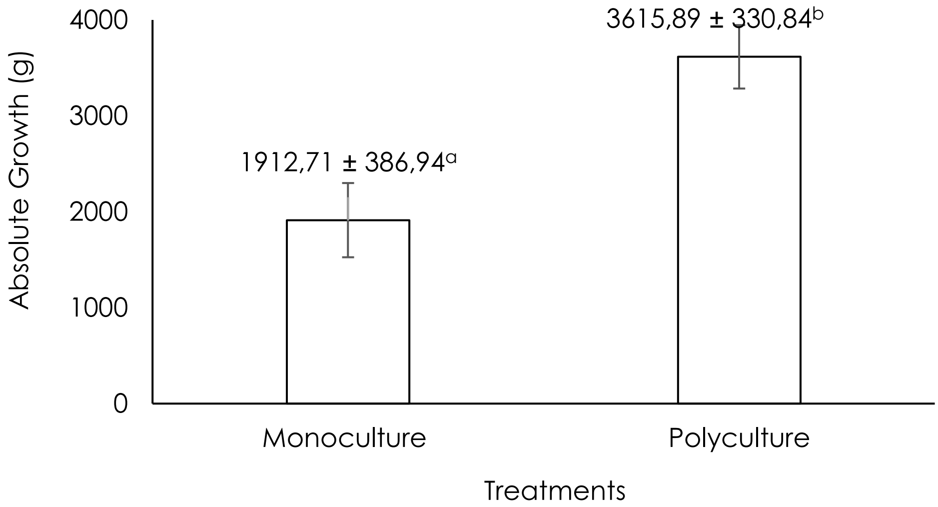


**Figure 2.** Absolute growth of *Gracilaria* sp. during the study
Notes: Values with different superscripts indicate significant differences (P<0.05).

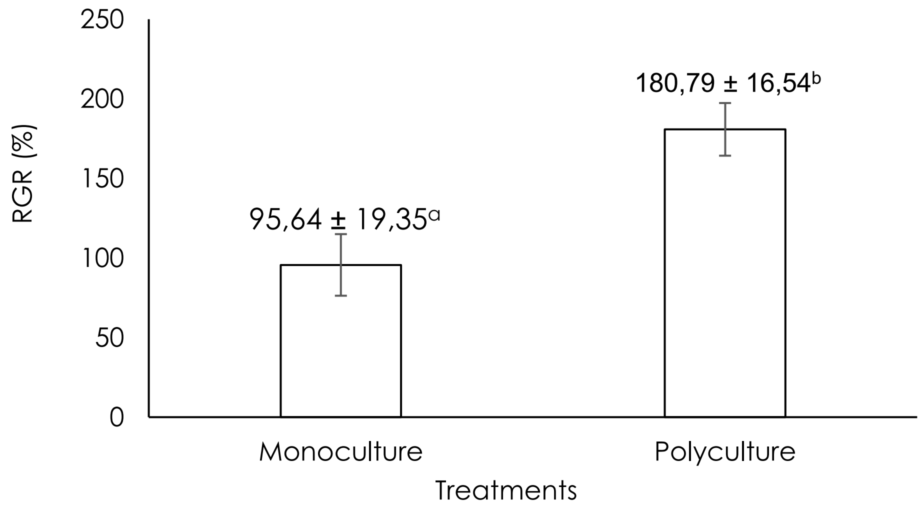


**Figure 3.** Relative Growth Rate (RGR) of *Gracilaria* sp. during the Study
Notes: Values with different superscripts indicate significant differences (P<0.05).

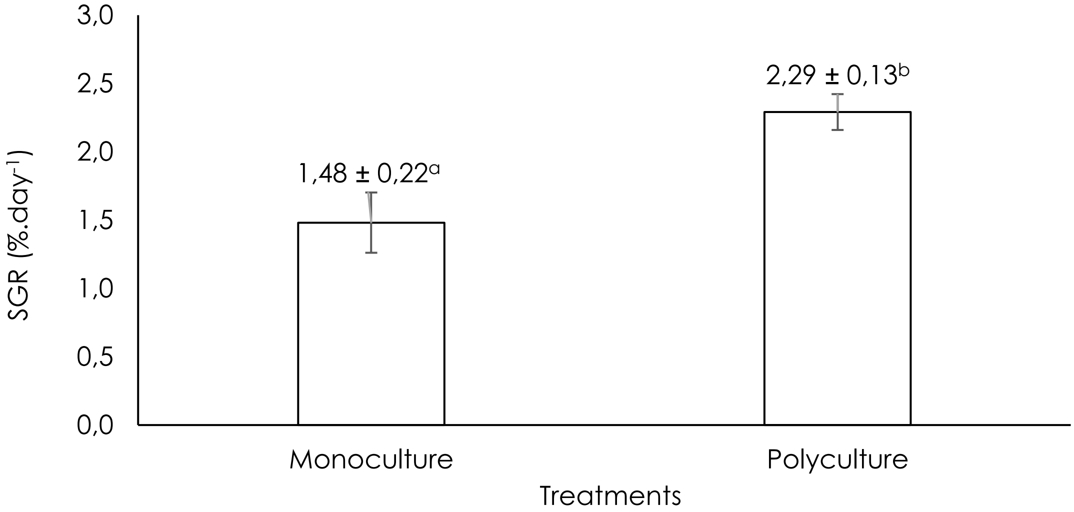


**Figure 4.** Specific Growth Rate (RGR) of *Gracilaria* sp. during the study
Notes: Values with different superscripts indicate significant differences (P<0.05).

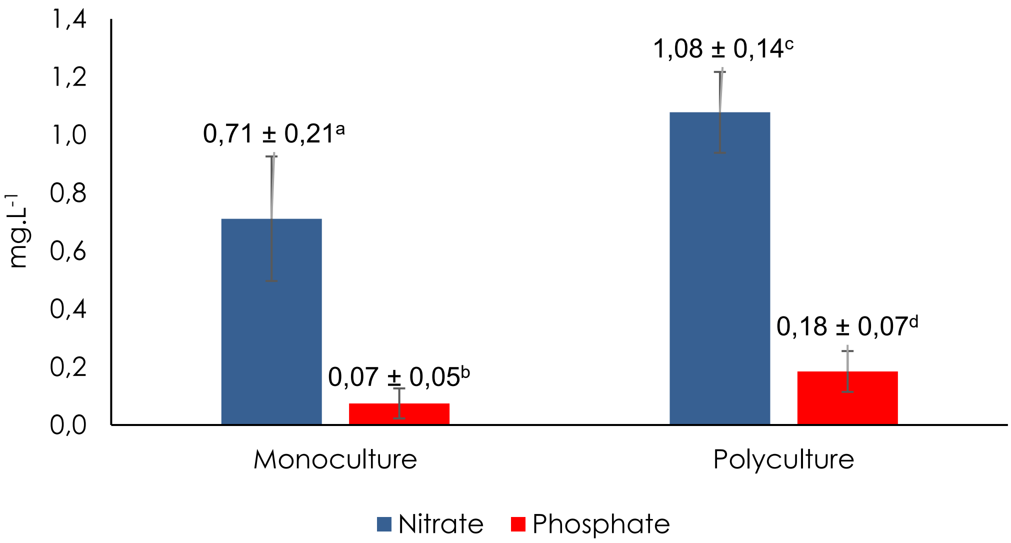


**Figure 5.** Nitrate and Phosphate Content in Monoculture and Polyculture Farming Systems
Notes: Values with different superscripts indicate significant differences (P<0.05).

The results of phosphate analysis in monoculture ponds amounted to 0.07 ± 0.04 mg.L$^{-1}$, while in polyculture ponds amounted to 0.18 ± 0.04 mg.L$^{-1}$. These results indicate that the cultivation of *Gracilaria* sp. monoculture and polyculture with milkfish (*Chanos chanos* Forsk) has a significant effect ($P < 0.05$) on the phosphate content in pond waters (Figure 5). The high phosphate value in the pond waters of polyculture systems is due to the decomposition of higher organic materials in polyculture system ponds. Phosphate values in the monoculture ponds are classified as unsuitable for *Gracilaria* sp. cultivation, while in the polyculture ponds, they are classified as suitable. The range of phosphate values suitable for seaweed growth is 0.1 - 3.5 mg.L$^{-1}$ (Nadlir *et al*. 2019). Phosphate in waters is naturally sourced from the weathering of mineral rocks and the decomposition of organic matter. Phosphate is a form of phosphorus that plants can utilize, both higher plants and algae, because it includes the essential ingredients for preparing proteins and forming chlorophyll in photosynthesis (Susanto *et al*. 2021). The photosynthesis process that occurs at a high rate also causes seaweed growth to be high (Fanni *et al.,* 2021).

**Table 1**. Water Quality of Monoculture and Polyculture System Ponds

| Variable | Treatments | | References | |
|---|---|---|---|---|
| | Monoculture | Polyculture | | |
| Temperature (˚C) | 28,7 – 30,98 | 28,57 – 30,87 | 20 - 34 [a] | 25 – 35 [b] |
| Salinity (‰) | 24,9 – 29,74 | 22,77 – 30,13 | 15 - 35 [c] | 30 – 35 [d] |
| pH | 7,71 – 8,03 | 7,54 – 7,84 | 6 - 8 [e] | 7,6 [f] |
| Brightness (%) | 48,48 – 56,63 | 37,85 – 41,54 | 80 - 100 [g] | - |
| Depth (cm) | 93,49 – 112,77 | 92,2 – 102,4 | 60 - 80 [h] | 50 [i] |
| Nitrate (mg.L$^{-1}$ ) | 0,49 – 0,93 | 0,99 – 1,17 | 0,1 – 3,5 [j] | 0,7 – 0,9 [k] |
| Phosphate (mg.L$^{-1}$ ) | 0,03 - 0,11 | 0,14 - 0,22 | 0,1 – 3,5 [j] | 0,03 – 0,05 [k] |
| Ammonia (mg.L$^{-1}$ ) | 0,01 - 0,07 | 0,07 - 0,11 | < 1,5 [l] | - |

Notes: a) Rejeki *et al*. (2018); b) Mulyaningrum & Suwoyo (2018) c) Rohman *et al.* (2018); d) Hastuti *et al*. (2022); e) Alda *et al.* (2022); f) Hidayat *et al*. (2021); g) Madina *et al.* (2022) and Ya'la (2022); h) Sukri *et al*. (2020); i) Ullah *et al*. (2023); j) Nadlir *et al*. (2019); k) Mujiyanto *et al*. (2020); l) Wahyuningsih & Gitarama (2020).

The results of water quality measurements in monoculture and polyculture ponds are presented in Table 1. Both cultivation systems showed decent values in terms of water quality, including temperature, salinity, pH, D.O., nitrate, and ammonia. In contrast, the value of phosphate in the monoculture system tends to need to be more suitable. In addition, the water depth and transparancy value in both cultivation systems are categorized as unsuitable for *Gracilaria* sp culture in ponds.

## CONCLUSION

The growth of *Gracilaria* sp. from the polyculture system with milkfish (2.29 ± 0.13 %.day$^{-1}$) was significantly higher (P<0.05) than monoculture *Gracilaria* sp (1.48 ± 0.22 %.day$^{-1}$). Nitrate and phosphate content in polyculture of *Gracilaria* sp. and milkfish are significantly higher (1.08 ± 0.09 and 0.18 ± 0.04 mg.L$^{-1}$, respectively) than in monoculture Gracilaria.

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