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Effects of Artificial LED Light on the Growth of Three Submerged Macrophyte Species during the Low-Growth Winter Season: Implications for Macrophyte Restoration in Small Eutrophic Lakes

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Abstract: Eutrophication of lakes is becoming a global environmental problem, leading to, among other things, rapid reproduction of phytoplankton, increased turbidity, loss of submerged macrophytes, and the recovery of these plants following nutrient loading reduction is often delayed. Artificial light supplement could potentially be a useful method to help speeding up recovery. In this study, three common species of submerged macrophytes, *Vallisneria natans*, *Myriophyllum spicatum* and *Ceratophyllum demersum*, were exposed to three LED light treatments (blue, red and white) and shaded (control) for 100 days (from 10 November 2016 to 18 January 2017) in 12 tanks holding 800 L of water. All the three LED light treatments promoted growth of the three macrophyte species in terms of shoot number, length and dry mass. The three light treatments differed in their effects on the growth of the plants; generally, the red light had the strongest promoting effects, followed by blue and white. The differences in light effects may be caused by the different photosynthetic photon flux density (PPFD) of the lights, as indicated by an observed relationship of PPFD with the growth variables. The three species also responded differently to the light treatments, *V. natans* and *C. demersum* showing higher growth than *M. spicatum*. Our findings demonstrate that artificial light supplement in the low-growth winter season can promote growth and recovery of submerged macrophytes and hence potentially enhance their competitiveness against phytoplankton in the following spring. More studies, however, are needed to elucidate if LED light treatment is a potential restoration method in small lakes, when the growth of submerged macrophytes are delayed following a sufficiently large external nutrient loading reduction for a shift to a clear macrophyte state to have a potential to occur. Our results may also be of relevance when elucidating the role of artificial light from cities on the ecosystem functioning of lakes in urban areas.

Keywords: submerged macrophytes; photosynthetic photon flux density; artificial light supplement; growth; eutrophication

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1. Introduction

Submerged macrophytes play an important role in aquatic ecosystems, particularly in shallow lakes [1,2]. Along with the intensification of lake eutrophication in recent decades, the abundance of submerged macrophytes in natural water has decreased in many lakes or even disappeared, often resulting in a transformation from a clear vegetated state with sound functioning into a non-vegetated turbid state with degraded functioning [2]. High turbidity as a result of extensive phytoplankton growth, stimulated by excessive discharge of nutrients, is widely believed to be the primary cause of the disappearance of submerged macrophytes [3–5]. Worldwide, close relationships between nutrients, phytoplankton chlorophyll and water transparency have been reported [6–8]. Enclosure experiments and field surveys conducted in Danish shallow lakes have suggested that submerged macrophytes tend to disappear when the concentrations of total nitrogen (TN) and total phosphorus (TP) exceed 1.2~2 mg L^{-1} and 0.1~0.2 mg L^{-1}, respectively [9]. A multi-lake comparison and long-term monitoring of Yangtze lakes suggested that a TP level of about 0.1 mg L^{-1} causes disappearance of submerged macrophytes [8]. Similar conclusions have been reached in global multi-lake comparisons [10].

As nutrients are a key mechanism for the loss of submerged macrophytes due to their promotion of growth and hence shading of phytoplankton [9,11,12], a reduction of nutrient loading has been widely applied in order to recover the vegetation and hence restore lake ecosystems [7,8,13]. However, because of nutrient release from the sediment, the concentrations of nutrients in the overlying water tend to show resilience to efforts of nutrient reduction [14,15]. Also, resilience in biological communities may contribute to a delayed response in both temperate and (sub)tropical lakes and appearance of submerged macrophytes [16]. A combination of a multi-lake comparison and long-term monitoring in Yangtze subtropical shallow lakes suggests that to recover submerged macrophytes, concentrations of total phosphorus must be diminished to levels as low as 50 μg L^{-1}, which is much lower than the level (100 μg L^{-1}) suggested to cause loss of vegetation [8]. To speed up the recovery after nutrient loading reduction lake managers may be required to use auxiliary approaches to stimulate vegetation recovery.

Lowering the water level has been suggested in practical lake management to improve the underwater light climate [17,18], but it may not always be a feasible method. Artificial light supplement has been proposed as a potential alternative method for recovery of submerged macrophytes as LED light has been widely used in the cultivation of terrestrial vegetables, medicinal herbs and ornamental waterweeds [19–23]. LED light also has the advantage that it is highly efficient, long-lived and stable. However, so far studies on the influence of LED on the growth of submerged macrophytes are scarce.

Artificial light in cities has been shown to affect aquatic ecosystems. Light near waterbodies can influence the behavior of insects, zooplankton and fishes, thus altering species interactions. Hölker et al. [24] found that high-pressure sodium lamps (70 W, 2000 K, 96 Lm W^{-1}) changed microbial community composition at the sediment surface, increasing the proportions of primary producers such as diatoms and cyanobacteria. Studies on periphyton demonstrate that nocturnal illumination by white LEDs (of around 20 lux) decreased periphyton biomass in both a subalpine stream and a lowland agricultural drainage ditch [25–27]. Only few studies of the effects of artificial light on primary producers in lakes are available, however.

In order to study the growth of submerged macrophytes under LED light, we selected three widely distributed species, *Vallisneria natans*, *Myriophyllum spicatum* and *Ceratophyllum demersum* for a 100-day experiment carried out in mesocosms exposed to three wavelengths of LED (red, white and blue). The objectives of this study were threefold: (1) To test the influence of different wavelengths on the growth of the three submerged macrophyte species; (2) to compare the differences among species and among wavelengths; (3) to explore possible mechanisms underlying the differences. We expected the results to provide valuable information for application in the restoration of submerged macrophytes in small eutrophic lakes and also on the role of artificial light in ecosystem functioning of city lakes.

2. Materials and Methods

2.1. Study Area and Experimental System

The experiment was carried out in an experimental facility comprising twelve cylindrical tanks (1.0 m in both height and diameter) placed under a shaded and breathable shed (<1% natural light).

The facility is located northeast of Lake Bao'an (30°17′17″ N, 114°43′45″ E) on the south bank of the middle Yangtze River. The region is dominated by a warm, humid subtropical climate with an annual mean air temperature of approximately 19 °C and an annual mean precipitation of 1030 mm. *V. natans*, *M. spicatum* and *C. demersum*, three widely distributed submerged macrophytes in the lakes along the mid-lower Yangtze River, were used for the experiment. On 30 October 2016, plants were collected from Lake Xiliang (29°51′ N~30°01′ N, 114°00′ E~114°10′ E), a middle Yangtze lake with a surface area of 80 km^2 and a mean water depth of 1.92 m. Similar-sized plants were selected and cut down to 20 cm before transport to the experimental area. We selected 20 plants from each of the three submerged macrophytes species to measure their growth value as the initial value at the beginning of experiment. All plants were planted in plastic pots (23 cm in top diameter, 13 cm in bottom diameter, 13 cm in height, five plants in each pot) filled with 10 cm sediment (taken from an experimental pond and mixed with washed sand. The ratio of sand to sediment was 1:3). Three pots were placed in each tank (Figure 1). The water used in the experiment was taken from Lake Bao'an. According to our survey from 2015 through 2016, annual mean water depth was 2.29 m, Secchi depth (ZSD) was 0.66 m, pH 8.54, dissolved oxygen (DO) 9.8 mg L^{-1}, total nitrogen (TN) 0.83 mg L^{-1}, total phosphorus (TP) 0.08 mg L^{-1} and chlorophyll a (Chl a) 36.7 μg L^{-1}; turbidity (Turb) was measured in nephelometric turbidity units (NTU) and was 14.7. The water depth was kept at 0.9 m by adding lake water to the tanks to compensate for the water loss caused by evaporation and sampling.

2.2. Experimental Treatments

There were four treatments with three replicates: Shaded (control), blue LED (450~460 nm), red LED (650~660 nm) and white LED. The LEDs were provided by Institute of Semiconductors, Chinese Academy of Sciences. The peak wavelength of LED light irradiation was measured by Institute of Semiconductors. Every treatment had three replicates. LED lights were regulated relative to the change in size of the plants during growth to ensure that the light was continuously about 20 cm above the plants (Figure 1). The light was restricted to 6 h per day (9:00~15:00) due to funding limitation. The experiment lasted for 100 days, from 10 November 2016 to 18 January 2017.

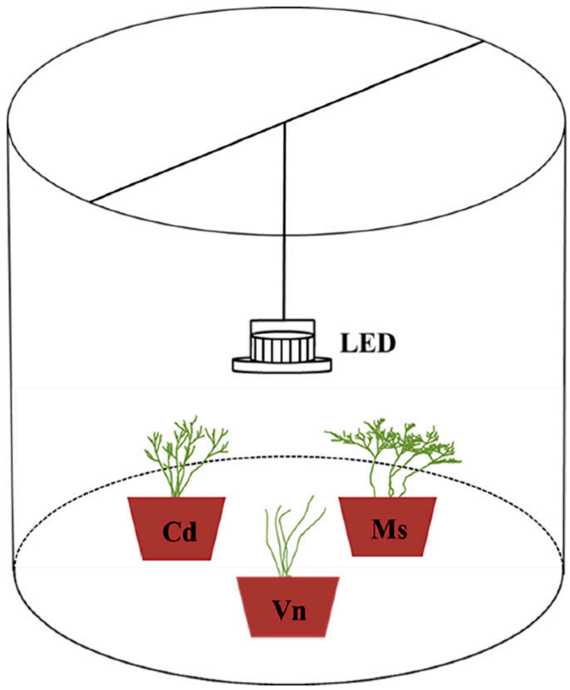

Figure 1. Experimental setup of three submerged macrophyte species and LED light in the tanks (Vn, *Vallisneria natans*; Ms, *Myriophyllum spicatum*; Cd, *Ceratophyllum demersum*).

2.3. Sampling and Measurement

During the experiment, environmental parameters were measured every twenty days. Water temperature, dissolved oxygen (DO), pH, and conductivity (Cond) were measured in situ at half water depth with a YSI ProPlus (Yellow Spring Inc., USA). Photosynthetic photon flux density (PPFD) was measured with an illuminometer (LI-192SA, LI-COR, Lincoln, NE, USA), just above the plant canopy. Water samples for chemical analysis were collected at half water depth in the center of each tank with a 5 L polymethyl methacrylate water sampler. TN was determined following an alkaline potassium persulphate digestion-UV spectrophotometric method (PERSEE, TU-1810, Beijing, China) and TP following an ammonium molybdate-ultraviolet spectrophotometric method after digestion with $K_2S_2O_8$ solution [28]. Phytoplankton Chl *a* was extracted using 90% acetone (at 4 °C for 24 h) after filtration through GF/C filters (Whatman, GE Healthcare UK Limited, Buckinghamshire, UK), and absorbance was then read at 665 nm and 750 nm both before and after acidification with 10% HCl using a spectrophotometer. Turbidity was measured with a turbidimeter (2100 Q, HACH, Loveland, CO, USA). At the end of the experiment, all plants were removed from the tanks and washed with tap water to record the number of plants and to measure the length of leaves. The plants in each pot were then separated into leaves and roots to measure dry mass with an electronic balance (0.0001 g, AUY220, Shimadzu Corporation, Kyoto, Japan) after drying at 80 °C (Jinghong, DHG-9071A, Shanghai, China) for 48 h to constant mass.

2.4. Calculation and Statistical Analyses

Microsoft Excel 2016, SPSS 25 and R 3.4.2 were used to process and analyze the data. Excel 2016 was used for data preprocessing. Spearman rank correlations were applied to test for relationships among environmental variables and macrophyte growth variables. Comparisons between groups were tested by One-Way (significant difference) range test with a significance level of 5% using the statistical analysis software SPSS 25 (IBM, Armonk, NY, USA). Relationships between PPFD and the growth variables of macrophytes were analysed applying the regression analysis in the statistical analysis software R 3.4.2 (R core team, 2018).

3. Results

3.1. Environmental Variables

The variables of water quality including TN, TP, WT and Turb were generally comparable among the treatments during the 100-day experiment (Table 1). The level of phytoplankton chlorophyll a (Chl *a*) was overall low, although the variations among treatments were relatively high. PPFD showed a clear gradient of Control < Blue < Red < White (Table 1). Spearman rank correlations among the environmental variables showed significant positive correlations between TN-TP and Turb-Chl *a* and negative correlations of TN with Turb and Chl *a* (Table 2).

Table 1. TN, total nitrogen (mg L^{-1}); TP, total phosphorus (mg L^{-1}); WT, water temperature (°C); Turb, turbidity (NTU); Chl a, chlorophyll a (mg m^{-3}) and PPFD, photosynthetic photon flux density (μmol m^{-2} s^{-1}) of the various treatments (mean ± SD).

Treatment	TN	TP	WT	Turb	Chl *a*	PPFD
Control 1	2.17 ± 1.21	0.19 ± 0.11	12.33 ± 3.59	1.47 ± 1.00	0.62 ± 0.36	4.20 ± 4.87
Control 2	2.38 ± 1.13	0.21 ± 0.11	12.30 ± 3.60	1.33 ± 1.08	0.36 ± 0.26	5.08 ± 6.03
Control 3	2.57 ± 1.11	0.19 ± 0.07	12.23 ± 3.62	1.01 ± 0.73	0.45 ± 0.46	4.60 ± 5.31
Blue 1	2.07 ± 0.87	0.18 ± 0.09	12.20 ± 3.67	1.56 ± 1.72	0.55 ± 0.39	91.75 ± 18.43
Blue 2	2.26 ± 1.02	0.19 ± 0.08	12.40 ± 3.57	1.43 ± 1.00	0.45 ± 0.26	94.75 ± 21.69
Blue 3	2.43 ± 1.16	0.16 ± 0.09	12.33 ± 3.59	1.45 ± 1.06	0.27 ± 0.22	92.25 ± 24.09
Red 1	1.90 ± 1.01	0.18 ± 0.10	12.23 ± 3.72	1.66 ± 1.51	2.18 ± 1.39	159.25 ± 22.17
Red 2	2.27 ± 1.16	0.16 ± 0.08	12.40 ± 3.57	1.21 ± 0.90	0.45 ± 0.46	159.75 ± 29.88
Red 3	1.94 ± 1.17	0.13 ± 0.07	12.27 ± 3.71	1.03 ± 0.71	0.18 ± 0.26	156.75 ± 19.82
White 1	1.88 ± 1.14	0.14 ± 0.09	12.27 ± 3.71	1.55 ± 0.87	1.00 ± 0.34	177.50 ± 35.80
White 2	1.52 ± 0.92	0.11 ± 0.07	12.20 ± 3.73	1.82 ± 1.29	0.64 ± 0.72	173.25 ± 35.38
White 3	2.35 ± 1.03	0.19 ± 0.10	12.40 ± 3.70	1.08 ± 0.94	0.27 ± 0.22	205.50 ± 42.85
Mean ± SD	2.14 ± 0.28	0.17 ± 0.03	12.30 ± 0.07	1.39 ± 0.25	0.62 ± 0.51	

Table 2. Spearman rank correlations (r, upper triangle; p, lower triangle; significant correlations are given in bold, * $p < 0.05$, ** $p < 0.01$, $n = 12$) between TN, total nitrogen (mg L^{-1}); TP, total phosphorus (mg L^{-1}); Turb, turbidity (NTU); Chl *a*, chlorophyll *a* (mg m^{-3}) and PPFD, photosynthetic photon flux density (μmol m^{-2} s^{-1}).

	TN	TP	Turb	Chl *a*	PPFD
TN		**0.63 ***	**−0.69 ***	**−0.63 ***	−0.46
TP	**0.03**		−0.34	−0.16	−0.53
Turb	**0.02**	0.29		**0.77 ****	0.15
Chl *a*	**0.03**	0.62	**0.003**		0.1
PPFD	0.13	0.08	0.64	0.76	

3.2. Growth Variables of Plants

The shoot number of plants at the end of the experiment remained at initial levels for *V. natans* (*Vn*) but decreased for *M. spicatum* (*Ms*) and *C. demersum* (*Cd*) (Figure 2a). The leaf number of *Vn* was clearly higher in the three light treatments than in the control (Supplementary Material, Table S1). For *Ms* and *Cd*, the blue light and red light treatments tended to produce higher shoot numbers than the control and white light treatments. No *Ms* survived in the control.

Maximum length and average length of shoots showed similar patterns (Figure 2b,c). Both increased somewhat or, in the case of *Vn*, remained at the start-up level. Both *Ms* and *Cd* increased after initial survival. For *Ms*, no clear difference was found in shoot length among the treatments. For *Cd*, shoot length was generally higher in the red and white light treatments than in the control and blue light treatments.

The dry mass of plants increased noticeably after initial survival compared with the start-up levels, except for *Ms* in the white light treatments (Figure 2d). For both *Vn* and *Ms*, dry mass was generally higher in the blue light and red light treatments than in the control and the white light treatments. For *Cd*, dry mass was highest in the red light and white light treatments, followed by the blue light treatments and, finally, the control.

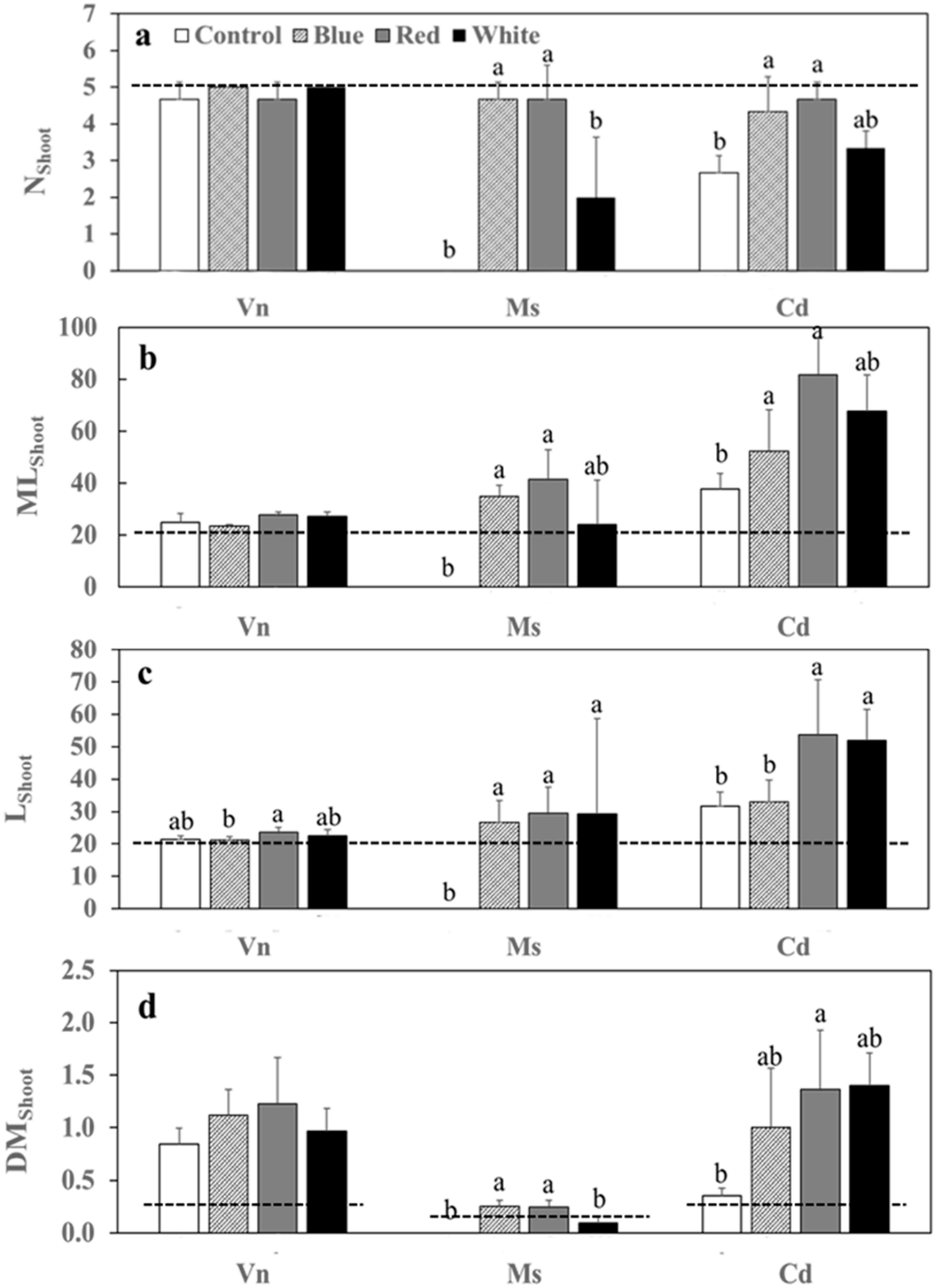

Figure 2. Histograms (mean + SD) of (**a**) shoot number (N_{Shoot}), (**b**) maximum length of shoot (ML_{Shoot}, cm), (**c**) average length of shoot (L_{Shoot}, cm) and (**d**) dry mass of shoot (DM_{Shoot}, g). Different letters indicate significant differences ($p < 0.05$) among treatments. Vn, *Vallisneria natans*; Ms, *Myriophyllum spicatum*; Cd, *Ceratophyllum demersum*. The dashed line indicates the initial value).

3.3. Relationships between Growth Variables and Environments

Spearman rank correlations (Table 3) suggested a significant negative relation between TN and ML_{Shoot} of *Vn* and significant positive relations of PPFD with N_{Leaf} of *Vn*, ML_{Shoot}, L_{Shoot} and DM_{Shoot} of *Cd*. Figure 3 shows the scatterplots of PPFD with growth variables of the three submerged macrophytes.

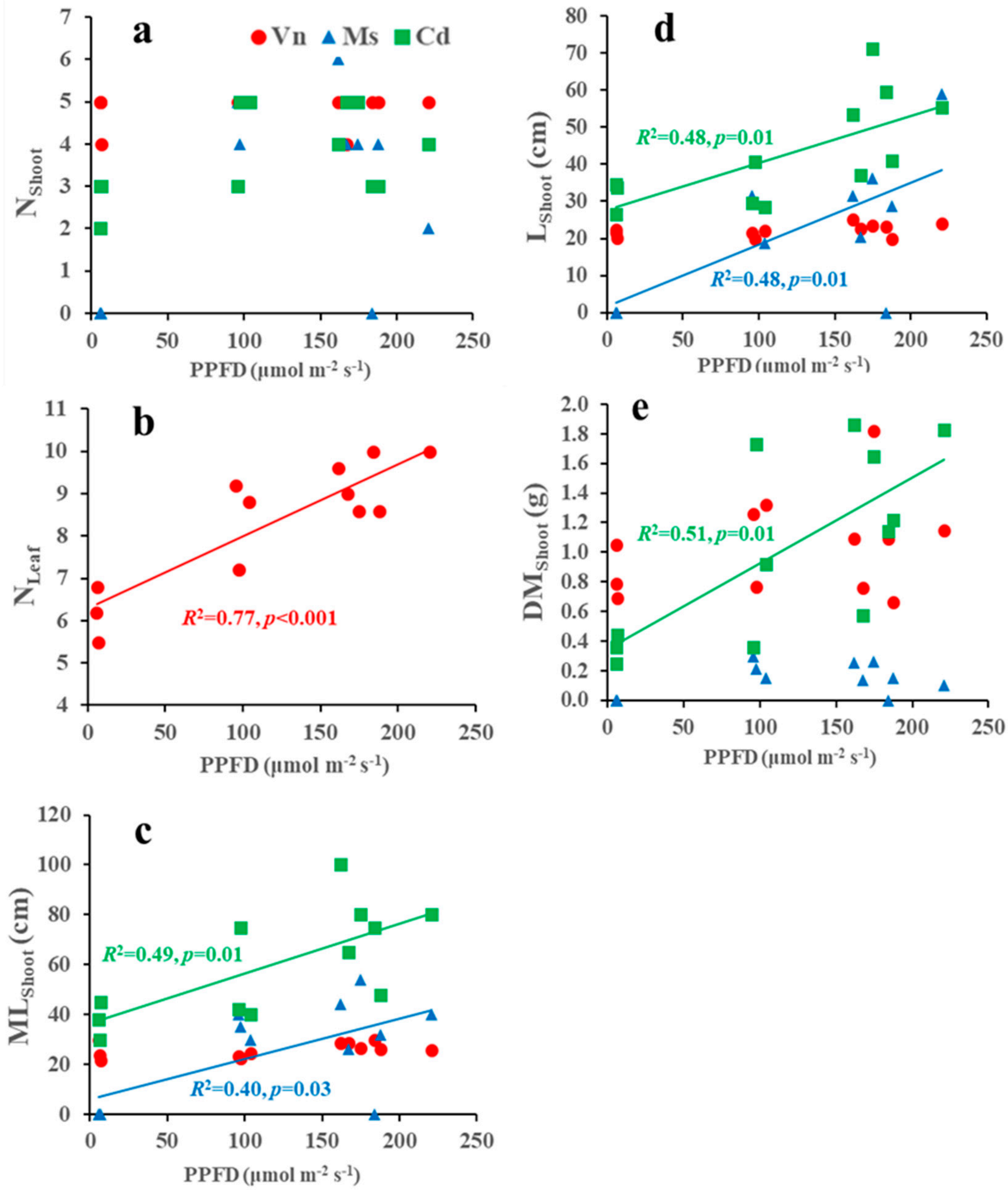

Figure 3. Relationships between (**a**) shoot number (N_{Shoot}), (**b**) leaf number (N_{leaf}), (**c**) maximum length of shoot (ML_{Shoot}), (**d**) average length of shoot (L_{Shoot}), (**e**) dry mass of shoot (DM_{Shoot}) and photosynthetic photon flux density (PPFD) Vn, *Vallisneria natans*; Ms, *Myriophyllum spicatum*; Cd, *Ceratophyllum demersum* (n = 12).

Table 3. Spearman rank correlations (*r* values) between morphological variables of three species of submerged macrophytes relative to the environment (significant correlations are given in bold, * $p < 0.05$, ** $p < 0.01$, $n = 12$).

		TN	TP	Turb	Chl *a*	PPFD
Vn	N_{Shoot}	0.00	−0.36	−0.19	−0.20	0.13
	N_{leaf}	−0.29	−0.49	−0.03	−0.15	**0.77 ****
	ML_{Shoot}	**−0.63 ***	−0.43	−0.22	−0.07	0.40
	L_{Shoot}	−0.08	−0.17	−0.43	−0.27	0.48
	DM_{Shoot}	0.34	−0.04	−0.46	−0.44	0.22
Ms	N_{Shoot}	−0.16	−0.48	0.03	−0.35	0.20
	ML_{Shoot}	0.01	−0.47	−0.21	−0.51	0.43
	L_{Shoot}	0.07	−0.36	−0.15	−0.47	0.51
	DM_{Shoot}	−0.01	−0.51	0.01	−0.37	0.19
Cd	N_{Shoot}	−0.27	−0.06	0.16	0.05	0.30
	ML_{Shoot}	−0.39	−0.49	−0.08	−0.20	**0.69 ***
	L_{Shoot}	−0.51	−0.55	0.10	0.10	**0.75 ****
	DM_{Shoot}	−0.45	−0.44	−0.04	−0.21	**0.64 ***

TN, total nitrogen (mg L^{-1}); TP, total phosphorus (mg L^{-1}); Turb, turbidity (NTU); Chl *a*, chlorophyll *a* (mg m^{-2}); PPFD, photosynthetic photon flux density (μmol m^{-2} s^{-1}); N_{Shoot}, shoot number; N_{leaf}, leaf number; ML_{Shoot}, maximum length of shoot (cm); L_{Shoot}, average length of shoot (cm); DM_{Shoot}, dry mass of shoot (g); Vn, *Vallisneria natans*; Ms, *Myriophyllum spicatum*; Cd, *Ceratophyllum demersum*.

4. Discussion

Our 100-day experiment revealed that artificial LED light supplement significantly promoted the growth of the three submerged macrophyte species. The leaf number of *V. natans* (*Vn*) and most of the growth indices of *M. spicatum* (*Ms*) and *C. demersum* (*Cd*) were significantly higher in the treatments with artificial light than in those without. Shoot dry mass of *Vn* also tended to be higher in the blue and red light treatment than in the control.

The response of various growth variables differed among species and among light treatments. In general, the selected growth variables responded most strongly in the red light treatments, although favorable responses of some of the growth variables were also observed in the blue and white light treatments. Better growth under red light has a physiological explanation. In a laboratory experiment with *Cd*, the activities of peroxidase, catalases and superoxide dismutase, the three enzymes closely related to plant respiration and photosynthesis, were noticeably higher under red light than under blue and white light [29]. Several species of ornamental waterweeds such as *Hydrocotyle vulgaris* and *Eichhornia crassipes* have been found to grow better in white, red and red/blue light treatments than under yellow, green light and ultraviolet light [22,30]. The close relationships of some growth variables with PPFD indicate that the different effects between the lights were caused by their different PPFD. Thus, red light seems to be the first choice for practical application, but also white light may be valuable, particularly for *Cd*.

The different species of submerged macrophytes have their specific strategy for strengthening their competitiveness and tolerance in lakes, as seen in our study. *Vn* tended to maintain a relatively high population size and accumulate mass, but length remained constant even when exposed to excess light. *Ms* selected for increasing the shoot length but did less for maintaining population size and mass accumulation than *Vn*. *Cd* performed better than *Ms* and *Vn* regarding both shoot elongation and mass accumulation, whereas population maintenance was comparably weak. Therefore, considering the general performance, *Vn* and *Cd* are the best target pioneer species when restoring submerged macrophytes by using artificial light.

Our results showed that *Vn* grew well in red light, and *Ms* and *Cd* grew well in both blue and red light. These results can probably be explained by the distribution zone of submerged macrophytes and light in natural lakes. In natural lakes, with the increases of water depth, the proportion of blue light decreases, while red light increases [2]. *Vn* is mainly distributed in the middle and lower layers in natural water bodies that receive more red light. However, *Ms* and *Cd* can grow to the surface of the water profiting from their long shoot length, and they can receive more blue light than *Vn*.

Whether artificial light can be used as a tool in lake restoration when plants are delayed after the external loading is reduced sufficiently to create a shift to a clear state, needs further studies. If not reduced sufficiently to help creating a shift to a macrophyte state the light may just enhance eutrophication by stimulating phytoplankton growth in winter. High costs may also limit the application of artificial light in natural waters. In our experiment, we provided about 100~200 μmol m^{-2} s^{-1} irradiance to the plants (light intensity in the natural lake being less than 30 μmol m^{-2} s^{-1} at a depth of 1 m during the experiment) at a cost of about \$30 m^{-2} in China. The cost in natural lakes could be lower, however, if using solar panels. Moreover, application of artificial LED light in aquatic ecosystems may have undesired side effects as it may alter species interactions and change community composition of primary producers, as seen with city light [25]. According to the study of Hölker et al. [24], illumination as slight as approximately 0.18 μmol m^{-2} s^{-1} may alter sediment microbial communities over time, this being far below the illumination that we added. Use of artificial LED light to promote recovery of submersed macrophytes is, however, aimed at eutrophic waters where undesired impacts are expected to be insignificant due to high turbidity. Light application should, however, be avoided in the night-time in order to mitigate potential adverse effects of nocturnal artificial illumination.

Our study was conducted in tanks, which have their limitations, such as absence of grazing by fish or crayfish and no wind disturbance. Therefore, extrapolation to natural lake ecosystems must be undertaken with caution. Moreover, we only covered the low-growth winter season and the effect might be different in summer for several reasons: more natural light, stronger photosynthesis activities of submerged macrophytes, higher growth rates and hence greater shading effects of phytoplankton because of more favorable nutrient source and temperature conditions. Therefore, further studies in natural systems and also during the high-growth summer season are needed to fully elucidate the effects of artificial light on the growth of submerged macrophytes and ecosystem consequences.

5. Conclusions

Based on the results of our 100-day experiment, the following conclusions can be drawn:

In the low-growth winter season, artificial LED light supplement promoted the growth of submerged macrophytes and led to an increase in shoot number, shoot length as well as biomass.

The effects by artificial LED light supplement on plant growth differed among the three light treatments. In general, red light seems to induce the best performance of growth variables, although good performance was found in the blue and white light treatments as well.

The different effects of the lights may be caused by their different photosynthetic photon flux densities.

The three tested species showed different responses to light treatments. As to their general performance, *Vn* and *Cd* can be considered as target pioneer species in the restoration of submerged macrophytes when using artificial LED light.

Side effects of artificial light on aquatic systems and studies at in situ conditions should be considered before its application as a restoration tool in lakes where the external loading has been sufficiently reduced to be able to push the system from a turbid to a clear state, but plant recovery is delayed.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/11/7/1512/s1, Table S1: Growth variables (average) of the three submerged macrophytes (different letters indicate significant differences at $p < 0.05$, $n = 3$).

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Conflicts of Interest: The authors declare no conflict of interest.

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