

EFFECTS OF CURLY-LEAF PONDWEED (*POTAMOGETON CRISPUS* L.) BIOMASS ON EUTROPHICATION IN BAIYANGDIAN LAKE

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Abstract. Aiming at improving water quality by avoiding eutrophication in Baiyangdian Lake, excessive growth of CLP (*Potamogeton crispus*) was treated by partial harvesting in spring, by means of an enclosure experiment. CLP was harvested in different proportions and water quality was monitored weekly for 46 days. TN concentrations and total bacterial counts reached a maximum on day 24 post harvest, after which they declined sharply. TP concentrations decreased first and then increased gradually. The highest values appeared in water without any CLP and also in plots where the maximum biomass had not been removed. In contrast, plots in which 20% of the biomass had been retained resulted in lowest TN and TP values. COD concentrations increased in mid-to-late May and this correlated with an increase of biomass. TLI of each treatment peaked at the end of May, and reached highest values in mid-June. The highest TLI value appeared in water without any CLP. The water of plots retaining 20% of maximum biomass resulted in medium nutrition levels, while the other treatments reached TLI levels indicative of light eutrophication. The best harvest time for CLP is mid-May and the best harvest rate, as suggested by this study, is approximately 80% of maximum biomass.

Keywords: CLP; total nitrogen; total phosphorus; COD; TLI

Introduction

Curly-leaf pondweed (*Potamogeton crispus* L., here abbreviated as CLP) is a submerged macrophyte commonly found in shallow lakes and a dominant plant in spring, displaying rapid growth and a high biomass production. It is common in many parts of the world including in China. The largest freshwater lake of China, Baiyangdian Lake (the name means “Pearl of North China”), contains areas that are seasonally covered for up to 95% by CLP. Although the plant can assist in natural purification of water, its excessive growth endangers local fishery industry. Moreover, nutrients released after death and decomposition of the plants cause secondary water pollution, and decaying plant parts result in a strong biological deposit-promoting effect that leads to rapid swamp formation. Especially the release of phosphorus is high per unit of plant mass (Tang et al., 2013), which is one of the key factors that causes eutrophication of lake water (Lu et al., 2011). Light intensity is one of the key factors in determining the fate of submerged macrophytes; thus, enhancement of water transparency can restore a healthy growth of submerged macrophytes in eutrophic lakes (Zhang et al., 2010; Xing et al., 2013). This can be achieved by timely harvesting of senescence CLP, in order to avoid a critical increase of nitrogen and phosphorus concentrations as a result of plant decay. Harvesting viable plants not only removes nutrients from the lake and limits nitrogen and phosphorus deposits in mud, but also controls CLP biomass and propagule

numbers (Yu et al., 2013; Jiang et al., 2012). Moreover, the harvested biomass can be utilized as feed for poultry, especially ducks. In most cases, the harvest of CLP begins when the submerged plants have reached maximum biomass.

Research has been conducted to determine the effect of harvesting aquatic plants on nitrogen and phosphorus cycles in aquatic ecosystems, their growth and recovery status after harvest and the optimal timing of this practice (Wu et al., 2012). Studies have shown that the concentrations of nitrogen and phosphorus in various forms increased compared to areas where harvesting was omitted, while the practice of manual CLP removal increased species diversity and abundance, although the reported effects were not statistically significant (Wang et al., 2013).

The work presented here aimed at improving the situation of eutrophication of Baiyangdian Lake as a result of excess CLP growth. An enclosure experiment was performed to study the effect of different quantities of CLP biomass removal on eutrophication levels. The analyses included determination of total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD), chlorophyll a (Chl-a), total bacterial numbers and calculation of the comprehensive nutrition state index (TLI). The results identified the optimum CLP biomass for managers and policy makers and provided a theoretical basis for the treatment of eutrophic shallow lakes and the management of these environments.

Materials and Methods

Sampling site and enclosure experiment setup

Samples were taken from Baiyangdian Lake in the north east of China (38.850°N, 116.000°E). The proportion of water with worse quality than class III in Baiyangdian Lake increased from 14.3% in 1973 to 83% in 2007, and the order of the comprehensive pollution index in the year 2006 was spring > summer > winter > autumn (Zhang et al., 2010). The eutrophic water of Baiyangdian Lake belongs to class five according to the standard limit values in "Environmental Quality Standard for Surface Water (GB3838-2002)". An enclosure experiment was conducted close to Datianzhuang village. The location of the study was selected to meet the following conditions: strong CLP growth, evenly distributed biomass and a surface coverage of over 95%. During the study, which was conducted in April-May 2012 according to *Table 1*, the average height of CLP plants was 149 cm, most of which was in blossom during the investigation, and the average biomass of CLP was 6.33 kg/m², with a water depth of 1.5~1.8 m. Water samples were also collected.

Table 1. Development stages of *P. crispus* (Ren et al., 2012)

| Development stages | Time | Temperature(°C) | Character |
|----------------------------------|-------------------------------|-----------------|--|
| Bud sprouting and growing period | Mid-August to early December | 16.6-7.3 | The breeding buds germinate and seedlings grow rapidly |
| Overwintering period | Early December to early March | 7.3-3.2 | Seedling live through the winter under water or ice |
| Recovering period | Early March to late May | 3.2-11.5 | CLP grows slowly and the leafage emerges |

| | | | |
|--|-------------------------|-----------|--|
| Exponential growth and flowering and fruiting period | Early April to mid-May | 11.5-15.2 | Biomass grows exponentially and <i>P. crispus</i> blooms and yields seed |
| Production of breeding bud | Early May to early June | 15.2-20.8 | The breeding buds produce |
| Death and rot period of plant | Mid-June to late August | 20.8-25.6 | Stems and leaves stop growing and die and rot. The breeding bud lives through summer |

The selected area was divided into 10 plots with impermeable canvas on April 23, 2012, (Fig. 1A). Each plot had a volume of $5 \times 10 \times 2 \text{ m}^3$ (length \times breadth \times depth) and the plots were separated by 2 m wide spaces. The canvas was covered by mud at the bottom to prevent translocation of fish. On April 27, CLP was harvested at various degrees, from 0% (no harvest, retaining 100%) in plot B1 to 80% removal of the content of the plot, resulting in 20% remaining CLP in B4. The other plots were harvested to maintain 60% (B2) and 40% CLP (B3), respectively. Each treatment was performed in triplicate. Control water samples (CK) were taken from an area without CLP growth positioned 2 m away from the enclosures. Every plot was divided into 5 parts (20% of the plot) to remove the required fraction of CLP, including the roots. (Fig. 1B). Water samples were taken in the middle of each plot at depths of 5, 15 and 25 cm. These samples were mixed and analysed for TN, TP, COD, Chl-a content and total bacterial counts on a weekly basis. At the same time, biomass of CLP in each plot was determined.



Figure 1. Photograph of the enclosure plots, showing CLP coverage to the left, and harvesting in action in the photo to the right.

Water analyses

TN concentrations, expressed in mg/L, were determined by alkaline potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) digestion followed by spectrophotometry (GB11894-89). The determination of TP concentrations (mg/L) was based on Mo-Sb spectrophotometry (GB1183-89) after potassium sulfate (K_2SO_4) digestion. COD content (mg/L) was determined by the potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) method, while the total number of

viable bacteria (expressed as $\text{cfu} \times 10^3/\text{mL}$) was determined by plate colony-counting methods. Hot ethanol spectrophotometry was used to determine Chl-a content, expressed in $\mu\text{g/L}$ (Zhang et al., 2008). The fraction of $\text{NH}_3\text{-N}$ was determined by Nessler's reagent colorimetric method (GB7479-87). Acidity of the water was measured with a pH meter and the water temperature was also recorded. Finally, transparency of the water was determined by Secchi disc (Gu et al., 2000) and expressed in m.

Sediment analyses

TN concentrations were determined by the Kjeldahl method after Sulfuric acid (H_2SO_4) digestion. The Mo-Sb colorimetric method was used to determine TP concentration after by NaOH melting. The content of organic matter was determined by titration with potassium dichromate.

Eutrophication assessment

The level of eutrophication was assessed by a nutrition state index, calculated according to Wang et al. (2002). The input parameters were the concentrations of Chl-a, TP, TN and SD. The comprehensive nutrition state index $\text{TLI}(\Sigma)$ was calculated as follows:

$$\text{TLI}(\Sigma) = \sum_{j=1}^n W_j \cdot \text{TLI}(j) \quad (\text{Eq.1})$$

with W_j is the weight of the nutrition state index of j 's parameter. $\text{TLI}(j)$ represents the nutrition state index of j 's parameter, calculated as follows:

$$\text{TLI}(\text{Chl-a}) = 10(2.5 + 1.086 \ln \text{Chl-a}) \quad (\text{Eq.2})$$

$$\text{TLI}(\text{TP}) = 10(9.436 + 1.624 \ln \text{TP}) \quad (\text{Eq.3})$$

$$\text{TLI}(\text{TN}) = 10(5.453 + 1.694 \ln \text{TN}) \quad (\text{Eq.4})$$

$$\text{TLI}(\text{SD}) = 10(5.118 - 1.94 \ln \text{SD}) \quad (\text{Eq.5})$$

$$\text{TLI}(\text{COD}) = 10(0.109 + 2.661 \ln \text{COD}) \quad (\text{Eq.6})$$

Data analysis

All statistical analyses were conducted using the software SPSS 19.0, DPS 7.5 and Excel 2007 software.

Results

Effects of CLP biomass on TN

The obtained values for TN, $\text{NH}_3\text{-N}$, TP, COD, pH and temperature in water and sediment at the beginning of the experiment are listed in *Table 2*.

Table 2. Indexes of water and sediment in the sampling sites

| Water | | | | | | Sediment | | |
|----------------------------------|-------------------|-------------------|-----------------------------|------|-----------|-------------------|-------------------|--------------------------|
| $\text{NH}_3\text{-N}$ (mg/L) | Total N (mg/L) | Total P (mg/L) | COD _{Cr} (mg/L) | pH | T (°C) | Total N (g/kg) | Total P (g/kg) | Organic matter (g/kg) |
| 1.066 | 3.32 | 0.33 | 65.44 | 8.99 | 14.9 | 1.20 | 0.52 | 23.29 |

Fig.2A shows the concentration of TN in water from each of the plots over time from the day of harvest (day 0). During the experiment, the concentration of TN declined in all samples, ending at levels approximately 5 times lower than at the start (the average removal rate of TN concentration was 79.2% at the end of the experiment). As can be seen from the figure, TN concentrations slightly decreased during the first 10 days post harvest, to increase to levels at day 24 that were similar to day 0. A significant drop followed, which at day 31 resulted in TN concentrations less than 0.3mg/L, the critical concentration required for eutrophication as determined elsewhere (Xie et al., 2011). The exception were B1 plots (where all biomass had remained), which did not quite reach below this critical concentration. After this, TN stayed at low levels till day 46, by which time highest levels were recorded in B1 plots where no CLP had been removed. The order of TN at the end of the experiment was B1 > CK > B3, B2 > B4 (*Fig. 2A*). Thus, although the different treatments did not have a significant effect on TN concentrations, there was a tendency of stronger TN decrease when more CLP was harvested.

Effects of CLP biomass on TP

Fig.2B shows the concentration of TP over time during the experiment. There was more variation between the plots for this parameter compared to TN. The plots from which no CLP had been removed (B1) resulted in a slow but steady increase in TP. In the other plots TP decreased to a minimum at day 10, followed by a gradual increase to approximately original levels at day 24. This trend was seen for the plots from which various fractions of CLP biomass had been removed as well as for the control in which CLP was absent. The content of TP then decreased in the plots in which 20% or 40% of CLP had been retained, and remained stable in the CLP-free control, but continued to increase for the other two plots. By day 31, TP concentrations were higher in B1 (100% CLP retained) and B2 (60% retained) than in the other plots, while the lowest value was found in B4 (20% CLP retained). The results obtained at day 46 were highly different in all plots. With the exception of B4, which remained low, TP concentration in water was now higher than for all previous time points. In particular the CLP-free CK control reached high levels (3 times that of T=0), followed by B3, B1, and B2. The order of TP at the end of the experiment was Ck >> B3 > B1 > B2 >> B4. There was a significant difference between the concentrations of TP in the B4 treatment compared to the other treatments ($P < 0.01$).

Effects of CLP biomass on COD

The concentration of COD in the CLP-free CK plots changed little and was initially higher than that of the other plots. The trends over time are shown in *Fig.2C*. The values for COD increased with time in all treatment plots, though this increase was lowest for the CLP-free control, which ended with the one-but lowest concentration. On day 10 after harvest, COD concentration had significantly declined in B1 plots, after which it considerably increased again, in particular after day 24. On that day, the water of B4 had the lowest COD concentration of all samples, which can be taken as a sign for the best water quality, and it remained as such at day 46. All samples showed an increase in the COD concentration from day 24 to day 46, with the strongest increase in B1 where no CLP had been removed. At the end of the experiment, COD concentrations were highest in B1 (nearly 4 times its original levels), followed by B2 > B3 > CK > B4. This indicated that the

more CLP biomass had remained present in the plot, the higher COD concentrations were produced at the end of the experiment, indicating poorest water quality.

Effects of CLP biomass on Chl-a

The content of Chl-a in the water remained relatively stable till day 31, as can be seen in Fig.2D. Only at day 46 were significant differences detected, with highest levels in CK (6 times that of T=0), followed by B2 > B3 > B1 > B4. This indicates that the lowest biomass of phytoplankton was obtained in plots where 80% of CLP had been harvested.

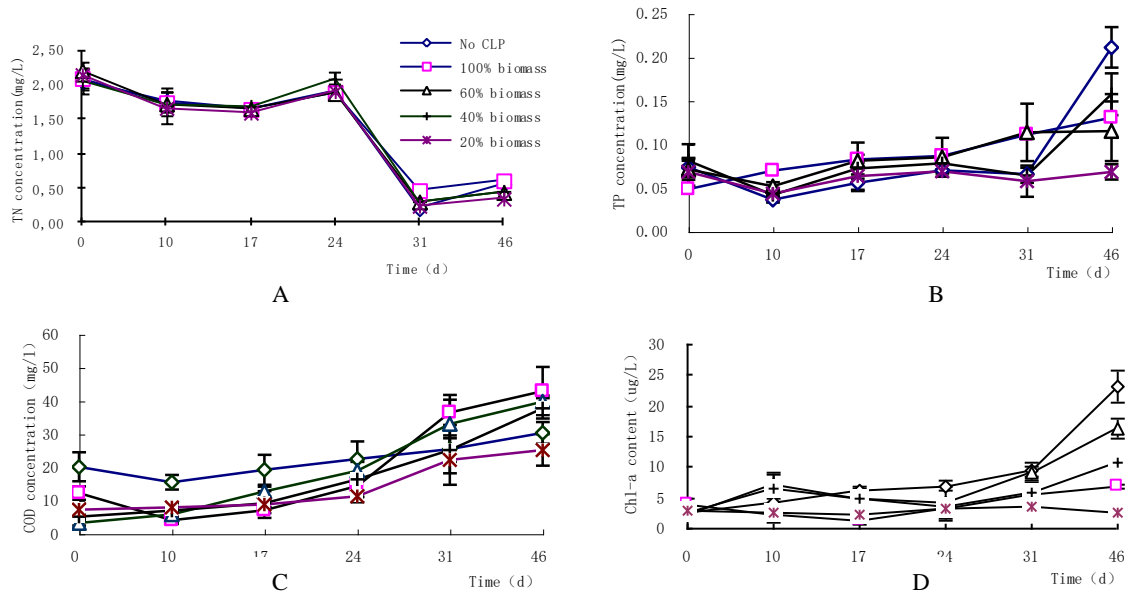


Figure 2. Changes of TN, TP, COD and chl-a concentration over time for plots. The same symbols were used in B, C, D as in A.

Effects of CLP biomass on total bacteria content

Total bacterial content in water was determined for each plot and shown in Table 3. The total bacterial numbers first increased and then decreased over time, while their numbers reached a maximum at day 24 in all samples. At the end of the experiment, the bacterial numbers were in the order of B3 > B2 > CK > B1 > B4. The counts at day 24 correlate with the order of total nitrogen content that had the highest value on that day after harvesting 60% CLP.

Table 3. Arithmetical means \pm SD of the bacterial counts after CLP harvest ($cfu \times 10^3 mL$)

| Days after harvest | 10 | 17 | 24 | 31 | 46 |
|---------------------------|------------------|------------------|-------------------|-------------------|------------------|
| CK, no CLP present | 14.0 \pm 0.71c | 52.5 \pm 0.71c | 564.0 \pm 5.09a | 99.5 \pm 7.07b | 16.1 \pm 2.71c |
| B1, 100% retained biomass | 4.8 \pm 0.14c | 30.0 \pm 0.71c | 225.0 \pm 1.41a | 180.0 \pm 1.27b | 11.3 \pm 2.12c |
| B2, 60% retained biomass | 8.5 \pm 0.14c | 35.5 \pm 4.24c | 392.0 \pm 8.49a | 265.0 \pm 7.05b | 31.5 \pm 0.71c |
| B3, 40% retained biomass | 16.1 \pm 0.49c | 26.9 \pm 2.51c | 680.0 \pm 5.66a | 295.0 \pm 2.12b | 69 \pm 3.17c |
| B4, 20% retained biomass | 24.0 \pm 0.34c | 23.8 \pm 1.41c | 212.8 \pm 6.12a | 85.0 \pm 4.24b | 7.6 \pm 0.55c |

Note: Different superscript letters in the same row indicates a significant difference among samples ($p < 0.01$)

The significance of the CFU counts at the different time points was determined by DPS software. The results showed that the CFU numbers were highly significantly different ($P < 0.01$) on day 24 compared to the other time points, and significantly different ($P < 0.05$) between day 31 and the other time points. This applied to all treatments. Nutrients released by CLP were at a maximum 24-31 days post harvest and during this period, the bacterial content in water was also highest and the eutrophication degree was the largest.

For those plots from which CLP was harvested, the maximum variation of total bacterial counts over time was observed in B3 plots, with standard deviation 300.71. The other plots showed less extensive variations, in the order of CK (SD 234.45) > B2 (172.39) > B1 (104.14). The smallest standard deviation, of 87.85, was observed in B4 plots.

Effects of CLP biomass on TLI

According to the comprehensive eutrophication index, the trophic status of a lake can be divided in five degrees: TLI < 30 indicates a poor nutrition status; a value between 30 and 50 indicates medium nutrition; between 50 and 60 is indicative of slight eutrophication; 60-70 is a sign of moderate eutrophication, while a TLI > 70 is an indicator of severe eutrophication. The mean values of the calculated comprehensive eutrophication index of each treatment are listed in *Table 4*. The eutrophication degree varied between 47.9 and 61.0 (slight to moderate eutrophication) and was highest for the periphery water (CK). The indices for B1, B2 and B3 treatment plots were very similar, at the slight eutrophication level. The lowest values of TLI were obtained in the plots remaining 20% CLP biomass, that were at medium nutrition levels. The comprehensive eutrophication indexes in water of all treatments reached a peak at days 24 and 46 after harvest. The reason may be that the CLP biomass in B4 plots was lowest but also the most suitable as a local carbon sources and carbon sink, which resulted in a balance.

Table 4. Effect of CLP biomass on comprehensive nutrition state index (TLI)

| Days after harvest | 0 | 10 | 17 | 24 | 31 | 46 | Mean value \pm SD |
|---------------------------|------|------|------|------|------|------|---------------------|
| CK, no CLP present | 56.5 | 54.1 | 58.4 | 58.9 | 55.2 | 65.5 | 58.1 \pm 4.1 |
| B1, 100% retained biomass | 56.5 | 51.2 | 50.5 | 57.3 | 58.5 | 61.0 | 55.8 \pm 4.2 |
| B2, 60% retained biomass | 51.5 | 53.0 | 56.0 | 57.4 | 56.4 | 60.1 | 55.7 \pm 3.1 |
| B3, 40% retained biomass | 50.7 | 52.7 | 53.9 | 57.0 | 54.2 | 60.1 | 54.8 \pm 3.3 |
| B4, 20% retained biomass | 49.5 | 47.9 | 50.0 | 52.3 | 48.3 | 50.7 | 49.8 \pm 1.6 |

Discussion

Effects of CLP biomass on main indexes of eutrophic water

If CLP biomass is at the hypersaturated state, it will affect photosynthesis and water purification efficiency. When CLP is artificially harvested, the nitrogen and phosphorus accumulated in the plants is removed from the system, which initially reduces the TN and TP concentration. As a result of the decrease of barriers formed by the stems and leaves, the bottom of the shallow water is now susceptible to disturbance. As a result, the dissolution of $\text{NH}_3\text{-N}$ and other nutrients in sediments accelerate, resulting in a subsequent rise in TN concentrations. Owing to the fact that the DO content is low in

the period from April to June, phosphorus bounded to metal ions in the sediment such as iron and aluminum start to be released, which increases the P content in water (Harvey et al., 2008). In the described experiment, after mid-May (day 24), TN concentration decreased rapidly, probably because the release levels of nutrients in sediment decreased and the massive reproduction of phytoplankton and microorganisms in the water absorbed more nutrients (Dong J, et al., 2014).

In June, CLP started to decline and decompose, with decomposing plant parts turning into a source of nitrogen and phosphorus from the original "sink" role that the plants had provided during their vigorous growth stage (Lu et al., 2006). The released TN content from a decaying plant is typically 0.064 mg/g, and less than that of TP (2.404 mg/g) (Wang et al., 2013). These effects explain why phosphorus content first increased slowly and then, after day 31, rapidly.

COD is a comprehensive indicator of the relative content of organic matter. At the beginning of the experiment, COD concentrations decreased because of CLP absorbing nutrients from the water. After mid May (day24) when CLP started to decline, the COD content gradually increased and reached the highest values in the plots containing maximum biomass. The determined COD content was higher here than for the other treatments by the end of the trial, while COD content was lowest in the plot retaining 20% biomass.

Chlorophyll occurs in all phytoplankton and is a reliable index to quantitate phytoplankton biomass. The content of chlorophyll can be used as an indicator of the degree of eutrophication. A positive correlation was observed between Chl-a content and the decay degree of CLP at locations with high water pollution (Wei et al., 2013). With a rise of temperature, the biomass of phytoplankton in the CLP-free water increased and the content of Chl-a was gradually rising. By the end of the experiment, it was higher in the CK plots than in the other treatments. At the end of May, the content of Chl-a increased with the increase of biomass in plots with CLP growth. The reason is that the decline and decay of CLP released more nutrients into the water, promoting phytoplankton growth. But in the plots from which no CLP was harvested, the plants provided shade that hindered the growth of plankton, therefore the concentration of Chl-a in those plots was close to that of the plots from which 80% CLP was harvested.

Some suggestions on CLP management

Submerged hydrophyte species, distribution and biomass play a very important role in ecological restoration of eutrophic lakes (Ginn, 2011; Dong et al., 2014) but multiple processes with conflicting outcome occur simultaneously. As a result, in our experiment there were no significant differences in the contents of some of the determined variables between plots that had retained different amounts of biomass. Nevertheless, the contents of all determined indexes in those plots where 80% of CLP had been removed invariably reached minimum values. This conclusion is similar to that of Li and colleagues, who reported that the optimal biomass harvest rate of *Hydrilla verticillata* Royle was 75- 87.5% (Li et al., 2014). Likewise Xu et al. (2013) concluded that the biomass of submerged plants should be reduced to 140-180 g/m². Therefore, we suggest that the most suitable biomass to be retained is 20% when the coverage of CLP reaches 95% or higher in freshwater lakes.

Conclusions

(1) TN concentrations and total bacterial counts rose to maximum values 24 days post CLP harvest conducted at the end of May, after which they declined sharply, while TP concentrations decreased first and then increased gradually. At the end of the experiment (mid-June), the changes of TN and TP concentrations in water were basically the same. The highest values appeared in plots without CLP and those where no biomass was removed; lowest values were recorded in plots that had retained 20% of the maximum biomass. The concentrations of TP displayed a significant difference between plots retaining 20% of biomass and the other treatments ($p < 0.05$). The total bacterial counts reached highest values at the end of May, and by day 24 produced a highly significant difference with the other time points ($P < 0.01$). COD concentration increased and this increase correlated with the increase of biomass. The contents of Chl-*a* in plots retaining 20% biomass remained constant at levels before the harvest, while the levels increased in the other treatments.

(2) The comprehensive nutrition state index (TLI) in each tested treatment peaked on day 24 post harvest (end of May) and again in mid-June (day 46) when it reached the highest values. The TLI value was highest in water without CLP and very similar TLI values were obtained in water containing maximum biomass, 60% and 40% of maximum CLP biomass treatments. The plot in which 20% of maximum biomass had remained resulted in water with medium nutrition levels, while the other treatments resulted in light eutrophication levels.

(3) The best harvest time of CLP is mid-May and the best harvest rate is about 80% of maximum CLP biomass.

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