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The time-effect model of nitrogen and phosphate uptake by *Potamogeton crispus* L in eutrophic water body

C Y Zhang[1,2], L P Wang[1], D L Xu[2], L Wan[2] and C B Yin[2,3]

[1]School of Environment Science and Spatial Informatics, China University of Mining and Technology, Xuzhou 221116, China
[2]School of Environment Engineering, Xuzhou Institute of Technology, Xuzhou 221111, China

E-mail: wlpcumt@126.com

Abstract. In this study, the effects of *Potamogeton crispus* L on total nitrogen (TN) and total phosphate (TP) in a eutrophic water body were explored and a time-effect model was developed for nitrogen and phosphate uptake by *P. crispus*. The results revealed that i) TN and TP in eutrophic water showed a trend of first decline and then increase at different *P. crispus* biomass levels; ii) at the same nutrient level, the amount of TN and TP uptake by *P. crispus* increased with increasing biomass; and iii) at the same biomass conditions, the amount of TN and TP uptake by *P. crispus* was the highest at nutrient levels of 13.95-20.56 mg/L for TN and 0.037-0.263 mg/L for TP. Moreover, a time-effect model was developed for TN and TP uptake by *P. crispus* in eutrophic conditions, as well as a TN and TP limit theoretical model. Using multiple sets of data, it was demonstrated that the models were well fitted with error rates of less than 10%. Overall, the study provides a strong basis for optimal *P. crispus* biomass input and harvest time in ecological restoration of eutrophic water bodies.

1. Introduction

Submerged plants are important regulators of lakes and other shallow water ecosystems and play key roles in improving water quality and stabilizing aquatic ecosystems. Reconstruction of aquatic vegetation, with submerged plants as dominant plants, has become an important research topic for improving water quality and restoring eutrophic water bodies [1-4]. *Potamogeton crispus L* of the family Potamogetonaceae is a widely distributed submerged perennial herbaceous plant and a dominant species in grass lakes [5,6]. It is often used as an important species for ecological restoration and water quality improvement in eutrophic lakes [7-9]. However, with increasing water pollution and eutrophication, *P. crispus* populations in many rivers and lakes worldwide have destroyed in recent years, leading to river blockages, decreasing landscape and sightseeing functions, limiting growth of other species and seriously affecting the ecological balance of water bodies [10-12]. Therefore, establishing the ecological effect model of *P. crispus* on eutrophic water bodies has become an urgent issue and is of great significance to use and manage *P. crispus*.

2. Materials and methods

2.1. Materials

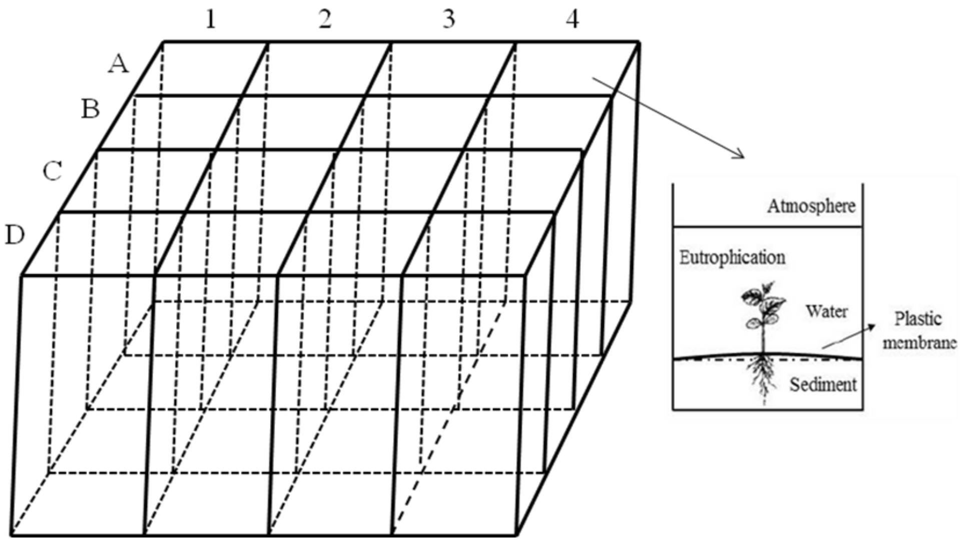
P. crispus was collected from the artificial lake in the campus of Xuzhou Engineering College

(Xuzhou Institute of Technology, China). A thousand healthy *P. crispus* turions with uniform size were selected, placed in four large plastic bins with water from the artificial lake in campus and cultured in a culture box at 25°C and grade IV light conditions. When the sprouting seedlings were about 2-4 cm, they were transplanted into the experimental apparatus.

The tested solutions were artificial nutrient solutions formulated with NH_4Cl and NH_4NO_3 at molar ratio of 1:1 for TN and with NaH_2PO_4 for TP. Lake sediment was taken from the areas where *P. crispus* perennially grows in the campus artificial lake.

2.2. Experimental methods

2.2.1. Experimental design. The experimental apparatus was a 2 m long, 2 m wide and 1.2 m high glass steel water tank consisting of 16 evenly separated sections of 0.5m x 0.5m x 1.2 m (sketch 1). Each section was covered with 2 kg lake sediment at the bottom. Different amount of well-developed healthy seedlings were planted in the sediment by hands, carefully put plastic membrane on the matrix, then slowly watered with the pre-prepared nutrient solution and cultured in an indoor environment with sufficient light and water temperature of 25±1°C. TN and TP concentrations were measured every three days. As shown in table 1, the density of *P. crispus* was set as 20 seedlings/m^2 (A), 80 seedlings/m^2 (B), 160 seedlings/m^2 (C) and 320 seedlings/m^2 (D), TN and TP contents set as four levels. During the experiment, algae were promptly removed using dense nylon mesh network to ensure experimental reliability.



Sketch 1. Experimental apparatus for planting *P. crispus*.

Table 1. Experimental design.

No.	Nutrient level	Nutrient content (mg/L) TN	Nutrient content (mg/L) TP	*P. crispus* biomass (seedlings/m^2)
A1	1	2.0	0.04	20
B1				80
C1				160
D1				320
A2	2	6.0	0.3	20

B2				80
C2				160
D2				320
A3	3	12.0	2.0	20
B3				80
C3				160
D3				320
A4	4	24.0	10.0	20
B4				80
C4				160
D4				320

2.2.2. Time-effect models of TN and TP uptake by P. crispus in eutrophic water. Based on the effects of *P. crispus* at a density level of 160 seedlings/m^2 on TN and TP contents in an eutrophic water body, three-dimensional diagrams and multiple linear regression equations were developed with MATLAB mathematical software. The initial TN and TP contents were used as the Y coordinate (the initial TN content was 3.58 ~ 21.58 mg/L and the initial TP content was 0.04 ~ 13.21 mg/L), time as the X coordinate (1~123 d) and measured TN and TP contents as the Z coordinate.

2.2.3. TN and TP limit theoretical models of P. crispus in restoring the water quality of eutrophic water. The TN and TP limit theoretical models of *P. crispus* in restoring ecology of eutrophic water bodies were established by using the initial TN and TP contents as the X coordinate, *P. crispus* biomass as the Y coordinate and maximum TN and TP uptake by *P. crispus* as the Z coordinate. Graphs were plotted using MATLAB and multiple linear regression equations were deduced using the step regression method.

2.3. Measurement methods
The TN and TP concentrations were measured based on the “Water and Wastewater Monitoring and Analysis Methods” [13]. Each indicator was measured in triplicates and averaged.

3. Results

3.1. Water quality effects of P. crispus on TN and TP

3.1.1. Effects of P. crispus density on TN. As shown in figure 1, TN concentration in all groups show a trend of first decreases and then increases, until eventually reaching its initial value. In groups with level 1 nutrient concentration (A1, B1, C1, and D1), the average initial TN concentration is 3.72 mg/L, and it required 66-73 days to reach the lowest average concentration of 1.65 mg/L.The TN uptake rate by *P. crispus* is average of 56%, and the final TN content is 3.73 mg/L. The average initial TN content in all groups with level 2 nutrient concentration (A2, B2, C2 and D2) is 7.12 mg/L and the time needs to reach the average minimum concentration, 1.75 mg/L, is 65-73 days. The final TN content is 5.41 mg/L and the TN uptake rate by *P. crispus* is 75% in average. The average initial TN content in all groups at level 3 nutrient concentration (A3, B3, C3 and D3) is 13.95 mg/L, reaching the average minimum of 3.53 mg/L in 65-77 days. The TN uptake rate by *P. crispus* is 75% in average, and the final TN content in average is 11.39 mg/L. The average initial TN content in all groups at level 4 nutrient concentration is 20.56 mg/L. After 65-77 days of uptake, TN content reaches the lowest in average of 7.49 mg/L, with an uptake rate by *P. crispus* of 64% in average, and the final TN content is 18.21 mg/L. Therefore, the above results showed that with increasing nutrient concentration, TN uptake by *P. crispus* gradually increased, and required slightly more time to reach the minimum concentration.

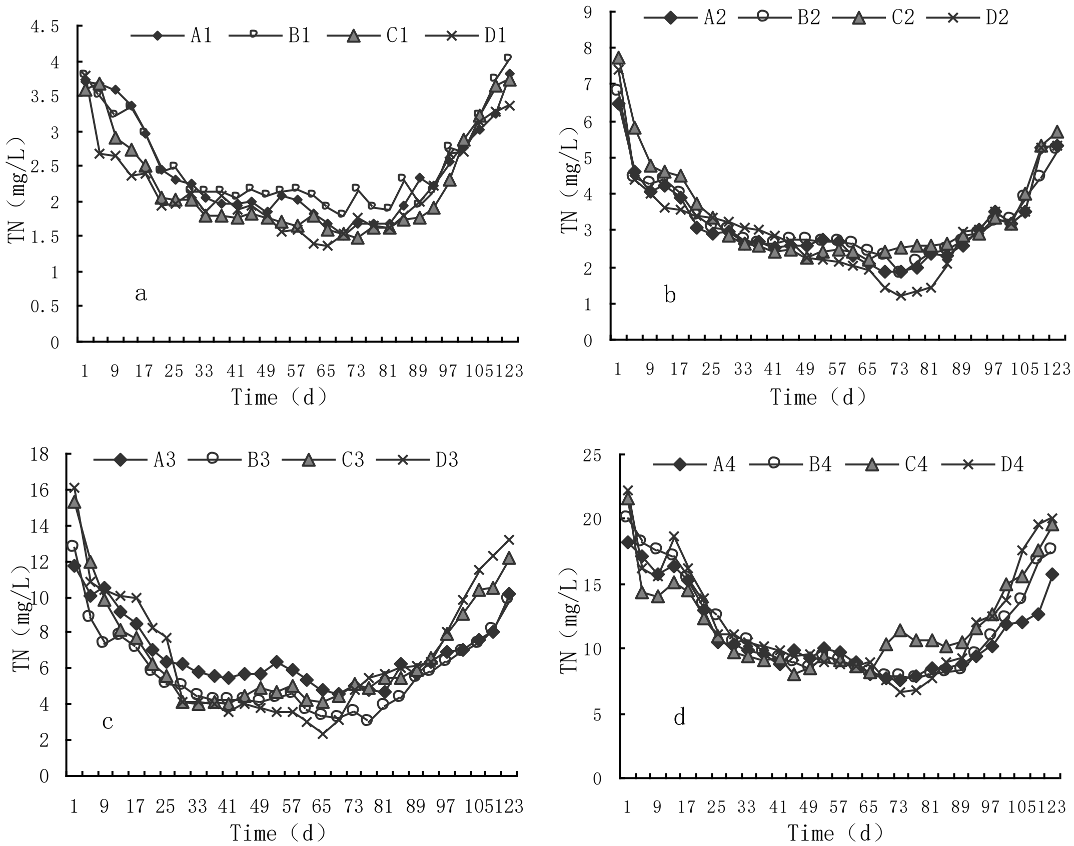


Figure 1. TN uptake by different densities of *P. crispus* in water bodies with different nutrient levels. a: level 1 (2.0 mg/L), b: level 2 (6.0 mg/L), c: level 3 (12.0 mg/L), d: level 4 (24.0 mg/L).

Table 2. Effect of *P. crispus* on TN content.

No.	Biomass (seedlings/m^2)	Maximum uptake (%)	Final content (%)	Maximum uptake time (d)
A1	20	48.04	98.80	69
A2		71.37	187.09	69
A3		61.17	124.27	69
A4		59.00	109.52	73
B1	80	52.00	120.56	69
B2		74.60	202.90	73
B3		76.05	224.37	77
B4		62.04	129.39	77
C1	160	58.39	149.83	73
C2		71.84	163.13	65
C3		73.20	197.81	65
C4		61.99	138.17	65
D1	320	63.94	145.93	65

D2	83.41	334.26	73
D3	85.05	451.38	65
D4	70.13	202.20	73

Table 2 shows that at the same nutrient level, TN uptake gradually increased with the density of *P. crispus*. At the same *P. crispus* density, TN uptake shows a similar order, where the uptake ranking is level 3 > level 2 > level 4 > level 1, except at the lowest *P. crispus* density. At 20 seedlings/m^2 TN uptake ranks as A2>A3> A4>A1 , and reaches its maximum of 13.95-20.56 mg/L between level 3 and level 4 nutrient concentration. This indicated that TN uptake by *P. crispus* increased with initial TN concentration.

3.1.2. Effects of P. crispus density on TP. Figure 2 shows that TP contents in each group also exhibite a trend of first decline and then increase, similar to that of TN. The final TP content in all groups is slightly lower than their initial TP content. The average initial TP content is 0.037 mg/L, 0.263 mg/L, 2.275 mg/L and 10.31 mg/L in groups at level 1, 2, 3 and 4 nutrient concentration, respectively. *P. crispus* needs 73-81 days, 73-81 days, 73-85 days and 81-85 days, respectively, to reach the minimum concentration; the average minimum TP concentration is 0.008 mg/L, 0.032 mg/L, 0.764 mg/L and 4.14 mg/L, respectively; and TP uptake rate is 78%, 88%, 66% and 60%, respectively; the final TP content is 0.027 mg/L, 0.168 mg/L, 1.843 mg/L and 8.597 mg/L, respectively. Ithad clearly showed that with increasing nutrient level, TP uptake by *P. crispus* was continuously reduced, and the time needed to reach the minimum TP concentration gradually increased and longer than that needed for TN. The results indicated that TP uptake by *P. crispus* was slightly slower than that of TN.

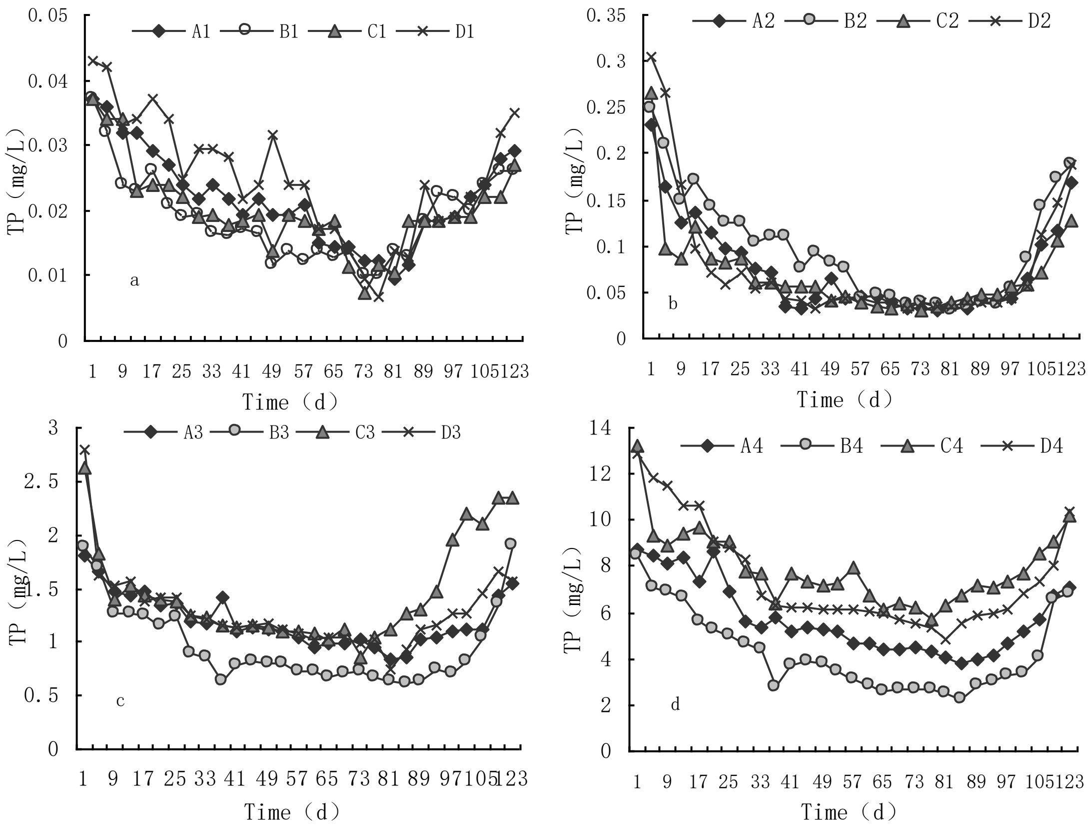


Figure 2. Effects of of *P. crispus* density on TP content in water bodies with different nutrient levels. a: level 1 (0.04 mg/L), b: level 2 (0.3 mg/L), c: level 3 (2.0 mg/L), d: level 4 (10.0 mg/L).

Table 3. Effect of *P. crispus* on TP content.

No.	Biomass (seedlings/m^2)	Maximum uptake (%)	Final content (%)	Maximum uptake time (d)
A1	20	70.00	222.22	81
A2		95.69	460.00	77
A3		54.09	87.23	81
A4		56.16	85.01	85
B1	80	72.97	160.00	77
B2		96.37	503.23	81
B3		66.97	206.46	85
B4		69.78	200.13	85
C1	160	81.08	285.71	73
C2		97.37	312.90	73
C3		67.15	172.80	73
C4		53.53	79.94	77
D1	320	83.72	400.00	77
D2		98.68	450.00	77
D3		73.40	110.80	81
D4		62.46	114.06	81

As showed in table 3, TP uptake by *P. crispus* at the same nutrient levels gradually increase with *P. crispus* density, except for the level 4 nutient concentration. The ranking of the average TP uptake at the same *P. crispus* density is A2> A1> A4> A3, B2> B1> B4> B3, C2> C1> C3 > C4 and D2> D1> D3> D4. Therefore, TP uptake by *P. crispus* at same biomass showed a trend of first increase and then decrease. The optimal limit of TP uptake was between level 1 and level 2 nutrient concentration, which was 0.037-0.263 mg/L.

3.2. Water quality effect models of P. crispus on TN and TP in eutrophic water bodies

3.2.1. The time-effect model of P. crispus on TN and TP. In natural conditions, the density of *P. crispus* is generally about 200 seedlings/m^2 but may reach up to 700-800 seedlings/m^2 [14]. Based on figure 1, a time-effect model of *P. crispus* is established at a density of 160 seedlings/m^2 on TN and TP contents in different nutrient levels (figures 3 and 4). The established multivariate regression equation is Z = 0.536Y - 0.001X - 0.305 (R^2 = 0.688, P <0.001) for TN and Z = 0.603Y - 0.004X +0.147 (R^2 = 0.943, P <0.001) for TP. Figures 3 and 4 better reflect the ecological effects of *P. crispus* on eutrophic water body at natural conditions. Within the listed initial TN and TP contents, TN and TP levels first decline and then increase (a periodical fluctuation), reach the minimum concentration at about 70 days and eventually increas close to the initial concentrations.

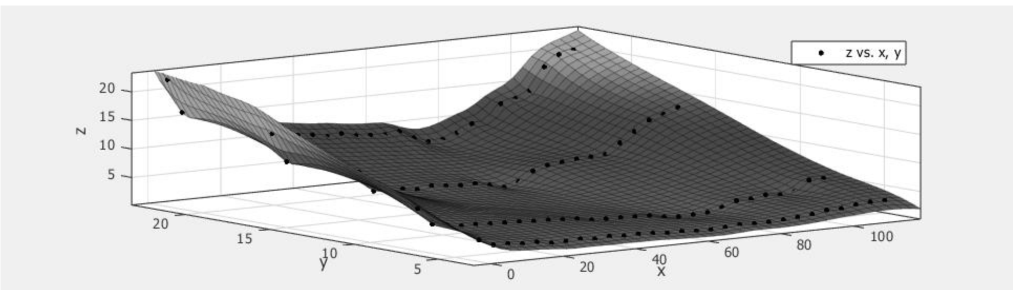


Figure 3. The time-effect model of *P. crispus* on TN.

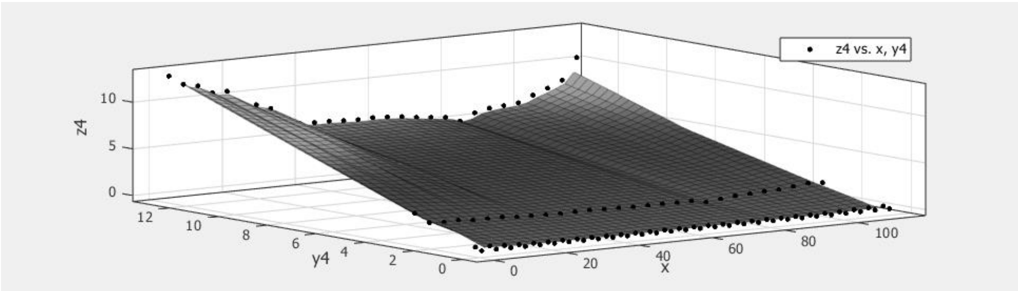


Figure 4. The time-effect model of *P. crispus* on TP.

3.2.2. TN and TP limit theoretical models of ecological restoration by P. crispus in eutrophic water. Based on the maximum TN and TP uptake of *P. crispus* in an eutrophic water body, the limit theoretical models between their maximum uptake and *P. crispus* density is established, as well as their initial nutrient concentration (figures 5 and 6). The model equations are Z = 0.006Y + 0.657X - 0.525 (R^2 = 0.964, P <0.001) for TN and Z = 0.001Y + 0.583X + 0.015 (R^2 = 0.987, P <0.001) for TP.

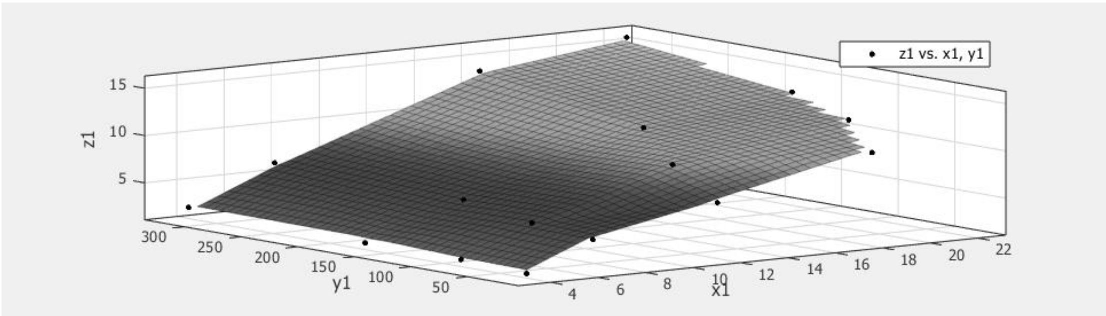


Figure 5. TN limit theoretical models of ecological restoration by *P. crispus* in eutrophic water.

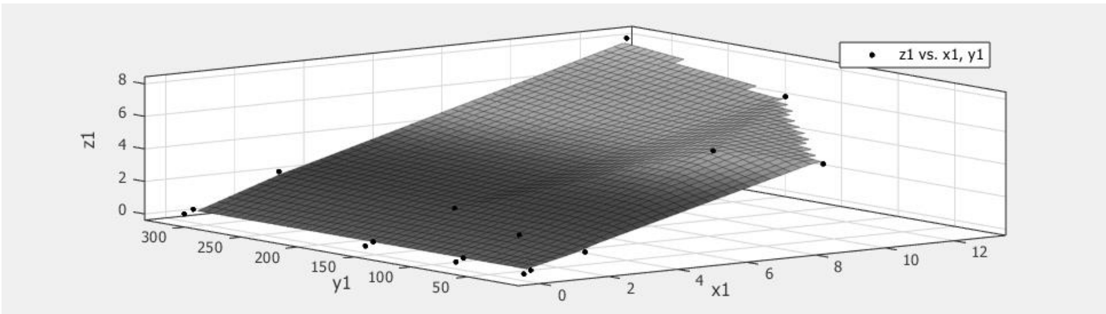


Figure 6. TP limit theoretical models of ecological restoration by *P. crispus* in eutrophic water.

3.3. Validation of the theoretical model

In order to test the accuracy of the established models, changes are monitored in TN and TP contents (initial and minimum) in the artificial lakes with different *P. crispus* biomass in Xuzhou Engineering College Campus from February 28 to June 6, 2013. These data are used to calculate, based on the model, and predict maximum uptake, which are further compared with the actual measured values. From tables 4 and 5, the error rate of the limit theoretical models is 2.17% ~ 8.34% for TN and 2.61%~8.65% for TP, which both are less than 10%. It indicated that the models also conformed well to natural conditions.

Table 4. Validation of the TN limitation model.

Monitoring regions	Density (seedlings/m^2)	Initial TN content (mg/L)	Minimum TN content (mg/L)	Measured maximum TN uptake (mg/L)	Predicted maximum TN uptake (mg/L)	Error rate (%)
Region A	100	1.93	0.71	1.27	1.38	8.34
Region B	70	2.03	0.77	1.26	1.23	2.48
Region C	50	1.85	0.90	0.95	0.99	4.26
Region D	40	2.16	1.05	1.11	1.13	2.17

Table 5. Validation of the TP limitation model.

Monitoring regions	Density (seedlings/m^2)	Initial TP content (mg/L)	Minimum TP content (mg/L)	Measured maximum TP uptake (mg/L)	Predicted maximum TP uptake (mg/L)	Error rate (%)
Region A	100	0.25	0.01	0.24	0.26	8.65
Region B	70	0.28	0.02	0.26	0.25	4.52
Region C	50	0.18	0.02	0.16	0.17	6.21
Region D	40	0.34	0.08	0.26	0.25	2.61

4. Discussion and conclusions

P. crispus has a strong uptake capacity to nutrients in eutrophic conditions and plays an important role in the ecological restoration of aquatic environments [15]. Under natural conditions, the life-cycle of *P. crispus* from turion germination to death is about four months. The plant generally grows slowly in early March and then rapidly in early April. After reaching its maximum biomass in mid-May, it begins to decline and senesces in late May and most of its turions drop to the sediment, resulting in decreased water transparency [16]. During our incubation period from February 28 to June 6, nutrients (TN and TP) in the water body first decreased then increased in all experimental group, like a big “U”. It is well known that *P. crispus* is a common aquatic plant used for water-restoration because of its super absorption capacity for nutrients in contaminated water, so *P. crispus* absorbs greater amount of nutrients for growing in the first half incubation, resulting TN and TP contents reduced quickly. But in the second half incubation, nutrients were constantly released into the water body with the decline and fall of *P. crispus*, resulting TN and TP contents continued to elevate. Moreover, *P. crispus* was revealed the potential ability of balance the nutrients including TN and TP in a good range. The study showed that *P. crispus* had best restoration ability in eutrophic water when TN was in the range of 13.95-20.56 mg/L and TP in the range of 0.037-0.263 mg/L. During these ranges, *P. crispus* has very good uptake ability. Its maximum uptake rate was 64% -75% for TN and 78% -88% for TP, indicating it has an important role in real practice.

The ecological effect model of *P. crispus* on eutrophic water body has two parts: i) the time-effect model of *P. crispus* on TN and TP, ii) the TN and TP limit theoretical model for ecological restoration. The former shows the real-time changes in water quality during the whole 123 days of life cycle of *P. crispus* from turions sprouting to the seedlings deteriorating and better reflects the ecological effects of *P. crispus* at natural conditions on different eutrophic water bodies. When TN and TP at the tested initial content, their levels showed a cyclical trend of first decline then increase and reached their minimum in about 70 days. The final TN and TP contents were relatively equal to their initial contents. The latter with low error rate (<10%) could be used to accurately calculate one of the parameter of the initial TN contents (or TP contents), the *P. crispus* density and the maximum TN uptake (or TP uptake), when the other two are acquired. Hence, it has the ability to predict the restoration function of *P. crispus* in eutrophic water body. The both models are also useful management tools for treating eutrophication water using *P. crispus*. On one hand, according to the level of nitrogen and phosphorus nutrients in eutrophic water body, reasonable planting density of *P. crispus* might be determined [17].

The other hand, *P. crispus* biomass could be adjusted (increase or decrease) through real-time prediction strategy.

In the practice of water quality and ecological restoration, the time-effect model of *P. crispus* on TN and TP could visually reflect the ecological effects of *P. crispus* on TN and TP in eutrophic water body and the TN and TP limit theoretical model of ecological restoration can predict its restoration capacity, such as the projected ecological restoration limits based on *P. crispus* biomass and nutrient content of the water body. Therefore, these models can be used to determine the best harvest time of *P. crispus*, and provide effective guidelines for ecological restoration.

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