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## Control of turion germination in *Potamogeton crispus*

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### Abstract

*Potamogeton crispus* is a cosmopolitan aquatic species occurring in a wide range of climatic habitats. The effects of water depth, light availability, substrate type and simulated predation (by removing germinated sprouts) on turion germination of *P. crispus* were monitored from 1999 to 2000 under field conditions in Hubei province, China. The germination rate of the turions decreased at a mean rate  $0.05\text{ m}^{-1}$  per day with increasing water depth irrespective of lake. There were significant negative linear relationships between germination rate and water depth both of the same lake and across the lakes. Turion germination was inhibited in the dark, but was little affected by types of substrate. Removing the germinated sprouts promoted germination of other dormant buds. Therefore, the potential to germinate further after sprout removal might be one mechanism by which *P. crispus* can dominate habitats with predation pressure from herbivorous fishes. The results may be used to predict turion germination dynamics of *P. crispus* from water depth of lakes providing light attenuation does not change, and to construct a population model.

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**Keywords:** Hubei; Light availability; *Potamogeton crispus*; Sprout removal; Turion germination; Water depth

### 1. Introduction

Curly pondweed (*Potamogeton crispus* L., Potamogetonaceae), a freshwater submerged species, with wide geographical range (Obermeyer, 1966), can be found in freshwater lakes, ponds, paddy fields and rivers throughout China. It not only can accumulate pollutants such as phenol from waters (Hafez et al., 1998), cerium, cobalt, cesium (Hafez et al., 1992),

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but also grows well in eutrophic lakes. Therefore, it is envisaged as an indicator species of eutrophication (Hamashima et al., 1977, cited in [Sastroutomo, 1980](#)). At the same time, *P. crispus* is an important primary producer in freshwater ecosystems, providing good fodder source for herbivorous fishes.

The life history of *P. crispus* differs from most of the submerged plants ([Kunii, 1982](#)). Its turions germinate in autumn and produce a thin rhizome with one or more stems ([Tobiessen and Snow, 1984](#)). When the water temperature begins to rise in the spring, it displays a rapid increase in vegetative growth ([Stuckey, 1979](#); [Rogers and Breen, 1980](#); [Sastroutomo, 1981](#)). Actually, the burst of growth occurs in late April and early May while the water temperature is rather low. Biomass production of *P. crispus* often reaches its maximum in early summer while flowering and fruiting occur from April to May. Such a growth pattern allows *P. crispus* to avoid competition from other species in the habitats primarily because they are still in dormant state ([Tobiessen and Snow, 1984](#)).

*P. crispus* can produce both numerous turions ([Yeo, 1966](#); [Rogers and Breen, 1980](#); [Chen, 1985](#)) and large numbers of seeds ([Waisel, 1971](#); [Teltscherova and Hejny, 1973](#); [Rogers and Breen, 1980](#)). Although seeds might be important in long distance dispersal ([Waisel, 1971](#)), seed germination of *P. crispus* is, however, extremely low, being 0.001% ([Rogers and Breen, 1980](#)). Therefore, local recruitment from seeds may play a minor role in its persistence and range expansion. Turions serve as a multi-functional organ for carbohydrate storage, propagation and dispersal, thus the understanding of their germination behavior under field conditions is essential to understanding natural population dynamics of *P. crispus*. A few studies (e.g. [Rogers and Breen, 1980](#); [Sastroutomo, 1981](#); [Kadono, 1982](#); [Li et al., 1989](#)) have been conducted to investigate its turion germination under controlled conditions, from which it is concluded that temperature, light intensity and photoperiod can all regulate turion germination, and lower temperatures can initiate turion germination.

In the field, water depth, light intensity, substrate and predation by fishes probably influence turion germination. However, to the best of our knowledge, up to now no formal studies have been conducted to investigate how these factors regulate the turion germination under the natural conditions. Therefore, the effects of water depth, light conditions, substrate type and simulated predation (by removing germinated sprouts) on the turion germination were considered in the study.

## 2. Materials and methods

### 2.1. Experiments 1 and 2: effects of water depth on turion germination

Experiment 1 was conducted from June 1999 to January 2000 in Liangzi Lake located in Hubei province of China, 30.1–30.3°N, 114.4–114.6°E. The water level of the lake remained relatively stable, with maximum fluctuation in water level being less than 0.5 m during that period. In order to examine the relationship between water depth and germination rate of the turions, six areas with different depths were chosen from the lake. Their water depths averaged 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 m, respectively. In each area, six sampling points of about 100 m<sup>2</sup> were chosen randomly, and were positioned by using GARMIN 12C GPS. GPS was used to navigate for further investigations. On 2nd of every month from July

1999 to January 2000, about 500 turions were randomly collected at each point from the lake bottom surface using a plastic net, and then germinated turions were counted. The germination percentage of turions was then calculated for each sampling point.

In order to further test the generality of the results from experiment 1, experiment 2 was carried out across five lakes in Hubei province that have different water depths, i.e. Liangzi Lake (30.1–30.3°N, 114.4–114.6°E), Xiliang Lake (29.9–30.1°N, 114.0–114.2°E), Changhu Lake (30.4–30.5°N, 112.3–112.5°E), Bailian Lake (29.7–30.7°N, 112.4–112.5°E), Haikou Lake (30.0–30.1°N, 115.2–115.3°E). In each lake, three transects were randomly established, along each of which two sampling points were randomly positioned. At each sampling point, about 500 turions were randomly sampled. The first survey was conducted on 15 June 1999; and subsequent surveys were carried out on 8th of every month from July 1999 to January 2000. Similarly, germination percentage of turions was calculated for each sampling point.

### 2.2. Experiment 3: effects of light and substrate on turion germination

This experiment was performed in a pond with a fixed depth of 120 cm at Wuhan University, from 10 June 1999 to 10 January 2000. The turions of *P. crispus* used here were collected on 8 June 1999 from Liangzi Lake. The substrates were also collected from the same lake.

The experimental design was factorial, in which two factors were considered, substrates (silt, river sand or no substrates) and light availability (natural light or dark), making a total of six treatments. Each treatment was replicated five times. Thirty-centimeter-diameter plastic pots (40 cm deep) were filled with one of the three types of substrate to a depth of 10 cm. Five-hundred freshly collected turions were placed on the substrate surface of each pot, and then slightly pressed into the substrate. At the same time, a fine iron net was put on the turions so that they would not move or float. The dark condition was created in the way that each pot was covered with a piece of black cloth with two 0.5-cm holes. All the pots were sunk into the pond, whose top was kept about 80 cm away from the water surface. During the experimental period, extra water was frequently added to the pond so as to make the water depth constant. On 10th of every month from July 1999 to January 2000, the turion germination was checked, and the germination percentage was then calculated.

### 2.3. Experiment 4: effects of sprout removal on further germination of turions

In order to simulate predation of sprouts by fishes, germinated sprouts were hand-removed by fine tweezers. The experiment was initiated on 10 June 1999, and terminated on 10 January 2000. Fifteen 30-cm-diameter plastic pots were filled with silt to a depth of 10 cm. Other preparations were made as in experiment 3. The pots were sunk into the pond, whose top was kept about 80 cm away from water surface. They were equally assigned to three removal treatments. In treatment 1, the germinated sprouts were not removed whereas the other two treatments were subjected either to removing the first sprouts or removing both the first and second sprouts. Turion germination was monitored for 7 months. Sprout removals were performed on 10th of every month during the study period when turion germination

was checked. The cumulative germination percentages of the first, second and third sprouts were recorded.

2.4. Data analysis

In order to examine the effects of water depth, light conditions, substrate, sprout removal on turion germination, analyses of co-variance (ANCOVA) were performed, in which  $\sin^{-1}$  transformed germination percentage of turions was treated as dependent variable while water depth or experimental treatments were treated as independent variable, and sampling date co-variate. In experiment 4, post hoc test was performed to further examine whether or not there were significant differences between the removal treatments.

In order to find how water depth affected the time course of turion germination, germination rate was calculated. The time required for half the final germination ( $T_{50}$ ) is believed to be a good estimator of the germination speed (Thornley, 1986; Li et al., 2000). It can be derived from a logistic equation:

$$G = \frac{k}{1 + a \exp(-rT)} \tag{1}$$

where  $G$  is germination percentage at time  $T$ ;  $k$  is the maximum germination capacity, i.e. the observed final germination percentage, or in most cases simply 100%; and  $a$  and  $r$  are estimated parameters. From the fitted equations, the time required to achieve 50% of germination ( $T_{50}$ ) can be calculated for each water depth by the following equation:

$$T_{50} = \frac{\ln a}{r} \tag{2}$$

Linear regression analysis can also be performed between  $T_{50}$  or  $1/T_{50}$  (the latter was here used) and water depth so that further predictions of the germination dynamics can be projected from the water depth.

3. Results

3.1. Effects of water depth on turion germination

The turion germination was greatly affected by the water depth (Table 1) of Liangzi Lake (Table 2, Figs. 1 and 2). Although the final germination percentage was unaffected

Table 1  
Major parameters of five lakes surveyed in experiment 2 located in Hubei, China

Parameters	Liangzi Lake	Xiliang Lake	Changhu Lake	Bailian Lake	Haikou Lake
Lake area (km <sup>2</sup> )	304.3	72.1	129.1	4.7	7.0
pH	5.5–6.5	5.5–6.5	5.5–6.5	5.5–6.5	5.5–6.5
Water transparency (m)	1.0 ± 0.27 <sup>a</sup>	1.1 ± 0.30	1.2 ± 0.28	0.8 ± 0.28	1.0 ± 0.27
Mean water depth (m)	5.2 ± 0.21	4.2 ± 1.32	3.1 ± 1.19	2.6 ± 1.21	1.8 ± 1.13

<sup>a</sup> Mean over seasons ± standard deviation.

Table 2

Summary of ANCOVAs for testing the effects of water depth on turion germination of *Potamogeton crispus* or the difference among the lakes

Major factor	d.f.	<i>F</i>	<i>P</i>	Co-variate	d.f.	<i>F</i>	<i>P</i>
Water depth	5281	31.1	***	Time	1281	2663.5	***
Lakes	4234	13.6	***	Time	1234	4673.2	***

Note: (1) the germination percentages were  $\sin^{-1}$  transformed; (2) time (months after 15 June 1999) was treated as con-variate.

\*\*\*  $P < 0.0001$ .

by the water depth, the time course of germination was significantly affected, which could be generally described by a logistic model (Fig. 1a). Similarly, the model also held true for the relationships between cumulative germination percentage and time across the lakes that are all located in Hubei province. Fig. 2 also shows that the germination rate, expressed as per day to 50% germination, decreased with increasing water depth. There were significant linear relationships between germination rate and water depth both of the same lake and across the lakes. According to the linear model, the calculated mean decrease rate was  $0.05 \text{ m}^{-1}$  per day (Fig. 2). Theoretically, the water depth over which the turion germination does not occur can be calculated by using this simple model. As a whole, the turion germination did not take place if the water depth was over 10.3 m (see dotted line in Fig. 2). Therefore, the dynamics of the turion germination could be predicted from the water depth.

### 3.2. Experiment 3: effects of light and substrate on turion germination

Light had profound influence on the turion germination (Table 3). Fig. 3 shows that under the light conditions, turions germinated more rapidly and had higher final germination percentages than under the dark conditions, indicating that dark conditions inhibited the germination. On the other hand, types of substrate did not affect the germination process of turions under the same light conditions (Table 3).

Table 3

A two-way ANCOVA on the effects of light conditions and substrates on the turion germination of *Potamogeton crispus*

Source of variation	d.f.	Mean square	<i>F</i>	<i>p</i>
Substrate (M)	2	24.5	0.3	NS
Light (L)	1	12,187.9	124.5	***
Substrate $\times$ light	2	1.4	0.01	NS
Time	1	16,1103.2	1645.4	***
Error	203	97.9		

Note: (1) the germination percentages were  $\sin^{-1}$  transformed; (2) time (months after planting) was treated as co-variate; (3) significance is given by NS,  $P > 0.05$ .

\*\*\*  $P < 0.0001$ .

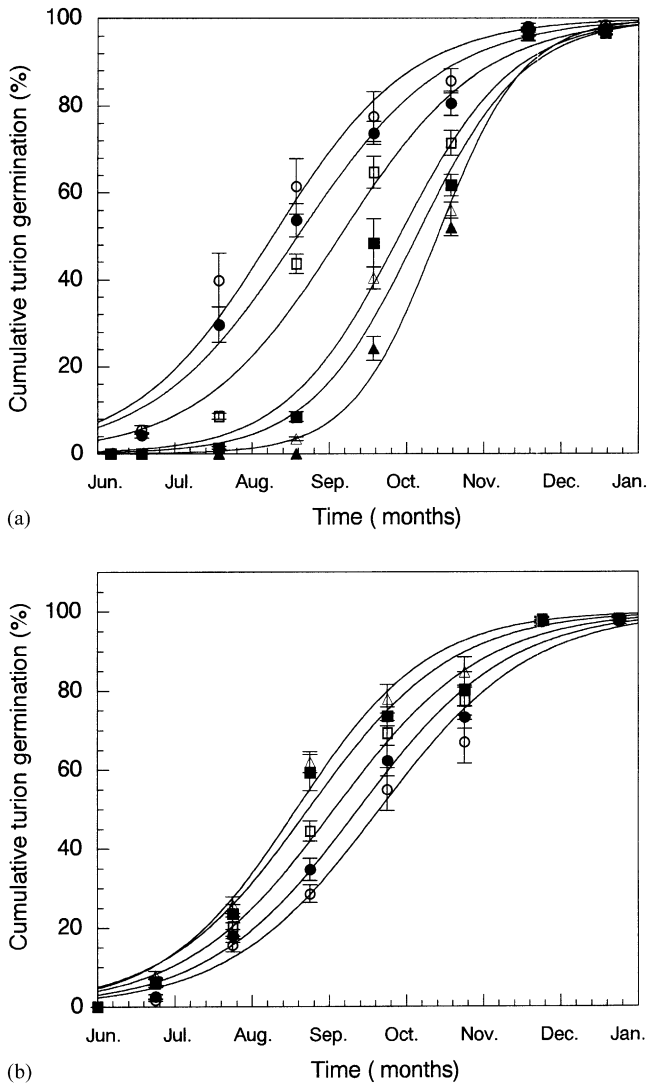


Fig. 1. The time course of the turion germination of *Potamogeton crispus* as influenced by water depth: (a) for Liangzi Lake (○) 3 m; (●) 3.5 m; (□) 4 m; (■) 4.5 m; (△) 5 m; (▲) 5.5 m; and (b) for different lakes with varying water depth (○) Liangzi Lake; (●) Xiliang Lake; (□) Changhu Lake; (■) Bailian Lake; (△) Haikou Lake. The curves represent the best fit of the data to Eq. (1). The vertical bars represent the standard deviation.

### 3.3. Experiment 4: effects of bud removal on further germination of turions

Removing the germinated sprout promoted further germination of dormant buds on the turions (Table 4). Fig. 4 shows that when no germinated sprouts were removed, few of the second and third dormant buds germinated. When the first germinated sprouts were removed,

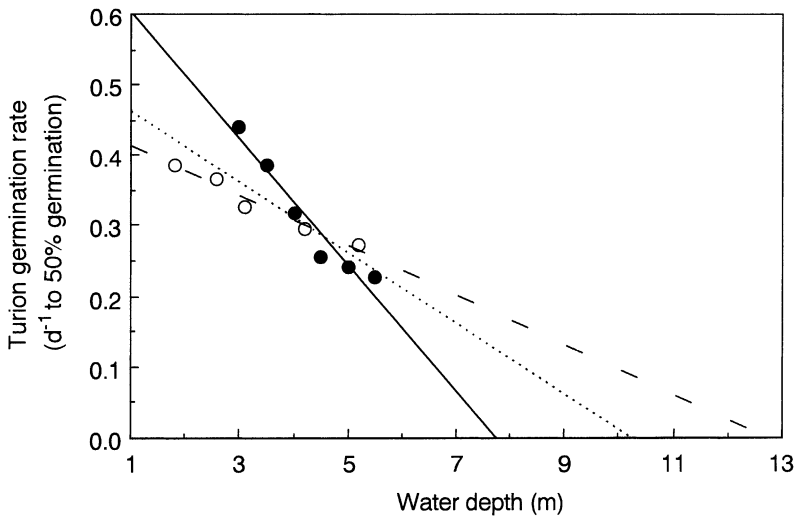


Fig. 2. The germination rate of *Potamogeton crispus* as a function of water depth. Solid line (●) for Liangzi Lake ( $y = 0.692 - 0.089x$ ,  $R^2 = 0.93$ ,  $n = 6$ ,  $P < 0.01$ ); broken line (○) for all five lakes ( $y = 0.450 - 0.035x$ ,  $R^2 = 0.96$ ,  $n = 5$ ,  $P < 0.01$ ); dotted line for pooled data ( $y = 0.515 - 0.050x$ ,  $R^2 = 0.73$ ,  $n = 11$ ,  $P < 0.001$ ).

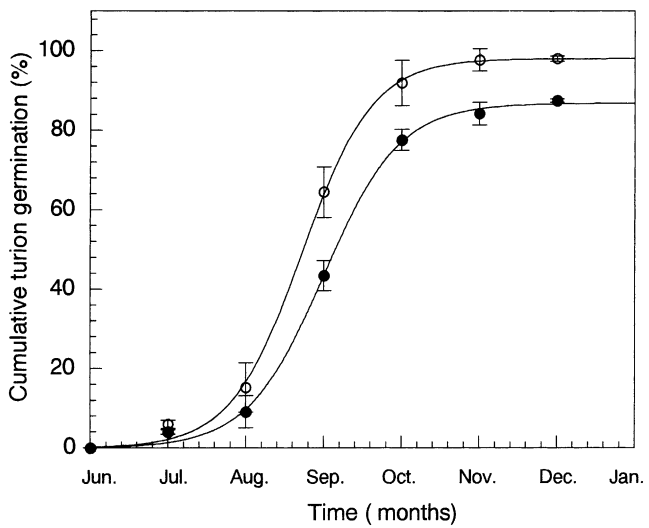


Fig. 3. The time course of the turion germination of *Potamogeton crispus* as influenced by the light conditions in a pond. The symbols are defined as (○) natural light; (●) dark. The experimental data were fitted to Eq. (1). Note that the data from substrate treatments were combined as substrates had no influence on germination. The vertical bars represent the standard deviations.

Table 4

Summary of ANCOVAs for testing the effects of sprout removal on successive sprout germination of *Potamogeton crispus*

Major factor	d.f.	<i>F</i>	<i>P</i>	Co-variate	d.f.	<i>F</i>	<i>P</i>
Second bud	2116	50.0	***	Time	1116	245.0	***
Third bud	2116	36.2	***	Time	1116	110.3	***

Note: (1) the germination percentages were  $\sin^{-1}$  transformed; (2) time (months after planting) was treated as co-variate.

\*\*\*  $P < 0.0001$ .

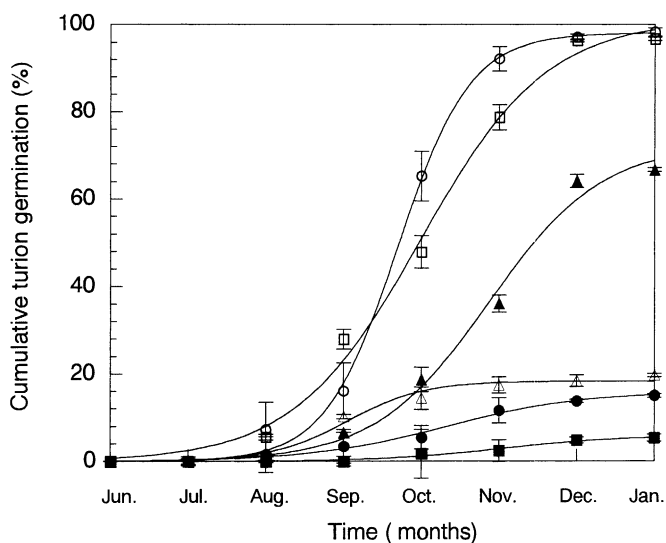


Fig. 4. The effects of sprout removals on successive bud germination of *Potamogeton crispus* in a pond. The symbols are defined as (○) germination percentage of the first bud; (●) germination percentage of the second bud when no sprout removal was performed; (□) germination percentage of the second bud when the first sprout was removed; (■) germination percentage of the third bud when no sprout removal was performed; (△) germination percentage of the third bud when the first sprout was removed; (▲) germination percentage of the third bud when the first and second sprouts were removed. The experimental data were fitted to Eq. (1). The vertical bars represent the standard deviations.

large proportion of the second buds germinated (post hoc test:  $P < 0.0001$ ). Similarly, when both the first and second germinated sprouts were removed, large proportion of the third buds germinated (post hoc test:  $P < 0.0001$ ).

#### 4. Discussion

Our study revealed that for both natural (experiments 1 and 2) and artificially-established (experiments 3 and 4) turion populations of *P. crispus*, massive germination occurred in autumn although a few turions were capable of germinating by mid-July. This may reflect



the phenomenon that germination of dormant buds on turion is regulated by photoperiod (Thomas and Vince-Prue, 1997). Therefore, about 50% germination of turions occurred in late September or even later (Figs. 1, 3 and 4), depending on the experimental conditions while turion formation normally takes place in summer. This illustrates that most of newly-formed turions might enter a period of dormancy as observed in an early study (Rogers and Breen, 1980), which normally lasts for the summer months. Previous studies (e.g. Waisel, 1971; Rogers and Breen, 1980) suggested that high temperature in summer might inhibit the turion germination. If this is the case, it is expected that the turion germination should start earlier in deeper lake than in shallower lakes as water temperature decreases with increasing water depth. However, our results clearly show that the rate of turion germination decreased with increasing water depth. Therefore, temperature may not be the only controller of the turion germination. It is also likely that short days and low temperatures may be the environmental clues to the turion germination whilst the turion formation of *P. crispus* can be triggered by long days (16 h or longer) and high temperatures (16 °C or higher) together (Chambers et al., 1985).

Another consequence of increasing water depth is the reduced light intensity. *P. crispus* was found to grow at low temperatures and very low light intensities, allowing it to exist in deeper or less transparent lakes than other aquatic plants. Nevertheless, the results from experiment 3 demonstrated that both the final germination percentage and rate were higher under natural light conditions than under dark conditions, indicating that its turion germination is a light-demanding process. This is perhaps the reason why the germination percentage and rate decreased with an increase in water depth of the same lake or five different lakes. A general negative linear relationship was established between the germination rate (per day to 50% germination of turions) and water depth, from which one can determine the threshold water depth over which turion germination does not take place. In practice, this model can be used to predict the germination dynamics of turions simply from the mean water depth of a given lake, as long as the light attenuation characteristics are constant. Such information can be potentially used for constructing population model or at least for projecting local recruitment from turion populations.

It is well documented that seed germination is sensitive to light quality (e.g. red/far-red ratio or R:FR) in many species. One may question at this point: what roles does the underwater light quality play in controlling turion germination as it may change with changing water depth. The underwater light quality is indeed different from that immediately above the surface (Smith, 1994) as clear water has strong absorption bands in the FR (far red) and in the near IR (infrared), and therefore the FR is selectively attenuated. In consequence, the increase in R:FR with depth underwater can be very large. On the other hand, the presence of vegetation, dissolved molecules and suspended particles in lakes largely offsets the effects of water on the underwater light quality (Smith, 1994). Thus, the nature of the underwater light quality can be very complicated. Although the changed light quality underwater may have influence on turion germination, we are not in a position to infer the effects of light quality on turion germination. In fact, there is also lack of published evidence of how turion germination responds to the changed light quality. Obviously, further studies are needed to demonstrate the effects of light quality on turion germination by deliberately separating the differential effects of light quantity and light quality.

Under field conditions, most turions gave rise to only one sprout, but when it was removed, another bud sprouted. Similarly, when both the first and second germinated sprouts were removed, a third dormant bud tended to sprout. This strategy is important in the species persistence in the habitats on which herbivorous fishes feed. The potential to germinate further might also be one of mechanisms that this species can be dominant in many lakes and ponds that experience high predation pressure from herbivorous fishes.

Finally, excessive human disturbance had seriously damaged aquatic vegetation in many lakes and ponds in China, leading to degradation of water quality and malfunction of freshwater ecosystems. In order to restore the damaged lakes and ponds, a common practice is to sow turions of *P. crispus*. According to the results obtained here, germination percentage and rate of turions can be predicted from both calendar time and water depth of lakes. For purpose of restoration, it is possible to achieve a given density of plant (sprout) density at a given time by manipulating turion density. Therefore, a full understanding of its turion germination dynamics may be useful to future restoration programs.

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