



Growth of *Potamogeton crispus* L. from turions in darkness: Implications for restoring submerged plants in eutrophic lakes



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ABSTRACT

In the turbid water of eutrophicated lakes, sunlight becomes the primary limiting factor in restoring submerged plants in such lakes. *Potamogeton crispus* L., a species of pondweed, is widely used in such restoration because it can be easily propagated through its turions. However, little is known so far of the growth characteristics of turions growing under limited light or even in total darkness. The growth and physiological responses of plants raised from small, medium, or large turions (categorized on the basis of their weight) and growing in total darkness in an aquarium showed that plants raised from small turions were significantly shorter (33.4 ± 4.8 cm) than those grown from medium or large turions (49.9 ± 4.3 cm and 46.9 ± 7.4 cm, respectively; the difference between the two categories was not significant) when measured on the average of 48th, 62nd and 69th days after planting. Although the plant biomass was significantly different among the three groups of turions, it remained virtually the same within each group throughout. Plants raised from the three categories of turions, however, did not differ significantly either in terms of root length or with respect to soluble protein contents and peroxidase activity; the last parameter increased significantly after 48 days in all the three categories, whereas the activity of superoxide dismutase increased significantly after 62 days. These observations indicate that *P. crispus* plants growing in total darkness suffer from stress due to antioxidants, especially after 48 days of culture. Therefore, it is recommended that larger turions be chosen for planting and that suitable measures be taken to ensure that at least some light reaches the plants 48 days after planting. Three formulas are presented, depending on the level of turbidity and the weight of turions, to calculate the maximum water depth at which *P. crispus* could survive for restoration of *P. crispus* in eutrophic lakes.

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1. Introduction

Eutrophication of lakes has become a serious environmental problem all over the world (Alimov and Golubkov, 2014). In China, such eutrophication began in the early 1970s (Cheng and Li, 2006; Jin et al., 2008), and in recent years many lakes are suffering from eutrophication (Jin et al., 2005; Liu et al., 2010), including Lake Taihu in the province of Jiangsu, Lake Chaohu in Anhui, and Lake Dianchi in Yunnan (Jin et al., 2005; Qin et al., 2004; Wang et al., 2012). With eutrophication, the entire lake ecosystem suffers from the growing biomass of phytoplankton and decreased water transparency, which often leads to degeneration – and

even disappearance – of the submerged macrophytes (Jupp and Spence, 1977; Qin et al., 2013). For deep lake restoration from eutrophication, engineering approaches (nutrient removal, sediment pumping, hypolimnion oxygenation, alum treatments) may be most appropriate where the aim is simply to reduce the production and crop of one component, the phytoplankton. They do not always give the desired results because the nutrient loading may only be reduced to a limited extent. However, restoration of shallow lakes is more difficult because it involves a change of state from phytoplankton to aquatic plant dominance (Moss, 1990). Usually, it is time-consuming and expensive to improve the water transparency and change back to the macrophyte-dominated state through the reduction of nutrient loadings and biomanipulation, because stabilising mechanisms that cause resilience may delay a response, and the clear water state is hard to maintain (Søndergaard et al., 2007). The maintenance and restoration of the stable state of macrophyte dominance should be one of the best choices for the

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management of eutrophic shallow lakes with reference to a better water quality because it is very difficult or even impossible to cut down the internal and external nutrient loadings of such shallow lakes (Qiu et al., 2001). Submerged macrophytes are known to be important in stabilizing the clear-water state in shallow lakes, and they are also crucial to preserving the ecological equilibrium of shallow lakes (Ciurli et al., 2009; van Donk and van de Bund, 2002). It has been proposed that these macrophytes be restored for the rehabilitation of lake ecosystems, along with a few other inexpensive, in-lake management strategies for shallow eutrophic lakes (Mitsch et al., 2014; Moss, 1990; Qiu et al., 1997).

However, restoring submerged vegetation by means of transplanting is difficult because transplanting is labor intensive and not particularly efficient (Li et al., 2008; van Donk et al., 1990). Using seeds for propagating the macrophytes may be a better option for some submerged macrophytes, such as *Vallisneria spirallis* and *Hydrilla verticillata*, known to produce seeds in large numbers (Barrat-Segretain, 1996). However, these seeds are usually very small and light, easily dispersed by wind, and most often fail to germinate (Brux et al., 1987). Among the various propagules produced by submerged plants, turions of *Potamogeton crispus* L. are large and store enough nutrients to support the plants to grow tall enough (Boedeltje et al., 2003). Moreover, because its turions are easy to obtain and plant, *P. crispus* is considered easy to restore over large areas and therefore widely used in rehabilitating degraded lakes (Lauridsen et al., 1994; Li, 2012; Wu et al., 2009).

In eutrophic lakes dominated by algal blooms, inadequate light often becomes a serious limiting factor for the growth of submerged macrophytes such as *P. crispus* (Bornette and Puijalon, 2011; Dijk and van Donk, 1991; Freedman and Lacoul, 2006). Many studies have shown that the turions of *P. crispus* not only germinate but also grow under darkness (Yang et al., 2009; Zhou et al., 2008); however if light intensity continues to be low, growth begins to suffer: the plants remain stunted and produce significantly fewer leaves and less biomass (Zhou et al., 2008). Yang et al. (2009) suggested that insufficient light over a long term could significantly reduce the ability of *P. crispus* to respond to light. Yet, few studies have tried to find out how tall the plants could grow under total darkness – information that is essential for determining the depth at which turions could grow. Such depth also depends on how clear or turbid the water is.

Any drastic decrease in light intensity underwater is a source of physiological stress on submerged plants. This stress manifests itself through decreased production of carbon-based secondary chemicals such as sugars and proteins (Cronin and Lodge, 2003) and overproduction of such reactive oxygen species (ROS) as superoxide radicals (O_2^-), hydroxyl radicals ($\cdot OH$), singlet oxygen (1O_2), and hydrogen peroxide (H_2O_2) (Fridovich, 1986), which could lead to further lipid peroxidation. Plants have developed suitable mechanisms to defend themselves against antioxidants. One such mechanism is to scavenge the ROS through such enzymes as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Wang et al., 2011): SOD converts the destructive superoxide radicals to less dangerous forms, namely hydrogen peroxide and molecular oxygen, and POD and CAT then convert hydrogen peroxide to water and oxygen. This conversion effectively prevents any damage to the cell membrane from the interaction between hydrogen peroxide and superoxide radicals (Monk et al., 1989). Therefore, physiological traits such as the level of activities of SOD and POD and the amount of soluble protein are generally used as physiological indicators of stress on macrophytes (Zhang et al., 2010).

The present study consisted of a three-month-long experiment with turions of *P. crispus* in an aquarium. Because mature turions from nature differ very much in weight, in order to test which ones (size/weight) are the best for re-introducing *P. crispus* in restored lakes, they were divided into three groups – small, medium, and

large – to study whether and how the size of turions affects the growth of *P. crispus* in terms of plant height, root length, and biomass as well as in terms of physiological variables including SOD activity, POD activity, and soluble protein content, under total darkness. The hypothesis was that heavier turions will lead to (1) taller plants and (2) plants with greater tolerance to stress. More specifically, the study sought to (1) measure the growth and maximum height attained by *P. crispus* growing under darkness; (2) examine physiological changes, especially the response of the plants to antioxidants during turion growth; and (3) ascertain the maximum depth of water at which it is possible to grow *P. crispus* from turions as part of the efforts to restore eutrophic lakes dominated by algal blooms.

2. Materials and methods

2.1. Experimental design

The experiment was conducted in a glass aquarium (105 cm × 45.5 cm × 110 cm) from January to April, 2015. Sediment and *P. crispus* turions were collected from Lake Donghu, in Wuhan, China. The aquarium was divided into three compartments separated by white gauze in order to allow water to pass through. The turions were divided into three categories by weight (average fresh weight), namely, small (0.6 ± 0.1 g), medium (1.1 ± 0.1 g), and large (1.8 ± 0.2 g), and each category was represented by 50 turions. The turions were planted 1 cm deep into the sediment, which was covered with a 10 cm column of water. After the turions had germinated and the plants had grown about 5 cm tall, more water was added to make the column of water about 100 cm high. During the course of the experiment, water parameters such as pH, temperature, and dissolved oxygen were measured using a YSI meter (YSI, Yellow Springs, Ohio, USA). A water pump ensured that the water did not remain stagnant (to simulate field conditions better) and that fluctuations in the aquatic environment remained within a narrow range. Average values of the parameter ranged as follows: temperature, 8.4–15.1 °C; pH, 7.7–8.4; and dissolved oxygen, 3.6–7.3 mg/L. The experiment was conducted in total darkness.

Plant height was measured first on the 24th day of the experiment and once a week thereafter (except between the 29th and 48th day) until the end of the experiment (that is, on the 29th, 48th, 56th, 62nd, 69th, and 76th day). From the 29th day, 4 individual plants were sampled from each group every two weeks, and their root length and total biomass (fresh weight, including the seedlings, turions, and roots) were recorded, followed immediately by the physiological parameters measuring (SOD activity, POD activity, and soluble protein contents) of the leaves from the sampled plants. Thus, these parameters were recorded four times in all, namely on the 29th, 48th, 62nd, and 76th day after planting. Each weight category had four replicates.

2.2. Analytical method

Plant height and root length were measured carefully with a ruler and total biomass, with an analytical balance. For determining the physiological parameters, samples (0.01 g each) of fresh leaves were homogenized using a tissue grinder in 6 mL of 0.05 mol/L ice-cold phosphate buffer (pH 7.8) containing 1% polyvinylpolypyrrolidone. The suspension was centrifuged at $12000 \times g$ for 20 min at 4 °C in centrifugal tubes (Wang et al., 2011), and the supernatant was used for determining the above-mentioned physiological parameters.

Soluble protein content was determined by the Coomassie brilliant blue method using bovine serum albumin as a standard

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