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ORIGINAL PAPER

Effects of water depth and substrate type on rhizome bud sprouting and growth in *Zizania latifolia*

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Abstract *Zizania latifolia* is a common emergent macrophyte that plays an important role in the ecological restoration of lakes in the middle and lower reaches of the Yangtze River basin. To understand the mechanisms behind the relative changes in the distribution of *Z. latifolia* in the field, we analyzed the effects of water depth and substrate type on rhizome bud sprouting (RBS) and growth in *Z. latifolia* through simulation experiments. Four water depths (0, 30, 60, and 90 cm) and three substrate types (sandy loam, clay loam, and silt) were used. The results showed that: (1) water depth significantly affected the RBS, and the RBS percentage significantly decreased with increasing water depth. However, substrate type and its interaction with water depth had no effect on RBS. (2) Two-way analysis of variance (ANOVA) showed that plant height, stem diameter, and root length were significantly affected by water depth, with the highest values observed at a water depth of 0 cm. Only root length was significantly affected by substrate type and its interaction with water depth, and it was highest in silt. (3) Both water depth and its interaction with substrate type had significant effects on total biomass and the root:shoot ratio in *Z. latifolia*, but no significant effect was found when it came to subtrate type. The results of this study will be useful for the ecological restoration of lakes in the middle and lower reaches of the Yangtze River basin.

Keywords *Zizania latifolia* · Water depth · Substrate type · Sprouting · Rhizome · Ecological restoration

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Introduction

The area encompassing the middle and lower reaches of the Yangtze River is one of the regions that has the most freshwater lakes in China. Historically, these lakes were all connected to the main flow of the Yangtze River, and supported abundant communities of aquatic plants (Wang and Wang 2009). However, sluices were built in most of the lakes in this region between the 1950s and the 1970s, which has led to large changes in the natural water-level fluctuations (Wang and Dou 1998). Some studies on these lakes have demonstrated that the water level fluctuations are the main factors influencing species composition and the distribution of aquatic plants (Fang et al. 2006; Zhang et al. 2014, 2015). Using long-term hydrologic data, Zhang et al. (2016) classified these disconnected lakes into two categories, and described the succession processes for aquatic plants in these two types of lakes.

Zizania latifolia (Griseb.) Turcz. ex Stapf is widely distributed in wetlands of the Yangtze River basin (Wang et al. 2014). However, the management of *Z. latifolia* in the two types of lakes described by Zhang et al. (2016) is totally different. Some lakes are mainly used for shipping and irrigation, and their mean water levels increased markedly after the sluices were built, leading to a decline or elimination of many emergent aquatic plants mainly due to failures in germination or sprouting. In these lakes, *Z. latifolia* is commonly cultivated for ecological restoration because of its high root activity in remediating contaminated sludge and its ability to remove nutrients from the sediment (Bai et al. 2013; Li et al. 2010). In addition, the well developed rhizome can also strengthen dykes, immobilize the sediment, and control sediment resuspension (Li et al. 2010).

The second lake type is mainly used for flood control and fishing, and the mean water levels decreased after the sluices were built, which accelerated the overgrowth of tall emergent macrophytes (Zhang et al. 2016). In these types of lakes, *Z. latifolia* has always been the single dominant species due to its strong morphological plasticity and flooding tolerance compared with other common emergent macrophytes (Yamasaki and Tange 1981; Wang et al. 2014; Zhang et al. 2016). The overgrowth of *Z. latifolia* not only increases the degree of terrestrialization through the deposition of plant residues in the lakebed, but also consumes large amounts of oxygen and releases nutrients as a result of residue decomposition (Li 1997; Gu et al. 2005). Therefore, controlling the spread of *Z. latifolia* has become the most important issue in these lakes.

At present, several studies have demonstrated that the successful sprouting of the rhizomes has significant implications for the establishment of some tall emergent macrophytes (Coops et al. 2004; Cao 2007). In addition, the resistance of aquatic plants to adverse environmental conditions was found to be significantly lower in the seedling stage than in other growth stages (Mauchamp et al. 2001; Nishihiro et al. 2004). Thus, understanding the environmental requirements for the successful bud sprouting and growth of *Z. latifolia* is of primary importance for the ecological restoration of lakes in the middle and lower reaches of the Yangtze River basin. However, research in this area has not been reported to date. The objective of this study was to determine the independent or combined effects of water depth and substrate type on rhizome bud sprouting (RBS) and growth of *Z. latifolia*.

Zizania latifolia

Zizania latifolia, commonly known as Manchurian wild rice, is a perennial emergent plant in the Poaceae. Plants can reach a maximum height of 3–4 m with well developed rhizomes, and can reproduce by seeds, tiller buds of the aboveground stems, and rhizomes (Wang 1991). In the middle and lower reaches of the Yangtze River basin, shoots emerge from rhizome buds from February to March (Zhang et al. 2015). The shoots can reach heights of 1.8 m by early May, and the maximum growth rate in summer can be up to 10 cm/day (Wang 1991).

Experimental design

Zizania latifolia rhizomes were collected in lakeside zone (N: 30°15′27.90″; E: 116°42′45.29″) of Wuchang Lake in Anhui Province, and transported immediately to the botany garden of Anqing Normal University (N: 30°15′27.90″; E: 116°42′45.29″) for cultivation on February 25th 2016. Rhizomes with stem diameters of $\sim$1.0 cm were selected, and cut into 8–10 cm segments with 2 tiller buds per segment. The old roots were removed from the rhizome sections which were then planted in plastic boxes (length 37 cm, width 28 cm, height 15 cm) at a depth of 3 cm, with four rhizome segments per box. Silt, sandy loam, and clay loam were used as the three substrate types. Twelve replicate boxes were filled with each substrate type to a depth of 12 cm. The total nitrogen (TN) and total phosphorus (TP) contents of each substrate were measured using standard methods (Lu 2000). TN contents of the silt, sandy loam, and clay loam were 1.52 ± 0.02, 0.44 ± 0.02, and 0.21 ± 0.04 mg/g, respectively, and TP contents were 0.63 ± 0.02, 0.12 ± 0.01, and 0.08 ± 0.03 mg/g, respectively. The 12 boxes of each substrate treatment were then suspended randomly in an outdoor concrete tank from a steel bar. The tank was 2.2 m in width by 3.5 m in length, and was filled with fresh water to depth of 1.0 m. Four water depth treatments were designed as 0, 30, 60, and 90 cm above the substrate surface, and each water depth had three replicates per substrate treatment. The water quality parameters at the beginning of the study were measured using standard laboratory procedures (Wei 2002). The original TN and TP contents of the water were 0.22 and 0.01 mg/L, respectively, and the turbidity of the water was 4 NTU. The number of sprouted rhizome buds was counted at five day intervals, and the percentage of RBS in each box and each treatment was then calculated by the following equation: $P = N_1/N_2$, where P is the percentage of RBS; N_1 is the number of RBS; N_2 is the total number of rhizome buds. In order to determine whether the rhizome buds had successfully sprouted from the substrate, the plastic boxes were raised to the water surface for observation in the 30, 60, and 90 cm water depth treatments and then returned to their original depths after assessment.

Data analyses

The experiments were terminated at day 40 on April 5th 2016. Plants were collected from the plastic boxes after determining the percentage of RBS in all treatments. The plant heights and root lengths of the *Z. latifolia* shoots were measured with a meter stick, and the stem diameter was measured with a vernier caliper. The plant height was defined as the distance from the soil line to the tip of the uppermost leaf, and the root length was defined as the length of the longest root. All plants were then divided into aboveground and belowground (not including the original rhizome segments) parts which were dried in an oven at 80 °C for three days. The individual weights of the roots, shoots, and whole plants were then determined. Two-way analysis of variance (ANOVA) was applied to analyze the effects of substrate type and water depth on the percentage of RBS, plant height, stem diameter, root length, and the root:shoot ratio in *Z. latifolia*. Before conducting the ANOVA, the normality and homogeneity of the variances were tested. Post-hoc multiple comparisons were conducted using Tukey's honestly significant difference for equal variances or Tamhane's T2 method for unequal variances (Kara et al. 2013). All data analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

Results

Rhizome sprouting

The *Z. latifolia* rhizomes began to sprout on day 5 of the experiment, and the percentage of RBS reached a peak on the day 15 of the experiment (Fig. 1). After that, there was little change. Based on the results of two-way ANOVA, the RBS was significantly affected by water depth ($F = 269.72$, $P < 0.001$), and the percentage of RBS decreased significantly with increasing water depth (Fig. 2a). The substrate type

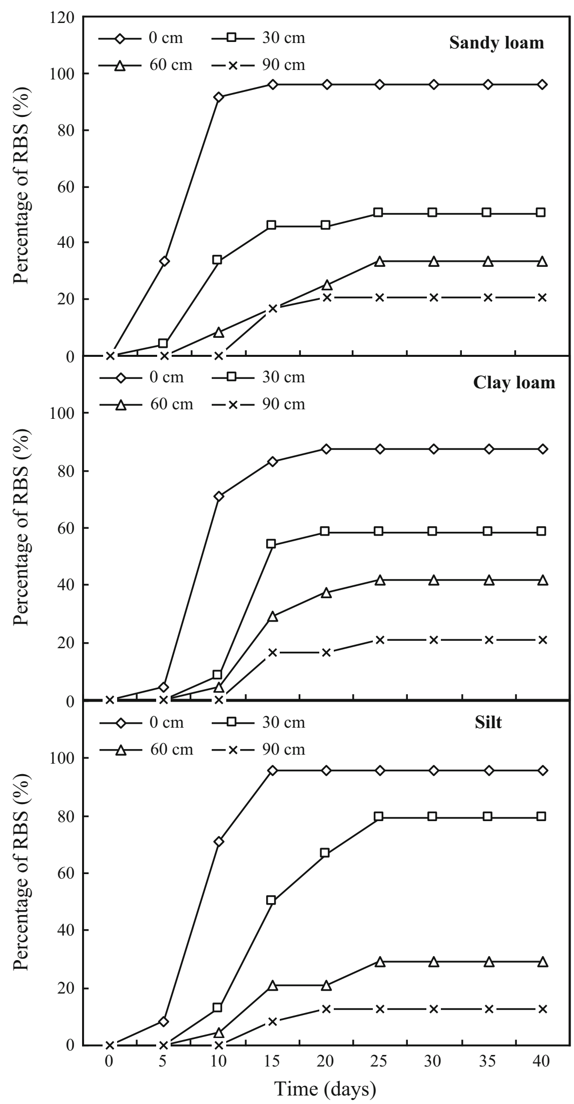

Fig. 1 Effect of water depth and substrate type on the RBS percentage in *Zizania latifolia*

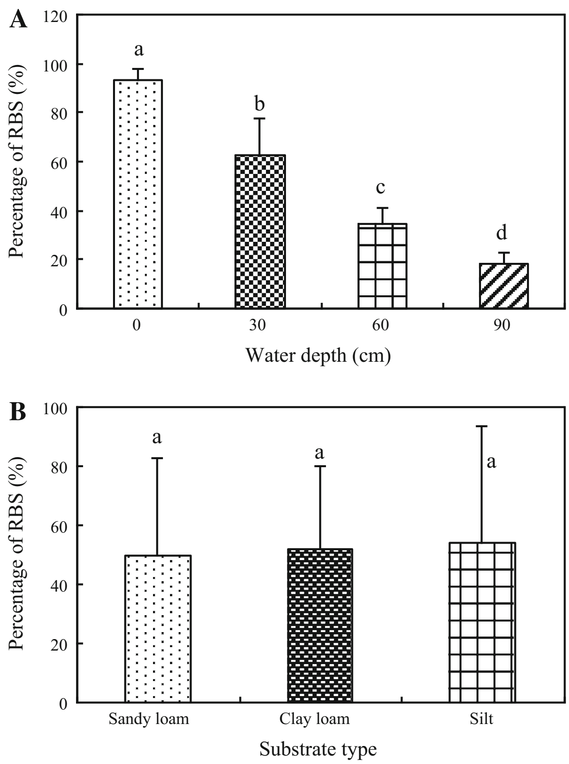

Fig. 2 Comparisons of water depth (**a**) and substrate type (**b**) on the RBS percentage in *Zizania latifolia* (mean ± SD). *Different lower case letters* indicate significant differences ($P < 0.05$)

and its interaction with water depth had no significant effects on RBS ($F = 2.09$, $P > 0.05$; $F = 4.05$, $P > 0.05$). During the course of the experiment, the average percentage of RBS in all three substrates was ~50% (Fig. 2b).

Morphological characteristics

Water depth had significant effects on the plant height, stem diameter, and root length of *Z. latifolia* shoots (Table 1). Plant heights in the 0 and 30 cm water depth treatments showed no significant differences, but they were significantly higher than those in the 60 and 90 cm water depth treatments (Fig. 3). Both the stem diameters and root lengths were highest at 0 cm water depth, and both were significantly higher than in the other three water depth treatments (Fig. 3). Substrate type and its interaction with water depth had a significant effect only on root length (Table 1), and it was highest in the silt treatment.

Biomass allocation

Water depth had significant effects on the total biomass and root:shoot ratios (Table 1), and both of these parameters were significantly higher in the 0 cm water depth treatment than in the other three treatments (Fig. 4). However, substrate type had no significant effect on either parameter.

The interaction between water depth and substrate type affected both total biomass and the root:shoot ratio significantly (Table 1). The total biomass in silt was significantly higher than it was in the other two substrates for the 0 cm water depth treatment, while it was only significantly higher in silt than it was in clay loam in the 30 cm water depth treatment. With further increases in water depth (the 60 and 90 cm water depth treatments), the total biomass showed no significant differences among the three substrate types (Fig. 5).

Discussion

Effects on RBS

Increasing water depth significantly inhibited RBS in *Z. latifolia*, which decreased from 93.1% at a water depth of 0 cm to 18.1% at 90 cm (Fig. 2a). This is similar to previous results related to RBS in *Acorus calamus* and *Phragmites australis* (e.g. Cao 2007). In

Table 1 Two-way analysis of variance (ANOVA) of the effects of water depth, substrate type, and their interaction on plant height, stem diameter, root length, total biomass, and the root:shoot ratio in *Zizania latifolia*

Source of variation	Plant height	Stem diameter	Root length	Total biomass	Root:shoot ratio
Water depth (W)	16.522***	85.987***	231.433***	100.442***	52.514***
Substrate type (S)	0.070	0.130	6.018**	1.475	0.858
W × S	2.283	1.162	30.623***	3.382**	3.315**

** $P < 0.01$; *** $P < 0.001$

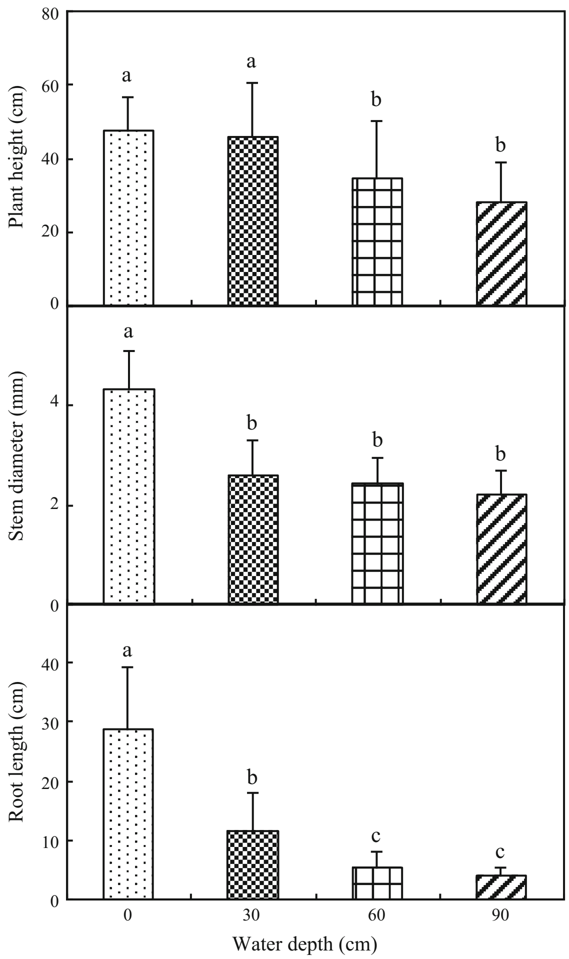

Fig. 3 Comparisons of the plant height, stem diameter, and root length in *Zizania latifolia* grown at different water depths (mean ± SD). *Different lower case letters* indicate significant differences ($P < 0.05$)

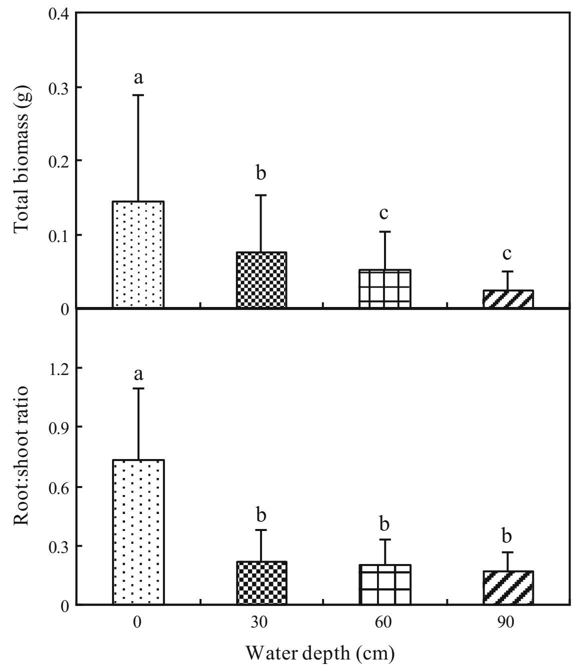

Fig. 4 Effects of water depth on total biomass and the root:shoot ratios in *Zizania latifolia*. *Different lower case letters* indicate significant differences ($P < 0.05$)

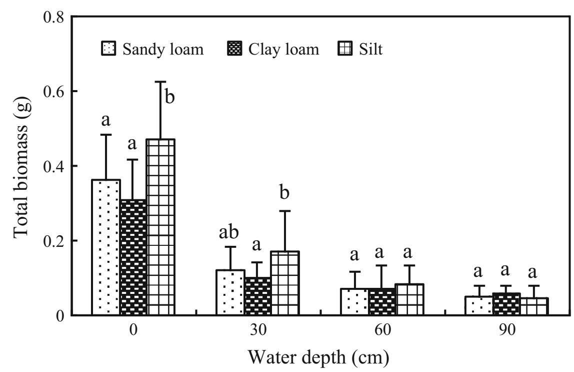

Fig. 5 Effects of the interaction between water depth and substrate type on total biomass in *Zizania latifolia*. *Different lower case letters* indicate significant differences ($P < 0.05$), and the significance values are within treatments

wetland ecosystems, deeper water always leads to failure or a decrease in the sprouting percentage of aquatic plants (Coops et al. 2004). This effect could result from decreases in light availability, oxygen content, and water temperature that correspond to increases in water depth (Zhang et al. 2013). However, the study of Jian et al. (2001) showed that an increase in water depth only significantly delayed the starting time of sprouting in *Potamogetom crispus* turions, but did not change the final percentage of sprouting. This indicates that the sprouting characteristics may vary remarkably among aquatic plants with different life forms. Otherwise, previous studies on rhizome sprouting in *Z. latifolia* in the field showed that the water depth threshold was ~1.0 m (Zhang et al. 2016), which was close to our findings in this study. However, it was far deeper than the threshold depth for rhizome sprouting in *Phragmites australis*, which was found to be only ~30 cm (Cao 2007). This explains why *Z. latifolia* is often distributed in areas of deeper water than is *P. australis* in the field, and is also indirectly related to the observation that *Z. latifolia* has a higher tolerance to flooding than does *P. australis*.

There have been relatively few studies focused on the effects of substrate type on rhizome sprouting in aquatic plants. In our study, we found that the substrate type had no effect on rhizome sprouting in *Z. latifolia*, which is consistent with studies on *Arundo donax* (Boose and Holt 1999) and *Potamogeton crispus* (Jian et al. 2001). According to Jian et al. (2001), because aquatic plant propagules contain enough nutrition to support their sprouting, water level is the main determining factor for sprouting. In the field investigation, we also found that rhizomes of *Z. latifolia* floating at the water surface could successfully sprout. This finding supported the results of Jian et al. (2001), and also indirectly suggested that substrate is not a necessary condition for rhizome sprouting in *Z. latifolia*. Otherwise, although the three substrates varied with respect to particle size, the oxygen contents in the three substrates may show little difference. Some studies have shown that the normal gas exchange between air and substrate is blocked under saturated conditions, and the substrate is usually maintained in an anaerobic state (Tian and Song 2002). Therefore, the oxygen content of the substrate may also not be the main factor affecting rhizome sprouting in this study.

Effects on growth

Periodic inundation is one of the most important factors affecting the growth and development of wetland plants (Zhang et al. 2015). Over the course of evolution, diverse wetland plant species have developed morphological characteristics, phenological rhythms, and life history strategies specific to the aquatic environment (Poff et al. 1997; Lytle and Poff 2004). According to Macek et al. (2006), emergent macrophytes, compared to submerged ones, usually cannot carry out photosynthesis under inundated conditions, and thus they have developed spatial and temporal strategies to avoid inundation, or they can tolerate being inundated through specific metabolic changes. The most common morphological adaption of emergent macrophytes to inundation is the promotion of stem elongation through cell growth or division, which reduces the investment to the underground parts (Cooling et al. 2001). Thus, many studies on emergent macrophytes suggest that with increases in submerged depth, stem diameters and root lengths decrease, while plant heights increase in order to allow the photosynthetic shoots to be in a more favorable light environment (Cao 2007; Wang et al. 2014). In the present study, *Z. latifolia* demonstrated decreased stem diameters and root lengths with the increase of the water depth, and both were significantly lower in the 30, 60, and 90 cm water depth treatments than in the 0 cm water depth treatment. However, the maximum plant height was not found in the maximum water depth treatment (90 cm). The main reason was that the water depth gradient in this study was large, and the *Z. latifolia* plants grown in the 60 and 90 cm water depth treatments were completely submerged throughout the course of the experiment. The limited morphological adaptation was not enough for them to rapidly avoid the adverse effects of submergence, and thus the plant height remained low. The substrate is the main source of nutrition during the growth of emergent plants (Lenssen et al. 1999). However, substrate type and its interaction with water depth had a significant effect on root length only, and had no effect on plant height and stem diameter in our study. This could be due to the fact that the duration of the experiment was not long enough for differences to develop between the groups. Otherwise, we found that the roots of *Z. latifolia* shoots were longest in the silt, and this was different from other studies. Root length in *Spartina alterniflora* showed no significant differences with increases in substrate nutrient levels (Deng et al. 2010), while root length in *Vallisneria natans* was significantly reduced (Wang et al. 2013). These findings indicate that the morphological responses of roots to nutrient content may differ among different species of aquatic plants.

Changing biomass allocation is one of the survival strategies of aquatic plants in response to submergence (Zhang et al. 2013; Wang et al. 2014). Several studies have demonstrated that the total biomass of aquatic plants will decrease as the submerged depth increases, and more biomass will be allocated to the aboveground parts to acclimate to the adverse environmental conditions (Coops et al. 1996; Cao 2007). In this study, the total biomass of *Z. latifolia* decreased significantly with increases in water depth, and the root:shoot ratio was significantly higher at 0 cm water depth than in the other treatments. This is consistent with the results of previous studies with *Z. latifolia*. However, the total biomass and the root:shoot ratio in *Z. latifolia* was not significantly affected by substrate type in our study, and this may also be because the duration of the experiment was not long enough. Analysis of the interaction between water depth and substrate type showed that the total biomass for plants grown in silt was significantly higher than in the other two substrates in the 0 cm water depth treatment. However, with increasing water depth, the differences among the three substrates decreased gradually and even disappeared. This observation shows that the effects of substrate type on growth of *Z. latifolia* shoots would be minimized or eliminated when the plants are completely submerged.

Implications for lake management

The middle and lower reaches of the Yangtze River basin encompass an area with abundant freshwater lakes that has also suffered serious wetland degradation (Fang et al. 2006). In the last few decades, the diversity of aquatic plants has decreased considerably in this region. In considering the important status of *Z. latifolia*, the results of this study will have implications for the ecological recovery of lakes in this part of China. In lakes where aquatic plants have been lost due to the increase in the mean water level, a limited number of factors should be considered for the recovery of the *Z. latifolia* community. Water depth is the major factor influencing RBS in *Z. latifolia*, and the highest percentage of RBS occurs at a water depth of 0 cm. The substrate type may play more important roles in the growth of *Z. latifolia* shoots than in the RBS.

In lakes where the overgrowth of *Z. latifolia* has become a nuisance, the inhibition of RBS is crucial to control the spread of *Z. latifolia*. As the water depth of most lakes can be controlled by sluices, increasing the water depth during the sprouting stage of *Z. latifolia* may be the most simple and effective method to control its growth. Thus, controlling the water depth to appropriate levels is a precondition for the ecological recovery process. If these measures can be implemented, the ecological health of lakes in the middle and lower reaches of the Yangtze River basin will be improved.

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