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Original Research Article

Impact of regulated water level fluctuations on the sexual reproduction of remnant *Myricaria laxiflora* populations

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ABSTRACT

The construction and operation of the Three Gorges-Gezhou cascade water conservancy and hydropower projects has dramatically changed downstream water level fluctuations. Remnant populations of *Myricaria laxiflora*, an aquatic endangered plant, have been severely affected by the regulated water level fluctuations. In this study, the impact of regulated water level fluctuations on sexual reproduction of *M. laxiflora* were assessed by investigating variations in the number of flowering branches, inflorescences, flowers, and fruits, seed vigor, and 1,000-seed weight of remnant *M. laxiflora* populations across a water level fluctuation gradient. Our findings indicate that the water level fluctuation gradient significantly influences the sexual reproduction of *M. laxiflora*. The flowering and fruit setting of *M. laxiflora* plants in the upper region of the habitat were superior to those in the middle and lower regions. The mean flowering branches, the number of inflorescences per branch, and the number of flowers of *M. laxiflora* plants in the upper region of the gradient were 150.0%, 356.7%, and 196.8% higher than those in the middle region, and 124.7%, 491.7%, and 173.6% higher than those in the lower region, respectively. The number and rate of fruit settings of *M. laxiflora* plants in the upper region of the gradient were 389.1% and 61.1% higher than those in the middle region, and 788.4% and 192.6% of those in the lower region, respectively. The number of seeds per fruit, 1,000-seed weight, and seed vigor of the *M. laxiflora* plants in the upper region of the gradient were 7.3%, 36.2%, and 181.6% higher than those in the middle region, and 10.9%, 31.6%, and 176.1% higher than those in the lower region, respectively. Further analysis indicated that flowering, fruit setting, and seed development of *M. laxiflora* were correlated with soil water content and duration of habitat exposure, which are aquatic ecological environmental factors that are related to water level fluctuations. Taken together, the findings of this study indicate that water level fluctuations regulated by the operation of the cascade water conservancy and hydropower projects affect the sexual reproduction of remnant *Myricaria laxiflora* populations.

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1. Introduction

Seeds are the important propagule resources for the recruitment of plant populations. Plant flowering and fruiting play vital roles in plant population regeneration (Hampe and Arroyo, 2002). Over time, plants have gradually adapted to their surrounding environment in their flowering and fruit settings (Kozlowski and Pallardy, 2002; Lambrecht et al., 2017). Ecological environmental changes can severely affect the flowering, fruit setting, and seed development, and restrict population regeneration of plants (Wiegand et al., 2009; Stinson et al., 2018). Water level fluctuations are key ecological processes that influence plant populations in the riparian zone (Ma et al., 2018; Zhang et al., 2017). The aquatic ecological environment in the riparian zone changes with the pattern of water level fluctuations (Webb et al., 2012; Wang et al., 2018). The processes of flowering and fruiting in many riparian plants are often compatible with water level fluctuation patterns, so normal water level fluctuations usually do not affect the sexual reproduction of these populations (Van der Sman et al., 1993; Chen and Xie, 2007). In some extreme cases, the individual growth and development and population formation and development of some species are completely dependent on water level fluctuations (Mony et al., 2010; Crosby et al., 2015). The flowering, fruit setting, and seed development of these riparian plants are often affected when subjected to alterations in water level fluctuations, which further poses constraints to population regeneration and lead to population degradation (Moxham et al., 2018; Satake et al., 2001). Thus, a few plants have been endangered for this reason (Kitamura et al., 2009).

Myricaria laxiflora (Franch) (P. Y. Zhang & Y. J. Zhang), a Tamaricaceae shrub with well-developed root system and high resistance to flooding and drought, is native to the river beach in the middle and lower reaches of the Yangtze River, which runs from Zhijiang, Hubei Province, to Yibin, Sichuan Province, China. The Three Gorges Reservoir Region serves as the main distribution area (Chen et al., 2005). With the construction of the Three Gorges Dam, the water level of this reservoir has substantially increased and submerged all habitats of *M. laxiflora* in the Three Gorges Dam Reservoir Region. Currently, *M. laxiflora* only remains in a small population in the Yangtze River in Yichang and Yibin downstream of the Gezhou Dam and Xiangjia Dam (Chen and Wang, 2015). Due to the influence of the Three Gorges-Gezhou Dam, large-scale water conservancy projects have been conducted for purposes of flood control and power generation on river regulation, and the water level fluctuation patterns in the habitat of remnant *M. laxiflora* have dramatically changed, i.e., significantly shortened flood periods in the summer, accelerated decline in water levels in autumn, and a significant drop in water levels during the drought period in winter (Duan et al., 2016). According to a field investigation on remnant *M. laxiflora* populations in Yichang conducted by Bao et al. (2010), seedling production by individuals of the populations is currently extremely low. This finding suggests that the regeneration of *M. laxiflora* population have been hampered. The remnant *M. laxiflora* populations suffer flooding every summer. After each flood, the plants begin to blossom and bear fruit immediately. *M. laxiflora* seeds with pubescence can be dispersed either by wind or river current. Wind-dispersed seeds usually settled within 25 m from parent plants, and water-dispersed seeds often landed and established on strands of firth (Chen and Xie, 2007). We hypothesize that the flowering, fruit setting, and seed development of *M. laxiflora* are influenced by water level fluctuations in the Yangtze River, and changes in downstream water level fluctuation patterns and the aquatic environment caused by large-scale hydropower projects influence its sexual reproduction and population regeneration to some extent. The present study examined the flowering, fruit setting, and seed development of remnant *M. laxiflora* populations distributed across a water level fluctuation gradient in Yichang downstream of the Gezhou Dam to uncover its effects on the sexual reproduction of this species. The emergence time, duration, and soil water content of different water level fluctuation gradients are related to the water level fluctuation pattern of rivers. We hereby examined the relationship between the aforementioned aquatic ecological environmental factors and the sexual reproduction characteristics of remnant *M. laxiflora,* and assessed the impact of the regulated water level fluctuations on the sexual reproduction of remnant *M. laxiflora* populations.

2. Materials and methods

2.1. Study sites and habitat

The remnant populations of *M. laxiflora* are located in the transitional zone between the middle subtropical zone and northern subtropical zone where the annual average temperature is between 17.1°C and 19.5 °C. The average temperature is between 28.0°C and 29.6 °C in July and 5.0°C–7.1 °C in January. The annual average precipitation of the area is between 1,000 and 1,220 mm. The non-frost period is longer than 300 d. Therefore, the area has a mild climate, which includes a warm winter, cool summer, and abundant precipitation (Wang et al., 2003). Downstream of the Three Gorges Dam, the remnant *M. laxiflora* is primarily distributed on the river beach of three islands in the middle of Yangtze River from Yichang to Zhijiang. The Yanzhiba island in Yichang (30°38′56″N, 111°19′10″E) was used as experimental plot. There are over 10,000 *M. laxiflora* plants in this island, which is also the largest remnant population of *M. laxiflora*. The soil of this area is primarily alluvial soil that is sandy, but varies extensively along the water level fluctuation gradient. There are sandy soil and pebbles near the bank; off the bank, the soil gradually turns into sandy loam. The pH of the soil is within the range of 7.0–8.4; its organic matter and nutrient content is low (Chen and Wang, 2015). Shrubs and grasses are common vegetation types on the beach, which include *M. laxiflora* + *Salix variegata* communities.

2.2. Experimental design

Due to the influence of seasonal fluctuations in the water level of the Yangtze River, the study plot is submerged during the annual flood season (June–September) and exposed during the drought season (September–May of the following year). There is a large discrepancy in the timing and duration of the submergence and exposure of different regions of the habitat of *M. laxiflora* due to the difference in altitude gradient. In the aforesaid distribution area of remnant *Myricaria laxiflora* populations, the experimental plot was divided into higher (altitude (above sea-level) ≥ 45.1 m), middle (45.1 m < altitude >42.8 m), and lower (altitude < 42.8 m) water level fluctuation gradients along the altitude gradient, on which line transects were set up. Quadrats of 5 m × 5 m were arranged at an interval of 10 m within each line transect; there were 10 quadrats per transect and 30 quadrats in the whole plot (Fig. 1).

2.3. Investigation of the aquatic ecological environment of the study sites

The water level and weather of the experimental plot were monitored and recorded once every five days from the time the habitat was exposed. Variations in the water level and exposure of various line transects were recorded by considering the altitudinal variations in the water level of the Yangtze River based on on-site investigations (Fig. 2). In addition, the phenological period of *M. laxiflora* populations distributing in various line transects were monitored. The upper region of the sample plot is exposed from September to June, where it is submerged in water until June 2 of the next year. The flowering period, fruiting period, and the overall exposed duration of this region were 48 d, 45 d, and approximately 250 d, respectively. During the exposed season, the upper region was reflooded from September 27 to 30, October 6 to 23, and from October 26 to 27 due an increase in water level. The exposed seasons of the middle and lower regions were generally similar because of the fast flood recession in the later flood season. Specifically, the middle region was exposed on November 4 and was submerged in water on May 17 of the following year. The flowering period, fruiting period, and the overall duration of exposure of this region were 14 d, 28 d, and approximately 190 d, respectively. The lower region was exposed on November 21 and submerged in water on May 2 of the next year. The flowering period, fruiting period, and the overall duration of exposure of this region were 15 d, 23 d, and approximately 160 d, respectively.

Soil samples were collected every week using a soil sampler (15 cm deep) at three randomly selected points in each quadrat on a line transect based on their exposure time during the experiment, and were then dried and weighed to measure their water content in the laboratory. Thirty soil samples were collected at each gradient each time. According to the test results, variations in soil water content in the habitat was consistent with the changes in the water level of the Yangtze River (Fig. 2). The soil water content in the upper region of the habitat was between 10.0 and 15.0% in the initial stage of the exposed season (mid-September). With the rise in the water level of the Yangtze River, the soil water content increased to 30.0–35.0% when the upper region was flooded and re-exposed. The soil water content in the middle region was within the range of 20.0–25.0% during the initial time of the exposed season (early November); the soil water content in the lower region was within the range of 10.0–15.0% during the initial time of the exposed season (mid-November). Afterwards, the soil water content at all three gradients decreased and coincided with the rapid recession of the water level of the Yangtze River. In late December, the soil water content at all these gradients decreased to around 5.0%.

2.4. Investigation of the flowering and fruit setting of the remnant population

Three *M. laxiflora* plants with heights 0.8–1.0 m and in their fertile period were randomly marked as experimental subjects within each quadrat in every line transect according to the timing of exposure at various water level fluctuation gradients. One of these three plants was used to assess flowering and fruit setting in *M. laxiflora*. The number of flowering branches and the number of inflorescences per branch of these plants were recorded once every three days from the initial flowering period until no new flowers or fruits were observed. The initial period, peak period (50.0% inflorescences bloomed or bore fruits), and

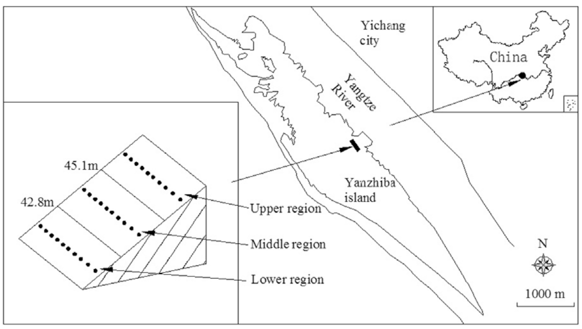

Fig. 1. The location of sample plot and the distribution of line transects and quadrats.

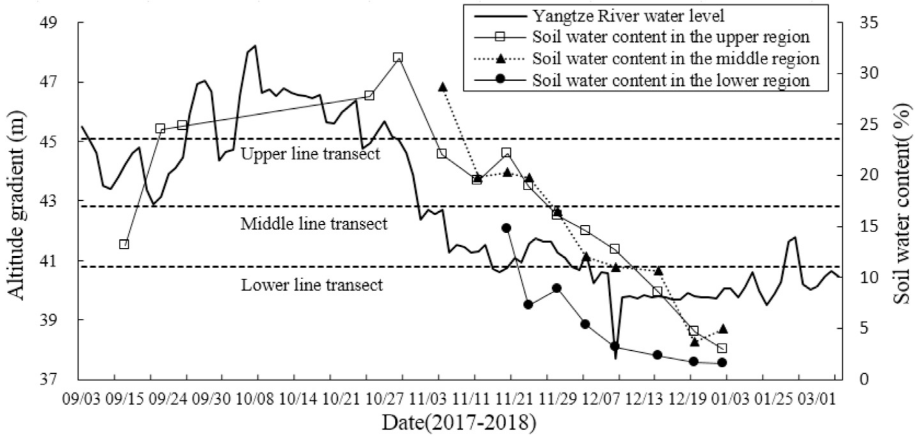

Fig. 2. Fluctuations in the water level and changes in soil water content along the altitudinal gradient within the Yichang section of the Yangtze River.

termination period of flowering and fruit settings were noted. Another plant was used to observe the process of flowering and fruit setting. Three flowering branches of this plant were randomly selected. The time of flowering, the number of flowers, and the number of fruit settings on each flowering branch were recorded every three days from the initial flowering period until the flowering and fruit setting of the marked branch had ended. The other plant was used to study fruit and seed development. One inflorescence was randomly selected at the upper, middle and lower regions of each plant; one ripe fruit from the upper, middle and lower regions of these three inflorescences was picked. In total, nine fruits were picked in each quadrat, totaling 90 from each gradient. The fruits were picked and marked before the capsules were cracked open. The number of seeds per fruit and 1,000-seed weight were measured as soon as these were transported back to the laboratory. Then, the seeds from each plant were mixed evenly, after which 100 seeds were randomly picked and pooled as one experimental unit for the seed germination experiment. The germination rate was counted to evaluate seed vigor. This experiment was performed in triplicate for each quadrat, with 30 replicates at each water level fluctuation gradient. Seed vigor was measured by the germination method. The germination conditions were set to 21 °C, 3,000 Lx, and 12 h illumination/12 h darkness, with a relative humidity of 75.0%, and the soil was managed each day and kept moist.

2.5. Statistical analysis

Data processing was conducted using Excel. SPSS (19.0) was used for one-way analysis of variance (ANOVA). Sexual reproduction characteristics, such as the number of flowering branches, the number of inflorescences, the number of flowers, the number of fruit setting, fruit set percentage, and the number of seeds per fruit, were used as dependent variables, and water level fluctuation gradients (upper, middle, and lower regions) were used as independent variables to assess differences in indicators using variations in fluctuation gradients. Multiple comparison (Tukey) was conducted to analyze differences in flowering and fruit settings of remnant *M. laxiflora* among various water level fluctuation zones when the factor processing effect reached significance. The correlations of the flowering period, fruiting period, and the number of flowers and fruits of remnant *M. laxiflora* to the timing of exposure, flowering period, fruiting period, and soil water content were obtained using Pearson correlation and used in the comprehensive analysis of the relationship between the flowering and fruit settings of remnant *M. laxiflora* and water level fluctuation patterns. Combined with changes in water level fluctuation patterns in the habitat, the effects of regulated level fluctuation on sexual reproduction of the species were analyzed.

3. Results

3.1. Variations in the number of flowering branches and inflorescences of plants across a water level fluctuation gradient

The number of flowering branches and inflorescences per branch of the remnant *M. laxiflora* plants exhibited significant spatial variations across the water level fluctuation gradient (Fig. 3). The number of flowering branches and inflorescences per branch in the upper region of the water level fluctuation zone were significantly higher than those in the middle and lower regions ($P < 0.05$), whereas no significant difference in the number of flowering branches and inflorescences per branch was observed between the middle and lower zones ($P < 0.05$). In particular, the number of flowering branches of plants in the upper region was 20 per plant, which was 150.0% and 124.7% higher than the middle and lower regions, respectively. The number of inflorescences per branch in the upper region was 121.3 per branch, which was 356.7% and 491.7% higher than the middle and lower regions, respectively.

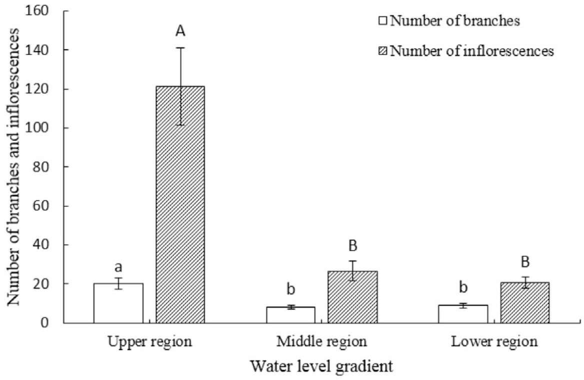

Fig. 3. Changes of the number of flowering branches and inflorescences of *M. laxiflora* across a water level gradient. Note: Different letters indicate significant differences ($p < 0.05$).

3.2. Variations in plant flowering and fruit setting across a water level fluctuation gradient

Significant differences in the flowering and fruiting periods, the number of flowers and fruits, and fruit set percentage of *M. laxiflora* plants among different water level fluctuation gradients were observed ($P < 0.05$) (Table 1). The flowering period, fruiting period, the number of flowers and fruit setting of plants in the upper region of the water level fluctuation zone were all significantly higher than those in the middle and lower regions ($P < 0.01$), whereas these did not significantly differ between the middle and lower regions. The flowering period, number of flowers, fruiting period, number of fruit settings, and the fruit set percentage of plants in the upper region of the water level fluctuation zone were 238.2% and 222.4% longer, 196.1% and 173.6% larger, 59.6% and 95.5% longer, 389.1% and 788.4% larger, and 61.1% and 192.6% higher than those of the middle and lower regions, respectively. Plants in the upper region of the water level fluctuation zone underwent repeated submergence during the flowering and fruiting periods. The number of flowers and fruit settings of no repeated flooded branches was 42.7% and 88.1% higher than branches underwent repeated submergence, respectively.

There were significant differences in plant flowering and fruiting process across the water level fluctuation gradient (Fig. 4). Plants in the upper region underwent long flowering and fruiting periods, the number of flowers and fruit settings increased gradually; whereas the flowering and fruiting periods of the plants in the middle and lower regions were short, and the number of flowers and fruit settings increased gradually rapidly. The flowering period of plants in the upper region of the water level fluctuation zone lasted for 76 d; this occurred from September 6 to November 21 (including the duration of reflooding) and peaked during the 6th to 14th d after exposure. The fruiting period lasted for 86 d, occurring from September 9 to December 14 (including the duration of reflooding); the peak period was from the 10th to the 26th d after exposure. During the flowering and fruiting periods, the upper region underwent reflooding and exposure thrice and fully emerged by October 28. The flowering period of plants in the middle region lasted for 14 days, occurring from November 19 to December 3 and peaked from the 15th to 23rd d after exposure. The fruiting period of plants in this region lasted for 28 d and took place from November 21 to December 19; the peak period was from the 35th to the 44th d after exposure. The flowering period of plants in the lower region lasted for 15 d, taking place from December 1 to 16 and peaking during the 10th to 18th d after exposure. The fruiting period of plants in this region lasted for 23 d, occurring from December 5 to 28 and peaking during the 24th to 30th d after exposure.

3.3. Variations in seed development of remnant M. laxiflora across a water level fluctuation gradient

Significant differences in seed development of *M. laxiflora* plants were observed across the water level fluctuation gradient ($P < 0.05$) (Table 2). The number of seeds per fruit, the 1,000-seed weight, and seed vigor were higher in plants from the upper

Table 1
Characteristics of flowering and fruiting across a water level gradient.

Water level gradient		Flowering		Fruiting		
		Flowering period (d)	Number of flowers	Fruiting period (d)	Number of fruits	Fruiting rate (%)
Upper region	No Reflooding	26.0 ± 1.6	41.8 ± 1.3	21.3 ± 1.2	30.2 ± 1.1	72.7 ± 1.9
	Reflooding	21.7 ± 1.5	29.3 ± 1.39	23.8 ± 1.28	16.0 ± 1.1	57.2 ± 3.7
	Total	47.7 ± 1.4a	37.0 ± 1.9a	50.1 ± 1.2a	26.1 ± 1.6a	71.1 ± 3.4a
Middle region		14.1 ± 1.2b	12.5 ± 0.8b	28.2 ± 4.5b	5.3 ± 0.8b	44.1 ± 5.2b
Lower region		14.8 ± 3.4b	13.5 ± 0.9b	23.4 ± 3.8b	2.5 ± 0.4b	24.3 ± 3.4c

Note: Different letters in the same column indicate significant differences ($p < 0.01$).

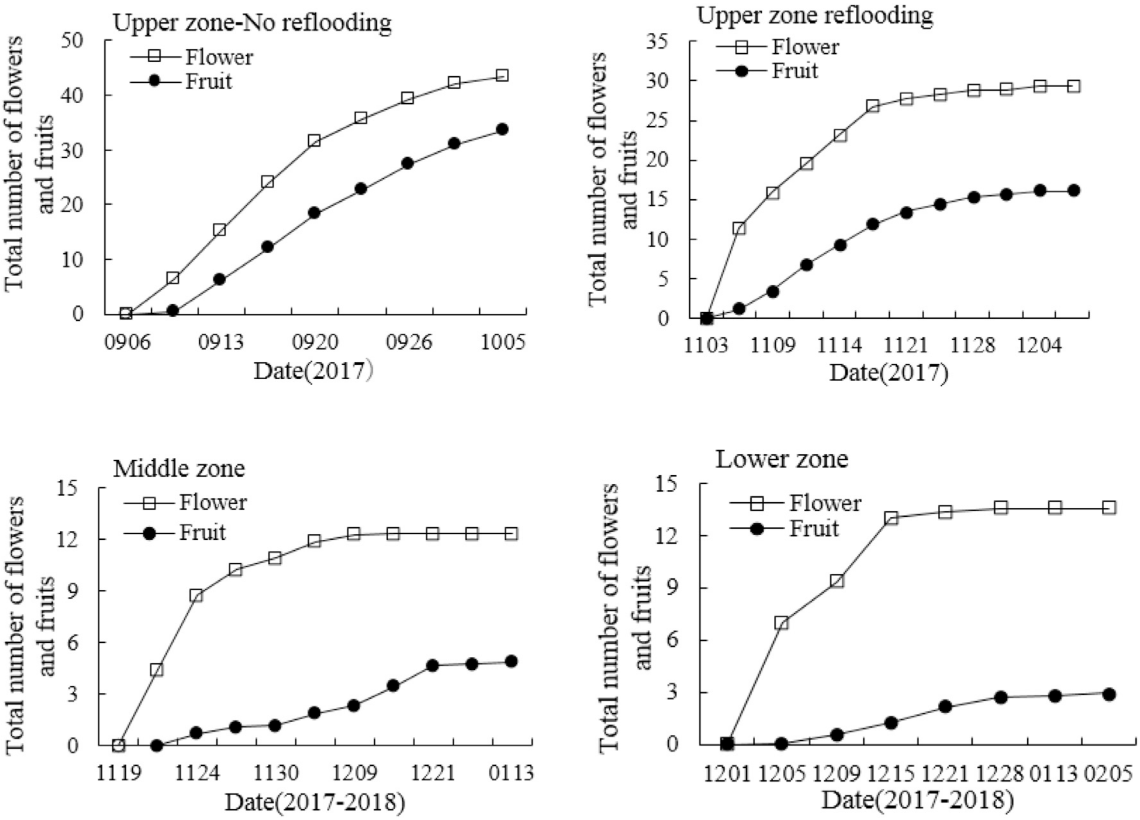

Fig. 4. Number of single inflorescences that generated flowers and fruits at different water level gradients.

Table 2
Seed production characteristics of *M. laxiflora* across a water level gradient.

Water level gradient		Seed number per fruit	Thousand seed weight (mg)	Seed vitality (%)
Upper region	No reflooding	89.4 ± 3.2 ab	160.2 ± 17.4 ab	45.9 ± 3.4b
	Reflooding	96.0 ± 4.4a	181.8 ± 6.4a	65.5 ± 3.6a
	Total	91.9 ± 3.1A	171.02 ± 9.7A	55.7 ± 5.0A
Middle region		85.7 ± 3.5AB	125.6 ± 10.6A	19.8 ± 1.2B
Lower region		82.9 ± 3.3B	130.0 ± 20.5A	20.2 ± 1.2B

Note: Different lowercase letters in the same column indicate significant differences between the No reflooding and Reflooding areas of the upper flooding region ($p < 0.05$); different capital letters indicate significant differences in the upper, middle, and lower regions ($p < 0.05$).

region of the water level fluctuation zone. The number of seeds per fruit, the 1,000-seed weight, and seed vigor of plants from the upper region were 7.3% and 10.9%, 36.2% and 31.6%, and 181.6% and 176.1% higher than those in the middle and lower regions, respectively. The plants in the upper region underwent reflooding during the flowering and fruiting periods. No significant differences between the number of seeds per fruit and 1,000-seed weight of plants with and without experiencing reflooding were observed, but seed vigor in these two conditions significantly differed ($P < 0.05$) because the seed vigor of plants that experienced reflooding was 42.6% higher than those that did not.

3.4. Correlation between flowering and fruit setting of remnant M. laxiflora plants and aquatic ecological features

Analysis using the Pearson correlation between the number of flowers, the number of fruit setting, flowering period, fruiting period, the number of seeds per fruit and seed vigor of *M. laxiflora* indicated a significant correlation between flowering, fruit setting, and seed development ($P < 0.01$) (Table 3). The number of flowers, the number of fruit setting, the number of seeds per fruit, flowering period and fruiting period was also significantly positively correlated with soil water content, the duration of exposure and water level fluctuation gradient ($P < 0.01$), respectively, suggesting that the flowering, fruit setting, and seed development of *M. laxiflora* plants were influenced by water level fluctuations.

Table 3
Pearson correlation coefficient of the number of flowers and fruits, flowering period, and fruiting period in relation to water level fluctuations.

Variable	Number of flowers	Number of fruits	Flowering time	Fruiting time	Number of seeds	Seed vitality	Soil water content	Duration of exposure	Water level gradient
Number of flowers	1	0.92**	0.85**	0.70**	0.43*	0.87**	0.50**	0.78**	0.83**
Number of fruits	0.92**	1	0.87**	0.75**	0.44*	0.84**	0.61**	0.86**	0.89**
Flowering time	0.85**	0.87**	1	0.73**	0.34	0.79**	0.60**	0.79**	0.82**
Fruiting time	0.70**	0.75**	0.73**	1	0.48**	0.70**	0.54**	0.67**	0.70**
Number of seeds	0.43*	0.44*	0.34	0.48**	1	0.42*	0.54**	0.39*	0.41*
Seed vitality	0.87**	0.84**	0.79**	0.70**	0.42*	1	0.57**	0.76**	0.80**
Soil water content	0.50**	0.61**	0.60**	0.54**	0.54**	0.57**	1	——	0.73**
Exposuring time	0.78**	0.86**	0.79**	0.67**	0.39*	0.76**	——	1	——
Water level gradient	0.83**	0.89**	0.82**	0.70**	0.41*	0.80**	0.73**	——	1

Note: **indicates a significant correlation at the 0.01 level (bilateral).

4. Discussion

4.1. Responses in sexual reproduction phonology of remnant M. laxiflora to water level fluctuations

Sexual reproduction is one of the periods when plants are the most sensitive to external environmental conditions during their life cycle (Mo et al., 2017). The sexual reproduction of terrestrial plants is primarily influenced by variations in air temperature and illumination (McBride et al., 2014), whereas sexual reproduction of riparian plants often depends on the timing of decreased river water levels in autumn (Satake et al., 2001; Castillo and Figueroa, 2009). Water level fluctuations are an important ecological process that influences the function of riparian ecosystem (Tonkin et al., 2018). Riparian plants are usually flooded in the summer, for which they start growing and sexually reproducing as water levels decrease in autumn (Blom et al., 1990; Jun and Kanodo, 2004). Previous studies on riparian plants distributed in the same region, such as *Salix variegate* and *Buxus ichangensis*, indicated that these plants remained in a dormant state during the flood season in the summer and started sexual reproduction after the flood receded (Chen et al., 2008; Xue et al., 2007). The flooding time and duration in summer directly determine the time and duration of riparian plant growth, flowing, and fruit setting after their emergence. Therefore, water level fluctuation patterns play a decisive role in the sexual reproduction of riparian plants.

Differences in the duration of exposure of riparian plants at various water level fluctuation gradients result in spatial variations in sexual reproduction (Chen and Wang, 2015; Kawano et al., 2010). The plants distributed in the upper region of the water level fluctuation gradient underwent a shorter duration of submergence in the summer, for which these plants are exposed and bloom early and their flowering and fruiting periods last for a longer period of time. However, plants distributed in the middle and lower regions of the gradient are submerged in water for a longer duration, which meant that these plants were exposed late and bloomed late. With poor climatic conditions after exposure, their flowering and fruiting periods are shorter than those in the upper region (Min, 2014; Su et al., 2010). When studying the responses in the phenology of flowering and fruit settings of *Melaleuca ericifolia* and *Xanthium sibiricum*, Salter et al. (2010) and Liu et al. (2017) found the spatial variations involving riparian plants during sexual reproduction. The sexual reproduction of remnant *M. laxiflora* was also directly influenced by water level fluctuations. *M. laxiflora* plants underwent sexual reproduction following short-term vegetative growth with the recession of water levels and the exposure of the riparian zone after surviving a long period of submergence that lasted from June to September every year in the dormant state. Due to differences in exposure time at different altitudinal gradients, the duration and timing of flowering and fruiting of plants may vary. Plants at the higher-altitude gradient are exposed the earliest and bloomed first, with the longest flowering period (47 d) and fruiting period (50 d). The flowering period and fruiting period of plants at middle and lower altitudes lasted for about 14 d and 24 d, respectively.

4.2. Quantitative responses of flowering and fruit setting of remnant M. laxiflora to water level fluctuations

Plant reproductive organs are highly sensitive to changes in the external environmental, often resulting in alterations in plant flowering and fruit setting (Zhang and Yang, 2001). Water level fluctuations also lead to spatial variations in the number of flowers and fruits of plants (Salter et al., 2010; Liu et al., 2017). Zeng et al. (2006) found that the sexual reproduction characteristics of *Arundinella hirta* exposed early at the upper region of the riparian zone such as the number of seeds per plant and the total weight of the seeds were superior to those in lower regions. They argued that it was the excessive time of submergence at a low altitude and a short time of air exposure that hampered reproductive growth and seed generation. A significant positive correlation between the number of flowering branches and the number of inflorescences and the duration of the flowering period has also been reported (Su et al., 2010; Jun and Kadono, 2004). Plants of the upper region of the water level fluctuation zone emerge and blossom early, their inflorescences and flowers develop fully as these have a higher seed setting rate, whereas plants of the lower region emerge and blossoms relatively late, large numbers of flower buds would fail to bloom normally at a low temperature, which in turn impacted the fruit setting (Fang et al., 2004; Gesti et al., 2005). Changes in riparian soil water content caused by water level fluctuations were also considered as an important factor leading to spatial variations in the number of flowers and fruits (Haukos and Smith, 2006; Mo et al., 2017). In this study, the sexual reproduction characteristics of remnant *M. laxiflora* in the upper region, such as the number of flowering branches, inflorescences, and seeds per fruit, were also far superior to those in the middle and lower regions indicating that water level fluctuations induce spatial variations in the number of sexual reproductive organs of remnant *M. laxiflora* plants.

Cho et al. (2018) argued that a high level of nutritional reserve within the plant would significantly boost the growth recover and the signal release related to blossoming, which in turn promotes flowering and fruit setting. Earlier and longer exposure would leave more time and a more suitable climate for plants to photosynthesize and accumulate nutrients, thereby facilitating their sexual reproduction (Xue et al., 2007; Robertson et al., 2001). In addition, drought often occurs in water level fluctuation zones when water levels receed in the autumn and winter, which in turn induces variation in the photosynthetic physiology of plants, leading to a decline in photosynthesis and nutrition (Mo et al., 2017). Thus, there would be undesirable changes in plant sexual reproduction, such as a decrease in the number of flowers, shortened inflorescence length, more abscission of flowers and fruits, lower fruit set percentage, and smaller seed weight (Lambrecht et al., 2017; Giayetto and Cerioni, 2002). The difference in the timing and duration of exposure and soil water content in the water level fluctuation zone at different fluctuation gradients could cause alterations in plant photosynthesis and nutrition within an altitude gradient, further leading to spatial variations in sexual reproduction (Chen et al., 2008; Darnell et al., 2015). The timing and duration of exposure of the habitat of remnant *M. laxiflora*, groundwater levels, and soil water content varied across the altitudinal gradient. The upper region of the water level fluctuation zone was exposed early and showed a longer duration; the soil of this region was fine with favorable water conservation effects and high water content. However, the middle and lower regions were exposed late and showed a shorter duration; the soil of these regions was coarse, indicating poor water conservation and low water content. Therefore, the plants in the upper region of the water level fluctuation zone in the habitat of remnnat *M. laxiflora* enjoyed the longest flowering period, better photosynthetic performance, and higher reserves of carbohydrates and sugars (Chen and Wang, 2015), which are also reasons why the flowering and fruiting of plants at this water level fluctuation gradient were higher than those in the middle and lower regions.

4.3. Effects of regulated water level fluctuation patterns on flowering and fruit setting of remnant M. laxiflora

Water level fluctuations are crucial ecological processes that influence the flowering and fruiting of riparian plants (Zeng et al., 2006). The variations in the timing and duration of exposure and soil water content at different water level fluctuation gradients caused by water level fluctuation directly act upon the phenology and quantity of sexual reproduction of riparian plant (Choo et al., 2014; Greet et al., 2013). In addition, these variations also alter plant photosynthesis and growth and indirectly influence sexual reproduction (Haukos and Smith, 2006). Therefore, there is strong association between the sexual reproduction of plants and variations in the aforementioned water level-induced factors (Fang et al., 2004; Kawano et al., 2010). The present study suggested an extremely significant correlation between the number of flowers and the number of fruit settings, the flowering period, and the fruiting period of *M. laxiflora*, as was the correlation of these traits of *M. laxiflora* to the variations of exposure duration, water level fluctuation gradient and soil water content caused by water level fluctuations. Therefore, the sexual reproduction of remnant *M. laxiflora* was notably subject to the influence of water level fluctuation patterns.

The growth and sexual reproduction of riparian plants generally adapt to a certain range of river water level fluctuations (Bovee and Scott, 2010; Campbell et al., 2016). However, when there is a dramatic change in the water level fluctuation pattern of the river, sexual reproduction of riparian plants suffers and population regeneration is hampered (Crosby et al., 2015). According to Bijarchi et al. (2011), the dams had reduced downstream peak discharge, intensified the drawdown rate of water level after the peak, and impeded the ecological demand for water of *Populus trichocarpa*, which led to a significant decline in its sexual reproduction rate.

Before the construction of the Three Gorges-Gezhou Dam hydropower projects, *M. laxiflora* was submerged by floods from June to September each year. As the water level receded in early September, *M. laxiflora* plants would start blooming and bear fruits within about one week after exposure. However, the construction of these dams has resulted in abrupt changes in the water level fluctuation patterns of the Yangtze River and its aquatic ecological environment. (1) The timing of exposure of the water level fluctuation zone is delayed. The timing of exposure of the whole fluctuation zone is concentrated in early to mid-November. The original fluctuation zone was exposed in late August, which meant the timing of exposure was delayed for about two months after the construction of the dams (Chen and Wang, 2015). (2) Water level recession is accelerated; the lowest water level significantly decreased. The fluctuation zone is exposed from early November and fully exposed around November 20, but its original recession usually lasted more than one month, which meant a higher water level recession rate. In addition, the riverbed had been significantly lowered due to sediment interception and clean water discharge from the upstream reservoir (Duan et al., 2016). These variations in water level fluctuation patterns caused a delay in exposure time, a decrease in exposure duration, and reduced groundwater levels and soil water content in the habitat of *M. laxiflora*. This study indicated that sexual reproduction of this species is significantly correlated with water level fluctuations and the aquatic ecological environment. Changes in the water level fluctuation pattern will inevitably affect the sexual reproduction of this species. To be specific, the delay in the timing of water level recession and the timing of exposure of the fluctuation zone delays and shortens the flowering and fruiting periods of remnant *M. laxiflora*. Accelerated water level recession and the significant decline of the minimum water level lead to drought, which impacts sexual reproduction. Moreover, prolonged submergence and an abrupt drop in soil water content caused by variations in water level fluctuation patterns also influence the photosynthetic physiology and nutrition storage of plants, which indirectly undermines the adaptability to submergence and sexual reproduction of plants in the following year. Taking the example of fruit set percentage alone, the fruit set percentages of remnant *M. laxiflora* in the upper, middle, and lower regions of the water level fluctuation zone were 71.1%, 44.1%, and 24.3%, respectively. In comparison, the fruit set percentage of the natural *M. laxiflora* population in the reservoir area was 95.1% before the Three Gorges Dam was built (Chen and Wang, 2015). This suggested that there was a sharp decline in the fruit set percentage of *M. laxiflora* after the dam was built.

Currently, remnant *M. laxiflora* plants can only be found in a small population in the Yangtze River in Yichang and Yibin downstream of the Gezhou and Xiangjia Dams (Chen and Wang, 2015). Water level regulation for purposes of flood prevention and power generation has delayed the timing of exposure of the habitat of residual *M. laxiflora* and reduced the soil water content, which has severely disrupted the sexual reproduction of remnant *M. laxiflora*. Population regeneration and development are further affected by the decline in sexual reproduction success. To protect this endangered species, the original water level fluctuation patterns should be restored. First, it is advisable to appropriately move the timing of reservoir interception earlier in the year and reduce the interception rate by changing the river regulation schedules of large-scale hydropower stations. This way the timing of exposure the habitat of residual *M. laxiflor* is earlier, and rate of water level recession is slower. Second, a small dam could be built in the habitat of remnant *M. laxiflora* to control the timing and rate of water level recession within a small range.

5. Conclusions

In this study, the sexual reproduction of remnant *M. laxiflora* across a water level fluctuation gradient was measured, which indicated significant differences in the average number of flowering branches, number of inflorescences per branch, number of flowers, number of inflorescences, number of fruit settings, seed vigor, and 1,000-seed weight. With the reduction in the fluctuation gradient, the sexual reproduction indicators of the aforementioned plant significantly decreased and exhibited spatial differences. Considering that different fluctuation gradients are influenced by water level fluctuations, there are significant differences in the timing and duration of exposure and soil water content. Therefore, the spatial difference in the sexual reproduction of remnant *M. laxiflora* is actually a spatiotemporal response to the water level fluctuations of the river. The flowering and fruiting periods of *M. laxiflora* in the lower region of the water level fluctuation gradient are shorter than those in the upper region because the timing and duration of exposure of the lower region is delayed and shorter than those of the upper region. In addition, the soil water content of the lower region is lower than that of the upper region, which may also influence the spatial changes in the sexual reproduction of *M. laxiflora.* Correlation analysis suggests that the key indicators of the sexual reproduction of this species are significantly correlated with the timing and duration of exposure, soil water content, and water level fluctuation gradient of the habitat. Therefore, the regulation of fluctuation pattern has a significant impact on the sexual reproduction of remnant *M. laxiflora* populations. The construction of the Three Gorges-Gezhou Dam for water conservancy and hydropower projects has changed the water level fluctuation pattern of the habitat of *M. laxiflora*, which has delayed and reduced the timing and duration of habitat exposure and decreased the soil water content. Such changes have negatively influenced the sexual reproduction of *M. laxiflora*. Our findings have proven our hypothesis that changes in the downstream water level fluctuation patterns and the aquatic ecological environment of the habitat of *M. laxiflora* caused by large-scale hydropower projects have impacted its sexual reproduction and population regeneration to some extent.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2019.e00628.

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