



The contribution of seeds to the recruitment of a *Nymphoides peltata* population

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ARTICLE INFO

Article history:

Received 18 June 2012

Received in revised form 7 January 2013

Accepted 6 May 2013

Available online 24 July 2013

Keywords:

Nymphoides peltata

Seed germination

Seed buoyancy

Seedling development

Seedling re-establishment

ABSTRACT

The *Nymphoides peltata* population expands quickly in Lake Taihu, China. One question addressed in this study is whether the seeds of *N. peltata* contribute to this expansion. The buoyancy and germination of *N. peltata* seeds and the development of *N. peltata* seedlings were studied, using seeds collected from Lake Taihu. The results indicated that a low wind velocity of $2.4\text{--}3.0\text{ m s}^{-1}$ had a slightly negative effect on seed buoyancy. After 19 and 67 h of gentle stirring, 50% and 90%, respectively, of the *N. peltata* seeds had sunk. Few seeds floated again after sinking, but these refloating seeds sank soon with the disturbance. The *N. peltata* seeds did not germinate without stratification, but the stratification of seeds for a two-week period resulted in a high germination rate (63.3%) at a light intensity of $20\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$. Both the light and stratification treatments stimulated the seeds germination. The seeds did not germinate in sediment at depths greater than 0.25 cm. A high germination rate (74%) was observed for the seeds that laid on the water–sediment interface; however, nearly all of the germinated seeds floated on the water surface after germination. Only a small fraction (14%) of the buoyant seedlings could re-establish in shallow water (less than 3 cm). In the eighth week of the experiments, the buoyant seedlings that failed to re-establish rotted. When grown in low light intensity conditions, the *N. peltata* seedlings had smaller cotyledons, shorter primary roots, and weak development of adventitious roots. Sufficient light was important for both seed germination and seedling development. It was found that sexual reproduction is likely to have little direct contribution to the rapid expansion of *N. peltata* towards the centre of this large shallow lake.

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Introduction

Nymphoides peltata (Menyanthaceae) is a wide-spread aquatic species that occurs in a wide range of climates and habitats, especially in temperate regions of the northern hemisphere (Li et al. 2010). In the past few decades, the distribution of *N. peltata* has drastically changed. This species is now listed as ‘vulnerable’ in the national Red Data Book of Japan (Ministry of the Environment, Japan 2002). In contrast, *N. peltata* is an invasive species of North America and New Zealand (Champion and Clayton 2003; Darbyshire and Francis 2008), and it is considered a nuisance in these areas. In China, this native species is expanding rapidly in waterways and lakes, such as Lake Taihu, in Jiangsu Province. *N. peltata* often grows in dense patches and competes with other species. This growth habit leads to stagnant areas with low oxygen levels (Caraco et al. 2006) and threatens commercial shipping and recreational vessels. Therefore, a study of the establishment and propagule dispersal of *N. peltata* is needed.

Lake Taihu is a shallow lake with a surface area of 2338 km² and a mean depth of 1.9 m. In the 1950s, the lake was oligotrophic;

however, since then, the water quality has decreased, and the lake is now eutrophic. Blue-green algae dominate the west basin of Lake Taihu, whilst the east basin is generally covered by vascular plants (Rose et al. 2004; Qin et al. 2007; Ye et al. 2007). Multi-temporal satellite imagery reveals that Lake Taihu area is dominated by floating-leaved macrophytes; the population of hydrophytes has been increasing rapidly since 2001 (Ma et al. 2008). According to a field survey conducted in the summer of 2010, the floating-leaved macrophytes of Lake Taihu were dominated by *N. peltata* plants.

The *N. peltata* plants colonise by vegetative means (i.e., by vegetative fragments) and by germination of seeds (Cook 1990). The structure of *N. peltata* seeds and the biology of its seed dispersal have been described in detail by van der Velde and van der Heijden (1981), Smits et al. (1989), and Cook (1990). The results reported by these authors are in agreement, but some aspects have not been addressed. For instance, quantitative description of seed buoyancy is lacking. Danvind and Nilsson (1997) proposed a method which can be used for assessing quantitatively the seed buoyancy of *N. peltata*. Furthermore, seed germination is a vital step in the process of population expansion. Takagawa et al. (2005) reported that safe-sites for *N. peltata* seeds germination were those less prone to inundation on bare ground exposed to sufficient light during the spring water-level drawdown. However, seeds located far from the shoreline might be faced with different conditions, such as

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low light intensity and high sediment deposition rate. Successful seedling establishment could lead to the recruitment of *N. peltata* population and contribute to its rapid expansion. In the centre of large, shallow, eutrophic lake, seeds are usually buried by sediment and exposed to low light intensity. However, seed germination and seedling development responses to these environmental stresses have seldom been considered, but are important for refining models of population recruitment and expansion.

In this paper, we hypothesise that seeds are a major contributor to the recruitment and rapid expansion of the *N. peltata* population. The successful recruitment of *N. peltata* from seeds in population expansion depends on three main characteristics: (1) long-distance seed dispersal capacity; (2) successful seed germination; and (3) survivorship of seedlings (establishment and development). Therefore, the aims of this paper are focused on examining these three characteristics to determine whether sexual reproduction plays an important role in the expansion of *N. peltata* populations in the centre of Lake Taihu. A detailed understanding of the reproduction and dispersal patterns will lead to the development of better models of vegetation dynamics and more predictable management results.

Materials and methods

Seed collection and storage

In October 2011, more than 950 ripe fruits were collected from several localities in a *N. peltata* stand of eastern Lake Taihu (31.112° N, 120.366° E). The fruits were stored in an open, plastic container filled with tap water (changed weekly) at room temperature (approximately 20 °C). The fruits were collected randomly from different colonies to minimise the bias for specific plants. In the laboratory, the seeds were released from the fruits naturally. According to Smits et al. (1989), when the fruits become detached from the mother plants at maturity in nature, the seeds within the fruits are stacked upon one another in a fashion analogous to piles of coins. When the stacked seeds are disturbed, they slide away from one another and rest on the water surface. The seeds used for our experiments are randomly selected from 100 fruits, and pre-treatment of seeds were described in every experiment below.

Seed buoyancy

For each experiment, 50 seeds were released from the 5 random fruits and immediately put into a glass beaker (1.5-L) filled with 1-L tap water (four replicates), gently stirred for 5 s, and then the number of floating seeds was counted. The number of floating seeds was counted every 4 min during the first hour, every hour for the next 7 h, thereafter every 12 h for another 15 days (Boedeltje et al. 2003). The beakers were placed in an unheated room in natural daylight. Before each count, a beaker was stirred for 5 s to reduce the influence of water surface tension (Danvind and Nilsson 1997). The effect of stirring beaker was similar to the action of flapping of leaves and wave. The time periods after which 50% (t_{50}) and 90% (t_{90}) of the seeds had sunk were recorded.

In a different treatment, the effect of wind of the seed buoyancy was assessed. The wind was simulated using an electric fan (2.4–3.0 m s⁻¹, AN400 Wind Anemometer, Extech Instruments, New Hampshire, USA) per container. Each experiment utilised 100 seeds in plastic containers (45 cm × 40 cm × 20 cm), with a water depth of 15 cm. Seed buoyancy was monitored for 2 months.

The control was 100 seeds that were left floating on the water surface in plastic containers without any treatment. This

experiment was performed for 3 months from October 10, 2011 to January 10, 2012 in triplicate.

Seed germination

The three factors studied were the following: light intensity, stratification (here defined as storage in demineralised water at 4 °C), and seed depth in sediment. The experimental design consisted of the two following treatments:

- (a) *Light intensity and stratification interaction*: After being stratified for 0, 1, 2, 3, or 4 weeks, the seeds were incubated in a petri dish and submerged by fresh tap water to a depth of approximately 0.2 cm. Seeds length and width were 2.37 ± 0.13 , and 4.19 ± 0.29 mm ($n = 389$, mean \pm S.D.), respectively. All the seeds used were submerged in 0.2 cm of the water. The light was provided by a fluorescent white tube for a photoperiod of 14 h. Each treatment was performed in the following three light regimes: 0, 5, and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The maximum light intensity of the light gradient in the area slightly above the sediment of the *N. peltata* stand in Lake Taihu in May was similar to the light intensity used in the laboratory experiments (LI-189, LI-COR, Inc., Lincoln, NE, USA). Batches of 30 seeds were used in each Petri dish for each experiment, and the experiments were performed in triplicate at 25 °C in a climate-controlled room. The experiment lasted four weeks. During the period, the water was replenished, and the germinated seeds were counted and removed every other day.
- (b) *Light intensity and seed depth interaction*: The seeds that were stratified for 4 weeks were used in the experiments. The substrates were collected from the *N. peltata* stand in Lake Taihu (31.112° N, 120.366° E). The average size of the particles in suspension was close to $9.5 \pm 0.0 \mu\text{m}$ ($n = 3$, mean \pm S.D.), measured by a laser diffraction instrument (Mastersize 2000, Malvern, UK). A stable weight of $4.5 \pm 0.3\%$ ($n = 3$, mean \pm S.D.) was obtained by performing loss on ignition to estimate sediment organic matter at 550 °C for 4 h. The concentration of total nitrogen and total phosphorus were 1.24 ± 0.09 , and $0.33 \pm 0.02 \text{ g kg}^{-1}$ ($n = 3$, mean \pm S.D.), respectively. The sediment was passed through a 1.5-mm sieve to remove the *N. peltata* seeds and then stirred in a mixer. The substrates were collected in 1-L black cylindrical polyethylene containers or 1-L rectangular glass containers that were filled to a height of 15 cm. Seed germination was examined using the two following experimental designs: (I) The seeds were horizontally sown (cotyledons emerged parallel to the water–sediment interface) at depths of 0, 0.25, 0.5, 1.0, and 1.5 cm in the black polyethylene containers. Each depth treatment was placed in a new container. The light was provided at the top of the containers by a fluorescent white tube at an intensity of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; (II) The seeds were vertically sown (cotyledons emerged perpendicular to the water–sediment interface) along the wall of the rectangular glass containers at depths of 0, 0.25, 0.5, 1.0, and 1.5 cm with reference to the longitudinal axis of the seeds. The light was provided by a laterally positioned, fluorescent white tube at an intensity of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. This approach allowed us to determine the effect of light on seed germination at different depths. All containers were placed in continuously circulating water at 25 °C, and performed in triplicate. For each experiment, 30 seeds were planted in each container; the water was replaced with aerated tap water once a week. If the radicle protruded at least 1 mm from the seed, the seed was scored as germinated.

To determine the effect of the oxygen concentration on the seed germination rate of seeds sown in sediment at different depths, we used an oxygen microoptode with a tip diameter of less than 0.1 mm (Presens PSt1, Munich, Germany) and determined the oxygen concentration profile of the sediment (Glud et al. 1996) at the beginning of the experiment.

Seedling development and re-establishment

The seeds, stratified for 4 weeks, were incubated in a climate-controlled room. The seeds that had a 2 mm radicle protruding from them were used for two main experiments. In Experiment 1, the germinated seeds were incubated in a beaker that was filled with fresh tap water to a depth of approximately 0.3 cm. The light for the experiment was provided by a fluorescent white tube for a photoperiod of 14 h, and each treatment was tested using the following three light regimes: 0, 5, and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Batches of 10 seeds were used for each treatment. All of these experiments were conducted at 25 °C in a climate-controlled room for a period of 2 weeks. The water was replaced every other day. After the seedlings were harvested, the WinRHIZO programme (WinRhizo Pro 2007, Régent Instruments, Québec, Canada) was used to analyse root morphology.

Preliminary seed germination experiments suggested that nearly all seedlings floated in the water, and floating seedlings would be transported to other water area. In Experiment 2, we performed an experiment to ascertain the re-establishment of seedlings that floated. We filled cylindrical containers (60 cm in height) with sediment to a substrate depth gradient of 60, 57, 50, and 10 cm, and then filled them with water to a water depth gradient of 0, 3, 10, and 50 cm, respectively. Batches of 30 seeds were used in each treatment in each container, and experiments were performed in triplicate. All containers were placed in continuously circulating water at 25 °C and exposed to a light intensity of 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The water was replaced weekly with aerated tap water. The experiments were conducted until all floating seedlings settled or decayed. Seedling root in the sediment being greater than 1 cm and sprouting a new leaf after cotyledons was defined as the successful establishment.

Data analysis

Considering nonlinearities, we first estimated a general additive model in which the effects of elapsed time on the buoyancy of *N. peltata* seeds were fitted in a nonparametric regression procedure, Loess. This method can capture the underlying patterns of the high variable data set. The loess procedure does not estimate a specific function linking elapsed time to seed buoyancy, so we used a generalised linear model to predict the time point at which 50% and 90% of the seeds had sunk. All of these analyses were performed using the SAS package (SAS 9.1, SAS Institute, Cary, NC, USA).

The germination data are binomial because they are not linear or additive. Hence, before performing the comparison of means or regression analysis, binomial data were transformed into linear data. For the response variable 'germination', we performed a generalised linear model analysis with a logit link function (McCullagh and Nelder 1989) using the SAS-procedure 'Logistic' (SAS 9.1, SAS Institute, Cary, NC, USA). Experimental parameters (e.g., stratification and light intensity) were included in the model as explanatory variables (i.e., factors).

The effects of various light conditions and water depths on seedling development and reestablishment, respectively, were compared using a one-way ANOVA analysis. For significant ANOVA results, Tukey tests were used for multiple comparisons. In all cases, the significance level was set at 5%.

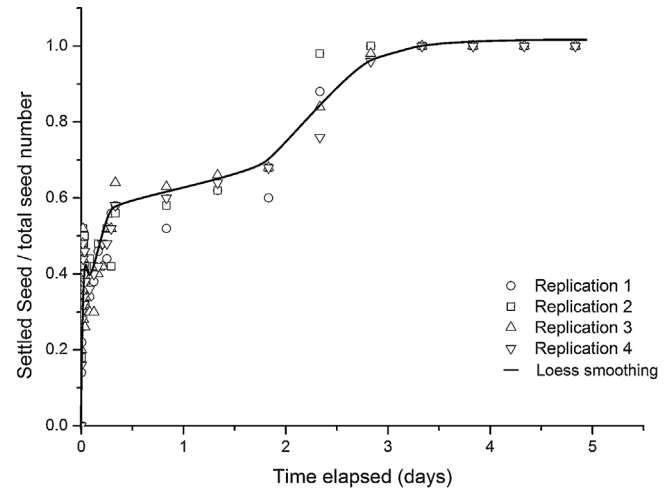


Fig. 1. The buoyancy of *Nymphoides peltata* seeds that were gently stirred to reduce the influence of water surface tension. The fitting plot for seed buoyancy using the SAS Loess Procedure. The Loess smoothing parameter is 0.15, and 19 points are used in the local neighbourhood.

Results

Seed buoyancy

If the seeds were allowed to float undisturbed on the water surface, none of the *N. peltata* seeds sank for 3 month during our experiment. Only $3.7 \pm 0.0\%$ of the *N. peltata* seeds sank in response to wind treatments. However, when these seeds were stirred, they sank rapidly. The *N. peltata* seeds that sank were able to float again during the 100 min after the seeds were freshly released from the capsules and first set onto the water surface. Loess smoothing analysis ($r^2 = 0.8119$) also revealed this phenomenon (Fig. 1); however, this seed buoyancy only lasted for short period of time. After this period, a few *N. peltata* seeds began floating again 100–3000 min after the end of the stirring treatment, but no seeds were observed to resurface more than 3000 min after. We calculated the seed buoyancy with a generalised linear model to be the following: $t_{50} \approx 0.8$ days and $t_{90} \approx 2.8$ days.

Seed germination

The seeds of *N. peltata* showed a rapid, uniform germination pattern. No seeds were observed to germinate before the fourth day of the experiment. Both of these results were found irrespective of the stratification and light conditions (Fig. 2). The germination rate of *N. peltata* seeds increased with the duration of stratification (Table 1; regression coefficient in the logistic model, $\beta_{\text{stratification}}$). No seeds were observed to germinate without stratification under the three light regimes. At a light intensity of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the percentage of *N. peltata* seeds that germinated increased gradually during one week of stratification, then increased sharply after

Table 1

The results of the Logistic regression analysis of the germination of *Nymphoides peltata* seeds.

Independent variables	Dependent variables		
	β	SE	p value
Light intensity	0.05	0.02	<0.05
Stratification	0.85	0.10	<0.05
Light intensity * stratification interaction	0.02	0.01	<0.05

β = regression coefficient; SE = standard error of β ; p = level of significance.

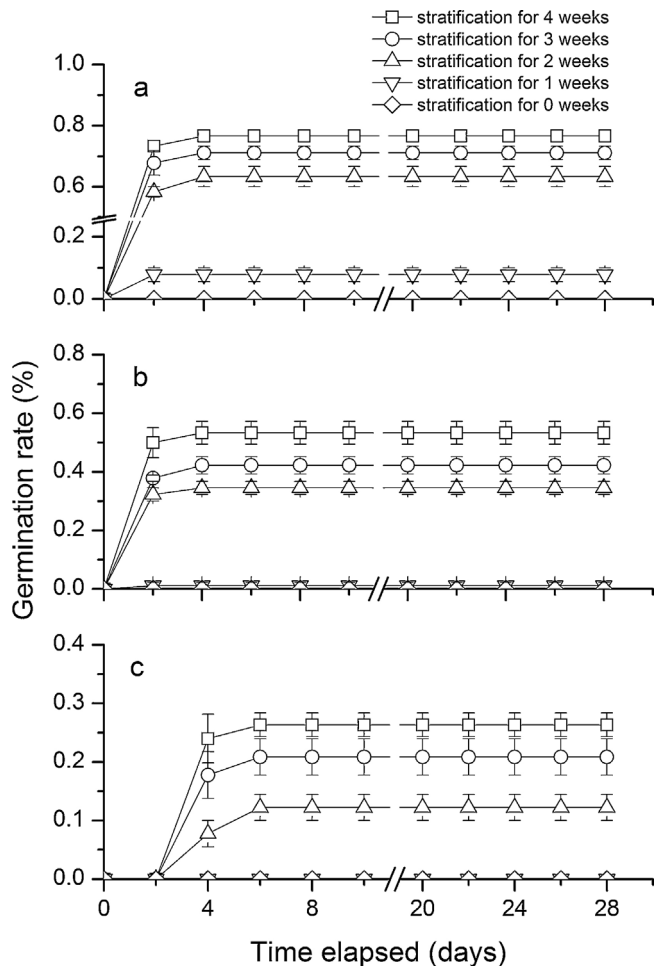


Fig. 2. The germination of seeds (mean \pm S.E.) subjected to different light intensities: (a) 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, (b) 5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, and (c) 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

2 weeks of stratification, and increased slowly thereafter. After 3 weeks and 4 weeks of stratification, the germination percentages were similar ($p=0.13$). The response of *N. peltata* seeds to stratification, which was observed as the percentage of germinated seeds, showed the same pattern at a light intensity of 0 and 5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

The light treatment stimulated the germination of *N. peltata* seeds ($\beta_{\text{light intensity}}$, $p<0.05$) (Table 1). When the seeds were exposed to the same period of stratification, the percentage of germinated *N. peltata* seeds increased with the light intensity. The percentage of germinated *N. peltata* seeds was greatest ($43.8 \pm 8.8\%$, mean \pm S.E.) for the 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ treatment and lowest ($11.9 \pm 3.0\%$, mean \pm S.E.) for the 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ treatment. The effect of light was significant for a given stratification treatment ($\beta_{\text{stratification} \times \text{light intensity}}$, $p<0.05$).

Five *N. peltata* samples were used for oxygen profile measurements. Because of sediment homogenisation, the data deviation was minor (Fig. 3). The results showed that oxygen concentrations decreased rapidly as soil depth increased; these concentrations approached hypoxic ($<2.0 \text{ mg L}^{-1}$) conditions at the 0.28 cm depth and anoxic ($<0.5 \text{ mg L}^{-1}$) conditions at the 0.38 cm depth.

When the seeds of *N. peltata* plants were sown horizontally in black polyethylene containers, $74.4 \pm 5.1\%$ (mean \pm S.E.) of these germinated at the 0 cm depth. In contrast, $36.7 \pm 6.7\%$ (mean \pm S.E.) of the *N. peltata* seeds that were vertically sown along the wall

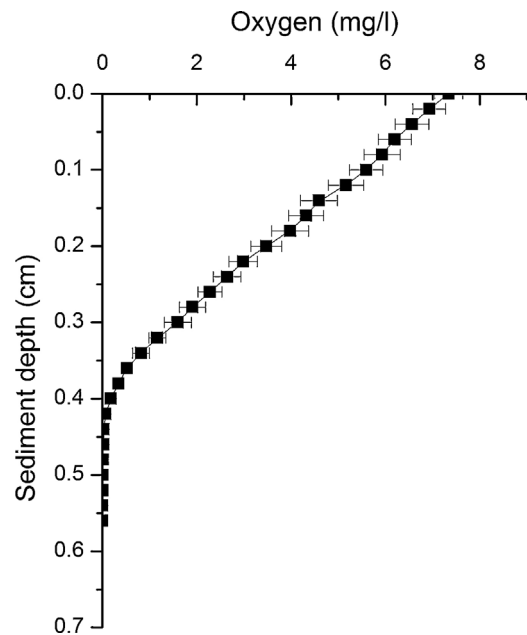


Fig. 3. The oxygen concentration (mean \pm S.E.) profile in sediment that was incubated at 25 $^{\circ}\text{C}$. $n=10$.

of glass containers at a 0 cm depth germinated. No germination was observed for the *N. peltata* seeds that were sown at depths of 0.25 cm or greater, where the oxygen concentration was less than 2.65 mg L^{-1} (Fig. 3). However, all *N. peltata* seedlings floated on the water surface within 4 days after germination, although a higher germination rate was observed for the seeds that were sown horizontally on the surface of the sediment. All seedlings successfully established when the seeds were sown vertically on the surface of the sediment.

Seedling development and re-establishment

The lengths, widths and areas of *N. peltata* cotyledons increased with increases in the light intensity ($p<0.05$) (Fig. 4). All of the cotyledons that were exposed to light were green, whereas the *N. peltata* hypocotyls that were grown under dark conditions were yellow. When the light intensity increased from 0 to 5 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the cotyledon area per seedling increased from 0.04 ± 0.02 to $0.15 \pm 0.05 \text{ cm}^2$ (mean \pm S.D., $p<0.05$), while the hypocotyl length per seedling decreased from 6.13 ± 1.32 to $1.13 \pm 0.21 \text{ cm}$ (mean \pm S.D., $p<0.05$). The hypocotyl lengths of *N. peltata* seedlings were similar for the light intensities between 5 and 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. When the light intensity was increased from 0 to 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the primary root lengths of *N. peltata* seedlings increased from 2.61 ± 0.73 to $4.53 \pm 0.83 \text{ cm}$ (mean \pm S.D., $p<0.05$), whereas the average diameters of these primary roots were similar. The length and average diameter of *N. peltata* adventitious roots increased with the light intensity in a similar manner as was observed for the primary root length (Fig. 4). Furthermore, some *N. peltata* seedlings did not develop adventitious roots (data not shown).

To test for the establishment of *N. peltata* seedlings that floated, an eight-week experiment was conducted. At the water depth of 0 cm, 100% of the seedlings were established; in contrast, $14.4 \pm 5.1\%$ (mean \pm S.E.) of the seedlings were established at the water depth of 3 cm. All of the floating seedlings decayed at the water depths of 10 cm and 50 cm.

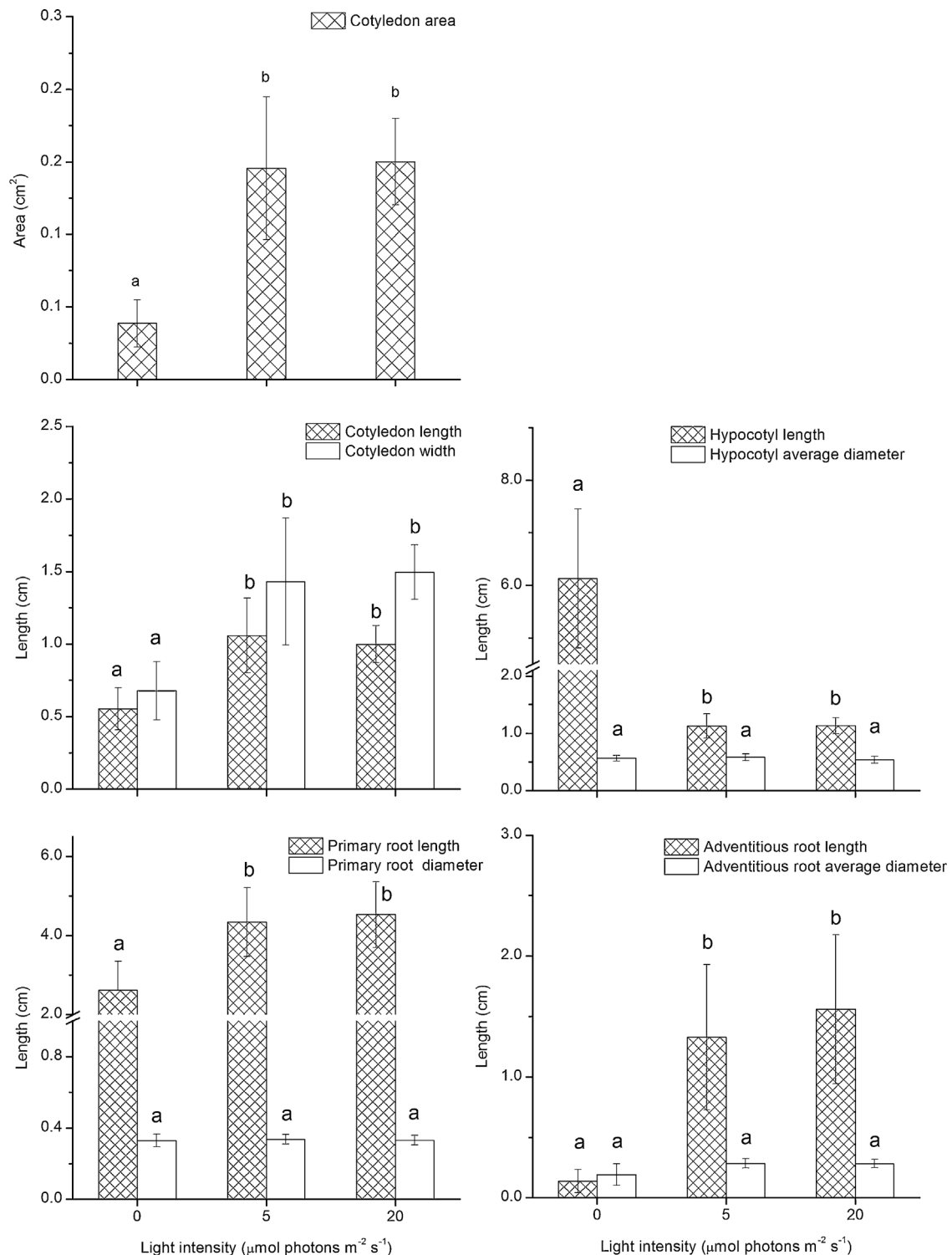


Fig. 4. The influence of different light intensities on the cotyledon area, cotyledon length, cotyledon width, hypocotyl length, average hypocotyl diameter, primary root length, average root diameter, adventitious root length, and average adventitious root diameter. Bars show the mean \pm S.E. ($n = 30$). The columns with the different letters are significantly different at $p < 0.05$.

Discussion

Seed buoyancy

In comparison to the data from the seed buoyancy studies of Boedeltje et al. (2003, and literature cited therein), the buoyancy of *N. peltata* seeds ($t_{50} = 0.8$ days, $t_{90} = 2.8$ days) is lower than for other species (Table 2). In the study of Boedeltje

et al. (2003) the seeds of most submerged species seeds from submerged aquatic species floated for a short period of time, whereas the buoyancies of *Potamogeton natans* seeds float much longer. If *P. natans* seeds is not considered, the values of seed buoyancy of submerged macrophytes decreased significantly from $t_{90} = 11.12 \pm 10.10$ (mean \pm S.E.) days to $t_{90} = 1.48 \pm 0.46$ (mean \pm S.E.) days (Boedeltje et al. 2003). The buoyancy of *N. peltata* seeds observed in the present study was greater than the one

Table 2

The seed buoyancy of different growth forms. The data were adapted from Boedeltje et al. (2003).

	t_{50}	t_{90}	n
Submerged aquatic species	10.91 (10.31)	11.12 (10.10)	11
Emergent aquatic species	53.52 (11.65)	66.80 (11.64)	18
Semi-aquatic and terrestrial bank species	23.28 (4.16)	20.28 (4.52)	80

t_{50} = time after which 50% seeds had sunk (SE in parenthesis); t_{90} = time after which 90% seeds had sunk (SE in parenthesis).

of most submerged aquatic species. Approximately two days of seed buoyancy are enough for dispersing these seeds over great distances even for a moderate water flow; this dispersal might promote the colony expansion of *N. peltata*.

The seeds that are heavier than water may float because of a hydrophobic surface (Cook 1990); if these seeds are forced underwater, they sink as mentioned by van der Velde and van der Heijden (1981). Cook (1990) first reported that seeds can float again after sinking. Our investigation also demonstrated that seeds that sank could float again, however, this seed buoyancy only lasted for short period of time and mainly occurred during the 100 min following the release of seeds from capsules. Based on the finding that the leaf cover was high in the dense patches of *N. peltata* plants, it was suggested by Brock et al. (1983) that these floating seeds encounter numerous leaves on the water surface. These encounters would result in the sinking of most *N. peltata* seeds within the stand by disturbances, such as leaf flapping induced by the wind and water currents that are similar to the effect of gentle stirring within the beaker. Other seeds that are released at the edge or outside of the main area colonised by plants are more likely to be dispersed over serve as long-distance seed dispersal events.

Smits et al. (1989) found that nearly all the seeds of *N. peltata* remained floating in open tanks after 2 weeks; van der Velde and van der Heijden (1981) reported that the seeds remained floating in undisturbed petri dishes after 2 months. We found that undisturbed seeds could float for more than 3 months. This result might be due to the following seed characteristics: (1) the flatness of *N. peltata* seeds; (2) the marginal bristles of these seeds, which increase water surface tension interactions; and (3) the hydrophobic substance in the coat of *N. peltata* seeds (Cook 1990). Smits et al. (1989) reported that pouring water into seed buoyancy experiments promoted the sinking of seeds. This finding suggests that simulated rain disturbs the balance of floating seeds. The action of wind and an accompanying water current are also common conditions in nature; our research suggested that a slow, continuous wind had a slightly negative effect on the buoyancy of *N. peltata* seeds compared with simulated rain disturbs (Smits et al. 1989). Thus, the wind will accelerate the dispersal of seeds. It is worth noting that wind-dispersal is a hazardous process that might be unfavourable for *N. peltata* seeds because they have a high probability of being transported to a terrestrial site if they do not sink while in open water or in the riparian zone. In addition, wind often causes sediment disturbance and favours seed burial. Therefore, we can assume that wind plays a minor role in the dispersal of seeds from *N. peltata* stands in open water where water depth is not low.

Seed germination

In our experiments, it is worth noting that the seeds of *N. peltata* had a rapid, uniform germination pattern under different treatments, which means all seeds were likely to germinate immediately when all requirements were met. A rapid and uniform germination of the seeds of *N. peltata* may have an important contribution to the expansion of *N. peltata* populations.

The establishment of seedlings is generally postponed by the dormancy of seeds. The stratification of seeds has been found to stimulate germination by Grime et al. (1981). These results confirmed that *N. peltata* seeds require cold exposure to overcome innate dormancy (Baskin and Baskin 1985). In our investigation, the seeds of *N. peltata* required more than 2 weeks of stratification to achieve germination percentages that were greater than 50%. This finding is consistent with the results of Smits et al. (1990). During cold stratification, the seeds become conditionally dormant (Probert 1992). A stratification requirement may represent a mechanism to ensure that germination occurs in the spring or early summer. Although *N. peltata* fruits set at July, released seeds cannot make a contribution to the recruitment until following year.

It should be emphasised that seed germination depends strongly on light conditions and cold treatments. This idea is consistent with the notion that the light is the most reliable environmental signal for the appropriate timing of seed germination (Baskin and Baskin 1998). Although exposure to light stimulated germination, some germination occurred at 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. When counting the number of germinated seeds, dark-treatment seeds were unavoidably exposed to low light intensity conditions. However, short exposures to low light appeared to have a negligible effect because most seeds did not germinate at later stages of the experiment.

In general, increasing in the soil depth favour the maintenance burial depth increases seed dormancy and survival (Conn and Farris 1987) and the suppression of seed germination. Our results suggest that seeds sown at a depth of greater than 0.25 cm fail to germinate. The light intensity did not have a positive effect on the germination of sown *N. peltata* seeds. The difference in the germination percentages of *N. peltata* seeds that were sown horizontally and vertically might result from the differences in oxygen concentrations and light availability. Smits et al. (1990) also reported that the seeds of *N. peltata* were unable to germinate under hypoxic conditions. Oxygen profiles of sediment in this experiment showed that, rapid, diminishing oxygen concentrations with increasing depths was probably an important factor causing germination failure of seeds buried by sediment.

If this hypothesis is applied to an aquatic habitat, it would indicate that sufficient oxygen levels are absolutely required for the *N. peltata* seeds to break dormancy. The *N. peltata* seeds that were situated in an aerobic environment germinated; only the seeds that are located slightly below or on top of the sediment will produce seedlings and beach along the shoreline or moist land. After the floating seeds sink in the mud, they are covered by vast amounts of inorganic sediment and macrophyte litter during the next year, which causes the oxygen content to decrease rapidly and produces anaerobic conditions. Based on the amount of ^{137}Cs in Lake Taihu, Xue et al. (2007) reported that the amount of sedimentation was 0.39 cm yr^{-1} . This report suggests that most *N. peltata* seeds will fail to germinate, and these non-germinated seeds will become part of persistent propagule banks (Thompson and Grime 1979). The consequence of seed dispersal is that new territories might be colonised, which leads to the expansion of the population. The reproductive success depends on the ability of *N. peltata* seeds to germinate and successfully establish in these areas. According to our results, only a few *N. peltata* seeds will germinate, most of these germinated seeds will float and few seedlings will successfully established in sediment.

Buoyant seedlings are also produced by other aquatic plants (Hutchinson 1975). Upon disturbance, these loosely rooted seedlings are easily detached and may float for a long time period before they are/can re-established; our experiments suggested that buoyant *N. peltata* seedlings could float for more than eight weeks. Thus, a secondary dispersal of this species within the same body of water could occur.

Seedling development and re-establishment

To adapt to low light conditions during photosynthesis, the seedlings allocate a large proportion of their resources to the rapid, vertical elongation of the hypocotyl. This allocation leads to smaller cotyledons, shorter primary roots and weak development of adventitious roots in the seedlings. By the end of the experiment, some seedlings of the $0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ light treatment appeared to exhaust the energy reserves of the seeds and decay. Sufficient light was important for seedling development of *N. peltata*.

Presumably physical factors such as water currents and other disturbances would also have an impact on seedling development; these disturbances lead to the uprooting of weak seedlings. The failure of seedlings to establish in existing populations seems to be a common feature of perennial species that form dense monospecific stands (McNaughton 1975; Eriksson 1992). Our research confirmed that most seedlings floated on the water surface and were failure to establish. Therefore, for these species, the suitable sites for recruitment of sexually produced offspring might be separated spatially from the sites where adult plants occur (Clevering 1995).

According to our investigation, the buoyant seedlings did not settle to the sediment surface and only re-established in shallow water or moist soil. This finding indicates that the re-establishment of buoyant seedlings mainly occurred in the land–water ecotone. This result was confirmed by Nishihiro et al. (2001); the authors reported that *N. peltata* seedlings were found along the shoreline near the *N. peltata* stand. No juvenile plants were established in the study area in Lake Kasumigaura, which covered approximately 2 ha. Recently, recruitment has been unsuccessful in the metapopulation of *N. peltata* in Lake Kasumigaura (Nishihiro et al. 2001). The studies conducted by Shinichi et al. (2009) elucidated that the bare ground areas that were exposed during periods of low water levels in the spring were considered to be ‘safe sites’ for *N. peltata* seedling establishment. However, even if these seedlings were established successfully, they retained their terrestrial form instead of elongating into floating leaf forms (Shinichi et al. 2009). Our subsequent experiment also revealed that the development of a floating leaf was very slow; two months after a seed germinated, the petiole length of a floating leaf was less than 10 cm (data not shown). This finding suggests that the expansion of *N. peltata* populations is probably maintained by the extension of stolons and dispersal of vegetative fragments. Larson (2007) also suggested that vegetative reproduction constituted an important part of the total reproduction at the level of genetic variation.

Conclusions

In summary, the seed buoyancy of *N. peltata* was greater than most submerged macrophytes. A disturbance of the seeds by a reduction in the water surface tension could promote the sinking of seeds. Most seeds within the *N. peltata* stand will sink when they are disturbed by leaf flapping. Stratification was obligatory for seed germination. A short period of stratification was able to break the innate seed dormancy. The germination rate increased with increases in the light intensity and duration of stratification, but this rate decreased with increases in the depth of sown seeds. When seeds sink to the water–sediment interface located far from the shoreline, the settlement of decayed aquatic plant and other inorganic matter will bury the seeds, thereby decreasing light intensity, oxygen availability and germination rates. Even if the seeds germinate at the water–sediment surface, most seedlings rise to float on the water surface after germination and will only establish if transported to shallow water. A few floating seedlings can settle and re-establish in deep water. Sexual reproduction might contribute little directly to the rapid expansion of *N. peltata* towards

the centre of large shallow lakes (or open water). The predominance of *N. peltata* clonal spread is also suggested to occur in many hydrophytes that exhibit a high frequency of flowering and a high seed set (Aarssen et al. 2006). The answer to the question of why *N. peltata* invests so many resources in sexual reproduction remains unanswered; thus, this question should be addressed further in future research.

Acknowledgements

The authors greatly appreciate Dr. Jingge Shang in providing experimental instruments, Dr. Xiang Bai in hosting this research. This research was supported by the National Natural Science Fund (41171413) and the National Chaohu Lake Special Project of China (2012ZX07103-002).

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