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Germination and early growth of *Nymphaea odorata* at different water depths

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ABSTRACT

We experimentally determined the effects of water depth on seed germination and seedling growth and morphology, and we documented the transition from submerged to emergent plants in the white water lily, *Nymphaea odorata*. Seeds of *N. odorata* were germinated at 30, 60, and 90 cm water depth in outdoor mesocosms and percent germination and morphology measured after a month. The presence of self-seeded seedlings in pots at the same 3 water levels was also recorded over two years. To examine juvenile growth, seeds planted in soil were placed at the same mesocosm depths; germination and growth were monitored for three months, when the plants were harvested for morphological and biomass measurements. *N. odorata* germinated equally well in 30, 60 and 90 cm water; seedlings grew as submerged aquatics. After one month, seedlings in 90 cm water had less biomass than those in 30 cm (1.1 vs. 3.3 mg and 1.0 vs. 1.8 mg for different seed sources, respectively) and allocated relatively more biomass to shoots (97.5 vs. 67.8% and 73.1 vs. 58.0%, respectively). Seedlings in 60 cm water were intermediate. After 3 months of submerged growth, plant biomass remained less in 90 vs. 60 and 30 cm water (22.5 vs. 36.4 and 33.3 mg, respectively). Plants in 90 and 60 cm water had greater biomass allocation to shoots than plants in 30 cm water (85.7 and 72.6% vs. 64.4%, respectively) and produced larger laminae on longer petioles (lamina length = 33.3 vs. 25.2 mm in 90 vs. 30 cm; petiole length = 99.0 vs. 36.0 mm, respectively). After about 3 months, submerged plants produced floating leaves that had 39% shorter laminae but 267% to 1988% longer petioles than submerged leaves on the same plant. Lamina length to width allometric relations of submerged leaves were >1 at all water levels, distinguishing them from the equal allometry of adult floating leaves. The switch from production of submerged to emergent leaves resembles submergence-escape growth in other aquatics, but because the seedlings have been submerged throughout their life, submergence itself cannot be the stimulus to produce emergent leaves in these totally immersed plants. Our data show that *N. odorata* plants can establish from seeds in up to 90 cm water and that seedlings grow as submerged aquatics until they switch abruptly to production of floating leaves.

1. Introduction

Emergent aquatic plants that occupy deeper water habitats can colonize these habitats by seed or by vegetative propagation (Combroux et al., 2002; Capers, 2003). Colonization by seed requires either that seeds germinate under water and grow through the water to the surface or that water levels fluctuate enough for seedlings to germinate and become established in shallow water. Many aquatic species utilize the latter strategy, needing shallow water or saturated soil and the light and temperature associated with these environments for their seeds to germinate, but some deeper water species germinate better under flooded conditions (Baskin and Baskin, 1998). Species of water lily in the genus *Nymphaea* are distributed world-wide in temperate and tropical wetlands (Borsch et al., 2007) and are important indicators for deeper wetland habitats (Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007). Wetland loss is a major problem worldwide, and restoration or mitigation efforts to re-introduce plants such as water lilies to former or newly created wetland habitats need to know the effects of water depth on water lily germination and seedling growth.

Our understanding of *Nymphaea* germination and seedling growth requirements comes primarily from laboratory experiments and seed bank studies. Seeds of *Nymphaea odorata* have a physiological dormancy that can be overcome by cold (Baskin and Baskin, 1998), and germination can be induced by crowding (Else and Riemer, 1984). In Else and Riemer's (1984) experiments, where *N. odorata* seeds were germinated in water-filled vials, germination increased linearly with seed number between 20 and 100 seeds per vial, with <20 seeds per vial showing no germination

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and ≥100 seeds per vial around 47% germination. This crowding response, which occurred without cold treatment, was found in light but not dark, and ethylene stimulated levels of germination similar to crowding (Else and Riemer, 1984). Germination of the closely related *Nymphaea alba* was induced by hypoxia and light after cold stratification; in this species very low levels of germination occurred in the dark under aerobic conditions (Smits et al., 1990). Under inductive conditions, *N. alba* released ethanol, and both ethanol and ethylene stimulated germination (Smits et al., 1995).

For wetland seed bank studies where *Nymphaea* species are present and often abundant in the vegetation, the species have been reported as present in the seed banks (Middleton et al., 1991; Van der Valk and Rosburg, 1997; Brock and Rogers, 1998; Mulhouse et al., 2005; Cherry and Gough, 2006; Miao and Zou, 2009) or absent (Smith et al., 2002; Johnson, 2004; Liu et al., 2005). Even when water lilies emerge in seed bank studies, they often are relatively rare (e.g., Gerritsen and Greening, 1989; Van der Valk and Rosburg, 1997; Miao and Zou, 2009). All of these studies used seedling emergence assays with water depths ≤ 10 cm except for Middleton et al. (1991), who used 20 cm, and none followed seedling growth after germination.

White water lily, *N. odorata*, is a characteristic species of deeper wetland habitats throughout North America (Woods et al., 2005) and is a target or indicator species for these habitats in wetland restorations (Leck and Simpson, 1995; McVoy et al., 2011). *N. odorata* has been identified as a species that germinates better under flooded conditions (Conard, 1905; Baskin and Baskin, 1998), but experiments showing this used flooded conditions that were only ≤5 cm deep (Gerritsen and Greening, 1989; Cherry and Gough, 2006). In the southern Florida Everglades, *N. odorata* seeds were abundant in some Everglades soils that were searched for seeds (mean density of 286 intact seeds per m^2), but only 20 seedlings emerged from the seed bank. If *N. odorata* germinates better under water, seedlings must be able to grow as submerged aquatics, but this phase of the life history has not been documented. Submerged seedling growth has been described and/or illustrated for other species of *Nymphaea* and for species of other genera in the Nymphaeaceae (*Victoria regia* (Gwynne-Vaughan, 1897); *Nymphaea lutea* (Arber, 1920); *Brasenia schreberi*, *Nuphar advena*, and *Nymphaea tuberosa* (Nieuwland, 1916); *N. alba* (Heslop-Harrison, 1955); *Nuphar luteum* and *Nymphaea coerulea* (Haines and Lye, 1975)). The effects of water depth on seedling growth, however, have not been documented for any of these species, while the transition from submerged to emergent floating leaves has not been studied in detail.

In order to better understand white water lily's life history characteristics, as well as to provide essential information for restoration of wetland habitats such at the Everglades ridge and slough (McVoy et al., 2011), we used mesocosm studies to experimentally investigate the effects of water depth on *N. odorata* germination and on seedling to emergent growth and morphology.

2. Materials and methods

2.1. Pollination and seed collection

Plants of *N. odorata* collected in southern Florida's Water Conservation Area 3A were grown outside on the Florida International University (FIU) campus in mesocosms (see Troxler and Richards, 2009; Richards et al., 2011 for collection locations and descriptions). The mesocosms were 3410 L (900 gal) round polypropylene cattle tanks that were 1 m deep × 2.1 m wide. Plants were grown in native peat in 25 cm diameter × 19 cm high pots on shelves suspended at 30 and 60 cm depths with an additional shelf on the bottom at 90 cm depth. Flowers of *N. odorata* are protogynous, opening as females in the morning of the first day, then closing in the afternoon and re-opening the next day in the male phase, which lasts 1–2 days, so flowering is complete in 2–3 days (Schneider and Chaney, 1981). In our mesocosms, flowering typically lasted 3 days. Because maternal seed source can have major effects on germination and seedling growth (Mousseau and Fox, 1998; Galloway and Etterson, 2007) and in order to generate enough seeds of known maternal source for use in germination experiments, we hand-pollinated receptive female flowers of plants growing in the experimental mesocosms in June 2009 and August to October 2010.

Several days after pollination, the flower peduncle coils and pulls the developing fruit underwater, where it matures over 3–5 weeks. Upon maturation, the carpel splits open, releasing the seeds. Like other species of *Nymphaea* (Arber, 1920; Smits et al., 1989), the seeds are covered by a loose aril that forms a sac around each seed, trapping gas and causing the seeds to float to the water surface after release from the fruit. In order to capture the seeds, about two weeks after pollination we placed plastic bags (2009) or muslin bags (2010) over the developing fruits; each bag was secured loosely at the base of the flower. Seeds were released into the bags about 4 weeks after pollination.

2.2. Germination and early seedling growth

In order to examine the effects of water on germination and the earliest stages of seedling growth, we assayed germination in glass vials suspended at different depths in the mesocosms. We used two maternal sources for these trials. In 2010 we used seeds from hand-pollinations of plants grown in the mesocosms (August to October 2010 pollinations, see Section 2.1, above), referred to as mesocosm-generated (MG) seeds or seedlings. In 2011 we used seeds from an open-pollinated fruit collected in the Everglades, referred to as field-generated (FG) seeds or seedlings. In both sets of experiments we germinated seeds in 25 ml glass vials placed at 30, 60 and 90 cm water depths in the mesocosms. Vials had no added soil, so seeds germinated and grew for a month in water. Vials were covered with plastic screening to retain the seeds but allow water and gas exchange.

The MG seed germination trials were conducted from September through December 2010, as seeds became available. Seeds were collected within a day of fruit dehiscence and were exposed to sunlight for at least 5 h while floating in water-filled containers. After exposure, 10 seeds were placed in each vial, and five vials were placed at each water level in a mesocosm. We repeated this procedure in five trials: two in different mesocosms in September, one in October, and two in the first two mesocosms in November. After 30–31 days, we counted the number of germinated seeds per vial, recorded the number of mature leaves and the shape of the most recently matured (MRM) laminae on germinated seedlings, and measured the length of the first internode, lamina length of the MRM leaf, and length of the longest root. Plants from the October trial were divided into shoots, roots and remnant seeds by vial, oven-dried at 70 °C, and weighed on a microbalance; biomass per individual was calculated by dividing this weight by the number of seedlings per vial.

To compare germination and early growth in seeds from plants in the mesocosms to seeds from open-pollinated plants in the field, we collected fruits from Water Conservation Area 3B (25° 46′ 4.712″ N, 80° 40′ 18.475″ W) on June 8 2011. Fruits were placed in beakers set in water in the mesocosm area; one fruit dehisced on June 9 2011, and the seeds were exposed to sunlight for a day, then were placed in glass vials suspended individually at 30, 60 and 90 cm water levels in two mesocosms ($n = 7$ vials per water level per mesocosm); vials at different water depths were interspersed. Seeds were allowed to germinate and grow for

a month. A subsample of 100 FG seeds was used to determine initial seed biomass; these seeds were divided into 10 groups of 10 seeds each, oven-dried at 70 °C, and weighed on a microbalance. Relative light levels in the mesocosms at 30, 60 and 90 cm depths were determined with a LI-COR underwater PAR sensor (LI-COR Biosciences, Lincoln, NE). Temperature at the three water levels was monitored throughout the 2011 experiment with thermochron iButtons (Maxim Integrated Products, Inc., Sunnyvale, CA) suspended at the same levels as the vials. The pH was measured with an Oakton series 10 pH meter (Eutech Instruments Pte Ltd., Singapore). Electrical conductivity and total dissolved solids were measured with a. HI 98311 EC/TSD and temperature meter (Hanna Instruments, Woonsocket, Rhode Island, USA). Air temperature was determined from data collected by Dr. Rene Price's FIU Weather Stations (http://www2.fiu.edu/~pricer/).

After 31 days, the number of germinated seeds per vial was determined, and leaf number, root length, lamina length and width and lamina shape were recorded for plants from 3 vials; these plants were dried and weighed as a bulk sample for each vial. Total biomass per individual was determined by dividing sample biomass by the number of plants per sample. Seedlings from the remaining 4 vials were separated into roots, shoots (=stems plus leaves) and remnant seeds, combining all the plant parts from one vial; samples were dried at 65 °C, weighed on a microbalance, and weight per individual determined by dividing the sample weight by the number of individuals per sample. Seedling biomass and biomass allocation were determined from these samples.

As a third measure of the effects of water depth on germination, we recorded *N. odorata* seedlings volunteering in pots of *N. odorata*, *Nymphoides aquatica* and *Eleocharis elongata* set at 30, 60 and 90 cm water levels in our nine mesocosms (9 pots per species per mesocosm). General environmental conditions for the site and tanks are given in Richards et al. (2011). Monthly observations on seedling presence were made over 2 years, beginning in October 2005 and ending in November 2007, except data collection was interrupted by a complete *N. odorata* harvest in November 2006 and re-introduction of plants in December 2006. Seedlings were removed after recording their presence. Because these experiments were conducted in isolated mesocosms on the FIU campus, in the first year of observation when plants were planted in commercial potting soil, there was no source for these volunteer seedlings other than the *N. odorata* plants in the mesocosms. In the second year, the newly collected *N. odorata* plants were planted in peat collected from the Everglades, so seedlings were potentially derived from the seed bank, as well as from plants in the mesocosms. Because water lily seeds float to the surface when their capsules split but then sink, our assumption was that soil surfaces of pots at all water levels were equally likely to intercept seeds; each water level had the same total pot surface area.

2.3. Submerged seedling and juvenile plant growth

In 2009 seeds were planted in 12 cm × 8 cm plastic inserts filled with commercial potting soil; each insert had six 4 cm × 4 cm subdivisions. Four seeds were planted per subdivision, resulting in 24 seeds per insert. We placed seeds from a single fruit in three inserts, and each insert was placed at one of three water depths (30, 60 and 90 cm) in a mesocosm. We had five blocks consisting of seeds from a single fruit; a sixth block combined seeds from two different flowers, but these two were represented in the same proportions in each insert. Planting took place as seeds from the June 2009 pollinations (see Section 2.1, above) became available, with a complete block being planted on a single date. Planting occurred between 13 July and 5 August 2009. The number of seeds germinated was recorded one and two months after planting and at about 3 months, when the juvenile plants were harvested between 20 October and 20 November 2009. Juveniles from one fruit were harvested within a single week. Juveniles from a single insert subdivision were gently washed and separated. Lamina length and width and petiole length of the leaf bearing the largest lamina of each juvenile plant was measured. These laminae were sagittate, so lamina length was measured from the lamina tip to the base of one of the lobes. Lamina width was measured across the lamina at the point of petiole attachment. Juvenile roots were separated from shoots, placed in open aluminum foil containers and dried at 70 °C for at least 72 h. Dried samples were weighed on a microbalance. Because we could not always accurately separate roots of seedlings in a single insert subdivision, when we calculated seedling weights and biomass, we averaged the biomass of seedlings in each insert subdivision and used this average in our analyses; an average seedling weight per insert was used in the final analyses. To estimate initial biomass of MG seeds, in 2011 we hand-pollinated 4 plants, collected the seeds, oven-dried 3–5 subsamples (10 seeds per subsample) at 70 °C, weighed them on a microbalance, and calculated weight per seed.

2.4. Transition from submerged to floating-leaved aquatic

Four seedlings in our 2009 experiment had produced emergent floating leaves at harvest. To further document this phenomenon, in June 2010 we planted germinated seedlings at 30, 60 and 90 cm water depths in three mesocosms and monitored production of floating leaves over the next three months. We harvested seedlings after the first floating leaf had matured and measured lamina length and width and petiole length of the floating leaf and the preceding submerged leaf. We also collected volunteer seedlings that had produced floating leaves from these same tanks and made similar measurements.

2.5. Statistical analyses

Results were analyzed with JMP 8.0 (SAS Institute Inc., Cary, NC, 2008) or with R (R-project, CRAN). For the 2009 germination experiment, effect of water depth and maternal genotype on seedling presence over time were analyzed with a generalized linear mixed-effects model that accounted for temporal pseudoreplication (Crawley, 2007, pp. 655–656), using a binomial error term. Water depth and maternal genotype were fixed effects, while time given insert was a random repeated effect. Model simplification was done based on an analysis of deviance between successively simpler models; if two models were not significantly different, the simpler model, based on the Akaike Information Criterion (AIC), was chosen to represent the data. Germination data for the first month of the 2009 experiment and for the 2010 and 2011 vial germination experiments was analyzed with a generalized linear model (GLM) using a binomial error term, with water depth and maternal genotype (2009), or water depth and trial (2010) or tank (2011) as fixed effects.

Biomass and morphological data for seedlings (2010, 2011 vial experiments) were not normally distributed, whether untransformed or ln transformed. The data were averaged by vial, and the vial means used to compare differences among water depths with Kruskall–Wallis (KW) nonparametric tests; Dunn's multiple comparisons test was used for post hoc comparisons among water depths (Dunn, 1964) to compare differences among water depths. Biomass and morphological data from the 2009 harvest were averaged by insert and log or square root transformed where necessary to satisfy assumptions of normality, then analyzed in a two-way ANOVA with water depth and maternal genotype as fixed effects. Tukey's HSD was used to test for differences among water depths. Data are presented as untransformed means ± SE. Differences were considered to be significant for $p \leq 0.05$.

Morphological data for juvenile leaves were further analyzed by fitting linear regressions to the data to examine scaling relationships at different water depths. Allometric relations of juvenile laminae were compared to those of adult laminae using data for adult leaves collected from plants grown in the same mesocosms and similar experimental conditions (Richards et al., 2011). Differences among slopes of regression lines were compared using *t*-tests.

3. Results

3.1. Tank environment

During the FG germination experiment from June 9 to July 11 2011, air temperature on the FIU campus averaged 27.6 ± 2.4 °C (range = 21.9–35.8 °C), while day length varied from 13 h 38 m to 13 h 45 m. The pH of the tanks averaged 7.08 ± 0.41, conductivity was 225.5 ± 12.4, and total dissolved solids were 448 ± 25. Water temperature in the mesocosms varied with season, time of day and water level (Richards et al., 2011), but in June/July 2011 median differences between water levels during the day (6 AM to 6 PM) were 1 °C for 30 to 90 cm, 0 °C for 30 to 60 cm and 0.5 °C for 60 to 90 cm; median differences between water levels at night (6 PM to 6 AM) were less (0 °C for 30 to 60 cm, and 0.5 °C for 30 to 90 cm and 60 to 90 cm). Average temperature at 90 cm approximated the average air temperature. Light levels at 30, 60 and 90 cm water depths did not differ significantly between tanks but did differ among water levels ($p < 0.0001$, ANOVA); photosynthetically active radiation (PAR) at 30 cm was 42.6 ± 2.4% of surface irradiance; PAR at 60 cm was 27.6 ± 1.9%; and PAR at 90 cm was 19.4 ± 1.7%.

During the September through December 2010 germination experiments, air temperature was 23.1 ± 4.8 °C (range = 6.1–32.9 °C), while day length varied from 12 h 21 m to 10 h 32 m. During the 3 month growth experiments from July 13 2009 to November 20 2009, air temperature averaged 27.4 ± 3.1 °C (range = 13.6–34.8 °C); day length varied from 13 h 37 m to 10 h 47 m. Although we did not measure water temperature during in these experiments, the data reported above for the June 2011 experiment, as well as in Richards et al. (2011), indicate that water temperature was within 1–2° of air temperature and was warmer at shallower depths.

3.2. Seed germination and seedling mortality

Percent germination from the June 2011 FG seeds was very high, ranging from 99% to 100% (30 cm = 99 ± 4%, 60 cm = 99 ± 3%, 90 cm = 100%) with no significant differences between tanks ($Pr > \chi^2 = 0.3561$, GLM) or among water levels ($Pr > \chi^2 = 0.8030$, GLM) and no significant interactions ($Pr > \chi^2 = 0.8106$, GLM).

Percent germination for the MG seeds in September through December 2010 was more variable, ranging from 57% to 91% averages in individual trials (30 cm = 68 ± 4%; 60 cm = 81 ± 3%; 90 cm = 77 ± 4%). There were significant differences among trials ($Pr > \chi^2 < 0.0001$, GLM), among water levels ($Pr > \chi^2 = 0.0015$, GLM) and significant interactions ($Pr > \chi^2 < 0.0001$, GLM). The significant differences in germination among water levels were between 30 cm water and the other two water levels, with germination at 30 cm being less in four of the five trials.

Fewer of the 2009 MG seeds planted in soil germinated; these seeds did not get a pre-planting sunlight exposure. In the 2009 experiment 49.3% of the seeds germinated in the first month, and only four more new seedlings appeared over the succeeding two months. Percent germination in the first month did not differ significantly among water depths ($Pr > \chi^2 = 0.4763$, GLM), but did differ significantly among maternal genotypes ($Pr > \chi^2 < 0.0001$, GLM).

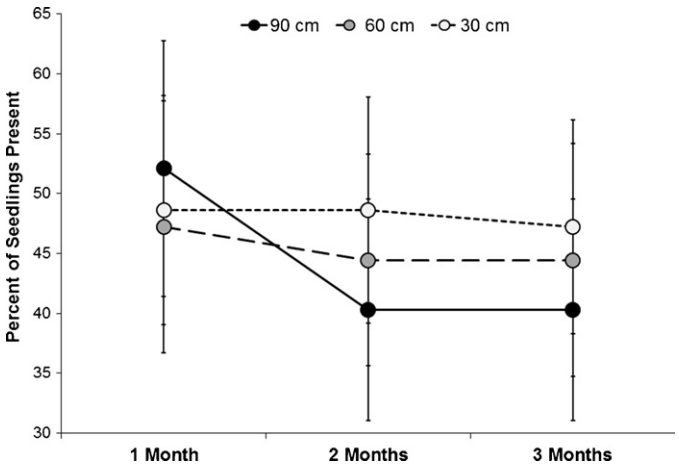

Fig. 1. Percent *N. odorata* juvenile plants seedlings present over time at 30, 60 and 90 cm water levels in outdoor mesocosms, 2009. Seed germination and seedling survival is similar among water depths over time but differs among genotypes. Data are mean ± SE for sample 1 (29–31 days after planting), sample 2 (61–74 days after planting) and sample 3 (harvest, 84–107 days after planting). $N = 18$ inserts with 24 seeds/insert.

Total plant number declined over the 3 months of the 2009 experiment (Fig. 1); decline was greatest in the 90 cm water level, but this decrease was not significant. The full model for the effects of water depth and maternal genotype on seedling number over time was not significantly different from a model that eliminated the temporal effect ($\chi^2 = 1.13$, df = 5, $Pr > \chi^2 = 0.9516$). In the simplified model seedling number did not differ significantly among water depths but did differ significantly among maternal genotypes: removing water depth did not produce a significantly different model ($\chi^2 = 0.84$, df = 2, $Pr > \chi^2 = 0.6570$), but removing genotype did ($\chi^2 = 36.88$, df = 5, $Pr > \chi^2 < 0.0001$).

In the monthly mesocosm observations of *N. odorata* seedling presence over two years, *N. odorata* seedlings were recorded 83 times. Forty-five percent of the records were of seedlings in pots in 90 cm water, 35% were of seedlings in pots in 60 cm water, and 20% were of seedlings from pots in 30 cm water.

3.3. Seedling and juvenile morphology and biomass

3.3.1. Seedling plants

Seedling growth differed among water depths in both MG and FG seedlings. Seeds initially produced a root, then an internode that carried the shoot apex away from the seed. For the seedlings growing in vials, this first internode was longer in deeper water (Table 1). Subsequent internodes were short (<2 mm) on all plants. Seedlings produced an acicular leaf initially, followed successively by leaves with elliptic, deltoid, and cordate to sagittate laminae. After a month, FG seedlings had matured 2 leaves (median; range = 0–4). Forty-four percent of FG seedlings at 30 cm water depth had deltoid laminae, compared to 31% at 60 cm and 12% at 90 cm. The MG seedlings also had 2 mature leaves (median; range = 0–3) after a month of growth. Fifty-nine percent of seedlings in vials in 30 cm water had elliptic laminae, compared to 72% in 60 cm water and 49% in 90 cm water; no MG seedlings had produced mature deltoid or sagittate laminae.

The length of the most recently matured leaf blades on FG plants differed among water levels, with longer laminae on plants in 30 cm water, and shorter laminae on plants in 60 cm and 90 cm water (Table 1). Petiole length was shorter in shallower water, but differences were not significant (Table 1). Although length of the longest root did not differ significantly among water levels, roots in deeper water tended to be shorter (Table 1). MG seedlings showed similar

Table 1
Morphological measurements in mm (mean ± SE) from *N. odoroata* seedling and juvenile plants grown in mesocosms at different water depths for one (seedlings) and three (juveniles) months after planting. Mesocosm-generated (MG) seedlings were grown from seeds collected from hand-pollinated plants growing in the mesocosms, while field-generated (FG) seedlings were grown from an open-pollinated fruit collected from the Everglades. Data are mean ± SE; *p*-values from KW tests (seedlings) or ANOVA (juveniles). Values followed by different letters are significantly different at $p \leq 0.05$.

	30 cm	60 cm	90 cm	*p*
MG seedlings				
First internode length	2.7 ± 0.3[a]	7.7 ± 1.0[b]	22.1 ± 2.3[c]	<0.0001
Root length	55.6 ± 3.7[a]	48.2 ± 5.2[a]	14.5 ± 3.1[b]	<0.0001
Lamina length	16.0 ± 0.3[a]	15.8 ± 0.3[a]	11.3 ± 0.4[b]	<0.0001
FG seedlings				
First internode length	2.5 ± 1.0[a]	5.5 ± 1.3[b]	12.4 ± 4.0[b]	0.0089
Root length	61.0 ± 4.6	51.5 ± 7.3	37.8 ± 7.8	0.0745
Lamina length	13.4 ± 0.3[a]	12.3 ± 0.2[b]	11.0 ± 1.0[b]	0.0215
Petiole length	21.4 ± 1.2	26.2 ± 5.8	34.4 ± 3.4	0.1090
Juveniles				
Lamina length	25.2 ± 1.0[a]	35.0 ± 1.8[b]	34.4 ± 3.5[b]	0.0220
Petiole length	36.3 ± 3.2[a]	76.6 ± 10.6[b]	110.4 ± 20.5[b]	0.0038

morphological differences between seedlings in 30 cm and 90 cm water, with differences in petiole and root length being significant in these trials (Table 1).

Dry weight of FG seeds prior to germination was 3.40 ± 0.04 mg. After a month at different water levels, total biomass of the seed remnants plus young plants was less than the original seed weight (Table 2) and differed among water depths, with less total biomass in 90 cm water (Table 2). Biomass of the plants (total mass minus seed remnant) was also less in 90 cm water (Table 2), while remnant seed mass did not differ among water levels ($p = 0.1377$, KW test). Seedlings in 90 cm water had less biomass in roots than those at 30 and 60 cm water, and they allocated relatively more biomass to shoots and less to roots than plants in 30 cm water (Table 2). The plants in 60 cm water were intermediate in shoot and root allocation between plants in 30 and 90 cm water (Table 2). The effect of water depth on biomass in the MG seedlings was similar to that for the FG seeds (Table 2) and correlated with the morphological data, e.g., the more extreme difference in root biomass in MG vs. FG seedlings (Table 2) was also seen in the data on root length (Table 1).

3.3.2. Juvenile plants

In the 2009 experiment after about 3 months of submerged growth, juvenile plants at all water levels had 3 (range = 0–7) mature leaves present, but leaf morphology differed among plants at different water levels (Table 1B). Laminae on plants in 90 and 60 cm water levels were larger than laminae on plants in 30 cm water (Table 1B). Although all leaves were submerged, petioles of plants in 90 cm water were longer than those of plants in 60 cm water levels, which were longer than those of plants in 30 cm water (Table 1B).

Leaves at different water levels also had different allometric relationships. Lamina length and width of juveniles at all water levels were highly positively correlated, and at all water levels, a given increase in width was accompanied by a greater increase in length (i.e., slope for regression line > 1, Fig. 2). Laminae in 90 and 60 cm water levels had similar slopes (Fig. 2, $t_{D\ vs.\ M} = 0.06$, df = 118, $p = 0.956$), while laminae in 30 cm water had a shallower slope (Fig. 2, $t_{M\ vs.\ S} = 2.02$, df = 128, $p = 0.045$; $t_{S\ vs.\ D} = -2.20$, df = 122, $p = 0.030$).

Seed biomass for MG seeds was 4.1 ± 0.2 mg. After 3 months of submerged growth, total plant biomass was much greater, averaging 30.9 ± 2.0 mg. Total biomass of juvenile plants differed with water depth, as juveniles from 90 cm water had less biomass than those in 60 cm water (Table 2). This difference was primarily a result of differences in root biomass, with juvenile plants in 90 cm water having less root biomass than juveniles in 30 or 60 cm water;

Table 2
Biomass and percent biomass allocation of *N. odorata* seedlings after 31 days growth in vials at different water depths and of juvenile plants after 3 months growth in soil at the same water depths. Seedling total weight includes remnant seed. MG = mesocosm-generated; FG = field-generated. *p*-Values from depth effects in GLM. Values followed by different letters are significantly different at $p \leq 0.05$.

	30 cm	60 cm	90 cm	*p*
MG seedlings				
Total weight (mg)	5.78 ± 0.19[a]	4.99 ± 0.12[ab]	4.69 ± 0.11[b]	0.0124
Weight of plant (mg)	3.25 ± 0.14[a]	2.45 ± 0.04[ab]	1.05 ± 0.06[b]	0.0019
Shoot Wt (mg)	2.21 ± 0.16[a]	1.77 ± 0.04[ab]	1.03 ± 0.06[b]	0.0019
Root Wt (mg)	1.03 ± 0.12[a]	0.69 ± 0.02[ab]	0.03 ± 0.01[b]	0.0019
Percent shoot	67.8 ± 3.2[a]	72.0 ± 0.9[a]	97.5 ± 0.4[b]	0.0060
Percent root	32.1 ± 3.6[a]	28.0 ± 0.7[a]	2.7 ± 0.5[b]	0.0060
FG seedlings				
Total weight (mg)	2.88 ± 0.09[a]	2.75 ± 0.10[a]	2.29 ± 0.10[b]	<0.0001
Weight of plant (mg)	1.81 ± 0.13[a]	1.58 ± 0.13[a]	0.97 ± 0.13[b]	<0.0001
Shoot Wt (mg)	1.07 ± 0.12[a]	1.08 ± 0.12[a]	0.72 ± 0.11[b]	0.0441
Root Wt (mg)	0.74 ± 0.04[a]	0.50 ± 0.07[b]	0.25 ± 0.04[c]	<0.0001
Percent shoot	58.0 ± 3.3[a]	67.3 ± 4.3[ab]	73.1 ± 3.9[b]	0.0179
Percent root	42.0 ± 3.3[a]	32.7 ± 4.3[ab]	26.9 ± 3.9[b]	0.0179
Juveniles				
Total Wt (mg)	35.6 ± 6.4[ab]	38.3 ± 5.1[a]	23.3 ± 5.1[b]	0.0396
Shoot Wt (mg)	23.0 ± 4.0	27.8 ± 2.9	19.3 ± 4.2	0.1049
Root Wt (mg)	12.8 ± 2.4[a]	10.5 ± 1.3[a]	4.1 ± 1.0[b]	0.0050
Percent shoot	64.4 ± 0.8[a]	72.6 ± 0.8[b]	85.7 ± 1.2[c]	<0.0001
Percent root	35.6 ± 0.8[a]	27.4 ± 0.8[b]	14.4 ± 1.2[c]	<0.0001

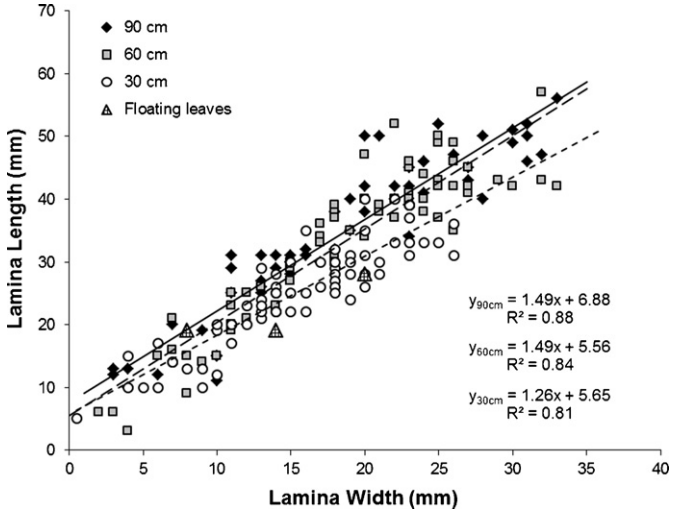

Fig. 2. Correlations of lamina length to lamina width for submerged juveniles of *N. odorata* growing in 30, 60 and 90 cm water levels for 3 months; data from the first floating laminae on three of the same plants included as triangles. $N = 68$ (30 cm), 64 (60 cm) and 58 (90 cm).

shoot biomass did not differ among water levels. Biomass allocation differed significantly among water levels, with relatively more allocation to shoots and less allocation to roots with increasing water level (Table 2).

3.4. Transition to floating-leaved plants

Submerged juvenile plants began to produce floating laminae after about 3 months of growth. Four of the 2009 juvenile plants had produced floating emergent laminae at the time of harvest; these plants had been planted between 93 and 100 days prior to harvest, three in 30 cm water, and one in 90 cm water. Plants produced floating laminae abruptly rather than gradually—i.e., one petiole elongated much beyond the other petioles on that plant, rather than successive leaves producing slightly longer petioles until one reached the surface. The laminae of the first floating leaves were smaller than laminae of submerged leaves on the same plants, but petioles of floating leaves were much longer than petioles of submerged leaves on the same plant. The lamina length to width ratio for these floating leaves fell within the range of lamina length to width ratios for the other harvested plants (Fig. 2).

Lamina shape in submersed juveniles was different from that of floating-leaved adult plants (Fig. 3). Adult plants increased lamina length and width approximately equally, producing a round lamina (Fig. 3, Richards et al., 2011). The slope of a regression line for juvenile lamina length vs. lamina width from all water levels was greater than the slope for similar data from adult leaves ($t_{A\ vs.\ S} = -10.43$, df = 342, $p < 0.0001$). The reduction in size of the first floating laminae produced on the juvenile plants put them at the intersection of the lines describing these two sets of scaling relationships (Fig. 3).

Submerged and floating leaf pairs sampled from the same plant showed these same scaling relationships (Fig. 3, inset). Floating leaves were produced from submerged plants by greatly increasing petiole length between successive leaves. The amount of elongation varied with water depth but ranged from the floating leaf having a petiole 267–1988% longer than the preceding submerged leaf on the same plant. A floating lamina was shorter by 39.0 ± 9.7% and narrower by 30.6 ± 9.7% than the preceding submerged lamina on the same plant. Floating and submerged laminae had similar allometric relations, but the floating laminae were smaller than the submerged laminae (Fig. 3, inset; t for difference between floating and submerged lamina slopes = −0.5178, df = 76, $p = 0.6061$).

4. Discussion

4.1. Seed germination and seedling growth

Although many aquatic plants need shallow water or saturated soil to germinate (Baskin and Baskin, 1998), our data show that *N. odorata* seeds can germinate at water depths up to 90 cm, and

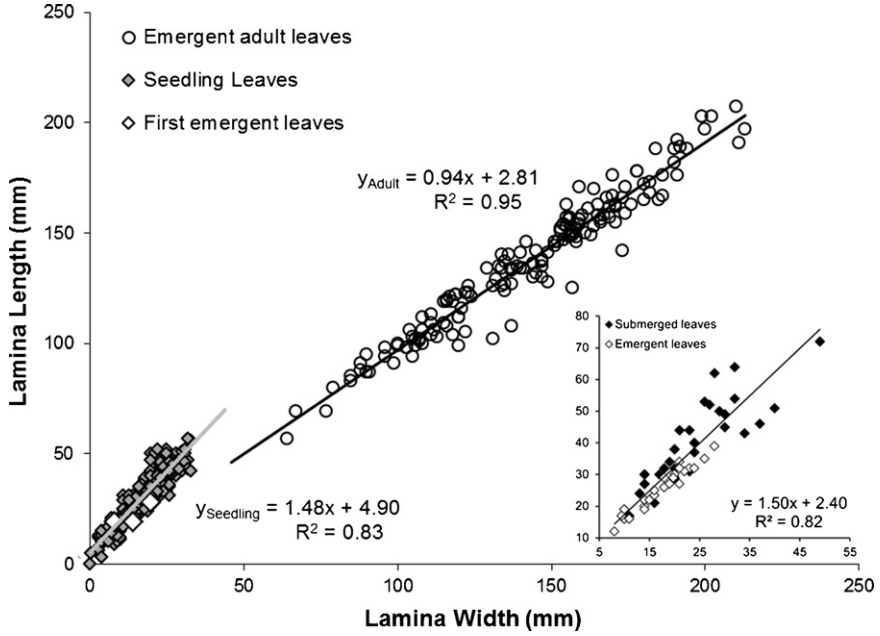

Fig. 3. Correlations of lamina length by width for juvenile and adult leaves from plants of *N. odorata* grown at 30, 60 and 90 cm water depths. Data for juvenile leaves from 2009 experiment in this study (Fig. 2); data for adult floating leaves from Richards et al. (2011). Juvenile leaves were submerged, while adult leaves had floating laminae. White diamonds show data from the first floating leaves produced by submerged plants. The inset shows data for submerged and floating leaf pairs harvested in 2010 from plants in the mesocosms.

seedlings and juveniles grow at these depths as submerged aquatics until they produce emergent floating leaves. Crowding of seeds has been shown to promote germination (Else and Riemer, 1984; Smits et al., 1995), but the presence of single seedlings volunteering in the mesocosms and germination of seeds under uncrowded conditions in all our experiments indicate that crowding is not required. Light stimulated germination of *N. alba* (Smits et al., 1990), and seeds of *N. odorata* are exposed to light under natural conditions when they are released from the fruit and float to the surface. The difference in germination percentages in our experiments when seeds were exposed to sunlight vs. when they were not suggests that sunlight greatly promotes germination.

In our experiment, water lily germination levels were similar among water depths, but even our shallowest water level was deeper than water levels in most wetland seed bank studies. Conard (1905) reported that seeds of water lilies needed to be submerged in 5–30 cm of water; our data show that *N. odorata* can germinate from much deeper. Aerobic conditions reduce germination in *N. alba* (Smits et al., 1995), and Else and Riemer (1984) had very low levels of germination of *N. odorata* when air was bubbled through water in their germination vials. The absence or relative rarity of *N. odorata* in seed bank studies thus may reflect unfavorable germination conditions rather than absence of seeds in the seed bank.

4.2. Seedling and juvenile morphology and growth

N. odorata seedlings and juvenile plants can grow and increase in biomass as submerged aquatics. Water depth affects the amount of biomass and plant morphology, but plants transitioned from submerged to emergent plants from 30 to 90 cm depths. *N. odorata*'s sudden change from submerged to emergent resembles the submergence-escape response or low oxygen escape syndrome (LOES (Pierik et al., 2009)) described for some wetland species (Jackson, 2008). In *N. odorata* seedlings, however, the morphological and physiological alterations occur as a life-history change in plants that are totally immersed and have been immersed throughout their growth, rather than as a response to submergence. The submergence-escape response typically involves ethylene induction of a signal transduction pathway that leads to increased cell elongation and/or cell division in the elongating stem or petiole, which elevates the submerged leaf or shoot above water (Voesenek et al., 2006; Jackson, 2008). The trigger for the suite of morphological, physiological and molecular genetic responses is submergence, which allows ethylene to be trapped within the tissues of the plant (Vriezen et al., 2000; Voesenek et al., 2004, 2006; Jackson, 2008). The juvenile plants of *N. odorata* described here, in contrast, were growing as submerged aquatics when they switched suddenly from the submerged to emergent, floating-leaved habit. Thus, the trigger for the submergence-escape response in *N. odorata* juvenile plants cannot be submergence. Petioles of the floating-leaved species *Nymphoides peltata* elongate in response both to ethylene and to applied tension; such tension would be expected to result from submergence of aerenchymatic laminae (Ridge and Amarasinghe, 1984). An increase in aerenchyma between successive laminae in submerged seedlings of *N. odorata* could provide such a buoyant tension and produce the petiole elongation that elevates the first emergent lamina.

Under mesocosm growth conditions, adult floating-leaved plants of *N. odorata* had similar total biomass at different water depths, but they changed biomass allocation with changes in water depth, allocating more biomass to leaves and roots in 90 cm water (Richards et al., 2011). Submerged juvenile plants of *N. odorata* differed from adults in that both seedlings and juveniles in 90 cm water had less total biomass than seedling and juvenile plants in 30 and 60 cm water and allocated less biomass to roots. The reduced allocation to roots was present at early seedling stages, when plants were growing at least partly on seed reserves. This allocation difference reflects differences in the submerged vs. emergent habit of the juvenile vs. adult plants. Because juvenile plants are completely submerged, they do not have access to the aerial environment and so cannot aerate their root systems with the pressurized ventilation system found in adult plants (Grosse and Bauch, 1991). Shipley et al. (1989) found a lack of association between juvenile and adult traits of wetland emergent plants, but they did not sample floating-leaved or submerged plants. Our data suggest a similar dissociation of juvenile and adult traits in white water lily.

The changes in juvenile leaf morphology with increased depth resembled shade responses, consistent with the reduction in light intensity seen with increased water depth in the mesocosms, as in other aquatic habitats (Voesenek et al., 2006). Laminae produced by juvenile and adult plants had different allometric relations, with submerged juvenile leaf blades longer than wide, whereas floating adult laminae were round (Richards et al., 2011). When juveniles first produced a floating lamina, they did not immediately switch to making rounded laminae. Instead, they produced smaller laminae, which reduced the length/width difference of the laminae and brought them closer to adult allometry. This change in allometric relations may be induced by different light levels, as the juvenile plants at 30 cm water had a lower length/width ratio than plants in deeper water.

Deeper water habitats have been lost in wetlands worldwide, and re-establishment of these habitats and their associated vegetation, such as water lilies, is often a restoration goal (Van der Valk et al., 1992; Leck and Simpson, 1995; Ogden, 2005; McVoy et al., 2011). Our results indicate that increasing water depth under restoration should not reduce, and may increase, seed recruitment and seedling establishment of *N. odorata*, a prominent slough species. Additionally, because seeds can both germinate under and grow to the surface through water up to 90 cm deep, if underwater light levels are sufficient to allow the plants to accumulate biomass, trial re-introductions of water lilies into deeper water habitats using seeds are warranted.

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