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Experimental determination of effects of water depth on *Nymphaea odorata* growth, morphology and biomass allocation

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ABSTRACT

Growth, morphology and biomass allocation in response to water depth was studied in white water lily, *Nymphaea odorata* Aiton. Plants were grown for 13 months in 30, 60 and 90 cm water in outdoor mesocosms in southern Florida. Water lily plant growth was distinctly seasonal with plants at all water levels producing more and larger leaves and more flowers in the warmer months. Plants in 30 cm water produced more but smaller and shorter-lived leaves than plants at 60 cm and 90 cm water levels. Although plants did not differ significantly in total biomass at harvest, plants in deeper water had significantly greater biomass allocated to leaves and roots, while plants in 30 cm water had significantly greater biomass allocated to rhizomes. Although lamina area and petiole length increased significantly with water level, lamina specific weight did not differ among water levels. Petiole specific weight increased significantly with increasing water level, implying a greater cost to tethering the larger laminae in deeper water. Lamina length and width scaled similarly at different water levels and modeled lamina area (LA) accurately ($LA_{modeled} = 0.98LA_{measured} + 3.96$, $R^2 = 0.99$). Lamina area was highly correlated with lamina weight ($LW = 8.43LA - 66.78$, $R^2 = 0.93$), so simple linear measurements can predict water lily lamina area and lamina weight. These relationships were used to calculate monthly lamina surface area in the mesocosms. Plants in 30 cm water had lower total photosynthetic surface area than plants in 60 cm and 90 cm water levels throughout, and in the summer plants in 90 cm water showed a great increase in photosynthetic surface area as compared to plants in shallower water. These results support setting Everglades restoration water depth targets for sloughs at depths ≥45 cm and suggest that in the summer optimal growth for white water lilies occurs at depths ≥75 cm.

1. Introduction

In wetland gradients from shallow to deep water, emergents dominate in shallow water, floating-leaved aquatics dominate deeper water, and submerged aquatics are found in the deepest water (Whittaker, 1967; Spence, 1982; Keddy, 2000). These patterns are hypothesized to represent either competitive exclusion at the shallower edge and physical constraints at the deeper edge of each zone (Keddy, 2000), or a trade-off between drought and flood tolerance (Luo et al., 2008). Givnish (2002) has argued that the trade-offs that result in zonation involve differences in biomass investment in lamina support: emergents invest relatively more in petioles or stems that support laminae, while floating-leaved plants invest relatively less in petioles, which function as tethers rather than supports. Data from leaves of emergent *Pontederia cordata* vs. floating leaved *Nymphaea odorata* supported this interpretation on a per-leaf basis (Givnish, 2002), but what differences these leaf modifications have on whole-plant biomass allocation of floating-leaved species is not known.

Numerous studies have examined changes in biomass and/or biomass allocation with water depth for emergent species (Leiffers and Shay, 1981; Grace, 1989; McKee and Mendelssohn, 1989; Kirkman and Sharitz, 1993; Coops et al., 1996; Lentz and Dunson, 1998; Blanch et al., 1999; Vretare et al., 2001; Edwards et al., 2003; Busch et al., 2004; Macek et al., 2006; Smith and Brock, 2007; Luo et al., 2008). These studies have found that emergent plants generally respond to increased water levels by growing more slowly, producing fewer but longer shoots, increasing biomass allocation to above-ground parts while decreasing allocation to roots or below-ground parts, and decreasing allocation to mechanical support. How water depth affects whole-plant biomass and biomass allocation of floating-leaved species is less well-studied (Brock et al., 1983; Brock et al., 1987; Sinden-Hempstead and Killingbeck, 1996).

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Historically, emergents and floating-leaved plants in southern Florida's Everglades marshes were organized in a patterned "ridge and slough" landscape. The shallow ridges were dominated by emergent sawgrass (*Cladium jamaicense*). Adjacent ridges were separated by deeper water sloughs dominated by floating-leaved water lilies (*N. odorata*; Science Coordinating Team, 2003; Ogden, 2005). Ridges and sloughs were elongated parallel to the direction of flow. Anthropogenic modifications of flow have reduced historic water levels in the Everglades, causing loss of the ridge and slough landscape as sawgrass has expanded in slough habitat (Science Coordinating Team, 2003; Ogden, 2005). A major goal of the Comprehensive Everglades Restoration Plan (CERP) in southern Florida is restoration of this patterning and the associated difference in water depth between ridges and sloughs.

N. odorata is the dominant macrophyte in Everglades sloughs (Gunderson, 1994; Givnish et al., 2008; Richards et al., 2008). This species was common throughout Everglades marshes prior to the 1900s (Saunders et al., 2008) but is currently unevenly distributed in the landscape with reduced abundance in the southern range, including Everglades National Park (Stober et al., 2001; Richards et al., 2008). Although this absence has been associated with hydrologic alterations of the landscape, specifically with decreased amounts of water and increased length of drydowns, white water lily's hydrologic optima and tolerances have not been investigated experimentally. These tolerances can be inferred from correlations of species presence with hydrologic measurements in the field (Duever, 1982; Wood and Tanner, 1990; David, 1996; Sinden-Hempstead and Killingbeck, 1996; Jordan et al., 1997; Olmsted and Armentano, 1997; King et al., 2004; Givnish et al., 2008; Richards et al., 2008) or from species presence in a slough community type that has a known hydrology (Loveless, 1959; Gunderson, 1994; White, 1994). Water depth tolerances for water lilies derived from such studies range from 0 cm to over 2 m, with annual averages from 30 to 67 cm. These data suggest that *N. odorata* can grow at shallower water levels and thus fail to provide insight into why emergent sawgrass (*C. jamaicense*) dominates ridges, while floating-leaved water lilies are found in sloughs, or why sawgrass has recently extended its dominance into habitats previously occupied by water lilies.

In order to understand trade-offs in biomass allocation for this floating-leaved species' responses to water depth and to predict water lily responses to hydrologic restoration in the Everglades, we used mesocosm experiments to study the effects of water depth on growth and biomass allocation of *N. odorata*. Specifically, we wanted to know how water depth affects rates of leaf production and senescence, leaf morphology, biomass allocation to leaves and whole plant biomass allocation. We also investigated how the scaling relations of leaf morphology and biomass vary with water depth in order to model photosynthetic surface area and leaf biomass from simple morphological measurements.

2. Materials and methods

2.1. Field collections

Plants of *N. odorata* used during this study were collected from Water Conservation Area 3A in southern Florida. Our primary collecting site was a slough at 25°47′8.881″N, 80°41′16.431″W; additional collections were made from sites within a 5 km radius of this primary site. The physical and biotic characteristics of these sites are described in Troxler and Richards (2009). Initial plant collections were made in July and August 2005 with supplemental collections made September and October 2005. Specimens were transported to Florida International University's Modesto Maidique campus (FIU MMC) and left in shallow ponds until planting within 48 h. Single unbranched rhizome tips ≥10 cm in length were planted in 25 cm diameter × 19 cm high plastic pots in peat humus organic growing medium (Greenleaf Products, Inc., Haines City, FL); each pot was labeled with a unique numbered tag so that individual plants could be followed over time.

2.2. Experimental mesocosms

Plants were grown outside in mesocosms. Day length varied from 10 h 31 m (winter solstice, December 21, 2005) to 13 h 45 m (summer solstice, June 21, 2006) (http://www.wunderground.com/history/). Air temperature averaged 24.5 ± 4.4 °C (avg. ± SD) with a range of 6.5–35.5 °C (data from a weather station on the FIU MMC campus, http://www2.fiu.edu/~pricer/). November through April were cooler (21.4 ± 4.0 °C), while May through October were warmer (27.2 ± 2.8 °C).

The nine experimental mesocosms were 3410 L (900 gal.) round polypropylene cattle tanks that had a 2.2 m inner diameter and were 1 m deep. We suspended shelves at 30 and 60 cm depths in each mesocosm, while an additional shelf sat on the bottom of the mesocosm at 90 cm depth, allowing us to elevate these deeper-water plants for observations and measurements. Shelves did not overlap each other. While nymphaeids grow in water depths ranging from 0.5 to 3 m (Sculthorpe, 1967; Den Hartog and Van der Velde, 1988), and white water lilies have been reported from water depths exceeding 2 m (Sinden-Hempstead and Killingbeck, 1996), mesocosm water levels were chosen to examine plant responses to levels typical of the southern Florida Everglades, where wet season water levels in water lily habitat sampled across the Everglades ecosystem averaged 73 ± 5 cm and dry season water levels averaged 29 ± 3 cm (J.H. Richards, unpublished data). Flooded sloughs in the central portion of the Everglades averaged 67.1 ± 1.7 cm, with an average maximum and minimum across sites of 101.9 ± 1.9 cm and 26.8 ± 1.5 cm, respectively (Givnish et al., 2008); water lilies were the dominant macrophyte in these sloughs.

Three *N. odorata* plants were placed at each water depth in each mesocosm (9 plants per mesocosm, 81 plants total). Water levels were held at 90 cm in each tank throughout the experiment. Rhizomes in the pots were positioned ~15 cm above the shelves on which they sat, so depth of the rhizome apices were ~15, 45 and 75 cm below the water surface. Water temperature was measured at the soil surface in one pot per level in all experimental tanks using thermochron iButtons (Maxim Integrated Products, Inc., Sunnyvale, CA) programmed to record temperature every half hour. Temperature differences between water levels within a tank were calculated from this data by subtracting temperatures recorded within a given half-hour period. Dissolved oxygen and pH of the water in the tanks were measured monthly at app. noon beginning in February 2006.

We put plants into the experimental mesocosms on 18 and 19 October 2005. Initial measurements of the number of leaves per main shoot apex, total number of leaves per plant, and lamina length and width of the most recently matured leaf showed no significant differences among water levels in any of these parameters (ANOVA, $p \geq 0.3401$). Five of 81 plants (6%) died in the first 2 months of the experiment; these were from different tanks but all at the 90 cm water level. Dead plants were replaced by plants growing at 90 cm or 60 cm water levels in reserve mesocosms. Although we began taking measurements in October 2005, quantitative morphological and phenological data reported here begin with January 2006, when plant mortality had declined, and continued through November 2006, when all *N. odorata* plants were harvested.

2.3. Monthly growth measurements

Each month we marked new *N. odorata* floating laminae with indelible ink, using a different letter per month in order

to distinguish newly produced leaves from older leaves. We also tagged the most recently matured leaf on each plant with a cable tie, using a different colored tie each month. To make measurements, plants were briefly removed from the water. We counted the number of rhizome apices, new leaves and live leaves; a leaf was counted as living if more than ½ of the lamina was green. Beginning in April 2006 we also recorded whether plants were flowering. On the most recently matured leaf we measured the lamina length to petiole, total lamina length from tip to lobe base, lamina width and petiole length. To calculate rates of leaf senescence, we added the total number of live leaves from the previous month to the number of new leaves in the current month, then subtracted the number of live leaves in the current month.

2.4. Biomass determination

After the November 2006 monthly growth measurements, we harvested all of the *N. odorata* plants ($N = 81$) on 6–8 November 2006. Plants were removed from pots, washed free of soil and periphyton, separated into roots, laminae, petioles, rhizomes, and flowering parts, placed in labeled paper bags and dried at 70 °C to determine total dry weight and biomass allocation to different plant parts. Total leaf weight was calculated as the sum of lamina and petiole weight; total shoot weight was calculated as the sum of leaf, rhizome and flower biomass; total plant weight was the sum of root weight and total shoot weight. Root:shoot ratios were calculated for each individual plant by dividing root weight by total shoot weight.

In order to determine the effect of water depth on morphology and biomass of individual leaves, the most recently matured leaf and one additional leaf were harvested separately from the rest of the plant. These leaves were measured as described above for the monthly samples, but in addition petiole width at the midpoint was measured with calipers, and lamina area was measured with a Delta-T leaf area meter (Delta-T Devices Ltd., Cambridge, England). The lamina and petiole of each of these leaves were dried and weighed separately.

2.5. Modeling monthly photosynthetic surface area

We used the correlations among linear lamina measurements and lamina area determined at harvest to estimate total lamina photosynthetic area per main shoot apex for each month based on the monthly lamina measurements and leaf counts. We modeled lamina area from the linear lamina measurements, then multiplied this lamina area by the total number of leaves per main shoot apex at each monthly measurement. We assumed that all leaves present at one sample time were equal in area.

2.6. Statistical analyses

The monthly growth data were analyzed for differences among water levels using a repeated measures analysis in SAS 9.2 (SAS Institute, Inc., Cary, NC). Because intervals between sampling dates were not always equal, monthly rates of leaf production were calculated by multiplying daily rates of leaf production by 30. Daily rates of leaf production were determined by dividing the number of new leaves at each sample by the number of days since the last sample. Differences among water levels in flower number were analyzed with contingency tables.The harvest biomass data were analyzed in SAS 9.2 and JMP 8 (SAS Institute, Inc., Cary, NC). Correlations among plant parts were determined for the entire sample and for individual treatments using Pearson's correlation coefficient. Differences among water level treatments were analyzed using a mixed model ANOVA for a randomized complete block experimental design with replication. Tanks were blocks and were modeled as random factors, water levels were fixed treatments within blocks, and individual plants were replicates within treatments. If necessary, data were log (biomass data), square-root or arcsine square root (proportional data) transformed to approximate normal distributions for analysis, but graphs and tables present means and standard errors of untransformed data. Post-hoc comparisons among treatments were made with Tukey's HSD tests. Differences were considered significant if $p \leq 0.05$.

Relations between morphological parameters (e.g., lamina length, petiole length, lamina area) or between morphological parameters and biomass were analyzed using linear regression. Slopes of regression lines were compared with Student's *t*-tests.

Table 1
Temperature differences (°C) at pot soil surface for plants at 30, 60 and 90 cm water depths in mesocosms for July 2006 and December 2006. Data are mean ± SD.

Depth contrast	30 vs. 60 cm	30 vs. 90 cm	60 vs. 90 cm
July 2006			
Noon to 6 PM	1.4 ± 1.1	2.0 ± 1.4	0.7 ± 1.1
Midnight to 6 AM	0.0 ± 0.4	0.2 ± 0.6	0.1 ± 0.5
December 2006			
Noon to 6 PM	0.6 ± 1.0	1.2 ± 0.9	0.6 ± 0.6
Midnight to 6 AM	−0.1 ± 0.5	0.0 ± 0.4	0.2 ± 0.5

3. Results

3.1. Tank environment

The midday pH of water in the tanks was 7.7 ± 0.5, while dissolved oxygen measured concurrently was 6.6 ± 2.0 mg/L. Water temperature varied among water levels and the degree of difference among water levels varied daily and seasonally. In general water at 30 cm was warmer than water at 60 cm, which was warmer than water at 90 cm, but differences among water levels were greater in the day than during the night and greater in the summer months than the winter months (Table 1).

3.2. Seasonality of N. odorata growth at different water depths

Leaf production varied significantly with season ($F = 25.25$, $p < 0.0001$, repeated measures ANOVA), as more leaves were produced in warmer months with longer days (March through July) at all water levels (Fig. 1A). The rate of leaf production was significantly less in deeper water ($F = 33.75$, $p < 0.0001$, repeated measures ANOVA) (Fig. 1A). Rates of leaf production declined and converged among water levels from August through November Although there were significant differences in rates of leaf production among tanks ($F = 3.30$, $p = 0.0014$, repeated measures ANOVA), no significant water level by tank interaction was found ($F = 0.93$, $p = 0.5361$, repeated measures ANOVA).

Seasonal variation in leaf number paralleled seasonal variation in rates of leaf production, so plants at all water levels increased leaf numbers from January through March, had the greatest numbers of leaves in June and July and had declining numbers of leaves in August through November (Fig. 1B). Differences in leaf number among water levels were also greatest from March through July, with plants in 60 cm and 90 cm of water resembling each other in the number of leaves on the main shoot apex and plants in 30 cm of water differing from these two (Fig. 1B). The differences among plants at different water levels decreased as the number of leaves present declined from August through November on plants at all water levels (Fig. 1B). Rates of leaf senescence also varied with water depth. More leaves died each month on plants in 30 cm water (3.9 ± 0.1, avg. ± SE) than on plants in 60 cm

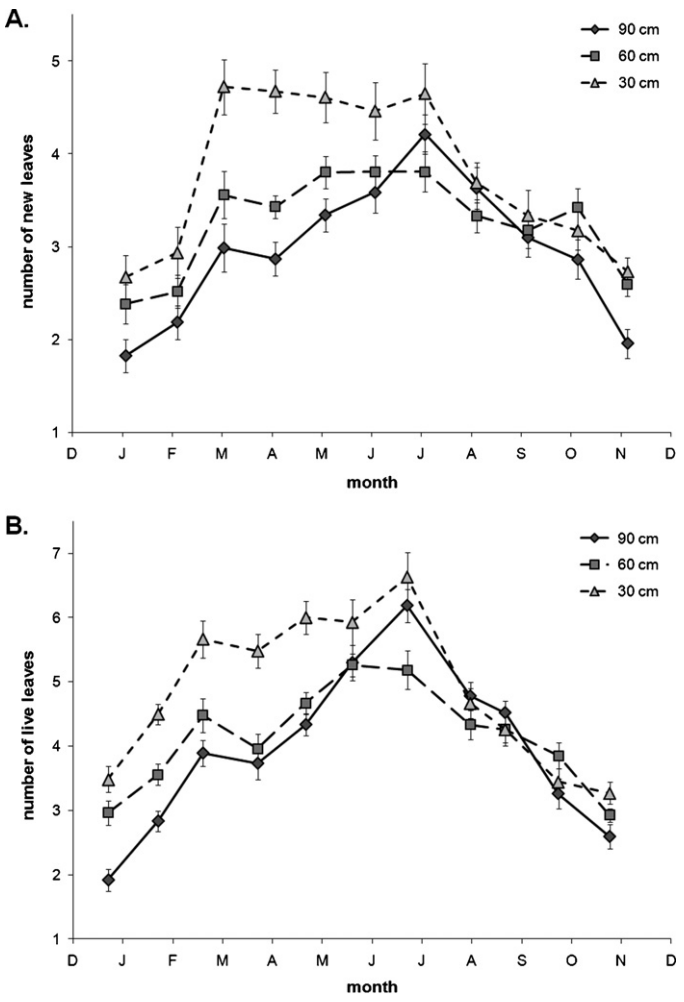

Fig. 1. Monthly rates of leaf production (A) and number of leaves on the main shoot apex (B) for plants in mesocosms at 30, 60 and 90 cm water depths. Data are means ± SE from January 2006 through November 2006.

(3.3 ± 0.1) or 90 cm (3.0 ± 0.1) water, especially in the warmer months.

Plants in 30 cm water branched more than plants in 60 cm and 90 cm water, resulting in more shoot apices per pot in shallow water (median number of apices = 2 in 30 cm water but 1 in 60 and 90 cm water). Leaves on plants in 30 cm water were smaller than leaves on plants in deeper water (Fig. 2) and had shorter petioles (30 cm = 26.9 ± 0.3 cm, 60 cm-level = 53.8 ± 0.3 cm, 90 cm = 82.4 ± 0.3 cm, mean ± SE). Leaves on plants at 60 cm depths were intermediate in size (Fig. 2). Differences among water levels in leaf measurements were significant ($p < 0.0001$ for all variables, repeated measures ANOVA). All leaf measurements also differed significantly among months ($p < 0.001$), indicating that leaf characteristics changed seasonally. Laminae size increased during the summer, peaking in July and August, then began to decrease (Fig. 2). This seasonal variation was greatest in plants in 90 cm water (Fig. 2). Variation in petiole length did not show as marked a seasonal pattern, as water depth dominated variation in this variable.

Plants produced more flowers from May through September. All three water levels showed this seasonality in flower production, although the 60 cm and 30 cm water levels began vigorous flowering earlier than the 90 cm water level. Flowering did not differ significantly among water levels (likelihood ratio $\chi^2 = 0.66$, $p = 0.72$).

Table 2
Morphological (A) and biomass (B) measurements for individual *N. odorata* leaves harvested in November 2006 from plants growing in mesocosms at 30, 60 and 90 cm of water. Data are mean ± SE for two leaves per plant (the most recently matured and one other) from 27 plants per water depth. Different letters to the right of values in a row indicate that these means were significantly different among water levels.

	90 cm	60 cm	30 cm
(A)			
Lamina length to petiole (cm)	9.6 ± 0.3[a]	8.6 ± 0.2[b]	6.2 ± 0.2[c]
Total lamina length (cm)	16.2 ± 0.3[a]	14.4 ± 0.3[b]	10.5 ± 0.3[c]
Lamina width (cm)	17.1 ± 0.3[a]	14.9 ± 0.2[b]	10.9 ± 0.3[c]
Lamina area (cm^2)	222.9 ± 8.3[a]	171.6 ± 5.7[b]	91.5 ± 4.5[c]
Petiole length (cm)	85.3 ± 0.9[a]	59.5 ± 0.9[b]	34.3 ± 0.9[c]
Petiole width (mm)	4.3 ± 0.1[a]	4.1 ± 0.1[a]	3.6 ± 0.1[b]
(B)			
Lamina weight (mg)	1801.1 ± 82.4[a]	1381.8 ± 51.1[b]	713.2 ± 36.2[c]
Specific lamina weight (mg/cm^2)	7.7 ± 0.2[a]	8.0 ± 0.1[a]	7.8 ± 0.1[a]
Petiole weight (mg)	1043.4 ± 33.4[a]	685.8 ± 23.8[b]	302.0 ± 13.6[c]
Petiole wt. per length (mg/cm)	12.3 ± 0.4[a]	11.6 ± 0.4[a]	8.9 ± 0.3[b]
Petiole wt. to total leaf wt.	0.37 ± 0.01[a]	0.33 ± 0.00[a]	0.30 ± 0.01[b]

3.3. Variations in *N. odorata* leaf morphology and biomass with water depth

Size of leaves harvested in November 2006 increased with increasing water depth, whether the variable measured was lamina length to petiole or total lamina length, lamina width, lamina area, petiole length or petiole width (Table 2). All of the leaf morphological variables differed significantly among water levels ($p < 0.0001$, ANOVA). Variables were significantly different between each water level except for petiole width, which was similar between 60 cm and 90 cm water levels, but petioles from these levels were significantly wider than petioles at the 30 cm water level (Table 2).

Measurements of lamina length to petiole attachment, total lamina length, lamina width, petiole length and petiole width for individual leaves were highly positively correlated ($R > 0.70$, $p < 0.0001$), so leaves with longer laminae tended to be wider and to have longer, thicker petioles. Laminae on plants in different water levels had similar scaling relationships, as reflected in similar slopes for lines fitted to data from each water level (Fig. 3, Table 3).

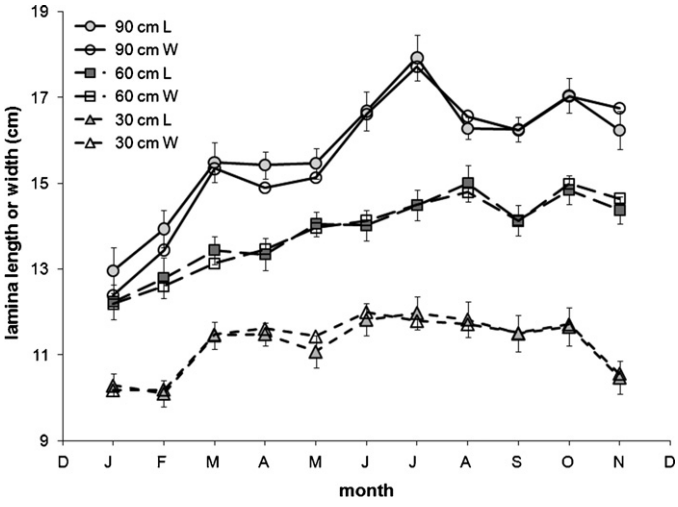

Fig. 2. Lamina length (solid symbols) and lamina width (open symbols) of the most recently matured leaf on *N. odorata* plants growing in mesocosms at 30, 60 and 90 cm water depths from January 2006 through November 2006. Data are means ± SE are shown for lamina length only.

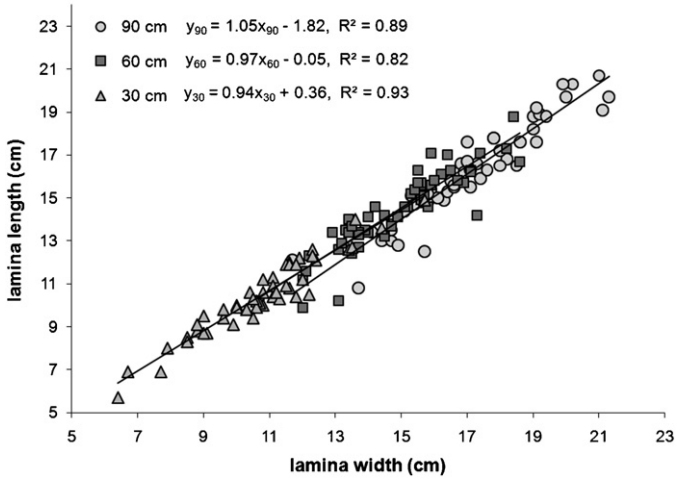

Fig. 3. Relation of lamina width to total lamina length for leaves of *N. odorata* grown for 13 months in mesocosms at 30, 60 and 90 cm water depths and sampled November 2006. Data are from the most recently matured leaf and one other leaf on the plant for each plant. Equations are for regression lines fitted to leaves from each water level.

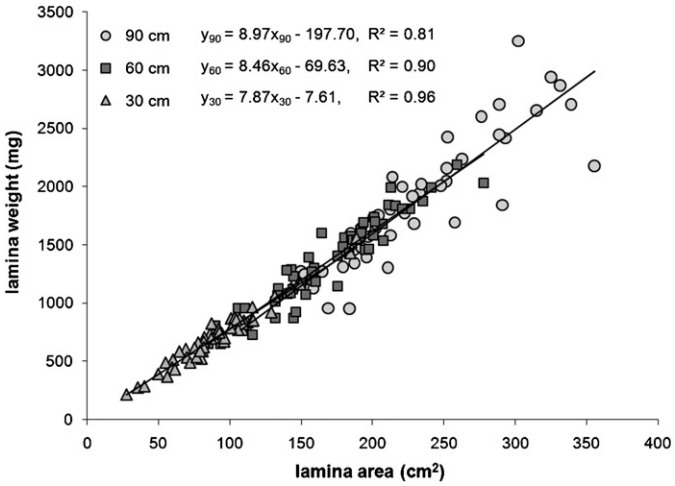

Fig. 4. Relation of lamina area to lamina weight for leaves of *N. odorata* grown in mesocosms at 30, 60 and 90 cm water depths and sampled November 2006. Data are from the most recently matured leaf and one other leaf on the plant for each plant. Equations are for regression lines fitted to leaves from each water level.

Correlations of lamina variables to petiole width also had similar slopes, although with more variation and lower correlation coefficients (range for $R = 0.48–0.74$, $p \leq 0.0002$ for all comparisons). When we modeled leaf area from lamina length and width, the modeled lamina area (LA_{mod}) was highly correlated with measured lamina area (LA_{mea}; $LA_{mod} = 0.98LA_{mea} + 3.96$, $R^2 = 0.99$), and this relationship was similar among water depths (Table 3).

Petiole length increased dramatically with increased water level, and this increase was correlated to increase in size of other parts of the leaf. Thus, the relationship of petiole length to lamina width for all plants showed a significant positive correlation ($R = 0.78$). At any one water level, however, variations in petiole length were not significantly correlated to variation in other parts of the leaf (range for $R = -0.08 – 0.27$, $p > 0.05$ for all comparisons). Linear regressions of petiole length vs. other leaf parts for plants from each water level had shallow (30 cm) or no (60, 90 cm) slopes and non-significant R^2. Thus, petioles scaled with other leaf measurements between water levels but not within water levels.

Petiole weight and lamina weight of individual leaves increased significantly with increasing water level ($p < 0.0001$, ANOVA) (Table 2B). Lamina weight (LW) was highly correlated with lamina area ($LW = 8.43LA – 66.78$; $R^2 = 0.93$), and this correlation was similar among water levels (Fig. 4, Table 3). Specific lamina weight (biomass/unit area) did not differ significantly among water levels ($p = 0.1997$, ANOVA), but petiole weight per unit length did ($p < 0.0001$, ANOVA). The petiole biomass per unit length in 30 cm water was significantly less than in deeper water (Table 2B).

Petiole weight of individual leaves was correlated with petiole length ($R^2 = 0.73$), although, as with the morphological variables, this correlation weakened or disappeared when correlations were examined separately at each water level. Petiole weight and length were weakly correlated in shallow water ($R^2 = 0.20$, $p = 0.0009$) but were not significantly correlated at the deeper water levels. As water depth increased, the petiole comprised a greater proportion of total leaf weight (Table 2B).

Table 3
Comparison of slopes for regressions of lamina measurements for leaves on plants grown for 13 months in 30, 60, and 90 cm of water, then harvested in November 2006. Data are Student's *t*-test followed in parentheses by *p*, the probability of observing a greater *t* when comparing slopes for regressions. Lamina $area_{mea}$ = measured lamina area; lamina $area_{mod}$ = lamina area modeled from lamina length and width measurements (see text). $N = 98–102$ leaves.

Depth contrast	30 vs. 60 cm	30 vs. 90 cm	60 vs. 90 cm
Lamina width × lamina length	0.44 (0.66)	−1.76 (0.08)	1.00 (0.32)
Lamina area × lamina weight	1.23 (0.22)	−1.65 (0.10)	0.69 (0.49)
Lamina $area_{mea}$ × lamina $area_{mod}$	−1.70 (0.09)	1.68 (0.10)	−0.29 (0.77)

3.4. Variation in N. odorata biomass in response to water depth

Plant total biomass at the November harvest did not differ significantly among water levels, but biomass allocation to different plant parts did (Table 4A). Plants in 90 cm and 60 cm water depths did not differ significantly from each other in any biomass variable for plant parts (total lamina weight, total petiole weight, total leaf weight, rhizome weight, root weight, and total shoot weight), but plants at these water levels did differ significantly from plants in 30 cm water in everything but root weight (Table 4A).

Plants in deeper water had significantly greater total leaf weight, comprised of heavier laminae and petioles, than plants in 30 cm water, but deeper water plants had fewer of these leaves (Table 4A, Fig. 1B). Although plants in 30 cm water had more leaves on the main shoot apex, part of the greater total number of leaves on these plants was a function of increased branching, as reflected in the

Table 4
(A) Biomass of different plant parts and counts of number of leaves and shoot apices and (B) biomass allocation for *N. odorata* plants grown for 13 months in 30, 60 and 90 cm of water then harvested in November 2006; $N = 81$ (27 for each water level). Data are means ± SE. Different letters to the right of values in a row indicate that these water levels were significantly different for this variable.

	90 cm	60 cm	30 cm
(A)			
Biomass (g)			
Total leaf weight	9.9 ± 0.7[a]	8.5 ± 0.4[a]	6.2 ± 0.5[b]
Lamina weight	6.1 ± 0.5[a]	5.5 ± 0.3[a]	4.2 ± 0.3[b]
Petiole weight	3.9 ± 0.3[a]	2.9 ± 0.1[a]	2.0 ± 0.1[b]
Rhizome weight	51.7 ± 3.0[a]	50.0 ± 1.7[a]	65.5 ± 3.2[b]
Total shoot weight	61.7 ± 3.4[a]	58.6 ± 1.8[a]	71.7 ± 3.3[b]
Root weight	38.8 ± 2.1[a]	34.8 ± 1.4[a]	33.1 ± 1.7[a]
Total plant weight	100.5 ± 4.5[a]	93.4 ± 2.6[a]	104.9 ± 4.7[a]
Number of leaves	3.3 ± 0.3[a]	4.1 ± 0.2[a]	6.2 ± 0.4[b]
Number of apices	2.1 ± 0.1[a]	2.0 ± 0.1[a]	2.8 ± 0.1[b]
(B)			
Biomass allocation (%)			
Leaves	10.0 ± 0.7[a]	9.2 ± 0.4[a]	6.0 ± 0.4[b]
Rhizomes	51.2 ± 1.4[a]	53.6 ± 1.0[a]	62.3 ± 0.9[b]
Roots	38.7 ± 1.3[a]	37.2 ± 1.1[a]	31.7 ± 0.9[b]
Root:shoot ratio	0.65 ± .04[a]	0.60 ± .03[a]	0.47 ± .02[b]

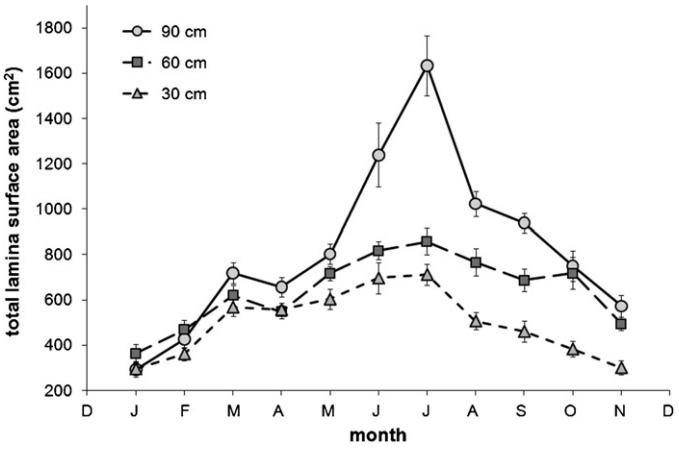

Fig. 5. Estimated monthly total lamina area present on the main rhizome of *N. odorata* plants grown in mesocosms at 30, 60 and 90 cm water depths. Data are means ± SE from January 2006 through November 2006.

greater number of apices on these plants (Table 4). Leaves on branch apices were usually much smaller than leaves on the main apex and thus weighed less.

Plants in 30 cm water had significantly heavier rhizomes than plants in deeper water (Table 4A). This difference in rhizome weight contributed to a greater total shoot biomass (leaves + rhizome + flowering parts) for plants in 30 cm water. Root weight differences among levels were not significant (Table 4).

Irrespective of treatment, plants had relatively more biomass allocated to rhizomes (>50%), followed by roots (30–40%), then leaves (<10%; Table 4B). Plants at 60 cm and 90 cm water depths, however, invested relatively more biomass in leaves and roots than plants at 30 cm depth (Table 4B). Root:shoot ratios summarized these differences in biomass allocation: plants in deeper water had greater root:shoot ratios than plants in 30 cm water (Table 2). All of these differences in relative biomass allocation were significant between plants in 30 cm water compared to plants in 60 and 90 cm water levels (Table 2).

3.5. Seasonal variations in photosynthetic surface area with water depth

Photosynthetic surface area per rhizome apex varied seasonally and with water depth (Fig. 5). The differences among water levels in lamina area and leaf longevity combined to produce much larger total lamina surface areas on plants in deeper water, especially from June through September for plants in 90 cm water (Fig. 5). Because the floating laminae were the major photosynthetic surfaces and because leaf specific weight was similar at all water depths, this greater surface area indicated a greater photosynthetic capacity and greater total productivity for plants in 90 cm water. Plants in 60 cm water levels had greater total lamina surface area than plants in 30 cm water for these same months, but they did not show the large peak in total leaf surface area exhibited by plants in 90 cm water (Fig. 5).

4. Discussion

Water depth affects rates of leaf production and senescence, leaf morphology, and plant biomass allocation in *N. odorata*. Leaves on plants in deeper water were larger, weighed more and lived longer, but plants produced fewer of them. Water depth did not affect total plant biomass in our experiment, but plants in deeper water allocated relatively more biomass to leaves and to roots, while plants in 30 cm water allocated more biomass to rhizomes. Increased allocation to roots differentiates this floating-leaved plant from many emergent aquatic plants, which allocate relatively less biomass to roots in deeper water (Grace, 1989; Coops et al., 1996; Lentz and Dunson, 1998; Blanch et al., 1999; Vretare et al., 2001; Edwards et al., 2003; Busch et al., 2004; Smith and Brock, 2007). In addition to varying with water depth, lamina size and photosynthetic surface area also varied seasonally. Because we harvested plants for biomass and biomass allocation in November, when leaf area was converging among depths, our data provide a conservative estimate of biomass differences with water depth.

Weight of individual laminae was greater in deeper water, but because specific lamina weight did not differ among water levels (Table 2B), the biomass investment per unit lamina area was the same in larger and smaller leaves. Laminae were necessarily connected to the submerged rhizome and roots by the petioles, and the cost of this connection increased with water depth, as reflected in petiole weight. Petiole biomass was greater not only because petioles in deeper water traversed a greater distance to the surface, but also because deeper petioles had more biomass per unit length as compared to petioles in shallow water. If plants made the same sized laminae in deeper water but had to produce longer petioles to tether the laminae, the cost of the tether relative to photosynthetic tissue would increase. Making larger laminae in deeper water helped maintain a relatively high investment in photosynthetic vs. tethering tissue. Because this lamina-to-petiole scaling is a general problem for floating leaved aquatics, we speculate that all floating-leaved species will produce larger laminae in deeper water.

Increased lamina size, however, had a cost in increased biomass per unit length of petiole, so *N. odorata* plants in our experiment did not maintain the same lamina:petiole biomass ratio as water depth increased (Table 2B). Tethering larger leaves required a greater investment per unit length of petiole, either to support additional mechanical forces from wave action on the larger leaf or additional compressive forces from the increased weight of water on the petiole. The increased cost of tethering leaves with increased water depth may contribute to depth limits for floating-leaved species.

Previous descriptions of how water lily lamina size varies with water depth have been derived from field studies. A clear relationship between the two variables has not emerged from these studies, perhaps because seasonal and plant developmental effects were not differentiated from depth effects in these studies. Sinden-Hempstead and Killingbeck (1996) did not find a strong association between *N. odorata* lamina surface area and water depth for leaves sampled on an area basis from 30, 60 and 100 cm depths in three Rhode Island USA ponds, although they did find relatively strong differences between mid bed and edge bed plants; the edges of beds were generally deeper than the middles. However, they sampled all leaves from ½ m^2 floating plots and thus could not associate leaves with individual plants. Their harvested laminae differed in surface area from 9.1 to 376 cm^2. This range suggests that their sampling method confounded water depth effects with plant developmental effects, as the lower end of this range probably included smaller leaves from seedlings or branches.

Kunii and Aramaki (1992) found that *N. tetragona* leaves in a Japanese pond showed an increase in leaf area from May to June, when water depth was close to 100 cm, then a decrease in area between June and October that paralleled a decrease in water depth to 30–40 cm. The spring increase in leaf area under constant water depths could have reflected renewed spring growth after a decrease in leaf area at the end of the previous growing season, while the mid to late season decrease in leaf area reflected the decrease in water depth.

Paillisson and Marion (2006) found that petioles of *N. alba* were longer but thinner in higher water levels but laminae showed no consistent pattern of change in surface area in relation to water level. Their data, however, were collected from wild-harvested

leaves taken from an unknown number of plants in m^2 plots and may not have reflected individual plant responses to relatively precisely known water levels. They reported a lamina to petiole biomass ratio ranging from 1.32 to 2.01, with no pattern in relation to season, and by extension, water level variation (Paillisson and Marion, 2006), whereas the lamina to petiole biomass ratio from our data ranged from 0.87 to 3.47 (mean = 2.04) and differed significantly with water level.

Like a number of other floating-leaved aquatics, white water lily has a temperature-driven pressurized ventilation system that forces air from younger leaves to older leaves, causing the rhizome and root system to be aerated and flushed in the process (Grosse et al., 1996). Differences among water levels in leaf size and number, as well as in relative biomass allocation to roots and rhizomes, may be associated with the effectiveness of this aeration system. As leaf size increases, so does thickness of the boundary layer at the surface of the leaf; the net result of this increase for leaves in sunny environments is an increase in leaf temperature (Givnish, 1987). Such an increase in temperature should increase the effectiveness of pressurized ventilation. Position of a leaf along a rhizome affects the pressurized ventilation system in floating-leaved aquatics: gas efflux from leaves of *N. alba* peaked in the 4th oldest leaf, then decreased through the 9th leaf (Grosse, 1996). Thus, in *N. odorata* the increase in lamina surface area of plants in deeper water may allow for increased pressurization of a single leaf, while the fewer leaves present on each rhizome apex may provide for more directed air flow. How differences in leaf size, number and position affect root and rhizome aeration is unknown; similarly, we do not know how the cooler temperatures in deeper water affect air flow, but we expect a decline. Understanding how morphological and growth differences affect pressurized ventilation will help predict water lily and other floating-leaved species' responses to variation in water levels.

Competitive exclusion by emergents that can shade out floating-leaved plants may be a direct cause of the sharp zonation found at the shallow end of wetland water depth gradients (Keddy, 2000). Our study shows, however, that *N. odorata* responded to water depth alone by allocating relatively more biomass to non-photosynthetic rhizomes in shallow water. In the natural ecosystem shallow environments are more likely to dry down completely; increased storage in rhizomes may be selected for in shallow environments in order to support regeneration after a complete dry-down. This change in photosynthetic allocation, however, may also contribute to a reduced competitiveness relative to emergents in these environments.

The allometric relationships developed in this paper provide a basis for estimating *N. odorata* lamina area and biomass from simple linear measurements, since lamina length and width can be used to accurately estimate lamina area, and lamina area is correlated with lamina biomass. The relationship between the modeled and measured lamina areas at different water depths was similar to the relationship for the entire data set and had similar high R^2. The model we developed used both lamina length and width to estimate lamina area. Alternatively, using lamina length alone to model a circular lamina ($LA_{mod} = \pi(LL/2)^2$) also provided a good estimate of lamina area with a relatively high correlation ($R^2 = 0.91$). Similar equations have been used to estimate lamina area and/or weight in other nymphaeids (Brock et al., 1983; Tsuchiya and Nohara, 1989; Kok et al., 1990; Kunii and Aramaki, 1992).

5. Conclusions

N. odorata plants have greater photosynthetic surface area and relatively greater biomass allocation to leaves and roots in deeper water. Although plants can grow and flower in shallow water, they allocate relatively more biomass to rhizomes under these conditions. The greatly increased summer photosynthetic surface area of *N. odorata* plants in our deepest water treatment suggests that during southern Florida's summer wet season, deeper water levels (≥75 cm) are closer to optimal growth conditions. These results support setting relatively deep (≥45 cm) annual water depth targets for slough restoration in southern Florida's Everglades.

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References

Blanch, S.J., Ganf, G.G., Walker, K.F., 1999. Growth and resource allocation in response to flooding in the emergent sedge *Bolboschoenus medianus*. Aquat. Bot. 63, 145–160.

Brock, T.C.M., Arts, G.H.P., Goossen, I.L.M., Rutenfrans, A.H.M., 1983. Structure and annual biomass production of *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae). Aquat. Bot. 17, 167–188.

Brock, T.C.M., Van der Velde, G., Van de Steeg, H.M., 1987. The effects of extreme water level fluctuations on the wetland vegetation of a nymphaeid dominated oxbow lake in the Netherlands. Arch. Hydrobiol. 27, 57–73.

Busch, J., Mendelssohn, I.A., Lorenzen, B., Brix, H., Miao, S., 2004. Growth responses of the Everglades wet prairie species *Eleocharis cellulosa* and *Rhynchospora tracyi* to water level and phosphate availability. Aquat. Bot. 78, 37–54.

Coops, H., Van den Brink, F.W.B., Van der Velde, G., 1996. Growth and morphological responses of four helophyte species in an experimental water-depth gradient. Aquat. Bot. 54, 11–24.

David, P.G., 1996. Changes in plant communities relative to hydrologic conditions in the Florida Everglades. Wetlands 16, 15–23.

Den Hartog, C., Van der Velde, G., 1988. Structural aspects of aquatic plant communities. In: Symoens, J.J. (Ed.), Vegetation of Inland Waters (Handbook of Vegetation Science, pt. 15/1). Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 113–154.

Duever, M.J., 1982. Hydrology-plant community relationships in the Okefenokee Swamp. Fl. Sci. 45, 171–176.

Edwards, A.L., Lee, D.W., Richards, J.H., 2003. Responses to a fluctuating environment: effects of water depth on growth and biomass allocation in *Eleocharis cellulosa* Torr. (Cyperaceae). Can. J. Bot. 81, 964–975.

Givnish, T.J., 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. New Phytol. 106, 131–160.

Givnish, T.J., 2002. Ecological constraints on the evolution of plasticity in plants. Evol. Ecol. 16, 213–242.

Givnish, T.J., Volin, J.C., Owen, V.D., Volin, V.C., Muss, J.D., Glaser, P.H., 2008. Vegetation differentiation in the patterned landscape of the central Everglades: importance of local and landscape drivers. Global Ecol. Biogeogr. 17, 384–402.

Grace, J.B., 1989. Effects of water depth on *Typha latifolia* and *Typha domingensis*. Am. J. Bot. 76, 762–768.

Grosse, W., 1996. Pressurised ventilation in floating-leaved aquatic macrophytes. Aquat. Bot. 54, 137–150.

Grosse, W., Jovy, K., Tiebel, H., 1996. Influence of plants on redox potential and methane production in water-saturated soil. Hydrobiologia 340, 93–99.

Gunderson, L.H., 1994. Vegetation of the Everglades: determinants of community composition. In: Davis, S.M., Ogden, J.C. (Eds.), Everglades, the Ecosystem and its Restoration. St. Lucie Press, Delray Beach, FL, pp. 323–340.

Jordan, F., Jelks, H.L., Kitchens, W.M., 1997. Habitat structure and plant community composition in a northern Everglades wetland landscape. Wetlands 17, 275–283.

Keddy, P.A., 2000. Wetland Ecology. Cambridge University Press, Cambridge, England.

King, R.S., Richardson, C.J., Urban, D.L., Romanowicz, E.A., 2004. Spatial dependency of vegetation—environment linkages in an anthropogenically influenced wetland ecosystem. Ecosystems 7, 75–97.

Kirkman, L.K., Sharitz, R.R., 1993. Growth in controlled water regimes of three grasses common in freshwater wetlands of the southeastern USA. Aquat. Bot. 44, 345–359.

Kok, C.J., Van der Velde, G., Landsbergen, K.M., 1990. Production, nutrient dynamics and initial decomposition of floating leaves of *Nymphaea alba* L. and *Nuphar lutea* (L.) Sm. (Nymphaeaceae) in alkaline and acid waters. Biogeochemistry 11, 235–250.

Kunii, H., Aramaki, M., 1992. Annual net production and life-span of floating leaves in *Nymphaea tetragona* Georgi: a comparison with other floating-leaved macrophytes. Hydrobiologia 242, 185–193.

Leiffers, V.J., Shay, J.M., 1981. The effects of water level on the growth and reproduction of *Scirpus maritimus* var. *paludosus*. Can. J. Bot. 59, 118–121.
Lentz, K., Dunson, W.A., 1998. Water level affects growth of endangered northeastern bulrush, *Scirpus ancistrochaetus* Schuyler. Aquat. Bot. 60, 213–219.
Loveless, C.M., 1959. A study of the vegetation in the Florida Everglades. Ecology 40, 1–9.
Luo, W.B., Song, F.B., Xie, Y.H., 2008. Trade-off between tolerance to drought and tolerance to flooding in three wetland plants. Wetlands 28, 866–873.
Macek, P., Rejmankova, E., Houdkova, K., 2006. The effect of long-term submergence on functional properties of *Eleocharis cellulosa* Torr. Aquat. Bot. 84, 251–258.
McKee, K.L., Mendelssohn, I.A., 1989. Response of a freshwater marsh plant community to increased salinity and increased water level. Aquat. Bot. 34, 301–316.
Ogden, J.C., 2005. Everglades ridge and slough conceptual ecological model. Wetlands 25, 810–820.
Olmsted, I., Armentano, T.V., 1997. Vegetation of Shark Slough, Everglades National Park. South Florida Natural Resources Center. Everglades National Park, Homestead, FL, 41 pp.
Paillisson, J.M., Marion, L., 2006. Can small water level fluctuations affect the biomass of *Nymphaea alba* in large lakes? Aquat. Bot. 84, 259–266.
Richards, J.H., Philippi, T.E., Kalla, P., Scheidt, D., 2008. Characterization of southern Florida marsh vegetation using a landscape scale random sample: R-EMAP Phase III vegetation sampling. In: GEER 2008—Greater Everglades Ecosystem Restoration: Planning, Policy and Science Meeting , Naples, FL, pp. 206–207.
Saunders, C.J., Jaffe, R., Gao, M., Anderson, W., Lynch, J.A., Childers, D.L., 2008. Decadal to millennial dynamics of ridge-and-slough wetlands in Shark Slough, Everglades National Park: Integrating Paleoecological Data and Simulation Modeling. Cooperative Agreement No. (GA) 5280-00-007. Everglades National Park, Homestead, FL.
Science Coordinating Team, 2003. The Role of Flow in the Everglades Ridge and Slough Landscape. United States Geological Survey, 62 pp.
Sculthorpe, C.D., 1967. The Biology of Aquatic Vascular Plants. Edward Arnold, London, UK.
Sinden-Hempstead, M., Killingbeck, K.T., 1996. Influences of water depth and substrate nitrogen on leaf surface area and maximum bed extension in *Nymphaea odorata*. Aquat. Bot. 53, 151–162.
Smith, R.G.B., Brock, M.A., 2007. The ups and downs of life on the edge: the influence of water level fluctuations on biomass allocation in two contrasting aquatic plants. Plant Ecol. 188, 103–116.
Spence, D.N.H., 1982. The zonation of plants in freshwater lakes. Adv. Ecol. Res. 12, 37–125.
Stober, Q.J., Thornton, K., Jones, R., Richards, J., Ivey, C., Welch, R., Madden, M., Trexler, J., Gaiser, E., Scheidt, D., Rathbun, S., 2001. South Florida Ecosystem Assessment: Phase I/II—Everglades Stressor Interactions: Hydropatterns, Eutrophication, Habitat Alteration, and Mercury Contamination (Summary). United States Environmental Protection Agency, Region 4 Science and Ecosystem Support Division, Water Management Division and Office of Research and Development, Athens, GA, 63 pp.
Troxler, T.G., Richards, J.H., 2009. delta C-13, delta N-15, carbon, nitrogen and phosphorus as indicators of plant ecophysiology and organic matter pathways in Everglades deep slough, Florida, USA. Aquat. Bot. 91, 157–165.
Tsuchiya, T., Nohara, S., 1989. Growth and life span of *Nelumbo nucifera* Gaertn. leaves in Lake Kasumigaura, Japan. Aquat. Bot. 36, 87–95.
Vretare, V., Weisner, S.E.B., Strand, J.A., Graneli, W., 2001. Phenotypic plastcity in *Phragmites australis* as a functional response to water depth. Aquat. Bot. 69, 127–145.
White, P.S., 1994. Synthesis: vegetation pattern and process in the Everglades ecosystem. In: Davis, S.M., Ogden, J.C. (Eds.), Everglades, the Ecosystem and its Restoration. St. Lucie Press, Delray Beach, FL, pp. 445–458.
Whittaker, R.H., 1967. Gradient analysis of vegetation. Biol. Rev. 42, 207–264.
Wood, J.M., Tanner, G.W., 1990. Graminoid community composition and structure within four everglades management areas. Wetlands 10, 127–149.