The effects of flooding regime on the rare Atlantic coastal plain species *Hydrocoytle umbellata*

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Abstract: The hydrological regime on many lakes is changing through climate change, construction of dams, and land-use changes. We examined the effects of flooding regime on growth and development of the endangered semiaquatic species, *Hydrocotyle umbellata* L. (water pennywort), in a greenhouse experiment. Growth was twice as fast under dry, above-water (terrestrial) conditions as under flood conditions. Depth of flooding (15 or 30 cm) had little impact. Short-term floods at either the middle or end of the growing season were just as damaging as continuous flooding. The decrease in growth under flood conditions was largely due to a temporary reduction in unit leaf rate (growth per unit leaf area) rather than to a reduction in leaf area ratio (leaf area per unit mass). Flooded plants allocated more resources to the production of petioles at the expense of reproductive structures and to a lesser extent, belowground structures. They also produced fewer, larger leaves with more stomata on the upper than on the lower surface. These changes in structure were largely restricted to new leaves produced under flood conditions; most of the mature leaves produced in a terrestrial environment senesced upon flooding. Chemical analysis of tubers produced by plants grown under terrestrial conditions indicates that these structures function in carbohydrate storage. The likely impact of changes in hydrological regime on this endangered species is discussed in light of these findings.

Key words: altered hydrological regime, flooding stress, tubers, pulsed resources, temporal environmental variability.

Résumé : Sous l'influence du changement climatique et de l'utilisation des terres, ainsi que de la construction de réservoirs, les régimes hydrologiques de plusieurs lacs sont modifiés. Les auteurs ont conduit une expérience en serre, pour examiner les effets des régimes de la submersion sur le développement de l'espèce semi-aquatique menacée *Hydrocotyle umbellata* L. Les taux de croissance sont deux fois plus rapides en conditions exondées qu'en état de submersion. La profondeur de submersion (15 vs. 30 cm) a peu d'impact. Les submersions de courte durée, au milieu ou à la fin de la saison de croissance, sont tout aussi dommageables que la submersion continue. La diminution de croissance par unité de surface foliaire) plutôt qu'à une réduction du ratio de surface foliaire (surface foliaire par unité de masse). Les plantes submergées allouent plus de ressources à la production des pétioles aux dépens de la reproduction et, à un moindre degré, aux structures hypogées. Elles produisent également moins de feuilles qui sont plus grandes, avec plus de stomates sur la face supérieure, que sur la face inférieure. Ces changements de structure sont surtout restreints aux nouvelles feuilles produites au cours de la submersion; la plupart des feuilles matures produites en condition exondée dépérissent en conditions submergées. L'analyse chimique des tubercules produits par les plantes venues sous des conditions exondées indique que ces structures fonctionnent comme réserve de glucides. À la lumière de ces résultats, les auteurs discutent des impacts des changements de régimes hydrologiques, sur cette espèce menacée.

Mots-clés : altération des régimes hydrologiques, stress par submersion, tubercules, ressources pulsées.

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Introduction

Hydrocotyle umbellata L. (water pennywort) is a member of the Atlantic Coastal Plain flora (ACPF). This is a unique flora that includes many rare or endangered species whose range extends along the eastern seaboard of North America and reaches its northernmost extension in southwestern Nova Scotia (Keddy 1985; Roland 1991). Species of the ACPF grow in moist and wet freshwater areas on gradual, sloping shorelines of sand or gravel and are usually associated with lakes that have large water-level fluctuations

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(Keddy and Wisheu 1989; Wisheu and Keddy 1989). In Canada, *H. umbellata* only occurs on limited sections on the shorelines of two lakes in southwestern Nova Scotia, Kejimkujik Lake in Kejimkujik National Park and Wilson's Lake in the Tusket River valley (Newell 1999; Vasseur 2005).

Although *H. umbellata* is endangered in Canada (Newell 1999), relatively little is known about its ecology and general biology (Wisheu and Keddy 1989; Sweeney and Ogilvie 1993; Vasseur et al. 2002; Vasseur 2005). It is a small perennial herbaceous plant of the family Apiaceae that produces elongate rhizomes with round or kidney-shaped leaves on long petioles. The primary form of reproduction is asexual by means of rhizome propagation (Vasseur 2005). Although *H. umbellata* may flower, it has not been observed to set viable seed in Nova Scotia (Vasseur 2005). Rhizome length, leaf size, and petiole length all exhibit a high degree

of phenotypic plasticity (Vasseur 2005). The species is known to produce tuberous growths on its rhizomes, but the function of these structures is unknown (Coulter and Rose1900).

Lakes where *H. umbellata* is found typically have water levels that peak in winter and gradually fall over the course of the spring and summer reaching minimum levels in late summer or early fall (Hazel 2004; Lusk 2006). Hydrocotyle umbellata occupies the lower shoreline in both the wet meadow and marsh zones (Keddy 1991) and are normally exposed to the atmosphere by July or August of most years, but heavy precipitation events can cause prolonged flooding at any time of the year (Lusk 2006). Thus, it is clear that H. umbellata can survive (at least temporarily) in both dry, above-water (hereinafter, terrestrial) and aquatic (flooded) habitats. However, it is unclear to what extent this species is capable of actually growing and propagating in each habitat and how switches between these two conditions impact success. Plants that can acclimate to either terrestrial or aquatic environments often differ in many respects when grown in these two habitats. These differences include the presence or absence of aerenchyma tissue, petiole orientation, stem/petiole elongation, leaf thickness, stomatal distribution, and allocation of energy to storage reserves (Kozlowski 1984; Crawford 1989; Insausti et al. 2001). In some species, leaves that are adapted to a terrestrial environment cannot change their morphology/physiology sufficiently to acclimate to an aquatic environment and must abort their mature leaves and produce a new set of leaves when subjected to flooding (Kozlowski 1984). Frequent fluctuations in water level, therefore, potentially have a marked negative effect on plant growth even in species that are well adapted to growing in either terrestrial or aquatic conditions. Given that the frequency, extent, and timing of flooding can be markedly altered by land-use changes in the surrounding watershed, by the construction of dams, and by climate change (O'Reilly Sternberg 1987; Keddy 1991; Hill et al. 1998), knowledge of how H. umbellata responds to waterlevel fluctuations is critical to the preservation of this endangered species.

This study examined the effect of flooding on the growth of *H. umbellata* in a controlled experiment. We addressed three questions: First, what is the rate of growth in an aquatic versus a terrestrial environment? Second, how does switching between an aquatic and a terrestrial environment affect the plant's growth? Third, does the timing of flooding have an effect on the capacity of the species to tolerate flooding? In addition to these three specific objectives we attempted to elucidate the mechanistic basis for the response of this species to flooding, using functional growth analysis (Hunt and Parsons 1974), assessing the effect of treatments (terrestrial vs. flooded) on dry-matter partitioning, and describing the changes in leaf morphology associated with treatments. We also noted over the course of this experiment that the production of underground tubers differed between treatments. As there is no information available in the literature on the function or composition of these structures, we determined the protein, lipid, nonstructural carbohydrate, structural carbohydrate, and mineral contents of these tubers to help elucidate their role.

Materials and methods

Standardized transplant plugs were prepared from H. umbellata material growing in the Harriet Irving Botanical Garden at Acadia University (material originally collected from Wilson's Lake, Yarmouth County, N.S.). Ten-centimetre long rhizome fragments that contained three to four ca. 10 mm diameter leaves were collected and planted in pots (8 cm \times 8 cm \times 5.5 cm deep) filled with a mixture of peat moss (3% by mass), sand (85%), and clayloam soil (12%). This soil mixture was chosen to simulate the soil composition at one of the two sites in Canada where H. umbellata grows naturally, Wilson's Lake. (Hazel 2004). One rhizome fragment was planted per pot and allowed to establish under terrestrial conditions in a greenhouse for 15 d. Once transplants were established, ca. 0.54 g of a slow-release fertilizer (Smart Cote, 4 month release, N-P-K 12-12-12 (Plant Products Co. Ltd., Brampton, Ont.)) was mixed with the surface soil in each pot. Fertilizer was applied at one-third of the manufacturer's recommended rate $(240 \text{ g}\cdot\text{m}^{-2})$ to simulate relatively low nutrient conditions.

A watertight stainless-steel bench, 1.8 m × 2.74 m × 0.41 m, equipped with a pressurized water supply and an adjustable-height water-overflow outlet was used to impose flooding treatments. The bench, located in a glasshouse that received natural light, was filled to a depth of 35.5 cm. The experiment ran from 1 June to 23 September 2005. An immersed thermocouple allowed the computerized glasshousecontrol program (Argus Controls, White Rock, B.C.), to monitor and control water temperature, opening a solenoid valve allowing cooler water into the bench whenever the desired water temperature was exceeded. The added water came from a well and had a temperature range of 11-13 °C. The desired water temperature in the bench was changed over the duration of the experiment to coincide with seasonal water temperature at Wilson's Lake, N.S. (Lusk 2006). At the beginning of the test period water temperature was set at 18 °C and was increased to a maximum temperature of 21 °C by day 56 of the experiment. The drain on the bench was set at the desired water level (35.5 cm) so that when the water-inlet valve was open, the overflow water drained off. To ensure adequate mixing and aeration of the water in the bench, a 2990 L/h submersible pump was placed at one end of the bench and 4 m of hose was run down the middle of the bench and attached such that the end of the hose emptied just above the desired water level.

The experiment contained six treatments as follows: three had constant water levels set at 0 cm (nonflooded), 15 cm (intermediate), or 30 cm (maximum) above the level of the soil in the pots, and three simulated floods in early, mid-, and late summer. Forty-five plants were assigned to each of the three constant water-level treatments and grew at their assigned depths for the entire test period (106 d). The earlysummer flood treatment had 45 plants that grew under water for 34 d and then were moved to the above-water position for the remaining 72 d. The midsummer flood treatment had 36 plants that grew above-water for 34 d, then were dropped to the maximum depth for 35 d and then brought back up to the above-water position for the remaining 37 d of the experiment. The late-summer flood treatment had 27 plants that grew at the highest level (water surface) for 69 d and then dropped to the maximum depth for the last 37 d of the experiment. The midsummer and late-summer flood treatments had fewer plants than the other treatments because they were identical to the nonflooded treatment prior to flooding.

The different flooding regimes were simulated in the bench by constructing platforms so that depth could be manipulated. At the beginning of the experiment each platform was 30 cm in height. The plants in the flooded treatments were gradually submerged by lowering the platforms on a weekly basis to ensure that the new transplants were not overly stressed by flooding. All plants started at the highest level, which was at the water surface. After day 6, trays that contained the intermediate, maximum, and early-summer flood treatments were lowered by 7.5 cm. At day 13, the intermediate, maximum, and early-summer flood treatments were lowered by another 7.5 cm. At day 20, trays that contained the maximum and early-summer flood treatments were lowered to the bottom of the bench. The mid- and late-summer flood treatments were lowered to the maximum depth in one move, as opposed to the gradual lowering of the early-summer and constant-flood treatments.

To avoid confounding differences in light level with flooding treatment, plants were arranged in groups within the bench such that the high-elevation plants were far enough away from the low-elevation plants not to shade them. These groups of low, mid- and high elevation pots were, in turn, arranged in three blocks based upon position within the bench. Each block contained plants from each of the three elevation groups and all six treatments. The positions of groups within each of these blocks were randomly assigned. Thirty-four days into the experiment, plants were re-randomized within each group and the positions of groups were rotated. At day 69 this procedure was repeated.

An initial harvest of 12 plants was taken prior to the treatments. Once treatments were imposed, three plants (one from each block) were harvested from the nonflooded, the intermediate flood, the maximum flood, and the earlysummer flood treatments every 7 d for 76 d; the harvest day was then moved by 2 d from day 83 to day 85. The last three harvests were conducted in 7 d intervals starting at day 85. Harvesting of plants from the midsummer and late-summer flood treatments did not commence until days 27 and 48, respectively, from which point three plants (one from each block) were harvested each week. At each harvest we counted the leaves, measured the length of the longest petiole, and determined leaf area using a LI-COR area meter (model 3100, LI-COR Inc., Lincoln, Nebr.). Plants were then separated into rhizomes/roots, live petioles, live laminas, dead leaves (petioles plus laminas), and live flowers/ buds and oven-dried at 50 °C before weighing.

The Hunt growth-analysis program (Hunt and Parsons 1974) was used to fit polynomial curves to the natural logarithm transformed dry mass (ln y) and leaf area (ln z) time (t) data. Either linear or quadratic equations were fitted to the data. In no case was there a significant (p < 0.05) cubic term. The program calculated relative growth rate ((1/y)(dy/ dt)), leaf area ratio (z/y) and unit leaf rate ((1/z)(dy/dt)) along with their 95% confidence intervals from the fitted relationships. Separate curves were fitted to each treatment, but the same 12 plants from the initial harvest were used in fitting each of these relationships (i.e., it was the first har-

vest for each treatment). Proportional allocation of biomass to various plant organs (roots plus rhizomes, petioles, and laminas) was calculated from the dry-mass data, and specific leaf area was calculated as leaf area/mass of lamina. The GLM procedure of SAS[®] version 8.02 (SAS Institute Inc., Cary, N.C.) was used to determine if treatment had a significant effect on any of these dependent variables at final harvest. A randomized complete block design was used with flooding treatment as the single independent variable. All dependent variables were tested for normality prior to analysis. In cases where there was a significant *F*-test for the effect of treatment, a Student–Newman–Keuls multiple-range test was used to compare the individual treatment means. The 0.05 level of probability was used for all tests of significance.

Stomatal density on both the upper and the lower surface of the leaf blade was determined by taking impressions with cellulose acetate (North 1956). Fifteen leaves were sampled, 7 from plants grown in terrestrial conditions and 8 from plants grown under aquatic conditions. Only fully emerged leaves (i.e., leaf expansion had ceased) that were initiated in their respective environments were sampled.

To determine the chemical composition of the tubers, material was collected from plants growing under terrestrial conditions and oven-dried at 100 °C. Six dried samples weighing 8 g each, were sent to the Nova Scotia Agriculture and Fisheries Quality Evaluations Division, Truro, for chemical analysis. Plant material was ground to pass through a 1 mm mesh screen. Total nitrogen (N) was determined by combustion in the presence of helium and oxygen and analysis of the combustion gas by thermal conductivity using a LECO food protein analyzer (Leco Corp., St. Joseph, Mich.). Crude protein content was calculated as N content × 6.25. Fiber content (i.e., cellulose, hemicellulose, and lignin) was determined using the neutral detergent fiber technique (Van Soest et al. 1991). Crude fat content was determined by extraction with petroleum ether. Ash content was determined by combustion at 600 °C for 3 h in a muffle furnace. Nonstructural carbohydrate content was determined by subtracting the crude protein, fiber, fat, and organic acid content from total dry mass.

Results

Relative growth rate (RGR) for the nonflooded treatment decreased in a linear fashion over time (Fig. 1). Its initial value, however, was much greater than that of the two constant-flood treatments (Fig. 1). The initial values for these two treatments were negative, but they increased rapidly over time, surpassing the rate for the nonflooded plants by day 62, before declining (Fig. 1). Depth of flooding had no effect on RGR (Fig. 1). For the flooded treatments, RGR peaked at approximately 0.06 $g \cdot g^{-1} \cdot d^{-1}$, which was slightly lower than the starting RGR of the nonflooded plants (Fig. 1). From day 62 onward, there was no significant difference in RGR among the three constant-flood treatments (Fig. 1). For plants flooded early or late in the summer RGR was lowest during the flooded phase. The lowest RGR was seen in plants flooded early in the summer, but later in the summer, when in a terrestrial environment, these plants

Fig. 1. Effect of flooding regime on relative growth rate (growth per unit mass), leaf area ratio (leaf area per unit mass), and unit leaf rate (growth per unit leaf area) of *Hydrocotyle umbellata*over time. Solid symbols represent plants that were growing above-water and open symbols represent plants growing below water. Circles represent the nonflooded treatment; squares represent the intermediate flooding depth; triangles represent the maximum flooding depth; upside-down triangles represent the the early-summer flood; diamonds represent the midsummer flood; hexagons represent the late-summer flood treatments. Error bars represent 95% confidence intervals.



also had the highest RGR (Fig. 1). Plants flooded in midsummer exhibited little variation in RGR.

Leaf area ratio (LAR) first increased and then declined with time in all treatments (Fig. 1). There was no difference in LAR among the constant treatments at any time over the course of the experiment. The switched treatments (plants moved between a terrestrial and an aquatic environment), on the other hand, did display some difference in LAR, but the differences were small. The early-summer flood treatment had a higher LAR than the late-summer flood treatment in the latter part of the experiment (Fig. 1). LAR for the midsummer flood treatments, and for the most part, was not significantly different from either (Fig. 1). The unit leaf rate (ULR) for the nonflooded treatment was higher than that of the constant-flood treatments for the first 27 d (Fig. 1). ULR for the nonflooded plants decreased with time until day 34, when ULR became more or less constant (Fig. 1). The constant-flood treatments had a negative ULR at the beginning of the experiment; however it increased rapidly until day 34, when ULR became relatively constant, and similar to that of the nonflooded treatment (Fig. 1). The pattern of ULR over time in the early-summer flood treatment was similar to that of the constant-flood treatments. ULR in the late- and midsummer-flood treatments tended to be lower during the period of time plants were flooded.

At the final harvest, nonflooded plants were twice as large as plants in either of the constant-flood treatments (Table 1).

Table 1. Effect of flooding treatme	ent on growth, leaf mo	rphology and dry-matte	r partitioning at the final harvest.
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Variable	Nonflooded	Intermediate depth (15 cm)	Maximum depth (30 cm)	Early-summer flood	Midsummer flood	Late-summer flood
Total mass (ln g)	4.78a	2.42bc	2.32bc	4.00ab	2.10c	3.37abc
Root/rhizome allocation (%)	55.0a	52.5ab	45.5bc	61.2a	50.2abc	38.0c
Petiole allocation (%)	13.7c	28.0ab	34.0a	18.6bc	17.3c	19.1bc
Lamina allocation (%)	10.1a	11.8a	13.6a	17.3a	17.7a	10.4a
Reproductive allocation (%)	19.7±4.5	0.09 ± 0.09	0	0.6±0.3	0	0
Proportion dead tissue (%)	1.52b	7.56b	6.84b	2.22b	14.7b	32.4a
Specific leaf area $(dm^2 \cdot g^{-1})$	408a	463a	445a	435a	478a	432a
Petiole length (mm)	186c	302b	423a	200c	417a	410a
No. of leaves	80b	64bc	50bc	117a	59bc	35c
Mean lamina mass (mg)	6.0ab	4.8b	6.3ab	5.9ab	5.9ab	9.9a
Mean petiole mass (mg)	8.2bc	11.8abc	15.8ab	6.4c	5.9c	18.8a

Note: Within a row, means followed by the same letter were not significantly different at p < 0.05. In the case of reproductive allocation, three treatments failed to flower, so means ± 1 SE are reported for those treatments that flowered.

Depth of flooding had no significant effect on final mass. Among the three switch treatments, plants which were flooded at midsummer were the smallest, approximately the same size as plants that were flooded for the entire experiment. On the other hand, plants that were flooded early in the experiment recovered well and had a final mass similar to the nonflooded plants. Plants that were flooded late in the summer were intermediate in size and not significantly different from the other two switch treatments, the constantly flooded plants, or the nonflooded plants.

Allocation to belowground structures (roots, rhizomes, and tubers) did not differ significantly between the nonflooded plants and plants flooded at the intermediate depth, but plants flooded at the greatest depth had a slightly lower resource allocation to their belowground structures (Table 1). On average, half of the plant mass was allocated to belowground structures in these three treatments. Allocation to belowground structures varied more markedly among the three switch treatments. Plants that were flooded early in the season allocated the largest proportion of their mass to belowground structures (61%), while plants flooded later in the season allocated the least (38%). Plants flooded midseason were intermediate between these two extremes and were the most similar to the constant treatments, allocating 50% of their mass to belowground structures. Plants flooded early in the season were most similar to the nonflooded plants and those subject to constant shallow flooding, while plants flooded late in the season were more similar to plants flooded at the maximum depth for the entire season. The only treatments that produced tubers were the nonflooded plants and plants flooded late in the season.

There were no differences among any of the treatments in the proportion of mass allocated to laminas, but there were differences in proportion of mass allocated to petioles (Table 1). Flooding increased mass allocation to petioles in the three constant-flood treatments. The flooded plants allocated more than twice as much mass to petioles as the nonflooded plants. There was no significant difference between the two constant-flood treatments in petiole allocation. Petiole allocation was the same among the switch treatments. Petiole allocation in these three treatments (switch treatments)was not significantly different from the nonflooded plants, but was lower than that of plants flooded at the maximum depth. It should be noted that the mass of the petioles was a substantial portion of the total plant mass. Averaged across treatments, 22% of total mass was allocated to petioles compared with only 13.5% for leaf laminas.

The only treatment in which plants flowered to any significant extent was the nonflooded treatment (Table 1). Nonflooded plants allocated about 20% of total mass to flower production at the final harvest. Although none of the plants had flowers at the final harvest in the mid- and late-summer treatments, plants in both of these treatments flowered prior to flooding earlier in the experiment, but all flowers aborted upon flooding (data not presented).

The proportion of total mass composed of dead tissue (laminas and petioles) at the final harvest did not vary among treatments with the exception that plants flooded late in the season had a higher proportion of dead tissue (Table 1). It should be noted that at earlier harvests, the proportion of total mass in dead tissue increased immediately after plants were subjected to flooding (data not presented). This was true regardless of when plants were flooded.

Specific leaf area did not vary among treatments at final harvest (Table 1) or at any of the earlier harvests (data not presented).

The differences in maximum petiole length among treatments were similar, but not identical to the difference in petiole allocation (Table 1). The shortest petioles were produced by the nonflooded plants and plants flooded early in the season. Petiole length increased with flooding, but unlike petiole allocation, depth of flooding also had an impact; petioles were longer when plants were flooded at a greater depth. The mid- and late-season-flooded plants had petioles as long as those produced by plants flooded constantly to the maximum depth.

Under aquatic conditions, plants tended to produce fewer leaves, but the individual leaves were larger, largely because of increases in petiole mass (Table 1). Among the constantflood treatments, this difference in number and size of leaves was most pronounced between the nonflooded plants and those flooded at a depth of 30 cm, but these differences were not significant at the 0.05 level. Among the switch treatments, the number of leaves at final harvest was greatest in those plants flooded early in the summer; these plants actually produced more leaves than plants that were not

Table 2. Proximate composition and mineral-element analysis of *Hydrocotyle umbellata* tubers collected from plants grown under nonflooding conditions (n = 6).

	Mean ± SE		
Crude protein (%)	8.61±0.49		
Crude fat (%)	0.81±0.05		
Structural carbohydrate (%)	15.43±3.17		
Nonstructural carbohydrate (%)	72.40±2.80		
Ash (%)	2.67±0.07		
Nitrogen (%)	1.38±0.08		
Phosphorous (%)	0.16±0.01		
Potassium (%)	1.12±0.02		
Calcium (%)	0.07 ± 0.01		
Magnesium (%)	0.09 ± 0.01		
Sodium (%)	0.11±0.01		
Iron (ppm)	30.25±12.48		
Manganese (ppm)	6.26±1.40		
Copper (ppm)	12.88±2.27		
Zinc (ppm)	34.39±12.11		
Boron (ppm)	2.65±0.70		

flooded at all. There was no difference in leaf number between plants that were flooded at mid- versus late summer, but plants flooded late in the summer had substantially larger petioles than those in either of the other switch treatments. Lamina mass in the late-summer flood treatment was also greater than that of plants flooded at a depth of 15 cm.

Leaves that developed in a terrestrial environment had equal numbers of stomata on the upper (18.2 stomata·mm⁻² ± 1 (mean ± SE)) versus lower (18.3·mm⁻² ± 1.2) surface. Aquatic leaves on the other hand, had fewer stomata on the lower surface (7.6·mm⁻² ± 1.5) and more on the upper surface (25.2·mm⁻² ± 2.6).

On average, 72.4% of tuber mass was composed of nonstructural carbohydrates compared with 15.4%, 8.6%, 0.8%, and 2.7% for structural carbohydrates (fiber), crude protein, crude fat, and ash, respectively (Table 2). After nitrogen, potassium was the most abundant mineral element followed by phosphorous, sodium, magnesium, and calcium. The tubers also contained significant amounts of iron, copper, boron, manganese, and zinc.

Discussion

Our study demonstrates that *H. umbellata* is capable of active growth in both terrestrial and aquatic environments, but the growth rate in a terrestrial environment is approximately double that in an aquatic environment. A recent field study by Lusk and Reekie (2007) provides a similar conclusion. By transplanting *H. umbellata* plants to different shore-line elevations at different times of the year and on lakes with different hydrological regimes, and regressing final mass on time spent above versus below water, it was concluded that growth rate above-water is roughly three times that below water.

In our study, ULR of the plant declined dramatically immediately upon submersion and RGR became negative. RGR and ULR eventually recovered, but this recovery took about 3 weeks and involved the sacrifice of most of the mature leaves on a plant and the production of a new set of leaves. This indicates that although *H. umbellata* is capable of actively growing in an aquatic environment, this requires a significant adjustment period. The postsubmersion leaf turnover was gradual enough, however, to produce little or no decrease in LAR; the decline in RGR with flooding was due almost entirely to the decrease in ULR.

The precipitous decline in ULR and the senescence of mature leaves upon flooding implies that leaves produced in a terrestrial environment differ sufficiently in structure and morphology from aquatic leaves that they cannot acclimate to aquatic conditions. Studies with other species indicate that leaves produced in aquatic conditions tend to have greater porosity, are thinner, less fibrous, tend to have fewer or no palisade layers, have more stomata on the upper leaf surface and may have longer petioles than terrestrial leaves (Kozlowski 1984; Insausti et al. 2001). In our study, little difference was found between the laminas of plants grown under terrestrial versus aquatic (flooded) conditions. If the leaves differed in thickness or porosity, one would expect differences in leaf area per unit mass, but we found no differences in specific leaf area among the treatments. The only differences we found between the laminas of terrestrial versus aquatic plants were an increase in stomatal density on the upper surface with a decrease in density on the lower surface under flood conditions, and an increase in lamina size in the late-summer flood treatment. The lack of any major difference in the structure of the laminas formed under terrestrial versus aquatic conditions reflects the fact that even under flood conditions, laminas were still found in a "terrestrial" environment in that they floated at the surface. The increase in stomatal density on the upper surface of these leaves facilitates gas exchange with the atmosphere and so would contribute to the increase in ULR observed as plants acclimated to the flood conditions. However, it is likely that most of this increase in ULR was simply due to the elongation of the petioles that allowed the leaves to float at the surface, enhancing light levels. In some species, this elongation occurs in organs that have already been formed and does not require the production of new stems or petioles (Kozlowski 1984). The fact that this elongation occurs largely in new leaves in H. umbellata indicates that this species requires a relatively long period (several weeks) to acclimate to flood conditions.

The increased petiole length of plants grown under submerged conditions comes at a cost in that biomass allocation to petioles must be increased substantially. Petiole allocation in the constant-flood treatment more than doubled relative to the nonflooded plants and petiole mass made up about onethird of total plant mass. Surprisingly, this substantial increase in petiole allocation had little effect on LAR. Rather, the increase in petiole allocation came almost entirely at the expense of flower production. The significance of this tradeoff is unclear. Field studies suggest that Canadian populations do not successfully set seed, and propagation is by clonal growth (Vasseur 2005). The reason(s) for the lack of sexual reproduction is (are) unclear. It may be due to the limited genetic variation in Canadian populations, or perhaps, our relatively short growing season does not provide sufficient time for seed maturation (Vasseur 2005). Regardless of the explanation, if Canadian populations never reproduce sexually, the trade-off between petiole allocation and reproduction in flooded plants will not have a significant impact on population growth.

Although the primary trade-off was between petiole allocation and sexual reproduction, there was some indication that flooding also reduced root/rhizome production, at least in those plants subjected to flooding late in the season. Particularly significant was the lack of tuber production in flooded plants. The chemical analysis of the tubers suggests that they function in energy storage, in that they contain a high proportion of nonstructural carbohydrates (72%). This value is comparable to those of other tubers/rhizomes known to have a role in carbohydrate storage including those of Cyprus esculentus L. (76%) (Umerie and Uka 1998), Typha latifolia L. (83%) (Kallman 1991), and Equisetum arvense L. (32%-37%) (Sakamaki and Ino 2004). Concentrations of other nutrients, including nitrogen, phosphorous, and potassium, are at the low end of the range for actively growing tissue (Fitter and Hay 2002), but this is to be expected given the high carbohydrate content of the tubers. Carbohydrate storage is critical for plants that tolerate the anoxia associated with prolonged flooding (Crawford 2003). The inefficiency of fermentation relative to aerobic respiration often means that the length of time an individual can tolerate flooding is directly related to the size of energy reserves. For *H. umbellata*, carbohydrate storage is likely to be particularly important for winter survival and production of new leaves in the spring. In the spring, before water levels drop, plants must produce leaves with long petioles that reach the surface to provide a diffusion pathway for oxygen to the roots/rhizomes. The initial production of these long petioles will be entirely dependent upon adequate energy reserves. This suggests that the current experiment may underestimate the negative effect of flooding on H. umbellata in that it does not assess over-winter survival.

One factor that may help reduce the cost of petiole production under flood conditions is an increase in lamina size. Plants flooded late in the season not only produced fewer leaves with longer petioles, but these leaves also had larger laminas. Since petiole size is be determined by water depth under flood conditions, producing fewer leaves with larger laminas reduces the resource requirement of the petioles relative to that of plants that produce a larger number of leaves with the same-sized lamina as terrestrial leaves. Field observations suggest that lamina size in *H. umbellata* varies a great deal from site to site (Vasseur 2005), so phenotypic plasticity in leaf size may be important for this species to acclimate to different flooding regimes.

One of the most important findings of this study was that plants subjected to temporary flooding in the middle of the growing season were as negatively impacted by short-term flooding as they were by continuous flooding. Given the relatively long 3 week acclimation period required for plants subjected to flooding, this is to be expected; rapid fluctuations in water level within the growing season are likely to be very detrimental for this species. There was little difference in resource allocation and physiology between plants flooded in the middle of the season (summer) and those flooded late in the season (summer). The major difference was the lower proportion of resources allocated to belowground structures in plants flooded late in the season. This may reflect the timing of resource allocation to roots/rhizomes. Allocation to belowground structures increased towards the end of the growing season in all of the treatments (data not presented). This resource allocation occurred at the same time plants in the late-summer flood treatment were subjected to flooding.

Plants flooded early in the season were less affected by flooding than those in the other switch treatments. Given that these plants were subjected to flooding gradually rather than abruptly as in the case of the other two switch treatments, we cannot assume that time of flooding was the sole factor responsible for reducing the impact of this treatment; the rate at which the water level changed may also have been critical. It is noteworthy that plants in this treatment produced more leaves than plants in any other treatment, including the nonflooding treatment. The increased leaf number probably reflects the negative effect flooding often has on apical dominance (Crawford 1989).

Implications for conservation

The conversion of lakes into headponds for dams, (Hill et al. 1998), and other changes in the watershed that minimize water-level fluctuation, will have a detrimental effect on H. umbellata. This conclusion is in accord with field studies; lakes with minimal water-level fluctuations support few Coastal Plain species (Keddy and Wisheu 1989; Keddy 1991). This distribution pattern has been interpreted as a consequence of the poor competitive ability of these species in that the lack of water-level fluctuation allows taller, more competitive plants such as shrubs to grow down to the water's edge (Sweeney and Ogilvie 1993). Our study suggests that in the case of H. umbellata, rate of growth under flooded versus terrestrial conditions is also important. Conversely, increases in the magnitude and frequency of waterlevel fluctuations could also be detrimental, particularly if fluctuations occur during periods of active growth and are abrupt. Deforestation tends to increase the magnitude of flood peaks in surrounding water bodies (O'Reilly Sternberg 1987), therefore deforestation in watersheds currently supporting H. umbellata is likely to have a negative impact. Winter flooding combined with an early but gradual drawdown of water in the spring such that plants are exposed to a terrestrial environment for most of the growing season, with a gradual increase in water levels in the fall and winter, would likely be the best scenario for this species. The period of terrestrial growth would allow H. umbellata to accumulate the necessary reserves to produce tubers, which are likely important both for winter survival and growth initiation in the spring, while flooding during the winter and spring would minimize competition from other species.

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