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GROWTH OF FLOATING-LEAVED AND SUBMERGED PLANTS IN ARTIFICIAL CO-CULTURED MICROCOSMS: MORPHOLOGICAL RESPONSES TO VARIOUS WATER FLUCTUATION REGIMES

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Abstract

Hydrocharis dubia can alternate between a rooted floating-leaved and a free-floating form, so given increasingly frequent precipitation extremes, it is not expected to be more negatively impacted by rapid water fluctuations than by gradual ones and may adapt water fluctuations by alteration of life forms. However, the opposite may be true for *Nymphoides peltata*, with only a rooted floating-leaved form. We designed an experiment combining six water depth treatments (constant shallow, constant deep, and two rapidly and two gradually fluctuating treatments) with three species-combinations (*N. peltata–H. dubia*, *N. peltata–Ceratophyllum demersum*, and *H. dubia–C. demersum*) to investigate plant responses to depth fluctuations and their co-cultured species. The total mass of *N. peltata* was considerably lower in the rapidly- than in the gradually-fluctuating treatments. However, total mass of *H. dubia* in the rapidly-fluctuating treatments was similar to or higher than in the gradually-fluctuating ones. Rapid fluctuations had a negative impact on the growth of *C. demersum* than gradual fluctuating. The floating-leaved species demonstrated divergent adaptive strategies to different water fluctuation patterns. In addition to expanding leaf blades, *H. dubia* can adapt to changing water depths by changing its life form. However, *N. peltata*, which mainly relies on morphological plasticity, such as petiole elongation, to adapt to water rise may reduce its abundance in communities subjected to increasingly frequent floods. The growth of submerged *C. demersum*, either co-occurring with *H. dubia* or with *N. peltata*, may be repressed by high flooding rates.

Key words: Aquatic macrophytes, Life form, Water level, Biomass.

Introduction

Heavy rainfall events are becoming more frequent with global climate changes (Gong & Wang, 2000; Tebaldi *et al.*, 2006; Ding *et al.*, 2007; Huang *et al.*, 2014), resulting in more rapid water level changes in aquatic plant habitats. When water depths fluctuate, plants are always sub-optimally acclimatized compared to plants at static water depths (Vretare *et al.*, 2001). Water level fluctuations involve frequency, depth, and duration. These different components of fluctuation affect plants in different and interactive ways (Smith & Brock, 2007; Wei *et al.*, 2014). Plant growth can be more negatively impacted at intermediate frequencies of fluctuation than at high or low ones because plants waste resources by continuously reallocating them (Vretare *et al.*, 2001). When water levels fluctuate, absolute depth is less important than the frequency of fluctuations and the duration of individual flooding events (Casanova & Brock, 2000). A few studies have demonstrated that here was more negative impact on aquatic plant growth with the rapid increases in water depth than slower increases (Yang *et al.*, 2004; Yu & Yu, 2011).

Floating-leaved macrophytes play important roles in aquatic ecosystems because of their high production of organic matter and their nutrient accumulation and cycling (Pieczynska, 1993; Marion & Paillisson, 2003; Yamaki &Yamamuro, 2013). They are rooted in sediment but their leaves float on the water surface, these plants cannot protect their leaves from flooding the way free-floating plants can, nor can they tolerate long periods of leaf submergence (Tsuchiya, 1991; Cooling *et al.*, 2001) like submerged plants. Generally, floating-leaved plants adapt to water fluctuations via morphological plasticity, such as rapid petiole elongation and continual leaf recruitment (Cooling *et al.*, 2001; Paillisson & Marion, 2006; Deegan *et al.*, 2007; Yu & Yu, 2011). However, growth-mediated morphological adaptations take time (Deegan *et al.*, 2007). Rapid water rises can submerge leaves, thus decreasing photosynthesis and biomass production in floating-leaved plants (Paillisson & Marion, 2006, 2011).When water fluctuates more quickly than a plant needs to respond, the negative impacts of floods on plants may be exacerbated (Vretare *et al.*, 2001).

The communities dominated by floating-leaved plants usually only have two layers in their vertical structures: the floating-leaved and submerged layers (Chen & Zhou, 1995; Zhou & Chen, 1996). The abundance of submerged plants is closely related to the coverage of the floating-leaved layer (Anon., 1980); where floating-leaved coverage is greater, submerged species are less abundant and vice versa. Both *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae) and *Hydrocharis dubia* (Bl.) Backer (Hydrocharitaceae) are floating-leaved species. Communities dominated by these two species commonly occur in shallow lakes (Anon., 1980; Zhou & Chen, 1996; Lacoul & Freedman, 2006), where *Ceratophyllum demersum* L. (Ceratophyllaceae) frequently dominates the submerged layer (Anon., 1980; Zhou & Chen, 1996; Ge *et al.*, 2003; Peng *et al.*, 2003). These communities experience increasingly frequent water level fluctuations (gradual or rapid) during summer.

In the floating layer, the dominant *N. peltata* and *H. dubia*, have different morphologies. Irrespective of water depth, *N. peltata* plants are always rooted in sediment, their stolons creep along the sediment surface, and their petioles hold the leaves at the water surface (Darbyshire & Francis, 2008). Therefore, *N. peltata* relies exclusively on morphological plasticity to adapt to water fluctuations (Yu & Yu, 2011). In contrast, *H. dubia* plants can root in sediment in shallow water or, in deep water, be free-floating with their roots hanging in the water column; their stems are either contracted, bearing rosette leaves, or elongate-stoloniferous and leafless; and their leaves are emergent or floating at the water surface (Tsuchiya, 1989). Therefore, *H. dubia* may respond to water fluctuations by alternating between rooted floating-leaved and free-floating forms. Based on their morphological differences, we hypothesized that *H. dubia* would not be affected more negatively by high flooding rates than low flooding, while the opposite would be true for *N. peltata*.

The objectives of this study were to answer the following questions: (1) Do the responses of *N. peltata* and *H. dubia* differ to rapid versus gradual water fluctuations in artificial co-cultured systems of both species or of one species growing with submerged *C. demersum*? (2) Do the responses of *C. demersum* to rapid and gradual water fluctuations differ when it is covered by a floating-leaved species? And (3) do the interactions between each floating-leaved species and submerged *C. demersum* vary with the patterns of water fluctuation?

Material and Methods

Plant materials: Vegetative fragments of *N. peltata*, *H. dubia*, and *C. demersum* were collected from Lake Donghu (N 30°38′, E 114°30′) in Wuhan City, Hubei Province, China, in the summer of 2010 and cultivated in a concrete pond (5 m × 3 m × 2 m deep) in the experimental garden at the South-Lake Campus of Central China Normal University, Wuhan (N 30°30′, E 114°21′). All three species overwintered as vegetative propagules, which germinated in the spring of 2011. After germination, the water depth in the pond was maintained at about 10 cm. Plant materials were collected from the pond on June 14, 2011. Each *H. dubia* sample included one rosette with intact leaves and adventitious roots, from which stolons were removed. Each sample of *N. peltata* was an apical fragment (3–4 nodes) with three fully-expanded leaves but without elongated adventitious roots at the nodes. Materials of *C. demersum* were apical fragments (about 9 cm long, 15–19 nodes) without ramifications. Dry weight and dry matter content per sample of each species were, respectively, 1.106 ± 0.005 g and 10.9% ± 0.3% (*N. peltata*), 0.370 ± 0.004 g and 5.2% ± 0.2% (*H. dubia*), and 0.030 ± 0.001 g and 5.8% ± 0.2% (*C. demersum*).

Experimental design: The experiment combined 6 water depth treatments with three species combinations in a factorial design that was replicated three times. Species combinations included three species pairs: *N. peltata*–*H. dubia*, *N. peltata*–*C. demersum*, and *H. dubia*–*C. demersum*. Each combination was cultivated in a concrete tank (50 cm × 40 cm × 80 cm deep). Plant quantities per tank were two fragments of *N. peltata*, three plants of *H. dubia*, or six fragments of *C. demersum*, as appropriate. The initial biomass densities (wet weight) at the beginning of experiment were similar to those of plants collected from the pond (*N. peltata*: *H. dubia*: *C. demersum* = 7:7:1). Plants of *H. dubia* and *C. demersum* were dropped into the ponds, but the lowermost node of each fragment of *N. peltata* was planted diagonally in sediment to favor the production of adventitious roots and allow nutrient absorption from the sediment. All plants were precultured at a water depth of 10 cm for 15 d, by which time all plants of *H. dubia* and *N. peltata* had successfully rooted in sediment.

Water depth treatments included shallow water of constant 10 cm depth (SC), deep water of constant 40 cm depth (DC), two rapidly-fluctuating treatments (R1 and R2), and two gradually-fluctuating treatments (G1 and G2). Water levels fluctuated in the range of 10–40 cm based on the findings of Xie (2003) and Yang (2003). Xie (2003) found that the deepest water in which *H. dubia* plants could root in sediment was 50 cm under nutrient-limited conditions. However, under fertilized conditions, *H. dubia* plants grew shorter and fewer roots (Yang, 2003). Therefore, in water with abundant nutrients, *H. dubia* should alternate between the rooted floating-leaved and free-floating forms when water levels fluctuate in the range of 10–40 cm.

After the preculture (on June 29) of the regulation water depth began to be regulated according to the experiment design. On the first day of treatment, depths increased from 10 cm to 40 cm in the DC, R2, and G2 treatments to create a deep-water condition; depths remained at 10 cm in the SC, R1, and G1 treatments. The DC treatment served as a deep-water control for the R2 and G2 treatments, and the SC treatment served as a shallow-water control for the R1 and G1 treatments. Furthermore, all six treatments could be compared because of their uniform precultured conditions. From the fourth day of treatment, depths in G1 and G2 were altered by 10 cm every 4 days to fluctuate between 10 and 40 cm. From the tenth day of treatment, depths in R1 and R2 were altered by 30 cm every 10 days until the 60th day and remained at the final depth until the end of the experiment on the 64th day. Water depths in G1 and G2 and in R1 and R2 fluctuated inversely to one another. In total, water depth increased three times and decreased twice in R1 and G1 and decreased three times and increased twice in R2 and G2 during the experimental period. This frequency was in the range of natural flooding (2–6 times per year, Ma *et al*., 2005) in the middle and lower reaches of the Yangtze River in China, where communities dominated by these floating-leaved species commonly occur.

To lower the water depth, plastic pipes 2.5 cm in diameter were used to draw water off the tanks to minimize disturbance. To increase the depth, basins 40 cm in diameter were used to pour water into the tank from 30 cm above the water surface to simulate forceful flooding disturbance. On rainy or sunny days, slight adjustments to water levels were made to maintain appropriate depths.

Fertile sediment (20 cm thick; exchangeable nitrogen 33.1 g kg^{-1}, exchangeable phosphate 0.51 g kg^{-1}, and organic matter 95.6 g kg^{-1}; 10.1% sand, 52.5% silt, and 37.4% clay) in tanks was collected from Lake Donghu. Tap water (50.4 μg L^{-1} N-NH_4^+, 168 μg L^{-1} N-NO_3^- and 50.1 μg L^{-1} P-PO_4^{3-}, pH 7.2) aerated for 3 days was used in the experimental tanks. We recorded water pH and temperature (Waterproof ECTestr, Eutech Instruments, Thermo Fisher Scientific, Waltham, MA, USA) and underwater light intensity (LI-193SA underwater irradiance sensor, LI-COR, Lincoln, NE, USA) at a depth of 5 cm around 13:00 every

4 days. Water pH, temperature, and surface light intensity in all tanks were in the range of 7.01–7.35, 28.0–34.1°C, and 679–861 μmol photons m^{-2} s^{-1}, respectively, throughout the experiment. Underwater light intensity decreased as the plants grew. At the beginning of the experiment, it was 505–510 μmol photons m^{-2} s^{-1} in all tanks. At the end of the experiment, under water light intensities in tanks containing the species pairs *N. peltata*–*H. dubia*, *H. dubia*–*C. demersum,* and *N. peltata*–*C. demersum* were 23–78, 60–94, and 89–112 μmol photons m^{-2} s^{-1}, respectively.

Measurements and calculations: Sixty-four days after treatment, we harvested all plants for measurement. Roots of *H. dubia* and *N. peltata* were carefully excavated and washed free of sediments. Because the plants of *H. dubia* and *C. demersum* in each tank were thoroughly entangled, we separated them by species rather than individual and measured species parameters on a total, per-tank basis. For comparisons, the parameters of *N. peltata* were also recorded by tank. We counted the nodes of *C. demersum* and *N. peltata* and the rosettes of *H. dubia*. We measured main stem length and length of each branch (including primary, secondary, and tertiary branches) of *C. demersum* and *N. peltata*, stolon length of *H. dubia*, and petiole length of *N. peltata* and *H. dubia* to the nearest millimeter. Plants of *H. dubia* and *N. peltata* were separated into the following parts: adventitious roots, stems, petioles, leaf blades, and reproductive organs (flowers and fruits, only for *H. dubia*) and dried at 72°C to weigh biomass. Specific leaf area (SLA) and leaf area ratio (LAR) were calculated as follows: SLA = leaf area/ leaf blade dry mass; LAR = leaf area/ total dry mass.

Data analysis: To achieve homoscedasticity, total biomass of the three species studied, leaf area of *H. dubia*, and total number of nodes of *C. demersum* were ln-transformed, and the root mass fraction of *H. dubia* was sqrt-transformed before analyses. For each species, two-way ANOVA tests were used to analyze differences in growth traits. Water depth and co-cultured species were treated as independent variables. For the traits in which significant differences ($p<0.05$) were detected, *t*-tests on independent samples between species combinations or Tukey's tests among water depth treatments were performed. The statistical package SPSS version 16.0 (IBM, Chicago, IL, USA) was used for all analyses. Figures were drawn in SigmaPlot version 10.0 (Systat Software, Chicago, IL, USA).

Results

Biomass production: Total biomass of the three species was significantly affected by both water depth and co-cultured species (Table 1). All three species accumulated the most total biomass in the SC treatment (Fig. 1). In the DC treatment, *H. dubia* mixed with *N. peltata* also produced a substantial amount of biomass. The total mass of *H. dubia* was greater than that of *N. peltata* in each treatment, both when the two floating-leaved species were co-cultured (ANOVA, $p< 0.05$) and in combinations of each with *C. demersum* ($p<0.05$). For *N. peltata*, total mass was considerably lower in the R1 than in the G1 treatments (ANOVA, $p<0.001$) and in the R2 than in the G2 treatments (ANOVA, $p< 0.01$). However, the total mass of *H. dubia* in the rapidly-fluctuating treatments was similar to or higher than that in the gradually-fluctuating treatments. In all water treatments, *H. dubia* produced significantly more biomass than *C. demersum* when co-cultured (ANOVA, $p<0.01$). Nevertheless, in four of six water treatments (excluding SC and R1) the total mass of *N. peltata* exceeded than that of *C. demersum*. Independent of water treatment, *C. demersum* produced significantly less total mass in mixture with *H. dubia* than in mixture with *N. peltata*. Among the six treatments, the total mass of *C. demersum* had following relationships: R1< G1 (ANOVA, $p= 0.061$) and R2< G2 ($p<0.01$), irrespective of co-cultured species.

Biomass allocation: Globally, biomass allocation to most organs of the two floating-leaved species was significantly affected by water depth and co-cultured species (Table 1). However, petiole mass fraction of *H. dubia* and leaf blade mass fraction of *N. peltata* were constant among treatments. For *H. dubia* in mixture with *C. demersum*, the root mass fraction was greatest in the DC treatment and greater in the gradually-fluctuating than in the rapidly-fluctuating treatments (Fig. 2). Its stem mass fraction was least in the DC treatment, and the leaf blade mass fraction was less in the gradually-fluctuating than in the rapidly-fluctuating treatments and in the SC than in the DC treatment. However, similar tendency was not observed in *H. dubia* mixed with *N. peltata*, in which the greatest root mass fraction was in the SC and G2 treatments and the stem mass fraction was constant among water treatments, excluding the G1 treatment. Among treatments, *N. peltata* had the least petiole mass fraction in the SC treatment, irrespective of co-cultured species. *Nymphoides peltata* co-cultured with *H. dubia* had greater petiole mass fraction but less stem mass fraction in the gradually-fluctuating than in the rapidly-fluctuating ones. For *N. peltata* mixed with *C. demersum*, however, differences in root mass fraction and in stem mass fraction were observed between G1 and R1, but not between G2 and R2.

Morphological traits: Except for the average length of *H. dubia* petioles and SLA and LAR of *N. peltata*, most morphological traits of the three species were significantly influenced by both water depth and co-cultured species (Table 1). Average length of petioles of *N. peltata* was significantly affected by water treatments, but not co-cultured species. *Hydrocharis dubia* had the greatest total number of rosettes and leaf area in the SC treatment among all water treatments, independent of co-cultured species (Fig. 3a, b). The SLA and LAR of *H. dubia* were significantly lower in the two constant treatments than in the four fluctuating treatments (data not shown). The average stolon length per rosette of *H. dubia* was greatest in the DC treatment and tended to be greater in the gradually-fluctuating than in the rapidly-fluctuating treatments (Fig. 3c). *Nymphoides peltata* produced the shortest petioles and largest leaf areas in the SC treatment and longer petioles and larger leaf areas in the gradually-fluctuating than in the rapidly-fluctuating treatments, irrespective of co-cultured species (Fig. 3e, f). *Ceratophyllum demersum* had the greatest total number of nodes but the shortest internodes in the SC treatment among water treatments (Fig. 3g, h).

Table 1. Summary of two-way ANOVA of the effects of water depth treatment, co-cultured species, and their interactions on growth traits of *Hydrocharis dubia*, *Nymphoides peltata* and *Ceratophyllum demersum*.

Species/trait	Water depth (W)	Co-cultured species (C)	W×C
	F	*F*	*F*
H. dubia			
Total biomass pond^{-1} (g)	623.6***	90.4***	76.0***
Root mass fraction	201.5***	672.1***	284.8***
Stem mass fraction	49.3***	1.2^{NS}	69.6***
Petiole mass fraction	0.4^{NS}	1.2^{NS}	1.7^{NS}
Leaf blade mass fraction	467.3***	1668.2***	426.2***
Total number of rosettes pond^{-1}	183.2***	5.2*	59.3***
Average stolon length ramet^{-1}(cm)	94.2***	9.7**	9.8***
Leaf area pond^{-1} (cm^{2})	173.0***	28.7***	46.8***
Specific leaf area (SLA) (cm^{2} mg^{-1})	167.5***	344.2***	30.9***
Leaf area ratio (LAR) (cm^{2} mg^{-1})	295.0***	986.2***	124.8***
Average length petiole^{-1} (cm)	2.1^{NS}	2.0^{NS}	1.5^{NS}
N. peltata			
Total biomass pond^{-1} (g)	104.1***	13.4**	2.4^{NS}
Root mass fraction	31.1***	71.3***	13.8***
Stem mass fraction	75.8***	56.5***	11.4***
Petiole mass fraction	474.8***	48.2***	30.4***
Leaf blade mass fraction	2.2^{NS}	1.6^{NS}	1.3^{NS}
Total number of nodes pond^{-1}	19.7***	12.3***	5.5**
Average length of internodes (cm)	18.6***	28.3***	14.5***
Leaf area pond^{-1} (cm^{2})	67.1***	5.0*	1.6^{NS}
Specific leaf area (SLA) (cm^{2} mg^{-1})	0.4^{NS}	0.1^{NS}	0.1^{NS}
Leaf area ratio (LAR) (cm^{2} mg^{-1})	0.2^{NS}	0.6^{NS}	0.8^{NS}
Average length petiole^{-1} (cm)	55.8***	0.7^{NS}	8.4***
C. demersum			
Total biomass pond^{-1} (g)	404.9***	659.6***	25.3***
Total number of nodes pond^{-1}	671.3***	1923.0***	270.0***
Average length of internodes (cm)	210.5***	27.9***	44.5***

***, $p<0.001$; **, $p<0.01$; *, $p<0.05$; NS, $p>0.05$.

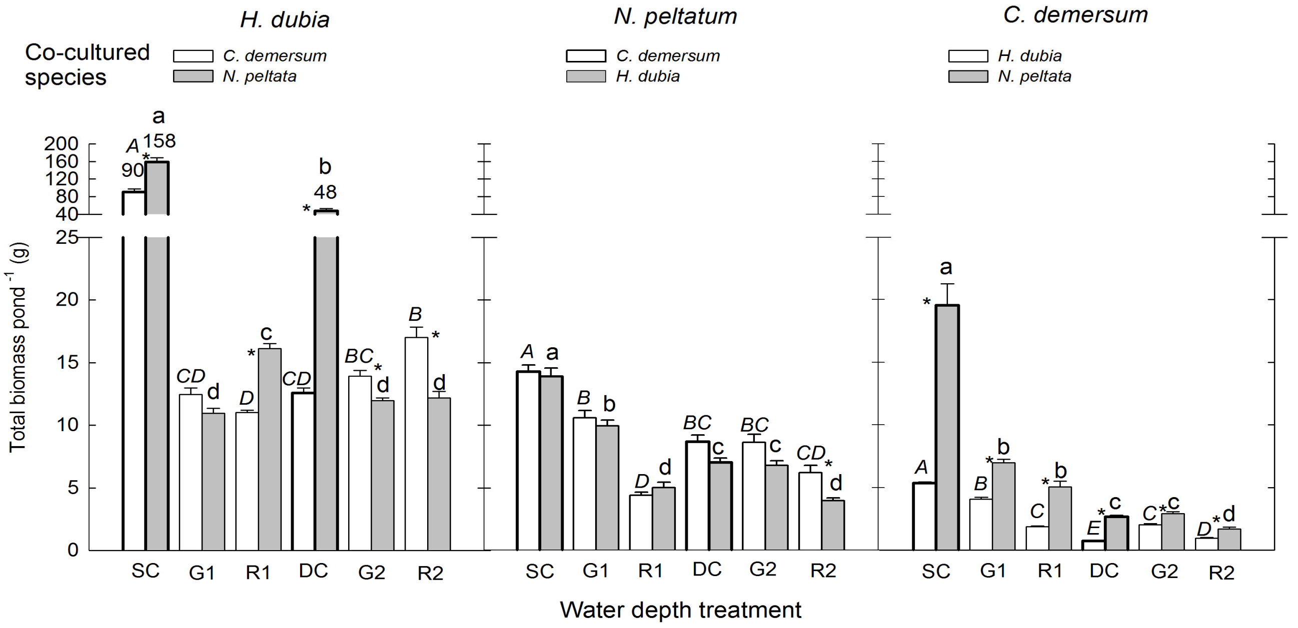

Fig. 1. Total biomass (means ± standard errors) of *H. dubia*, *N. peltata*, and *C. demersum* co-cultured with another species in six water treatments (SC, shallow water of constant depth; DC, deep water of constant depth; G1 and G2, gradual water-level fluctuation; R1 and R2, rapid water-level fluctuations). Different letters (capital or lowercase) above the bars denote significant differences among water treatments for each species in each co-culture mixture (Tukey's tests, $p<0.05$). Asterisks denote significant differences between species-combination treatments in each water treatment (*t*-tests, $p<0.05$).

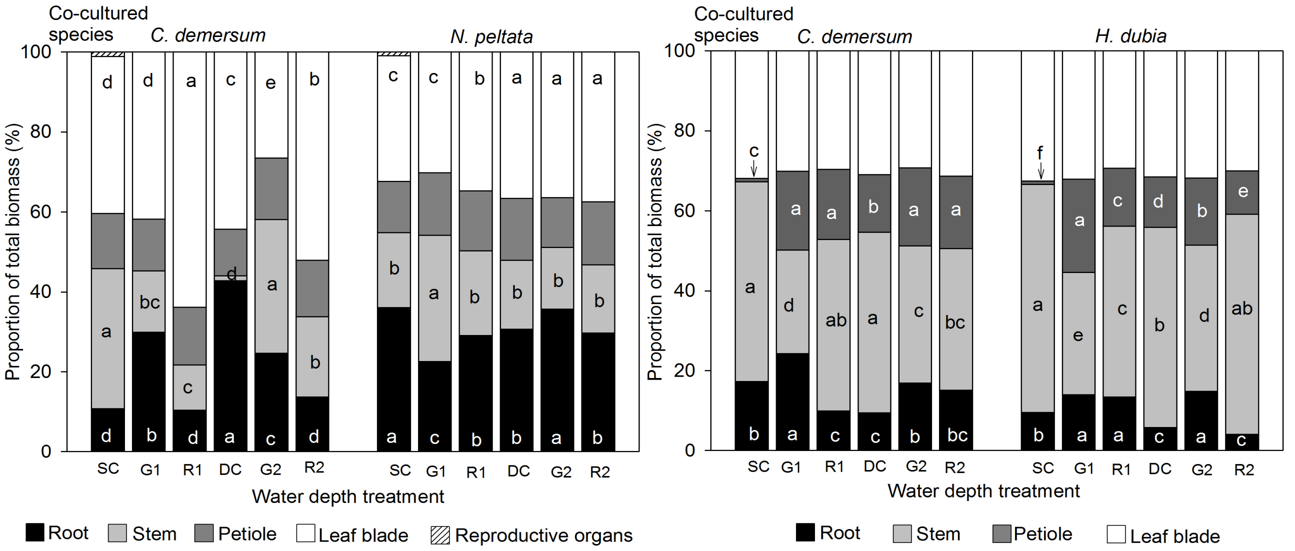

Fig. 2. Biomass allocation of *H. dubia* and *N. peltata* co-cultured with another species in six water treatments (SC, shallow water of constant depth; DC, deep water of constant depth; G1 and G2, gradual water-level fluctuation; R1 and R2, rapid water-level fluctuation). For traits in which significant differences ($p<0.05$) were detected by two-way ANOVA, different letters in the bars denote significant differences among water treatments for each species in mixture with each co-cultured species (Tukey's tests, $p<0.05$).

Hydrocharis dubia co-cultured with *C. demersum* had a greater leaf area than when co-cultured with *N. peltata* in the four fluctuating treatments (ANOVA, $p<0.001$), while this was not the case in the two constant treatments (ANOVA, $p= 0.148$) (Fig. 3b). In most cases, the SLA and LAR of *H. dubia* mixed with *C. demersum* was significantly greater than when it was mixed with *N. peltata* (data not shown). *Ceratophyllum demersum* mixed with *N. peltata* had a greater total number of nodes than when co-cultured with *H. dubia*, independent of water treatment (ANOVA, $p<0.001$) (Fig. 3g). The leaf area of *H. dubia* was considerably larger than that of *N. peltata* when the two floating-leaved species were co-cultured (ANOVA, $p<0.001$), as well as when each of these species are grown with *C. demersum* ($p<0.001$), irrespective of water treatment (Fig. 3b, e; note the different scales in the two histograms).

Discussion

Growth responses of the two floating-leaved species to water fluctuations: Fluctuating or increased water depth reduced the growth of the two floating-leaved species, *H. dubia* and *N. peltata*, in terms of biomass accumulation and leaf area, compared with shallow constant depth. The depressive effect of these treatments on the floating-leaved species was similar to that on the emergent *Phragmites australis* (Cav.) Trin. ex Steud. in the study by Vretare *et al.* (2001). *Nymphoides peltata*, which relies on morphological plasticity to adapt water fluctuations, was more negatively impacted by rapid fluctuations than by gradual fluctuations. In contrast, *H. dubia*, which can alter its life form in addition to its morphology to respond to water-level fluctuations, demonstrated similar or stronger tolerance to rapid versus gradual fluctuations. These results were consistent with our hypothesis.

The most conspicuous response of *N. peltata* plants to fluctuating water depths was having longer petioles. Petiole elongation, as well as continual leaf recruitment, is the most important way in which floating-leaved plants adapt to deepening water (Cooling *et al.*, 2001; Paillisson & Marion, 2011; Yu & Yu, 2011). High flooding rates combining with high water levels resulted in a high rate of petiole elongation and a low rate of leaf recruitment; as the water depth fell, new leaves had shorter petioles that matched the water depth (Cooling *et al.*, 2001). *Nymphoides peltata* plants produced longer petioles in gradually-fluctuating depths than in rapidly-fluctuating ones in this study, unlike in the report by Yu & Yu (2011). In that study, they measured each plant's longest petiole every 10 days and found that *N. peltata* produced longer petioles when water depth was increased rapidly (once) than when the change was gradual and stepwise. The difference between these two studies indicated that repeated fluctuations affected plants differently than unidirectional depth increases.

Accompanying petiole elongation, *N. peltata* responded to changes in water depth by altering biomass allocation among organs. In fluctuating and increased water depths, the greater allocation to petioles occurred at the expense of roots and/or stems but not leaf blades, implying that *N. peltata* plants incur greater costs to tethering blades in deeper water but that petiole growth compensates for depth without impacting leaf blades. The increased cost of tethering leaves in deeper water in *N. peltata* was similar to that in floating-leaved *Nymphaea odorata* Aiton (Sinden-Hempstead & Killingbeck, 1996; Richards *et al.*, 2011). The constant percentage of *N. peltata* biomass in leaf blades in different water treatments resembled the response of emergent *Typha domingensis* Pers. to water increases (Grace, 1989). A fixed percentage of biomass in photosynthetic tissues across water depths may maximize energy accumulation by aquatic plants. Simultaneously, *N. peltata* plants, similar to plants of *N. odorata* in different water depths (Richards *et al.*, 2011; their lamina specific weight is the reciprocal of SLA), had constant SLA among water treatments.

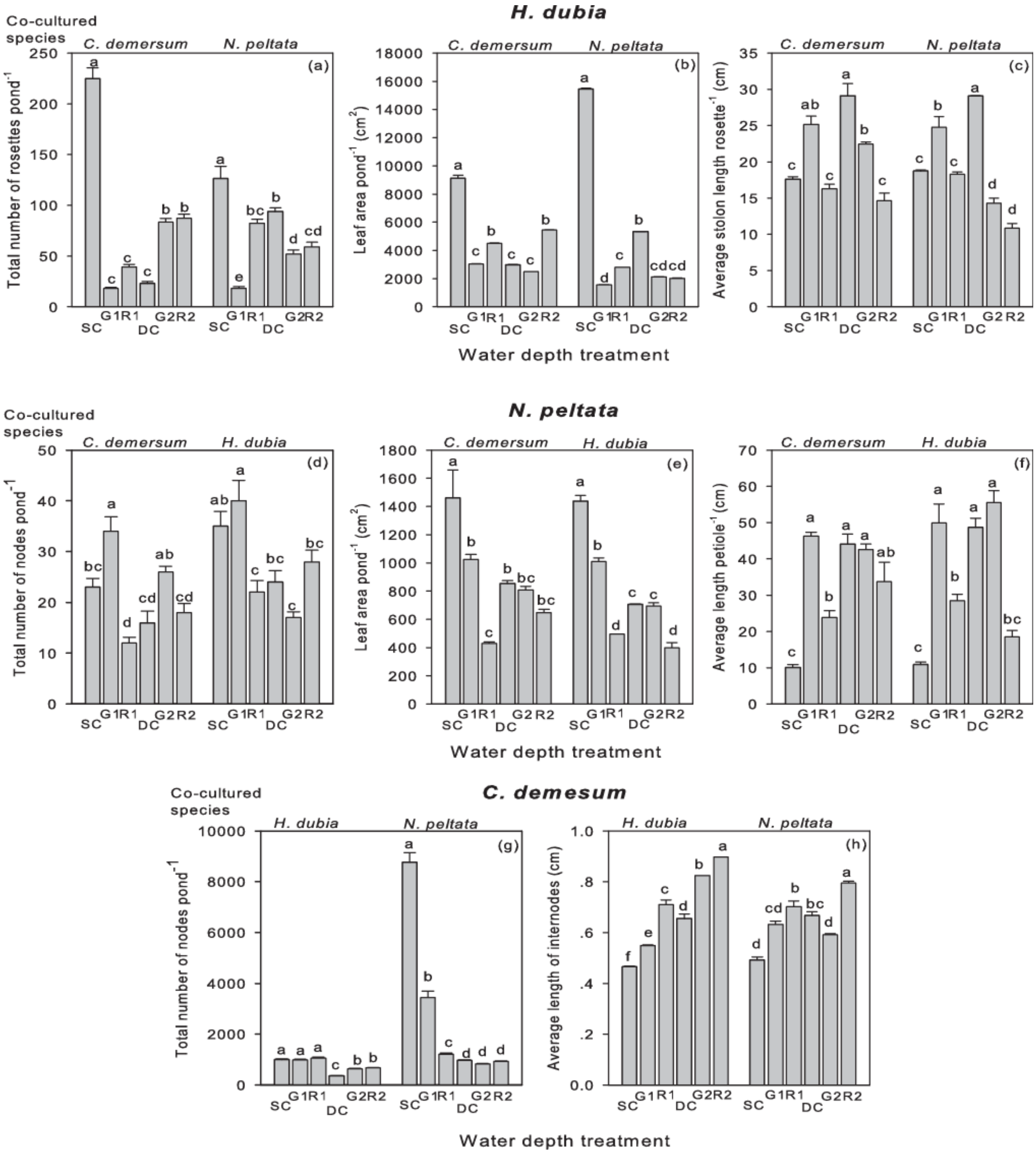

Fig. 3. Morphological traits of *H. dubia*, *N. peltata*, and *C. demersum* co-cultured with another species in six water depth treatments (SC, shallow water of constant depth; DC, deep water of constant depth; G1 and G2, gradual water-level fluctuation; R1 and R2, rapid water-level fluctuations). Different letters above the bars indicate significant differences among water treatments for each species in mixture with each co-cultured species (Tukey's tests, $p<0.05$).

Hydrocharis dubia responded differently to water rise by altering from a rooted floating-leaved form to a free-floating one. Therefore, the typical response of floating-leaved plants to water increase, i.e., petiole elongation (Cooling *et al*., 2001; Paillisson & Marion, 2011; Richards *et al*., 2011; Yu & Yu, 2011), was not observed in *H. dubia* plants. The length and proportional biomass allocation of their petioles were insensitive to depth. When they altered forms, we observed that submerged leaves of *H. dubia* were re-exposed to air more quickly in rapidly-increasing than in gradually-increasing depths, which might result from the higher water rise rate and the stronger force disturbance in the rapid fluctuating treatments. The briefer leaf submergence of *H. dubia* during rapid fluctuations maximized photosynthesis and biomass accumulation. In addition, *H. dubia* plants expanded their leaves by increasing SLA and LAR to promote light capture in fluctuating water, similar to the increased SLA reported in several species of *Potamogeton* in response to shade (Spence & Chrystal, 1970; Spence *et al*., 1973).

Growth responses of submerged *Ceratophyllum demersum* to water fluctuations: Irrespective of co-cultured floating-leaved species, *C. demersum* growth was generally repressed by high water levels and fluctuations. Furthermore, rapid fluctuations tended to impact *C. demersum* more negatively than gradual ones. *Ceratophyllum demersum*, which are adapted to high-light conditions (Su *et al*., 2004), elongated their internodes to mitigate the negative effect of low light availability in deeper water and shading by the floating-leaved species, as did *Potamogeton amplifolius* Tuck. under shaded conditions (Cronin & Lodge, 2003). The strategy of allocating more resources to length growth was also observed by Larson (2007), in whose study three submerged species, including *C. demersum*, shaded by different densities of floating *N. peltata* showed a positive relative elongation rate, whereas the rate of weight accumulation was negative. The more vigorous growth of *C. demersum* in mixture with *N. peltata* than in mixture with *H. dubia* may be related to the higher underwater light availability resulting from the lower leaf area of *N. peltata*. Besides reducing light levels underwater, a floating layer can cause a drop in dissolved oxygen concentration in water (Netten *et al*., 2010; Yamaki &Yamamuro, 2013).

Comparison of responses between the floating-leaved and submerged species: Independent of water depth, *H. dubia* dominated over co-cultured submerged *C. demersum*. However, the relative performances of *N. peltata* and *C. demersum* when grown together depended on the water treatment. When co-cultured with *C. demersum*, *H. dubia* increased biomass allocation to roots at the expense of aboveground organs when water levels fluctuated gradually, but not rapidly. When water levels were constantly high, free-floating *H. dubia* mixed with *C. demersum* allocated much more biomass to roots than did the rooted floating-leaved form of this species in constant shallow water. This biomass reallocation by *H. dubia* might be due to better nutrient uptake caused by having more roots hanging in the deep water column, where the two species compete for nutrients (Cao & Wang, 2012). To increase nutrient acquisition, *H. dubia* plants growing under nutrient-poor conditions have a relatively large proportion of root biomass (Tsuchiya, 1989), similar to submerged (Angelstein *et al*., 2009; Xie *et al*., 2013), emergent (Lorenzen *et al*., 2001), and terrestrial (Poorter & Nagel, 2000) plants. However, even in constant deep water, *N. peltata* maintained the rooted floating-leaved form, thus capturing enough nutrients from sediment (Darbyshire & Francis, 2008) and avoiding competition with *C. demersum* for nutrients in the water column (Lombardo & Cooke, 2003). In this case, efficient light capture rather than nutrient uptake may be the biggest challenge facing *N. peltata*, so increasing its resource allocation to aboveground organs at the expense of roots was the best strategy.

The outstanding performance of *H. dubia* in this study may be related to the phenologies of the three species studied. In natural communities dominated by the two floating-leaved species, *N. peltata* and *H. dubia* accumulate the most biomass in May and August, respectively; *C. demersum* is one of the dominant submerged species throughout the growth season (Zhou & Chen, 1996; Li *et al*., 2012; Flora of China, http://frps.plantphoto.cn). Therefore, during the season of this experiment (June–August), when natural floods occur frequently, *H. dubia* is normally dominant, while *N. peltata* is subordinate.

Conclusion

By responding differently to rapid versus gradual fluctuation patterns, the floating-leaved species demonstrated divergent adaptive strategies. *Hydrocharis dubia*, in addition to expanding leaf blades, can adapt to different water depths by alternating life forms (Tsuchiya, 1989) and is thus influenced less negatively by rapid water fluctuations. However, *N. peltata*, which is mainly dependent on morphological plasticity (Yu & Yu, 2011), such as elongated leaf petioles, adapt to water rise may become less abundant in communities subjected to increasingly frequent floods. The growth of the submerged *C. demersum*, either co-occurring with *H. dubia* or with *N. peltata*, may be repressed by high flooding rates. Small water fluctuations in nature, such as in the range we studied, might affect the structure of communities containing these three species that experience high flooding rates.

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