

# Changes in morphological and physiological traits of the freshwater plant *Ranunculus peltatus* with the phosphorus bioavailability

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**Abstract** Plastic adjustments in response to P availability were investigated for a freshwater, submerged plant, *Ranunculus peltatus* Schrank at various stages in its life cycle. A series of three, nine-day-experiments was performed under controlled conditions in April, June and August. *R. peltatus* plasticity was tested in response to three phosphorus water concentrations (50, 100, 300  $\mu\text{g/l PO}_4^{3-}\text{-P}$ ) and was evaluated using eight morphological and ecophysiological traits related to plant nutrient uptake and use. Some plastic adjustments in *R. peltatus* were found to be significant mostly for the April experiment. *R. peltatus* displayed higher nutrient uptake and lower NUE when P availability was high. It tended also to adapt its P uptake rate, which decreased during the experiment. When P availability was low, reverse results were found in the four ecophysiological traits. No morphological plastic adjustments in response to P enrichment were identified in *R. peltatus* concerning branching or creation of roots. However, the production of sexual organs tended to be enhanced by low P

concentrations in the water. Ecophysiological rather than morphological adjustments were highlighted in *R. peltatus* with respect to P availability. Plasticity was found to depend strongly on the particular phenological stage. These results may constitute valuable information concerning the evaluation and ecological significance of plasticity in freshwater plants

**Keywords** Controlled conditions · Ecophysiological adjustments · Macrophytes · Morphological traits · Phosphorus

## Introduction

Plasticity has been widely recognized as an important aspect of how organisms develop, function and evolves in their environments (Sultan 2000). This biological characteristic corresponds to the ability of an organism to adjust its performance by altering its morphology, physiology and life-history in response to varying environmental conditions (Bradshaw et al. 1964; Schlichting 1986; Sultan 1995; Sultan 2000). Possibly, the best known functional patterns of morphological plasticity for two essential resources—nutrients and light—involve increased biomass allocations to roots in low-nutrient soils (Dale 1986; Sultan and Bazzaz 1993) or greater leaf area relative to plant biomass under low

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photon flux density (Smith 1982; Reich et al., 1998; Poorter 2001; Navas and Garnier 2002; King 2003; Steinger et al. 2003). Some physiological adjustments also consist in varying minimum requirements of nutrients and light efficiencies (Tjoelker and Luxmoore 1991; Raghothama 1999), or changes in pigment concentrations associated with light capture (Larcher 2003). Focusing on plant adjustments to nutrient stress, plasticity in terrestrial plants consists of low nutrient requirements, slow growth rates, high nutrient-use efficiency and uptake capacity and several modifications in root system morphology and biomass in order to improve nutrient uptake from the soil (Loneragan and Asher 1967; Drew and Saker 1978; Keerthisinghe et al. 1998; Raghothama 1999).

While such global trends have been confirmed in several plastic terrestrial species in response to nutrient stress, fewer studies of freshwater plants have been undertaken to investigate their plasticity in relation to nutrient availability. The main reason is the difficulty of evaluating such adjustments in aquatic situations. According to Schlichting (1989), traits related to the capture and/or use of a particular resource—or traits known to be closely related to them—are good candidates for estimating the plasticity of a species relative to this resource, since those changes are likely to be of functional value. However, care must be taken when extrapolating the role of traits used in terrestrial environments to aquatic ones, because the same biological trait may not have the same functional value in both environments. One striking example is the use of the root to shoot ratio in terrestrial plants as a general indicator of plastic adjustments in response to nutrient availability. In submerged freshwater plants, especially those found in flowing systems, the root to shoot ratio is enhanced for the most part by disturbance (Barrat-Segretain 2001) due to the primary anchoring function of roots which prevent plants from being washed away (Arber 1920; Sculthorpe 1967). Aquatic vascular plants are able to attain nutrients via uptake across shoot and leaf surfaces, as well as from adventitious and original roots, depending on environmental conditions and plant specific strategies (Agami and Waisel 1986; Pelton et al. 1998). Therefore, no single

morphological trait related to the capture of nutrients may be selected. In this case, particular attention must be paid to physiological traits which have been poorly investigated in aquatic situations thus far.

In this study, a controlled condition experiment, designed to study plant plasticity as influenced by nutrient availability in the laboratory, was performed. Special attention was paid to Phosphorus (P), recognised as the most common limiting nutrient for freshwater plant growth (Carr and Chambers 1998). The model system *Ranunculus peltatus* Schrank was selected for its high morphological plasticity (Cook 1966; Garbey et al. 2004a, 2006). This submerged, rooted macrophyte is widely distributed in European rivers and streams (Haslam 1978) where it tends to dominate and form extensive stands in open water areas (Cook 1966; Spink et al. 1997; Thiébaud and Muller 1999). As found with other *Ranunculus* species, *R. peltatus*' life-cycle consists of four main stages: an extension stage in early spring a consolidation and flowering stage in late spring, a decline stage in late summer and a regeneration stage in early autumn (Dawson 1976; Garbey et al. 2006). Consequently, the same experiment was replicated at each of the first three distinct life-cycle stages in order to take this phenology (except regeneration stage) into account. The following questions were addressed: (1) Does *R. peltatus* exhibit physiological or morphological responses to P uptake and use along a P-availability gradient? and (2) To what extent does phenology influence *R. peltatus*' plastic responses?

## Methods

A series of three, nine-day experiments (April 24 to May 2; extension stage; June 7 to 16; consolidation phase; August 26 to September 4; decline stage) were performed to study *R. peltatus* responses to phosphorus availability. Experiments were performed during short periods as we wanted to grow *Ranunculus* in the river water where fragments were collected. This poorly buffered water made it difficult to grow plants during long periods without strong fluctuation

in water parameters at long-term; these were supposed to potentially alter plant growth. A 9-day duration was assumed to be sufficient to detect a significant growth as *R. peltatus*, as other *Ranunculus* species are characterised by a rapid growth (Eichenberger and Weilenmann 1982; Dawson 1976). The experiment starts at the time fragments are put into aquaria as physiological acclimation often occurs at short-term within the first few days. *R. peltatus* stem apices collected in the field were transplanted to laboratory glass aquaria filled with water enriched, when necessary, with phosphorus. Three concentrations were tested, corresponding to the following Soluble Reactive Phosphorus (SRP) concentrations: 50 µg/l (concentration A), 100 µg/l (concentration B) and 300 µg/l (concentration C). Only the impact of P water concentrations was tested on *R. peltatus* morphological and physiological variability

Plant and water samples were collected in two oligotrophic streams located in the Northern Vosges Man and Biosphere Reserve (49°1' N, 7°23' E). Both streams are characterised by weakly mineralised, slightly acidic water (Garbey et al. 2004a, 2006). For each experiment, 120 l of water were collected in the Schwartzbach stream and analysed, before setting-up the aquaria, according to the following parameters: Acid Neutralising Capacity (ANC) (Gran's titration; NFT 90-035, AFNOR 1986), Conductivity (C), pH, SRP (single reagent ascorbic acid technique for phosphorus, NFT 90-023, AFNOR 1986) and Ammonia (NH<sub>4</sub><sup>+</sup>-N) (indophenol technique for ammonia, NFT 90-015, AFNOR 1986). Plants were collected in the Falkensteinbach stream at a site known for a particular abundance and dominance of *R. peltatus*. Each plant cut, including apex and some adventitious roots, measured between 30 cm to 50 cm from the apex and was originated from the same clone. The specimens chosen did not exhibit buds or flowers

For each of the P water concentrations tested, six aquaria measuring 30 × 15 × 25 cm<sup>3</sup> were used; three replicates with plants and three replicates without plants (control). The aquaria were filled with 6.5 l of water enriched with a P-addition solution to attain the P concentration

needed for testing. Three *R. peltatus* individuals were selected at random from the stock of plants collected in the field, washed carefully and put into the aquaria replicates designated to contain plants. Fertilisation treatments followed a block design including all combinations of phosphorus, with or without plants. The 18 aquaria were then placed in a refrigeration tank and aerated to ensure mixing and sufficient carbon supply. Water temperature in the main tank was fixed at 15 ± 2°C. Light was provided by banks of 120 cm-long fluorescent tubes (58 watt Grow Lux broad-spectrum tubes) following a 14-h day/10-h night photoperiod. Conductivity and pH were measured in each aquarium both 6 h after the beginning and at the end of each experiment.

The P-addition solution was added every 3 days as needed to replace depleted phosphorus. Thus three time periods, T1, T2 and T3, were identified in each experiment. During T1, samples of 10 ml of water were taken every 6 h and stored at 4°C. Because this 6-h step survey did not bring significantly more information than a 12-h step survey, the sampling was subsequently reduced to a 12-h step survey during T2 and T3. All water samples were analysed for SRP within 24 h. P-depletion was assumed to be caused by either P-adsorption on the aquaria walls or by plant uptake. Therefore, P-depletion in the aquaria without plants was due solely to P-adsorption. Plant P-uptake was calculated for each concentration as the amount of P depleted in the aquaria with plants (Aq + veg) and P depleted in the control aquaria (Aq) without plants using the following formula:  $P_{\text{uptake}} = \left[ (\text{SRP}_i - \text{SRP}_f)_{(\text{Aq} + \text{veg})} - (\text{SRP}_i - \text{SRP}_f)_{\text{Aq}} \right] \times V_{\text{aq}}$  with SRP<sub>i</sub> and SRP<sub>f</sub> being initial and final SRP in the water respectively, and V<sub>aq</sub> as the water volume in the aquaria. Plant P-uptake in a given aquarium was calculated as the mean P-depletion among the three controls corresponding to the same P-concentration.

A total of eight traits were selected: four physiological and four morphological traits assumed to reflect plant nutritional and functional changes according to nutrient availability. Physiological traits concerned Relative Growth Rate (RGR), total plant phosphorus accumulation (tQa), Nutrient Use Efficiency (NUE) and plant

adaptability in P-uptake (ADAPT). RGR ( $d^{-1}$ ) was calculated for each individual from initial (day 0) and final (day 9) plant dry weights for the experimental period ( $DW_i$  and  $DW_f$ ) and experiment duration in days ( $T$ ) using the following formula:  $RGR = \frac{\ln(DW_f) - \ln(DW_i)}{T}$ . Initial dry weights were defined multiplying freshwater weight of each fragment used with average water content of fragments of similar size collected in the same conditions. The tQa (gP) was calculated for each aquarium as the sum of P accumulated during T1, T2 and T3 in the three individuals placed in the aquarium with respect to their initial dry weight ( $DW_{iT}$ ):  $tQa = \frac{(P_{uptake})_{T1} + (P_{uptake})_{T2} + (P_{uptake})_{T3}}{DW_{iT}}$ . NUE (gDW/gP) was determined at the level of total plant as dry matter production on nutrient absorption using the following equation (Bridgham et al. 1995):  $NUE = \frac{DW_{fT} - DW_{iT}}{P_{uptake}}$  with  $DW_{iT}$  and  $DW_{fT}$  as initial and final Dry Weight for the three plants in the aquaria. This parameter will precisely reflect what productivity may be linked with the P uptook by the plants. NUE was expected to decrease with increasing nutrient availability, thus pointing to a dilution effect. The reverse is found normally for plants developing in low nutrient availability situations. Finally, ADAPT was evaluated by comparing the absolute mean P-uptake rate during T1 and T3. Indeed, in nutrient-poor environments, plants were expected to exhibit increased P-uptake rates in order to improve their nutrient uptake efficiency. In contrast, no specific change in uptake rates was expected to occur in plants from nutrient-rich environments. This was evaluated using the following ratio:  $ADAPT = \left| \frac{(v_{uptake})_{T3}}{(v_{uptake})_{T1}} \right|$ . The  $v_{uptake}$  corresponded to the mean of six, instantaneous absorption rates which were calculated every 12 h in each period. Therefore, ADAPT may be greater than 1 in nutrient-poor situations and less than 1 in nutrient-rich situations.

The four morphological traits chosen concerned potential changes in *R. peltatus* in response to nutrient availability: the increase of plant photosynthetic area ( $\Delta(L + Sec)$ ); the proportion of rooted internodes ( $\Delta ROOT$ ); the change in

mean length of internodes ( $\Delta LNODE$ ); and the production of buds and flowers ( $B + F$ ).  $\Delta(L + Sec)$  was evaluated using two descriptors of general plant growth: the length of the plant's main shoot ( $L$ ) and the length of the longest secondary ramet ( $Sec$ ). Both parameters have been used successfully to describe local *R. peltatus* expansion in two previous studies (Garbey et al. 2004a, 2006).  $L$  and  $Sec$  were summed in order to evaluate total plant photosynthetic surface, which was expected to increase in nutrient-rich situations and remain stable in nutrient-poor ones. Changes in this trait were calculated as the difference between initial (day 0) and final (day 9) measurements for each *R. peltatus* individual.  $\Delta ROOT$  was calculated as the difference between the final and initial proportions of rooted internodes. These proportions were evaluated as the number of internodes with adventitious roots as compared to the total number of internodes. This trait was selected to examine the hypothesis that root creation is enhanced in nutrient-poor situations.  $\Delta LNODE$  was quantified as the change in mean internode length from the beginning to the end of the experiment. Mean internode length was calculated as  $L$  divided by the total number of internodes. Finally, sexual reproduction was evaluated using the total number of buds and flowers produced ( $B + F$ ). All four morphological traits were calculated for each *R. peltatus* individual, resulting, therefore, in nine measurements per P-concentration. The expected correlations, based on the theories presented in the Introduction section, between these eight traits and nutrient availability are synthesised in Table 1

**Table 1** Expected relationships between nutrient availability and variation in the selected traits

Plant attribute	Nutrient-poor situations	Nutrient-rich situations
<i>Physiological traits</i>		
RGR	Low	High
TQa	Low	High
NUE	High	Low
ADAPT	Above 1	Approximately 1
<i>Morphological traits</i>		
$\Delta(L + sec)$	Low	High
$\Delta ROOT$	Positive	Negative
$\Delta LNODE$	?	?
(B + F)	?	?

A two-way ANOVA test [factors: time (April, June, August), trophic level (A, B, C)] was achieved to detect significant impacts of phenology and trophy. For each experiment, one-way anovas were carried out in order to focus on the effect of trophy on plant traits. Newman–Keuls post hoc-tests were used to test differences between treatments (Winer 1962). Data were log-transformed whenever necessary to ensure normality of residuals and homogeneity of variances. When conditions for using Anova test were not fulfilled, non-parametric test were used. Trend results significant at  $P < 0.1$  level were also taken into account in the analyses. All statistical analyses were performed using STATISTICA software.

## Results

Initial water taken in the field and where plant fragments were grown was characterised by the following parameters:  $[\text{PO}_4^{3-}\text{-P}] = 26 \pm 15 \mu\text{g/l}$ ,  $[\text{NH}_4^+\text{-N}] = 48 \pm 19 \mu\text{g/l}$ ,  $\text{pH} = 6.56 \pm 0.23$ ; Conductivity =  $58 \pm 3 \mu\text{S/cm}$  and Alkalinity =  $235 \pm 33 \mu\text{eq/l}$ . In each aquaria, phosphorus was therefore added to reach the tested concentration. At the beginning of the three experimental periods (To), *R. peltatus* samples had similar mean morphological traits ( $P < 0.05$ ). Neither deficiency symptoms nor senescence of plant tissue were observed during the experimental periods. Further, slight variations in pH (6.6–7.2) and conductivity (63–69) were noticed between the beginning and end of each experiment. Mean phosphorus adsorbed on the aquaria walls was evaluated to approximately 21  $\mu\text{g/l}$  to 28  $\mu\text{g/l}$ , which corresponded to 30, 80 and 270  $\mu\text{g/l}$ , respectively, as the remaining P-concentration available for plants.

Highly significant time effects were identified for all morphological and physiological traits. Further, weaker effects of trophy and the interaction Period  $\times$  P-concentration were found for traits and especially for morphological traits (Table 2). The highest RGR, tQa and NUE were found in April and the lowest in June. Intermediate values were found in August (Fig. 1). NUE was even negative in June (mean NUE:

**Table 2** *F*-ratios and *P*-values obtained with a two-factor ANOVA [period: April, June, August, nutrient availability: A, B, C] on physiological and morphological traits

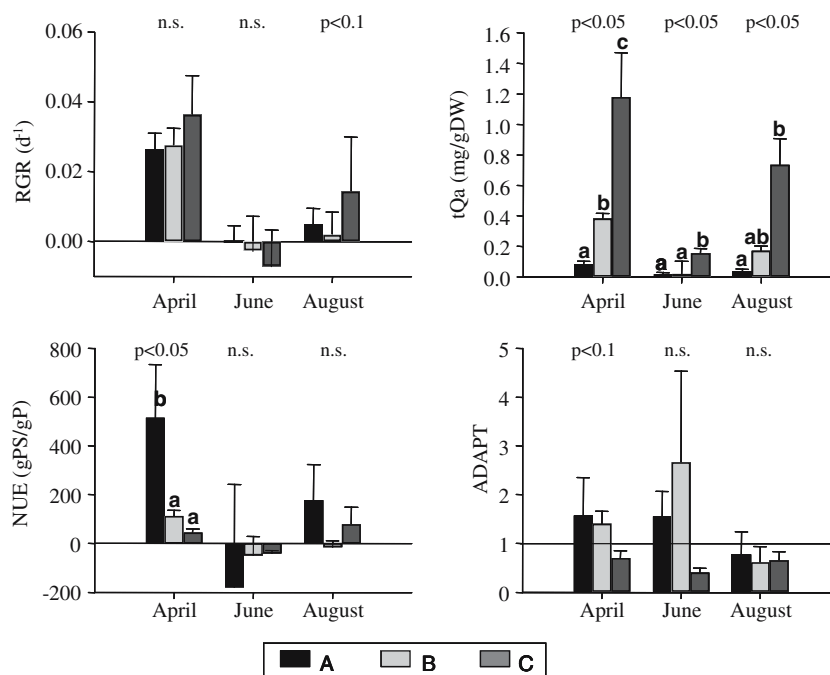
	Period	Trophic level	Period $\times$ Trophic level
<i>Physiological traits</i>			
RGR	84.21***	2.36 (t)	2.82*
tQa	38.87***	75.60***	13.54***
NUE	7.71**	2.35 (n.s.)	3.00*
ADAPT	4.25*	3.13 (t)	2.06 (n.s.)
<i>Morphological traits</i>			
$\Delta(L + \text{Sec})$	95.30***	1.21	1.30
$\Delta\text{ROOT}$	4.83*	2.59 (t)	0.97
$\Delta\text{LNODE}$	14.81***	1.66	1.91
(B + F)	12.19***	2.48 (t)	2.48 (t)

n.s.: non-significant; t: trend ( $P < 0.1$ ), \*: significant ( $P < 0.05$ ), \*\*: very significant ( $P < 0.01$ ), \*\*\*: highly significant

–89.5 gDW/g). ADAPT was also influenced significantly by the experimental period, the ratios being greater than 1 in April and June and less than 1 in August. The greatest elongation of *R. peltatus* photosynthetic area and the highest  $\Delta\text{LNODE}$  were found in April (mean  $\Delta(L + \text{Sec})$ : 22.4 cm; mean  $\Delta\text{LNODE}$ : 0.9), whereas similar low values of  $\Delta(L + \text{Sec})$  and  $\Delta\text{LNODE}$  were found in June and August (mean  $\Delta(L + \text{Sec})$ : 1.1 and 4.2 cm respectively; mean  $\Delta\text{LNODE}$ : –0.27 and –0.14 respectively).  $\Delta\text{ROOT}$  was also related to phenology, being close to zero for April and June (mean  $\Delta\text{ROOT}$ : 0.01 and –0.003 respectively) and near 10% for August (mean  $\Delta\text{ROOT}$ : 0.09). These results indicate that the proportion of rooted internodes was stimulated naturally at the end of the summer.

Through one-way anovas, significant differences relative to water P concentration were detected for most physiological traits: tQa (April, June and August) and NUE (April); RGR (August: trend) and ADAPT (April: trend). Higher values for tQa and NUE were found for concentration A than for concentration C whereas the reverse is true for RGR (Fig. 1). ADAPT also tended to vary according to P-concentration in April, with mean ratios ranging from 0.58 for concentration C to 1.30 for A and 1.56 for B. Therefore, an increase in mean uptake rate was found for concentrations A and B, whereas a decrease was found for C. No clear pattern of

**Fig. 1** Changes in the physiological traits RGR, tQa, NUE and ADAPT as a function of nutrient availability and experimental periods. Bars represent mean of three values for RGR, tQa and ADAPT and nine values for NUE. Error bars show standard error. A, B and C correspond to the initial nutrient concentrations (respectively, 50  $\mu\text{g/l}$ ; 100  $\mu\text{g/l}$ ; 300  $\mu\text{g/l}$ ). The letters “a, b, c” indicate significant differences between the monthly measurements as detected by Newman-Keuls tests ( $P < 0.05$ )

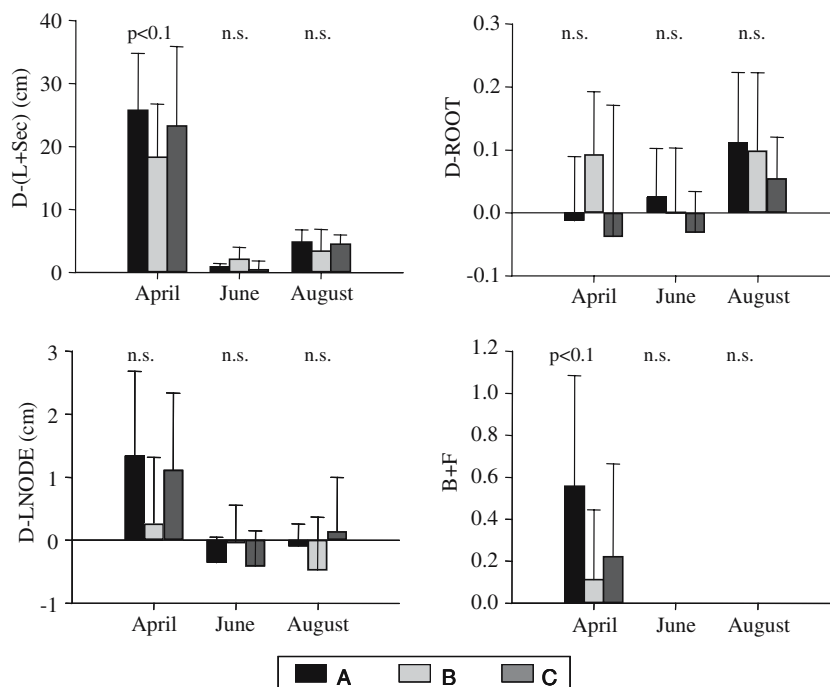


variation was shown however relative to SRP concentrations for morphological traits. Trend variations were though found in April for  $\Delta(L + \text{Sec})$  and B + F (Fig. 2)

## Discussion

Plant responses differed widely in intensity and quality as a function of the season. As previously

**Fig. 2** Changes in the morphological traits  $\Delta(L + \text{Sec})$ ,  $\Delta\text{ROOT}$ ,  $\Delta\text{LNODE}$  and (B + F) as a function of nutrient availability and experimental periods. Bars represent mean of nine values. Error bars show standard error



defined through field observations and experiments, *R. peltatus* phenology was clearly confirmed in the laboratory conditions of the present study. Indeed, changes in physiological and morphological traits pointed to active growth in April, which became less intensive in August. In June, no growth was underlined as an earlier flowering activated the senescing process (Dawson 1980). Maximum *R. peltatus* RGR obtained through this study was found to be slightly lower than RGR found in hypereutrophic conditions in *Ranunculus species* (Eichenberger and Weilenmann 1982; Sand-Jensen and Madsen 1991; Madsen and Brix 1997) but comparable to highly competitive species (Van et al. 1999). Experimental duration may therefore enable to highlight both potential physiological plasticity, which usually takes place within a few hours and morphological plasticity, which may develop at a longer term and should depend on plant growth rate.

Physiological traits depended widely on P-availability. Our results supported most of the hypotheses addressed in the introduction as far as the elongation and regeneration stages are concerned. However, most selected traits were not able to illustrate plastic adjustments in June. RGR increased with P-availability only during the regeneration stage: RGR in hypertrophic water was 2.9 times higher respectively in August than RGR in oligo/mesotrophic waters. Such a high multiplying coefficient resembles highly plastic species. For instance, Van et al. (1999) reported a RGR increasing 1.6 times from low to high fertility for *Hydrilla verticillata* (Lf.) Royle. A low multiplying coefficient of 1.1 was found for another plastic species, *Elodea canadensis* Rich., while nutrient availability increased (Madsen and Cedergreen 2002). In April, *R. peltatus* took up from 0.01–0.17 mgP g<sup>-1</sup> day<sup>-1</sup> from the water, depending on the amount of available P, a finding congruent with other studies (Pelton et al. 1998). These authors also noted that the rate of removal of P from the water increased with an increase in the nutrient loading-rate, which is similar to our findings. Bassirrad (2000) predicts however that nutrient-loading rate follows a Michaelis–Menten equation and reaches a stable nutrient uptake rate for high concentrations. This saturation was not reached in the present experiments, which sug-

gests that in nutrient-rich situations, passive diffusion through macrophyte stems and leaves may preferentially be used rather than less rapid and less efficient uptake in roots via facilitated diffusion with other cations (Carignan 1982; Madsen and Cedergreen 2002). This increase in P-uptake rate and therefore in P-tissue content with P-water is shown in field situations for *R. peltatus* and seems to follow an exponential law (Garbey et al. 2004b). In the field, *R. peltatus* displayed a less efficient ability to integrate rapidly P in plant structures in eutrophic conditions. This ability to store nutrients may be though an adaptive response of species whose range includes systems characterised by fluctuating nutrient supply (Madsen and Cedergreen 2002). In addition, our results indicated, through the calculation of ADAPT, that *R. peltatus* tended to adapt its P-uptake rate as a function of nutrient availability during the elongation stage. Indeed, in April, an increasing uptake rate was found for low P-concentrations while, surprisingly, the reverse was found for high P-availability. Such results suggest that *R. peltatus*' affinity for P was modified, with an increase in transport through the roots when the nutrient was limited. As expected, NUE decreased as a function of nutrient availability in April. Relatively low biomass was produced per milligram of P absorbed when a large P-supply was provided, highlighting a dilution effect (Best et al. 1996). This trend was mitigated much more in June and August as negative values were found for the intermediate P-concentration. The suitability of NUE as an indicator of plant plastic adjustments may be discussed especially when low growth is achieved. Indeed, the selected definition of NUE will give very high values in nutrient-deficient cultures with very low yields and therefore may not be a good indicator of efficient production at low growth and nutrient input (Gauley et al. 1994). The high variability noticed in June for the low P concentration reflected this bias. Regarding the period where maximum growth was achieved, our results supported Bridgham et al.'s model (1995) predicting that nutrient efficiency increased as nutrient availability decreased along a Monod function. However, the model predicts that beyond an optimum resource level, further

nutrient deficiency causes a decrease in nutrient efficiency. Therefore, such an optimum level may not have been exceeded

The lack of morphological functional changes in response to P-enrichment may be explained by three hypotheses. Firstly, *Ranunculus peltatus* may be nutrient-saturated in growth even at the lowest concentrations. Macrophytes are indeed nutrient-saturated with low amounts of nutrients (Gerloff and Krombholz 1966; Dawson and Kern-Hansen 1978). However, plastic adjustments may be however highlighted in saturated nutrient conditions (Van et al. 1999; Madsen and Ceder-green 2002). Another reason is that the selection of traits could have been inappropriate and weakly indicative of plant plastic adjustments according to nutrient stress. Indeed, plasticity of a species in response to a change in environment strongly depends on which trait is measured (Navas and Garnier 2002). This difference in the magnitude of plastic response to varying resources results in a hierarchy of plasticity among traits (White 1979) at the plant level. For example, in the freshwater plant, *Potamogeton pectinatus*, the number and length of branches are more sensitive to sediment characteristics than shoot length (Ideham-Almquist and Kautsky 1995). However, in the present study, variations recorded for  $\Delta(L + Sec)$ ,  $\Delta(LNODE)$  and  $\Delta(ROOT)$  for the three P-concentrations tended to correspond to random variation around an optimal trait value suggesting their independence from P-concentrations. *R. peltatus* morphology may hence not be responsive to P-availability under the experimental conditions considered. An exception is the B + F trait that was assumed at first to vary independently from P-concentration. Thus, contradicting plant ecology strategy schemes, which predict that flowering is enhanced by disturbance (Grime et al. 1988; Westoby 1998), sexual reproduction in these experiments was in fact enhanced in low nutrient availability. Seed production would enable plants to regenerate when conditions became more favourable and would consist of a long-term strategy. Such hypothesis may be however discussed as no information is available yet on the duration between the induction of flower primordia and the development of visible flowers in *R. peltatus*.

This effect may also reflect partly the impact of previous environmental conditions and not only the 9-day experimental conditions. To contain this bias, we used fragments collected in the same conditions which relative differences in their traits should be only depending on the concentrations tested

Two main aspects of *R. peltatus* plasticity according to nutrient availability were therefore highlighted. (i): Plasticity in *R. peltatus* is closely dependent on its phenology. Two hypotheses can explain this aspect. The first is the occurrence of a minimum metabolism activity to achieve plastic adjustments. Indeed, plants have to face the trade-off between costs induced by plasticity and benefits for the plant. Costs induced by plasticity may thus be only worth investing when plants may support them and benefit most from the competitive advantage plasticity gives. The second hypothesis is that the plasticity capacity of a plant may change during ontogeny. Meristems produced at different stages may respond differently to the same environmental trigger. This phenomenon, referred to as “ontogenetic contingency”, has been found to occur in some terrestrial plants (Diggle 1994; Watson et al. 1995) and, indirectly, in some aquatic species (Barko et al. 1982). At present, we can offer no conclusion about which hypothesis may provide the best interpretation of our results. (ii) According to nutrient availability, physiological rather than morphological adjustments were recorded. This strategy, already noted in terrestrial situations, is the result of the trade-off previously mentioned. Physiological plasticity is usually associated with a change in properties brought about by reversible sub-cellular rearrangements, and represents lower costs and a more rapid response to environmental stress, whereas morphological plasticity, involving replacement of existing tissues by new plant parts with different characteristics, appears to represent a high-cost solution to a change in environment (Bradshaw 1965). Nevertheless, physiological adjustments may act as a primary signal that could lead to longer-term responses in morphology. Further experiments should be conducted on longer periods to verify whether morphological plasticity would occur over the long term.

The evaluation of plasticity is therefore highly dependent on the time and duration of experiments. Most studies carried out in aquatic situations focus on short, punctual experiments which may underestimate plant plastic potential if done during a non-active growth stage. A solution would be (as we chose) to replicate these short experiments for each main stage in plant life cycle in order to integrate plant biology and therefore extrapolate plant response throughout the entire vegetative phase of plant growth and development.

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