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Article

Mineral nutrition in aquatic carnivorous plants: effect of carnivory, nutrient reutilization and K^+ uptake

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With 4 tables

Abstract: Processes of mineral nutrition were studied in the shoots of 5 rootless species of aquatic carnivorous plants. *Aldrovanda vesiculosa* and *Utricularia australis* shoots pre-cultivated with or without prey exhibited zero or very low NO_3^- uptake (for 4 h) in both prey variants, while NH_4^+ uptake in both species was around 1 mmol kg^{-1}_{FW} h^{-1} and was slightly higher in the –Prey variants. Thus, both species preferred NH_4^+ uptake over NO_3^-. Phosphate uptake ranged between 26–91 µmol kg^{-1}_{FW} h^{-1} and was significantly higher in the +Prey variant only in *A. vesiculosa*. K^+ uptake by shoots at 15 µM NH_4^+ was very low or rather distinctly negative due to an uptake interference with NH_4^+. K^+ uptake in light from a NH_4^+-free medium was always greatest in the apical (or photosynthetic in *U. stygia*) shoot segments, and ranged from 0.07–0.61 mmol kg^{-1}_{FW} h^{-1} in 5 aquatic carnivorous species, while it was always the lowest or even negative (in *U. vulgaris* and *U. stygia*) in basal or carnivorous shoot segments. *A. vesiculosa* and *U. australis* shoots grown at high (83 µM) and very low K^+ concentration (2–4 µM) showed no growth decrease at the low K^+ concentration. Relatively efficient N and P reutilization (28–62 %) but very low or negative K reutilization (5.3 to –8.5 %) was found in senescent shoot segments in both species. The results confirmed that prey capture in aquatic carnivorous plants has significant effects on the uptake of mineral N and P from the ambient water. It is not clear whether or not these changes are due to alterations in the shoot N and P content. However, it is apparent that the physiological responses of aquatic species to prey capture are highly species-specific.

Key words: *Aldrovanda vesiculosa*; *Utricularia* spp.; mineral nutrient uptake; feeding on prey; shoot polarity; N; P and K reutilization; shoot nutrient content; K^+ uptake

Introduction

Within the ecological group of carnivorous plants, around 50 species from the monotypic *Aldrovanda* (Droseraceae) and *Utricularia* (Lentibulariaceae) genera are submerged aquatic or amphibious plants (Juniper et al. 1989; Taylor 1989; Guisande et al. 2007). Aquatic carnivorous plants usually grow in shallow, standing humic (dystrophic) waters which are usually poor in inorganic N and P, but sometimes also in K (see Adamec 1997a; Adamec 2011; Guisande et al. 2007). On the other hand, their typical habitats have relatively high free CO_2 concentrations (Adamec 1997b; Adamec 2008a; Adamec 2009; Adamec 2011). Aquatic carnivorous plants are rootless and take up all necessary nutrients either directly from the ambient water or from animal prey by traps. In *Utricularia* spp., they can also utilise trap associations with different microbial commensals (Richards 2001; Sirová et al. 2009). They usually have linear shoots composed of regularly repeating modules consisting of leaf nodes and internodes. These maintain a high level of physiological polarity along the shoot. Under favourable conditions, even in nutrient-barren waters, they ex-

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hibit very rapid apical shoot growths of 1–4 leaf nodes d^{-1} while their senescent shoot bases decay at the same rate ("conveyer-belt" shoot growth system; Friday 1989; Adamec 2000; Adamec 2008a; Adamec 2008b; Adamec 2009; Adamec 2011; Adamec & Kovářová 2006; Adamec et al. 2010); the new biomass is allocated mainly to branches. This observed very rapid growth of aquatic carnivorous plants in nutrient-poor waters requires a combination of several ecophysiological adaptations that enable the plants to acquire the very limited supplies from the ambient water. The principal adaptations of the shoots include a very high net photosynthetic rate, prey capture, efficient mineral nutrient reutilization (resorption/recycling) from senescent shoots (efficient nutrient economy) and a very high nutrient uptake affinity from water (Friday 1989; Adamec 1997a; Adamec 1997b; Adamec 2000; Adamec 2006; Adamec 2008a; Adamec 2008b; Adamec 2009; Adamec 2011; Adamec 2013a; Adamec 2014; Englund & Harms 2003).

The very rapid apical shoot growth and the distinct growth polarity in *Aldrovanda* and *Utricularia* are associated with a steep physiological polarity of linear shoots. This includes a gradient in aerobic dark respiration and net photosynthetic rate, chlorophyll content, total starch and sugar contents, cytokinin content, the proportion of shoot dry weight (DW) to fresh weight (FW) and shoot mineral nutrient content (Friday & Quarmby 1994; Adamec 2000; Adamec 2008a; Adamec 2011; Adamec 2013a; Adamec 2013b; Adamec 2014; Adamec unpubl. res.). The foliar and/or shoot N and P contents steeply decrease from the shoot apex to the senescent shoot base, while the opposite occurs with K and Ca shoot contents, which are greatest at the aged shoot base. Thus, in aquatic carnivorous plants, N and P are efficiently reutilized from senescent shoot bases to the growing shoot apices or branches but Ca and K are not (Adamec 2000; Adamec 2008a; Adamec 2011; Adamec 2014) and must be permanently taken up from the ambient water or from prey. The fact that the same pattern of N, P and K reutilization has also recently been found in a rootless submerged macrophyte *Ceratophyllum submersum*, but not in five terrestrial *Utricularia* species, suggests that the inability of K reutilization may be general for all submerged plants (Adamec 2014). In general, the efficiency of N and P reutilization in senescent leaves/shoots is considered a significant ecophysiological trait and an adaptation to nutrient-poor environments (Killingbeck 1996; Aerts et al. 1999; Adamec 2002). However, the topic of mineral nutrient reutilization in aquatic plants clearly requires further study.

Mineral nutrient uptake by leaves or shoots in aquatic carnivorous plants is still veiled in mystery, and much of this is caused by methodical difficulties. Prey feeding, both in *Aldrovanda* and *Utricularia* spp., markedly enhanced their growth in many experiments (for the review see Adamec 1997a; Adamec 2011) but the nutrient uptake efficiency from prey is almost unknown. In one rare example covering only *U. vulgaris*, Friday & Quarmby (1994) found an 83 % efficiency of N uptake from young mosquito larvae. Moreover, no available results allow the determination of whether captured prey leads to a stimulation of nutrient uptake by shoots (*sensu* Adamec 1997a; Adamec 2011; Adamec et al. 2010). Aquatic carnivorous plants are adapted to growing in oligo- to mesotrophic, humic, slightly acidic waters in which NH_4^+ concentration usually prevails markedly over NO_3^- (Adamec 1997a; Adamec 2011). In a couple of uptake experiments, three species clearly preferred NH_4^+ over NO_3^- from 15–34 μM NH_4NO_3 (Adamec 2000; Fertig 2001). However, it is possible to assume from the growth in very nutrient-poor waters with zero prey availability that shoots of *Utricularia* species possess a very high uptake affinity for mineral nutrients: at least 0.4 μM for NH_4^+ and 0.1 μM for phosphate (e.g., Adamec 2009). Surprisingly, a ca. 1000 times lower NH_4^+ uptake affinity occurs inside prey-free *Utricularia* traps (Sirová et al. 2014).

Even less is known on the K^+ uptake characteristics in aquatic carnivorous plants. Even though rapidly growing species exhibit a rather high shoot K content of 1.5–5 % of DW (Adamec, 1997a; Adamec 2008a), they lose all the K in their senescent shoots. This is in marked contrast to the very efficient K reutilization found in terrestrial *Utricularia* species (Adamec 2000; Adamec 2014). Prey is a relatively poor K source so aquatic carnivorous plants must rely mainly on constant, rapid K^+ uptake by shoots from the ambient water. The K^+ concentration in some humic habitats can, however, be as low as 0.01 mg l^{-1} (i.e., 0.25 μM; see Adamec 1997a). *A. vesiculosa* shoots only took up K^+ by their basal and not their apical shoot segments, which need a permanent K^+ supply to maintain their rapid apical growth (Adamec 2000). However, repeated experiments in *Aldrovanda* and three *Utricularia* species failed to estimate any positive medium-term K^+ uptake from diluted media in light conditions (Adamec, unpubl.). These results may indicate that K^+ uptake occurs only under conditions of high photosynthetic rate, or rapid apical shoot growth or in the absence of NH_4^+ (Szczerba et al. 2008a; Szczerba et al. 2008b).

The aim of this work was to investigate how the processes of mineral nutrient uptake (NO_3^-, NH_4^+, HPO_4^{2-}, K^+) by shoots of *Aldrovanda vesiculosa* and *Utricularia* spp. from the ambient growth medium depend on prey capture. As found recently in aquatic *Utricularia reflexa* shoots (Adamec 2014) no K^+ is reutilised from senescent shoots and is thus lost to the plant. As K^+ concentration in ambient waters may sometimes be very low and thus growth-limiting for rootless aquatic carnivorous plants (Adamec 1997a), another two experiments focused on K^+ uptake by the shoots of 5 aquatic carnivorous species and on the character of K reutilization in *A. vesiculosa* and *U. australis* shoots raised at different K^+ concentrations.

Material and methods

Experiment A: Measurement of nutrient uptake by shoots of *A. vesiculosa* and *U. australis*

Mineral nutrient uptake was measured in shoots which were pre-cultivated with or without prey for 10–11 d. Adult stock plants of *Aldrovanda vesiculosa* L. (collected from E Poland) were grown outdoors in a 300 l laminate container which approximately simulated natural conditions and litter of robust *Carex* species was used as a substrate (Adamec 1997b; Adamec 2008b; Adamec 2009). Subadult stock plants of *Utricularia australis* R.Br. (from Třeboň Basin, Czech Republic) were grown in a 0.8 m^2 plastic container, which stood in a naturally lit greenhouse with open walls for cooling, and the growth conditions were similar to that above (Adamec et al. 2010). In both stock containers, plants were able to capture fine zooplankton.

On the 12th of June, twelve randomly selected, homogeneous non-branched *A. vesiculosa* plants were shortened to 8 mature apical leaf nodes (mean shoot length 5.3 ± 0.2 cm) and 12 non-branched *U. australis* plants were shortened to 16 mature apical leaf nodes (mean shoot length 9.7 ± 0.3 cm). These shortened shoots were allowed to grow in a 0.8 m^2 (40 cm high) plastic container, which stood in the naturally lit greenhouse. The container contained 300 l of tap water and 120 g (DW) of *Carex acuta* litter as substrate on the bottom (Adamec et al. 2010) and its water chemistry was similar to that in stock cultures. Six randomly selected shortened shoots of each species were placed into the container and could freely capture the added prey (variant +Prey) whereas the other 6 shoots of each species were transferred to each of two floating frames located in the same container (Adamec 2008b). The plastic floating frames were 0.35 m × 0.35 m by about 7 cm depth. Mesh with a pore size of 150 µm was attached to the bottom of the frames, to exclude zooplankton capture but still maintain unimpeded water exchange (variant –Prey). During the whole experimental cultivation period of 10–11 days, the pH of the water was within 7.54–7.65, electrical conductivity 38.7–39.2 mS m^{-1}, total alkalinity 1.21 meq l^{-1} and the free CO_2 concentration was ca. 0.06–0.08 mM. There was no indication that the pH inside and outside the floating frames differed by more than by 0.2 units. The water was considered slightly humic and oligotrophic: 0.53 µM NO_3^-, NH_4^+ below detection limit, 0.43 µM phosphate, ca. 90 µM K^+. Every two days, the meshes were thoroughly cleaned by tap water to further exclude any zooplankton inside the frames. Small zooplankton (undetermined ostracods, *Chidorus* sp., *Cyclops* sp., *Eudiaptomus* sp., size 0.6–1.5 mm) was added regularly to the container to feed the control plants. A submersible temperature data logger (Minikin T, EMS, Brno, Czech Rep.) monitored the water temperature at plant level. During the growth period, daily temperature maxima were within 19.7 to 28.3 °C and night minima were within 16.4 to 23.8 °C; daily oscillations were within 3 to 5 °C. The irradiance (PAR) at plant level ca. 1 mm below the water surface was ≈ 40 % of that in the open, which is an optimum level for both species (Adamec 1997b; Adamec et al. 2010).

After 10 days of the pre-cultivation with or without prey, 5 shoots of *A. vesiculosa* of each variant were shortened to 12 mature leaf nodes (shoot length 7.0–7.5 cm) for the measurement of nutrient uptake. A binocular loupe was used to check the traps and for the +Prey variant, ca. 35.3 % traps from the 6th–12th leaf node had animal prey. Virtually no prey was found in the –Prey variant. Similarly, after 11 days of pre-cultivation, 5 shoots of *U. australis* of each variant were shortened to 25 mature leaf nodes (shoot length 14–16 cm). The +Prey variant contained prey in their 10th–11th mature leaf nodes in ca. 32.8 % traps (> 50 % in 19th–20th nodes), while no prey was observed in the –Prey variant. Shoot FW was within 130–175 mg in *A. vesiculosa* and 230–480 mg in *U. australis*.

Shortened shoots were thoroughly washed with tap water, blotted dry and rinsed with distilled water. No macroscopic attached algae were present on the shoots. Firstly, shoots were placed in an experimental medium and exposed in a low-volume growth chamber at ca. 180 µmol m^{-2} s^{-1} (PAR) of fluorescent light at 25 ± 1 °C for 3 h as a pretreatment to stabilise ion fluxes (Adamec 2000). The medium contained (in µM): 15 NH_4NO_3, 4 K_2HPO_4, 52 KCl, 100 $CaCl_2$, 10 $MgSO_4$, 200 $NaHCO_3$ and ca. 100 CO_2. The initial pH ranged from 6.6 to 6.7. Then the shoots were blotted dry and each was put in 100 ml of fresh medium to estimate the ion uptake rate for 4 h. After 4 h, the media were sampled and analysed colorimetrically for NO_3^-, NH_4^+ and phosphate by an automatic FIAstar 5010 Analyzer (Tecator, Sweden), while K^+ concentrations were estimated by atomic absorption flame spectrometry using a Varian AA240FS (Agilent, Santa Clara, CA, USA). The ion uptake rate was calculated from the decrease in the initial ion concentration. FW of each shoot and DW (80 °C) of mixed samples were estimated. All variants consisted of 5 replicates. Results are expressed in mmol kg^{-1}_{FW} h^{-1}.

Experiment B: Measurement of K^+ uptake by shoots of 5 aquatic carnivorous species

K^+ uptake by apical and basal parts of shoots (or by photosynthetic and carnivorous shoots) or intact shoots was measured in *A. vesiculosa, U. australis*, *U. vulgaris* L., *U. reflexa* Oliver, and *U. stygia* Thor within 25th June –2nd July 2014. Plants of *A. vesiculosa* (collected from SW Hungary) were grown in an outdoor 1 m^2 laminate container at 0.13 mM K^+ in the ambient water and *U. australis* in an outdoor 3 m^2 laminate container at 0.026 mM K^+. *U. vulgaris* (from S Moravia, Czech Rep.), *U. reflexa* (from Okavango Delta, Botswana) and *U. stygia* (from Třeboň Basin) were grown in the same outdoor 2.5 m^2 laminate container at 0.15 mM K^+. Generally, water in the containers simulated natural conditions as above (see Exp. A) and all plants were able to capture small zooplankton. All containers

were partly shaded to ca. 30–50 % of the irradiance of that in the open. In *A. vesiculosa*, K^+ uptake was measured by apical shoot segments (4 cm long, 8 mature leaf nodes), basal shoot segments (4–5 cm, 9th–16th leaf nodes) and by whole, intact shoots (7–7.5 cm, 15–16 leaf nodes). In *U. australis*, by apical shoot segments (15–16.5 cm, 20 leaf nodes), basal shoot segments (12–17 cm, 21st–38th leaf nodes) and whole shoots (20–30 cm, 37–40 leaf nodes); in *U. vulgaris*, by apical shoot segments (13.5–26 cm, 25 leaf nodes), basal shoot segments (11–20 cm, 26th–56th leaf nodes) and whole shoots (25–34 cm, 51–55 leaf nodes); in *U. reflexa*, by apical shoot segments (16–22 cm, 15 leaf nodes) and basal shoot segments (17–24 cm, 16th–33rd leaf nodes), while in *U. stygia*, by photosynthetic shoots (7–17 cm) and pale carnivorous shoots (5–14 cm). Senescent parts of shoot bases were always removed.

Thoroughly washed shoots were placed in 500 ml of an experimental medium in the growth chamber and exposed under Exp. A conditions for 3 h as a pre-treatment. Due to fact that K^+ uptake could be inhibited by the simultaneous NH_4^+ uptake (see Szczerba et al. 2008a; Szczerba et al. 2008b), NH_4^+ ions were omitted from the medium. The medium contained (in µM): 5 K_2HPO_4, 70 KCl, 10 $CaCl_2$, 10 $MgSO_4$ and 300 $NaHCO_3$. The growth chamber was gently shaken. Then the shoots were blotted dry and each was put in 40–60 ml of fresh medium to estimate the K^+ uptake rate under the same conditions for 6 h. For each species, one shoot segment was used as one replicate but in *U. stygia*, 2–3 shoots were used. Overall, FW of the replicates was within 0.1–1.6 g for all species. As K^+ uptake by leaves of aquatic plants depends strongly on light conditions and net photosynthetic rate (Jeschke 1976; Adamec 1997c), CO_2 was permanently added to the medium to enhance net photosynthetic rate during the experiment. The 60 ml plastic jars with the medium and plants were placed in a small empty aquarium, which stood in the shaken growth chamber. The aquarium was covered by a plexiglas pane and CO_2 from a cylinder was very gently added by PVC tubing to the aquarium air. The CO_2 dosing was based on the continuous measurement of pH (by a combined pH electrode) in the same medium with another shoot placed in a parallel, 'testing' plastic jar and on the calculation of free CO_2 concentration according to carbonate equilibria (Helder 1988). Generally, the pH in the 'testing' jar was kept within 6.10–6.55 in all experiments and the free CO_2 concentration was thus ca. 0.2–0.5 mM, which enabled the plants very high photosynthetic rate (cf. Adamec 1997b; Adamec 2013a). After 6 h, the media were sampled and analysed for K^+ using ion chromatography (881 Compact IC pro-Cation; Metrohm AG, Herisau, Switzerland; cation column Metrosep C4-150/4.0). FW of each shoot and DW (80 °C) of mixed samples were estimated. All variants consisted of 6 replicates.

Experiment C: Reutilization of N, P and K in shoots of *A. vesiculosa* and *U. australis* raised at different K^+ concentrations

This experiment followed-up on the finding of efficient reutilization of N and P but zero K reutilization in senescent shoots of two rootless submerged plants (Adamec 2014). A special growth experiment was conducted which enabled *A. vesiculosa* and *U. australis* to grow under nearly natural conditions in humic water at very different K^+ concentrations. Twelve 3.5 l glass miniaquaria ('cucumber bottles'; e.g. Adamec 2014), each containing *Carex acuta* litter (6 g dry weight, but presoaked for 2 days) and 2.5 l of growth solution were set up 5 days before the experiment. The miniaquaria were covered with Petri dishes and floated in a 0.8 m^2 plastic container (40 cm high, 300 l), which stood in the naturally lit greenhouse (see above), for cooling. The experimental solution contained (in µM): 300 $NaHCO_3$, 20 $NaNO_3$, 2 K_2HPO_4, 100 $CaCl_2$, 40 $MgSO_4$, 6 FeNa-EDTA and 40 µl l^{-1} of the Gaffron microelement stock solution. In 6 miniaquaria, the K^+ concentration was only 3.9 µM ('low K^+' variant) and in the other 6 miniaquaria, KCl was added to make a final concentration of 128 µM ('high K^+' variant). At the start of the growth experiment on 11th June, 5 days after adding the soaked litter, the water chemistry in both variants changed. The water became brownish and could be considered mesotrophic: 5.7 µM NH_4^+, NO_3^- below detection limit, 1.17–1.84 µM $H_2PO_4^-$, pH 6.53–6.62, total alkalinity 409 µeq l^{-1}, 225–277 µM CO_2; K^+ concentration in the high K^+ variant was 83 ± 1.8 µM, while only 3.9 ± 0.2 µM in the low K^+ one.

A. vesiculosa (from E Poland) was grown in the outdoor 2.5 m^2 laminate container (see above) at 0.31 mM K^+ in the water. Twenty-four randomly selected, homogeneous plants were shortened to 12 mature apical leaf nodes (shoot length 8–11 cm) and all visible branches were excised. Subadult plants of *U. australis* 35–55 cm long were collected freshly from a mesotrophic, slightly humic forest fishpond in the Třeboň Basin where they grew with 0.15 mM K^+ in the water. Twenty-four randomly selected plants were shortened to 35 mature apical leaf nodes (shoot length 17–27 cm) and all visible branches were excised. Shoot lengths of both species was measured with a ruler. To estimate the apical shoot growth rate (as formation of new leaf nodes d^{-1}), internodes between the third and fourth leaf node in both species were tagged by a fine thread (Friday 1989; Adamec 2008b; Adamec 2009). Shoots were then thoroughly washed in tap water, rinsed in distilled water and blotted dry. Two randomly selected shoots of both species were put in each of the 6 miniaquaria within one variant (n = 12) on 11 June. The growth in the greenhouse lasted for 11 days. The container with the shoots was shaded by a neutral-density nylon filter and the irradiance at plant level was ca. 25 % of that in the open. A submersible data logger (see above) monitored water temperature in the container. During the growth period, the daily temperature maxima were within 21.4 to 35.2 °C and night minima within 18.2 to 28.5 °C with a total mean temperature of 27.0 °C; daily oscillations were within 3 to 5 °C.

Chemical analyses of water samples in the miniaquaria at the end of the growth period revealed that the pH in both variants was within 6.75–7.03, total alkalinity 0.37–0.41 meq l^{-1}, free CO_2 concentration 0.08–0.15 mM, NH_4^+ concentration 1.0–3.0 µM, NO_3^- concentration was below the detection limit and $H_2PO_4^-$ concentration 0.34–0.76 µM. No apparent differences occurred between both variants. In the high K^+ variant, the final K^+ concentrations were within 85 ± 11 µM (SD, n = 6), whereas in the low K^+ variant, they were consistently below the detection limit (ca. < 2 µM). The main shoot length, number of adult leaf nodes, position of the tag and number of branches were estimated in both species. To assess a reutilization efficiency, tissue N, P and K content was estimated in 4th–5th mature leaf nodes with traps in *A. vesiculosa*, in 9th–10th nodes (deprived of all traps) in *U. australis*, and also in two last living leaf nodes in both species (cf. Adamec 2000; Adamec 2008a; Adamec 2013a; Adamec 2014). The dried material (80 °C) of both parallel plants within each species in each miniaquarium was pooled together (n = 6) and ground by forceps into small pieces. Contents of N, P and K of leaf nodes were estimated in diluted samples after acid mineralization (for all details see Adamec 2002; Adamec

2008a) using the same technique as per Exp. A above. The results of tissue nutrient contents in mature and senescent shoot segments are always expressed in % DW and presented as such. As no data are available to determine the relative DW decrease in senescent shoots of aquatic carnivorous species (cf. Adamec 2000; Adamec 2008a; Adamec 2014), FW and DW (80 °C) were estimated in mature as well as senescent shoot segments of adult plants of *A. vesiculosa* (field collected) and *U. vulgaris* (grown outdoors). In the former species, the DW decrease in senescent shoot segments was 17.3 ± 1.7 % (SE, n = 6), while it was inconsistent in the latter species. Thus, the calculated correction factor of 0.827 (i.e., 1–0.173) was used for both species used to multiply the presented estimated values of nutrient content for senescent shoots to calculate the true N, P and K reutilization efficiency (ratio), which is defined as the decline of total nutrient content relative (in %) to that of the adult organ (Killingbeck 1996; Adamec 2002; Güsewell 2005; cf. Adamec 2014). Negative values of nutrient reutilization ratio indicate higher nutrient content in old shoots after the correction.

Statistical treatment

All data were checked for normality by a Kolmogorov-Smirnov test and were not transformed. The data in Exps. B and C were evaluated for significant differences using 1-way ANOVA (Tukey HSD test for multiple comparisons). All other data were evaluated using a two-tailed Student t-test. Means ± 1 SE are shown.

Results

The measurements of mineral nutrient uptake by *A. vesiculosa* and *U. australis* shoots pre-cultivated with or without prey, showed zero or very low NO_3^- uptake in both prey variants; in the latter species, the uptake in the –Prey variant was positive and differed significantly from the +Prey variant (Table 1). NH_4^+ uptake in both species was around 1 mmol kg^{-1}_{FW} h^{-1} and was slightly higher in the –Prey variants, but was only significant in *A. vesiculosa.* Phosphate uptake was about 10–40 times lower than that of NH_4^+, ranged between 26–91 µmol kg^{-1}_{FW} h^{-1}, and was significantly higher in the +Prey variant only in *A. vesiculosa*. K^+ uptake by shoots in 15 µM NH_4^+ was very low but positive only in the +Prey variant of *U. australis,* while a net efflux (0.15–1.06 µmol kg^{-1}_{FW} h^{-1}) was found in all other variants. In *A. vesiculosa*, the K^+ efflux was 2.4 times higher and significantly different in the –Prey variant.

K^+ uptake from a NH_4^+-free medium was always greatest in the apical (or photosynthetic in *U. stygia*) shoot segments and ranged from 0.07–0.61 mmol kg^{-1}_{FW} h^{-1} in 5 aquatic carnivorous species (Table 2). Whereas the K^+ uptake rate was always lowest or even negative (in *U. vulgaris* and *U. stygia*) in basal or carnivorous shoot segments and this difference was always statistically significant. The K^+ uptake rate by whole shoots in each species was around the mean of the uptake rate by the apical and basal segments.

The growth analysis of *A. vesiculosa* shoots at high and very low K^+ concentration showed no growth enhancement at high K^+ concentration (Table 3); greater

Table 1. Uptake of mineral ions by shoots of *Aldrovanda vesiculosa* or *Utricularia australis* from a diluted mineral medium in light for 4 h as dependent on prey feeding. Means ± 1 SE intervals are shown; n = 5. Within each species, asterisk denotes significant difference ($p < 0.05$; t-test) between both variants. Negative values indicate K^+ efflux from the shoots.

Species	Prey	DW (% FW)	Uptake of ions (in mmol/kg_{FW} h)			
			NO_3^-	NH_4^+	HPO_4^{2-}	K^+
A. vesiculosa	+	10.5	0.0	0.61 ± 0.02*	0.048 ± 0.007*	–0.445 ± 0.060*
–“”–	–	9.02	0.0	1.03 ± 0.03	0.026 ± 0.003	–1.06 ± 0.090
U. australis	+	6.36	0.0*	0.97 ± 0.20	0.091 ± 0.011	0.023 ± 0.049
–“”–	–	6.75	0.054 ± 0.015	1.36 ± 0.13	0.071 ± 0.003	–0.146 ± 0.058

Table 2. K^+ uptake by apical or basal shoot segments or by whole shoots from a solution of 80 µM K^+ (without NH_4^+) in light for 6 h. In *U. stygia,* the apical shoot segments were trap-free photosynthetic shoots, while basal shoot segments were pale carnivorous shoots with traps. Uptake rates are expressed in mmol kg^{-1}_{FW} h^{-1}. The negative sign indicates K^+ efflux from the shoots. Means ± 1 SE are always shown; n = 6. Different letters within each species denote significant difference at $p < 0.05$ (1-way ANOVA).

Species	Apical shoot segments		Basal shoot segments		Whole shoots	
	DW (% FW)	Uptake of K^+	DW (% FW)	Uptake of K^+	DW (% FW)	Uptake of K^+
A. vesiculosa	10.3	0.607 ± 0.036^a	9.01	0.176 ± 0.022^b	8.83	0.186 ± 0.024^b
U. australis	7.32	0.455 ± 0.016^a	6.76	0.181 ± 0.032^b	6.65	0.319 ± 0.033^c
U. vulgaris	7.32	0.067 ± 0.010^a	7.28	–0.085 ± 0.026^b	7.90	0.000 ± 0.033^{ab}
U. reflexa	6.19	0.218 ± 0.012^a	7.84	0.083 ± 0.016^b	–	–
U. stygia	9.78	0.387 ± 0.026^a	5.46	–0.048 ± 0.038^b	–	–

Table 3. Results of a growth experiment on *A. vesiculosa* (AV) and *U. australis* (UA) during a 10–11 d cultivation at a high (ca. 83 μM) or a low (ca. 4 μM) K^+ concentration. Within the same species, the symbols indicate a statistically significant difference (t-test): $^{**}p < 0.01$; $^{ns}p > 0.05$. Means ± 1 SE are always shown; n = 12.

Spec.	K+ conc.	Initial shoot length (mm)	Final shoot length (mm)	Final leaf nodes	Branches	Apical shoot growth rate (node d^{-1})
AV	high	90.0 ± 3.2	187 ± 8^{ns}	22.0 ± 0.6^{ns}	0.67 ± 0.14^{ns}	1.16 ± 0.03^{ns}
AV	low	88.8 ± 2.9	180 ± 7	21.3 ± 0.6	0.36 ± 0.15	1.11 ± 0.02
UA	high	229 ± 9	457 ± 24^{ns}	71.8 ± 2.2**	1.67 ± 0.26^{ns}	3.94 ± 0.07^{ns}
UA	low	209 ± 7	495 ± 11	78.6 ± 0.7	1.33 ± 0.26	4.02 ± 0.06

Table 4. The comparison of tissue N, P and K content in mature and old, senescent shoot segments of *A. vesiculosa* (AV) and *U. australis* (UA) after a cultivation at a high (ca. 83 μM) or a low (< 4 μM) K^+ concentration. RE-, true reutilization ratio of the given nutrient in senescent organs including the correction factor of 0.827. Means ± 1 SE are shown; n = 6. Within each species and the same K^+ variant, the different letters (on the right side) denote a statistically significant difference between mature and old organs; the asterisks on the left side denote a significant difference between the K^+ variants within each species and the same organ age ($p < 0.05$, 1-way ANOVA).

Spec.	Organ age	K^+ conc.	N content (% DW)	RE-N (%)	P content (% DW)	RE-P (%)	K content (% DW)	RE-K (%)	N:P	N:K
AV	mature	high	1.95 ± 0.10^a	–	0.276 ± 0.016^a	–	*3.88 ± 0.16^a	–	7.11 ± 0.17^a	*0.510 ± 0.038^a
AV	old	high	0.950 ± 0.045^b	59.8	0.127 ± 0.008^b	61.9	2.31 ± 0.27^b	33.3	7.50 ± 0.23^a	0.433 ± 0.040^a
AV	mature	low	1.84 ± 0.07^a	–	0.258 ± 0.012^a	–	1.86 ± 0.10^a	–	7.20 ± 0.31^a	1.01 ± 0.073^a
AV	old	low	0.886 ± 0.029^b	60.3	0.123 ± 0.007^b	60.5	2.14 ± 0.13^a	5.3	7.26 ± 0.30^a	0.423 ± 0.030^b
UA	mature	high	*1.39 ± 0.09^a	–	*0.200 ± 0.010^a	–	*3.42 ± 0.25^a	–	7.03 ± 0.55^a	*0.419 ± 0.043^a
UA	old	high	1.06 ± 0.07^b	36.6	0.098 ± 0.007^b	59.6	*4.04 ± 0.20^a	2.1	11.1 ± 0.82^b	*0.264 ± 0.014^a
UA	mature	low	1.16 ± 0.06^a	–	0.157 ± 0.009^a	–	1.75 ± 0.10^a	–	7.40 ± 0.31^a	0.674 ± 0.056^a
UA	old	low	1.01 ± 0.08^a	27.7	0.094 ± 0.002^b	50.4	2.29 ± 0.11^a	−8.5	10.8 ± 0.94^b	0.449 ± 0.047^b

branching was not statistically significant. However, in *U. australis,* the shoots grown at the low K^+ concentration (< 3.9 μM) lost significantly less senescent leaf nodes than in the high K^+ variant, which corresponded to slightly (and non-significantly) increased shoot length in the low K^+ variant. Branching in the low K^+ variant non-significantly decreased.

As follows from tissue N and P contents in adult and senescent shoot segments in both species, relatively efficient N and P reutilization was proven in senescent segments in both species (Table 4). In *A. vesiculosa*, N and P contents in senescent segments were around two times lower in both K^+ variants than those in adult segments and, after the correction for DW decline in senescent segments, N and P reutilization efficiency was 60–62 %. In *U. australis*, a similarly efficient P reutilization (50–60 %) but much less efficient N reutilization of 28–37 % was found in both K^+ variants. In *A. vesiculosa*, the pattern of K content in adult and senescent shoot segments was opposite in both K^+ variants: the K content in senescent segments decreased significantly by 40 % in the high K^+ variant and the calculated K reutilization efficiency reached formally 33 %, while the K content rose non-significantly by 15 % in senescent segments in the low K^+ variant and the corrected K reutilization efficiency was only 5.3 %. A similar trend of very low or even negative K reutilization efficiency (2.1 to −8.5 %) occurred in *U. australis* in both K^+ variants. The high K^+ concentration in the medium resulted in around a two times higher tissue K content in adult *A. vesiculosa* shoot segments when compared to those growing at the low K^+ concentration (Table 4). Similarly in *U. australis*, K content in both adult and senescent shoot segments growing in the high K^+ variant was ca. 1.9 times higher as compared to the same segments growing in the low K^+ variant. Moreover, the high K^+ concentration in the medium also led to a significant increase of N and P content in adult *U. australis* shoot segments as compared to the low K^+ variant. An almost constant N:P ratio (weight ratio) of ca. 7.3 in both shoot ages and K^+ variants in *A. vesiculosa* confirmed the equal reutilization efficiency of N and P, and the same N:P ratio was also found in adult *U. australis* shoot segments. However, due to a lower N reutilization efficiency in the latter species, the ratio was significantly greater in senescent segments. In both species, the N:K ratio differed significantly between adult and senescent shoot

segments only within low K^+ variants, with the higher ratio in adult segments.

Discussion

In spite of the many growth experiments on this plant group assessing the importance of prey capture for plant growth, there are only a few studies that aim to measure the medium-term nutrient uptake rate from the water by shoots or even by shoots grown either with and without prey (Adamec 2000; Fertig 2001). Present results on two species show usually a zero NO_3^- uptake but a consistent NH_4^+ uptake and, thus, a great preference for NH_4^+ uptake (Table 1). A similar distinct NH_4^+ uptake preference was reported by Adamec (2000) for *A. vesiculosa* (5–7 times) and by Fertig (2001) for *U. gibba* and *U. geminiscapa* (2.8–3.3 times), while no preference occurred in *U. macrorhiza*. Overall, the NH_4^+ and phosphate uptake rates by *A. vesiculosa* and *U. australis* shoots in the present study are similar to those reported for *A. vesiculosa* apical and basal shoot segments and intact plants (Adamec 2000) and for three *Utricularia* species (Fertig 2001). Here, in both species, NH_4^+ uptake by non-fed plants was greater when compared to fed plants, but the opposite was true for phosphate uptake; the differences were significant only for *A. vesiculosa.* It is a matter of discussion as to what the role of shoot N and P contents for these differences in nutrient uptake is. A similar growth experiment for both species (Adamec 2008b) found that feeding on prey led to a greatly decreased shoot N content. This was partly explained by a more rapid apical shoot growth and branching. Very low shoot N contents in the prey-fed variant should therefore rather stimulate (by a negative feedback mechanism) NH_4^+ uptake in both species. In conclusion, the present results do not support the idea that prey capture in aquatic carnivorous plants stimulates NH_4^+ uptake by shoots from the ambient water, as it occurs for the root nutrient uptake in terrestrial carnivorous plants (see Adamec 1997a; Adamec 2002), but such a phosphate uptake stimulation is not excluded in aquatic species.

When K^+ uptake was measured in the presence of 15 µM NH_4NO_3 (Exp. A) by *A. vesiculosa* and *U. australis* shoots, the K^+ uptake rate was almost zero or distinctly negative (Table 1) and similar results were confirmed repeatedly under similar conditions (Adamec, unpubl.). In both species, K^+ efflux was much greater in –Prey variants, although this difference was significant only in *A. vesiculosa*. Thus, K^+ efflux was much greater at a higher NH_4^+ uptake rate. However, without NH_4^+ in the medium, K^+ uptake by apical shoot segments in 5 species was always positive (0.07–0.61 mmol kg^{-1}_{FW} h^{-1}) and, except for *U. vulgaris* and the carnivorous shoots of *U. stygia,* it was also positive in basal shoot segments or whole shoots (Table 2). The difference in the character of K^+ uptake can be interpreted as an interference of K^+ uptake with NH_4^+ uptake as found in the roots of terrestrial plants (Szczerba et al. 2008a; Szczerba et al. 2008b; Coskun et al. 2014). As follows from comparing the results in different species (cf. Tables 1, 2 and also Adamec 2000), this K^+ uptake interference with NH_4^+ is rather different, not only in the various aquatic carnivorous species, but also in the apical and basal parts of the shoots within each species. Of all the species used in this study, *A. vesiculosa* proved to be the most sensitive to NH_4^+ uptake interference and, moreover, the very peculiar traits of K^+ uptake by its apical and basal shoot segments (Adamec 2000) could be explained in this way. K^+ is a nutrient necessary for dynamic apical shoot growth and, at a zero K^+ reutilization efficiency in senescent shoots of aquatic carnivorous plants (Table 4; Adamec 2014), all K^+ must be taken up *de novo*. In this study, the K^+ uptake can be considered short-term growth uptake. A simple model of growth and K^+ accumulation in aquatic carnivorous plants (the doubling time of biomass was 15 d or daily biomass increase was 4.7 %, the mean shoot K content was 3 % DW, DW:FW ratio 8 %; see Adamec 2011a) suggests that 1 g of shoot FW takes up ca. 113 µg K^+ (= 2.90 µmol) to cover the daily net growth increment. Thus, considering only uptake of K^+ from the water for 12 h of daylight, a mean light uptake rate of 0.24 µmol kg^{-1}_{FW} h^{-1} can be estimated (if the shoots contain also senescent segments, due to zero reutilization efficiency, this value might be much higher). Such a model of K^+ uptake rate was really measured in 5 aquatic carnivorous species, with the uptake rate by apical shoot segments being at least 2.5 times greater than by basal segments (Table 2). This means that in rootless aquatic carnivorous plants, apical shoot parts are autonomous in their K^+ gain and a substantial acropetal K^+ transport from basal parts does not occur.

The growth experiment on *A. vesiculosa* and *U. australis* conducted at markedly different K^+ concentrations did not reveal any significant growth changes except faster shoot senescence in the *U. australis* high K^+ variant (Table 3). This indicates that in both species, both K^+ uptake affinity and capacity from the ambient water are high enough to acquire a sufficient K^+ amount for very rapid growth even at

a low K^+ concentration of only ca. 2 µM. However, some field data show a K^+ concentration threshold for the growth of aquatic *Utricularia* species could be as low as ca. 0.25 µM (see Adamec 1997a). The growth experiment proved relatively high N and P reutilization efficiencies in senescent shoot segments, but the low K^+ concentration led also to a significant decrease in the tissue N and P contents in mature leaf nodes in *U. australis* (Table 4). Using the correction factor for DW decline in senescent shoot segments, the concept of zero K reutilization in senescent shoots was confirmed in both K^+ variants in *U. australis* and in the low K^+ variant of *A. vesiculosa* (cf. Adamec 2014). However, the interpretation of apparently efficient K^+ reutilization (33 %) in the high K^+ variant of *A. vesiculosa* is doubtful. Stock shoots of *A. vesiculosa* grew at a rather high K^+ concentration of 0.31 mM and their K content in mature leaf nodes (which became senescent and were sampled as such) at the time of the shoot transfer to 83 µM K^+ to miniaquaria could be at least 3.9 % (see Table 4). Due to the fact that senescent leaf nodes in both K^+ variants had nearly the same tissue K^+ content (2.1–2.3 % DW), it is more probable that highly mobile K^+ ions were not reutilized in senescent shoots, but had been released to the ambient medium before the nodes senesced. Moreover, about a two-times higher K^+ content in mature, younger shoot nodes in the high K^+ variants, as compared with the low K^+ variant in both species, confirm that the K^+ content in mature shoot segments depends strongly on the ambient K^+ concentration. However, in a field study on *U. australis,* the K shoot content correlated more strongly and statistically significantly with the quantity of captured prey rather than with the ambient K^+ concentration (Adamec 2008a).

In conclusion, the results confirmed that prey capture in ACPs has significant effects on the uptake of mineral N and P from the ambient water. It is not clear whether or not these changes are due to alterations in shoot N and P content in both prey variants. The changes of mineral nutrient uptake were partly different in *A. vesiculosa* and *U. australis*. Moreover, a markedly different photosynthetic response was found in these species in a similar experiment (Adamec 2008b). It is therefore probable that the physiological responses of aquatic species to prey capture are highly species-specific. Much more attention should be paid to the ambiguous effect of prey capture on shoot N and P content and its possible signaling role for physiological responses.

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References

Adamec, L., 1997a: Mineral nutrition of carnivorous plants: A review. – Bot. Rev. **63**: 273–299.

Adamec, L., 1997b: Photosynthetic characteristics of the aquatic carnivorous plant *Aldrovanda vesiculosa*. – Aquat. Bot. **59**: 297–306.

Adamec, L., 1997c: Relations between K^+ uptake and photosynthetic uptake of inorganic carbon by aquatic plants. – Biol. Plant. **39**: 599–606.

Adamec, L., 2000: Rootless aquatic plant *Aldrovanda vesiculosa*: physiological polarity, mineral nutrition, and importance of carnivory. – Biol. Plant. **43**: 113–119.

Adamec, L., 2002: Leaf absorption of mineral nutrients in carnivorous plants stimulates root nutrient uptake. – New Phytol. **155**: 89–100.

Adamec, L., 2006: Respiration and photosynthesis of bladders and leaves of aquatic *Utricularia* species. – Plant Biol. 8: 765–769.

Adamec, L., 2008a: Mineral nutrient relations in the aquatic carnivorous plant *Utricularia australis* and its investment in carnivory. – Fundam. Appl. Limnol. **171**: 175–183.

Adamec, L., 2008b: The influence of prey capture on photosynthetic rate in two aquatic carnivorous plant species. – Aquat. Bot. **89**: 66–70.

Adamec, L., 2009: Photosynthetic CO_2 affinity of the aquatic carnivorous plant *Utricularia australis* (Lentibulariaceae) and its investment in carnivory. – Ecol. Res. **24**: 327–333.

Adamec, L., 2011: Ecophysiological look at plant carnivory: Why are plants carnivorous? –In: Seckbach, J. & Dubinski, Z. (eds): All Flesh is Grass. Plant-Animal Interrelationships. – Cellular Origin, Life in Extreme Habitats and Astrobiology Vol. 16. – Springer Science+Business Media B. V., Dordrecht, pp. 455–489.

Adamec, L., 2013a: A comparison of photosynthetic and respiration rates in six aquatic carnivorous *Utricularia* species differing in morphology. – Aquat. Bot. **111**: 89–94.

Adamec, L., 2013b: Foliar mineral nutrient uptake in carnivorous plants: what do we know and what should we know? – Front. Plant Sci. **4**: 10.

Adamec, L., 2014: Different reutilization of mineral nutrients in senescent leaves of aquatic and terrestrial carnivorous *Utricularia* species. – Aquat. Bot. **119**: 1–6.

Adamec, L. & Kovářová, M., 2006: Field growth characteristics of two aquatic carnivorous plants, *Aldrovanda vesiculosa* and *Utricularia australis*. – Folia Geobot. **41**: 395–406.

Adamec, L., Sirová, D. & Vrba, J., 2010: Contrasting growth effects of prey capture in two aquatic carnivorous plant species. – Fundam. Appl. Limnol. **176**: 153–160.

Aerts, R., Verhoeven, J. T. A. & Whigham, D. F., 1999: Plant-mediated controls on nutrient cycling in temperate fens and bogs. – Ecology **80**: 2170–2181.

Coskun, D., Britto, D. T. & Kronzucker, H. J., 2014: The physiology of channel-mediated K^+ acquisition in roots of higher plants. – Physiol. Plant. **151**: 305–312.

Englund, G. & Harms, S., 2003: Effects of light and microcrustacean prey on growth and investment in carnivory in *Utricularia vulgaris*. – Freshw. Biol. **48**: 786–794.

Fertig, B., 2001: Importance of prey derived and absorbed nitrogen to new growth; preferential uptake of ammonia or nitrate for three species of *Utricularia*. – Student Rep., Brandeis Univ., Waltham, MA, USA, pp. 1–13.

Friday, L. E., 1989: Rapid turnover of traps in *Utricularia vulgaris* L. – Oecologia **80**: 272–277.

Friday, L. E. & Quarmby, C., 1994: Uptake and translocation of prey-derived ^{15}N and ^{32}P in *Utricularia vulgaris* L. – New Phytol. **126**: 273–281.

Guisande, C., Granado-Lorencio, C., Andrade-Sossa, C. & Duque, S. R., 2007: Bladderworts. – Funct. Plant Sci. Biotechnol. **1**: 58–68.

Güsewell, S., 2005: Nutrient resorption of wetland graminoids is related to the type of nutrient limitation. – Funct. Ecol. **19**: 344–354.

Jeschke, W. D., 1976: Ionic relations of leaf cells. – In: Lüttge, U. & Pitman, M. G. (eds.): Transport in Plants II. Part B. Tissues and Organs. – Encyclopedia of Plant Physiology, New Series, Vol. 2. – Springer-Verlag, Berlin, pp. 160–194.

Juniper, B. E., Robins, R. J. & Joel, D. M., 1989: The Carnivorous Plants. – Academic Press, London.

Richards, J. H., 2001: Bladder function in *Utricularia purpurea* (Lentibulariaceae): is carnivory important? – Am. J. Bot. **88**: 170–176.

Killingbeck, K. T., 1996: Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. – Ecology **77**: 1712–1727.

Helder, R. J., 1988: A quantitative approach to the inorganic carbon system in aqueous media used in biological research: dilute solutions isolated from the atmosphere. – Plant Cell Environ. **11**: 211–230.

Sirová, D., Borovec, J., Černá, B., Rejmánková, E., Adamec, L. & Vrba, J., 2009: Microbial community development in the traps of aquatic *Utricularia* species. – Aquat. Bot. **90**: 129–136.

Sirová, D., Šantrůček, J., Adamec, L., Bárta, J., Borovec, J., Pech, J., Owens, S. M., Šantrůčková, H., Schäufele, R., Štorchová, H. & Vrba, J., 2014: Dinitrogen fixation associated with shoots of aquatic carnivorous plants: is it ecologically important? – Ann. Bot. **114**: 125–133.

Szczerba, M. W., Britto, D. T., Ali, S. A., Balkos, K. D. & Kronzucker, H. J., 2008a: NH_4^+-stimulated and -inhibited components of K^+ transport in rice (*Oryza sativa* L.). – J. Exp. Bot. **59**: 3415–3423.

Szczerba, M. W., Britto, D. T., Balkos, K. D. & Kronzucker, H. J., 2008b: Alleviation of rapid, futile ammonium cycling at the plasma membrane by potassium reveals K^+-sensitive and -insensitive components of NH_4^+ transport. – J. Exp. Bot. **59**: 303–313.

Taylor, P., 1989: The Genus *Utricularia*: A Taxonomic Monograph. – Kew Bulletin, Additional Series, XIV.

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