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Mineral nutrient relations in the aquatic carnivorous plant *Utricularia australis* and its investment in carnivory

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With 4 tables

Abstract: Aquatic rootless carnivorous plants usually grow in nutrient-poor waters and take up all nutrients through their shoots, from either water or prey. The carnivorous plant *Utricularia australis* was sampled from 30 locations in the Třeboň basin, Czech Republic, with the aim of investigating the plant's mineral nutrient economy in relation to its carnivorous habit. Relationships were sought between, firstly, mineral nutrient levels in the ambient water together with prey quantity captured in traps and, N, P, K, Na, Ca, and Mg contents of shoot tissues, and, secondly, the proportion of total plant biomass attributable to traps (i.e., investment in carnivory). Even at very oligotrophic sites with low prey capture rates, shoot N and P content was always well above the level below which growth limitation could occur. Plants recycled at least 57 % of their N and as much as 81 % of their P from senescent shoots, although they lost all of the K, Na, Ca, and Mg from senescing tissues. The P and K content of traps was much greater than that in leaves. Regression analyses revealed much greater uptake of N, P, and K from prey than from the ambient water. The proportion of total biomass invested in traps (range 23–61 %) was positively correlated with CO_2 concentration, but negatively with shoot N content. It is suggested that shoot N content acts as a key endogenous factor regulating investment in trap biomass through a negative feedback mechanism. Any decline in shoot N content, for whatever reason, stimulates an increase in trap production, resulting in enhanced prey catching and, concomitantly, an increase in shoot N content. A reduction in trap proportion then follows and the cycle starts again.

Key words: Aquatic carnivorous plants, water chemistry, catching of prey, tissue nutrient content, regulation of investment in carnivory.

Introduction

The ecological grouping known as 'carnivorous plants' comprises about 600 species, 50 of which belong to the submerged aquatic or amphibious genera *Aldrovanda* and *Utricularia* (Juniper et al. 1989, Taylor 1989). Aquatic carnivorous plants usually grow in shallow, static, dystrophic waters, which are predominantly nutrient poor in (inorganic) N and P and commonly also deficient in K (see Adamec 1997a, Guisande et al. 2007). They take up all necessary nutrients through their shoots, either directly from water or from prey. Very rapid growth of rootless aquatic carnivorous plants in nutrient-poor habitats requires ecophysiological adaptations that enable the plants to access the very limited supplies of mineral nutrients. These adaptations include carnivory, efficient nutrient re-utilization (recycling) from senescent shoots, and a very high capacity for bioconcentration of nutrients from water (Sorenson & Jackson 1968, Kamiński 1987, Kosiba 1992a, b, Adamec 2000, Englund & Harms 2003).

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Whilst considering photosynthetic cost-benefit relationships, Givnish et al. (1984) postulated that, for terrestrial carnivorous plants, carnivory is only beneficial in nutrient-poor, moist, and sunny habitats. It is evident though that many aquatic carnivorous plant species in their typical habitats do not comply, as irradiance is often low and sometimes also free CO_2 is sub-optimal, though [CO_2] is commonly high (> 0.1 mM; see e.g., Hough & Fornwall 1988, Adamec 1997b, Adamec & Lev 2002). In aquatic *Utricularia* species, the structural and maintenance costs of traps are considerable (Friday 1992, Knight 1992, Adamec 2006, Porembski et al. 2006), but the plants are able to change the proportion of their resources invested in traps (their 'investment in carnivory') to match variations in habitat factors: particularly water chemistry, prey availability, and level of irradiance (Knight & Frost 1991, Knight 1992, Guisande et al. 2000, 2004, Richards 2001, Englund & Harms 2003, Manjarrés-Hernández et al. 2006). In three aquatic *Utricularia* species with homogeneous, non-differentiated shoots (*U. australis, U. gibba, U. reflexa*) and in the epiphytic *U. quelchii* with carnivorous shoots living in the leaf water reservoirs of bromeliads, Porembski et al. (2006) found the contribution of traps to the total plant biomass to be 11–20 % and 30 %, respectively. However, this proportion was only 0.14–0.85 % in six terrestrial *Utricularia* species.

There is certainly good evidence that carnivory in aquatic *Utricularia* species greatly enhances growth (Sorenson & Jackson 1968, Kosiba 1992a,b, Englund & Harms 2003) and attempts have been made to quantify the relative importance of prey capture in uptake of N or P (Knight 1988, Bern 1997). Of all the mineral nutrients taken up from prey by carnivorous plants, N and P have the greatest potential value for the plants, because of the relatively high N and P tissue content of prey carcasses (see Adamec 1997a). Although the catching of prey markedly enhances the growth of carnivorous plants, the relationship between the tissue nutrient content of plant shoots and carnivory is ambiguous and this parameter may not actually be a reliable predictor of plant 'fitness' or growth rate (Adamec 1997a, 2000, 2002). However, it may well be possible to correlate growth rate of (aquatic) carnivorous plants, or their 'fitness', with tissue nutrient content under the special conditions imposed by the significant nutrient limitations which plants can face in oligotrophic habitats (*sensu* Gerloff & Krombholz 1966). In spite of the generally low signalling value of tissue nutrient content for many plant physiological responses, tissue nutrient content still reflects both mineral nutrient availability in ambient media (soil or water) and also some important features of plant ecological behaviour (e.g., Dykyjová 1979, Aerts et al. 1999, Aerts & Chapin 2000). That is why this parameter is still commonly used in nutritional studies on carnivorous plants (for review see Adamec 1997a). Moreover, not only do absolute values of tissue nutrient contents give insight into the mineral nutrient dynamics of carnivorous plants in their natural environment, but also nutrient concentration ratios (nutrient stoichiometry) can reflect mineral nutrient balances in the environment (Méndez & Karlsson 2005, Ellison 2006).

Utricularia australis R.Br. (Lentibulariaceae) is a free floating, rootless, submerged aquatic carnivorous plant with homogeneous shoots bearing thousands of traps (Taylor 1989). The shoots have a regular, modular structure comprising leaf whorls separated by internodes. Like other aquatic *Utricularia* species, *U. australis* exhibits continuous, rapid apical shoot growth during the growing season, while progressively ageing and decomposing at the base. Under optimal summer conditions, it can produce 2.6–3.5 new leaf whorls a day and propagate rapidly by branching (Adamec & Kovářová 2006, cf. Friday 1989, 1992). The Třeboň basin in Southern Bohemia is one of the centres of its distribution in the Czech Republic. Here, it is widespread at hundreds of sites in different habitats. *U. australis* is considered a eurytopic species (Adamec & Kovářová 2006) and shows a very wide ecological tolerance of water chemistry (Kosiba 1993, 2004, Kosiba & Sarosiek 1993, Hofmann 2001, Navrátilová & Navrátil 2005, Adamec & Kovářová 2006). So, it can be expected that *U. australis* should each season acquire significantly more N and P from prey than it does of K, Ca, and Mg as prey is relatively a poor source of K, Ca, and Mg (Adamec 1997a). This might indicate that shoot tissue N and P content will correlate much more closely with prey capture than tissue K, Ca or Mg. In this study, *U. australis* has been chosen as a 'typical' aquatic carnivorous plant for its widespread distribution in different habitats in the Třeboň basin and for its great ecological plasticity.

The following questions were addressed in 30 field micropopulations of Třeboň *U. australis*: i) what is the efficiency of mineral nutrient re-utilization from senescent shoots? ii) to what extent does the shoot tissue nutrient content (N, P, K, Na, Ca, Mg) depend, on the one hand, on nutrient concentrations in the ambient water and, on the other, nutrient absorption from prey? iii) which external nutrient source (water chemistry or prey capture) determines the degree of investment in carnivory? iv) with which endogenous factors are in-

vestment in carnivory coupled and how is it regulated within plant shoots? For reasons discussed above, principal emphasis has been placed on N and P in this study.

Material and methods

Field sites

The field work and collection of plant material were conducted during the height of the 2004 summer from 29 June to 4 August. *U. australis* was studied at 29 field sites in the Třeboň Basin Biosphere Reserve and Protected Landscape Area, S Bohemia, Czech Republic (approx. 49° N, 14° 45′ E) and also in an artificial culture in a 2.5 m^2 outdoor plastic tank at the Institute of Botany at Třeboň. The 29 field sites were chosen non-randomly, in order to represent all distinct habitats in which *U. australis* grows commonly in the region and also to cover the widest possible range of water chemistry factors (pH, total alkalinity, nutrient concentrations, etc.). Based upon the principal water chemistry factors, these 30 sites represented four main types of shallow, static dystrophic waters:

1. strongly dystrophic waters of cyperaceous bogs or fens, usually in the littoral area of fishponds (14 sites; the concentration of the sum of humic acids and tannins (HAT) is usually > 6 mg l^{-1});
2. eutrophic, slightly dystrophic fishpond littoral areas (9 sites; the concentrations of both NH_4^+-N and PO_4-P usually > 30 µg l^{-1}, blooms of cyanobacteria);
3. mesotrophic, slightly dystrophic waters in sand-pits (2 sites; the concentration of both NH_4^+-N and PO_4-P usually 20–30 µg l^{-1});
4. oligotrophic, slightly dystrophic waters in sand-pits (4 sites plus outdoor artificial culture; the concentration of NH_4^+-N usually < 20 µg l^{-1} and PO_4-P < 15 µg l^{-1}).

All sites had water depths of 3–50 cm and, except for the oligotrophic sand-pits, bottom sediments were highly organic.

Field samples and processing of plants

At each site, four randomly selected, adult non-flowering *U. australis* plants were collected, from a typical microsite, and placed in a 1-litre plastic bottle with a moist atmosphere. In the laboratory, plants were washed thoroughly with tap water and cut into five segments: A, shoot apex with non-adult leaf whorls, B, subapical shoot segment with adult leaf whorls 1–6 (i.e., those with the youngest functioning traps), C, 11th adult leaf whorls, D, 12th adult leaf whorls, E, remaining senescent but still living whorls – in fact mostly stem material (see Adamec 2000). The A, B, and E segments were briefly rinsed with distilled water, blotted for surface water, and dried at 80 °C for tissue nutrient analyses. A check with a binocular loupe established that all these segments were relatively free from periphyton.

Due to considerable habitat variability and the shallowness of many sites, prey availability (*sensu* Harms 1999) was not investigated in the field. Instead, as a measure of percentage prey capture by plant traps (and, thus, of prey availability in the water), a binocular loupe was used to identify any prey in all C segment traps (11th adult leaf whorls) on the four plants sampled from each site. Leaf whorls in this position contain fully adult traps which have usually functioned for 4–6 days (Adamec & Kovářová 2006) and would therefore represent prey capture well during this period. D segments (12th adult leaf whorls) were excised exactly in the middle of internodes, and traps were separated using fine forceps. The traps, and the trapless D segments, of the four plants were then dried and weighed to estimate investment in carnivory (percentage trap biomass to total biomass of D segments).

In order to estimate mineral investment in carnivory, tissue mineral nutrient content was measured in traps devoid of prey and also in D stem segments without traps. To obtain orientation results, five replicates were used only from an oligotrophic sampling site, Hadí blato sand-pit. Before drying the traps, trap fluid was expressed using soft paper tissue.

Analytical procedures

Once at each site, pH and electrical conductivity were measured at the time of plant sampling (10:00–17:00 hr). All measurements were taken 2 cm below the water surface, in the plant zone. Water samples collected from each site were filtered (0.7 µm or 44 µm) and analysed for total alkalinity (TA), macronutrients (NO_3^--N, NH_4^+-N, PO_4-P, K, Na, Ca, Mg, total N (N_t), and total P (P_t), and humic acid + tannin concentration (HAT). For further details of analytical methods see Adamec (1997b) and Adamec & Lev (1999). CO_2 concentration was calculated from total alkalinity and pH (Helder 1988). Estimates of the level of shading cast by emergent vegetation or cyanobacterial blooms were made at 1-cm water depth using a submersible PAR sensor (Adamec 1997b, Adamec & Lev 1999) at the four eutrophic sites where the lowest irradiances occurred. The irradiance at all other sites was known to be non-limiting for *U. australis* growth (Adamec & Kovářová 2006).

The dried shoots of four plants selected from each of the 30 sites were mixed (A, B and E segments separately) and ground in a minimortar. Plant material was digested and mineralised using concentrated acids, diluted and analysed for N, P, K, Na, Ca, and Mg content. Subscripts are used in the results section to denote A, B and E samples. For further sample preparation and analysis details available see Adamec (2002).

Statistical procedures

For every investigated parameter of water or tissue chemistry, one value obtained was used for each of the 30 sites for correlation studies. To show whether the tissue chemistry depended on the trophic level of the sites, principal component analysis (PCA) was used to subdivide all sites into three distinct groups (each with 10 sites), oligo-, meso-, and eutrophic (data of PCA not shown). N_t and P_t were used as the only criteria for the PCA as they expressed the trophic level of the sites best. These three groups of sites did not correspond exactly to the same hydrobiological categories of sites when also the HAT concentration was included (see above). Differences in tissue nutrient content between the different-aged A, B, and E segments were tested by two-way ANOVA with trophic level (i.e., habitats) and segment age as factors and sites as covariate, to exclude the interrelationship between the A, B, and E segments at each site. One-way ANOVA was used to compare the differences in tissue chemistry for certain age of segments between habitats (Tukey HSD-test for multiple comparisons). Linear regression models were used to look for statistically significant relation-

ships between dependent variables (tissue nutrient content and trap biomass proportion, denoted as % traps) and independent variables (water chemistry, proportion of occupied traps) and between pairs of independent or dependent variables. pH values were wide-ranging, so they were not transformed. Ten linear regression models were identified, including expected and ecologically important relationships, and they are included in the results. One outlying value was excluded for two parameters. In order to minimize the influence of interrelated factors in these regression models and keep the critical probability level as high as possible, tissue chemistry parameters were reduced only to B segments and water chemistry factors to most important items. Bonferroni correction was used and, therefore, P = 0.005 is used as the critical probability level in the regression models.

Results

The 30 sites of *U. australis* selected in the Třeboň basin, representing three main trophic levels, represented a wide range of the parameters of water chemistry studied (Table 1). Some sites, especially in the younger, shallow sand-pits, had very soft and oligotrophic water, contrasting with the hard waters of limed eutrophic fishponds usually containing blooms of cyanobacteria in the water column. Most sites were characterized by NH_4^+-N as the dominant form of mineral N, by high concentrations of CO_2 (usually > 50 μM), and by the sum of humic acids + tannins generally being > 5 mg l^{-1}. Statistically highly significant differences were found in tissue nutrient contents (N, P, K, Na, and Ca) and their N : P and K : Ca ratios between shoot segments, while the interaction with trophic levels (habitats) was insignificant (Table 2). Apart from P and K, significant differences were also found between habitats within A segments; oligotrophic sites usually differed from eutrophic ones. Tissue N and P content declined markedly from the shoot apices towards the bases, whereas this was not the case for the other nutrients, suggesting that they are not being re-utilized from the basal segments. Mean tissue N : P content (w/w) was 6.7 in the apical segments and rose to 15.9 in the senescent ones, indicating much better re-utilization of P than N. In contrast, the mean tissue K : Ca content ratio (w/w) declined greatly from apical to basal segments (from 27.5 to 6.0), mainly due to the significant accumulation of Ca in basal segments. Individual *U. australis* micropopulations varied greatly in their proportions of traps with prey: totally from 0.8 % at very oligotrophic sites to 88 % at eutrophic ones (Table 2). Mean proportion of trap biomass to total plant biomass in D segments was 38.4 % (total range 22.9–60.5 %) and did not differ significantly between habitats.

Empty traps of plants from Hadí blato sand-pit had greater tissue P and K contents than trapless leaves from the 12th whorls (D), although the leaves had more N, Ca, and Mg (Table 3). Thus, the mean N : P ratio for leaf tissue was 16.8, but only 6.3 for traps; on the other hand, the K : Ca ratio was opposite.

The linear regression models show that success in prey capture (% of traps with prey – a measure of prey availability) relates significantly to external P_t (i.e., to trophic level; Table 4, No. 1). Tissue content of K_B was not correlated with K concentrations in the ambient water (Table 4, No. 2). Tissue N and P content from segments B had only weak, non-significant negative

Table 1. Mean parameters of water chemistry estimated at 30 sites of *U. australis* subdivided into three main trophic levels. HAT, sum of humic acids and tannins. SD and range of values are shown; n = 10.

		Oligotrophic sites			Mesotrophic sites			Eutrophic sites		
Parameter	Unit	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
pH	–	6.51	1.09	4.49–8.33	6.81	0.43	5.94–7.37	6.80	1.02	5.01–8.35
Conduct.	μS cm^{-1}	149	121	34–420	188	88	73–321	199	81	77–335
Tot. alk.	meq l^{-1}	0.60	0.63	–0.03–1.82	1.03	0.61	0.23–2.08	1.12	0.66	0.04–2.00
CO_2	mM	0.26	0.20	0.02–0.59	0.33	0.16	0.13–0.61	0.54	0.56	0.02–1.77
HAT	mg l^{-1}	6.9	7.0	1.5–24.3	11.7	8.7	3.4–31.6	13.5	9.9	4.8–34.8
NO_3^--N	μg l^{-1}	3.6	4.1	0.0–14.6	5.1	4.2	1.6–16.4	8.0	7.1	2.0–27.0
NH_4^+-N	μg l^{-1}	27	10	17–46	45	40	18–155	102	135	36–467
PO_4-P	μg l^{-1}	11.7	5.2	1.8–19.7	23.3	11.6	11.3–45.4	71.3	86.1	11.9–297
N_t	μg l^{-1}	930	250	410–1160	1660	220	1270–1930	2500	570	2000–3710
P_t	μg l^{-1}	75	10	61–94	100	20	69–125	199	172	75–639
K	mg l^{-1}	2.5	1.2	0.48–4.8	4.6	3.7	1.6–10.2	4.7	2.3	1.3–8.0
Na	mg l^{-1}	6.0	8.1	0.66–27.2	4.9	2.5	2.8–9.3	6.1	3.5	2.5–12.0
Ca	mg l^{-1}	14.5	13.5	2.6–43.5	20.4	10.3	6.7–36.4	21.8	12.1	4.6–40.3
Mg	mg l^{-1}	3.6	2.4	0.84–7.6	5.1	2.4	1.5–8.9	4.9	2.0	1.7–7.3

Table 2. Tissue nutrient content in *U. australis* shoot segments in % of DW, proportion of traps with prey, and investment in carnivory. A, apex with non-adult leaf whorls; B, 1st-6th adult leaf whorls; C, 11th adult leaf whorls; D, 12th adult leaf whorls; E, senescent but still living whorls, mostly stems. Hab., habitats; OL, oligotrophic; ME, mesotrophic; EU, eutrophic. Statistically significant difference between habitats within A or B, or E segments (one-way ANOVA) is shown on the bottom of each segment A, B, or E (F, and P). Different letters within each column and segment denote statistically significant difference (Tukey HSD test) between the habitats at $P < 0.05$. Statistically significant difference between A, B and E segments within all habitats (S) and interaction with habitats (S×H; F, P, two-way ANOVA) are shown at the bottom of the table.

		Tissue nutrient content (% DW)								Traps with prey in C (%)	Trap prop. in D (% DW)
Hab.	Param.	N	P	K	Na	Ca	Mg	N : P	K : Ca		
Apical segments (A)											
OL	Mean	3.17^{ab}	0.51	3.35	0.25^{a}	0.13^{a}	0.31^{a}	6.34	26.8	11.1a	36.3
OL	SE	0.20	0.04	0.12	0.03	0.004	0.02	0.32	1.4	3.4	2.8
ME	Mean	2.91^{a}	0.47	2.94	0.14b	0.12^{ab}	0.26^{b}	6.65	25.5	17.2^{ab}	42.4
ME	SE	0.18	0.06	0.24	0.02	0.005	0.009	0.47	1.8	6.4	3.4
EU	Mean	3.80^{b}	0.62	3.12	0.14^{b}	0.10^{b}	0.24^{b}	7.23	30.1	38.9^{b}	36.5
EU	SE	0.29	0.11	0.29	0.02	0.004	0.01	0.76	2.5	9.6	3.3
	$F_{2,27}$	4.07	1.01	0.80	6.15	6.99	6.76	0.68	1.53	4.42	1.24
	P	0.028	0.38	0.46	0.006	0.004	0.004	0.52	0.23	0.022	0.31
Subapical segments (B)											
OL	Mean	2.62	0.35	4.13	0.79	0.20	0.39^{a}	7.81	21.3	–	–
OL	SE	0.17	0.03	0.18	0.10	0.01	0.03	0.69	1.2		
ME	Mean	2.35	0.29	4.11	0.52	0.19	0.33^{ab}	8.58	22.0		
ME	SE	0.19	0.04	0.39	0.06	0.01	0.02	0.72	2.0		
EU	Mean	3.06	0.41	4.25	0.59	0.17	0.28^{b}	9.06	25.9		
EU	SE	0.27	0.07	0.37	0.09	0.009	0.02	1.22	3.0		
	$F_{2,27}$	2.71	1.27	0.058	2.41	1.42	4.74	0.48	1.26		
	P	0.084	0.29	0.94	0.11	0.26	0.017	0.62	0.30		
Basal senescent segments (E)											
OL	Mean	1.15	0.08	4.86	1.54	0.84	0.37	17.0	6.11	–	–
OL	SE	0.12	0.01	0.58	0.24	0.06	0.04	2.5	0.85		
ME	Mean	1.19	0.09	4.44	1.26	0.82	0.43	13.9	5.82		
ME	SE	0.24	0.02	0.59	0.20	0.05	0.05	2.1	1.05		
EU	Mean	1.85	0.12	4.54	1.45	0.76	0.32	16.9	6.07		
EU	SE	0.29	0.03	0.53	0.24	0.04	0.03	1.8	0.72		
	$F_{2,27}$	2.97	1.22	0.15	0.37	0.63	1.75	0.69	0.032		
	P	0.068	0.31	0.86	0.69	0.54	0.19	0.51	0.97		
	F (S)	264	123	10.0	329	1270	6.54	57.0	177	–	–
	P (S)	0.001	0.001	0.001	0.001	0.001	0.055	0.001	0.001	–	–
	F (SxH)	0.21	0.38	0.10	0.17	0.33	1.45	0.64	0.68		
	P (SxH)	0.93	0.82	0.98	0.95	0.86	0.23	0.63	0.60		

Table 3. Tissue nutrient content in leaves without traps and in traps without prey from the 12th adult leaf whorls in *U. australis* plants collected from Hadí blato sand-pit. Means ± 1.SE are shown; n = 5. Different letters denote statistically significant difference (two-tailed t-test) between leaves and traps at $P < 0.05$.

	Tissue nutrient content (% DW)							
Organ	N	P	K	Na	Ca	Mg	N : P	K : Ca
Leaves	2.35 ± 0.15^{a}	0.14 ± 0.01^{a}	5.20 ± 0.24^{a}	1.50 ± 0.07^{a}	0.91 ± 0.05^{a}	0.52 ± 0.02^{a}	16.8 ± 1.7^{a}	5.73 ± 0.21^{a}
Traps	1.63 ± 0.07^{b}	0.26 ± 0.01^{b}	8.68 ± 0.05^{b}	1.31 ± 0.07^{a}	0.52 ± 0.06^{b}	0.43 ± 0.01^{b}	6.25 ± 0.28^{b}	17.1 ± 1.53^{b}

Table 4. Statistically significant or ecologically important linear regression models of important parameters as dependent on variable factors; n = 29–30. As a result of Bonferroni correction, only values of $P < 0.005$ represent significant correlation (indicated by asterisk). Different sets of functional relationships are separated by dotted line. For units and explanation of variables see Tables 1 and 2. The subscript B for tissue nutrient contents refers to subapical B shoot segments. % prey, % of traps with captured prey in C segments; % traps, % of trap biomass in D segments; r^2, coefficient of determination.

No.	Linear regression model	r^2	P
1	% prey = 7.54 + 0.119 P_t	0.295	0.002*
2	K_B = 3.35 + 0.190 K	0.216	0.011
3	N_B = 3.00 – 0.861 CO_2	0.187	0.017
4	P_B = 0.430 – 0.208 CO_2	0.214	0.010
5	N_B = 1.53 + 3.25 P_B	0.533	<0.0005*
6	N_B = 2.25 + 0.0189 % prey	0.403	<0.0005*
7	P_B = 0.251 + 0.0045 % prey	0.462	<0.0005*
8	K_B = 3.64 + 0.0199 % prey	0.270	0.0039*
9	% traps = 32.2 + 14.3 CO_2	0.328	0.001*
10	% traps = 54.7 – 6.38 N_B	0.258	0.0049*

correlations with ambient free CO_2 (Table 4, Nos. 3–4). Within the B segments, tissue N content correlated highly significantly with that of P (Table 4, No. 5). N, P, and K tissue contents in B segments are correlated with prey capture (Table 4, Nos. 6–8). There was a highly significant positive relationship between the proportion of trap biomass in D segments and ambient CO_2 concentration, but it was negative with tissue N content for B segments (Table 4, Nos. 9–10). There are no indications that the relatively low irradiance (mean 5–10 % of that at un-shaded sites) at the four eutrophic sites influenced trap proportion in any way.

Discussion

This study has shown that water chemistry parameters such as pH, TA, [CO_2], HAT, N, P, K, Na, Ca show considerable variation throughout 30 sampled sites of *U. australis* (Table 1) and this confirms the species as being highly eurytopic (cf. Kosiba 1993, 2004, Kosiba & Sarosiek 1993, Hofmann 2001, Navrátilová & Navrátil 2005, Adamec & Kovářová 2006).

Tissue nutrient contents of A and B segments in *U. australis* (Table 2) were comparable with literature values for the genus (cf. Dykyjová 1979, Moeller 1980, Kosiba 1992a,b, Friday & Quarmby 1994), but most authors did not specify which parts of the shoots had been sampled for nutrient analyses. In this study, the tissue N and P contents of A and B segments of *U. australis* were always greater than those reported as growth-limiting for submerged plants (1.3 % DW for N, 0.13 % DW for P; Gerloff & Krombholz 1966). The relatively high tissue N and P content in this species, even at oligotrophic sites with low prey capture rates, suggests a very high nutrient-accumulation efficiency. As found by Friday & Quarmby (1994) in the very similar *U. vulgaris*, a very steep gradient of tissue N content occurred, from the 1st to the 7th adult leaf whorls (3.7 down to 1.3 % N per DW). Similar, but less distinct, gradients of almost all nutrients were found in the much slower growing aquatic *U. purpurea* (Moeller 1980). Assuming that the tissues of the last living E segments of *U. australis* shoots, about 1–3 days before their decay, represent a nutrient pool potentially lost to dead biomass, in effect the re-utilization efficiency for N may be as much as 57 % (as compared with A segments), and 81 % for P (Table 2). In fact, because there is a certain amount of organic matter loss from senesced organs (see e.g., Adamec 2002), the real values are slightly higher still. N : P stoichiometry suggests much higher re-utilization efficiency of P than that of N (Table 2). Friday & Quarmby (1994) also came to this conclusion for the ecologically similar *U. vulgaris,* and (Moeller 1980) for the quite different *U. purpurea.* The latter species only re-utilized 37 % of N, but 71 % of the P. However, the ecologically related *Aldrovanda* exhibited a re-utilization efficiency of as much as 92 % for N (Adamec 2000), which helps to explain this plant's very rapid growth rate (Adamec & Kovářová 2006).

U. australis shoots seem do not re-utilize K or Mg, and Na and Ca were significantly accumulated in the senesced biomass (Table 2), so were presumably not recycled either. These traits of nutrient loss were also found in *U. purpurea* (Moeller 1980) and *Aldrovanda* (Adamec 2000). However, tissue contents of no nutrients of *U. australis* senescent shoot segments depended significantly on the trophic level of sites (Table 2).

A significant difference was found between the tissue nutrient contents of traps alone and that of leaf whorls without traps (Table 3): N, Ca, and Mg tissue levels were higher in leaves, while P and K levels were higher in empty traps. Guisande et al. (2004) also found a low N content in *U. foliosa* traps. The extremely high tissue K content in traps (8.7 % DW) has never been reported before in aquatic plants (cf. Dykyjová 1979) but is probably due to the high K^+ concentrations one would expect in the internal glands of traps.

To demonstrate *U. australis* investment in carnivory, take the example of the 12th leaf whorls: the mean proportion of trap material in the total whorl biomass

is 38 % (Table 2). This is the structural investment in carnivory, but there is also a "mineral" investment in carnivory in that about 30 % of the plant's total N, 53 % of its P and 51 % of K is located in the traps. Of course, this means that there must be a considerable concomitant metabolic cost of maintaining these traps and this is indeed reflected in a high respiration rate of the traps (Adamec 2006): in fact, about 67 % of the plant's total respiration for *U. australis.* Adamec (2007) obtained similar values (60–68 %) for traps of *U. intermedia* and *U. stygia* with dimorphic shoots.

The tissue nutrient contents in A, B, and E segments have been used in this study to typify the overall nutrient economy of the plants, irrespective of their size or biomass. There does not appear to be any more effective, more available or simpler means of characterizing nutrient relations in micropopulations of rootless aquatic plants. Yet, tissue nutrient content can also be inversely related to plant growth rate, as rapid growth can result in a "dilution" of mineral nutrients (in relation to organic carbon) in the younger parts of shoots (Adamec 2000).

U. australis is, of course, a rootless aquatic plant and it can take up all required nutrients from both water and prey. Correlations between tissue nutrient contents and ambient water, on the one hand, and tissue nutrient contents and the percentage of traps with prey, on the other, show that shoot N and P contents in B segments clearly do not relate to N or P concentrations (incl. N_t and P_t) in the water (data not shown), but only to the successful catching of prey (Table 4). Potassium behaved similarly as K_B did not correlate with the K^+ concentration in the water but, at the same time, K_B was linked with prey capture. Thus, *U. australis* plants obviously seem to derive most of their seasonal N and P gain and also a substantial deal of K from prey.

Free CO_2 is the only inorganic carbon form available to aquatic *Utricularia* species (Adamec 1995, 1997a) and $[CO_2]$ increases greatly accelerate their growth (McDermott & Darnowski 2002, Pagano & Titus 2004). The weak negative correlation between tissue N_B and P_B content and $[CO_2]$ (Table 4) indicates that CO_2 can be partly the co-limiting factor in plant growth. Organic carbon is also taken up from prey (for review see Adamec 1997a) but the above correlations show that the main source of C for *U. australis* is free ambient CO_2 whilst N, P, and K are very largely acquired from prey.

One of the main aims of this study has been to identify the exogenous and endogenous controls over trap formation as a measure of carnivory investment in *U. australis*. Porembski et al. (2006) selected ten *Utricularia* species belonging to different ecological groups (aquatic, terrestrial, epiphytic) and showed, using the same measure, that the amount of investment in carnivory varied consistently between species of different ecological groups. For *U. australis* 12th leaf whorls, the proportion of trap biomass ranged from 23–61 % (Table 2). Other aquatic species have similar proportions in the field: 40–60 % (Friday 1992), or 10–25 % (Englund & Harms 2003) for *U. vulgaris*, ca. 26 % for *U. purpurea* (Richards 2001), ca. 28 % for *U. intermedia* and 24 % for *U. stygia* (Adamec 2007). Ambient CO_2 concentration was the only exogenous factor showing a positive correlation with trap proportion (Table 4). This suggests a direct relationship between photosynthetic rate and trap production. However, in strongly shaded *U. vulgaris*, trap production was blocked completely by prey addition (Englund & Harms 2003), even though carbon availability must have been better than that in the unfed control. Thus, it is probable that high $[CO_2]$ increased trap proportion in *U. australis* indirectly, due to increasing net photosynthetic rate, by decreasing tissue N content in A and B segments where the traps originate and mature.

Considering all endogenous nutrient factors investigated for *U. australis*, only N_B showed really significant negative correlations with trap proportion (Table 4). Thus, tissue N content in apical and subapical shoot segments appears to perform a key regulatory role in trap production. This finding is consistent with the suggestion of Guisande et al. (2004) that ambient N sources (NO_3^- and prey) are a limiting factor regulating investment in carnivory. So results obtained consistently support the hypothesis that all external nutrient factors which decrease tissue N content in young shoot segments (e.g., no catching of prey, low levels of NH_4^+-N, NO_3^--N, N_t, etc.) increase the proportion of total plant biomass allocated to traps. This regulation system is clearly a negative feedback mechanism (see also Ulanowicz 1995): a decline in external N concentrations and/or catching of prey causes a rapid decrease in shoot N content which diverts biomass production in the shoot apex towards a greater production of traps. This obviously increases prey catch and shoot N content rises, thereby suppressing trap production. This negative feedback also helps to stabilize the tissue contents of most other mineral nutrients. As prey catch also promotes plant growth (Kosiba 1992b, Englund & Harms 2003), growth rate itself is obviously a component of the endogenous regulatory system for trap production. However, in oligotrophic habitats with a very poor prey catch, the relative growth rate of *U. australis* is obviously very low and trap produc-

tion may be blocked by other endogenous factors (e.g., shortage of photosynthate, or of mineral nutrients). Kibriya & Jones (2007), studying *U. vulgaris*, proposed a central regulatory role for phosphorus in determining structural investment in carnivory, but the present study does not support this contention.

Conclusions

It is clear that a knowledge of both growth rates and nutrient relations is essential in understanding the 'cost-benefit relationships' involved in the allocation of resources by carnivorous plants, both terrestrial and aquatic (Ulanowicz 1995, Méndez & Karlsson 2005, Adamec & Kovářová 2006). Prey catch has always been known to be important (and the present study provides considerable additional insight here), but the present study has gone further and shown that there are two critical regulatory parameters in trap production: tissue N content in young shoots and ambient [CO_2] in the water. However, carnivorous plant behaviour is so variable across species and sites that measurements such as plant size, density, standing biomass, nutrient standing stock, etc., are not very helpful at all in explaining the physiological regulation of structural investment in carnivory. Furthermore, Hanslin & Karlsson (1996) and Adamec (2002) showed that, in the case of terrestrial carnivorous plants, the physiological consequence of carnivory appears to be determined by stimulation of nutrient uptake by the roots. Logically, the same mechanism – stimulation of mineral nutrient uptake by shoots from the ambient water as a result of catching prey, should also occur in rootless aquatic carnivorous plants.

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