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## Photosynthetic CO<sub>2</sub> affinity of the aquatic carnivorous plant *Utricularia australis* (Lentibulariaceae) and its investment in carnivory

Received: 7 December 2007 / Accepted: 2 May 2008 / Published online: 10 June 2008  
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**Abstract** Aquatic carnivorous plants usually grow in shallow dystrophic waters poor in inorganic N and P. *Utricularia australis* was chosen as a model plant for its prolific distribution and great ecological plasticity. The photosynthetic CO<sub>2</sub> compensation point and factors associated with investment in carnivory and capture of prey were measured in 17 *U. australis* micropopulations in Třeboň basin, Czech Republic, together with water chemistry factors at these sites differing greatly in their trophic level, water hardness, and prey availability. Apical shoot growth rate was estimated at some oligotrophic sites. The micropopulations differed greatly in the proportion of traps with animal prey (2.7–70%, mean 26%), trap proportion to total biomass (1.4–42%, mean 26%), mean trap biomass (0.7–63 µg trap<sup>-1</sup>, mean 19 µg), and maximum trap size (1–3 mm, mean 2.0 mm). CO<sub>2</sub> compensation points ranged from 0.7 to 6.1 µM (mean 2.6 µM). A weak HCO<sub>3</sub><sup>-</sup> use (compensation point 0.51 mM) was found in plants growing in alkaline water. Trap biomass proportion did not correlate significantly with prey capture and CO<sub>2</sub> compensation points with ambient [CO<sub>2</sub>]. A very rapid apical growth (2.5–4.2 new nodes day<sup>-1</sup>) occurred in sand pits. Thus, HCO<sub>3</sub><sup>-</sup> use in *U. australis* can be induced by growing at very high pH. CO<sub>2</sub> compensation points resembled those known in other aquatic non-carnivorous plants. They did not reflect carnivory. In spite of very rapid apical shoot growth, the relative growth rate of *U. australis* can be zero in oligotrophic habitats without prey.

**Keywords** *Utricularia australis* · CO<sub>2</sub> compensation point · Investment in carnivory · Catch of prey · Apical shoot growth

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### Introduction

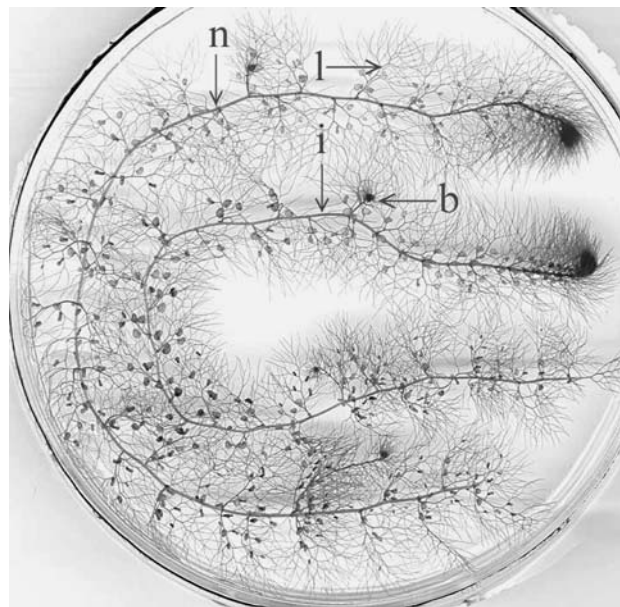
Within carnivorous plants, about 50 species of the genera *Aldrovanda* and *Utricularia* are submerged aquatic or amphibious plants (Juniper et al. 1989; Taylor 1989; Guisande et al. 2007). Aquatic carnivorous plants usually grow in shallow standing dystrophic (humic) waters that are usually poor in inorganic N and P, but commonly also in K (see Adamec 1997a). They are rootless and take up all necessary nutrients through their shoots, either directly from water or from prey. Very rapid growth of aquatic carnivorous plants in nutrient-poor habitats requires ecophysiological adaptations that enable the plants to gain limiting mineral nutrients. These adaptations include carnivory, efficient nutrient re-utilization (recycling) from senescent shoots, and very high affinity for mineral nutrients during their uptake from water (Kamiński 1987; Kosiba 1992a, 1992b; Adamec 2000; Englund and Harms 2003).

Aquatic carnivorous plants in their typical habitats may also face shortages of light (only 2–20% of incident PAR irradiance) and sometimes also free CO<sub>2</sub> (below 0.02–0.05 mM), though [CO<sub>2</sub>] is commonly high in their habitats, >0.1 mM (e.g., Hough and Fornwall 1988; Adamec 1997b, 2007; Adamec and Kovářová 2006). Therefore, the net photosynthetic rate of aquatic carnivorous plants in standing waters, like that of other submerged non-carnivorous plants generally, is limited by unfavorable physical and chemical factors, which are attributes of the aquatic environment: low diffusion rate of CO<sub>2</sub>, variable [CO<sub>2</sub>] strongly dependent on water pH and total alkalinity (TA), and, also, shortage of light (e.g., Maberly and Madsen 2002). It follows clearly, even though available data are limited, that the maximum net photosynthetic rate of aquatic carnivorous plants (per unit biomass) is usually higher than that known in aquatic non-carnivorous plants (Adamec 1997b, 2006). It has been shown that *Aldrovanda vesiculosa* and several aquatic *Utricularia* species use only CO<sub>2</sub> for

photosynthesis (Moeller 1978; Adamec 1995; Adamec and Kovářová 2006). For these species, estimated values of CO<sub>2</sub> compensation point of photosynthesis (CP CO<sub>2</sub>) range between 1.5 and 13 μM. Similar values within 1.4–11 μM have also been reported for aquatic non-carnivorous plants (Maberly and Spence 1983). Most species of aquatic carnivorous plants grow very rapidly due to both rapid apical growth and frequent branching (Friday 1989, 1992; Adamec 1999, 2000, 2007; Adamec and Kovářová 2006). Their doubling time of biomass can be from 8 to 20 days and their apical growth rate from 1 to 3 new nodes with leaves day<sup>-1</sup>. Obviously, their high net photosynthetic rate is a prerequisite for their very rapid growth (see Adamec 1997b) as this growth pattern is associated with a significant loss of carbohydrates in senesced shoot segments (Adamec 2000). Therefore, high [CO<sub>2</sub>] in the water is one of the most important ecological requirements for rapid growth of aquatic carnivorous plants (Adamec 1999).

In aquatic *Utricularia* species, the structural and energetic costs of traps are considerable (Friday 1992; Knight 1992; Adamec 2006, 2007; Porembski et al. 2006), and the plants change the proportion of trap biomass as their investment in carnivory according to some habitat factors including prey availability (Knight 1992; Guisande et al. 2000, 2004; Richards 2001; Englund and Harms 2003; Manjarrés-Hernández et al. 2006; Adamec 2007; Kibriya and Jones 2007). In aquatic *Utricularia* species with homogeneous shoots, about 10–55% of the total plant biomass is formed by traps (Friday 1992; Knight 1992; Richards 2001; Englund and Harms 2003; Porembski et al. 2006; Adamec 2007; Kibriya and Jones 2007). While Kibriya and Jones (2007) found a significant, inversely proportional relationship in *U. vulgaris* between phosphate concentration in the ambient water and the trap proportion, an inversely proportional relationship between trap proportion and shoot N content was proven by Adamec (2008a) for *U. australis*. As the latter internal factor correlated in a highly significant way with the abundance of trapped prey, the trap proportion depended in an inversely proportional way on trapped prey. However, the investment in carnivory also depended significantly on [CO<sub>2</sub>] in the water.

*Utricularia australis* R.Br. (Lentibulariaceae) is a free-floating, submerged aquatic carnivorous plant with homogeneous shoots. Its green, photosynthetic shoots with thousands of traps (bladders) 1–4 mm large have a regular and modular structure composed of leaves separated by internodes (Fig. 1). Like other aquatic *Utricularia* species, *U. australis* exhibits continuous, rapid apical shoot growth during the growing season, with progressive aging and decomposition at the base. Under optimum summer conditions, it can produce 2.6–3.5 new nodes with leaves a day and propagate rapidly by branching (Adamec and Kovářová 2006; cf. Friday 1989, 1992). It is common on all continents of the Old World (Taylor 1989). The Třeboň basin in S Bohemia is one of the centers of its distribution in the Czech Republic. Here, it is spread at hundreds of sites in



**Fig. 1** Scanned plants collected from oligotrophic Cep sand pit, big transparent pool, on 10 September 2006. The diameter of the dish is 12 cm. *l* leaf, *i* internode, *n* node, *b* branch. Note the small size and low quantity of traps, very thin stems, and short, non-growing branches

different habitats—bog and fen pools, fishpond littorals, and shallow sand pits. *U. australis* is considered a eurytopic species (Adamec and Kovářová 2006) and shows a very wide ecological tolerance of many water chemistry factors (Kosiba 1993, 2004; Kosiba and Sarosiek 1993; Adamec and Kovářová 2006). Based on the concentrations of mineral N and P in the water at its sites, these waters can be characterized as oligo- to eutrophic. At markedly oligotrophic sites of *U. australis* in Třeboň basin (sand pits), its growth may clearly be co-limited not only by low mineral N and P concentration in the water, but also by very low prey availability (Adamec 2008a). Therefore, one can assume a very low relative growth rate including a low apical shoot growth rate as a response to these stressful conditions.

In this study, *U. australis* was chosen as a model aquatic carnivorous plant for its prolific distribution at different habitats in the Třeboň basin and for its great ecological plasticity. The photosynthetic CO<sub>2</sub> compensation point and factors associated with investment in carnivory and catch of prey were measured in an outdoor collection and 16 field micropopulations of *U. australis* in Třeboň basin together with water chemistry factors at these sites differing greatly in their trophic level. Observations of growth were performed at some oligotrophic sites to estimate apical shoot growth rate. The following questions were addressed: (1) What are the values of CP CO<sub>2</sub> in plants growing in natural waters differing greatly in their CO<sub>2</sub> and mineral nutrient? (2) To what extent is the estimated photosynthetic CO<sub>2</sub> affinity correlated with carnivory or water chemistry? (3) What is the apical shoot growth rate at very oligotrophic sites with a low prey capture?

## Materials and methods

### Field sites

The field work and collections of plant material were conducted during the peak summer season from 8 July to 14 August 2007. Overall, *U. australis* was investigated at 14 field sites covering 16 microsites in the Třeboň Basin Biosphere Reserve and Protected Landscape Area, S Bohemia, Czech Republic (approx. 49°N, 14°45'E; ca. 430 m a. s. l.) and in one 2.5-m<sup>2</sup> plastic container in an outdoor culture in the Institute of Botany at Třeboň. The 14 field sites were selected non-randomly to represent all habitats in which *U. australis* grows commonly in the region and to cover as wide a set of ranges of factors of water chemistry (pH, TA, nutrient concentrations, etc.) as possible. One field site included three microsites. According to dominant vegetation or water chemistry factors, the total of 17 microsites can be subdivided into three main types of shallow standing waters (see Adamec 2008a), (1) oligotrophic, slightly dystrophic waters usually in sand pits (eight microsites including the outdoor culture); (2) dystrophic waters of Cyperoid-dominated bogs or fens usually near fishpond littorals (four microsites); (3) eutrophic, slightly dystrophic fishpond littorals or old sand pits (five microsites). At investigated microsites, water depth was 5–50 cm. Except for sand pits, the bottom sediments were usually organic.

### Processing of plants

At each site, 6–12 haphazardly selected, adult non-flowering *U. australis* plants were collected from a typical microsite and put in a 1-l plastic bottle with humid air. In the laboratory, the plants were washed thoroughly with tap water and cleaned of sessile organisms. For estimation of CP CO<sub>2</sub> using the final-pH method (Maberly and Spence 1983), apical parts of 1–2 shoots about 6–7 cm long, each consisting of a shoot apex and 4–6 nodes with mature leaves (i.e., with functioning traps), were put in 10-ml test tubes in a solution of 1 mM NaHCO<sub>3</sub> + 0.1 mM KCl (pH ca. 7.65; Adamec and Kovářová 2006). The whole internal volume was filled evenly with the plants. An air volume of about 1 ml was let in the closed tubes to reduce the final [O<sub>2</sub>] in the water. The tubes were exposed to natural light in water at 21–25°C and ca. 350–450 μmol m<sup>2</sup> s<sup>-1</sup> PAR for 5 h and final-pH values were measured. Values of CP CO<sub>2</sub> were calculated from pH and TA ( $TA = [HCO_3^-] + 2 \cdot [CO_3^{2-}] + [OH^-] - [H^+]$ ) after Helder (1988). All measurements were performed in six replicates.

As a measure of prey capture by plants and an assessment of the potential prey availability at the microsites, the presence of any distinguishable prey inside all traps was evaluated within the 11th nodes with mature leaves of four adult plants (in % of all traps) using a binocular loupe at a 25× magnification. Leaves in this

position contain fully mature traps, which could function for 4–6 days (Adamec and Kovářová 2006) and integrate prey capture during this period. The resolution limit for prey was about 0.2 mm and all microorganisms such as protozoa, algae, or debris were excluded as prey. Prey consisted of arthropods and worms. To estimate structural investment in carnivory, evaluated as percentage of trap biomass relative to shoot biomass, 12th nodes with mature leaves of the four plants were excised from the adjacent parts of stems exactly in the middle of internodes. All traps were counted, separated from this material using fine forceps, and the traps and trapless segments were dried and weighed (80°C; dry weight, DW). We assumed that DW of prey was negligible. Mean DW of one trap was calculated. Maximum trap size was measured in this material using a ruler (to the nearest 0.5 mm).

### Observation of growth parameters

To estimate apical shoot growth rate in natural *U. australis* micropopulations in oligotrophic habitats, growth parameters were observed in two large dystrophic and transparent pools of the Cep sand pit from 17 to 25 July and a similar one in one pool of Suchdol sand pit from 5 to 14 August 2007. The plants growing in these barren sand pits (Fig. 1) branch infrequently and have small traps compared to plants growing at other sites in this study. At the start, 12 adult, non-flowering *U. australis* plants were selected randomly in both pools in Cep sand pit. Plant length was measured and all distinguishable branches of the main shoot were counted. To estimate the formation rate of new nodes with leaves at the shoot apex, the internode between the second and third mature node was tagged by a fine thread (Friday 1989; Richards 2001; Adamec and Kovářová 2006). Nodes with young leaves were counted as mature if they bore functional traps (i.e., if they were able to suck in air bubbles, or if they contained prey/detritus). The same procedure was also performed with 16 plants in Suchdol sand pit. The tagged plants were returned to their natural microsite and allowed to grow for 8 days (Cep sand pit) or 9 days (Suchdol sand pit). Then, the apical shoot growth rate, plant length, number of branches and internodes between two successive branches, and percentage of traps with prey within the traps of the 11th nodes with leaves were estimated. Due to damage to some plants caused by herbivores, the final plant counts were usually lower than the initial number tagged.

### Analytical and statistical procedures

Once at each microsite, pH and electrical conductivity were measured at the time of plant sampling (at 10:00–13:00 of local time) or at the start and at the end of the growth experiment. All measurements were taken 2 cm below the water surface, at the zone with plants, within a

typical plant stand of *U. australis*. Water samples collected from the microsites (one sample from each microsite) were filtered (10 µm) and analyzed for TA and macro-nutrients (NH<sub>4</sub><sup>+</sup>-N, PO<sub>4</sub>-P). NO<sub>3</sub><sup>-</sup>-N was not determined as its concentration had been previously determined at the same or similar sites to be almost zero (Adamec 1999, 2007, 2008a; Adamec and Kovářová 2006). For all analytical details, see Adamec (1997b, 1999). [CO<sub>2</sub>] was calculated from TA and pH (Helder 1988).

For every investigated parameter of water chemistry or of the plants (pooled data from four plants), one value for each of the 17 microsites was obtained for correlation studies. pH values were used as such and were not transformed. The data expressed in percentage (% of traps with prey, % of trap DW) were tested for normality by Kolmogorov–Smirnov test. Linear regression models were used to find statistically significant relationships between variables. Due to collinearity of some variables, multiple regression analysis could not be used. All statistical tests were performed using Statistica software (version 5.5). Mean values ± 1 SE intervals are shown for the data from the observation of growth.

## Results

The 17 microsites with *U. australis* exhibited a very wide range of all factors of water chemistry studied (Table 1).

Some microsites, especially in younger shallow sand pits, had very soft and oligotrophic water, as opposed to harder waters of limed eutrophic fishponds. Summarily, *U. australis* was found in waters ranging in electrical conductivity from 27 to 394 µS cm<sup>-1</sup>, pH 4.93–9.20, TA –0.01 to 2.5 meq l<sup>-1</sup>, calculated values of [CO<sub>2</sub>] 0.001–1.08 mM, NH<sub>4</sub><sup>+</sup>-N 0.0–476 µg l<sup>-1</sup>, and PO<sub>4</sub>-P from 1.5 to 88 µg l<sup>-1</sup>. Individual *U. australis* micropopulations also differed greatly in the proportion of traps with animal prey, from 2.7% in very barren habitats up to 70% in others (Table 1); the mean was 26%. Trap proportion to total biomass in the 12th nodes with leaves was on average 26% but was also very variable among micropopulations and ranged from 1.4 to 42%. Similarly, mean trap DW varied greatly from 0.7 to 63 µg trap<sup>-1</sup> and the mean was about 19 µg trap<sup>-1</sup>. The maximum size of bladders in the 12th nodes with leaves varied from 1 to 3 mm, with the median of 2.0 mm. At 16 microsites, values of CP CO<sub>2</sub> ranged from 0.7 to 6.1 µM and the mean was about 2.6 µM (Table 1). All these values can be accepted as true CP CO<sub>2</sub> (cf. Maberly and Spence 1983; Madsen et al. 1996). However, in plants from the alkaline water in the fishpond Stare jezero (pH 9.2), only 0.13 ± 0.04 µM was found. It can indicate that in this micropopulation, weak HCO<sub>3</sub><sup>-</sup> use occurs. The apparent compensation point of HCO<sub>3</sub><sup>-</sup> was 0.51 ± 0.06 mM.

Linear regression models show that both mean trap DW and maximum trap size correlated statistically in a

**Table 1** Water chemistry factors at *Utricularia australis* sites at Trebon basin, S Bohemia, Czech Rep., features of carnivory on four plants of *U. australis* collected from these sites, and CO<sub>2</sub> compensation point of photosynthesis (CP CO<sub>2</sub>; mean ± 1 SE is shown) of apical shoot segments; n = 6

Site	G (µS cm <sup>-1</sup> )	pH	TA (meq l <sup>-1</sup> )	[CO <sub>2</sub> ] (mM)	NH <sub>4</sub> -N (µg l <sup>-1</sup> )	PO <sub>4</sub> -P (µg l <sup>-1</sup> )	% Prey	% Trap DW	Trap DW (µg trap <sup>-1</sup> )	Trap size (mm)	CP CO <sub>2</sub> (µM)
Oligotrophic sites											
Cep sand pit, big dystr. pool	27	5.34	0.033	0.39	25.1	12.2	5.88	41.4	17.0	2.0	3.56 ± 0.21
“-”, small dystr. pool	72	4.93	-0.014	0.39	0.0	1.5	12.8	25.6	12.2	1.5	5.00 ± 0.13
“-”, big transpar. pool	75	6.15	0.061	0.099	3.3	2.3	3.13	16.7	6.19	1.5	2.22 ± 0.12
Hradecek-Trebon sand pit	97	7.13	0.40	0.067	25.1	21.5	4.76	25.0	8.24	1.5	2.02 ± 0.14
Rozvodi sand pit	30	6.13	0.11	0.19	0.2	11.4	2.94	23.2	7.78	1.2	1.46 ± 0.10
Suchdol sand pit	108	7.01	0.46	0.11	18.5	16.2	5.02	36.6	15.9	2.0	2.74 ± 0.08
Ruda sand pit	120	7.80	0.83	0.030	22.0	11.1	43.2	2.22	1.52	1.5	1.87 ± 0.16
Aquatic plant collection	394	7.64	2.51	0.13	0.0	12.8	2.69	42.4	11.0	2.0	4.95 ± 0.09
Dystrophic sites											
Majdalena, forest dystr. pool	120	5.92	0.27	0.76	40.6	37.1	15.4	1.64	0.65	1.0	1.66 ± 0.15
Branna sand pit	40	6.25	0.26	0.34	0.0	12.8	70.4	37.8	63.0	2.5	1.69 ± 0.13
Fishp. Rod, dystrophic canal	221	6.32	0.96	1.08	40.6	45.8	21.0	31.4	30.6	2.5	1.19 ± 0.22
Peat bog near fishp. Nadeje	220	6.54	0.76	0.52	0.0	69.9	69.1	33.3	50.6	3.0	0.70 ± 0.14
Eutrophic sites											
Spoli sand pit	34	5.93	0.27	0.76	3.3	52.9	14.6	35.6	18.6	2.0	6.13 ± 0.20
Fishp. Maly Dubovec	302	7.15	1.84	0.30	476	43.3	35.2	13.8	17.6	2.0	1.36 ± 0.18
Fishp. Ptaci blato, first lagoon	252	7.15	0.71	0.12	0.0	58.6	62.5	25.8	25.8	2.0	2.31 ± 0.11
Fishp. Nove jezero	223	7.37	1.47	0.15	12.6	20.4	55.8	42.4	29.8	2.5	2.08 ± 0.09
Fishp. Stare jez., dystr. pool	161	9.20	0.76	0.001	20.0	88.2	28.6	1.38	1.10	2.0	0.13 ± 0.04?
Mean or median	147	6.70	0.69	0.32	40.4	30.5	26.4	25.7	18.7	2.0	2.56

G electrical conductivity, TA total alkalinity, % prey percentage traps with captured prey in the 11th mature nodes with leaves, % trap DW proportion of trap DW to the total biomass in the 12th mature nodes with leaves as “investment in carnivory”, trap DW mean DW of one trap, trap size maximum trap size in the 12th nodes, ? not valid as true CO<sub>2</sub> compensation point. Mean or median values for all 17 microsites are shown on the bottom

highly significant manner with successfulness of prey capture (% of traps with prey;  $r^2 = 0.39\text{--}0.51$ ;  $P < 0.007$ ; Table 2, Nos. 1, 2). The former two parameters correlated highly significantly with each other (Table 2, No. 3) and, therefore, trap proportion in the 12th nodes with leaves, as a measure of investment in carnivory, correlated significantly with both mean trap DW and maximum trap size ( $r^2 = 0.33\text{--}0.34$ ;  $P < 0.016$ ; Table 2, Nos. 4, 5). However, no correlation was found between trap proportion in the 12th nodes with leaves and prey capture ( $r^2 = 0.0020$ ;  $P = 0.87$ ; data not shown). CP CO<sub>2</sub> correlated only weakly, non-significantly, and negatively with pH of the ambient water ( $r^2 = 0.19$ ;  $P = 0.08$ ; Table 2, No. 6) but did not correlate at all with ambient [CO<sub>2</sub>] ( $r^2 = 0.014$ ;  $P = 0.63$ ; data not shown). Out of all other parameters, CP CO<sub>2</sub> correlated only weakly and non-significantly with trap proportion relative to total biomass ( $r^2 = 0.21$ ;  $P = 0.06$ ; Table 2, No. 7) and negatively with % of traps with prey ( $r^2 = 0.17$ ;  $P = 0.10$ ; No. 8).

The observation of growth in two pools of the Cep sand pit revealed a relatively poor shoot branching (0.9–2.0 branches plant<sup>-1</sup>; Table 3) but very high apical growth rate (2.9–4.2 new nodes with leaves day<sup>-1</sup>), although shoots slightly shortened simultaneously. Although the microclimatic conditions in two experimental pools in the Cep sand pit were very similar, the values of apical growth rate in both pools differed highly significantly from each other ( $t$  test,  $P < 0.005$ ). Experimental plants in the Suchdol sand pit were much longer and robust and branched much more (4.0–6.2 branches plant<sup>-1</sup>; Table 3), but their apical growth rate was

**Table 2** Linear regression models between variables showing all statistically significant ( $P < 0.05$ ) or weakly significant ( $0.05 < P < 0.1$ ) correlations;  $n = 17$ ;  $r^2$ , coefficient of determination

No.	Linear regression model	$r^2$	$P$
1	Trap DW = $5.50 + 0.495$ % prey	0.506	0.001
2	Trap size = $1.58 + 0.0131$ % prey	0.389	0.007
3	Trap size = $1.47 + 0.245$ trap DW	0.659	0.0001
4	% trap DW = $16.7 + 0.481$ trap DW	0.341	0.014
5	% trap DW = $-4.60 + 15.7$ trap size	0.331	0.016
6	CP CO <sub>2</sub> = $26.9 - 3.65$ pH	0.188	0.082
7	CP CO <sub>2</sub> = $-3.90 + 0.246$ % trap DW	0.214	0.061
8	CP CO <sub>2</sub> = $6.67 - 0.160$ % prey	0.168	0.103

**Table 3** Results of growth observation of *Utricularia australis* in shallow, oligotrophic pools in sand pits in Trebon basin, S Bohemia, Czech Rep. Non-modified adult plants were used at the start of the experiment

Site	Time	Initial data			Final data					
		$n$	Length (cm)	Branches (plant <sup>-1</sup> )	$n$	Length (cm)	Branches (plant <sup>-1</sup> )	NBB	% Prey	AGR (nod. day <sup>-1</sup> )
Cep, distr. pool	17–25 July	12	34.3 ± 1.9	2.00 ± 0.28	6	–	1.50 ± 0.62	–	–	2.92 ± 0.25
Cep, transpar. pool	17–25 July	12	36.8 ± 1.5	0.92 ± 0.23	9	30.1 ± 2.7	1.67 ± 0.33	22.1 ± 1.2	22.6 ± 7.3	4.22 ± 0.13
Suchdol	5–14 August	16	62.6 ± 3.1	4.00 ± 0.32	16	55.6 ± 3.3	6.19 ± 0.40	10.5 ± 0.4	24.0 ± 4.0	2.53 ± 0.05

NBB number of nodes between two adjacent branches, % prey percentage of traps on the 11th node with any captured prey, AGR apical growth rate, number of newly formed nodes with leaves day<sup>-1</sup>,  $n$  number of plants. Mean ± 1 SE are shown

somewhat lower (2.5 nodes day<sup>-1</sup>) than at Cep. The number of internodes between two successive shoot branches was found to be rather stable at each microsite.

## Discussion

### Investment in carnivory

Seventeen strongly contrasting microsities of *U. australis* were selected in the Třeboň basin, Czech Rep., to study the relationship of photosynthetic CO<sub>2</sub> affinity with external habitat factors or internal factors associated with carnivory. The range of all water chemistry factors at the microsities, however, much the selection of all microsities was biased and non-random, confirms clearly that this species is typically eurytopic, withstanding both strongly oligotrophic and strongly eutrophic conditions (Table 1; cf. Kosiba 1993, 2004; Kosiba and Sarosiek 1993; Adamec and Kovářová 2006; Adamec 2008a). No correlation was found between concentrations of NH<sub>4</sub><sup>+</sup>-N and PO<sub>4</sub>-P in the waters ( $r^2 = 0.020$ ;  $P = 0.58$ ; data not shown). The data obtained clearly support the general view that aquatic carnivorous plants prefer waters with high [CO<sub>2</sub>] but that they can also withstand waters with very low [CO<sub>2</sub>] (Moeller 1978; Adamec 1997a, 1997b, 1999, 2008a).

At the 17 microsities selected, *U. australis* plants also differed greatly in the proportion of traps with animal prey (2.7–70%; Table 1). This parameter was very variable for individual plants within each microsite at a given time (variation coefficient was 97% for experimental plants at Cep and 67% for those at Suchdol; Table 3) or at different times (cf. Tables 1, 3 for Cep and Suchdol). However, it shows what was the true prey utilization by the plants (Richards 2001; Adamec and Kovářová 2006; Adamec 2008a), as a marked prey selectivity (i.e., species and size of prey) occurs in *Utricularia* traps (Harms 1999; Darnowski et al. 2007; Guiral and Rougier 2007). In spite of the very variable microsities, the proportion of traps with prey correlated highly significantly with both mean trap DW or maximum trap size (Table 2, Nos. 1, 2), but not at all with proportion of trap DW as investment in carnivory. Thus, the amount of captured prey can influence the size and the DW of some traps rather than the investment in

carnivory. Yet, the data show that investment in carnivory is associated with trap size or DW more than with the number of traps (Table 2, Nos. 4, 5). Similarly, a direct relationship between the proportion of traps with prey and the proportion of trap DW was not proven by Adamec (2008a) in a similar study in *U. australis*. However, the author proved a significant, positive relationship between the proportion of traps with prey and the shoot tissue N content on one hand and a negative feedback of this shoot N content on the proportion of trap DW on the other. Overall, catch of prey is one of the important factors affecting—directly or indirectly—investment in carnivory in aquatic *Utricularia* species (Knight 1992; Guisande et al. 2000; 2004; Richards 2001; Englund and Harms 2003; Manjarrés-Hernández et al. 2006; Kibriya and Jones 2007).

### Photosynthetic CO<sub>2</sub> affinity

In field-grown *U. australis* shoots, CP CO<sub>2</sub> values varied between 0.7 and 6.1 μM (Table 1). These values are consistent with several data known in aquatic carnivorous plants, mostly *Utricularia* spp. (Moeller 1978; Adamec 1995, 2008b; Adamec and Kovářová 2006). Moreover, these values are well within the range of CP CO<sub>2</sub> values reported for many aquatic non-carnivorous plants (Maberly and Spence 1983; Madsen et al. 1996) but lie within lower values of the range. Thus, carnivory in itself in aquatic plants does not represent a marked deviation of CO<sub>2</sub> affinity from non-carnivorous plants. At all microsites but one, *U. australis* plants grew in the ambient water at the pH of 4.9–7.8 and at a relatively high [CO<sub>2</sub>] of 0.03–1.1 mM (Table 1). For all these 16 microsites, the estimated values of CP CO<sub>2</sub> represent true CO<sub>2</sub> compensation points of photosynthesis as in all typical strict CO<sub>2</sub> users. In contrast, the micropopulation in the dystrophic but eutrophic pool in the fishpond Stare jezero grew at a very low [CO<sub>2</sub>] in a dense stand of *Ceratophyllum demersum* which is known to be an efficient water alkalizer and HCO<sub>3</sub><sup>-</sup> user (Maberly and Madsen 2002). Under conditions of high pH and a shortage of CO<sub>2</sub>, *U. australis* was acclimated to weak HCO<sub>3</sub><sup>-</sup> use; the apparent HCO<sub>3</sub><sup>-</sup> compensation point was 0.51 mM and the final pH 10.0 ± 0.09. As the [HCO<sub>3</sub><sup>-</sup>] in the pool was ca. 0.67 mM a low HCO<sub>3</sub><sup>-</sup> use could occur. Maberly and Spence (1983) report a range of HCO<sub>3</sub><sup>-</sup> compensation points between 0.05 and 0.8 mM for several higher aquatic plants. Thus, HCO<sub>3</sub><sup>-</sup> use in *U. australis* appears to be inducible by a very low [CO<sub>2</sub>] or very high pH, like in the aquatic moss *Fontinalis antipyretica* (Peñuelas 1985; Adamec, unpublished data).

It does not follow clearly from the present results which external or internal factors regulate CO<sub>2</sub> affinity in field-grown *U. australis* (Table 2). In aquatic non-carnivorous plants with strict CO<sub>2</sub> use, CO<sub>2</sub> affinity depends on CO<sub>2</sub> availability during their growth (Madsen et al. 1996). However, in *U. australis* shoots,

no correlation was found between CP CO<sub>2</sub> and the ambient [CO<sub>2</sub>] and only a weak one between CP CO<sub>2</sub> and the ambient pH (Table 2). Nevertheless, CP CO<sub>2</sub> correlated weakly with the proportion of trap DW. This relationship could indicate that a greater proportion of traps with a much lower photosynthetic efficiency but a greater respiration rate (Adamec 2006) will result in reducing the CO<sub>2</sub> affinity of shoots. As carnivorous plants also take up organic carbon from prey (Juniper et al. 1989; Adamec 1997a), it would be possible to expect a partial compensation of CO<sub>2</sub> fixation by the uptake of organic carbon and, thus, an increase of CP CO<sub>2</sub>. Although this has recently been proven in outdoor-grown *U. australis* (Adamec 2008b), the present results (Table 2, No. 8) rather contradict this explanation. It may therefore be assumed that several factors are responsible for regulating CO<sub>2</sub> affinity in aquatic carnivorous plants, e.g., shoot N or P content, which correlated significantly with catch of prey (Adamec 2008a).

### Apical growth rate

In some sand pits, *U. australis* grew in oligotrophic waters, its prey capture was low, (Table 1), and the plants were weak, with very thin stems and leaves. Under these unfavorable trophic conditions, it might be expected that apical growth rate is very low to minimize losses of growth-limiting mineral nutrients (N, P, K) from senescent shoot segments (Adamec 2000, 2008a). However, a very rapid apical growth from 2.9 to 4.2 nodes with leaves day<sup>-1</sup> occurred in the Cep sand pit during very warm summer weather (maximum air temperatures 29–33°C) and about 2.5 nodes day<sup>-1</sup> in the Suchdol sand pit during colder summer weather (maximum air temperatures 18–25°C; Table 3). The former values from Cep significantly extended even those found in this species in a eutrophic pool with good prey availability in the same region during the warmest part of summer (3.2–3.5 nodes day<sup>-1</sup>; Adamec and Kovářová 2006). Present data showed an inversely proportional relationship between apical growth rate and frequency of branching and confirmed growth competition between the growth of shoot apices and branches (Adamec and Kovářová 2006). Although the relative growth rate of the experimental plants in the sand pits was not measured, it is possible that it was extremely low or even zero, in spite of very rapid apical shoot growth, as the shoot size decreased slightly during the experiment. Moreover, newly formed shoot branches usually stayed very short (0.5–2 cm) and did not grow (Fig. 1). As in *Aldrovanda* (Adamec 1999), branches in *U. australis* formed regularly at each site (Table 3) but, unlike *Aldrovanda*, they did not grow (cf. Adamec 1999; Adamec and Kovářová 2006).

Thus, the investigation of branching and the growth of branches should be an attribute in all growth studies on aquatic carnivorous plants. Moreover, the fact that

aquatic *Utricularia* species can also grow in oligotrophic habitats with very low prey availability evokes questions on trap functioning and ecological benefit of traps under these barren conditions.

**Acknowledgments** This paper is dedicated to Prof. Dr. Katsuhiko Kondo, Hiroshima University, Higashi-Hiroshima, Japan, for the long-term merits of his studies on carnivorous plants. Sincere thanks are due to Dagmara Sirová for critically reading the manuscript and to Prof. Douglas W. Darnowski for correction of the language. This study was partly funded by the Research Project of the Academy of Sciences of the Czech Republic No. AV0Z60050516.

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