

Investment in carnivory in *Utricularia stygia* and *U. intermedia* with dimorphic shoots

Investice do masožravosti u *Utricularia stygia* a *U. intermedia* s dvojtvarými prýty

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Utricularia stygia Thor and *U. intermedia* Hayne are aquatic carnivorous plants with distinctly dimorphic shoots. Investment in carnivory and the morphometric characteristics of both types of shoots of these plants were determined in dense stands growing in shallow dystrophic waters in the Třeboň basin, Czech Republic, and their possible ecological regulation and interspecific differences considered. Vertical profiles of chemical and physical microhabitat factors were measured in these stands in order to differentiate key microhabitat factors associated with photosynthetic and carnivorous shoots. Total dry biomass of both species in dense stands ranged between 2.4–97.0 g·m⁻². The percentage of carnivorous shoots in the total biomass, which was used as a measure of the investment in carnivory, ranged from 40–59% and that of traps from 18–29% in both species. The high percentage of total biomass made up of carnivorous shoots in both species indicates both a high structural investment in carnivory and high maintenance costs. As the mean length of the main carnivorous shoots and trap number per plant in carnivorous shoots in both species differed highly significantly between sites, it is probable that the investment in carnivory is determined by ecological factors with low water level one of the potentially most important. Marked differences were found only in [O₂] between the 1–3 cm deep free-water zone with green photosynthetic shoots of both species and the 10 cm deep loose sediment with chlorophyll-free carnivorous shoots with traps (range 1.7–7.2 vs. 0.0–0.8 mg·l⁻¹). The waters can be characterized as mesotrophic. Though anoxia occurred consistently at a depth of 10 cm in loose sediment at all *U. stygia* and *U. intermedia* sites the carnivorous shoots of both species growing in this microhabitat are able to survive and do not avoid this microhabitat.

Key words: aquatic carnivorous plants, carnivorous and photosynthetic shoots, investment in carnivory, *Lentibulariaceae*, water chemistry

Introduction

In terms of the photosynthetic cost-benefit relationship, Givnish et al. (1984) postulated that carnivory for terrestrial carnivorous plants is only beneficial in nutrient-poor, moist and sunny habitats. It is evident that this is not the case for many aquatic carnivorous plant species in typical habitats, which are short of light (only 2–20% of incident PAR) and sometimes also free CO₂ (below 0.05 mmol·l⁻¹) though [CO₂] is commonly high, > 0.1 mmol·l⁻¹ (e.g. Hough & Fornwall 1988, Adamec 1995b, 1997, Adamec & Lev 2002). In aquatic *Utricularia* species, the structural and maintenance costs of traps are considerable (Friday 1992, Knight 1992, Adamec 1995a, 2006, Porembski et al. 2006) and plants are able to change the proportion of resources invested in traps depending on habitat factors: 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 shoots (*U. australis*, *U. gibba*, *U. reflexa*) and the epiphytic *U. quelchii* with carnivorous stolons, which occur in aquatic tanks of bromeliads, Porembski et al. (2006) record that the percentage of the biomass of traps to the total plant biomass ranges from 11–20% and 30%, respectively. This percentage is only 0.14–0.85% in six terrestrial *Utricularia* species. Thus, an aquatic habit predetermines a relatively great investment in carnivory in *Utricularia*, which may be associated with a greater availability of prey or likelihood of capturing prey in aquatic environments.

Utricularia stygia Thor, *U. ochroleuca* R. Hartm. and *U. intermedia* Hayne (*Lentibulariaceae*) are rootless, aquatic to amphibious carnivorous plants with distinctly dimorphic shoots (Thor 1988, Taylor 1989). These species are intermediate in body plan between the aquatic *Utricularia* species with non-differentiated, homogeneous green shoots with traps (e.g. *U. vulgaris*) and terrestrial species (e.g. *U. uliginosa*) with above-ground green leaves and below-ground, chlorophyll-free carnivorous shoots bearing traps. Their green, more or less horizontal, photosynthetic shoots bear at most only a few traps, while their usually colourless, carnivorous shoots with strongly reduced leaves bear numerous traps and are immersed deeply in a loose organic substrate. In these aquatic *Utricularia* species with dimorphic shoots, either their investment in carnivory or the water chemistry in the zones with different types of shoots, have not been studied.

Utricularia stygia, *U. ochroleuca* and *U. intermedia* grow in very similar habitats in shallow, standing, dystrophic (humic), acidic waters, in fen lakes, bogs and peaty fishponds. However, they very rarely grow together in the same wetland (Adamec & Lev 2002, Płachno & Adamec 2007). Generally, the species show a rather wide ecological tolerance of many water chemistry factors (Melzer 1976, Pietsch 1977, Dierssen & Dierssen 1984, Schäfer-Guignier 1994, Harms 1999, Hofmann 2001) and their ecological limits are similar (Kosiba & Sarosiek 1989, Kosiba 1993, 2004, Adamec & Lev 2002). Based on the concentrations of mineral N and P, these waters can usually be characterized as oligomesotrophic. However, growth of both species may be limited not only by low N and P, but also very low K^+ concentration (Melzer 1976, Kosiba & Sarosiek 1989, Kosiba 1993, 2004). At five to 11 sites for the species in the Třeboň basin, S Bohemia, Czech Republic, Adamec & Lev (2002) revealed a statistically significant difference between the species only in $[K^+]$, with much lower values for *U. intermedia*. However, no field study on these species has shown a difference in microhabitat conditions occurring in the very shallow, free-water zone near the water surface, in which photosynthetic shoots grow, or in the loose, slowly decomposable, organic sediment, consisting of peaty or cyperoid litter, in which the carnivorous shoots with traps grow. Ecological characteristics of the microhabitat in the substrate with carnivorous shoots could indicate the conditions for prey capture, because the aquatic *Utricularia* species with homogeneous shoots capture their prey only in the free-water zone (Harms 1999).

The following questions were addressed in this study: (1) Does the investment in carnivory, measured as the percentage of carnivorous shoots and traps to the total biomass, as well as biometric parameters in *U. stygia* and *U. intermedia*, differ between different sites? (2) Are there interspecific differences in these parameters? (3) Are there differences in the physical and chemical factors in the vertical profile of free water, where photosynthetic shoots grow, and the loose organic substrate, in which carnivorous shoots with traps grow? The study was performed in stands of both species at very favourable sites in the Třeboň basin, Czech Republic.

Species studied

Morphologically, *U. stygia*, *U. ochroleuca*, and *U. intermedia* are very similar. As shown by Adamec & Lev (2002) the carnivorous shoots of these species can be as long as 14–18 cm and reach depth of 13–15 cm in loose anoxic substrates. Like all native European *Utricularia* species they overwinter as turions (i.e. winter buds). While *U. intermedia* is a fertile species setting seeds, *U. stygia* and *U. ochroleuca* are sterile, considered to be of hybrid origin, and their populations polymorphic (Thor 1988, Taylor 1989). On the basis of fine floral, foliar and quadrifid gland differences, Thor (1988) differentiated a separate species from *U. ochroleuca* s.l., which he named *U. stygia* Thor. As shown over the last decade this taxon, though reluctantly accepted by botanists, is more common in Europe than *U. ochroleuca* s. str. (Hofmann 2001, Schlosser 2003, Płachno & Adamec 2007). In this paper, the name *U. ochroleuca* will be used only for *U. ochroleuca* s. str. Taxonomically, within the genus *Utricularia*, the three species belong to the most abundant section *Utricularia*, which comprises 34 aquatic or amphibious species with both homogeneous and dimorphic shoots (Taylor 1989).

Study sites

In the Czech Republic, *U. ochroleuca* and *U. intermedia* are considered to be critically endangered but *U. stygia* is not officially mentioned (Holub & Procházka 2000). The Třeboň basin in S Bohemia is the centre of their recent distribution in the Czech Republic (Płachno & Adamec 2007). Here, 11 sites for *U. ochroleuca* or *U. stygia* and six for *U. intermedia* were verified within the period 2003–2005 (L. Adamec, unpublished data). As few as 20 shoots, to as many as one million occur at each site.

The field work and collection of plant material were carried out during the peak summer season, 10–15 July 2001. Water chemistry was investigated at four *U. stygia* sites and three *U. intermedia* sites in the Třeboň Basin Biosphere Reserve and Protected Landscape Area, S Bohemia, Czech Republic (approx. 49° N, 14° 45' E). In 2003–2005 that the taxon *U. stygia* occurred at all investigated sites was checked microscopically (Płachno & Adamec 2007). The sites were all peat bogs situated in the littorals of eutrophic fishponds (Table 1). For both species, there are large and very dense stands at all the sites selected. Thus, ecological optimum for both species may be assumed at these sites. Two typical, very dense stands of *U. stygia* or *U. intermedia* were selected at each site for measuring the physical and chemical factors, sampling water and collection of plant material for estimating stand biomass. The two stands selected were only 5–15 m apart, but could be inhabited by different dominant or subdominant vegetation (Table 1). In these stands, the depth of where the photosynthetic shoots occurred was usually only 1–3 cm, above 5–30 cm of organic, loose waterlogged sediment. Due to drought in some stands (Hliníř and Nový vđovec fishponds), there was no water above the sediment, and the photosynthetic shoots were terrestrial.

Table 1. – Characteristics of microsites in the Třeboň basin, Czech Republic, where *Utricularia stygia* (US) or *U. intermedia* (UI) were sampled and studied. These study sites were located at fishponds: SV – Švarcenberk; HL – Hliníř; VI – Vizír; RO – Rod; NV – Nový vdovec; PR – Příbrazský; SJ – Staré jezero.

Site	Free-water depth (cm)	Litter depth (cm)	Plant dominants (cover > 40% of total)	Plant subdominants (cover 10–40% of total)
SV1	4–8	18–19	<i>Carex rostrata</i>	<i>Eriophorum angustifolium</i> , <i>Calamagrostis canescens</i> , US
SV2	4–10	18–20	<i>Carex rostrata</i>	<i>Eriophorum angustifolium</i> , US, <i>Utricularia australis</i>
HL1	0	5	<i>Eriophorum angustifolium</i>	US
HL2	0	4–5	<i>Eriophorum angustifolium</i>	<i>Carex panicea</i> , <i>C. rostrata</i> , <i>Juncus bulbosus</i> , US
VI1	1–3	12–15	<i>Juncus bulbosus</i> , US	<i>Eriophorum angustifolium</i>
VI2	1–3	9–11	–	<i>Eriophorum angustifolium</i> , US, <i>Juncus bulbosus</i> , <i>Sphagnum</i> sp.
RO	1–2	20–25	<i>Carex rostrata</i>	<i>Carex acuta</i> , US, <i>Sphagnum</i> sp.
NV1	0–0.5	32	<i>Phragmites australis</i>	UI, <i>Carex acuta</i> , <i>C. canescens</i> , <i>Sphagnum</i> sp.
NV2	0	30	<i>Phragmites australis</i>	<i>Carex canescens</i> , <i>C. acuta</i> , UI
PR1	18–20	2	UI	<i>Eriophorum angustifolium</i> , <i>Phragmites australis</i> , <i>Carex acuta</i>
PR2	15–20	4–18	UI	<i>Eriophorum angustifolium</i> , <i>Carex lasiocarpa</i>
SJ1	1–2	28–29	–	<i>Sphagnum</i> sp., <i>Phragmites australis</i> , <i>Carex lasiocarpa</i> , <i>C. acuta</i> , UI, <i>Eriophorum angustifolium</i>
SJ2	1–2	30	–	<i>Phragmites australis</i> , <i>Carex acuta</i> , <i>Sphagnum</i> sp., <i>C. lasiocarpa</i> , UI, <i>Eriophorum ang.</i> , <i>C. rostrata</i> , <i>Comarum palustre</i> , <i>Salix aurita</i>

Methods

Investment in carnivory

In both of the dense stands of *U. stygia* and *U. intermedia* at each site (except Rod peat bog), the *U. stygia* and *U. intermedia* plants in the quadrats of 0.2 × 0.2 m or 0.5 × 0.5 m were separated from the surrounding plants by cutting with a knife and harvested. After thoroughly cleaning the plant material from the sediment, it was separated into green, photosynthetic shoots and usually colourless, carnivorous shoots with traps, dried (80 °C) and weighed to determine the dry weight (DW). From a dense stand close to one of the harvested stands at each site, 12 adult intact non-flowering plants of *U. stygia* or *U. intermedia* were randomly selected and carefully collected. A greater number of plants was not collected because both species are rare. Great attention was paid to collecting all of the carnivorous shoots and traps. In the laboratory, the following biometric data were estimated in each of all 12 plants: the length of main photosynthetic shoot, the number of isolated photosynthetic and carnivorous shoots (criteria for carnivorous shoots: minimum length 5 mm or presence of at least one trap), the length of main carnivorous shoots (crite-

ria: primary or secondary branches bearing at least three shoot apices), the number of internodes between two successive branches on each type of shoot, and the total number of traps on each type of shoot per plant. The differentiation of shoots into photosynthetic and carnivorous in both species was based on the number of traps, colour of shoots and shape of leaves (fork-shaped and flattened vs. reduced and filamentous). The length of shoots was measured to the nearest 1 mm using a ruler. Within each stand, the largest trap size was measured to the nearest 0.5 mm using a ruler. Of the 12 intact plants, 5 were randomly selected, all traps on both shoot types were cut-off, pooled, and weighed to determine the DW (80 °C), which was used as a measure of the plants' investment in carnivory. Both photosynthetic and carnivorous shoots without traps were also weighed and their DW determined.

Chemical analyses

In both stands at each site, between 9:00 and 15:00 local summer time, the dissolved O₂ concentration, pH, conductivity, and water temperature was measured at a depth of 1–2 cm in the water surrounding the photosynthetic shoots of both species and at a depth of ca 10 cm in the sediment at the same place. Filtered water samples (44 µm mesh size) were used to estimate total alkalinity (TA), total nitrogen (N_t) and phosphorus (P_t), and the sum of humic acid and tannin concentration was measured in the water and sediment collected using a syringe. Other water samples for NO₃⁻, NH₄⁺, PO₄, K, Ca, and Mg analyses were filtered through a 0.7 µm filter. Electrical redox potential (E_{red}) was measured using a Pt electrode and an Ag/AgCl reference electrode placed at a depth of 10 cm in the sediment, and recorded as the mean of six values taken at the same place. An estimate of the level of shading of the water surface by emergent vegetation was obtained in *Utricularia* stands by using a submersible pyranometric sensor, which measured photosynthetically active radiation (PAR; 400–700 nm; Adamec 1997, 1999). The sensor was positioned about 1 cm below the water surface. Ten measurements were made in various parts of each stand in order to compensate for the great variation within a stand. Readings are expressed as percentage of incident PAR penetrating to a depth of 1 cm (photosynthetic shoot level). All the analytical details are described by Adamec (1999, 2000) and Adamec & Lev (1999). Potassium persulphate mineralization of water samples was used for the N_t and P_t estimates (Adamec 2000). CO₂ concentration was calculated from total alkalinity and pH (Helder 1988). As the sensitivity of the oxygen sensor used was cca. 0.05 mg·l⁻¹ lower oxygen concentrations could not be distinguished from zero.

Statistical analysis

Mean values for the chemistry of the water and sediment at the two stands at each site are entered in the results. Due to the very low number of *U. stygia* (4) and *U. intermedia* (3) sites, absence of data for some sites, non-random selection of the sites and stands, and a great similarity of the habitats (Adamec & Lev 2002), statistical significance of the difference in habitat factors of both species was not tested. Instead, the means of all sites for both species for either water or sediment were pooled and the difference between water and sediment tested using a two-tailed t-test for independent samples. No transformation of pH values was used. Mean biometric data for each of 12 plants at each site were tested by two-way ANOVA (nested design) for significant differences between the species.

Results

Total dry biomass of *U. intermedia* in dense stands ranged from 14.7 to 97.2 g·m⁻² and from 2.4 to 35.9 g·m⁻² of *U. stygia* (Table 2). The percentage of carnivorous shoots in the total biomass was about 51% in *U. intermedia* and 46% in *U. stygia*. For five adult plants, the percentage was about 51% in *U. intermedia* and 49% in *U. stygia*. Trap biomass made up about 55% and 49% of the carnivorous shoots in *U. intermedia* and *U. stygia*, respectively, and about 28% and 24% of the total plant biomass in *U. intermedia* and *U. stygia*, respectively (Table 2).

The mean length of the main photosynthetic shoot was about 19 cm in both species (Table 3). The mean number of photosynthetic shoots in *U. stygia* (2.5) was not statistically significantly fewer than in *U. intermedia* (3.2), and the reverse was the case for carnivorous shoots. For each species, between-site differences in the mean length of the main carnivorous shoots were highly significant (one-way ANOVA; $P = 0.0003$), but means for both species were exactly the same (11 cm). The longest carnivorous shoots reached 10–19 cm in *U. stygia* and 12–14 cm in *U. intermedia* (Table 3). Both species differed significantly in the number of internodes between successive branches of photosynthetic as well as carnivorous shoots. In *U. stygia*, the relatively greater frequency of branching in the photosynthetic shoots was associated with fewer branches on carnivorous shoots. The reverse was the case in *U. intermedia*. Generally, however, the frequency of branching was about 1.8–2.8 times greater for carnivorous than photosynthetic shoots in both species. No significant difference ($P = 0.068$) was found in number of traps on photosynthetic shoot per plant between species (Table 3). Although the mean number of traps on carnivorous shoots differed highly significantly between sites within each species (one-way ANOVA; $P = 0.0001$), the means for both species were similar. The proportion of biomass made up of traps is statistically significantly correlated with the number of traps per plant in both species ($n = 7$, $r = 0.79$, $P = 0.05$), but not the for carnivorous shoots alone ($n = 7$, $r = 0.55$, $P > 0.05$).

Of all the water chemistry factors investigated, the only significant difference was in [O₂] in free water and the loose sediment (Table 4). [O₂] content of the free water was high and ranged between 1.7–7.2 mg·l⁻¹, while in the 10 cm deep sediment it was close to zero (0.0–0.8 mg·l⁻¹). Values of some factors (TA, [CO₂], NH₄⁺-N, PO₄-P, humic acids + tannins) were considerably higher in the sediment than in the water, but due to the variation the differences were not statistically significant. Mean values of incident irradiance at the level of the photosynthetic shoots at the sites ranged between 17–31%.

Discussion

Investment in carnivory and shoot differentiation

Even in very dense stands of *U. stygia* and *U. intermedia* the total dry biomass (2.4–97.2 g·m⁻², means of from 20–30 g·m⁻²; Table 2) was one to two orders of magnitude lower than that of dense stands of other submerged or emergent plants (Pokorný & Ondok 1991). Thus, the biomass of *U. stygia* and *U. intermedia* always contains very low amounts of nutrients. Both methods of estimating the percentage of the total plant biomass made up of carnivorous shoots gave similar results, between 40–59% in both species (Table 2). How-

Table 2. – Total biomass (DW) of *Utricularia intermedia* and *U. stygia* in dense stands at three sites of each species in the Treboň basin and percentage of total biomass allocated to carnivorous shoots. Biomass allocated to carnivorous shoots with traps, the traps as percentage of the total biomass and of carnivorous shoots based on measurement of five plants. Where possible two parallel values at each site were pooled together; n – number of samples.

Species	Parameter	DW (g·m ⁻²)	DW allocation to carnivorous shoots (%)	Percentage of biomass of 5 plants to		
				carnivorous shoots (%)	traps (% of total biomass)	traps (% of carnivorous shoots)
<i>U. intermedia</i>	Mean (n)	40.2 (6)	50.6 (6)	50.8 (4)	27.5 (4)	54.8 (4)
	Range	14.7–97.2	39.8–59.0	45.3–57.8	25.8–28.9	44.9–63.4
<i>U. stygia</i>	Mean (n)	17.1 (6)	45.7 (6)	48.5 (3)	23.8 (3)	48.9 (3)
	Range	2.4–35.9	41.9–51.5	39.7–54.0	18.0–27.5	45.3–53.2

Table 3. – Biometric data on *Utricularia stygia* and *U. intermedia* plants collected from dense stands. For the site names see Table 1. PS – photosynthetic shoots; CA – carnivorous shoots. Means per plant, 1SE intervals and range of values are shown for each site. P – probability of agreement between sites. For each species, total mean includes all three sites (n = 34–36). Statistically significant differences between the species (F and P parameters) were evaluated using two-way ANOVA, nested design.

Site	Parameter	Main PS	No. of isolated shoots		Length of main CA shoots (cm)	No. of internodes between two successive branches		No. of traps in shoots per plant		
		shoot length (cm)	PS	CA		PS	CA	PS	CA	
<i>Utricularia stygia</i>										
SV	Mean ± SE	22.6±2.8	2.75±0.39	4.08±0.43	13.8±0.8	11.3±0.7	6.2±0.2	8.3±1.3	151±24	
	Range	8.4–37.1	1–5	2–7	8.7–19.4	6.0–15.0	5.0–6.8	1–16	75–284	
HL	Mean ± SE	16.9±1.2	2.33±0.14	4.08±0.36	7.9±0.3	13.0±0.4	7.1±0.6	2.5±0.6	35.7±4.2	
	Range	12.0–24.3	2–3	2–7	6.6–9.8	11.0–14.3	5.5–11.0	0–7	19–74	
VI	Mean ± SE	18.5±2.6	2.50±0.23	3.33±0.41	10.6±0.6	12.4±0.6	6.8±0.3	3.5±1.4	81.3±8.3	
	Range	8.2–38.4	1–4	1–6	8.1–13.3	9.0–15.0	5.2–8.0	0–15	48–133	
Total mean ± SE		19.3±1.4	2.53±0.16	3.83±0.23	10.8±0.5	12.2±0.4	6.7±0.2	4.8±0.8	89.5±11.6	
<i>Utricularia intermedia</i>										
NV	Mean ± SE	20.8±1.9	3.08±0.31	3.75±0.33	9.9±0.4	17.4±0.9	5.8±0.2	0.25±0.25	102±12	
	Range	11.7–30.0	2–5	2–6	7.4–11.5	12.7–23.0	4.5–6.8	0–3	40–171	
PR	Mean ± SE	20.0±1.0	3.83±0.37	4.08±0.31	12.5±0.4	16.6±0.6	5.9±0.1	0.58±0.42	163±17	
	Range	13.8–25.9	2–6	3–6	9.3–13.8	14.5–22.0	5.0–6.5	0–5	70–254	
SJ	Mean ± SE	15.8±1.6	2.67±0.19	2.67±0.22	10.6±0.3	16.3±0.7	6.1±0.2	0	78.3±5.2	
	Range	8.8–26.5	2–4	2–4	8.4–12.4	11.0–20.0	5.2–7.8	–	49–101	
Total mean ± SE		18.8±0.9	3.19±0.19	3.50±0.19	11.0±0.3	16.8±0.4	5.9±0.1	0.28±0.16	114±9	
F _{1,4}		0.044	3.39	0.45	0.015	51.4	7.75	6.19	0.35	
P		0.84	0.14	0.54	0.91	0.0020	0.050	0.068	0.59	

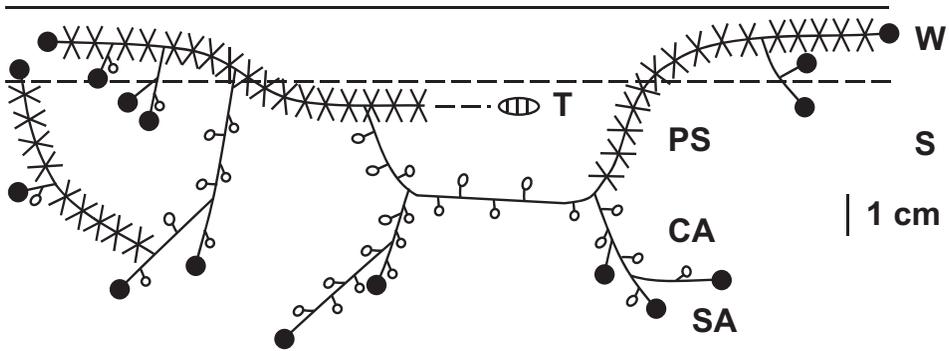


Fig. 1. – Schematic lateral view of a typical adult intact plant of *Utricularia stygia* or *U. intermedia* growing in very shallow free water (W) with shoots penetrating the loose sediment (S). T – old turion; PS – photosynthetic shoot; CA – carnivorous shoot with traps; SA – shoot apex.

ever, as an unknown but low number (and biomass) of traps were lost during harvesting, both the number and biomass of traps reported were slightly underestimated (Tables 2, 3). Except for *U. stygia* at Hliníř, the percentage of the total plant biomass that consisted of traps was within the narrow range of 26–29% in both species. At Hliníř, the low percentage (18%) of trap biomass, very low number of traps per plant and short carnivorous shoots were due to the very low water level at that site. Thus, low water level can reduce the extent and investment in carnivory in these species.

The percentage of trap biomass in *U. stygia* and *U. intermedia* with dimorphic shoots is comparable with that reported for other aquatic *Utricularia* species with homogeneous shoots. For example, Knight & Frost (1991) found the investment in carnivory to be 38–48% in *U. macrorhiza*, Friday (1992) 40–60% in *U. vulgaris*, Richards (2001) about 25% in *U. purpurea*, Adamec (unpublished data) on average 34% in *U. australis* and Porembski et al. (2006) 11–20% in three aquatic species with homogeneous shoots. In *U. stygia* and *U. intermedia*, with differentiated shoots, the investment in carnivory includes the production of carnivorous shoots as well as traps. The high percentage of these chlorophyll-free shoots, of between 40–59% of the total plant biomass (Table 2), is within the upper range of the percentage of traps in terms of total biomass in aquatic *Utricularia*. This indicates not only a high structural investment in carnivory but also high maintenance (metabolic) costs. As recorded by Adamec (2006) for both species the DW-based respiration rate of traps was 1.9–3.0 times greater than that of leaves without traps. Thus, the total dark respiration of carnivorous shoots could amount to 60–68% of the total respiration.

In both *U. stygia* and *U. intermedia*, the boundary between the two types of shoots is usually distinct and is mostly, but not strictly, separated by a branch (Fig. 1). Traps occurred regularly on photosynthetic shoots not only in *U. stygia* (Table 3) but some traps were also found in these shoots in *U. intermedia*, usually near the boundary with carnivorous shoots, as opposed to literature data (Thor 1988, Taylor 1989). As mean length of main carnivorous shoots and trap number per plant on carnivorous shoots in both species differed highly significantly between sites (one-way ANOVA; $P = 0.0003$), it is likely that the quantitative development of these structures is under ecological regulation. However,

except for low water level (at Hliníř), no other ecological regulatory factors were identified by this study. Theoretically, the concentration of growth limiting mineral nutrients (N, P, K) in the water, light, $[\text{CO}_2]$, and prey availability together with $[\text{O}_2]$ (and/or E_{red}) in the sediment, or sediment structure, could be the key ecological factors regulating investment in carnivory in both species (Knight & Frost 1991, Knight 1992, Guisande et al. 2000, 2004, Richards 2001, Englund & Harms 2003, Manjarrés-Hernández et al. 2006). Maximum trap size may also reflect the fitness of plants and investment in carnivory. The maximum trap size in *U. stygia* was 4.5 mm but only 4.0 mm at Hliníř, while it was 5.0 mm at all *U. intermedia* sites. These large traps allow both species to capture relatively large prey up to 4 mm in size (Harms 1999). A large number of traps of both *U. stygia* and *U. intermedia* in the field often contain brown detritus (probably precipitated humic acids and tannins) and may partly digest these substances instead of prey to obtain nutrients.

In both species, the frequency of branching was much greater of carnivorous than photosynthetic shoots (Table 3). Within each species, the mean values for each type of shoot did not differ between sites (data not shown) and the variation was very low. This implies that the frequency of branching of shoots in both species is very regular and does not depend much on habitat factors. The same phenomenon is reported in the aquatic carnivorous plant *Aldrovanda vesiculosa* (Adamec 1999). Although the mean frequency of branching is species specific in *U. stygia* and *U. intermedia* it cannot be used for plant determination due to overlapping values. The usual shape and branching of adult *U. stygia* and *U. intermedia* plants are shown in Fig. 1. Germinating turions always form a photosynthetic shoot. In adult plants, photosynthetic shoots grow horizontally or upwards and the branches grow usually downwards as carnivorous shoots. Long carnivorous shoots, growing deep in the sediment, form photosynthetic shoots that grow upwards into shallow water. This may facilitate the diffusion of oxygen from photosynthetic to carnivorous shoots. Long carnivorous shoots in both species grow at an angle of about 70–75 degrees downwards to the sediment. Thus, it is possible that carnivorous shoots can penetrate on average about 7.5–13.5 cm (upper range to 19 cm) into loose anoxic sediments (cf. Adamec & Lev 2002).

Ecological features of the microhabitats of Utricularia stygia and U. intermedia

In this study, ecological habitat factors were studied in very dense stands of *U. stygia* and *U. intermedia* at sites in the Třeboň basin where plant fitness was highest. Therefore, the ecologically optimal conditions for both species were recorded rather than their ecological amplitudes. Free water in dense stands of *U. stygia* and *U. intermedia* can be characterized as mesotrophic (Table 4). A highly significant linear correlation was found between N_t values (in $\text{mg}\cdot\text{l}^{-1}$) pooled for the water and sediment, and the concentrations of humic acids and tannins (HAT; $N_t = 0.016 \text{ HAT} + 0.39$; $n = 11$, $r = 0.81$, $P < 0.01$). It indicates that the main pool of N_t consisted of organic nitrogen contained in humic acids. No significant correlation was found between N_t and P_t ($n = 11$, $r = -0.081$, $P > 0.05$).

A highly significant and striking difference was found between $[\text{O}_2]$ in free water and that at a depth of 10 cm in the sediment, whereas other water chemistry factors were similar (Table 4). Thus, the free water near the water surface in dense *Utricularia* stands is relatively well mixed with the interstitial water 10 cm deep in the sediment. The steep gradients in $[\text{O}_2]$ and opposite gradients in $[\text{CO}_2]$ found in dense stands are caused by respira-

tion and decomposition of partly decomposed litter or peat. As in our previous study (Adamec & Lev 2002), $[O_2]$ in the free water never fell below $1.7 \text{ mg}\cdot\text{l}^{-1}$ and did not limit plant growth (Adamec 1997). Concentration of O_2 in the sediment usually ranged only from 0.03 to $0.08 \text{ mg}\cdot\text{l}^{-1}$. The usually positive values of E_{red} indicate there were traces of oxygen of an order of magnitude of about 0.5% of saturation in the sediments. Though these low values are only approximate assessments, they indicate that carnivorous shoots in the sediment zone may have suffered from an O_2 shortage. Carnivorous shoots of *U. stygia* and *U. intermedia* contain large gas spaces, as do the closely related *Genlisea* plants (Adamec 2003). If there is sufficient $[O_2]$ in the free water surrounding the photosynthetic shoots or around the terrestrial growth, O_2 can diffuse from photosynthetic to carnivorous shoots via gas spaces (see e.g. Sand-Jensen et al. 2005). Simultaneously, CO_2 can diffuse in the opposite direction from carnivorous photosynthetic shoots as in isoetids. However, $[CO_2]$ in the free water is usually so high that it is unlikely to limit photosynthetic activity (Adamec 1995b).

In general, the occurrence of *U. stygia* and *U. intermedia* at sites depends very weakly on chemical (and physical) qualities of the water. On the other hand, reduced $[O_2]$ in the free water (mean about 60–70% of saturation), relatively high free $[CO_2]$ ($> 0.02 \text{ mM}$, mean 0.17 – 2.3 mM) and high concentrations of humic acid + tannin (range 4–63, mean 15–24 $\text{mg}\cdot\text{l}^{-1}$) may be considered as typical of the water chemistry in *U. ochroleuca/U. stygia* and *U. intermedia* habitats (Table 4, Melzer 1976, Adamec & Lev 2002). The species are poor competitors and sensitive to habitat eutrophication. Therefore, marked seasonal fluctuations in the water level at *U. ochroleuca/U. stygia* and *U. intermedia* sites and associated temporary high water level are recognized as crucial in preventing the development of dense stands of wetland graminoids and cyperoids, which would outcompete these species (Navrátilová & Navrátil 2005). In this respect, the mean optimum level of PAR irradiance in *U. ochroleuca/U. stygia* and *U. intermedia* stands should not decline below about 9% of that in the open.

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Souhrn

Utricularia stygia a *U. intermedia* jsou vodní masožravé rostliny s výrazně dvojitvarými prýty. Investice do masožravosti jako podíl pastí a masožravých prýtů v celkové biomase rostlin a morfometrické charakteristiky obou typů prýtů byly zjišťovány v hustých porostech těchto druhů v mělkých dystrofních vodách na nejbohatších lokalitách v Třeboňské pánvi v ČR a byly zvažovány jejich možné ekologické regulace a mezidruhové rozdíly. V těchto porostech byly měřeny ve vertikálním profilu chemické a fyzikální faktory, aby se rozlišily klíčové mikrostanovištní faktory pro zelené fotosyntetické prýty a nezelené masožravé prýty s pastmi.

Celková sušina obou druhů v hustých porostech dosahovala $2,4$ – $97,2 \text{ g}\cdot\text{m}^{-2}$. Podíl masožravých prýtů jako investice do masožravosti z celkové biomasy byl v rozsahu 40–59% a podíl pastí 18–29% u obou druhů. Průměrná délka hlavních masožravých prýtů a počet pastí na rostlinu u masožravých prýtů obou druhů se vysoce statisticky

průkazně odlišovaly mezi lokalitami, a je tak možné uzavřít, že kvantitativní vývoj těchto masožravých orgánů jako investice do masožravosti je ekologicky regulován. Avšak z této studie nevyplývá, jaké konkrétní mikrostanovištní faktory regulují investici do masožravosti. Jako potenciální regulační faktor byla zjištěna pouze nízká hladina vody. Ačkoliv průměrná frekvence větvení fotosyntetických prýtlů se lišila statisticky průkazně u obou druhů, tento rozdíl nemůže být využit k určování obou druhů vzhledem ke značnému překrývání hodnot.

Výrazné rozdíly byly zjištěny v koncentraci O₂ mezi 1–3 cm hlubokou zónou volné vody se zelenými fotosyntetickými prýtlů a 10 cm hlubokým řídkým sedimentem s nezelenými masožravými prýtlů s pastmi (rozsah 1,7–7,2 vs. 0,0–0,8 mg·l⁻¹). Ze všech sledovaných faktorů chemismu vody byl zjištěn statisticky průkazný rozdíl mezi těmito dvěma zónami pouze v [O₂]. Přesto se převážná většina pastí u obou druhů vyskytuje na masožravých prýtech rostoucích v anoxii. Vody mohou být charakterizovány jako mezotrofní.

References

- Adamec L. (1995a): Oxygen budget in the traps of *Utricularia australis*. – Carniv. Plant Newslett. 24: 42–45.
- Adamec L. (1995b): Photosynthetic inorganic carbon use by aquatic carnivorous plants. – Carniv. Plant. Newslett. 24: 50–53.
- Adamec L. (1997): Photosynthetic characteristics of the aquatic carnivorous plant *Aldrovanda vesiculosa*. – Aquat. Bot. 59: 297–306.
- Adamec L. (1999): Seasonal growth dynamics and overwintering of the aquatic carnivorous plant *Aldrovanda vesiculosa* at experimental field sites. – Folia Geobot. 34: 287–297.
- Adamec L. (2000): Rootless aquatic plant *Aldrovanda vesiculosa*: physiological polarity, mineral nutrition, and importance of carnivory. – Biol. Plant. 43: 113–119.
- Adamec L. (2003): Zero water flows in the carnivorous genus *Genlisea*. – Carniv. Plant. Newslett. 32: 46–48.
- Adamec L. (2006): Respiration and photosynthesis of bladders and leaves of aquatic *Utricularia* species. – Plant Biol. 8: 765–769.
- Adamec L. & Lev J. (1999): The introduction of the aquatic carnivorous plant *Aldrovanda vesiculosa* to new potential sites in the Czech Republic: A five-year investigation. – Folia Geobot. 34: 299–305.
- Adamec L. & Lev J. (2002): Ecological differences between *Utricularia ochroleuca* and *U. intermedia* habitats. – Carniv. Plant Newslett. 31: 14–18.
- Dierssen B. & Dierssen K. (1984): Vegetation und Flora der Schwarzwaldmoore. – Veröff. Natursch. Landschaftspfl. Bad.-Württ., Beih. 39: 1–512.
- Englund G. & Harms S. (2003): Effects of light and microcrustacean prey on growth and investment in carnivory in *Utricularia vulgaris*. – Freshwat. Biol. 48: 786–794.
- Friday L. E. (1992): Measuring investment in carnivory: seasonal and individual variation in trap number and biomass in *Utricularia vulgaris* L. – New Phytol. 121: 439–445.
- Givnish T. J., Burkhardt E. L., Happel R. E. & Weintraub J. D. (1984): Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. – Amer. Natur. 124: 479–497.
- Guisande C., Andrade C., Granado-Lorencio C., Duque S. R. & Núñez-Avellaneda M. (2000): Effects of zooplankton and conductivity on tropical *Utricularia foliosa* investment in carnivory. – Aquat. Ecol. 34: 137–142.
- Guisande C., Aranguren N., Andrade-Sossa C., Prat N., Granado-Lorencio C., Barrios M. L., Bolívar A., Núñez-Avellaneda M. & Duque S. R. (2004): Relative balance of the cost and benefit associated with carnivory in the tropical *Utricularia foliosa*. – Aquat. Bot. 80: 271–282.
- Harms S. (1999): Prey selection in three species of the carnivorous aquatic plant *Utricularia* (bladderwort). – Arch. Hydrobiol. 146: 449–470.
- 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.
- Hofmann K. (2001): Standortökologie und Vergesellschaftung der *Utricularia*-Arten Nordwestdeutschlands. – Abhandl. Westfäl. Mus. Naturk. (Münster) 63: 1–106.
- Holub J. & Procházka F. (2000): Red list of vascular plants of the Czech Republic – 2000. – Preslia 72: 187–230.
- Knight S. E. & Frost T. M. (1991): Bladder control in *Utricularia macrorhiza*: lake-specific variation in plant investment in carnivory. – Ecology 72: 728–734.
- Kosiba P. (1993): Ekologiczna charakterystyka populacji *Utricularia ochroleuca* Hartman i *Utricularia neglecta* Lehmann oraz warunków ich występowania w Węglińcu [Ecological characteristics of the population of *Utricularia ochroleuca* Hartman and *Utricularia neglecta* Lehmann as well as their conditions of occurrence in Węglińiec]. – Acta Univ. Wratisl. 1443, Pr. Bot. 52: 25–31.

- Kosiba P. (2004): Chemical properties and similarity of habitats of *Utricularia* species in Lower Silesia, Poland. – Acta Soc. Bot. Pol. 73: 335–341.
- Kosiba P. & Sarosiek J. (1989): Stanowisko *Utricularia intermedia* Hayne i *Utricularia minor* L. w Strzybnicy koło Tarnowskich Gór [The site of *Utricularia intermedia* Hayne and *Utricularia minor* L. in Strzybnica near Tarnowskie Mts.]. – Acta Univ. Wratisl. 973, Pr. Bot. 39: 71–78.
- Manjarrés-Hernández A., Guisande C., Torres N. N., Valoyes-Valois V., González-Bermúdez A., Díaz-Olarte J., Sanabria-Aranda L. & Duque S. R. (2006): Temporal and spatial change of the investment in carnivory of the tropical *Utricularia foliosa*. – Aquat. Bot. 85: 212–218.
- Melzer A. (1976): Makrophytische Wasserpflanzen als Indikatoren des Gewässerzustandes oberbayerischer Seen. – Diss. Bot. 34: 1–191.
- Navrátilová J. & Navrátil J. (2005): Stanovištní nároky některých ohrožených a vzácných rostlin rašelinišť Třeboňska [Habitat requirements of some endangered and rare plants in mires of the Třeboň region]. – Zpr. Čes. Bot. Společ. 40: 279–299.
- Pietsch W. (1977): Beitrag zur Soziologie und Ökologie der europäischen *Littorelletea*- und *Utricularietea*-Gesellschaften. – Feddes Repert. 88: 141–245.
- Plachno B. J. & Adamec L. (2007): Differentiation of *Utricularia ochroleuca* and *U. stygia* populations in Třeboň basin, Czech Republic, on the basis of quadrid glands. – Carniv. Plant Newslett. 36 (in press).
- Pokorný J. & Ondok J. P. (1991): Macrophyte photosynthesis and aquatic environment. – Academia, Praha.
- Porembski S., Theisen I. & Barthlott W. (2006): Biomass allocation patterns in terrestrial, epiphytic and aquatic species of *Utricularia* (*Lentibulariaceae*). – Flora 201: 477–482.
- Richards J. H. (2001): Bladder function in *Utricularia purpurea* (*Lentibulariaceae*): is carnivory important? – Am. J. Bot. 88: 170–176.
- Sand-Jensen K., Pedersen O., Binzer T. & Borum J. (2005): Contrasting oxygen dynamics in the freshwater isoetid *Lobelia dortmanna* and the marine seagrass *Zostera marina*. – Ann. Bot. 96: 613–623.
- Schäfer-Guignier O. (1994): Weiher in der Franche-Comté: eine floristisch-ökologische und vegetationskundliche Untersuchung. – Diss. Bot. 213: 1–239.
- Schlosser E. (2003): *Utricularia stygia* in California, USA, and *U. ochroleuca* at its southern range. – Carniv. Plant Newslett. 32: 113–121.
- Taylor P. (1989): The genus *Utricularia*: a taxonomic monograph. – Kew Bulletin, addit. ser., 16: 1–724.
- Thor G. (1988): The genus *Utricularia* in the Nordic countries, with special emphasis on *U. stygia* and *U. ochroleuca*. – Nord. J. Bot. 8: 213–225.

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