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Enzyme production in the traps of aquatic *Utricularia* species

Lubomír Adamec[1], Dagmara Sirová[2, 3], Jaroslav Vrba[2, 3], & Eliška Rejmánková[4]

[1] *Institute of Botany AS CR, Section of Plant Ecology, Dukelská 135, CZ-37982 Třeboň, Czech Republic; e-mail: adamec@butbn.cas.cz*
[2] *University of South Bohemia, Faculty of Science, Department of Ecosystem Biology, Branišovská 31, CZ-37005 České Budějovice, Czech Republic*
[3] *Biology Centre AS CR, Institute of Hydrobiology, Na Sádkách 7, CZ-37005 České Budějovice, Czech Republic*
[4] *University of California Davis, Department of Environmental Science and Policy, One Shields Avenue, Davis, CA 95616, USA.*

Abstract: We studied the influence of habitat and increased mineral phosphorus and nitrogen loading on the extracellular activity of five selected hydrolases and pH in the trap fluid of the aquatic carnivorous plants, *Utricularia vulgaris, U. australis*, and *U. foliosa* (Lentibulariaceae). Enzyme activities in the trap fluid were determined using fluorometry. Phosphatase exhibited the highest activities in the traps of the European species as well as field-grown tropical *U. foliosa.* Trap enzyme production appeared to be uninfluenced by elevated dissolved mineral N or P concentrations both in the trap and ambient environment and thus, it seems to be constitutive. Enzyme activity in the trap fluid was determined by species and environmental conditions and varied significantly among sites within a single species. Trap fluid pH was between 4.2–5.1 in *U. vulgaris* and *U. australis* but between 5.7–7.3 in *U. foliosa* and seems to be regulated by the traps.

Key words: aquatic carnivorous plants; extracellular enzyme activity; phosphatase; aminopeptidase; trap fluid pH; growth experiment; nutrient enrichment

Abbreviations: SRP – soluble reactive phosphorus, DM – dry mass

Introduction

About 50 species of the carnivorous rootless genus *Utricularia* (Lentibulariaceae) are aquatic or amphibious plants usually growing in standing, nutrient-poor, and humic waters (Taylor 1989; Guisande et al. 2007). *Utricularia* supplements normal photoautotrophic nutrition by trapping and utilizing animal prey (Friday 1989). The trap of aquatic *Utricularia* is a hollow utricle usually 1–4 mm long, mostly two cells thick, and filled with water. It is the most sophisticated trap among those of carnivorous plants (Juniper et al. 1989), with a variety of glands and trichomes both on the inner and outer surfaces. After the prey irritates trigger hairs situated close to the trap door, it is sucked in as a result of underpressure maintained inside the utricle. As the trap can be fired by any mechanical irritation (wind, larger invertebrates, fish etc.), the "prey" frequently includes various detritus and various particles including algae and bacteria (Richards 2001). After firing, the trap restores underpressure by rapidly removing ca. 40% water from the lumen. This process lasts about 30 min and the trap is ready to fire again (Sydenham & Findlay 1975).

Little is known about the mechanisms of digestion in *Utricularia*. Extracellular hydrolytic enzymes in the trap fluid likely play an important role in nutrient acquisition of *Utricularia*. Evidence was provided for presence of proteases in the traps using standard biochemical techniques (for review see Juniper et al. 1989). Protease (Parkes 1980; Vintéjoux 1973, 1974; Vintéjoux & Shoar-Ghafari 2005), acid phosphatase, and esterase (Heslop-Harrison 1975; Parkes 1980) were localized cytochemically in the quadrifid glands. The above studies, however, did not determine extracellular enzyme activities directly in the trap fluid. Recently, Sirová et al. (2003) investigated activities of five extracellular enzymes (aminopeptidase, phosphatase, β-hexosaminidase, α- and β-glucosidase) in the trap fluid from four aquatic *Utricularia* species. Phosphatase always exhibited the highest activity, while the activities of the other enzymes were usually lower by one or two orders of magnitude. As the activities of the other enzymes were usually higher in the ambient culture water than in traps (without macroscopic prey), the enzymes could have entered the trap from the ambient water. Phosphorus (P) uptake therefore seems to be a key process within *Utricularia* traps, as P is a (co)limiting nutrient in most waters inhabited by *Utricularia* species (Friday 1989; Adamec 1997; Guisande et al. 2007). The importance of phosphatase activity in trap digestion was also supported by Płachno et al. (2006), who showed the presence of phosphatases using the ELF (enzyme-labelled fluorescence) method both

inside the terminal gland cells and on the surface of quadrifid glands in traps of 26 *Utricularia* species, including some species grown *in vitro*.

Results from the previous work (Sirová et al., 2003) suggest a decrease in phosphatase activity in the traps in some *Utricularia* species after the capture of prey. However, these data do not provide exact information on whether the activity of trap enzymes is constitutive or prey-induced and whether it is influenced by changes in mineral nutrient concentration, both in the ambient environment and in the trap fluid.

Utricles also support diverse communities of microorganisms (e.g. Richards 2001). Considering that aquatic *Utricularia* species frequently grow in P-limiting conditions, that trap phosphatase activity is invariably high (Sirová et al. 2003), and that the affinity of phosphate uptake of some microorganisms (bacteria) is at least one order of magnitude greater than that of aquatic plants (Currie & Kalff 1984), it is highly probable that this microbial community enhances P acquisition for the trap under nutrient-poor growth conditions. Determining the role of utricles in the nutrition of *Utricularia* is further complicated by the rapid ageing of traps. In *U. vulgaris*, for example, most utricles complete their life cycle in 30 days or less, and during this time, continuous changes in trap function occur (Friday 1989). The age of traps is therefore a vital consideration in any study of the functional ecology of *Utricularia* (Friday 1989; Sirová et al. 2003).

The aim of our study was to compare the trap enzyme activities of *U. australis* growing *in situ* in two different freshwaters. To determine whether trap enzyme production is constitutive or inducible, we conducted a two-day trap-loading experiment, where both N and P were applied directly into the traps of *U. vulgaris*. The effect of a nutrient enrichment of the ambient culture medium on the trap enzyme activity and plant growth patterns in *U. foliosa* was assessed in a mesocosm growth experiment. In our experiments, we also estimated pH in the trap fluid.

Material and methods

Plant material

Adult plants of *U. australis* R.Br. were collected from a meso-eutrophic fen pool Ptačí Blato and a mesotrophic shallow humic sand-pit Branná (both sites in the Třeboňsko Biosphere Reserve, Czech Republic; Adamec 2005), 2 h before experimental use. Both sites had comparable pH (6.4 and 6.6, respectively) and nutrient contents (NO_3^- 0.48 and 0.58 μM; NH_4^+ 5.4 and 5.2 μM; soluble reactive phosphorus (SRP) 0.78 and 0.66 μM), but substantially differed in the concentration of total humic acids and tannins (6.1 and 25.5 mg l^{-1} for Branná and Ptačí Blato, respectively).

Adult plants of *U. vulgaris* L. (collected in the Czech Republic) were cultivated outdoors in a plastic container which approximately simulated natural conditions (for details see Sirová et al. 2003). At the time of the experiments (July 2004), pH of the cultivation medium was approximately 7.0, total alkalinity 0.62 meq l^{-1}, free CO_2 concentration 0.14 mM, and humic substances (humic acids + tannins) 4.2 mg l^{-1}. The medium was considered oligotrophic (NH_4^+ 2.4 μM, NO_3^- 0.29 μM, SRP 65 nM). Addition of fine zooplankton prey to the container was interrupted approximately 2 weeks before the start of the experiments.

U. foliosa L. plants for the long-term nutrient enrichment experiment were collected from a shallow, oligotrophic, carbonate-based marsh "Hidden" (Orange Walk province, Belize, Central America; Rejmánková et al. 1996) in August 2004. The following water chemistry parameters were measured at the site by standard methods (see Rejmánková et al. 1996) at the time of plant collection: pH 7.8, electrical conductivity 14.3 mS m^{-1}, total alkalinity 1.14 meq l^{-1}, NH_4^+ 0.43 μM, $NO_3^- < 0.5$ μM, SRP 65 nM.

All *Utricularia* plants were transported submerged in filtered water (mesh size 44 μm) collected at the sites to prevent the trapping of air and detritus by the traps. The above *Utricularia* species were selected for experiments as they have relatively large traps (> 2.5 mm), they were used in our previous study (Sirová et al. 2003), and were available in large biomass. The use of temperate and subtropical *Utricularia* species has allowed us to compare ecophysiological trap characteristics of plants from quite different habitats and climatic conditions.

Collection of trap fluid, pH measurements

Trap fluid from utricles of the same size class (> 2 mm) and age was collected using a glass pipette with a fine, 0.4–mm wide tip, attached to a plastic syringe (Sirová et al. 2003). In all experiments, only empty traps without visible prey were selected for analysis. Fluid collected from 200–500 traps (∼0.5 ml) of each of the *Utricularia* species in various treatments was pooled into Eppendorf vials. Samples for enzyme assays were filtered through 0.2 μm filter to remove bacterial and algal cells as well as detritus, and were frozen at –20 °C till analysis. Ambient water samples were processed in the same way. The pH of trap fluid was estimated using a pH paper (Lachema, Brno, Czech Rep.; for detailed description, see Sirová et al. 2003).

U. australis in the field

U. australis plants from the two sites described above were used to collect trap fluid for enzyme (phosphatase, aminopeptidase) activity assay and pH measurement. Freshly collected leaves with functional, medium-old traps from 6^{th} to 12^{th} adult leaf whorls were used.

Trap-loading experiment on U. vulgaris

Leaves with functional traps were cut from adult plants of *U. vulgaris* and placed into plastic jars (0.2 l). The leaves were collected from the middle parts of the shoots (5^{th}–10^{th} adult leaf whorls), their traps were rose to pink, and corresponded to the traps of intermediate age. A part of the trap-bearing leaves was used immediately to collect trap fluid from empty traps without macroscopic prey for estimating enzyme activity and pH prior to nutrient loading. Control leaves were placed into filtered (mesh size 44 μm) culture water, the +P variant leaves into a solution of 1 mM NaH_2PO_4 (pH 5.54) and that of +N variant into a solution of 1 mM NH_4Cl (pH 5.85). We preferred to use an NH_4-rather than NO_3-based salt, as there are indications in literature that aquatic carnivorous species with ecophysiology similar to *U. vulgaris* preferentially utilize NH_4^+ (Adamec 2000; Fertig 2001). Based on potential prey size, prey N and P content, and trap volume, the 1 mM concentrations of N and P were considered to be present, in reality, in the trap fluid with prey in the process of digestion. Traps in the +N and +P variants were loaded by the solutions twice (at

1.5 h intervals) by mechanical irritation. After this loading, the leaves were transferred back to the nutrient-poor filtered culture water. Plastic jars with leaf cuttings were placed into the same light and temperature conditions as those under which the plants were cultivated. In this way, leaf cuttings were exposed at 20–24 °C for two days. It is reasonable to assume that two-day incubation is sufficiently long, considering trap physiology as well as the relatively fast trap aging rate.

Trap fluid from traps without macroscopic prey in each treatment was collected after 1- and 2-day exposure in the culture water. Activities of aminopeptidases, phosphatases, β-hexosaminidases, α- and β-glucosidases in trap fluid were determined together with pH. Excised leaves with traps of *U. vulgaris* are relatively autonomous organs and are able to survive in ambient water for several weeks and regenerate. Thus, the 2-day exposure of the excised leaves in the ambient water in the light should not reduce substantially their carnivorous reactions (see also Sirová et al. 2003).

Nutrient enrichment experiment on U. foliosa

The 30–40-cm long apical parts of *U. foliosa* plants were transferred into 9 outdoor experimental containers (0.9 × 0.9 × 0.4 m) at a field station in Orange Walk, Belize. The containers were placed approximately 0.5 m apart and shaded with screens to simulate natural light conditions. Each container received 120 l of rainwater at an electrical conductivity of 12 mS m^{-1}, 100 g of dry *Eleocharis cellulosa* litter as substrate, and 15 randomly selected *U. foliosa* plants. Note that the rain water had relatively elevated levels of nutrients due to intensive burning of sugar cane fields in the area. Each plant was tagged with a string below the shoot apex for the estimation of growth rate. Three containers received the addition of P (16.1 μM KH_2PO_4 final concentration) and three were enriched in N (357 μM NH_4NO_3 final concentration) in four successive additions four days apart. The remaining three containers did not receive any nutrients and served as a control. Rain water was added to compensate for evaporative losses.

Samples of trap fluid for the estimation of phosphatase and aminopeptidase activity, trap pH measurement, as well as samples for nutrient analyses of plant tissue and culture water were taken prior to the first nutrient addition (assigned as 0 d), one day after the first nutrient addition (1 d), and two days after the last nutrient addition, i.e., day 15 (End). All parameters were analysed independently for each tank. Growth rate, expressed as biomass increase, and flowering of the plants were estimated two days after the last nutrient addition. Shoot parts grown and maturated after the experiment start were termed "new" and parts that originally grew and maturated in the field were termed "old". Both parts of plants separated by the tag were used for sample collection; samples for plant biomass and tissue nutrient content determination were subdivided into traps (without macroscopic prey), leaves, and stems. The whole new parts of shoots were used for these analyses. Samples for trap fluid enzyme analyses were collected from the first four mature segments on the new shoots. Initial pH in all containers was approximately 7.5 and water temperature (measured at 13:00) ranged from 32.0 to 37.7 °C during the experiment.

Enzyme assay

A common fluorometric method was adopted for the microplate assay and modified to determine enzyme activity in both trap fluid and ambient water (Sirová et al. 2003). The 7-amino-4-methylcoumarin and 4-methylumbelliferyl fluorogenic substrates (Glycosynth, Warrington, UK) were used. Samples were analysed in black 96-well microplates for fluorescence detection (Nunc, Roskilde, Denmark) and fluorescence was measured at 365 nm excitation and 445 nm emission wavelengths with FluoroMax-3/MicroMax (Jobin Yvon/Spex Horiba, Irvine, CA, USA) or Thermo Labsystems Fluoroskan (Ascant, Florida). Trap fluid samples were buffered at the pH 4.7, and those of ambient water at 7.0. For detailed description of microplate set-ups, buffering, calibration method, and enzyme activity calculations see Sirová et al. (2003). In the trap-loading experiment on *U. vulgaris*, we analyzed aminopeptidases, phosphatases, β-hexosaminidases, α- and β-glucosidases. Only aminopeptidases and phosphatases were analyzed in the nutrient enrichment experiment on *U. foliosa*, as preliminary results showed no detectable activity of the other three enzymes under the conditions of the study.

Other methods

Plants of *U. foliosa* from each tank were pooled in a single sample for shoot nutrient analyses in the long-term nutrient experiment. Total carbon (C) and total N were analyzed using the Carlo-Erba series 5000 CHNS analyzer (Milano, Italy). Total P was measured spectrophotometrically after combustion and consequent acid digestion (McNamara & Hill 2000). Ambient water samples for NH_4^+ , NO_3^- and SRP analyses were filtered through a 0.45 μm membrane filter and analyzed colorimetrically according to standard procedures (Hunter et al. 1993).

Statistical treatment

The particulars associated with trap fluid collection and processing (see Sirová et al. 2003) did not allow us to work with independent samples but only with pseudoreplicates in the case of enzyme activity measurements. Thus, the variability of enzyme activities shown (±2 SD) represents only the analytical variability, not the variability of the material used. Standard statistical analyses were performed using STATISTICA 6.0 (StatSoft Inc.) and Prism 4.0 (GraphPad Software Inc.) software; non-parametric tests (Mann-Whitney and Kruskal-Wallis) and Sheffé's test were used to compare parameters among two or more treatments.

Results

U. australis in the field

Though nutrient concentrations were similar in both Branná and Ptačí Blato sites, we observed marked differences between the *U. australis* plants from either site in both aminopeptidase and phosphatase activities, as well as in the pH of the trap fluid (Tab. 1). Traps at the more eutrophic Ptačí Blato had considerably higher activities of both enzymes; the pH of the trap fluid was slightly lower. The phosphatase activity in trap fluids markedly exceeded that in the ambient waters, while the aminopeptidase activity in the trap fluids and the ambient water was roughly comparable and generally rather low. At the two field sites, tissue N content (1.92 and 2.35% dry mass (DM)) and P content (0.31 and 0.29% DM, respectively) in young shoots of *U. australis* was similar.

Table 1. The comparison of enzyme activity (μmol l^{-1} h^{-1}) and pH in the trap fluid from *U. australis* plants and in the ambient water; the plants grew in the field at Ptačí Blato and Branná, Czech Republic.

Parameter	Trap fluid		Ambient water	
	Ptačí blato	Branná	Ptačí blato	Branná
Aminopeptidase	0.630 ± 0.004	0.0	0.103 ± 0.003	0.315 ± 0.021
Phosphatase	17.7 ± 1.1	10.6 ± 0.4	1.98 ± 0.06	0.0
pH	4.5 (4.5–4.8)^a	5.1 (5.1–5.1)^b	6.6	6.4

Explanations: Means (± 2SD) of four (trap enzyme activity) or eight (water enzyme activity) parallel determinations are shown. Medians and ranges of values are shown for fluid pH; $n = 5$. Different letter indicates statistically significant difference within each parameter between trap fluid and ambient water at $P < 0.05$.

Table 2. Comparison of enzyme activity (μmol l^{-1} h^{-1}) and pH in the *U. vulgaris* trap fluid and in the ambient culture water during two days following the loading of traps by N or P.

Enzymes	Ambient water	Control traps			Traps +P		Traps +N	
	0 d	0 d	1 d	2 d	1 d	2 d	1 d	2 d
Aminopeptidase	0.071±0.006	0.019±0.001	0.033±0.002	0.057±0.002	0.0	0.0	0.0	0.020±0.001
Phosphatase	3.33±0.11	11.9±0.4	15.2±1.3	19.2±1.3	9.13±0.36	10.4±0.70	8.20±0.32	9.31±0.38
β-hexosaminidase	1.69±0.11	0.0	0.0	3.30±0.11	0.0	0.999±0.04	0.0	0.0
α-glucosidase	2.67±0.12	0.953±0.067	0.0	3.51±0.17	0.0	0.0	0.0	1.67±0.05
β-glucosidase	1.18±0.04	1.22±0.09	0.0	4.77±0.30	0.0	1.02±0.08	0.0	2.91±0.14
pH	7.0	5.1 (5.1–5.1)^a	5.1 (5.1–5.1)^a	5.1 (4.8–5.1)^a	4.2 (3.9–4.2)^b	4.5 (4.2–4.8)^a	5.1 (4.8–5.1)^a	4.5 (4.2–4.8)^a

Explanations: 0 d, traps prior to the experiment start; 1 d, 2 d, traps 1 day and 2 days following nutrient loading into the traps. Means (± 2SD) of four (for traps) or eight (for water) parallel determinations are shown. Medians and ranges of values are shown for trap fluid pH; $n = 5$. Different letter indicates statistically significant difference at $P < 0.05$ within the control or between the control and a variant for the same time period.

Table 3. Enzyme activity (μmol l^{-1} h^{-1}) and pH in the trap fluid of *U. foliosa* in the nutrient enrichment growth experiment; nutrient concentrations (μM) in the corresponding ambient culture water.

Parameter	Field*	Control treatment			+P treatment		+N treatment	
	0 d	0 d	1 d	End	1 d	End	1 d	End
Aminopeptidase	0.0	0.0	0.0	10.1±1.2	0.0	6.21±2.70	0.0	4.36±1.89
Phosphatase	0.29±0.23	3.81±1.15	3.71±0.25	2.74±1.24	3.02±0.12	3.12±1.48	4.84±1.11	2.81±1.41
Trap fluid pH	7.0 (6.4–7.5)	6.6 (6.1–7.2)	6.2 (5.7–6.5)	7.2 (6.5–7.8)	5.7 (5.4–7.1)	6.5 (5.6–7.0)	6.1 (5.7–6.5)	7.3 (6.4–7.7)
SRP in the water	0.53	0.36	0.54	0.92	1.75	2.20	0.53	0.97
NH_4^+ in the water	1.46	3.05	3.48	2.45	3.49	1.77	28.4	0.74

Explanations: Means (± 2SD) of four (enzyme activity) or three (water chemistry) parallel determinations are shown. Medians and ranges of values are shown for fluid pH; $n = 8$. No statistically significant difference at $P < 0.05$ was found for pH values between the control plants and the +P or +N variant, respectively, for the same time period. *, data from the plants collected in the field.

Trap-loading experiment

Prior to the start of the experiment (0 d), all enzymes, with the exception of phosphatase, were more or equally active in culture water than in the trap fluid (Tab. 2). Trap phosphatase exhibited the highest activities (8.2–19.2 μmol l^{-1} h^{-1} of all enzymes) in all experimental treatments. In the control, we detected a gradual increase in aminopeptidase and phosphatase activity both one (1-d) and two (2-d) days after the experiment start. β-hexosaminidase, α- and β-glucosidase were zero in 1-d control traps. The 1-d and 2-d traps loaded with P showed a decrease in the activity of phosphatase compared to the control, with a slight rise in the activity 2 days after P loading. The α- and β-glucosidase activity in the P treatment followed the same pattern as in the control traps, although the activity increase in the 2-d traps was lesser. Phosphatase activity decreased markedly in N-loaded traps compared to the control traps, but there was no obvious difference in activity between the N and P treatments. Trap fluid pH remained unchanged in control treatment and N-loaded traps during the experiment, but decreased significantly in the P treatment.

Nutrient enrichment experiment

Traps from field-collected *U. foliosa* plants exhibited zero aminopeptidase and a very low phosphatase activity (0.29 μmol l^{-1} h^{-1}; Tab. 3) compared to those found in the European species. After transferring the plants from the field to the experimental containers, phosphatase activity rose substantially (3.81 μmol l^{-1} h^{-1}). Aminopeptidase trap activity was zero in all treatments

including the control 1 day after the first nutrient addition to the tanks. However, we detected a dramatic increase in its activity at the end of the experiment in all the treatments, the enzyme being most active (10.1 μmol l^{-1} h^{-1}) in the control. Phosphatase activity remained relatively constant throughout the experiment in all treatments (2.74–4.84 μmol l^{-1} h^{-1}). The activity of both enzymes in the ambient water was below the detection limit throughout the experiment duration. A slight decrease in trap fluid pH in P-enriched tanks was not statistically significant (Tab. 3).

There were no significant differences in final biomass of *U. foliosa* plants among the treatments (data not shown), however, plants from the P treatment flowered significantly more often than plants from control or N treatments (Scheffé; $P < 0.05$). The proportion of trap DM to the total plant biomass (i.e. the investment in carnivory) was markedly lower in the new parts of plants grown in the N treatment. Tissue P and N content varied considerably among treatments, plant organs, and plant age (Fig. 1). It was always somewhat greater in new shoot segments as compared to the old ones. However, due to the great variance in the data, no difference in N or P nutrient content for any organ was statistically significant between the controls and the N- or P-enriched variants at $P < 0.05$ though the trap and stem P content in the P-enriched variant markedly exceeded that in the controls and so did the foliar and stem N content in the N-enriched variant.

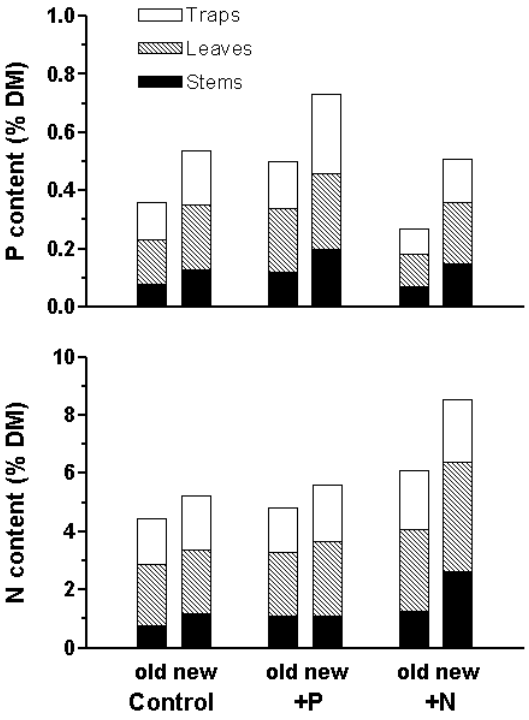

Fig. 1. Tissue P and N content in *U. foliosa* shoots (in % dry mass) from the nutrient enrichment experiment. Means of three independent determinations are shown for each old and new organ.

Discussion

Our results indicate that extracellular enzyme activities in *Utricularia* traps are not directly affected either by direct trap loading with mineral P or N or by mineral N or P enrichment of the ambient culture water. Thus, the trap enzyme production in aquatic *Utricularia* species seems to be constitutive as opposed to that inducible in Droseraceae traps (cf. Juniper et al. 1989). Moreover, there is no obvious difference in hydrolytic enzyme activity in trap fluid between empty traps and those with captured prey (Sirová et al. 2003). However, the results of both experiments suggest that *Utricularia* traps can react rapidly to certain changes in the ambient environment but, presumably, also spontaneously in the course of trap ageing. This can be demonstrated by the marked increase in phosphatase activity of *U. foliosa* control traps following the transfer of plants from the field to culture water with lower concentration of nutrients (at the beginning of the long-term experiment; Tab. 3). A similar increase in phosphatase activity of the *U. vulgaris* control traps was observed in the short-term experiment after the leaf cuttings were placed into filtered water without nutrient-rich detritus (Tab. 2). As shown also by Sirová et al. (2003) trap age is one of key factors influencing the extracellular enzyme activity in the trap fluid of aquatic *Utricularia* plants. Vintéjoux & Shoar-Ghafari (2005) have observed ultrastructural changes in the quadrifid glands within *Utricularia* traps at different stages of development. It is therefore possible that old traps, though still fully functional, shift their function from enzyme production towards nutrient uptake (*sensu* Owen et al. 1999).

Plants of the same species growing at different sites can differ substantially in trap enzyme production as well as in the pH of the trap fluid, as was demonstrated in field-grown *U. australis* (Tab. 1). The ability to rapidly change hydrolytic activities within the traps according to the changes in the ambient environment may have contributed to the success of *Utricularia* within the carnivorous plant group and to the ubiquitous distribution of the genus in a variety of environments (Taylor 1989; Müller & Borsch 2005). Our data indicate that the most active enzyme present in the trap fluid depends on the species and environmental conditions – phosphatase always exhibited the highest activities in the traps of field grown subtropical *U. foliosa* as well as of European species in all treatments and sites studied so far (see also Sirová et al. 2003), while aminopeptidase was the most active enzyme only in the *U. foliosa* traps in variants of the nutrient-enrichment experiment. Our data (Tabs. 1, 2) support the view of Sirová et al. (2003) that activities of some hydrolytic enzymes in trap fluid are derived from those in the ambient water due to incidental trap firings. In spite of certain differences in trap fluid pH within each species (Tabs. 1–3; also Sirová et al. 2003), trap fluid pH has no relationship to that in the ambient medium and seems to be regulated by the trap. Yet, the main buffering system of the trap fluid is unknown.

Repeated N or P enrichment of the culture water

in the growth experiment on *U. foliosa* led to ambiguous results. Although the concentration of SRP or NH_4^+ in the culture water in +P or +N variants usually exceeded several times those in controls during the experiment (Tab. 3), growth of new parts of shoots (or separately of stems, leaves, and traps) was not significantly different at $P < 0.05$ from the controls (data not shown). However, the proportion of trap DM to the total plant DM as investment in carnivory in the new biomass was markedly reduced in the +N variant as compared to the controls (20.1% vs. 29.2%, respectively) and, simultaneously, the foliar and stem N content in the +N variant was markedly increased (by 69% for leaves and by 122% for stems; Fig. 1). Thus, this decrease in trap DM proportion in the +N variant is consistent with the finding of Bern (1997) on the same species and with that of Adamec (2008) on *U. australis* that the investment in carnivory is inversely proportional, as negative feed-back, to shoot N content.

The only growth process, which significantly differed between the nutrient-enriched variants of *U. foliosa*, was higher flowering frequency in the +P variant. It may thus be concluded that P enrichment specifically stimulates flowering in this species, unlike terrestrial carnivorous plants (cf. Adamec 1997).

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