Enzymatic activities in traps of four aquatic species of the carnivorous genus *Utricularia*

Dagmara Sirová[1], Lubomír Adamec[2] and Jaroslav Vrba[1,3]

[1]Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, CZ–37005 České Budějovice, Czech Republic; [2]Institute of Botany AS CR, Section of Plant Ecology, Dukelská 135, CZ–37982 Třeboň, Czech Republic; [3]Hydrobiological Institute AS CR, Na Sádkách 7, CZ–37005 České Budějovice, Czech Republic

Author for correspondence:
Lubomír Adamec
Tel: +420 384 721156
Fax: +420 384 721156
Email: adamec@butbn.cas.cz

Received: *31 March 2003*
Accepted: *14 May 2003*

doi: 10.1046/j.1469-8137.2003.00834.x

Summary

• Here, enzymatic activity of five hydrolases was measured fluorometrically in the fluid collected from traps of four aquatic *Utricularia* species and in the water in which the plants were cultured.

• In empty traps, the highest activity was always exhibited by phosphatases (6.1–29.8 μmol l^{-1} h^{-1}) and β-glucosidases (1.35–2.95 μmol l^{-1} h^{-1}), while the activities of α-glucosidases, β-hexosaminidases and aminopeptidases were usually lower by one or two orders of magnitude. Two days after addition of prey (*Chydorus* sp.), all enzymatic activities in the traps noticeably decreased in *Utricularia foliosa* and *U. australis* but markedly increased in *Utricularia vulgaris*.

• Phosphatase activity in the empty traps was 2–18 times higher than that in the culture water at the same pH of 4.7, but activities of the other trap enzymes were usually higher in the water. Correlative analyses did not show any clear relationship between these activities.

• Trap comensals (*Euglena*) could be partly responsible for production of some trap enzymes. The traps can produce phosphatases independently of catching prey. Taking into account the enzymatic activities in traps, phosphorus uptake from prey might be more important than that of nitrogen for the plants.

Key words: aquatic carnivorous plants, trap fluid pH, extracellular enzymatic activity, phosphatase, aminopeptidase, glucosidase, β-hexosaminidase, chitinase.

Introduction

The genus *Utricularia* (Lentibulariaceae), with over 210 species, is the most widespread of the carnivorous plants (Juniper *et al.*, 1989; Taylor, 1989). Many species from this rootless genus are aquatic and form floating shoots in quiet, pollution-free ponds and humic waters where they are important components of the freshwater flora. Like other carnivorous plants, *Utricularia* supplements normal photolithotrophic nutrition by trapping and utilizing prey, typically aquatic crustaceans, mites, rotifers and protozoa (Jobson & Morris, 2001; Richards, 2001). The trap is a hollow utricle, mostly two cells thick, filled with water. Traps are usually 1–4 mm long. They have a doorway obstructed by a trapdoor which opens inwards upon irritation. The trapdoor is surrounded by trigger hairs and other appendages. After the prey brushes against the trigger hairs, it is sucked in because of the underpressure maintained inside the utricle. After firing, the trap restores the underpressure by removing water from the lumen until the original compressed shape is reached. After this process, which lasts about 30 min, is completed, the trap is ready to fire again (Sydenham & Findlay, 1975).

Little is known about the mechanisms of digestion in *Utricularia*. There have been no recent or detailed studies on *in situ* enzymatic activity in the actual trap fluid. Standard biochemical techniques have provided evidence for the presence of proteases in the traps (von Luetzelberg, 1910; Adowa, 1924; Hada, 1930). Protease (Parkes, 1980; Vintéjoux, 1973, 1974), acid phosphatase and esterase (Heslop-Harrison, 1975; Parkes, 1980) were localized cytochemically in the quadrifid digestive glands. Utricles also support a diverse community

of microorganisms, including many species of living bacteria, algae, rotifers and protozoa (Cohn, 1875; Hegner, 1926; Schumacher, 1960; Botta, 1976; Jobson & Morris, 2001; Richards, 2001). Species of *Euglena* (Euglenophyta) apparently even reproduced in this environment (Hegner, 1926; Botta, 1976). It is therefore reasonable to assume that a considerable proportion of enzymatic activity in the trap fluid is derived from this community. This supports the hypothesis that *Utricularia* plants benefit more from the byproducts of this community than from carnivory itself (Richards, 2001).

The aim of our study was to measure *in situ* activity of five common hydrolases in the trap fluid collected from four aquatic *Utricularia* species and in the water in which the plants were cultured, in order to estimate pH optima and changes in activity of these enzymes at time intervals following prey capture. In this way, we determined the direct availability of the enzymes in the traps for prey digestion.

Materials and Methods

Plant material

Adult plants of *Utricularia vulgaris* L. and *Utricularia australis* R.Br. (collected in the Czech Republic) were cultivated outdoors in plastic containers (area 2 m^2, 750 l for the former species; area 0.8 m^2, 220 l for the latter species; Adamec, 1997a,b). *Utricularia foliosa* L. (collected in northern Florida, USA) was grown in a glasshouse in a similar container to that used for *Utricularia australis*. In these cultures, water depth was kept at 25–35 cm and tap water (NO_3-N 0.7–1.1 mg l^{-1}; NH_4^+-N < 10 μg l^{-1}; PO_4-P < 10 μg l^{-1}) was added to compensate for water loss. *Utricularia aurea* Lour. (collected in Malaysia) was grown indoors in 3-l aquaria (Adamec, 1999). Litter of robust sedge species was used as a cultivation substrate in all of the above cultures, and approximately simulated natural conditions. From the concentrations of NH_4^+ (NH_4^+-N 5–10 μg l^{-1}), NO_3^- (NO_3^--N 28–40 μg l^{-1}), HPO_4^{2-} (PO_4-P 12–15 μg l^{-1}) and humic substances (humic acids + tannins 4–10 mg l^{-1}), the water in these cultures was considered oligotrophic and humic (Adamec, 1997b). The pH of the cultivation media ranged from 7.3 to 7.9, dissolved oxygen concentration from 0.22 to 0.3 mM, and free CO_2 concentration from 0.03 to 0.1 mM. Cultivation methods used were analogous to those described for the aquatic carnivorous plant *Aldrovanda vesiculosa* (Adamec, 1997a,b, 1999). Fine zooplankton (*Chydorus* sp., *Bosmina* sp. and *Cyclops* sp.) was added repeatedly to the cultures to promote plant growth. Feeding was interrupted approximately 2 wk before trap sampling.

Collection of trap fluid, pH measurements

Trap-bearing leaves of the four *Utricularia* species studied were cut from adult plants and placed into plastic jars (0.2 l) with filtered (mesh size 44 μm) culture water. The cuttings were collected from younger parts of the plants to ensure full trap functionality. A portion of the material was used immediately to collect trap fluid for estimation of enzymatic activity in empty traps. Fine zooplankton, commonly found in habitats of all aquatic *Utricularia* species (*Chydorus* sp., size 0.5–0.6 mm, fresh weight *c.* 50–100 μg), was added to the remaining material and was carefully removed after 8 h. By this time, most of the traps had fired and contained prey. Trap fluid from the fed traps of *U. foliosa*, *U. australis* and *U. vulgaris* was collected 2 d later (and also after 4 d in *U. vulgaris*), during which the plastic jars were placed in the same light and temperature conditions as those under which the plants were cultivated. No zooplankton was added to traps of *U. aurea*.

Trap fluid from the largest traps (usually > 2 mm) was collected by glass pipettes with fine, 0.4-mm wide tips attached to a plastic syringe. The trap door was carefully pushed inwards by the pipette tip to avoid damage to plant cells and consequent contamination. The fluid was sucked into the tip by capillary action and forced into plastic 0.5 ml Eppendorf filtration vials placed on ice. The fluid (volume *c.* 0.5 ml) collected from 90 to 300 traps of each *Utricularia* species was pooled into a vial equipped with a filter (0.2 μm) and centrifuged at 600–1200 ***g*** for 10–15 min. This procedure filtered out thousands of algal cells, mainly *Euglena* sp., which could burst upon freezing and contaminate the samples. Several vials with filtrate were prepared in this way and frozen at −20°C. The abundance of *Euglena* cells in parallel fluid samples was estimated using a Petroff–Hausser counting chamber (depth 0.04 mm) and a light microscope (magnification ×200). This was repeated 10 times for each *Utricularia* species.

The pH of the trap fluid was estimated roughly using a pH paper (Lachema, Brno, Czech Rep.). A piece of the pH paper, 3 × 3 mm, was placed on clean white plastic pad and soaked in the fluid pooled from 10 traps (volume *c.* 20 μl). The colour change of the pH paper was compared with the standard colour scale. This was repeated five times for each of the *Utricularia* species.

Enzymatic assay

A common fluorometric method (Hoppe, 1983, 1993) was adopted for the microplate assay (Marx *et al.*, 2001) and modified to determine enzymatic activity in both trap fluid and ambient water samples. We used five fluorogenic substrates: L-leucine 7-amino-4-methylcoumarin hydrochloride, 4-methylumbelliferyl (MUF) phosphate, MUF *N*-acetyl-β-D-glucosaminide, MUF α-D-glucoside, and MUF β-D-glucoside (Glycosynth, Warrington, UK), to estimate the activity of aminopeptidases, phosphatases, β-hexosaminidases, α-glucosidases, and β-glucosidases, respectively. We used special, black 96-well microplates for fluorescence detection (Nunc, Roskilde, Denmark) in two different microplate

set-ups as follows. For activity in traps, all wells were filled with 300 µl of 2 mM acetate buffer (pH 4.7). Then, 24 wells were filled with the same trap fluid at 10 µl each. For activity in ambient water, 300 µl of an ambient water sample and 20 µl of 100 mM acetate buffer (pH 4.7) were added in each of 48 wells. Then, tetrads or octets of the wells were supplemented with one of the five above substrates at 50 µl each (300 µM final concentration) and mixed afterwards. Distilled water at 50 µl each was added to one tetrad or octet of control wells to measure background fluorescence.

Immediately after adding all substrates, the microplate was inserted into the microplate reader to detect the rate of hydrolysis of each substrate during the next 2 h (= incubation time, t). Relative fluorescence in each well was measured (excitation/emission wavelengths were 365 nm and 445 nm, with 1 nm and 5 nm band passes, respectively) at 5-min intervals with FluoroMax-3/MicroMax spectrofluorometer (Jobin Yvon/Spex–Horiba, Irvine, CA, USA). A linear increase in fluorescence over time indicated hydrolysis of a particular substrate. Using linear regression of the mean of four or eight replicates, corrected for the average background fluorescence, we determined a relative fluorescence rate (r_{RF}) as a slope of the regression line.

For calibration of the method, we filled a microplate according to either set-up with appropriate amounts of sample and buffer but, instead of the corresponding substrate, we added 50 µl of 7-amino-4-methylcoumarin or 4-methylumbelliferone as the standard at the following final concentrations: 0, 10, 20, 40, 60 and 80 µM. We determined a calibration factor (f_C) as a slope of the regression line. Finally, we calculated the rate of hydrolysis (v, in µmol l^{-1} h^{-1}) according to the following equation:

$$v = r_{RF}/(f_C \times t)$$

Enzyme-labelled fluorescence was used to visualize phosphatase activity in the traps of eight aquatic *Utricularia* species. The method was modified after Nedoma *et al.* (2003) as follows: traps isolated in a small volume of tap water were filled, after firing, with a solution of ELF97 phosphate (*c.* 20 µM final concentration; Molecular Probes, Eugene, OR, USA), incubated for 3 h, and then inspected in an epifluorescence microscope (Olympus BH-60, magnification ×200).

Determination of pH optimum

To determine enzymatic pH optimum, we estimated activities of aminopeptidase and phosphatase both in the fluid from the traps and in the ambient water. The media were buffered to pH values between 4 and 9. We adjusted acetate buffers to pH values of 4.0, 4.7 and 5.5, and Tris-HCl buffers to pH 7.0 and pH 9.0 and used them at the same concentrations as those described in the previous section. We also used the corresponding microplate set-ups and substrates for detection of r_{RF}. Values measured at each pH were expressed as percentage of the maximum r_{RF} within the pH range of 4–9.

Statistical treatment

The small volume of trap fluid collected from single traps and the method of fluid filtration allowed us to collect only one mixed sample for each *Utricularia* species and time point. Thus, it was not possible to collect independent samples of the fluid and the tetrads of single mixed samples (i.e. pseudoreplicates) therefore represented only the variability of activity measurements, not the variability of the material used. In consequence, we could not evaluate differences among species or variants statistically. Relationships between the enzymatic activities in the empty traps and culture water were tested using the nonparametric Spearman correlation (Prism 3.0; www.graphpad.com) (San Diego, CA, USA).

Results

Estimated pH values in trap liquids varied between 4.9 and 5.4 in both empty and fed traps of all four *Utricularia* species, although old plants of *U. vulgaris* had a lower pH (4.2; Table 1). Tables 2 and 3 present hydrolysis rates for five artificial substrates estimated in traps of four *Utricularia* species and in ambient water from the particular cultures, respectively, expressed in the same units (enzymatic activity per litre of trap fluid or water). Among empty traps of growing plants, phosphatases always exhibited the highest activity (6.1–29.8 µmol l^{-1} h^{-1}) followed by β-glucosidases (1.35–2.95 µmol l^{-1} h^{-1}), while the activities of α-glucosidases, β-hexosaminidases, and aminopeptidases were usually lower by one or two orders of magnitude (0–1.60, 0–0.356 and 0.076–0.116 µmol l^{-1} h^{-1}, respectively; Table 2). Empty

Table 1 Estimated pH values of the fluid sucked out from *Utricularia* traps

Plant material	*U. aurea*	*U. foliosa*	*U. australis*	*U. vulgaris*
Empty traps	5.25 (5.1–5.4)	5.06 (5.0–5.1)	4.95 (4.9–5.1)	4.97 (4.9–5.1)
Fed traps after 2 d	n.d.	5.05 (5.0–5.1)	4.93 (4.9–5.1)	4.99 (4.9–5.1)
Fed traps after 4 d	n.d.	n.d.	n.d.	4.95 (4.9–5.1)
Empty traps (old plants)	n.d.	n.d.	n.d.	4.24 (4.2–4.3)

Means and ranges of five parallel determinations are shown; n.d., not determined.

Table 2 Activity of five enzymes at pH 4.7 in the fluid sucked out from *Utricularia* traps (after filtration)

	Activity in the fluid from the traps (μmol l^{-1} h^{-1})								
Enzymes	*U. aurea* 0 d	*U. foliosa* 0 d	2 d	*U. australis* 0 d	2 d	*U. vulgaris* 0 d	2 d	4 d	*U. vulgaris* old plants, 0 d
Aminopeptidase	0.076 ± 0.010	0.022 ± 0.001	0.000	0.055 ± 0.003	0.026 ± 0.001	0.048 ± 0.002	0.116 ± 0.007	0.066 ± 0.006	0.000
Phosphatase	29.6 ± 8.2	6.12 ± 0.28	0.511 ± 0.021	7.62 ± 0.31	0.521 ± 0.018	29.8 ± 4.1	99.9 ± 6.9	65.3 ± 9.2	0.933 ± 0.050
β-Hexosaminidase	0.356 ± 0.015	0.000	0.000	0.096 ± 0.003	0.000	0.286 ± 0.008	1.95 ± 0.07	2.19 ± 0.09	0.000
α-Glucosidase	0.000	1.60 ± 0.07	0.847 ± 0.048	0.774 ± 0.053	0.000	0.043 ± 0.001	0.821 ± 0.062	2.85 ± 0.22	0.000
β-Glucosidase	1.81 ± 0.23	2.95 ± 0.17	1.53 ± 0.10	1.35 ± 0.06	0.000	1.40 ± 0.05	15.0 ± 0.6	4.77 ± 0.26	0.000

Old plants of *U. vulgaris* formed turions; 0 d, empty traps; 2 d, traps with prey after 2 d; 4 d, traps with prey after 4 d. Means (± 2 SD) of four parallel determinations are shown.

Fig. 1 Correlation of all enzymatic activities in the empty traps of four *Utricularia* species and in culture waters (both in μmol l^{-1} h^{-1}). Spearman rank coefficient and solid regression line for all data (n = 25); solid symbols are the phosphatases, dashed regression line applies only for the other enzymes (open symbols, n = 20).

functioning traps of old *U. vulgaris* plants (forming turions) exhibited only phosphatase activity, which was 30 times lower than in the empty traps of growing plants of the same species. While phosphatase activity expressed per unit volume in the trap fluid was always lower than that in the ambient water, activities of the other enzymes at the same pH of 4.7 were usually higher in the ambient water than in the trap fluid (cf. Tables 2 and 3). Correlation analyses did not show any clear relationship between the activities in the trap fluid and those in the ambient water, although an overall correlation of all enzymatic activities in empty traps of all four species with those in ambient waters was significant (r_s = 0.65; P = 0.0004; n = 25). This significance was, however, mainly due to the high phosphatase activities in the traps (Fig. 1). Both correlations of all five enzymes (with phosphatase being an outlier) for one *Utricularia* species (i.e. one cultivation container or aquarium) and of one enzyme for all four species were insignificant in all but one case.

Aminopeptidases in the empty traps of *U. vulgaris* and in its ambient water responded similarly to increasing pH, while phosphatases showed very distinct pH optima (Fig. 2). Phosphatase activity in traps exhibited maximum activity (12.6 μmol l^{-1} h^{-1}) at pH 5.5; the values at pH 4.0 and pH 9.0 were close to zero. By contrast, in the culture water, no phosphatase activity was detected at low pH (4.0–5.5) and 13% of the maximum activity (7.4 μmol l^{-1} h^{-1}) was detected at pH 7.0 (pH close to that in the culture water), while considerable activity (58 μmol l^{-1} h^{-1}) was observed at pH 9.0 (Fig. 2a). The comparison of data in Table 3 and Fig. 2a shows that activity of phosphatases (at pH 4.7) in the culture water was greatly variable in time. The aminopeptidases, both in the trap fluid and in the ambient water, were inactive at lower pH values (4.0 and 4.7) but showed high activity at higher pH values (Fig. 2b).

Table 3 Activity (at pH 4.7) of five enzymes in ambient samples of the water in which *Utricularia* plants were cultured

	Activity in the water samples (μmol l^{-1} h^{-1})				
Enzymes	*U. aurea*	*U. foliosa*	*U. australis*	*U. vulgaris*	*U. vulgaris* old plants
Aminopeptidase	0.000	0.117 ± 0.005	0.088 ± 0.003	0.085 ± 0.003	0.000
Phosphatase	1.82 ± 0.27	3.17 ± 0.12	3.63 ± 0.15	3.11 ± 0.11	0.425 ± 0.063
β-Hexosaminidase	0.104 ± 0.007	1.44 ± 0.05	2.57 ± 0.10	3.40 ± 0.11	0.000
α-Glucosidase	0.000	0.841 ± 0.058	2.53 ± 0.10	3.52 ± 0.25	0.000
β-Glucosidase	0.289 ± 0.119	1.92 ± 0.07	2.10 ± 0.09	5.04 ± 0.20	0.045 ± 0.003

Means (± 2 SD) of eight parallel determinations are shown.

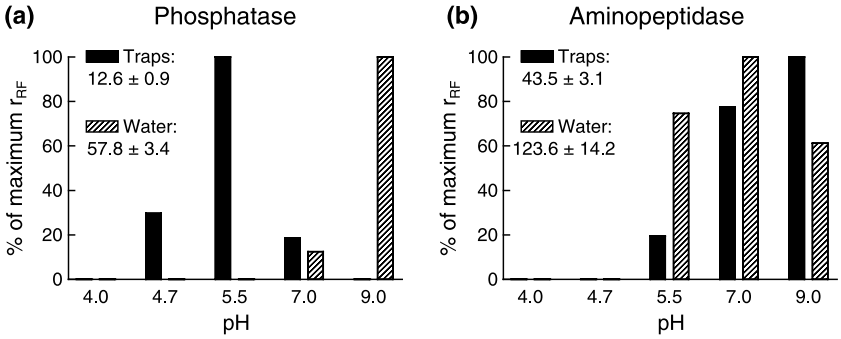

Fig. 2 The pH optima of phosphatases (a) and aminopeptidases (b) in the trap fluid (closed bars) of *Utricularia vulgaris* and its ambient water (hatched bars). Absolute values (mean ± 2 SD, in μmol l^{-1} h^{-1}) of each maximum activity are: (a) traps 12.6 ± 0.9, water 57.8 ± 3.4; (b) traps 43.5 ± 3.1, water 123.6 ± 14.2.

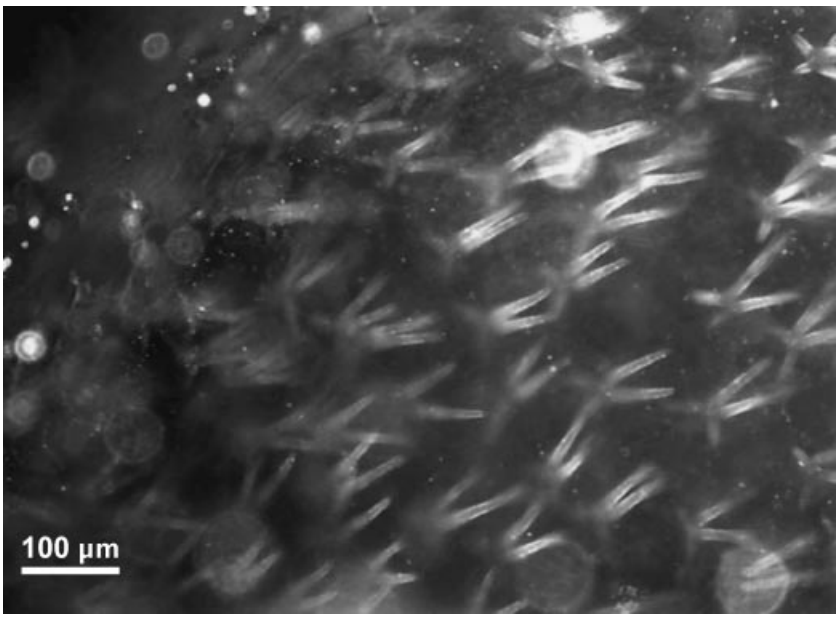

Fig. 3 Photomicrograph of the trap of *Utricularia australis* showing localization of phosphatase activity in the quadrifid glands using enzyme-labelled fluorescence.

After the addition of prey, the fed traps of *U. foliosa* and *U. australis* showed conspicuous decreases in all enzymatic activities after 2 d. By contrast, all activities increased remarkably in the fed traps of *U. vulgaris* after 2 d, although activities of some enzymes subsequently declined (Table 2).

We also tried to detect directly the activity of phosphatases in the quadrifid glands of eight aquatic *Utricularia* species (*U. aurea*, *U. australis*, *U. foliosa*, *U. vulgaris*, *U. bremii*, *U. floridana*, *U. ochroleuca* and *U. purpurea*). Using enzyme-labelled fluorescence, phosphatase activity in the glands was clearly detected only in *U. australis* and *U. ochroleuca* (Fig. 3). The estimated abundance of *Euglena* cells in the fluid of the four *Utricularia* species averaged about 610 cells per trap (range 550–720; data not shown). No significant differences ($P > 0.05$) in cell numbers were found between empty and fed traps.

Discussion

Among digestive enzymes of carnivorous plants, acid peptidases and acid phosphatases have been commonly reported to occur in traps of various terrestrial or aquatic species (Tökés *et al.*, 1974; Clancy & Coffey, 1976; Robins & Juniper, 1980). Yet, this study is the first attempt to measure in parallel the activities of five hydrolytic enzymes both in the trap fluid collected from aquatic *Utricularia* species and in the water in which the plants were cultured. We did not observe substantial aminopeptidase activities in either empty or fed traps of the four *Utricularia* species (Table 2). Moreover, aminopeptidase activity in the culture water was usually several times higher than that in the trap fluid in the plants (cf. Tables 2 and 3). This fact, together with the similar pH optima of the aminopeptidases both in the culture water and in the empty traps of *U. vulgaris*, suggested that production of aminopeptidases in the *Utricularia* traps was very low. Furthermore, considering that about 40% of the resting trap volume was sucked in from the culture water as a result of firing (Juniper *et al.*, 1989) before trap collection, a portion of the aminopeptidase activity in the traps could come from the culture water. The enzymes in the culture water could concentrate in the traps even to activities slightly higher than in the environment. On average, peptidase activities found in *Utricularia* traps were one to two orders of magnitude lower than those of 50–175 mg l^{-1} h^{-1} of bovine albumin (i.e. 0.76–2.65 μmol l^{-1} h^{-1}, determined in the pitcher fluid of carnivorous *Heliamphora tatei*; Jaffe *et al.*, 1992).

Our results clearly showed significant release of acid phosphatase and its accumulation in the empty traps of all the *Utricularia* species tested. The activity of this enzyme in the trap fluids exceeded the phosphatase activity in the culture water (as measured at pH 4.7) by a factor of about 2–18 (Tables 2 and 3), or the latter activity was even zero (Fig. 2a). Clancy & Coffey (1976) reported release of various acid phosphatases by a terrestrial carnivorous plant, *Drosera rotundifolia*; some of these enzymes had a pH optimum around 5.0. However, these authors detected initial enzyme release 1–2 d after feeding, with maximum activity on day 4, followed thereafter by a gradual decrease in activity. By contrast, in traps of two aquatic *Utricularia* species, we usually observed a marked decrease in the activities of all five enzymes studied 2 d after feeding. The only exception was *U. vulgaris*, which showed a similar response to that in *D. rotundifolia* after feeding (Table 2). No information exists as to whether the enzymatic secretion by *Utricularia* traps is constitutive or stimulated by prey (Juniper *et al.*, 1989). However, the former case is much more probable, as suggested by the relatively high enzymatic activities in empty traps (Table 2, Fig. 3). Despite a rather inaccurate method for estimating pH in trap liquids (*c.* ±0.3), the results suggest that the pH in both empty and fed traps is the same (Table 1) and confirm the concept of permanent enzymatic secretion in the traps. Such a distinct variation of enzyme activities among *Utricularia* species after feeding might be due to a quite different digestion rate among the species or a different abundance of commensals in the traps (e.g. *Euglena*) facilitating prey digestion. Determination of enzyme activities in the trap fluid does not prove the plant origin of these enzymes, although it does show the *in situ* enzymatic capacity for prey digestion. Jobson *et al.* (2000) observed that feeding on *Euglena* sp. significantly decreased the growth of terrestrial *U. uliginosa*. This might indicate that *Euglena* in the traps competed for limited nutrient sources, thus behaving as parasites rather than commensals.

The very low activity of aminopeptidases in traps, in contrast to the activity of phosphatases found in all four *Utricularia* species under study, is in disagreement with a greatly efficient and rapid uptake of both N and P from prey in *U. vulgaris* traps (Knight, 1988; Friday & Quarmby, 1994). However, *U. foliosa* under natural conditions took up 1.75% of its seasonal P gain, though only 0.44% of its N gain, from carnivory (Bern, 1997). This implies that, in P-limited waters, uptake of P from prey in *Utricularia* traps is more important than that of N. Comparison of the activities of three glycolytic enzymes (β-hexosaminidase, α- and β-glucosidase) in empty traps and in the culture water shows that these enzymes were produced, at least partly, inside the traps. Although β-hexosaminidase (i.e. chitinase) was detected in traps of some carnivorous plant species, the origin of this activity and its physiological importance for prey digestion remain uncertain (Juniper *et al.*, 1989).

Acknowledgements

This study was partly funded by the GA AS CR project (No. A6017202, awarded to J. V.) and by the Research Programme of the Academy of Sciences of the Czech Republic (No. AV0Z6005908). Thanks are due to K. Beneš and V. Němec for helpful comments and to M. Studnička for providing experimental plant material. Sincere thanks are also due to D. W. Darnowski for linguistic correction and to J. R. Gallon and three anonymous referees for their valuable critical comments.

References

Adamec L. 1997a. Photosynthetic characteristics of the aquatic carnivorous plant *Aldrovanda vesiculosa*. *Aquatic Botany* **59**: 297–306.

Adamec L. 1997b. How to grow *Aldrovanda vesiculosa* outdoors. *Carnivorous Plant Newsletter* **26**: 85–88.

Adamec L. 1999. The biology and cultivation of red Australian *Aldrovanda vesiculosa*. *Carnivorous Plant Newsletter* **28**: 128–132.

Adowa AN. 1924. Zur Frage nach den Fermenten von *Utricularia vulgaris* L. II. Der relative Gehalt der Blasen und Zweige von *Utricularia vulgaris* an Proteoklastischen Fermenten. *Biochemische Zeitung* **153**: 506–509.

Bern AL. 1997. Studies on nitrogen and phosphorus uptake by the carnivorous bladderwort, *Utricularia foliosa*, in south Florida wetlands. PhD thesis. Miami, FL, USA: Florida International University.

Botta SM. 1976. Sobre las trampas y las víctimas o presas de algunas especies argentinas del género *Utricularia*. *Darwiniana* **20**: 127–154.

Clancy FGA, Coffey MD. 1976. Acid phosphatase and protease release by the insectivorous plant *Drosera rotundifolia*. *Canadian Journal of Botany* **56**: 480–488.

Cohn F. 1875. Über die Function der Blasen von *Aldrovanda* und *Utricularia*. *Beiträge Zur Biologie der Pflanzen* **1**: 71–92.

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

Hada Y. 1930. The feeding habits of *Utricularia*. *Transactions of the Sapporo Natural History Society* **11**: 175–183.

Hegner RW. 1926. The interrelationships of protozoa and the utricles of *Utricularia*. *Biological Bulletin* **50**: 239–270.

Heslop-Harrison Y. 1975. Enzyme release in carnivorous plants. In: Dingle JT, Dean RT, eds. *Lysozymes in biology and pathology, Vol. 4*. Amsterdam, The Netherlands: North Holland Publishing, 525–578.

Hoppe HG. 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Marine Ecology Progress Series* **11**: 299–308.

Hoppe HG. 1993. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ, eds. *Handbook of methods in aquatic microbial ecology*. Boca Raton, FL, USA: Lewis Publishing, 423–431.

Jaffe K, Michelangeli F, Gonzales JM, Miras B, Ruiz MC. 1992. Carnivory in pitcher plants of the genus *Heliamphora* (Sarraceniaceae). *New Phytologist* **122**: 733–744.

Jobson RW, Morris EC. 2001. Feeding ecology of a carnivorous bladderwort (*Utricularia uliginosa*, Lentibulariaceae). *Australian Ecology* **26**: 680–691.

Jobson RW, Morris EC, Burgin S. 2000. Carnivory and nitrogen supply affect the growth of the bladderwort *Utricularia uliginosa*. *Australian Journal of Botany* **48**: 549–560.

Juniper BE, Robins RJ, Joel DM. 1989. *The carnivorous plants*. London, UK: Academic Press.

Knight SE. 1988. The ecophysiological significance of carnivory in *Utricularia vulgaris*. PhD thesis. Madison, WI, USA: University of Wisconsin.

von Luetzelberg P. 1910. Beiträge zur Kenntnis der *Utricularia*. *Flora* **100**: 145–212.

Marx MC, Wood M, Jarvis SC. 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry* **33**: 1633–1640.

Nedoma J, Štrojsová A, Vrba J, Komárková J, Šimek K. 2003. Extracellular phosphatase activity of natural plankton studied with ELF97 phosphate: fluorescence quantification and labelling kinetics. *Environmental Microbiology* **5**: 462–472.

Parkes DM. 1980. Adaptive mechanisms of surfaces and glands in some carnivorous plants. MSc thesis. Clayton, Victoria, Australia: Monash University.

Richards JH. 2001. Bladder function in *Utricularia purpurea* (Lentibulariaceae): is carnivory important? *American Journal of Botany* **88**: 170–176.

Robins RJ, Juniper BE. 1980. The secretory cycle of *Dionaea muscipula* Ellis. IV. The enzymology of the secretion. *New Phytologist* **86**: 401–412.

Schumacher GJ. 1960. Further notes on the occurrence of desmids in *Utricularia* bladders. *Castanea* **25**: 62–65.

Sydenham PH, Findlay GP. 1975. Transport of solutes and water by resetting bladders of *Utricularia*. *Australian Journal of Plant Physiology* **2**: 335–351.

Taylor P. 1989. *The Genus* Utricularia: *a taxonomic monograph*. *Kew Bulletin Additional Series XIV*. London, UK: HMSO.

Tökés ZA, Woon WC, Chambers SM. 1974. Digestive enzymes secreted by the carnivorous plant *Nepenthes macfarlanei* L. *Planta* **119**: 39–46.

Vintéjoux C. 1973. Études des aspects ultrastructuraux de certaines cellules glandulaires and raport avec leuc activité sécrétive chez l' *Utricularia neglecta* L. (Lentibulariaceae). *Comptes Rendues* **277D**: 2345–2348.

Vintéjoux C. 1974. Ultrastructural and cytochemical observations on the digestive glands of *Utricularia neglecta* L. (Lentibulariaceae). Distribution of protease and acid phosphatase activities. *Portugaliae Acta Biologica Seriea A* **14**: 463–471.