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Faculty of Science

Hunters or gardeners? Plant-microbe interactions in rootless carnivorous *Utricularia*

Summary of Ph.D. thesis

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Annotation

Various aspects of the ecophysiology of rootless carnivorous *Utricularia* plants were assessed, with focus on nutrient acquisition and mutualistic interactions within trapping organs. The study includes extracellular enzyme measurements, the evaluation of commensal microbial community structure and function, stable isotope labelling to determine plant carbon allocation, and ion chromatography analyses of trap fluid composition.

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List of papers and manuscripts with author's contribution

The thesis is based on the following manuscripts:

I. **Sirová D**, Adamec L, Vrba J, 2003. Enzymatic activities in traps of four aquatic species of the carnivorous genus *Utricularia*. New Phytol. 159: 669–675.
DS designed the experiment setup, optimized the sample collection methods, helped with extracellular enzyme analyses and with manuscript preparation.

II. **Sirová D**, Borovec J, Cerná B, Rejmánková E, Adamec L, Vrba J, 2009. Microbial community development in the traps of aquatic *Utricularia* species. Aquat. Bot. 90: 129–136.
DS was the author of experimental design, collected samples, performed microscopy analyses, helped with nutrient and PLFA measurements and wrote the manuscript.

III. **Sirová D**, Borovec J, Šantrucková H, Šantrucek J, Vrba J, Adamec L, 2010. *Utricularia* carnivory revisited: plants supply photosynthetic carbon to traps. J. Exp. Bot. 61: 99–103.
DS was the author of experimental design, collected samples, helped with stable isotope measurements and wrote the manuscript..

IV. Adamec L, **Sirová D**, Vrba J, Rejmánková E, 2010. Enzyme production in the traps of aquatic *Utricularia* species. Biologia 65: 273–278.
DS helped with experimental design and sample collection participated in manuscript preparation and revision.

V. Adamec L, **Sirová D**, Vrba J, 2010. Contrasting growth effects of prey capture in two aquatic carnivorous plant species. Fundam. Appl. Limnol. 176: 153–160.
DS helped with experimental design, participated in manuscript preparation and revision.

VI. Adamec L, Vrba J, **Sirová D**, 2011. Fluorescence tagging of phosphatase and chitinase activity on different structures of *Utricularia* traps. Carniv. Plant Newslett. 40: 68–73.
DS helped with microscopic assessments participated in manuscript preparation and revision.

VII. **Sirová D**, Borovec J, Picek T, Adamec L, Nedbalová L, Vrba J, 2011. Ecological implications of organic carbon dynamics in the traps of aquatic carnivorous *Utricularia* plants. Funct. Plant Biol. 38, 583–593.
DS was the author of experimental design, collected samples, performed microscopy analyses, helped with microbial respiration measurements and ion chromatography, wrote the manuscript.

VIII. Borovec J, **Sirová D**, Adamec L, 2012. Light as a factor affecting the concentration of simple organics in the traps of aquatic carnivorous *Utricularia* species. Submitted to Fundam. Appl. Limnol.
DS helped with experimental design and ion chromatography participated in manuscript preparation and revision.

INTRODUCTION

The presented thesis is based on eight attached research publications or manuscripts, which are referred to in the text by roman numerals. All deal with a common theme – the ecophysiology of bladderworts (*Utricularia*, Lentibulariaceae), and are a part of long-term research covering this topic, carried out by a team of collaborators. Beginning with a paper published in 2003, we have focused mainly on the internal environment of the trapping organs or bladders of aquatic *Utricularia*, the structure and function of the associated microbial assemblages, and how this might relate to plant nutrient acquisition as well as ecological success of the genus.

As a member of the research team, I have been involved in most of the tasks, from hypothesis generation, experimental design, and sampling, through to sample analysis, data evaluation, and the writing of manuscripts.

The general background and aims of the study, the summary of the main results, and the conclusions reached are presented in the following chapters.

GENERAL BACKGROUND

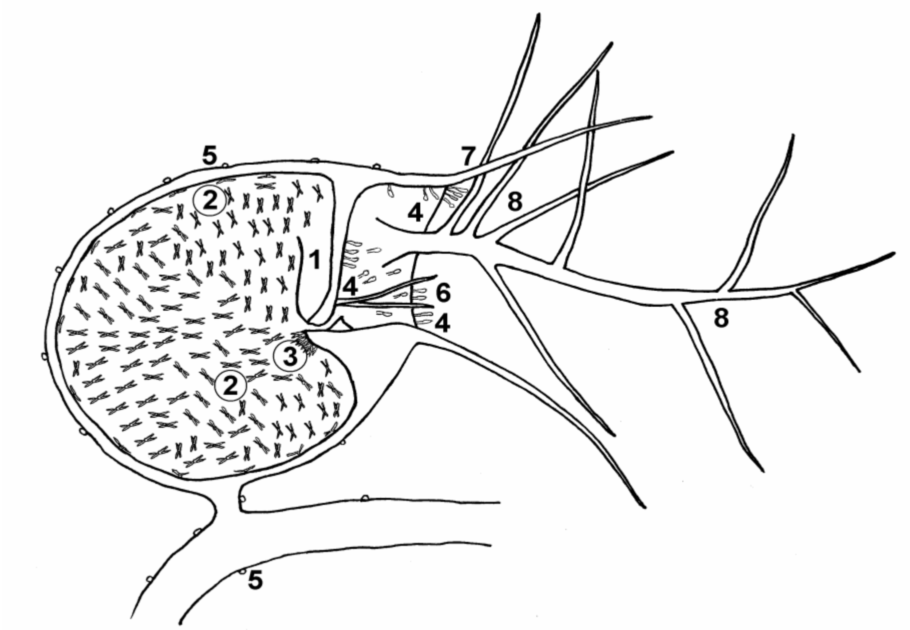
The genus *Utricularia*

Of the approximately 250,000 species of angiosperms, less than 1% are classified as carnivorous and about one-third of these belong to *Utricularia* (Lentibulariaceae) (Juniper et al. 1989; Taylor 1989). Bladderworts are not only the largest, but also the most cosmopolitan plant carnivorous genus, with a distribution ranging from high latitudes and boreal environments to the tropics. Their ecological success is further underscored by the great variety of life forms which include aquatic (lentic and lotic), terrestrial, epilithic, and epiphytic species (Taylor 1989; Guisande et al. 2007). All are characterized by numerous trapping organs or utricles, and by the absence of roots. The genus is also very interesting at the molecular level. Some species are known to have minute genomes: *U. gibba* has the smallest known plant genome, which, at ca. 80 megabases, is approximately half, that of Arabidopsis (Greilhuber et al. 2006). In addition, highly enhanced molecular evolutionary rates have been observed in chloroplast, mitochondrial and nuclear ribosomal sequences.

Trap structure and function

Although the *Utricularia* traps are the smallest among those of carnivorous plants, they are arguably the most sophisticated and intricate ones. They have fascinated scientists since the times of Darwin.

Figure 1.



Schematic longitudinal section through a trap of *Utricularia* with glands and other structures: 1, trap door; 2, quadrifid glands; 3, bifid glands; 4, stalked mucilage glands; 5, spherical sessile glands; 6, trigger hairs; 7, rostrum; 8, antennae. Figure by J. Vrba reproduced from Adamec et al. 2011.

These oval-shaped, fluid-filled bladders of foliar origin are typically 1–6 mm long with elastic walls two cell layers thick and a mobile trap door (Fig. 1). The inner part of the trap is densely lined by large glands of two types: quadrifid glands cover almost the whole inner surface and take part in the secretion of digestive enzymes (Adamec et al. 2011) and in the resorption of released nutrients, while the smaller bifid glands, which are located near the door, take part in pumping the water out of the trap lumen (Sydenham and Findlay 1975). The function of many other gland types on the trap surface and around trap door opening has not been fully elucidated yet.

After the irritation of trigger hairs situated close to the trap door (Fig. 1), surrounding medium is sucked in as a result of negative pressure maintained inside the utricle. As the trap can be fired by any mechanical irritation (wind, larger invertebrates, fish, etc.), 'prey' frequently includes various detritus and particles of suitable size, including algae and bacteria (Richards 2001). After firing, the trap restores negative pressure by rapidly removing ca. 40 % water from the lumen (while leaving dissolved organic matter behind) until the original compressed shape is reached. This process lasts about 30 min and the trap is ready to fire again (Sydenham and Findlay 1975). The trap environment is completely sealed for particles, therefore once inside the trap, prey cannot leave the lumen unless trap walls are damaged or the trap is no longer functional. Recently, it has been shown that *Utricularia* traps are capable of spontaneous firing as frequent as once in every couple of hours (Adamec 2011).

Utricularia traps were found to support diverse communities of bacteria and algae (e.g. Plachno et al. 2012), and organisms previously considered as prey, specifically rotifers and various protozoa, have been found alive, even reproducing within the trap lumen (e.g. Jobson and Morris 2001, Richards 2001, Plachno et al. 2012).

In addition, Adamec (2007) found that the steady-state O_2 concentration in the traps of *Utricularia* always approached zero and the redox potential was low (–24 to –105 mV). It seems that any oxygen entering or produced in the trap is rapidly respired, either by the highly active cells of the internal glands or the microbial community within the trap.

Utricularia *ecophysiology*

In this thesis, due to methodological reasons, we have concentrated exclusively on the aquatic species from tropical or temperate regions, which generally grow in shallow, standing, dystrophic waters (Juniper et al. 1989, Taylor 1989). These waters are usually nutrient poor, sometimes low in K, but especially low in N and P (Adamec 1997a, 2008, Guisande et al. 2007). The most typical feature of these dystrophic waters is a high concentration of free CO_2 (Adamec 1997a, b, 2008).

Although growing in nutrient poor waters, *Utricularia* exhibit very rapid apical shoot growth (Adamec 2009, Adamec et al. 2010) of several nodes per day under favorable temperature conditions. Ecophysiological adaptations that enable the plants to gain limiting mineral nutrients are commonly thought to include carnivory, efficient nutrient re-utilization (recycling) from senescent shoots, and very high affinity for mineral nutrients during their uptake from water (Kaminski 1987, Adamec 2000, Englund and Harms 2003).

The importance of carnivory

In the wide-spread *U. australis*, traps usually represent only about one-third of dry biomass of mature shoot segments as a structural investment in carnivory (i.e., structural cost), but the total trap dark respiration (RD) rate amounted to almost 70 % of the total shoot RD. This high value is most probably due to the high oxygen demand by the metabolically very active internal glands. The net photosynthetic rate of traps of aquatic *Utricularia* (per unit fresh weight), however, reaches only 7–10 % of the values for leaves or shoots (Adamec 2006). This combination means that traps represent, apart from high mineral and structural cost, a very high energetic (metabolic) cost for the plants and therefore the investment into trap biomass is under a purposeful ecological regulation. It has been shown that plants are able to change the proportion of resources invested in traps depending on numerous habitat factors, including water chemistry (often mineral N availability), prey availability, and the level of irradiance (Knight and Frost 1991, Richards 2001, Englund and Harms 2003, Adamec 2008).

Carnivory – the trapping and utilizing of microcrustacean prey – is widely thought to significantly supplement *Utricularia* mineral nutrition. The importance of carnivory for the nutrition of *Utricularia* has, however, been debated, especially in ultraoligotrophic environments, where prey capture rates are negligible (Richards 2001). Despite the virtual absence of prey at these locations, such as the Florida Everglades or the wetlands of the Yucatan peninsula, species growing here make significant investments into traps, which contain large amounts of organic detritus and associated microorganisms.

AIMS

In the light of the results of previous studies, there arose the question of whether *Utricularia* traps might have an additional ecophysiological role, other than carnivory. This thesis focuses on the following topics related to trap function in general:

- The chemical characteristics of the inner trap environment, specifically the trap fluid
- The characteristics of trap microbial communities and their potential role in *Utricularia* nutrition
- The role of trap age and mineral nutrient availability

METHODOLOGY

The *Utricularia* traps represent a rather difficult system to work with, mainly due to their small size, fragility, firing properties, and the small volume of trap fluid found in each trap. In our analyses, we have mostly worked with extracted trap fluid which we obtained by carefully inserting a thin glass capillary, attached to a peristaltic pump, under the trap door to avoid the damage to and contamination from trap tissues (Manuscript I). In this way we have collected a sample pooled from traps of the same age, which was then subjected to various analyses, such as extracellular enzyme and nutrient assessment, epifluorescence microscopy, ion chromatography or mass spectroscopy. Plants from greenhouse and laboratory cultivations as well as those collected in the field were used.

BRIEF SUMMARY OF MAIN RESULTS

Chemical characteristics of the trap fluid

Prior to this work, the chemical composition of the trap internal environment was unknown. The trap lumen contains large absorptive surfaces, comparable in area to those of the leaves. These surfaces are completely sealed from the surrounding water for most of the time. The characterization of the trap fluid is therefore an important step in elucidating *Utricularia* trap function.

pH and extracellular enzyme activities

The results show that pH of the *Utricularia* trap fluid (Paper I and II) is tightly regulated, but dependent on species and trap age. Measured values ranged between 4.9 and 5.4 in most European species, and between 6.8 and 7.9 in tropical *U. foliosa*. In all species, the pH values in traps close to senescence were found to be lower than those found in younger traps.

Traps are thought to be primarily digestive and absorptive organs; one of the first steps was therefore the assessment of the activity of various extracellular enzymes (Paper I, II, IV, and VI). A combination of fluorometric methods and enzyme-labelled fluorescence revealed the presence of alkaline phosphatases, aminopetidases, a-glucosidases, ß-glucosidases, and ß-hexosaminidases.

Alkaline phosphatase always exhibited the highest activity, significantly larger than that of the ambient medium. The activities of the other enzymes were usually lower by one or two orders of magnitude. Furthermore, the trap enzyme activities are apparently independent of prey digestion and the enzyme production is constitutive, i.e., not induced by the presence of prey. More detailed analysis focused on the alkaline phosphatase confirmed that it is of trap origin, as the trap enzymes have a distinct pH optimum from enzymes in the ambient water, and the activity was found on the trap internal glands as well as on commensal microbial cells. The proportion of these two different activities changes with the ageing of traps: the plant-derived activity is more important in young traps and decreases significantly with increasing trap age, i.e., with growing numbers of colonizing microbes.

Concentrations of nutrients in the trap fluid

Utricularia species thrive in nutrient poor environments, it was therefore important to find out what is the concentration of mineral and organic nutrients inside the traps compared to that in the ambient water. The amount of nutrients increases with increasing trap age, and the total amounts of carbon, nitrogen, and phosphorus accumulated within traps during their lifetime are of the order of 100 mg L^{-1} for carbon and nitrogen, and between 0.2 and 0.6 mg L^{-1} for phosphorus (Paper II). A significant part of this nutrient pool is present in the dissolved form. The nutrient concentrations in the trap fluid without any metazoan prey are surprisingly high, even comparable to those commonly found in waste waters. These surfaces are therefore permanently in contact with a medium where the concentrations of potentially utilizable nutrients exceed those in the ambient water by 2–3 orders of magnitude. Once inside, nutrients accumulated in the closed trap are no longer available for *Utricularia* competitors. For a rootless 'planktonic' plant, this undoubtedly is an advantage that 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.

The concentration, composition and origin of trap fluid organic compounds

The large amounts of carbon found in the trap fluid made us consider the potential role played by C exudation in enhancing plant–microbe interactions. A ^{13}C-labelling experiment was conducted (Paper III) in greenhouse conditions to determine the C allocation between plant tissues of increasing age and the trap fluid in two *Utricularia* species. Both species allocated a majority of the newly fixed carbon into the fast growing shoot apex and its allocation rapidly decreased with increasing age of the shoot, constituting only less than 10 % of the total newly fixed carbon in the oldest analyzed segments. In the trap-bearing shoot segments, the ratio of carbon exuded into the trap fluid to that in plant tissues increased markedly with age – in the oldest analyzed segments twice as much newly fixed carbon was allocated into the trap fluid than the plant tissue. Overall, a significant amount of the newly fixed carbon, approximately up to 25 %, was allocated into the trap fluid. This value is comparable to the amount of carbon exuded into the rhizosphere by rooted plants, where it is known to fuel a variety of mutually beneficial plant–microbe interactions (Marschner 1995, Flores et al. 1999). The composition of organic carbon in the trap fluid, its availability for microbial uptake, and the influence of plant nutrient status and trap age on its biodegradability was investigated to assess the possibility of similar interactions taking place in *Utricularia* (Paper VII, Manuscript VIII). Up to 30 % of total dissolved organic carbon in *Utricularia* trap fluid in oligotrophic conditions was determined as easily biodegradable compounds, commonly found in plant root exudates (mainly glucose, fructose and lactate). The proportion of these compounds and their microbial utilization decreased with increasing mineral nutrient supply and trap age.

Trap microbial community

Traps were previously found to support diverse microbial communities, however a more detailed analysis about the structure and development of these communities was lacking. Microscopy and PLFA analyses conducted as part of this thesis (Manuscript II) revealed that trap fluid contains all components of a complex microbial food web, from bacteria, fungi, and algae to bacterivorous protists and nematodes, with bacteria forming more than 58 % of the viable microbial biomass in the trap. Of these, alpha- and beta-Proteobacteria were the dominant group (Paper VII). Microorganisms found inside the trap are most probably derived from the periphyton growing on *Utricularia*, although Archaea and some species of algae (especially desmids and *Euglena* spp.) were only found inside the trap lumen. Trap age and mineral nutrient concentrations in the ambient growth medium seem to be key factors in determining the patterns of microbial community development (Paper II and VII).

A growth analysis was performed under greenhouse conditions on two aquatic, carnivorous plants in order to determine growth effects caused by supplemental feeding on zooplankton (Manuscript V). The two species surveyed were *Aldrovanda vesiculosa* (Droseraceae) with open snapping traps and *Utricularia australis*. The species were found to have markedly different growth responses to the presence of zooplankton prey. For example, while a highly significant increase in shoot biomass (by 60 %) was found in *Aldrovanda* plants supplemented with prey, no significant effect of prey addition occurred in *Utricularia*. The difference was attributed to by the operation of a complex food web in *Utricularia* traps, which possibly substitutes for prey capture in barren waters.

CONCLUSIONS

Utricularia are plants with a terminal position in the phylogeny of the eudicots with some of the smallest angiosperm genomes found (Greilhuber et al. 2006). One possible explanation for this phenomenon lies in selective constraints on a wide range of genomic regions that may have been lowered due to the use of an alternative mode of acquiring nutrients (Müller et al. 2006). This ‘alternative mode’ does not necessarily need to be the capture of live microzooplankton prey, but can also include the exploitation of a close plant–microbe interaction analogous to the interactions in the rhizosphere of rooted plants.

This work has shown, contrary to what is believed about nutrition of carnivorous plants in general, that phosphorus uptake through traps is more important than that of nitrogen for aquatic *Utricularia*. Their traps harbor a complex microbial food web, with microorganisms producing significant amounts of extracellular enzymes to aid in organic matter degradation within the trap lumen. Supplying readily available carbon to the trap microbial community can increase turnover rates of the accumulated organic matter (including N and P) and will help to facilitate fast apical growth of the plants in oligotrophic conditions. Such an arrangement could enable the rootless ‘planktonic’ *Utricularia* to grow and utilize both inorganic and organic nutrients (either dissolved or particulate, i.e. from plankton as well as detritus) in the water column without the need for attachment to a substrate or sediment. Inhabiting this ecological niche provides advantages such as reduced competition with rooted aquatic macrophytes for nutrients and light and also increased diffusivity of available nutrients. This undoubtedly is an advantage that may have contributed to the success of *Utricularia* within the carnivorous plant group and to the ubiquitous distribution of the genus in a wide variety of nutrient-poor environments.

The traps of carnivorous plants have often been successfully used as model systems in ecological studies (e.g. Cochran-Stafira and von Ende 1998; Sota et al. 1998; Mouquet et al. 2008). The traps of aquatic *Utricularia* with the associated microbial communities can be relatively easily manipulated for experimental purposes. We believe they are useful model study systems which can help uncover novel aspects of plant–microbe interactions in relation to plant nutrient acquisition. Due to the small genome size of many species within the genus and with the onset of the high-throughput sequencing methods, they may be especially useful systems for combining genomic and transcriptomic analyses with ecological studies.

REFERENCES

Adamec L, 1997a. Relations between K+ uptake and photosynthetic uptake of inorganic carbon by aquatic plants. Biol. Plant. 39: 599–606.

Adamec L, 1997b. Mineral nutrition of carnivorous plants: A review. Bot. Rev. 63: 273–299.

Adamec L, 2000. Rootless aquatic plant *Aldrovanda vesiculosa*: physiological polarity, mineral nutrition, and importance of carnivory. Biol. Plant. 43: 113–119.

Adamec L., 2006. Respiration and photosynthesis of bladders and leaves of aquatic *Utricularia* species. Plant Biol. 8: 765-769.

Adamec L, 2007. Investment in carnivory in *Utricularia stygia* and U. intermedia with dimorphic shoots. Preslia 79: 127–139.

Adamec L, 2008. Mineral nutrient relations in the aquatic carnivorous plant *Utricularia australis* and its investment in carnivory. Fund. Appl. Limnol. 171: 175–183.

Adamec L. 2009. Photosynthetic CO2 affinity of the aquatic carnivorous plant *Utricularia australis* (Lentibulariaceae) and its investment in carnivory. Ecol. Res. 24: 327–333.

Adamec L. 2011. The comparison of mechanically stimulated and spontaneous firings in traps of aquatic carnivorous Utricularia species, Aquatic Botany 94 (2011) 44–49.

Adamec L, Sirová D, Vrba J, 2010. Contrasting growth effects of prey capture in two carnivorous plant species. Fundam. Appl. Limnol. 176: 153-160.

Adamec L, Vrba J, Sirová D, 2011. Fluorescence tagging of phosphatase and chitinase activity on different structures of *Utricularia* traps. Carniv. Plant Newslett. 40: 68-73.

Cochran-Stafira DL, von Ende CN, 1998. Integrating bacteria into food webs: studies with *Sarracenia purpurea* inquilines. Ecol. 79: 880–898.

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.

Flores HE, Vivanco JM, Loyola-Vargas VM,1999. 'Radicle' biochemistry: the biology of root-specific metabolism. Trends in Plant Science 4: 220–226.

Greilhuber J, Borsch T, Müller K, Worberg A, Porembski S, Barthlott W, 2006. Smallest angiosperm genomes found in Lentibulariaceae, with chromosomes of bacterial size. Plant Biol.: 770–777.

Guisande, C, Granado-Lorencio, C, Andrade-Sossa, C, Duque SR, 2007: Bladderworts. Funct. Plant Sci. Biotechnol. 1: 58–68.

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

Kaminski R, 1987b. Studies on the ecology of *Aldrovanda vesiculosa* L. II. Organic substances, physical and biotic factors and the growth and development of A. vesiculosa. Ekol. Pol. 35: 591–609.

Knight SE, Frost TM, 1991. Bladder control in *Utricularia macrorhiza*: lake-specific variation in plant investment in carnivory. Ecol. 72: 728–734.

Marschner H, 1995. Mineral nutrition of higher plants, 2nd edn. London: Academic Press.

Mouquet N, Daufresne T, Gray SM, Miller TE, 2008. Modelling the relationship between a pitcher plant (*Sarracenia purpurea*) and its phytotelma community: mutualism or parasitism? Funct. Ecol. 22: 728–737.

Plachno BJ., Lukaszek M, Wolowski K, Adamec L, Stolarczyk P, 2012. Aging of *Utricularia* traps and variability of microorganisms associated with that microhabitat. Aquat. Bot. 97: 44-48.

Richards JH, 2001. Bladder function in *Utricularia purpurea* (Lentibulariaceae): is carnivory important? Amer. J. Bot. 88: 170–176.

Sota T, Mogi M, Kato K, 1998. Local and regional-scale food web structure in *Nepenthes alata* pitchers. Biotropica 30: 82–91.

Taylor P, 1989. The Genus *Utricularia*: A Taxonomic Monograph. Kew Bulletin, Additional Series, XIV.

Paper I

Sirová D, Adamec L, Vrba J, 2003. Enzymatic activities in traps of four aquatic species of the carnivorous genus *Utricularia*.
New Phytol. 159: 669–675.

• 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 b-glucosidases (1.35–2.95 μmol l^{-1} h^{-1}), while the activities of a-glucosidases, b-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.

Paper II

Sirová D, Borovec J, Cerná B, Rejmánková E, Adamec L, Vrba J, 2009. Microbial community development in the traps of aquatic *Utricularia* species. Aquat. Bot. 90: 129–136.

We examined trap fluid of three aquatic carnivorous species of *Utricularia* (Lentibulariaceae) to assess the role of microbial community within their traps in plant nutrient acquisition. In the context of increasing trap age, we characterized microbial community composition using phospholipid fatty acid (PLFA) analysis and microscopy. Nutrient content in various fractions of the trap fluid was analyzed and the abundance of free-suspended bacteria estimated. The activities of extracellular phosphatase in the trap fluid were determined using fluorometry and the contribution of the microbial community to phosphatase production assessed by epifluorescence microscopy. The trap microbial community seems to be largely derived from *Utricularia* associated periphyton. PLFA analysis revealed that trap fluid contained all components of a complex microbial food web with bacteria forming more than 58% of the viable microbial biomass in the trap. Trap age seems to be the key factor in determining the patterns of microbial community development as well as enzyme production. The amount of nutrients increases with increasing trap age, and the total amounts of C, N, and P accumulated within traps during their lifetime are relatively large—of the order of 100 mg L^{-1} for C and N, and between 0.2 and 0.6 mg L^{-1} for P. A significant part of the nutrient pool is present in the dissolved form. Trap fluid stoichiometry (molar N:P ratios about 100) as well as the presence of nutrient limited microbial cells (molar N:P ratios 25–61) indicates the importance of phosphorus rather than nitrogen for the nutrition of *Utricularia*. Our findings support the hypothesis that mutualism, apart from the predator–prey interaction, is an important association in aquatic *Utricularia* traps and that the trap-associated microbial community may be of benefit to the rootless aquatic *Utricularia* species facing problems with P acquisition due to the loss of roots in their evolution.

Paper III

Sirová D, Borovec J, Šantrucková H, Šantrucek J, Vrba J, Adamec L, 2010. *Utricularia* carnivory revisited: plants supply photosynthetic carbon to traps. J. Exp. Bot. 61: 99–103.

The rootless, aquatic *Utricularia* species belong to the largest and most cosmopolitan carnivorous plant genus. Populations of *Utricularia* plants are an important component of many standing, nutrient-poor, and humic waters. Carbon (C) allocation is an aspect of *Utricularia*'s ecophysiology that has not been studied previously and there is considerable uncertainty about the functional and ecological benefit of the trap-associated microbial community and the potential role played by C exudation in enhancing plant–microbe interactions. A ^{13}C-labelling experiment was conducted in greenhouse conditions to determine the C allocation between plant tissues of increasing age and trap fluid in two *Utricularia* species. Both species allocated a majority of the newly fixed C into the fast growing shoot apex (46.1±8.6% in *U. vulgaris* and 56.1% in *U. australis)*. Carbon allocation rapidly decreased with increasing age of the shoot, constituting only 8.0±4.0% and 6.7% of the total newly fixed C in the oldest analysed segments in *U. vulgaris* and *U. australis*, respectively. In the trap-bearing shoot segments, the ratio of C exuded into the trap fluid to that in plant tissues increased markedly with age—in the oldest analysed segments twice as much newly fixed C was allocated into the trap fluid than the plant tissue. Overall, a significant amount of the newly fixed C, approximately 25% (*U. vulgaris*) and 20% (*U. australis*), was allocated to the trap fluid. The importance of C exudation for the development of the microbial community associated with the traps as well as for the growth and ecology of aquatic *Utricularia* is discussed.

Paper IV

Adamec L, Sirová D, Vrba J, Rejmánková E, 2010. Enzyme production in the traps of aquatic *Utricularia* species.
Biologia 65: 273–278.

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.

Paper V

Adamec L, Sirová D, Vrba J, 2010. Contrasting growth effects of prey capture in two aquatic carnivorous plant species.
Fundam. Appl. Limnol. 176: 153–160.

A detailed 11-day growth analysis was performed under greenhouse conditions on two aquatic, carnivorous plants in order to determine growth effects caused by supplemental feeding on zooplankton. The two species surveyed were the rare, stenotypic *Aldrovanda vesiculosa* and the more common, eurytopic *Utricularia australis*. While a highly significant increase in shoot biomass (by 60 %) was found for Aldrovanda plants supplemented with prey, no significant effect of prey addition occurred in *Utricularia*. However, main shoots of fed plants of both species were significantly longer, had more mature leaf nodes, and their mean apical growth rate was higher than in unfed plants. Branching was markedly supported by prey in *Aldrovanda* only. Feeding significantly increased the structural investment in carnivory in *Utricularia* but had no effect on *Aldrovanda*, although significantly increasing maximum trap length in both species. Measurements of total nutrient concentrations in fed *Aldrovanda* plants revealed much more total N, P, K, and Mg at the end of the experiment, compared with unfed plants. In *Utricularia*, however, fed plants contained only more N than unfed plants but the opposite was true for the total amount of P, K and Mg. In *Aldrovanda*, a large proportion of N, P, K, and Mg consumption could be covered from prey. The different growth effect of prey addition in the two species could be explained by the operation of a complex food web in *Utricularia* traps, which possibly substitute for prey capture in barren waters.

Paper VI

Adamec L, Vrba J, Sirová D, 2011. Fluorescence tagging of phosphatase and chitinase activity on different structures of *Utricularia* traps. Carniv. Plant Newslett. 40: 68–73.

Technical Refereed Contribution

Paper VII

Sirová D, Borovec J, Picek T, Adamec L, Nedbalová L, Vrba J, 2011. Ecological implications of organic carbon dynamics in the traps of aquatic carnivorous *Utricularia* plants.
Funct. Plant Biol. 38, 583–593.

Rootless aquatic carnivorous *Utricularia* exude up to 25 % of their photosynthates into the trap lumen, which also harbours a complex microbial community thought to play a role in enhancing *Utricularia* nutrient acquisition. We investigated the composition of organic carbon in the trap fluid, its availability for microbial uptake, the influence of plant nutrient status and trap age on its biodegradability, and the composition of prokaryotic assemblages within the traps of three aquatic *Utricularia* species. Using ion chromatography and basal respiration rate measurementsweconfirmed that up to 30 % of total dissolved organic carbon in *Utricularia* trap fluid in oligotrophic conditions was easily biodegradable compounds commonly found in plant root exudates (mainly glucose, fructose and lactate). The proportion of these compounds and their microbial utilisation decreased with increasing mineral nutrient supply and trap age. Fluorescence in situ hybridisation analyses showed that microbial trap assemblages are dominated by alpha and beta Proteobacteria, and that the assemblage composition is affected by changes in the ambient mineral nutrient supply. We suggest that organic carbon dynamics within the traps, involving both the plant and associated microbial assemblages, underlies the acquisition of key nutrients by *Utricularia* and may help explain the evolutionary success of the genus.

Manuscript VIII

Borovec J, Sirová D, Adamec L, 2012. Light as a factor affecting the concentration of simple organics in the traps of aquatic carnivorous *Utricularia* species. Submitted to Fundam. Appl. Limnol.

Rootless aquatic carnivorous *Utricularia* plants exude significant amounts of photosynthates into the trap fluid, where they have been shown to support complex microbial commensal communities. Using ion chromatography, the composition of four groups of easily metabolised, carbon-rich organic compounds (sugars, sugar alcohols, amino acids and organic acids) was investigated in trap fluid collected from three aquatic *Utricularia* species, *U. vulgaris*, *U. reflexa* and *U. stygia*, for different trap ages, irradiance levels during plant growth and for exposure to periods of darkness. The total sum of the concentrations of the four groups of organic compounds in the trap fluid ranged within 14-42 mg l^{-1} in greenhouse-grown *U. vulgaris*, compared to 9.0-14 mg l^{-1} in *U. reflexa*. The concentrations of organic compounds were significantly higher in younger traps than in the older traps of *U. vulgaris* grown at high irradiance. Within the same trap age categories in *U. vulgaris*, the group concentrations of sugars, organic acids, and total sums of analysed compounds were significantly higher in plants growing at high irradiance when compared with those grown in the shade. Dark exposure of cut traps for 1-2 d significantly decreased the concentrations of sugars and organic acids in the fluid. It may be concluded that the concentrations of organic compounds in the trap fluid of aquatic *Utricularia* are species specific, subject to rapid turnover and depend significantly on various endogenous (trap age) or exogenous factors (water chemistry, irradiance).