

NEWS IN ECOPHYSIOLOGICAL RESEARCH ON AQUATIC *UTRICULARIA* TRAPS

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This paper is dedicated to the memory of Dr. Peter Taylor who contributed invaluable to the knowledge of the genus *Utricularia*.

Introduction

Aquatic carnivorous plants comprise the species *Aldrovanda vesiculosa* L. (Droseraceae) and about 50 species of the genus *Utricularia* L. (Lentibulariaceae; Taylor 1989). Aquatic *Utricularia* species usually grow in shallow, standing humic waters which are usually poor in N and P, but occasionally also in K (Adamec 1997; Guisande *et al.* 2007). Although they take up all necessary nutrients either directly from the water by their rootless shoots or from animal prey by traps, under favorable conditions, they exhibit very rapid apical shoot growth of 3-4 leaf nodes d⁻¹, while their shoot bases decay at this same high rate (Friday 1989; Adamec 2009).

The *Utricularia* traps have always fascinated scientists: they are the smallest among those of carnivorous plants but are arguably the most sophisticated and intricate. One composed leaf of aquatic *Utricularia* usually bears dozens to hundreds of oval-shaped, fluid-filled traps of foliar origin (see Figs. 1, 2). As opposed to their terrestrial counterparts having only tiny traps (0.5-1.5 mm), aquatic or amphibious *Utricularia* species have much larger traps which have exclusively been used for physiological research (Taylor 1989; Adamec 2011a). These bladders are typically 1-6 mm long with elastic walls two cell layers thick and have a mobile trap door (see *e.g.*, Juniper *et al.* 1989 or Adamec *et al.* 2011 for detailed structure). 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 and in the resorption of released nutrients, while the smaller bifid glands,



Figure 1: Traps of *U. arnhemica* ca. 2 mm large from a tissue culture. All photos L. Adamec.



Figure 2: Traps of *U. foliosa* are very slimy.

which are located near the door, take part in pumping out the water. The traps capture small aquatic animals, usually 0.5-2 mm long, and these are mostly zooplankton. These basic characteristics of *Utricularia* trap functioning based on several “classic” studies have been known and compiled in a stagnant form for decades. Nevertheless, within the last five years (2007-2011) with the boom in 2011, many novel physiological, biophysical, and ecological works have been published and crucially extended our knowledge of the trap functioning, often changing our basic understanding of these processes. These recent studies based on very advanced methods partly answer the following questions: How do these perfect traps function, then kill and digest their prey? How do they provide ATP energy for these demanding physiological functions? What are the nature of the interactions between traps and the mutualistic microorganisms living inside them as commensals? In this mini-review, all of these questions are addressed from an ecophysiological’s point of view, based on the most recent literature, data, and unpublished results. Inspirations for further research on this fascinating topic are also suggested.

Trap Functioning in “Classic” Studies

Almost all of the knowledge of the biophysical nature of *Utricularia* trap functioning was published in a series of only four famous studies conducted between 1973-1985 (Sydenham & Findlay 1973, 1975; Sasago & Sibaoka 1985a,b). These studies are based on tricky measurements of negative pressures inside the trap and water outflow by piercing the isolated trap with fine glass capillaries (outer tip diameter 20-50 μm). The trap thickness was simultaneously optically or mechanically monitored as a reliable measure of trap water flow and the negative pressure. It is very curious that nobody (except for Singh *et al.* 2011) has successfully repeated this simple capillary method due to water leakage.

The trap is hermetically sealed and a negative pressure of about -16 kPa (*i.e.* 1/7 bar) relative to the ambient water is maintained inside a fully reset trap. When a prey species touches sensory hairs situated on the trap door it opens, the small animal is aspirated into the trap and the door closes again. This process of firing is complete within 10-15 ms and is the most rapid plant movement known (Sydenham & Findlay 1973). Immediately after firing, the negative pressure inside the trap drops to zero, but is soon restored by the rapid removal of *ca.* 40% of the water from the fired trap until the original concave shape is reached. This aspect of trap resetting lasts about 25-30 min and the trap is ready to fire again; the full resetting process lasts much longer. Using very inventive methods, the authors (Sydenham & Findlay 1973, 1975; Sasago & Sibaoka 1985a,b) have also found that water is pumped out of the traps by an active process associated with a consumption of the metabolic energy of ATP. When inhibitors of aerobic respiration were added to the trap fluid, the water pumping and trap narrowing processes were very markedly blocked.

How is water pumped out of the traps? The authors have found that bifid glands attached close to the trap door take part in water pumping and that water is exuded from the pavement epithelium close to the door. Electrophysiological measurements have led to the hypothesis (Sydenham & Findlay 1975; Sasago & Sibaoka 1985a) that Cl^- ions are actively taken up from the trap fluid by the bifid glands and, due to their movement, osmotically drag water molecules. Monovalent cations (Na^+ , K^+) accompany the Cl^- fluxes, while divalent cations (Ca^{2+} , Mg^{2+}) rather inhibit them. The second part of the water pathway is still, however, veiled in mystery as it appears that the water is expelled from the cells of the pavement epithelium by the turgor pressure through a very leaky plasmalemma (for solutes <600 Daltons; Sydenham & Findlay 1975). Traps can also pump out water in moist air or when immersed in a liquid paraffin oil (Sasago & Sibaoka 1985a).

Food Web Inside the Traps

Traps of aquatic *Utricularia* possess one significant ecological trait which is their principal difference from snapping *Aldrovanda* traps. This could offer unexpected ecological possibilities for the plants. In spite of its tiny volume, the trap fluid in *Utricularia* plants is inhabited permanently by various commensal microorganisms – bacteria, cyanobacteria, microfungi, algae, euglens, dinophytes, protozoa (ciliates), and rotifers – which live in a mutualistic interaction with the plant (e.g., Richards 2001; Peroutka *et al.* 2008; Alkhalaf *et al.* 2009; Sirová *et al.* 2009; Płachno *et al.* 2012). These organisms enter the traps from the ambient environment, propagate inside the traps and take part in prey decomposition. Recently, evidence for the formation of a miniature food web has been provided in two Central American *Utricularia* species (Sirová *et al.* 2009). Similar food webs have also been described in digestive fluids of large pitcher-shaped traps of several species and genera of terrestrial carnivorous plants (*Darlingtonia californica*, *Sarracenia purpurea*, *Nepenthes* spp.; Juniper *et al.* 1989). In *Utricularia* traps, due to methodical limitation, it may not be clear which microorganisms found inside the traps are living as commensals and which are prey (Peroutka *et al.* 2008; Alkhalaf *et al.* 2009, 2011; Płachno *et al.* 2012).

Oxygen Regime Inside The Traps and Trap Respiration

Oxygen concentration in the *Utricularia* trap fluid may be considered the crucial characteristic, not only for trap respiration and water pumping but also for life and propagation of the commensal communities and the death of the prey. Using a miniature oxygen sensor, almost zero $[O_2]$ were measured in the trap fluid of mature empty traps of 6 aquatic *Utricularia* species with larger traps (Adamec 2007). The median steady-state $[O_2]$ values were within 0.0-4.7 μM , but usually only within 0.0-1.4 μM (i.e., 0.00-0.04 $\text{mg}\cdot\text{l}^{-1}$ or 0.0-0.5% of O_2 saturation), both in isolated traps and those on intact shoots bathed in stirred, aerated media. These results are independent of irradiance and O_2 oversaturation in shoot tissues. Thus, even in spite of some leakage of oxygenated water into the measured traps and O_2 diffusion from the trap wall intercellulars, the internal trap structures are able to consume the O_2 rapidly to cause anoxia. After the minisensor tip had been inserted into the trap lumen, the trap fluid $[O_2]$ declined linearly almost to zero within 10-100 min. The linear rate of the decline is a measure of the respiration rates of internal trap structures and commensal organisms offset by O_2 influx from the trap walls. It may be inferred that under natural growth conditions, long periods (hours to 20 h) of anoxia inside the traps are interrupted by short periods (20-100 min) of higher $[O_2]$ after stimulated or spontaneous firings. Therefore, captured organisms either die of O_2 deprivation and are prey, or are able to tolerate anoxia and are commensals. *Utricularia* traps likely kill their prey by suffocation. Prey digestion inside the traps did not increase the “external” dark respiration rate (R_D) of traps (Adamec 2006). Though photosynthesis in *Utricularia* shoots (i.e., O_2 oversaturation in the shoot tissues) leads to a much greater O_2 influx to the trap lumen than from a stirred, aerated ambient medium in darkness, the steady-state $[O_2]$ in the trap fluid is zero in both cases (Adamec 2007). In conclusion, the anoxic trap fluid of intact traps is well isolated from the oxygenated ambient water from the trap walls such that O_2 from the ambient water is not transferred inside the traps. As opposed to these isolated traps, open pitchers of *Sarracenia purpurea* exhibit only a reduced $[O_2]$ in their viscous fluid (Wakefield *et al.* 2005) but their prey dies in the same way of suffocation.

The discovery of internal trap anoxia raises some important ecophysiological questions. Due to

their very demanding functions, *Utricularia* traps are metabolically very active and their R_D per unit fresh or dry weight is 2-3 times greater than that of leaves or shoots bearing these traps (Adamec 2006). Moreover, as found in the four classic studies, pumping the water out of the traps strictly requires the participation of aerobic respiration inside the traps. It can be assumed that it is the high R_D of internal bifid and quadrifid glands and pavement epithelium that are responsible for the high trap R_D due to the presence of abundant mitochondria and transfer cells in all these structures (Sasago & Sibaoka 1985a; Plachno & Jankun 2004). From a biochemical point of view, however, it is unclear how the internal trap glands provide ATP energy to maintain their demanding physiological functions (water pumping) under (almost) anoxic conditions for many hours or even permanently (Adamec 2011b,c; Vincent *et al.* 2011). Based on molecular findings, a link between faster reaction kinetics of *Utricularia* traps and a mutation occurring in the mitochondrial respiratory chain enzyme cytochrome *c* oxidase has been suggested (Jobson *et al.* 2004). Within Lentibulariaceae, this mutation has only been detected in *Utricularia*, but not in the sister genera *Pinguicula* and *Genlisea* with immobile traps. The authors (Laakkonen *et al.* 2006) further hypothesize the decoupling of mitochondrial proton pumping from electron transfer, which could be a rich source of ATP energy after trap firing during the aerobic period. Such decoupling would allow the traps to optimize power output during times of need, although with a 20% decrease in total energy efficiency of the respiratory chain. For some important reasons, it seems improbable that the traps provide most of their ATP energy need from anaerobic fermentation. On the contrary, it is highly probable that the inner trap structures possess an extremely high O_2 affinity (well below 0.5-1 μM , which is beyond the resolution of the Clark-type O_2 sensor), using the permanent O_2 influx from the trap wall intercellulars. This suggestion has recently been supported by the finding of very high O_2 affinity of respiratory enzymes in terrestrial plant leaves (0.3-1.1 μM ; Laisk *et al.* 2007). The above mentioned mutation in the cytochrome *c* oxidase could account for such a high O_2 affinity. Finally, transcriptomic global gene expression analysis in *U. gibba* has recently confirmed that traps significantly over-express genes involved in respiration (Ibarra-Laclette *et al.* 2011).

It seems the extremely low $[O_2]$ in the trap fluid is thus a result of a functional compromise: it must be very low to reliably kill the captured prey (requiring *ca.* >15-30 μM) but higher than a threshold for an effective aerobic R_D (>*ca.* 0.4 μM). Following from direct gasometric measurements, even abundant microbial commensal communities in old *Utricularia* traps do not affect crucially the O_2 regime inside the traps: their R_D was between *ca.* 5-50% of the total "internal" trap R_D and their net photosynthetic rate in light did not extend the R_D of the inner trap structures, either (Adamec, unpubl.).

Regulation of the Investment in Carnivory

In 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; Adamec 2008) but the total trap R_D amounted to 67% of the total shoot R_D (Adamec 2006). If the net photosynthetic rate of traps of aquatic *Utricularia* (per unit fresh weight) reached only 7-10% of the values for leaves or shoots even under optimum conditions (Adamec 2006), then this combination means that traps represent a very high energetic (metabolic) cost for the plants. Moreover, as found recently (Adamec 2008, 2010), the traps also represent a high mineral cost, especially for N, P, and K, and can contain 12-69% of the total plant amount of these nutrients. Therefore, the proportion of trap biomass to the total plant biomass is under a purposeful ecological regulation. The regulation includes two steps: the supreme step is high $[CO_2]$ as a prerequisite for high photosynthetic rate (posi-

tive feed-back), while the second step is tissue N or P content in young shoot segments as a negative feed-back. When [CO₂] is short (e.g., in oligotrophic sand-pits) the mineral regulation is inefficient (Adamec 2008, 2009). However, in different species, the regulation depends on different nutrients: in *U. australis*, it is only N (Adamec 2008; Sirová *et al.* 2011), but only P in *U. vulgaris* (Kibriya & Jones 2007), while both N and P in *U. foliosa* (Bern 1997).

Production of Digestive Enzymes

Little is still known about prey digestion in *Utricularia* traps though standard biochemical techniques revealed the presence of proteases in the trap fluid as early as the 1920s (Juniper *et al.* 1989). Later, protease, (acid) phosphatase, and esterase were localised cytochemically in the digestive quadrifid glands (Heslop-Harrison 1975; Vintéjoux & Shoar-Ghafari 2005) with phosphatase also on their surfaces (Sirová *et al.* 2003; Plachno *et al.* 2006). Activities of 5 hydrolases (phosphatase, aminopeptidase, β -hexosaminidase /chitinase/, α - and β -glukosidase) were measured microfluorimetrically directly in the filtered trap fluid collected from both empty traps of four aquatic *Utricularia* species and their culture water (Sirová *et al.* 2003). Phosphatase invariably 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 in the trap fluid were usually lower than in the ambient culture water, the enzymes could have entered the trap from the ambient water. However, the trap fluid phosphatase exhibited its highest activity at a pH between 4.7-5.5, while that in the ambient water occurred at a higher pH of 9.0. It has been confirmed recently for some *Utricularia* species that traps produce the phosphatase continuously and independently of prey capture (Sirová *et al.* 2003), loading with N or P salts, or enrichment of the culture water in mineral N and P (Adamec *et al.* 2010). As follows from both latter papers, trap age is the key factor in the patterns of phosphatase production. It is possible that old, still functional traps shift their function from enzyme production towards nutrient uptake (Sirová *et al.* 2009). Trap fluid pH in most of the examined species was usually within a narrow range from 4.8 to 5.1 and seemed to be regulated by the traps (Sirová *et al.* 2003, 2009; Adamec *et al.* 2010).

One surprising aspect of these enzyme studies should be pointed out. In all species studied (excepting *U. foliosa*) and under all experimental conditions, very low or even zero activity of aminopeptidase (*i.e.*, protease) was found in the fluid in traps with or without prey (Sirová *et al.* 2003, 2009; Adamec *et al.* 2010). However, on the basis of a very efficient total N uptake from prey in *U. vulgaris* traps (Friday & Quarmby 1994), it is evident that proteinaceous N as the main N source from prey must also be effectively digested and absorbed. Moreover, the aminopeptidase found inside the traps had its optimum pH between 7.0 to 9.0, but zero activity at pH 4.7 and much resembled that found in the ambient water; the activities in both environments were also very similar. Thus, a great deal of the very low aminopeptidase activity enters the traps from the ambient water. The absence of aminopeptidases in the fluid could be compensated for by the autolysis of dead prey tissues. Yet the discrepancy between the findings of very low or zero protease activity in the trap fluid with or without prey, and in the presence of large secretory vesicles (Golgi apparatus) rich in proteases in quadrifid glands (Heslop-Harrison 1975; Vintéjoux & Shoar-Ghafari 2005) still cannot be explained. As proteases commonly have a very diverse substrate specificity, it is possible to suspect the used microfluorimetric method involves only a minor part of all present proteases in the fluid.

Similarly, the same discrepancy exists between the invariably high phosphatase activity found in the trap fluid and a very low activity of enzyme labeled fluorescence (ELF) of phosphatase usually detected on the surface of quadrifid glands (Sirová *et al.* 2003; Adamec *et al.* 2011). At least a part of this

discrepancy might be explained by methodical limitation of the ELF method (*cf.* Plachno *et al.* 2006). Moreover, it is not clear which proportion of any enzyme activity in the filtered trap fluid is produced by trap glands alone or various trap commensals, in addition to an unknown part of the activity gained from the ambient water. Evidence has recently been provided using the ELF method that some commensals in the trap fluid (algae, bacteria) exhibit a considerable phosphatase activity on their surface (Plachno *et al.* 2006; Sirová *et al.* 2009). Consistently high trap fluid activities of phosphatases in all species imply that P uptake from prey or detritus might be more important than that of N for the plant.

The Importance of Commensals in *Utricularia* Traps

As mentioned above, an abundant community of commensal microorganisms occurs inside aquatic *Utricularia* traps and is denser in older traps. Due to green euglens or other algae, they are greenish. Though the first data on species composition of the community are over 130 years old their importance for *Utricularia* ecophysiology is still quite unclear. One must emphasise that, in spite of their undeniable perfect functional features, aquatic *Utricularia* traps capture relatively little animal prey in barren, nutrient-poor waters though a high abundance of commensal organisms also occur in empty traps (Richards 2001; Peroutka *et al.* 2008; Adamec 2008, 2009; Sirová *et al.* 2009; Plachno *et al.* 2012). Starting with the novel study by Richards (2001) it has therefore been assumed in these studies that a mutualistic interaction between the plant and trap commensal community prevails over catching animal prey for mineral nutrient uptake in these barren waters.

Trap commensal microorganisms are not strictly specialised to living inside the traps. They can live either as periphyton on the external plant surface or freely as plankton in the ambient water and may be considered generalists (Peroutka *et al.* 2008; Alkhalaf *et al.* 2009, 2011; Sirová *et al.* 2009). All of these studies have also suggested a considerable potential importance of phytoplankton for mineral nutrition of (European) aquatic *Utricularia* species. A high proportion of traps contained the same planktonic algae as in the ambient water but about 90% of them were dead and thus served as prey (Peroutka *et al.* 2008). They entered the traps due to an incidental firing. That is why the authors have named their paper appropriately: “*Utricularia* – a vegetarian carnivorous plant?” In the study from northeastern Germany, 60% of all animals found inside traps were ciliates (Alkhalaf *et al.* 2009; *cf.* Plachno *et al.* 2012). In species with dimorphic shoots (*U. intermedia*, *U. floridana*), the carnivorous shoots of which grow down to a loose peaty substrate, an incidental aspiration of brown detritus (rich in humic acids) could also be of a similar nutritional importance (see Fig. 3).

A great accumulation of nutrients inside the traps without prey has been proven. In *U. foliosa* and *U. purpurea* growing at an oligotrophic site in Belize, surprisingly high concentrations of both organic and mineral dissolved substances were found in the filtered trap fluid in all trap age categories (in mg.l^{-1}): C, 64-307; N, 7-25; P, 0.2-0.6 (Sirová *et al.* 2009). Nevertheless, the total content of both C, N, and P in the trap fluid, including mainly the particulate form (*i.e.*, the commensal organisms and detritus), was several times greater (in mg.l^{-1}): C, 632-1570; N, 21-81; P, 0.9-4.2. The total nutrient content usually correlated with the increasing trap age. On the basis of phospholipid fatty acid analysis of the trap commensal biomass, the occurrence of a complex microbial food web in the trap fluid was revealed, with bacteria forming >75% of the viable microbial biomass. The authors thus assumed that trap commensals could play a role in N and P uptake by the traps in barren waters without prey.

In a two-day experiment on *U. australis* and *U. vulgaris* using ^{13}C , a great proportion of newly fixed CO_2 was allocated from shoot bases not only to shoot apices, but also to mature shoot segments (Sirová *et al.* 2010). Total carbon allocation in plant tissues rapidly decreased with increasing age of the shoot segments but the ratio of C exuded into the trap fluid to that in plant tissues

increased markedly with age – twice as much newly fixed C was allocated into the trap fluid than the plant tissue in the oldest analyzed segments. Overall, 20-25% of the newly fixed C was allocated into the trap fluid. Sirová *et al.* (2011) have recently proven that C exudates fuel respiration of the prevalingly heterotrophic microbial commensal community within the empty traps of three aquatic *Utricularia* species. Up to 30% of the total dissolved organic C analyzed in the trap fluid were easily metabolized compounds (mainly glucose, fructose, sucrose, and lactate) and the proportion of exuded compounds, as well as their microbial utilization, decreased with increasing mineral nutrient supply (N, P) and trap age. The total concentration of 46 analyzed organic compounds in the trap fluid ranged between *ca.* 30-150 mg.l⁻¹ (*i.e.*, 1.2-8.7 mM of organic C); comparable values between 9-78 mg.l⁻¹ were found in another study in three *Utricularia* species (Borovec *et al.*, unpubl.). Moreover, shaded plants exhibited lower concentrations of organics in their trap fluid. This extensive C supply to the trap fluid is undoubtedly an important additional maintenance (energetic) cost of traps. It is thus possible to conclude that the dominant part of C in the commensal organisms is provided by the plant (Sirová *et al.* 2009, 2010, 2011). Yet, on the basis of record high photosynthetic rate of photosynthetic *Utricularia* shoots (Adamec 2006) the plants can afford such “gardening” despite their very rapid growth. Beneficially, the plant could gain growth-limiting N and P from phytoplankton and detritus decomposed in the fluid of traps without any animal prey. Nevertheless, this N and P input from the ambient medium to the traps has never been experimentally quantified (but see below). It can be concluded that the concentration of organic compounds in the trap fluid is partly species specific and depends on cultivation conditions (irradiance) and water chemistry. High concentration of organic acids in the fluid could determine the low pH values of about 5 (Sirová *et al.* 2003, 2009; Adamec *et al.* 2010) indicating also a high buffering capacity.

Rapid Trap Movement, Spontaneous Firings, and Permanent Water Flow

In 2011, several papers were published which crucially changed and specified our understanding of the biophysical processes associated with *Utricularia* trap firing. The unique use of a high-speed camera has revealed that the essence of the reversible trap door opening and closing is the rapid curvature inversion called buckling and unbuckling (Joyeux *et al.* 2011; Singh *et al.* 2011, Vincent *et al.* 2011a,b; Vincent & Marmottant 2011). As a result of the negative pressure inside the trap, the curved trap door is metastable and any tiny mechanical perturbation (mediated by prey through the trigger hairs) triggers the curvature inversion (buckling) leading to trap opening and inflation. When the negative pressure is gone, the trap door spontaneously returns to its initial curvature, closing the door again. Thus, the negative pressure inside the traps is an essential component part of the opening and closing mechanism. Moreover, the complete process of trap firing was specified to last only 2-5 ms in several species.

New important challenges for *Utricularia* trap ecophysiology have very recently arisen from the discovery of spontaneous firing in *Utricularia* traps. Using both a high-speed camera for intact shoots (Vincent *et al.* 2011a,b) and a linear position sensor for excised traps (Adamec 2011b,c), a more or less regular trap firing without any mechanical stimulation was confirmed in several aquatic *Utricularia* species and in two trap age categories. Spontaneous firings occurred 0.3-2.4 times during the 1-day resting period and the mean time between two spontaneous firings varied between 5-16 h. Quantitatively (trap thickness increase due to firing, resetting rate), spontaneous firings resembled mechanically stimulated ones (Adamec 2011b). In two *Utricularia* species, Vincent *et al.* (2011b) subdivided the traps after the time patterns of spontaneous firings into metronomic, random and bursting. It is possible that a spontaneous firing occurs as a “safety valve” protecting the integrity of the trap door. It was also found that the traps pumped water out after firing for at least 5-10 h until a steady-state was attained (Adamec 2011b).



Figure 3: Traps of *U. intermedia* are born only on carnivorous shoots. Some traps are full of dark peaty detritus.



Figure 4: Traps of African *U. reflexa* (from Botswana) can be to 6.5 mm large in an indoor culture and are conveniently used for experiments.

As opposed to Sydenham & Findlay (1973) who postulated a concept of an internal negative pressure sensor regulating water pumping in the trap, the most recent data (Adamec 2011c) suggest a permanent pumping out of water from the traps. These data are based mainly on the fact that no lag-period in water pumping from the trap was detected within the first 2 s after trap firing, which occurred after a 3-h resetting period. This can mean that either the water permanently recirculates through some leaks under the trap door or the mechanism of water pumping becomes thermodynamically inefficient at high negative pressure though it runs permanently.

Taxonomic aspects and basic functional characteristics of trap firing (magnitude of firing and resetting rate as “trap efficiency”) were compared in 13 aquatic *Utricularia* species of the mean trap length between 2.0 to 5.3 mm (Adamec 2011c). On the absolute scale, the 13 species differed considerably in their firing and resetting rates. Significant interspecific differences were also found in the magnitude of firing (in total 3.7-4.2 times) and resetting rates (10-24 times) per unit trap thickness or length. Overall, traps of *U. australis*, *U. stellaris*, and *U. inflata* showed the greatest firing and resetting rates. The relative magnitude of firing per unit trap thickness or length showed a highly significant negative correlation with both trap thickness and length and the same also held for the relative resetting rates. Smaller and narrower traps are thus relatively more effective at trap firing and resetting than larger traps. Neither firing nor resetting characteristics were significantly different between unfed and prey-fed traps of *U. reflexa* (see Fig. 4) and this was also true for the occurrence of spontaneous firings.

Ecological Consequences of Water Flow

Obviously, the most recent findings on trap operation – spontaneous firing and water recirculation – can better explain how growth-limiting N and P enter the traps from the ambient medium and become a substrate for the microbial food web. It is therefore evident that spontaneous firing of each trap, which occurs on average several times a day, could lead to a substantial gain of N and P for the

traps. During its three-week life-span, each trap can aspirate around 15 times its own volume in surrounding water (40% trap volume \times 2-daily spontaneous firings \times 20-day life-span). If the trap commensals help to decompose this “non-animal” prey, it could theoretically imply a significant N and P gain for the plant, especially for those plants in barren waters which trap few animals. The literature assumed that this N and P gain by empty traps could be ecologically important but no quantification has been given so far (Richards 2001; Peroutka *et al.* 2008; Alkhalaf *et al.* 2009, 2011; Sirová *et al.* 2009, 2010; Adamec 2011b). When, however, one considers the published data on the mean total N and P content (both dissolved and particulate, see above) within a middle-aged trap of *U. purpurea* and *U. foliosa* (Sirová *et al.* 2009) and the mean total N and P content at natural oligo- and mesotrophic sites of *U. australis* in the Czech Republic (Adamec 2008), a simple budget model of N and P gain can be made. When water recirculation through the traps is accounted for then, surprisingly, only a very slow accumulation of total N and P within empty traps can occur (see Adamec 2011c for all details). [Model data: trap volume 5 μ l, water recirculation rate 1 μ l.h⁻¹ for 20 h a day, only NH₄-N and PO₄-P influx from the ambient water, mean natural concentrations of NH₄-N and PO₄-P at the sites; Sasago & Sibaoka 1985a,b; Adamec 2008, 2011c]. Simply, it would take between 40-70 days for an empty trap to accumulate the measured N or between 15-23 days for them to accumulate the P if there were only spontaneous firing and water recirculation mechanisms occurring. By the application of the model data for the relationship between trap volume and biomass and for shoot N and P content in *U. australis*, one can further estimate that the total N and P amount inside the traps represents only about 3.5% of total plant N and 1.2% of P (Adamec 2011c).

Several Conclusions Can Be Drawn From These Model Data.

1. The calculated N and P input rates from the ambient water due to both spontaneous firings and hypothetical water recirculation are so low that each trap without prey can only reach these nutrient levels after a long time, which is comparable with or even longer than the trap life-span. Thus, such a low N and P gain cannot be ecologically important for the plant mineral nutrition at all but the rapid turnover of N and P inside the traps can be.
2. Since even young *Utricularia* traps (*ca.* 1 week old) contained a relatively high total N and P content comparable with that in the older traps (Sirová *et al.* 2009), the traps could take up neither N nor P from the trap fluid for the plant itself as the total N and P content in the trap fluid would have been much lower.
3. To account for all of these findings, it is evident that the traps exude not only organic C into the fluid (Sirová *et al.* 2010), but also N and P to enhance the microbial community. This idea can be supported by the finding of a growth reduction of terrestrial *U. uliginosa* after addition of euglens to peaty soil (Jobson *et al.* 2000). It is possible that inoculation of young traps by microorganisms stimulates the traps to exude both organic C and also N and P. Therefore, it seems that *Utricularia* traps without animal prey have no nutritional benefit from the trap commensal community and that the trap microorganisms behave rather as parasites than commensals. The extent of this N and P exudation to the trap fluid can only be a few % of the total plant N and P amount.
4. *Utricularia* species commonly grow in very oligotrophic, barren waters with very low prey availability (Friday 1989; Adamec 1997, 2008, 2009; Richards 2001; Guisande *et al.* 2007; Peroutka *et al.* 2008; Sirová *et al.* 2009). It can be inferred that the N and P uptake affinity of *Utricularia* shoots is very high (the limits for uptake should be below 1 μ M for NH₄⁺ and 0.1 μ M for phosphate), while that for N- and P-containing substances in the traps without prey can be very low or zero (*cf.* Sirová *et al.* 2009).

5. If the trap microorganisms are beneficial for the plant, the ecological benefit can occur only in traps with captured prey to facilitate prey digestion. The trap microorganisms in traps represent additional ecological costs for trap maintenance. It may be hypothesized that plants keep up their trap commensal community (“gardening”) by exudation of organic and mineral nutrients into all traps, as an offset for better prey digestion only in the traps with captured animal prey and, thus, for greater uptake of mineral nutrients from prey. This should lead to a final ecological nutritional benefit for the plant. The real cost:benefit ratio depends on the proportion of traps which capture animal prey during their life.
6. Aquatic *Utricularia* species invest more in trap production at low tissue N and/or P content in shoots (Bern 1997; Kibriya & Jones 2007; Adamec 2008; Sirová *et al.* 2011). As the greater proportion of trap biomass usually leads to increasing the total prey capture, ecological benefit associated with the greater proportion of trap biomass should obviously prevail over the cost associated with the structure and maintenance of the traps, including the support of the commensal community.
7. If capturing animal prey is crucially important for the nutritional benefit of carnivory in *Utricularia*, the strategy of prey capturing could “drive” the evolution of traps in aquatic *Utricularia* species, while the interaction with the trap commensal organisms could be more marginal.

Concluding Remarks: Inspiration For Further Research

It is undeniable that the newest data on the water flow in the traps (trap door buckling, spontaneous firings, and water recirculation) have markedly changed the classic view of trap function. It follows from this review, that it is necessary to reject the commonly accepted view that there is a great nutritional importance on the trap commensal community for the plant in barren waters with low prey availability (*e.g.* Richards 2001). The microbial trap community in traps without animal prey may now be considered as parasites rather than commensals. However, this revised view on the role of trap microorganisms in *Utricularia* traps does not contradict the commonly accepted role of these microorganisms in facilitating prey digestion as commensals but only clearly excludes a nutritional benefit for the plant from their living in traps without prey.

To obtain further insight into the ecophysiology of trap functioning in (aquatic) *Utricularia* species, the following directions of research could be considered and the questions raised could be answered.

1. Electrophysiological studies should resolve the crucial question as to whether *Utricularia* traps are stimulated to fire via the conventional electrophysiological signalling pathway (including a rise in action potential in the sensory hairs) or purely mechanically (*cf.* Sydenham & Findlay 1973; Vincent *et al.* 2011a).
2. As the dominant part of ecophysiological research on *Utricularia* traps has been conducted on aquatic species (they have much larger traps) do the same processes run also in the terrestrial species (*i.e.*, water flows, secretion of enzymes, respiration characteristics, interaction with the commensals, *etc.*)?
3. The discrepancy between very low protease activity found in the trap fluid and high activity found inside secretory vesicles in the quadrifid glands should be elucidated. Moreover, what is the role of commensal microorganisms in prey digestion for different enzyme classes? What is the uptake efficiency of the main mineral nutrients (N, P, K) from prey in the traps?
4. As opposed to the almost zero [O₂] in the trap fluid, the internal trap glands can provide sufficient ATP energy for their demanding functions. Which adaptation allows these glands to provide sufficient ATP energy?

5. The biochemical mechanism of water pumping out of the traps has been insufficiently studied. Can this mechanism be determined using modern experimental approaches (e.g., patch-clamp, vibration probe, ion-sensitive microelectrodes)?
6. Biophysical aspects of trap firing and resetting should be studied in association with the measurement or setting of negative pressure inside the traps as a possible regulatory factor for water pumping. The study should verify the recent concept of continuous water pumping from the traps and water recirculation.
7. Is the pattern of the spontaneous firing associated rather with the mechanical properties of the trap walls or metabolic characteristics of traps?
8. The importance of phytoplankton and detritus as a potential nutrient (N, P, K) source for *Utricularia* in barren waters could be elucidated on the basis of estimation of the matter in the trap fluid and modelling.
9. What is the role of the commensal community in the nutrient interactions within the plant having traps with or without prey? Obviously, the use of sterile plants and their inoculation with commensal microorganisms could be one of the promising approaches.

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