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# Functional characteristics of traps of aquatic carnivorous *Utricularia* species

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ABSTRACT

Firing and resetting of traps in aquatic *Utricularia* species are associated with water flow and trap volume changes. In this study, trap thickness was used as a measure of water flow and was monitored automatically using an electronic position sensor. The basic characteristics of mechanically stimulated firing and resetting were measured in isolated traps from 13 aquatic *Utricularia* species and in two trap size categories in *Utricularia reflexa*. This allowed to study the relationship between these trap functions and trap thickness and length (criteria of trap size). Additionally, the characteristics of spontaneous firings (without any mechanical stimulation) were compared for *U. reflexa* traps fed or denied prey during a 1-day period. On the absolute scale, the 13 *Utricularia* 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 *Utricularia australis*, *Utricularia stellaris* and *Utricularia 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* and this was also true for the occurrence of spontaneous firings. A strict linear resetting rate, without any lag-period, was found during the first 3 min after trap firing in *U. reflexa*. This suggests that water is pumped out of the trap continuously and probably recirculates. Given the concepts of spontaneous firing and water recirculation, an ecological model based on the literature data has been devised to quantify the daily N and P gain from the ambient water by *Utricularia* traps devoid of animal prey. The model shows that the total N and P content estimated in the trap fluid is too high to be accumulated from only the ambient water. This implies that the prey-free traps do not take up N or P from the trap fluid but rather exude a quantity of these nutrients to the fluid to support the microbial community. Therefore, the trap microorganisms behave more as parasites than commensals and represent an additional ecological cost for trap maintenance.



## 1. Introduction

*Utricularia* L. (bladderwort, Lentibulariaceae) is the largest of the carnivorous plant genera, holding about 220 species. This rootless genus contains around 50 species of aquatic or amphibious plants which usually grow in standing, nutrient-poor humic waters (Taylor, 1989; Guisande et al., 2007). Aquatic *Utricularia* species exhibit all the principal features of the carnivorous syndrome and meet all of the demands required to be considered carnivorous (Juniper et al., 1989; Adamec, 1997a; Rice, 2011). The plants supplement photoautotrophic nutrition by trapping and utilizing aquatic prey such as small crustaceans, mites, nematodes, rotifers and protozoa (*e.g.*, Richards, 2001; Gordon and Pacheco, 2007). The discoid suction traps are of foliar origin and are hollow, water-filled bladders, typically 1–5 mm long with a wall thickness of two cells. They contain a variety of glands and trichomes on both the inner and outer surfaces and the function of these is still partially unresolved (Sydenham and Findlay, 1975; Sasago and Sibaoka, 1985a; Juniper et al., 1989). When fully prepared for firing, a negative pressure of ~−16 kPa relative to the ambient medium is maintained within the trap (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985a). Trigger hairs are situated on the external side of the trap door and when these are touched by a prey species the door opens, the animal is aspirated into the trap and the door closes again forming a watertight seal. Using an electrophysiological method, Sydenham and Findlay (1973) estimated the duration of this complete process to be within 10–15 ms. However, using a recent high-speed video imaging technique, Vincent et al. (in press) reported the duration of the whole firing process to be around only 2–5 ms in three aquatic *Utricularia* species. Immediately after firing, the pressure within

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the trap is equalized with the ambient medium, but the negative pressure is soon restored by the rapid removal of *ca.* 40% of the water from the trap lumen until the original compressed shape is reached. This initial process usually lasts only about 25–30 min and the trap is then ready to fire again (Sydenham and Findlay, 1973, 1975; Sasago and Sibaoka, 1985a). The complete process of water pumping (resetting), however, lasts at least 6–10 h (Adamec, 2011).

During studies of water pumping from *Utricularia* traps (Sydenham and Findlay, 1973, 1975; Sasago and Sibaoka, 1985a,b), the authors measured trap thickness (i.e., the distance between the two parallel walls) and also quantified trap water pumping and pressure changes. The trap thickness, as an easily and accurately measured parameter, correlated closely with the magnitude of the negative pressure during trap resetting. The negative pressure within the traps approached its minimum, stable value just 25–30 min after firing, while the thickness decreased exponentially and water removal continued for several hours (Sydenham and Findlay, 1973, 1975; Sasago and Sibaoka, 1985a). At any given time, trap thickness is therefore a better measure of the quantity of water pumped out of the trap than the internal pressure. As the negative pressure inside the traps might determine the extent of water pumping, Sasago and Sibaoka (1985a) suggested the concept of a physiological negative pressure sensor in the traps.

In their natural environment, traps can be fired by any mechanical stimulus (e.g., wind, fish, water movement). Nevertheless, automatic monitoring of the trap thickness using a position sensor, as a reliable method of measuring water flow, has recently confirmed that isolated traps in aquatic *Utricularia* also fire spontaneously i.e. without any mechanical stimulation (Adamec, 2011). There was no quantitative difference between spontaneous and mechanically stimulated firings. The mean time between two spontaneous firings was between 5 h and 15 h in three *Utricularia* species but these firings were highly irregular. Spontaneous firing was also demonstrated in traps on intact *Utricularia* shoots using a high-speed camera (Marmottant et al., 2009; Vincent et al., 2011, in press). This camera has also enabled the trap door motion during firing to be described, with the authors observing a transient buckling/unbuckling of the trap door associated with a convex/concave door inversion (Vincent et al., 2011, in press; Joeyux et al., 2011). Using a mechanical model of a dynamic trap simulation (including trap geometry and Young's elasticity modulus of the trap door), the authors calculated that the negative trap pressure critical for trap-door buckling was 15.5 kPa. This is in perfect agreement with the measured value of the negative pressure in the traps (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985a). When the pressure difference is slightly lower than this critical value, the trap is stable but the energy barrier to trigger the buckling is low. This makes the trap door extremely sensitive to mechanical stimulation. It is, however, still not clear if the trap firing is triggered by an electrophysiological signal arising from the trigger hairs, like in Droseraceae, or purely mechanically (see Sydenham and Findlay, 1973; Juniper et al., 1989; Vincent et al., 2011, in press).

Aquatic *Utricularia* traps are usually inhabited by diverse communities of microorganisms as commensals i.e. bacteria, algae, protists and rotifers (e.g., Mette et al., 2000; Richards, 2001; Sirová et al., 2009). In Belizean *Utricularia* species, Sirová et al. (2009) found high amounts of organic C and mineral N and P in the filtered fluid as well as the particulate fraction collected from traps free from macroscopic prey. Phospholipid fatty acid analysis of the community suggested the existence of a microbial food web inside the traps. A prolific occurrence of phytoplanktonic algae inside *Utricularia* traps, especially in nutrient-poor waters, has also been confirmed (Peroutka et al., 2008; Alkhalaf et al., 2009). Most algal cells found in the trap fluid were aspirated in from the ambient water and most of these microscopic organisms likely die of anoxia in the trap fluid (Adamec, 2007a). These then decompose and may serve as a nutrient source for *Utricularia*. Richards (2001) suggested that these commensal–trap interactions in *Utricularia* plants growing in nutrient-poor waters with low prey availability may be of greater nutritional importance to the plants than prey capture alone. The potential nutritional benefit of the commensal community for the plant could consist of facilitated digestion of animal prey or phytoplanktonic algae and/or detritus (Richards, 2001; Peroutka et al., 2008; Alkhalaf et al., 2009; Sirová et al., 2009; Adamec, 2011). This concept of "vegetarian" nutrition in nutrient-poor waters has yet a major question to answer: how does a sufficient quantity of N and P in the form of microrganisms and/or detritus enter the traps from the ambient medium? This could be either due to incidental, mechanical irritation of the trigger hairs or to spontaneous firings but no budget of such a N and P gain has been estimated so far.

The aim of this study was to automatically monitor trap thickness as a measure of water flow in 13 aquatic *Utricularia* species, the traps of which differed greatly in their size and the species in their world spread, ecology or shoot morphology. As in our previous study (Adamec, 2011), an electronic position sensor was used to describe the basic comparative characteristics of trap firing and resetting which were evaluated by regression analyses. The basic characteristics of spontaneous firings of *Utricularia reflexa* traps either fed or denied prey over a period of 24 h were also compared. As the results of our previous study (Adamec, 2011) suggested that traps may pump out water continuously, leading to a futile water recirculation within reset traps, a detailed resetting time-course was measured over the first few minutes following trap firing to determine if there was a lag-period in water pumping. The trap water pumping is very demanding on ATP energy (Sasago and Sibaoka, 1985b; Adamec, 2006, 2007a). For isolated traps, aerobic dark respiration rates ($R_D$) of both intact and partly sliced traps were compared over 24 h to determine if they have sufficient energy to cover the continuous water pumping. Given the concepts of spontaneous firing and water recirculation, an ecological model based on literature data has been devised to quantify the daily N and P gain from the ambient water by *Utricularia* traps without animal prey. This was used to establish if the trap microorganisms behave more as commensals or parasites.

## 2. Materials and methods

### 2.1. Plant material

Adult plants of *U. australis* R.Br., *U. vulgaris* L., *U. stygia* Thor (syn. *Utricularia ochroleuca* R.Hartm. *sensu lato*), *Utricularia intermedia* Hayne (all of them collected from the Czech Republic), *Utricularia geminiscapa* Benj. (from Massachusetts, USA), *Utricularia bremii* Heer *ex* Kölliker (from NW Russia), and *U inflata* Walt. (from New Jersey, USA) were grown outdoors in five plastic containers which simulated their natural conditions (area 0.25–2 $m^2$, 90–750 L). Adult plants of *U. stellaris* Linn.f. (from NE N.S.W., Australia) and *Utricularia hydrocarpa* Vahl (from E Nicaragua) were grown outdoors in 3–6 L aquaria standing in cooling water in a 1.5 $m^3$ plastic container. Adult plants of *Utricularia floridana* Nash., *Utricularia purpurea* Walt. (both from Florida, USA), *U. striata* Le Conte *ex* Torrey (from Georgia, USA), and *U. volubilis* R.Br. (from SW Australia) were grown in a naturally lit greenhouse in 3–20 L aquaria floating or standing in a 300 L plastic container. *U. reflexa* Oliver (from Okavango Delta, Botswana) and *Utricularia aurea* Lour. (from Cambodia) were grown indoors under natural light in 3 L aquaria. Out of all species, *U. stygia*, *U. intermedia* and *U. floridana* have distinct dimorphic shoots (photosynthetic and carnivorous; Taylor, 1989). All outdoor and greenhouse cultures were partly shaded. The plants in all cultures were grown in tap water with a litter of robust *Carex* species used as a substrate. The pH of the cultivation media

was ~7.0, dissolved oxygen concentration ranged from 0.15 mM to 0.30 mM and the free $CO_2$ concentration was 0.10–0.15 mM (for all details, see Adamec, 1997b, 2007a, 2011; Sirová et al., 2003). From the concentration of nutrients and humic substances, the water in these cultures was considered oligotrophic and humic. Small zooplankton species were added weekly to most cultures to promote plant growth.

### 2.2. Measurement of trap thickness

As a measure of water flow and trap volume change for the correlative study, trap thickness was measured in isolated traps of the 15 *Utricularia* species. For most species, young traps from the 4th to 6th mature leaf node, as counted from the plant apex, were used. In *U. stygia*, *U. intermedia*, *U. floridana* and *U. volubilis*, they were young to intermediate in age. The traps were usually functional and their length was 2.0–6.1 mm (see Table 1). In *U. purpurea* and *U. striata*, however, their ability to fire and reset was greatly limited to ~10–20% and the results for both species were hence discarded. For *U. reflexa*, two trap size categories ("large" 4.4–5.8 mm long; "small" 3.0–3.8 mm long) were used. The intact trap-bearing leaves were excised directly under water in the cultivation containers or aquaria and trap firing was induced by shaking the leaves. They were then transferred to the laboratory, washed in tap water, and a trap was excised and carefully transferred to a 10 mL perspex chamber containing a solution composed of 0.1 mM KCl, 0.05 mM $CaCl_2$ and 0.2 mM $NaHCO_3$ of pH 7.3–7.4 (Adamec, 2011).

A freshly excised trap at the initial stage of resetting (2–5 min) and without any macroscopic animal prey or air bubbles, was carefully inserted into the holder of an electronic position sensor to measure trap thickness changes (for all technical details, see Adamec, 2011). The position sensor had a 1 μm resolution and pressed against the fixed trap by a force not exceeding the weight of 0.4 g. In most cases, trap thickness was monitored at 1 min intervals and the chamber was partly covered by a perspex lid to minimize water evaporation. Water temperature was relatively stable during the measurements; mean values were within 22.0–25.5 °C for the 15 species, with the total temperature range <2 °C for each species. During the measurements, the temperature change was usually <1.0 °C and the traps were in natural dim daylight. With the aid of a loupe, the fixed traps were mechanically stimulated to fire using a very fine brush which gently touched the sensitive trigger hairs but the position of the fixed trap in the holder remained unchanged (Adamec, 2011). Measurements of trap thickness were made according to the following schedule: 60 min after the insertion of a fired trap into the holder (resetting period), the trap was re-fired by mechanical irritation and this step was conducted a total three times, each after a further 60 min. Thus, three sets of firing – resetting records were usually obtained for each trap. Within each species, 5–8 parallel traps of the same age (or size) category and originating from different plants, were used for the measurements. After all measurements, trap length and width were estimated to the nearest 0.1 mm by measuring with a rule, again viewed under a loupe. For each species, between 9 and 18 firing – resetting records were obtained.

In another set of experiments, the basic characteristics of spontaneous firings (after Adamec, 2011) were compared between the *U. reflexa* traps without prey and those freshly fed on prey over a period of 24 h. Young traps without prey from the 4th to 5th mature leaf node, 4.5–6.1 mm long, were used. One hour after the insertion of a fired trap into the holder, the control trap was fired by mechanical irritation (denoted as time 0), while the other trap was fed on 1 mm large dead ostracod as prey (fed variant). The ostracod was killed by warm water (45 °C) immediately before the feeding and inserted into a tip of a fine, thin-walled plastic tube, which gently touched the trigger hairs. Thus, the firing caused aspiration of the ostracod. The trap was then allowed to rest for 24 h and spontaneous firings were recorded. After 24 h, the trap was further fired by mechanical irritation and the next 30 min of the trap resetting process were recorded. In the fed variant, feeding sometimes caused the position of the fixed trap to change and, thus, the trap thickness change data for such a firing were discarded. During all measurements, the temperature was 23.5 ± 1.5 °C, with the temperature change usually <1.2 °C during each measurement. 10 or 12 parallel traps from different plants were used for control or fed variant experiments.

To confirm that traps pump out water continuously and a futile water re-circulation might occur within the reset traps, detailed resetting time-course data of trap thickness were measured during the first 10 min after trap firing. The aim was to decide whether there is a lag-period in water pumping just after firing, due to a possible cessation of water pumping. Young *U. reflexa* traps without prey from the 4th mature leaf node, 3.6–4.7 mm long, were used. 3 h after the insertion of a fired trap into the holder (denoted as time 0), the reset trap was fired by mechanical irritation and trap thickness was monitored at 2 s intervals for the next 10 min. During the measurements, the temperature was 22.5 ± 0.7 °C, with the temperature change <0.8 °C during each measurement. Twelve parallel traps from different plants were used. The resetting rate of the traps was measured for the last 10 min before the firing (between 170 min and 180 min) and for the first 3 min after the firing; the latter rate was evaluated and plotted at 10 s intervals.

To evaluate the plasticity of the trap wall structures with a mechanical impact less than that imparted by the position sensor (*cf.* Adamec, 2011), a 1-cm piece of nylon line (diameter 0.25 mm) was first inserted into the trap door of a freshly separated trap to keep the trap door permanently open. The trap was inserted into the holder after 5 min and the first phenomena of plasticity could manifest themselves without any other external force. Trap thickness was recorded for 4 h after the trap insertion. This was done with young *U. reflexa* traps (4th–5th mature leaf node, 4.8–6.0 mm long) at 23.0 ± 1.0 °C; $n = 9$.

### 2.3. Measurement of trap respiration

Aerobic dark respiration rates of isolated intact and partly sliced traps of *U. vulgaris* were compared over 24 h to determine if the traps can have sufficient energy to enable continuous water pumping. The $R_D$ of 6 large young traps of *U. vulgaris* from the 6th to 7th mature leaf node, 3.5–4.0 mm long (fresh weight/FW/12–18 mg, dry weight ~4.5% fresh weight), was measured at 25.0 ± 0.1 °C using an oxygen sensor in a solution of 0.5 mM KCl with 0.1 mM $CaCl_2$ (for all technical details, see Adamec, 1997b, 2005). $R_D$ data of intact, freshly collected traps were measured for 15 min in the dark ('external' $R_D$) and the traps were then partly sliced with a razor blade, to cancel the negative pressure inside the traps and allow the stirred solution to flow into the trap lumen ('total' $R_D$ incorporating 'internal' $R_D$; see Adamec, 2007a). $R_D$ measurements were then repeated and the used traps were stored in the unstirred solution at 25.0 ± 1.0 °C in the dark for 24 h. Both intact traps and traps on separated leaves were also stored in this way for 24 h and the $R_D$ was then measured in these traps. All measurements were repeated six times under the same conditions with traps originating from different shoots. $R_D$ is expressed in mmol $kg_{FW}^{-1}$ $h^{-1}$.

### 2.4. Evaluation of data and statistical analysis

The following parameters were evaluated from the measurements (after Adamec, 2011): initial maximum (fired) trap thickness just after insertion into the holder; change of trap thickness due to mechanically stimulated firing 1 h after insertion, after 24 h, and during spontaneous firings; initial resetting rate after all types of

**Table 1**
Trap length, initial trap thickness after fired trap insertion, and firing and resetting characteristics of separated traps in aquatic *Utricularia* species.

| Species | Length (mm) | Thickness (μm) | Firing (μm) | Resetting (μm) after | |
|---|---|---|---|---|---|
| | | | | 10 min | 30 min |
| *U. australis* | 2.7 ± 0.04 | 798 ± 27 | 276 ± 15 | 120 ± 6 | 257 ± 13 |
| *U. vulgaris* | 3.5 ± 0.07 | 1160 ± 24 | 276 ± 12 | 80.7 ± 6.8 | 199 ± 17 |
| *U. stellaris* | 2.2 ± 0.02 | 671 ± 12 | 215 ± 6 | 108 ± 5 | 208 ± 8 |
| *U. geminiscapa* | 2.4 ± 0.09 | 706 ± 51 | 174 ± 8 | 73.7 ± 4.6 | 155 ± 14 |
| *U. aurea* | 3.9 ± 0.06 | 1527 ± 14 | 282 ± 17 | 76.5 ± 3.3 | 191 ± 11 |
| *U. bremii* | 2.8 ± 0.06 | 886 ± 41 | 255 ± 9 | 79.1 ± 2.9 | 202 ± 6 |
| *U. hydrocarpa* | 2.0 ± 0.03 | 792 ± 17 | 158 ± 5 | 46.1 ± 3.1 | 116 ± 6 |
| *U. inflata* | 2.5 ± 0.04 | 787 ± 24 | 274 ± 9 | 70.8 ± 4.8 | 193 ± 11 |
| *U. reflexa* – large tr. | 5.3 ± 0.24* | 1665 ± 62* | 252 ± 9ns | 45.2 ± 1.5* | 123 ± 4* |
| *U. reflexa* – small tr. | 3.4 ± 0.14 | 1021 ± 51 | 230 ± 8 | 60.1 ± 1.5 | 149 ± 5 |
| *U. stygia* | 3.3 ± 0.28 | 1125 ± 26 | 160 ± 7 | 38.2 ± 2.4 | 97.3 ± 5.8 |
| *U. intermedia* | 3.1 ± 0.08 | 923 ± 53 | 173 ± 9 | 43.1 ± 2.0 | 106 ± 5 |
| *U. floridana* | 2.6 ± 0.05 | 745 ± 57 | 169 ± 7 | 36.1 ± 2.5 | 91.0 ± 3.6 |
| *U. volubilis* | 3.0 ± 0.08 | 1078 ± 27 | 90.2 ± 6.6 | 6.64 ± 1.59 | 28.8 ± 3.3 |
| Total mean | 3.0 ± 0.09 | 983 ± 33 | 216 ± 5 | 62.0 ± 2.2 | 150 ± 5 |

Means ± S.E. intervals ($n$ = 9–18) are always shown. For two *U. reflexa* trap size categories, the asterisk denotes statistically significant difference at $P < 0.0003$ (one-way ANOVA); ns, non-significant difference at $P > 0.05$.

firing over 10 min (expressed as trap thickness decrease between 1 min and 11 min) and over 30 min (0–30min); frequency of spontaneous firings within the 24-h resting period with no mechanical irritation; and time between two spontaneous firings (and/or the time between the last mechanically stimulated firing and the first spontaneous firing).

Within the correlative study on 13 *Utricularia* species, the data for the same and different traps were pooled, with outlying values discarded, and presented as such. Repeated measures design of ANOVA could not be used for data evaluation as this step would lead to a substantial reduction of data. Moreover, as the traps – partly within each species but mainly between the species – differed greatly in their thickness and length (Table 1), all parameters were also expressed per initial, fired trap thickness and length as measures of trap size. Trap length correlated very strongly with trap width ($r^2 = 0.94$, $P < 0.0001$, $n = 80$) within the whole trap dataset. As the measured parameters usually correlated with trap length more strongly than with trap width (data not shown), only trap length was used. Linear regression models, including the data from all 13 species, were used to look for statistically significant relationships between the measured parameters of trap firing and resetting and trap thickness and length and the same models were used for evaluation of the data after being related to trap thickness or length. Only expected and physiologically important relationships are included in the results. Due to interrelated factors in these regression models, Bonferroni correction was used. Significant differences in the parameters between two trap size categories of *U. reflexa* and also between un-fed and fed traps of *U. reflexa* were evaluated by 1-way ANOVA (Tukey HSD test for unequal $n$). In other cases, the two-tailed $t$-test was used. Mean ± S.E. values are shown where possible.

## 3. Results

The 13 *Utricularia* species used differed considerably in their trap length and thickness (Table 1). On the absolute scale, firing of traps of *U. aurea*, *U. australis* and *U. vulgaris* led to the greatest trap thickness increase, while the highest 10-min and 30-min resetting rate occurred in *U. australis* and *U. stellaris*. In these firing and resetting characteristics, *U. volubilis* was evidently the least effective species. The magnitude of firing of significantly larger and thicker *U. reflexa* traps was similar to smaller traps but both resetting rates were significantly higher (at $P < 0.05$) in larger traps. Linear regression models revealed a very significant correlation between trap thickness and trap length within the whole dataset (Table 2). Trap thickness increase due to firing correlated significantly and positively with both trap length and thickness. The 10-min resetting rate correlated significantly but negatively with trap thickness but non-significantly with trap length ($r^2 = 0.040$, $P = 0.0063$, $n = 185$). Although the 10-min and 30-min resetting rates correlated significantly with each other, the 30-min resetting rates did not correlate significantly with either trap thickness or trap length. A significant correlation was found between the magnitude of firing and both resetting rates.

On the relative scale there were still great interspecific differences (in total 3.7–4.2 times) in the magnitude of firing per unit trap thickness or length (Table 3). For both relative parameters of firing, *U. australis, U. inflata* and *U. stellaris* traps had the highest values of all species. Similarly, the highest 10-min and 30-min relative resetting rate occurred in *U. australis* and *U. stellaris*, followed by *U. geminiscapa* and *U. inflata*. *U. volubilis* was again the least effective species in all parameters as its values ranged markedly below the average. Large traps of *U. reflexa* were found to be significantly (at $P < 0.01$) less effective at all relative firing and resetting parameters than small traps. It follows from preliminary data (not shown) that the absolute and relative values of both resetting rates for *U. purpurea* traps were also very low and comparable with those for *U. volubilis*. Relative values of the magnitude of firing, when expressed per unit trap thickness or length, correlated significantly but negatively with both trap thickness and length. This was also true for the relative resetting rates (Table 4). Therefore, smaller traps with lower trap thickness are relatively more effective in both trap firing and resetting than larger traps. Neither firing nor resetting characteristics were significantly different (at $P < 0.05$) between un-fed and prey-fed traps of *U. reflexa* during the 24 h resting period and this was also true for the occurrence of spontaneous firings (Table 5). Isolated traps thus appear to be rather autonomous units the firing of which does not much depend on prey digestion.

As shown in Fig. 1, the detailed time-course of resetting small *U. reflexa* traps, plotted at 10 s intervals, revealed a strict linear resetting rate during the first 3 min immediately after firing ($r^2 = 0.998$, $P \ll 0.0001$, $n = 19$). This initial resetting rate was 5.75 μm min$^{-1}$ (*cf.* Table 1), while the preceding resetting rate after 3 h of resetting (170 min and 180 min) was only 0.392 ± 0.056 μm min$^{-1}$ (data not shown). No lag-period thus occurs within the first several seconds after firing which suggests that water is pumped out of the trap continuously.

After young opened traps of *U. reflexa* had been inserted into the holder, a logarithmic-type increase in trap thickness over time was recorded for 4 h (data not shown), indicating a marked effect

**Table 2**
Statistically significant or physiologically important linear regression models of trap parameters on the basis of the dataset from Table 1 as dependent on variable factors.

| No. | Linear regression model | *n* | $r^2$ | *P* |
|---|---|---|---|---|
| 1 | Length = 0.0437 + 1.33 width | 80 | 0.935 | <0.0001* |
| 2 | Thickness = 0.0188 + 0.318 length | 80 | 0.837 | <0.0001* |
| 3 | Firing = 148 + 21.9 length | 202 | 0.089 | <0.0001* |
| 4 | Firing = 160 + 56.3 thickness | 208 | 0.070 | 0.00013* |
| 5 | Reset 10 min = 82.7−6.63 length | 185 | 0.040 | 0.0063 |
| 6 | Reset 10 min = 84.7−22.3 thickness | 185 | 0.053 | 0.0016* |
| 7 | Reset 30 min = 173−7.44 length | 179 | 0.013 | 0.132 |
| 8 | Reset 30 min = 173−22.7 thickness | 179 | 0.014 | 0.115 |
| 9 | Reset 30 min = 29.8 + 1.94 reset 10 min | 177 | 0.873 | <0.0001* |
| 10 | Firing = 125 + 1.50 reset 10 min | 185 | 0.434 | <0.0001* |
| 11 | Firing = 88.5 + 0.866 reset 30 min | 175 | 0.616 | <0.0001* |

Trap length, width, and thickness are in mm, while firing, reset 10 min, and reset 30 min in μm. As a result of Bonferroni correction, only values of $P < 0.0045$ represent significant correlation (indicated by asterisk). $r^2$, coefficient of determination.

**Table 3**
Firing and resetting characteristics of separated traps in aquatic *Utricularia* species expressed per initial trap thickness after fired trap insertion or per trap length.

| Species | Firing/thickness (μm/mm) | Firing/length | Reset 10′/thickness | Reset 10′/length | Reset 30′/thickness | Reset 30′/length |
|---|---|---|---|---|---|---|
| *U. australis* | 350 ± 24 | 103 ± 6 | 153 ± 10 | 44.7 ± 2.4 | 329 ± 23 | 95.7 ± 5.3 |
| *U. vulgaris* | 239 ± 12 | 79.1 ± 4.1 | 69.8 ± 6.6 | 23.2 ± 2.2 | 172 ± 17 | 57.4 ± 5.5 |
| *U. stellaris* | 318 ± 9 | 97.6 ± 2.8 | 161 ± 8 | 49.1 ± 2.4 | 308 ± 12 | 94.0 |
| *U. geminiscapa* | 250 ± 11 | 72.9 ± 3.3 | 100 ± 8 | 30.8 ± 2.3 | 224 ± 17 | 67.4 ± 6.4 |
| *U. aurea* | 185 ± 11 | 73.1 ± 4.6 | 50.2 ± 2.3 | 19.8 ± 1.0 | 125 ± 7 | 49.4 ± 3.2 |
| *U. bremii* | 288 ± 11 | 89.1 ± 2.6 | 89.0 ± 2.9 | 27.6 ± 0.9 | 226 ± 7 | 70.0 ± 1.4 |
| *U. hydrocarpa* | 202 ± 8 | 79.5 ± 2.9 | 59.3 ± 4.3 | 23.2 ± 1.6 | 149 ± 8 | 58.5 ± 3.0 |
| *U. inflata* | 349 ± 9 | 111 ± 3 | 89.5 ± 5.1 | 29.1 ± 2.0 | 246 ± 9 | 78.8 ± 3.8 |
| *U. reflexa* - large tr. | 152 ± 6* | 48.1 ± 1.6* | 27.2 ± 1.2* | 8.59 ± 0.37* | 73.9 ± 2.5* | 23.3 ± 0.8* |
| *U. reflexa* - small tr. | 226 ± 7 | 67.0 ± 2.0 | 58.8 ± 1.7 | 17.5 ± 0.5 | 145 ± 4 | 43.0 ± 1.1 |
| *U. stygia* | 142 ± 6 | 49.9 ± 2.4 | 34.0 ± 2.2 | 11.9 ± 0.7 | 86.4 ± 5.2 | 30.0 ± 1.5 |
| *U. intermedia* | 189 ± 10 | 54.9 ± 2.8 | 47.2 ± 2.3 | 13.7 ± 0.6 | 115 ± 5.4 | 33.6 ± 1.5 |
| *U. floridana* | 229 ± 8 | 65.3 ± 2.5 | 51.3 ± 5.8 | 13.9 ± 1.1 | 127 ± 8 | 35.0 ± 1.3 |
| *U. volubilis* | 84.3 ± 6.4 | 29.9 ± 2.2 | 6.31 ± 1.53 | 2.28 ± 0.57 | 27.1 ± 3.3 | 9.58 ± 1.14 |
| Total mean | 229 ± 6 | 72.9 ± 1.7 | 68.4 ± 3.2 | 21.7 ± 1.0 | 163 ± 6 | 51.9 ± 1.9 |

Trap length and thickness are in mm, while firing, reset 10 min, and reset 30 min in μm. Mean ± S.E. intervals ($n = 9$–18) are always shown. For two *U. reflexa* trap size categories, the asterisk denotes significant difference at $P < 0.0001$ (one-way ANOVA).

of trap wall plasticity. Trap thickness increased on average by 54 ± 3 μm (S.E., $n = 9$) after 30 min, by 76 ± 4 μm after 2 h, and only by 87 ± 5 μm after 4 h. The 'external' $R_D$ of fresh, intact *U. vulgaris* traps (in mmol $kg_{FW}^{-1}$ $h^{-1}$) was 10.2 ± 0.7 (S.E., $n = 6$; data not tabulated), while slicing the traps increased the $R_D$ by 55% to 15.8 ± 0.7 (i.e., 'total' $R_D$), indicating a great proportion of internal trap structures on the 'total' $R_D$. The 'external' $R_D$ of separated intact traps decreased by 39% (6.20 ± 0.24) after 24 h in the dark and the same 'external' $R_D$ (6.02 ± 0.25) was measured in intact traps attached to leaves after 24 h in the dark. The 'total' $R_D$ of sliced traps decreased by 53% (7.45 ± 0.74) after 24 h in the dark.

## 4. Discussion

The position sensor was used to quantify trap thickness changes due to mechanically stimulated firing and the subsequent resetting in 13 aquatic *Utricularia* species differing greatly in their world spread and ecological and morphological traits (see Taylor, 1989; Guisande et al., 2007). As the traps of the different species differed greatly in their size (i.e., trap length and thickness; Table 1) one could expect that the 'absolute' interspecific differences in firing and resetting parameters found might depend much more on the trap size parameters rather than on the interspecific differ-

**Table 4**
Statistically significant and physiologically important linear regression models of relative trap parameters on the basis of the dataset from Table 3 as dependent on trap thickness and length.

| No. | Linear regression model | *n* | $r^2$ | *P* |
|---|---|---|---|---|
| 1 | Firing/thickness = 375 − 146 thickness | 202 | 0.299 | <0.0001* |
| 2 | Firing/thickness = 353 − 40.1 length | 202 | 0.190 | <0.0001* |
| 3 | Firing/length = 109 − 36.0 thickness | 202 | 0.207 | <0.0001* |
| 4 | Firing/length = 113 − 13.0 length | 202 | 0.226 | <0.0001* |
| 5 | Reset 10′/thickness = 149 − 79.0 thickness | 185 | 0.314 | <0.0001* |
| 6 | Reset 10′/thickness = 141 − 23.2 length | 185 | 0.230 | <0.0001* |
| 7 | Reset 10′/length = 44.1 − 22.0 thickness | 185 | 0.274 | <0.0001* |
| 8 | Reset 10′/length = 44.7 - 7.35 length | 185 | 0.261 | <0.0001* |
| 9 | Reset 30′/thickness = 322 − 157 thickness | 179 | 0.333 | <0.0001* |
| 10 | Reset 30′/thickness = 311 − 47.2 length | 179 | 0.251 | <0.0001* |
| 11 | Reset 30′/length = 95.4 − 42.8 thickness | 179 | 0.271 | <0.0001* |
| 12 | Reset 30′/length = 99.3 − 15.1 length | 179 | 0.288 | <0.0001* |

Trap length and thickness are in mm, while firing, reset 10 min, and reset 30 min in μm. As a result of Bonferroni correction, only values of $P < 0.0042$ represent significant correlation (indicated by asterisk). $r^2$, coefficient of determination.

**Table 5**
Thickness changes of separated *U. reflexa* traps with or without prey during trap firing (stimulated by mechanical irritation or induced spontaneously) or trap resetting during the 24-h resting period.

| Initial max. trap thickness (μm) | Trap thickness increase (μm) during firing within | | | Resetting rate (μm) after firing during | | | | | | Spontaneous firing | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 10 min within | | | 30 min within | | | Freq. per 24 h | Time between SFs (min) |
| | INS | 24 h | SF | INS | 24 h | SF | INS | 24 h | SF | | |
| (A) Traps without prey | | | | | | | | | | | |
| 1831 ± 50 | 272 ± 20 | 444 ± 42 | 416 ± 42 | 48.4 ± 2.9 | 74.1 ± 5.8 | 66.9 ± 4.0 | 135 ± 7.8 | 183 ± 12 | 167 ± 9.7 | 1.22 ± 0.40 | 734 ± 130 |
| (B) Traps with prey | | | | | | | | | | | |
| 1875 ± 25 | 329 ± 21 | 535 ± 23 | 467 ± 35 | 47.6 ± 4.0 | 65.5 ± 6.1 | 75.5 ± 6.0 | 126 ± 9.1 | 163 ± 13 | 182 ± 13 | 1.09 ± 0.34 | 586 ± 117 |

Traps were fired mechanically both 1 h after trap insertion (INS) and 24 h afterwords (24 h); spontaneous firing (SF) was associated with no type of trap irritation. A part of traps (traps with prey) were fed on prey 1 h after trap insertion, while the others were without prey. Frequency of spontaneous firings is shown for the period of 24 h. Means ± S.E. intervals are shown; $n = 8$–12. No statistically significant difference was found between the values for traps with and without prey (at $P < 0.05$, 1-way ANOVA).




**Fig. 1.** The plot of the trap thickness changes of the first 3 min after firing of *U. reflexa* traps preceded by 3 h resetting. The resetting rate after the firing was monitored at 2 s intervals and plotted at 10 s intervals (±S.E., $n = 12$). The linear regression of the plot ($r^2 = 0.998$, $P \ll 0.0001$, $n = 19$) revealed the mean resetting rate 5.75 μm min$^{-1}$, while the preceding resetting rate was 0.39 ± 0.06 μm min$^{-1}$.

ences themselves. This view was further supported by the finding of statistically significant correlations (at $P < 0.05$) between the magnitude of trap firing or the 10-min resetting rate on the one hand and the trap length and thickness on the other (Table 2). However, while the magnitude of firing showed a highly significant positive correlation with the trap size parameters, the 10-min resetting rate did so negatively with trap thickness and only at $P < 0.05$; moreover, the slope of this relationship was low. It may therefore be suggested that larger and thicker traps exhibit greater firing but reset slightly more slowly.

On the relative scale, when expressed per unit trap thickness or length, smaller and thinner traps both fire more and reset (both 10-min and 30-min rate) faster than larger traps do (Table 4) and this effect is significant ($P < 0.0001$). The observed relationship between trap size and 'relative' firing and resetting rates is clearly supported by significant differences between larger and smaller *U. reflexa* traps (Table 3). One of the reasons for the markedly higher 'relative' firing effectiveness of smaller traps is probably the mechanical properties of trap walls (*cf.* Vincent et al., 2011, in press) with smaller traps being more elastic and mobile. The reason for achieving lower resetting rates in larger traps may be geometric as the lower trap volume:trap surface or trap volume:trap thickness ratio in larger traps should be a disadvantage. On the basis of the 'relative' firing and resetting rates, great interspecific differences are evident (Table 3). *U. australis*, *U. stellaris* and *U. inflata* are the three species that consistently show the highest 'relative' firing and resetting rates. These species, with monomorphic shoots, belong to the generic section *Utricularia.* They grow over vast territories, are usually found on several continents (Taylor, 1989), and may be considered very successful species of the genus. The ability of their traps to aspirate in a great water volume and reset rapidly gives them the chance to be very effective prey hunters provided sufficient prey are available (e.g., Adamec, 2008).

In contrast, relatively low trap effectiveness was found in three species with distinctly dimorphic shoots, *U. stygia*, *U. intermedia* and *U. floridana*. The former two amphibious species usually grow in very shallow, strongly dystrophic waters, with their carnivorous shoots bearing numerous traps which penetrate into a loose, peaty anoxic substrate (Taylor, 1989; Adamec, 2007b). Their traps commonly contain dark detritus (Adamec, unpubl.). Thus, the traps of these species may be less adapted to capturing highly mobile pelagial zooplankton but more to utilization of detritus and inhabiting organisms (but *cf.* Mette et al., 2000). *U. volubilis* (section *Pleiochasia*; Taylor, 1989) traps are clearly the least effective of all species

studied (Table 3), closely followed by *U. purpurea* (section *Vesiculina*) traps (data not shown). Moreover, as opposed to traps of all other *Utricularia* species with the walls two cell layers thick (*e.g.*, Juniper et al., 1989), traps of *U. volubilis* are formed by four cell layers (Adamec, unpubl.). In this way, their mechanical stiffness (Young's elasticity modulus) should be much higher on account of trap elasticity, rendering their relative firing/resetting volume changes small. Although the phenomenon of trap wall plasticity was also confirmed in traps having been opened before insertion into the holder (see results, *cf.* Adamec, 2011), due to the very rapid movement during the trap firing, such a slow movement does not apply for either trap firing or resetting. Therefore, only trap wall elasticity applies for trap movement (Vincent et al., 2011, in press; Joeyux et al., 2011).

The occurrence of reproducible spontaneous firings (i.e., without any mechanical stimulation) has recently been described in intact or isolated traps of four *Utricularia* species (Marmottant et al., 2009; Vincent et al., 2011, in press; Adamec, 2011). The spontaneous firings and the trap resetting rates agreed quantitatively with those stimulated mechanically after the 24-h resting period (Adamec, 2011). In *U. reflexa*, no significant difference in firing/resetting parameters or in the occurrence of spontaneous firings was found between traps supplied with or denied prey (Table 5). This finding, together with the results by Vincent et al. (2011), supports the established view that spontaneous firing does not depend on captured prey and that *Utricularia* traps are autonomously working organs (Sirová et al., 2003; Adamec, 2011). Using a high-speed camera and a mechanistic model, Vincent et al. (in press, 2011) suggest that spontaneous firing occurs when the negative pressure inside the trap slightly exceeds the critical value for the buckling of the trap door. Moreover, they also distinguished three patterns of spontaneous firing according to their frequency of occurrence which were characteristic for single traps. In conclusion, spontaneous firings are presumably a native component of trap function in all aquatic *Utricularia* species.

Sasago and Sibaoka (1985a) suggested the presence of a physiological negative pressure sensor in the traps which should switch on and off the water pumping mechanism. Recent findings no longer support this idea. Trap thickness measurements in three *Utricularia* species revealed that a complete trap resetting process runs for at least 6–10 h but usually even much longer (Adamec, 2011). This might indicate that water pumping out of the traps is not terminated. Detailed measurements of the resetting rate in *U. reflexa* trap estimated the linear resetting kinetics during the first few seconds after firing (Fig. 1). After 3 h of resetting and subsequent firing, the resetting rate increased suddenly from $0.39 \pm 0.06\ \mu m\ min^{-1}$ to $5.8\ \mu m\ min^{-1}$. It is conceivable that if the water pumping mechanism had been regulated by the trap, the resumption of water pumping after the new firing would have been delayed and a lag-period would have been evident.

The new concept of continuous water pumping out of the traps does raise some new questions. Water pumping out of the traps is an energetically demanding process, strongly dependent on the ATP energy from aerobic dark respiration (Sydenham and Findlay, 1975; Sasago and Sibaoka, 1985a,b; Adamec, 2006; Laakkonen et al., 2006). Moreover, dark respiration of all inner trap structures occurs in the (almost) anoxic trap fluid (Adamec, 2007a). Thus, if the trap pumps out the water continuously for 5–20 h until a new firing event (stimulated or spontaneous), this implies that the respiration of bifid glands under the (almost) anoxic conditions is extremely efficient (see Laakkonen et al., 2006) and also that the trap energy consumption is permanently very high. The measurement of $R_D$ for sliced traps confirmed a high proportion of the 'internal' $R_D$ to the 'total' $R_D$ (see Section 3) in support of Adamec (2007a) but the reduced $R_D$ rate of sliced traps after 24 h is probably sufficient to cover the ATP consumption for trap resetting (*cf.* Sasago and Sibaoka, 1985b; Adamec, 2007a). These findings may explain why isolated traps can fully fire and reset for at least 24 h (Table 5; Adamec, 2011). There is another question on the character of the continuous water flow. The water could either permanently recirculate through some leaks at the trap door or the mechanism of water pumping could become thermodynamically inefficient at high negative pressure although it could run permanently.

### 4.1. Ecological model of nutrient gain by Utricularia traps from the ambient water

In nutrient-poor waters with low prey abundance, the nutritional value of *Utricularia* carnivory can be minimal (Richards, 2001; Adamec, 2008; Peroutka et al., 2008; Sirová et al., 2009). In such cases, commensal–trap interactions in prey-free traps could be of a greater nutritional importance for the plants than prey capture alone but the importance has never been quantified. For the possible trap uptake of growth-limiting N and P through the microbial food-web, it is crucial how much particulate matter (phytoplankton, bacteria, detritus; Peroutka et al., 2008; Alkhalaf et al., 2009) and dissolved mineral nutrients are the traps able to draw in from the ambient water. The most recent findings on trap functioning – 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. A simple budget model based on literature data may be devised (Table 6) to quantify the daily N and P gain from the ambient water by *Utricularia* traps without animal prey. This can be used to decide whether the trap microorganisms can behave as commensals rather than parasites. The essence of the model is the combination of the published data on the mean total N and P content (both dissolved and particulate) in the trap fluid of a middle-aged trap of two Belizean *Utricularia* species growing in a very oligotrophic wetland (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).

Let us assume that a 3.0 mm trap (post-firing volume 5.0 μl) of *U. australis* can fire spontaneously (40% trap volume) twice a day (Adamec, 2011). Thus, it can aspirate a daily total of 4.0 μl of the ambient water with all its suspended particles; each resetting lasts for 2 h. Assuming the published values of total N and P at oligo- and mesotrophic sites (see Table 6; Adamec, 2008), the trap can gain 3.72–6.64 ng total N and 0.30–0.40 ng total P per day exclusively through the process of spontaneous firing. Assuming that trap water recirculation occurs at a rate of $1.0\ \mu L\ h^{-1}$ (Sasago and Sibaoka, 1985a) for 20 h a day and that only dissolved and mineral N and P forms ($NH_4^+$-N and $PO_4$-P) can be gained by this ultrafiltration (this is evidently a simplification and underestimation as soluble organic forms of N and P/humic acids, phosphoesters/can also be gained), the trap can gain 0.54–0.90 ng $NH_4^+$-N and 0.26–0.46 ng $PO_4$-P daily (Table 6). Therefore, when combined, each day the prey-free trap could potentially obtain totally 4.26–7.54 ng N and 0.54–0.86 ng P from the ambient water. A middle-aged trap of volume 5 μL should contain 300 ng of total N and 12.5 ng of total P in the trap fluid (Sirová et al., 2009). Theoretically, such a high total N amount in the trap fluid is accumulated for 39.8–70.4 d or P amount for 14.5–23.1 d. Considering that the age of the traps was only *ca.* 8–12 d the model clearly shows that the total N and P content estimated in the traps was too high to be accumulated from the ambient water, even when no N and P is taken up by the trap.

What conclusions can be drawn from this model? First, the calculated N and P input rates from the ambient water due to both spontaneous firings and 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-

**Table 6**
Model daily gain of N and P (in ng) from the ambient dystrophic natural water by an aquatic *Utricularia* trap due to the uptake of both $NH_4^+$-N and SRP (soluble reactive phosphorus) and TN (total N) and TP (total P) as a result of spontaneous trap firing and water recirculation through the trap.

| Hab. type | Ambient water concentration ($\mu g L^{-1}$) | | | | Model daily gain of | | | | Trap fluid cont. | | Days needed to cover | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_4$-N | SRP | TN | TP | $NH_4$-N | TN | SRP | TP | TN | TP | TN | TP |
| OL | 27 | 12 | 930 | 75 | 0.54 | 3.72 | 0.24 | 0.30 | 300 | 12.5 | 70.4 | 23.1 |
| ME | 45 | 23 | 1660 | 100 | 0.90 | 6.64 | 0.46 | 0.40 | 300 | 12.5 | 39.8 | 14.5 |

See the text for the model assumptions. It is assumed that due to firings, the trap can gain both N and P as TN and TP from the ambient water, while only $NH_4^+$-N and SRP due to water recirculation. Data for the ambient water concentration were taken from Adamec (2008) for *U. australis* sites; OL, oligotrophic sites; ME, mesotrophic sites. Data for the trap fluid content (in ng) were taken from Sirová et al. (2009). Days needed to cover TN and TP in the trap fluid are based on the model daily N and P gain.

span (*ca.* 20 d; Friday, 1989). Thus, such a low N and P gain cannot be ecologically important for the plant mineral nutrition at all. Moreover, the model shows that the prey-free traps do not take up any N and P from the trap fluid but rather exude a certain amount of N and P to the trap fluid to support the microbial community. This idea can be supported by the reported finding of a reduction in growth of terrestrial *U. uliginosa* after addition of euglens to peaty soil (Jobson et al., 2000). A similar extensive exudation of organic substances into the trap fluid has recently been found in two aquatic *Utricularia* species (Sirová et al., 2010). Therefore, it seems that *Utricularia* traps without animal prey gain no nutritional benefit from the trap microbial community, the trap microorganisms behave rather as parasites than commensals and represent additional ecological costs for trap maintenance. If the trap microorganisms are beneficial for the plant, the ecological benefit can occur only in traps with captured prey by facilitating prey digestion (Płachno et al., 2006; Sirová et al., 2009).

It may be hypothesized that plants maintain their trap commensal communities ("gardening") by exuding organic (Sirová et al., 2010) and mineral nutrients into all traps to ensure better prey digestion for those that have captured animal prey. The real cost:benefit ratio depends on the proportion of traps which capture animal prey during their life. That is why capturing animal prey always leads to a growth enhancement in aquatic *Utricularia* species (*cf.* Adamec, 1997a; Adamec et al., 2010). As 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 microbial organisms could be more marginal.

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