Aquatic Botany 133 (2016) 10–16

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Measurement of the critical negative pressure inside traps of aquatic carnivorous *Utricularia* species

Lubomír Adamec[a,*], Simon Poppinga[b]

[a] Institute of Botany of the Czech Academy of Sciences, Section of Plant Ecology, Dukelská 135, CZ-379 82 Třeboň, Czech Republic
[b] Plant Biomechanics Group, Botanic Garden, Faculty of Biology, University of Freiburg, Schänzlestr 1, D-79104 Freiburg im Breisgau, Germany

ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 18 April 2016
Accepted 27 April 2016
Available online 3 May 2016

Keywords:
Lentibulariaceae
Aquatic carnivorous plants
Trap functioning
Spontaneous trap firing
Taxonomic differences
Test of trap firing

ABSTRACT

Firing and resetting of aquatic *Utricularia* traps are associated with water flows and pressure changes. A negative pressure of ca. −0.16 bar is formed in reset traps, but its direct measurement is very difficult. We present a method of a gradual external application of negative pressure of −0.56 to −0.84 mbar s^{-1} through a fine capillary to cut off aquatic *Utricularia* traps to determine the critical negative pressure (CNP) at which the traps (located in air) fire and aspirate an air bubble. Using an electronic pressure sensor, we simulated the physiologically formed negative pressure needed for spontaneous trap firing in 15 aquatic *Utricularia* species of four generic sections. Mean CNP values ranged from −0.069 bar in giant traps of *U. reflexa* to −0.346 bar in *U. dichotoma*. The average in all 20 species or variants tested was −0.195 ± 0.018 bar, while that in 13 species or variants of the generic section *Utricularia* was −0.165 ± 0.015 bar and significantly differed from that of three populations of two species (*U. dichotoma, U. volubilis*) of the generic section *Pleiochasia* (−0.335 ± 0.006 bar). CNP differed significantly between giant and smaller traps of *U. reflexa* and young and old traps of *U. vulgaris*. Pooled data for 20 species or variants showed a significant negative linear correlation between trap length and CNP value. Within each species, high variability of the CNP was found: the lowest values were usually 2–3 times lower than the highest ones. This variability can represent three types of spontaneous firings described in the literature.

1. Introduction

The rootless carnivorous genus *Utricularia* L. (Lentibulariaceae) is the largest carnivorous plant genus and comprises ~240 species (Fleischmann, 2015). Around 60 species are of aquatic or amphibious life form and usually grow in standing, nutrient-poor humic waters (Taylor, 1989; Jobson et al., 2003; Guisande et al., 2007). The plants are able to capture animal prey, typically small zooplankton, by their traps of foliar origin and to utilise mineral nutrients from prey carcasses (*e.g.*, Friday and Quarmby, 1994; Adamec, 1997a, 2008; Harms, 1999; Richards, 2001). These lenticular traps are hollow bladders, usually 1–5 mm long, with walls typically constituted of two cell layers, and are filled with trap fluid. They contain a variety of glands and trichomes on both the inner and outer surfaces, the function of which is still partially unresolved (Sydenham and Findlay, 1973, 1975; Sasago and Sibaoka, 1985a,b; Juniper et al., 1989; reviewed by Poppinga et al., 2016).

In a set state, when the trap is deflated and ready for firing, a negative pressure ("underpressure") of ~−0.12 to −0.16 bar relative to the ambient water is formed and maintained inside the trap as found in three *Utricularia* species of the generic section *Utricularia* (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985a; Singh et al., 2011). When trigger (sensory) hairs situated externally on the trap door are touched by prey the door opens, the animal is aspirated into the trap lumen and the door closes the trap watertight again, all completed within 3–5 ms. The complex door motion comprises a reversible buckling/unbuckling process associated with a convex/concave door curvature inversion (Joeyux et al., 2011; Singh et al., 2011; Vincent and Marmottant, 2011; Vincent et al., 2011a,b). The negative pressure is partly restored by pumping out of ca. 40% of the water from the trap fluid within 25–30 min, after which the trap is ready to fire again (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985a; Singh et al., 2011). However, the complete process of trap resetting lasts at least 6–10 h (Adamec 2011a,b). In studies of water pumping from *Utricularia* traps (Sydenham and Findlay, 1973, 1975; Sasago and Sibaoka, 1985a,b), the trap thickness as an easily and accurately measured parameter, correlated closely with the magnitude of the negative pressure only during the first 30 min of trap resetting. The stable negative pressure inside the

* Corresponding author.
E-mail addresses: lubomir.adamec@ibot.cas.cz, lubo.adamec@seznam.cz (L. Adamec).

http://dx.doi.org/10.1016/j.aquabot.2016.04.007
0304-3770/

traps was reached within 30 min after firing, while trap thickness decreased and water pumping continued for several hours. In line, Adamec (2011b) found a strict linear resetting rate of trap thickness changes, without any lag-period, during the first 3 min after firing in *U. reflexa* (generic section *Utricularia*) traps and suggested that water is pumped out of the trap continuously and probably recirculates through leaks in the reset state (see also Vincent et al., 2011b). As Płachno et al. (2015) have recently reported a distinct 4–6 min lag-period of trap resetting in two populations of *U. dichotoma* from the Australian generic section *Pleiochasia*, the regulation of water pumping out of the traps may not be unified within the whole genus. Although some *Utricularia* species from the section *Pleiochasia* possess 3–5 cell layers thick trap walls, no interrelationship was found between this parameter and the presence of the lag-period of water pumping.

It has recently been found that both cut off and intact traps can also fire spontaneously in the course of time, *i.e.*, without any mechanical stimulation by prey (Adamec, 2011a,b; Vincent and Marmottant, 2011; Vincent et al., 2011a,b; Płachno et al., 2015). There was no quantitative difference between spontaneous and mechanically stimulated firings and subsequent resetting rates (Adamec 2011a,b; Płachno et al., 2015). To explain this phenomenon, Vincent et al. (2011b) suggest that spontaneous firing occurs when the internal negative pressure equals to the 'critical value' (*i.e.*, threshold) for the buckling of the trap door. After the frequency of spontaneous firings, the latter authors described in intact traps of *U. australis* and *U. inflata* three types of spontaneous firings (random, metronomic, bursting), which were inherent to each single trap and were usually not changing during three weeks. The periods between two subsequent firings ranged from a few dozens of min (bursting type) to 1–8 h within the other types. The authors assume (see Fig. 1 therein) that each spontaneous firing type operates at distinctly different negative pressure values inside the trap: the shorter is the period between two firings, the lower is the absolute value of the negative pressure. The authors suggest that the different firing types are due to different mechanical properties (stiffness) of trap doors, but it is also plausible that the diversity of the reactions is due to a varying localization of the trap door margin on the sealing pavement epithelium, which could differ after each firing (Poppinga et al., 2016). Spontaneous firings occur commonly in both main generic sections (*Utricularia*, *Pleiochasia*) comprising aquatic or amphibious species (Adamec 2011b; Płachno et al., 2015).

The initial aim of this study was to apply an electronic pressure sensor for measuring the negative pressure inside large traps of several aquatic *Utricularia* species (*sensu* Sydenham and Findlay, 1973; Singh et al., 2011). Since the direct measurement of the negative pressure was not successful, we have developed a modified method for a gradual external application of negative pressure in cut off aquatic *Utricularia* traps to determine the negative pressure (critical or threshold negative pressure, CNP) at which the trap located in air fires and aspirates an air bubble. In this way, we simulated the physiologically formed negative pressure needed for spontaneous firing. These comparative measurements were conducted on traps in 15 aquatic or amphibious *Utricularia* species of four generic sections. As cut off traps can normally and repeatedly fire in moist air (Adamec, 2012), we additionally present here a very simple test demonstrating the ability of traps to fire spontaneously and after a mechanical stimulation in moist air.

2. Materials and methods

2.1. Plant material

All plants used in this study were kept in the collection of aquatic carnivorous plants in the Institute of Botany of the Czech Academy of Sciences at Třeboň, S Bohemia, Czech Republic. Adult plants of *Utricularia australis* R.Br., *U. vulgaris* L., *U. stygia* Thor (syn. *U. ochroleuca* R.Hartm. *sensu lato*), *U. ochroleuca* R.Hartm. *sensu stricto*, *U. intermedia* Hayne (all of them collected from the Czech Republic), *U. macrorhiza* Le Conte (from Canada), *U. stellaris* Linn.f. (from NE N.S.W., Australia), and *U. inflata* Walt. (from New Jersey, USA) were grown outdoors in six plastic containers which simulated their natural conditions (area 0.25–2 m^2, 90–750 L). Adult plants of *U. australis* (from Kashmir, NW India), *U. purpurea* Walt. (from Florida, USA), and *U. volubilis* R.Br. (from SW Australia) were grown in a naturally lit greenhouse in a 300 L plastic container, while two aquatic clones of *U. dichotoma* Labill. (smaller from Katoomba, N.S.W., Australia; robust from Newcastle, N.S.W., Australia), *U. humboldtii* Schomb. (from N Brazil), and *U. reniformis* A.St.Hil. (syn. *U. cornigera*, from N Brazil) were grown in 3 L aquaria floating in the same container for cooling. Adult plants of two populations of *U. reflexa* Oliver (from Okavango Delta, Botswana, and with giant traps from Zambia) and *U. aurea* Lour. (from Cambodia) were grown indoors under natural light in 3 L aquaria. Out of all species, *U. stygia, U. intermedia* and *U. ochroleuca* have dimorphic shoots differentiated into photosynthetic and carnivorous, while *U. humboldtii* and *U. reniformis* are epiphytic species forming markedly different terrestrial and aquatic shoots; the latter shoots bear relatively large traps (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 0.15–0.30 mM and the free CO_2 concentration was 0.10–0.15 mM (for all details, see Adamec, 1997b, 2011a,b; 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. All measurements were conducted on growing specimens between 10 June – 9 July 2015.

2.2. Measurement of critical negative pressure in traps

For the measurements, fully functional, mature young traps from the 4th to 6th mature leaf nodes (as counted from the shoot apex) were used in *Utricularia* species with linear shoots, while mature young to medium-old traps were used in all other species. For *U. vulgaris*, both young, rose-greenish traps from the 5th mature leaf node and old, pink traps from the 25th leaf node were used for comparison. Firstly, the direct measurement of the endogenous negative pressure inside traps was tested on large cut off traps of *U. vulgaris* (> 4.5 mm) and *U. reflexa* (>5 mm) using the published capillary method (see Sydenham and Findlay, 1973, 1975; Sasago and Sibaoka, 1985a; Singh et al., 2011), but in contrast to these former attempts, the negative pressure was continuously monitored by an electronic pressure sensor (JUMO dTRANS p30, type 404366, JUMO GmbH & Co. KG, Fulda, Germany). This pressure transducer with a piezo-resistive element and a voltaic output had a full range of 0 to −1 bar (relative to the atmospheric pressure), reproducibility ≤0.05% of full scale, response time ≤10 ms, and a thermal error of ≤0.04% K^{-1}. To reduce an electrical noise, the sensor's output was stabilised by a capacitator. The output was recorded using a 16-bit datalogger (DRAK5, Papouch Ltd., Prague, Czech Rep.) connected to a personal computer. The total accuracy of the whole measuring and recording system was ca. ±0.2 mbar with the half-time of the reaction ≤0.5 s.

Thin-walled glass capillaries for impaling the traps were around 2 cm long, 1.10 or 1.35 mm wide, and their tip (outer diameter) was 80–100 μm wide. The sensing cavity of the pressure sensor was carefully filled with a working solution (degased 0.1 mM KCl + 0.05 mM $CaCl_2$ + 0.2 mM $NaHCO_3$, pH 7.3–7.4; Adamec, 2011a,b) and the capillary filled with the same solution was

inserted watertight into the sensor's input pin using a 8 mm piece of a tygon tubing so that the capillary tip was immersed ca. 3–5 mm in the solution in a 50 mL perspex chamber. The signal of the sensor with the inserted capillary filled with the water was recorded as a baseline for the zero negative pressure. A trap excised under water in a container or aquarium was fired by shaking, transferred under water to the laboratory, and rinsed by tap water. Under a loupe with two times magnification, the bubble-free trap was carefully impaled by hand under water in the dorsal part of the trap with the capillary tip. Then, the impaled trap was immediately elevated above the water and carefully blotted dry superficially with a narrow strip of a soft paper tissue at the place surrounding the capillary insertion area. Simultaneously, the air from an air pump (RH ca. 50–60%) was blown onto the trap to dry the contact of the capillary with the trap for ca. 2 min. A droplet (ca. 1–2 μL) of a quickly solidifying cyanoacrylate adhesive normally used for attachment of aquarium plants (PlantaFix liquid, Dohse Aquaristik GmbH & Co. KG, Gelsdorf, Germany; www.dupla.com) was applied to the dried contact area using a nylon thread, and the adhesive was spread on the surface of the trap to ca. a 1.5 mm distance from this area. The adhesive solidifies quickly by the contact with water. Then, the sealed and impaled trap was slightly submerged again and the monitoring of the negative pressure started at 21–23 °C and in natural dim daylight. However, in 10 large traps (>4 mm) of *U. reflexa* (Botswana) or *U. vulgaris*, the maximal absolute value of the negative pressure of only 0.01–0.04 bar was formed after 2–4 h, which is only a fraction of the values reported in the literature (ca. −0.16 bar; Sydenham and Findlay, 1973, 1975; Sasago and Sibaoka, 1985a; Singh et al., 2011). The estimated membrane flexibility of the pressure sensor of 24 μL per 0.16 bar pressure change could theoretically only slow down the formation of the negative pressure, but not cease it. To test the quality of the trap wall-capillary contact, the connecting system between the impaled trap and the sensor's input pin was provided with a flexible PVC tubing and screwing clip allowing to manually set the negative pressure stepwise to speed up the attaining of the final "stable" negative pressure. After a negative pressure of −0.10 to −0.14 bar was preset by the screwing clip in a sealed impaled trap in the water, the absolute value of the negative pressure inside the trap was gradually decreasing (i.e., the negative pressure was getting weaker over time). In conclusion, these results suggested that the sealed contact between the trap wall and the capillary tip was leaky. It is not clear why Sydenham and Findlay (1973, 1975), Sasago and Sibaoka (1985a) and Singh et al. (2011) could measure directly the negative pressure in *Utricularia* traps without using any adhesive. As they used finer capillary tips (only 20–50 μm outer diameter) it is possible to assume that the tips were sealed by mucilage secreted by the traps themselves when in contact with the trap walls.

The above described experimental setup was then modified to allow for a slow and exact external application of the negative pressure to an impaled trap to determine the critical (or threshold) negative pressure at which the trap (located in air) fires and aspirates an air bubble like during a spontaneous firing; the trap firing was under visual control. A cut off and impaled trap without air bubbles, which was sealed with the capillary (filled with water as described above), was connected to an arm of a narrow Y–shaped glass fork, the base of which was inserted into the sensor's input pin. The other arm of the glass fork was connected to a regulated peristaltic pump (PCD 22 M, Kouřil Co., Kyjov, Czech Rep.) by a narrow, thick-walled tygon tubing (Fig. 1). This connecting system was filled only with air. After the cyanoacrylate adhesive had been applied onto the impaled trap (capillary tip diameter 80–120 μm) as described above, the trap was immersed in the water for at least 8 min to allow the adhesive to solidify properly. Afterwards, the impaled and sealed trap was put out of the water, carefully blotted dry, then the connecting system was inserted into the sensor,

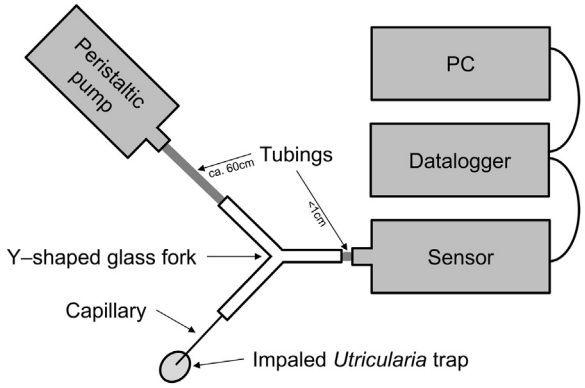

Fig. 1. Schematic drawing of the experimental setup used for the CNP measurements. Note that length ratios are not correct.

and the signal was recorded by an electronic datalogger (see above) every 1 s. The negative pressure was gradually applied by the peristaltic pump in two steps. Firstly, to attain ca. −0.10 to −0.12 bar, the pressure was changed rapidly at a rate of −5.1 to −6.1 mbar s^{-1} followed by a low rate of −0.56 to −0.84 mbar s^{-1}. The wet trap in the air was observed under a loupe with 2 times magnification. The gradual application of negative pressure to the impaled trap was monitored also by checking the moving meniscus in the capillary to exclude that the capillary tip is plugged. It was checked experimentally that the capillaries used had sufficient a hydraulic conductivity so as not to delay the spreading of the applied negative pressure into the trap interior. As soon as the applied and recorded negative pressure inside the trap caused the first bubble aspiration due to spontaneous firing, the pump was stopped. The values of the negative pressure recorded just after the firing and before were averaged and taken as the CNP needed for spontaneous trap firing. The whole measurement usually lasted 2–3 min. As based on the application rate of the negative pressure and its recording rate, it may be considered that the total inaccuracy of the estimation of the CNP did not exceed 0.6–0.8 mbar. Trap length was approximated to the nearest 0.1 mm by measuring with a small ruler, again viewed under a loupe with 5 times magnification. Generally, the measurements could be conducted on traps larger than 2 mm. For methodical reasons, it has not been possible to conduct the CNP measurements in terrestrial *Utricularia* traps which are smaller. The CNP measurements were conducted on cut-off traps as we could not manipulate with intact plants/leaves above water. We decided to conduct the CNP measurements in the air for methodical reasons as it would be hardly possible to determine the moment of trap firing under water. All measurements were conducted at 22–27 °C. Within each species, the temperature varied only within ±1 °C.

2.3. Statistical analyses

For each plant species (and/or trap age or size group or population), 12 measurements on traps from different leaves usually on different shoots were conducted and means ± SE intervals were calculated. The statistically significant differences between giant and small traps of *U. reflexa* (Zambia), young and old traps of *U. vulgaris*, two populations of *U. australis*, between *U. stygia, U. ochroleuca* s. str. and *U. intermedia* (this subgroup comprises very similar three species with markedly differentiated shoots into photosynthetic and carnivorous), and between two *U. dichotoma* populations, were tested using 1-way ANOVA (Tukey HSD test). Linear regression models were used to look for statistically significant relationships between the critical negative pressure and trap size within each species (and/or trap age or size group or population; $n = 12$) and for all 20 variants pooled together ($n = 240$).

Table 1
Values of externally applied, critical negative pressure necessary for *Utricularia* trap-door opening.

Generic section	Species	Trap length (mm)	Critical negative pressure (bar) (range in parentheses)	Comp.items
UTR	*U. reflexa* (Botswana)	4.0–5.7	−0.170 ± 0.009 (−0.125 to −0.229)	
–“”-	*U. reflexa* (Zambia): giant traps	6.7–7.2	−0.069 ± 0.007 (−0.039 to −0.112)*	A
–“”-	– “” –: small traps	3.4–3.9	−0.122 ± 0.005 (−0.092 to −0.144)*	A
–“”-	*U. macrorhiza*	3.0–3.4	−0.129 ± 0.008 (−0.080 to −0.182)	
–“”-	*U. vulgaris*: young traps	3.3–4.2	−0.117 ± 0.010 (−0.057 to −0.165)*	B
–“”-	*U. vulgaris*: old traps	3.1–4.3	−0.188 ± 0.006 (−0.154 to −0.218)*	B
–“”-	*U. stellaris*	2.1–2.8	−0.193 ± 0.007 (−0.167 to −0.253)	
–“”-	*U. australis*: Czech Republic	2.5–3.0	−0.176 ± 0.006 (−0.139 to −0.214)*	C
–“”-	*U. australis*: Kashmir, NW India	2.9–3.9	−0.148 ± 0.006 (−0.110 to −0.194)*	C
–“”-	*U. aurea*	2.1–2.8	−0.143 ± 0.013 (−0.078 to −0.217)	
–“”-	*U. inflata*	2.6–2.9	−0.157 ± 0.005 (−0.131 to −0.187)	
–“”-	*U. stygia*	3.3–4.0	−0.217 ± 0.007 (-0.175 to −0.252)ns	D
–“”-	*U. ochroleuca* s. str.	3.4–3.8	−0.252 ± 0.010 (−0.208 to −0.332)ns	D
–“”-	*U. intermedia*	2.8–3.7	−0.252 ± 0.020 (−0.114 to −0.366)ns	D
VES	*U. purpurea*	2.1–2.6	−0.109 ± 0.009 (−0.071 to −0.189)	
PLE	*U. dichotoma* (NSW, Katoomba)	1.9–2.6	−0.327 ± 0.021 (−0.178 to −0.449)ns	E
–“”-	*U. dichotoma* (NSW, Newcastle)	2.4–2.9	−0.346 ± 0.011 (−0.276 to −0.412)ns	E
–“”-	*U. volubilis*	2.8–4.2	−0.332 ± 0.014 (−0.227 to −0.398)	
IPE	*U. humboldtii*	2.9–4.0	−0.261 ± 0.014 (−0.157 to −0.339)	
–“”-	*U. reniformis* (=*U. cornigera*)	3.0–3.6	−0.184 ± 0.016 (−0.093 to −0.278)	

UTR, *Utricularia*; VES, *Vesiculina*; PLE, *Pleiochasia*; IPE, *Iperua*. Means ± SE intervals and the range of values shown; $n = 12$. The statistically significant difference (1-way ANOVA) between giant and small traps of *U. reflexa* (Zambia), young and old traps of *U. vulgaris*, Czech and Indian plants of *U. australis*, between *U. stygia, U. ochroleuca* s. str. and *U. intermedia*, or between two *U. dichotoma* populations is indicated by asterisk ($P < 0.01$); *ns*, $P > 0.05$. The compared species or variants are labelled by the same letters in the right column.

2.4. Test of the ability of traps to fire

A simple test was designed to demonstrate the ability of cut off *Utricularia* traps to fire spontaneously and after a mechanical stimulation in moist air. The test is an extremely simple but efficient method to demonstrate spontaneous and stimulated firing in many traps simultaneously without a need of any costly and complicated technique, which is otherwise concentrated only on one trap (cf. Adamec, 2011a; Vincent et al., 2011b). The test is based on the recent observation that cut off traps can normally and repeatedly fire in moist air (Adamec, 2012). Leaves of *U. vulgaris* from the 5th and 6th leaf nodes with young rose-coloured traps 3–4 mm large were excised under water in the cultivation container and transferred to the laboratory. The leaves were shaken to stimulate the traps to fire, the traps were excised, shortly rinsed by the working solution (see above), and put on a filter paper wetted slightly with distilled water in 6 cm plastic Petri dishes. Twenty traps without air bubbles from one plant were put into each of 5 Petri dishes. The Petri dishes were exposed in a thermostatted growth chamber at 25 ± 1 °C in darkness for 5 h. After the exposure of 3.5 and 5 h, traps with an air bubble were counted under a loupe with 5 times magnification. After 5 h, the remaining traps without any air bubble were gently stimulated to fire by a fine brush (Adamec, 2011a,b, 2012). Firing was scored as an aspiration of an air bubble inside the trap (see Adamec, 2012).

3. Results

Mean values of the externally applied CNP differed in various species or variants up to 5 times from each other and ranged from −0.069 bar in giant traps of *U. reflexa* (Zambia) to −0.346 bar in *U. dichotoma* (N.S.W., Newcastle; Table 1). Within each species or variant, high variability was found: the lowest values were usually 2–3 times lower than the highest ones. The mean value in all 20 species or variants tested was −0.195 ± 0.018 bar, while that in all young traps in 13 species or variants of the generic section *Utricularia* was −0.165 ± 0.015 bar. Giant traps of *U. reflexa* (Zambia) 6.7–7.2 mm large attained a significantly lower absolute value of the negative pressure (−0.069 ± 0.007 bar) than small traps (3.4–3.9 mm) of the same plants (−0.122 ± 0.005 bar). However, young traps of *U. vulgaris* attained a significantly lower absolute value of the CNP than old ones though the trap sizes were the same. Traps of two *U. australis* populations from the Czech Republic and NW India also differed significantly in the CNP. Within the generic section *Utricularia*, the trinity of very similar species with dimorphic shoots (*U. stygia, U. ochroleuca* s. str., *U. intermedia*) attained the highest absolute values of the CNP between −0.217 and −0.252 bar. Within this subgroup, the values for single species did not significantly differ from the other ones at $P < 0.05$. From all species tested, *U. dichotoma* and *U. volubilis* with three populations ($n = 36$) from the generic section *Pleiochasia* attained the highest absolute values of the CNP (−0.327 to −0.346 bar). The two different *U. dichotoma* populations did not differ significantly from each other in the parameter.

Out of all linear regression models relating the CNP with trap size within each species or variant, only that for *U. dichotoma* (N.S.W., Katoomba) was significant at $P = 0.015$ ($r^2 = 0.462$; $n = 12$), while all the other species were not ($P > 0.10$; data not shown). However, all pooled data for 20 species or variants ($n = 240$) showed a highly significant linear correlation (Fig. 2): absolute value of the CNP (bar) = −0.0277 trap length (mm) + 0.289 ($r^2 = 0.125$; $P < 0.0001$) and a similar significant correlation was also found for 13 species or variants ($n = 156$, excluding only old *U. vulgaris* traps) of the *Utricularia* section. Thus, larger traps attain a lower absolute value of the CNP than smaller traps. However, the correlation between the CNP and trap length was not significant in three populations of the *Pleiochasia* section.

The test with young cut off *U. vulgaris* traps kept in moist air clearly demonstrated that on average 34.0 ± 6.6% traps fired spontaneously by 3.5 h and 54.0 ± 9.4% traps by 5 h. On average after 5 h, the other remaining 43.0 ± 10.3% reset (unfired) traps fired after the mechanical stimulation and also 79.0 ± 3.7% traps with a bubble, which had fired spontaneously formerly, fired repeatedly after the stimulation. Thus, totally 97.0 ± 2.0% (range 90–100%) traps fired either spontaneously or after the stimulation after 5 h, and the visual detection of all firings in moist air was very simple.

4. Discussion

A negative pressure ranging from −0.12 to −0.17 bar was measured directly inside 2–3 mm large traps of three aquatic *Utricularia* species in three studies (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985a; Singh et al., 2011). Surprisingly, glass capillaries

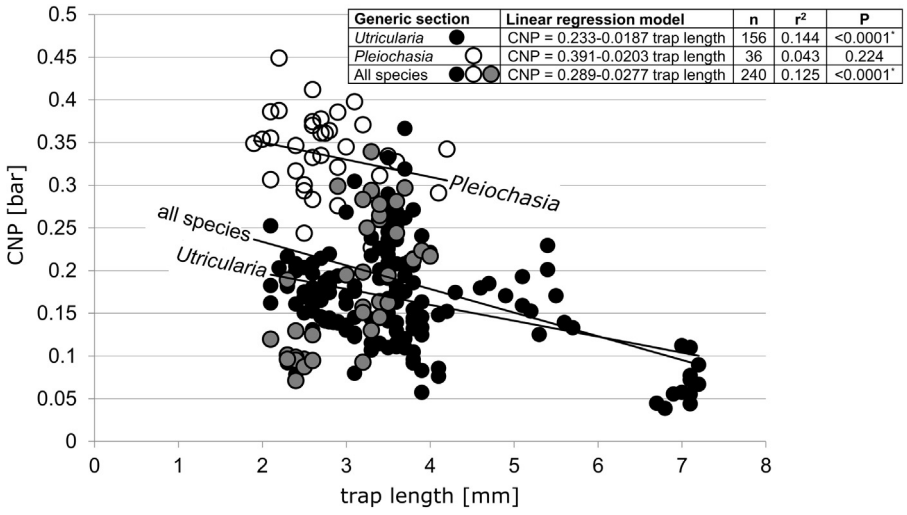

Fig. 2. Relationships between trap lengths and CNPs for generic sections *Utricularia* (black dots), *Pleiochasia* (white dots) and for all species (all dots), presented as scatter plot with regression lines. The grey dots belong to the "all species" group and comprise species from generic sections *Iperua* and *Vesiculina*. The linear regression models between CNP and trap length for different species groups are indicated as inlet (above right). Absolute values of CNP in bar, trap length in mm. As a result of Bonferroni correction, only values of $p < 0.0167$ represent significant correlation (indicated by asterisk); r^2, coefficient of determination.

with outer tip diameter of 20–50 μm used for trap impalement were *not* sealed in these studies by any adhesive, but no apparent leakage occurred. In contrast, during our direct measurements on much larger traps (>4 mm) by using capillaries with 80–100 μm wide tips and by sealing the impalement area with an underwater adhesive, leakage of the trap wall evidently occurred. However, this small leakage could not influence the results of short-term measurements of the CNP. In the experimental equipment for measuring the CNP inside traps, the only limiting step in transmitting the generated pressure changes to the trap lumen was the hydraulic conductivity of the capillary tip for water, but not inertia of the sensor. It was verified, however, that the applied low rate of pressure changes from −0.56 to −0.84 mbar s^{-1} was transmitted into the impaled trap rapidly enough, with the delay not exceeding ca. 0.5–1 s.

Spontaneous trap firings occur more or less commonly in all aquatic or amphibious *Utricularia* species (Adamec 2011a,b; Vincent et al., 2011a,b; Płachno et al., 2015). Therefore, the estimation of the CNP needed for trap firing simulates the physiologically formed negative pressure needed for spontaneous firing. Using a dynamic model simulation, Vincent et al. (2011a) calculated from the mechanical properties and geometry of the trap door that the CNP theoretically is −0.155 bar; trap-door buckling and opening then occurs spontaneously. When the pressure difference (absolute value) is lower in a resetting trap, the trap door is in a metastable state and sensitive to any above-threshold external mechanical stimulation (*e.g.*, zooplankton) to trigger its buckling. The sensitivity depends on the negative pressure inside the trap and the water pressure acting on the door. Values very similar to the theoretical CNP (-0.12 to −0.17 bar) were measured directly in three aquatic *Utricularia* species (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985a; Singh et al., 2011) and in the present study (Table 1).

In spite of the very accurate CNP measurements (totally < ± 0.8 mbar), the values are highly variable within each species or variant (Table 1). Accepting the concept by Vincent et al. (2011b) on the three types of spontaneous firings inherent to each single trap, we suggest that the high variability of values in our measurements could be the physical basis for these three types of spontaneous firings. The lowest absolute values of the CNP measured within each species or variant could correspond to the bursting type (characterized by a short sequence of several spontaneous firings), the medium values to the metronomic one (characterized by relatively long and constant periods between each spontaneous firing), while the highest absolute values to the random and bursting ones. The biophysical diversification of *Utricularia* traps in the three types can result in a diversification of prey capture. A frequently firing trap of the bursting type would generate only a comparably lower pressure difference (Vincent et al., 2011b), aspirate a lower volume of the water, and persist much shorter period in the reset state. Thus, on the one hand, their efficiency to capture prey is probably restricted to small animals, but on the other hand, they would aspirate totally a great volume of the ambient water with phytoplankton and other suspended particles as a potential nutrient source for trap microbial commensals or directly for the trap (see Richards, 2001; Peroutka et al., 2008; Sirová et al., 2009; Adamec, 2011b). In contrast, traps belonging to the random and metronomic types would generate higher pressure differences, persist in the reset states for much longer time, aspirate greater water volumes, and thus could potentially capture also fast or larger prey animals trying to escape the aspiration zone (cf. Vincent et al., 2011a). The comparably long waiting periods, with the traps becoming more and more sensitive to mechanical perturbations, would even suggest that also much smaller (weaker) prey is capable of triggering the door, given that triggering is indeed purely mechanical.

Generally, this biophysical trap type diversification together with different trap sizes could contribute to a wide range of prey selection in environments with various prey availability instead of possessing a narrow prey spectrum which could be disadvantageous under certain circumstances. Mean CNP values in traps of 15 aquatic *Utricularia* species cover a wide interval from −0.069 to −0.346 bar (Table 1) with a mean of −0.195 ± 0.018 bar for all species and variants. Also, some differences between the generic sections were found. The mean values of young traps of 10 species of the *Utricularia* section (−0.165 ± 0.015 bar; $n = 156$) were significantly different ($F = 239.4$; $P < 0.0001$; 1-way ANOVA) from those in two species of the *Pleiochasia* section (−0.335 ± 0.009 bar; $n = 36$). Trap walls of all species from the *Utricularia* section tested so far contain only two cell layers, while several species from the Australian *Pleiochasia* section (including *U. dichotoma* – Newcastle and *U. volubilis*) possess 3–4 cell layers (Reifenrath et al., 2006; Płachno et al., 2015). Yet, the relationship between the number of cell layers in trap walls (or trap wall thickness) and the CNP may not exist as

the smaller clone of *U. dichotoma* (from Katoomba) with only two cell layers and thinner trap walls (Płachno et al., 2015) did not differ in the CNP from that from Newcastle with 3–4 cell layers and thicker walls. Similarly, no relationship was found between the number of cell layers and trap resetting rates as a criterion of trap efficiency in *U. dichotoma* (two clones) and *U. volubilis*, either (Adamec, 2011b; Płachno et al., 2015). However, traps of *U. dichotoma* and *U. volubilis* exhibited ca. 2–5 times lower firing magnitudes and resetting rates than was the total average of 13 species mainly of the *Utricularia* section, they showed a distinct lag-period after firing (unlike the *Utricularia* section; Adamec, 2011b; Płachno et al., 2015), and also generated much greater pressure difference in their traps than the *Utricularia* section species (Table 1).

It can be concluded that these biophysical trap differences between the dissimilar generic sections are not due to different numbers of trap cell layers but probably rather to different mechanical properties of trap walls and doors (stiffness, elasticity) and/or different physiological activities of the traps (water pumps). According to the theory of Vincent et al. (2011a), traps of the *Pleiochasia* section could possess not only stiff trap walls (*i.e.,* low firing magnitude and resetting rate; see Adamec, 2011b; Płachno et al., 2015) but also a very stiff trap door allowing to buckle (fire) only at very high absolute values of the CNP. In line, the thickness of the central part of the trap door should be crucial for the trap-door stiffness and the magnitude of the CNP. Anyway, the ratio between the stiffness (~thickness) of the lateral walls of the trap body and that of the trap door is decisive for the biophysical properties of traps concerning the firing (*i.e.*, the fired volume and rate of water aspiration is high when the trap door is considerably thinner than the trap body; Vincent et al., 2011a) and could vary in the phylogeny of different taxa to meet species-specific needs for prey capture. The results of CNP estimated both for all 20 *Utricularia* species and 13 species of the *Utricularia* section significantly ($P < 0.0001$) confirm the trend that larger traps attain lower absolute values of the CNP. This relationship was also striking in the Zambian clone of *U. reflexa*: the absolute value of the CNP in its giant traps was almost two times lower than that in much smaller traps. The marked difference in the CNP between young and old traps of the same size in *U. vulgaris* probably indicates some stiffness changes during the trap life-span.

Direct instrumental demonstration of *Utricularia* trap firing (esp. in small traps) is relatively difficult, complicated and costly (Adamec, 2011a; Vincent et al., 2011a). The simple test of trap firing with cut off *Utricularia* traps kept in a moist air for several hours was shown to be very reliable and instructive and could clearly demonstrate spontaneous and stimulated firing without any demanding facilities (*cf.* Adamec, 2011a; Vincent et al., 2011a,b) and could also be used for smaller traps (1-1.5 mm). Many endogenous or external factors (*e.g.*, trap age and size, captured prey, growth conditions, effect of various substances) could be tested in regard to spontaneous firings in this way.

One out of the crucial questions on the trap functioning is whether *Utricularia* traps are stimulated to fire via the electrophysiological signalling pathway (including a rise in action potentials in the trigger hairs) or purely mechanically by a force on the trigger hairs which act as levers (see Sydenham and Findlay, 1973; Vincent et al., 2011a,b; Adamec, 2012). As reviewed by Juniper et al. (1989), all other carnivorous plant genera with rapid trap movements exhibit the electrophysiological way of regulation. However, the integration of the all above recent findings on *Utricularia* trap functioning – mainly spontaneous firings and use of metabolic inhibitors and low temperature – rather supports indirectly the mechanical concept of trap triggering (Adamec, 2012).

In conclusion, spontaneous trap firing in aquatic *Utricularia* species can be considered a universal and (most presumably) essential phenomenon in all aquatic/amphibious species contributing to a greater diversity of captured prey or aspirated suspended particles and, probably, to a greater benefit of traps. Further studies could specify on which environmental (*e.g.*, prey availability, water chemistry and temperature, irradiance) or endogenous factors (*e.g.*, trap N and P content, captured prey, trap size and age) the characteristics of the spontaneous firing and the magnitude of the CNP depend. Moreover, spontaneous firings triggered by physical forces exclude the electrophysiological concept on trap triggering (Adamec, 2012). At least within the *Utricularia* generic section with a high firing-resetting trap efficiency, universal spontaneous firings also clearly support the concept of continuous water pumping out of the traps (Adamec, 2011b). A great variability of CNP values by the factor of 5 was found among various *Utricularia* species in the present study; the mean value for the *Pleiochasia* generic section was two times higher than that for 13 species of the *Utricularia* section. This great difference raises the question whether the biochemical mechanism of water pumping out of the traps is unified for all *Utricularia* species.

Acknowledgements

This study was supported (for LA) partly by the Research Programme of the Czech Academy of Sciences (No. RVO 67985939). The contribution of SP was supported by the Innovationsfonds Forschung of the University of Freiburg. Sincere thanks are due to Prof. Thomas Speck for critically reading the manuscript. Thanks are also due to Prof. Elisabeth M. Gross and two anonymous referees for valuable comments.

References

Adamec, L., 1997a. Mineral nutrition of carnivorous plants: a review. Bot. Rev. 63, 273–299.
Adamec, L., 1997b. How to grow *Aldrovanda vesiculosa* outdoors. Carniv. Plant. Newslett. (Fullerton) 26, 85–88.
Adamec, L., 2008. Mineral nutrient relations in the aquatic carnivorous plant *Utricularia australis* and its investment in carnivory. Fundam. Appl. Limnol. 171, 175–183.
Adamec, L., 2011a. The comparison of mechanically stimulated and spontaneous firings in traps of aquatic carnivorous *Utricularia* species. Aquat. Bot. 94, 44–49.
Adamec, L., 2011b. Functional characteristics of traps of aquatic carnivorous *Utricularia* species. Aquat. Bot. 95, 226–233.
Adamec, L., 2012. Firing and resetting characteristics of carnivorous *Utricularia reflexa* traps: physiological or only physical regulation of trap triggering? Phyton 52, 281–290.
Fleischmann, A., 2015. Taxonomic *Utricularia* news. Carniv. Plant. Newslett. (Fullerton) 44, 13–16.
Friday, L.E., Quarmby, C., 1994. Uptake and translocation of prey-derived 15 N and 32 P in *Utricularia vulgaris* L. New Phytol. 126, 273–281.
Guisande, C., Granado-Lorencio, C., Andrade-Sossa, C., Duque, S.R., 2007. Bladderworts Funct. Plant Sci. Biotechnol. 1, 58–68.
Harms, S., 1999. Prey selection in three species of the carnivorous aquatic plant *Utricularia* (bladderwort). Arch. Hydrobiol. 146, 449–470.
Jobson, R.W., Playford, J., Cameron, K.M., Albert, V.A., 2003. Molecular phylogeny of Lentibulariaceae inferred from *rps16* and *trnL-F* chloroplast gene regions: implications for character evolution and biogeography. Syst. Bot. 28, 157–171.
Joeyux, M., Vincent, O., Marmottant, P., 2011. Mechanical model of the ultrafast underwater trap of *Utricularia*. Phys. Rev. Lett. E 83, 021911.
Juniper, B.E., Robins, R.J., Joel, D.M., 1989. The Carnivorous Plants. Academic Press, London.
Płachno, B.J., Adamec, L., Kamińska, I., 2015. Relationship between trap anatomy and function in Australian carnivorous bladderworts (*Utricularia*) of the subgenus *Polypompholyx*. Aquat. Bot. 120, 290–296.
Peroutka, M., Adlassnig, W., Volgger, M., Lendl, T., Url, W.G., Lichtscheidl, I.K., 2008. Utricularia: a vegetarian carnivorous plant? Algae as prey of bladderwort in oligotrophic bogs. Plant Ecol 199, 153–162.
Poppinga, S., Weisskopf, C., Westermeier, A.S., Masselter, T., Speck, T., 2016. Fastest predators in the plant kingdom: functional morphology and biomechanics of suction traps found in the largest genus of carnivorous plants. AoB Plants 8, plv140.
Reifenrath, K., Theisen, I., Schnitzler, J., Porembski, S., Barthlott, W., 2006. Trap architecture in carnivorous *Utricularia* (Lentibulariaceae). Flora 201, 597–605.
Richards, J.H., 2001. Bladder function in *Utricularia purpurea* (Lentibulariaceae): is carnivory important? Am J. Bot. 88, 170–176.

Sasago, A., Sibaoka, T., 1985a. Water extrusion in the trap bladders of *Utricularia vulgaris* I: a possible pathway of water across the bladder wall. Bot. Mag. 98, 55–66.
Sasago, A., Sibaoka, T., 1985b. Water extrusion in the trap bladders of *Utricularia vulgaris* II: a possible mechanism of water outflow. Bot. Mag. 98, 113–124.
Singh, A.K., Prabhakar, S.P., Sane, S.P., 2011. The biomechanics of fast prey capture in aquatic bladderworts. Biol. Lett. 7, 547–550.
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.
Sirová, D., Borovec, J., Černá, B., Rejmánková, E., Adamec, L., Vrba, J., 2009. Microbial community development in the traps of aquatic *Utricularia* species. Aquat. Bot. 90, 129–136.
Sydenham, P.H., Findlay, G.P., 1973. The rapid movement of the bladder of *Utricularia* sp. Aust. J. Biol. Sci. 26, 1115–1126.
Sydenham, P.H., Findlay, G.P., 1975. Transport of solutes and water by resetting bladders of *Utricularia*. Aust. J. Plant Physiol. 2, 335–351.
Taylor, P., 1989. The Genus *Utricularia*: A Taxonomic Monograph. Kew Bulletin, Additional Series, XIV.
Vincent, O., Marmottant, P., 2011. Carnivorous *Utricularia:* the buckling scenario. Plant Signal. Behav. 6, 1752–1754.
Vincent, O., Weisskopf, C., Poppinga, S., Masselter, T., Speck, T., Joyeux, M., Quilliet, C., Marmottant, P., 2011a. Ultra-fast underwater suction traps. Proc. R. Soc. B 278, 2909–2914.
Vincent, O., Roditchev, I., Marmottant, P., 2011b. Spontaneous firings of carnivorous aquatic *Utricularia* traps: temporal patterns and mechanical oscillations. PLoS One 6, e20205.