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Physiological characterisation of aquatic traps in the epiphytic carnivorous plant *Utricularia humboldtii*

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ABSTRACT:

The suction traps of aquatic *Utricularia* species are discoid bladders, 1–6 mm long, with their lumen isolated by a mobile trapdoor from the ambient medium. Water is pumped out of the traps to maintain the negative pressure in the reset traps. When a prey animal touches the sensory hairs on the trapdoor it opens, the ambient water along with the prey is sucked inside and the trapdoor is closed again. *Utricularia humboldtii* is a robust, semiaquatic–epiphytic or terrestrial species from the generic section *Orchidioides* from South America. The efficiency of its aquatic traps was measured based on trap firing and resetting rates as well as changes in trap thickness due to both mechanically stimulated and spontaneous firings using an electronic sensor. The *U. humboldtii* traps exhibited relatively low firing and resetting rates. These values are 2 to 20 times lower than those in other aquatic *Utricularia* species reported in the literature. These results together with the low aerobic respiration rate of sliced traps indicate that the less efficient aquatic traps in *U. humboldtii* are specialised for catching fine prey items in bromeliad water tanks. A marked lag-period in trap resetting was found to occur during the first 30 min after firing, contradicting the accepted concept of continuous water pumping. Spontaneous trap firings were also observed with the same magnitude as that in stimulated firings. This implies that spontaneous firings occur consistently in all the tested aquatic *Utricularia* species. However, although they relate to the continuous water pumping mechanism of the traps, their physiological importance has not been fully elucidated.

INTRODUCTION

The suction traps of aquatic *Utricularia* (Lentibulariaceae) species are hollow oval or discoid bladders (utricles), 1–6 mm long, and their lumen is hermetically closed by a flexible mobile trapdoor from the ambient medium (JUNIPER *et al.* 1989; TAYLOR 1989; POPPINGA *et al.* 2016; WESTERMEIER *et al.* 2017; ADAMEC 2018). Four types of glands occur in the traps, with numerous quadrifid and bifid glands being of crucial importance for trap physiology: they play an active role in prey digestion, nutrient uptake and water pumping. The bifid glands situated on the trapdoor actively pump water out of the trap, thereby maintaining a negative pressure relative to the ambient medium of ca. -0.16 bar in a fully reset trap. When a prey animal touches the sensory hairs on the trapdoor, it opens, the ambient water along with the prey is sucked inside and the trapdoor is closed again. The whole trap firing lasts only 3–5 mins. Commonly, traps also fire spontaneously when the negative pressure generated inside the trap reaches the critical value required for trapdoor opening. Water pumping out of the trap is generally believed to occur continuously but is not regulated biophysically. Significant trap volume changes are caused by trap firing and can reach up to 40% of the volume of reset traps. Since the trap thickness (the distance

between the two parallel trap walls) changes markedly due to trap firing and resetting, measuring trap thickness is frequently used for the quantification of water flow or firing/resetting. After firing, trap resetting lasts ca. 25–40 min until the trap can fire again, but full resetting takes much longer (Adamec 2011a, b, 2018; Poppinga *et al.* 2016; Westermeier *et al.* 2017).

The traps of aquatic *Utricularia* species execute demanding physiological functions (the secretion of digestive enzymes, the uptake of nutrients from digested prey carcasses and water pumping) and exhibit an intensive energy metabolism despite their inner trap fluid being almost anoxic (Adamec 2007). Their aerobic dark respiration (RD) rate (5.1–10.2 mmol O_2 $kg^{-1}h^{-1}$ per unit fresh weight, FW), measured in seven aquatic species, was 1.7–3.0 times higher than that in leaves and/or shoots (Adamec 2006, 2011b, 2014, 2018). However, the net photosynthetic rate of the traps is relatively low, approximately one order of magnitude lower than that of leaves/shoots, thus illustrating the high maintenance and photosynthetic costs of these mechanisms (Adamec 2018).

Utricularia humboldtii Schomb. is a robust, perennial, semiaquatic–epiphytic or terrestrial species from the northern tropical part of South America (Taylor 1989). Based on morphological traits and life form, Taylor (1989) classified this 'orchid-like' species within the *Utricularia* generic section *Iperua,* which was closely related to the section *Orchidioides.* Recently, however, based on both molecular markers and morphological and anatomical traits, Rodrigues *et al.* (2017) included the species in the unified section *Orchidioides,* joining both sections into one. This concept has been accepted in subsequent taxonomic studies (e.g. Silva *et al.* 2018).

Utricularia humboldtii has distinctly dimorphic leaves: the aerial ones are cuneate or reniform, coriaceous, usually at least 2–6 cm wide and long petiolate, while the submerged aquatic leaves on special (semi) aquatic stolons are much smaller, membraneous and divided into linear or filiform segments bearing abundant aquatic traps (Fig. 1; Taylor 1989). This species grows in water-filled tanks in the leaf axils of bromeliads and also as epiphytes on tree trunks or in very wet soils in open savanna, commonly in tepuis (Taylor 1989). The discoid aquatic traps can be 3–12 mm long (Fig. 1) and belong to the largest traps within the genus. However, as seen in Fig. 1, the trap size in cultured plants is usually only 2–4 mm.

Very little is known about trap functioning traits in *U. humboldtii* (and relative species from the section *Orchidioides*) since most knowledge on trap functioning has been obtained from the evolutionary advanced generic section *Utricularia* with efficient traps (Adamec 2006, 2011a, b, 2013, 2018; Poppinga *et al.* 2016; Westermeier *et al.* 2017). Using a fine pressure sensor, Adamec & Poppinga (2016) measured the critical negative pressure in *U. humboldtii* aquatic traps when the trapdoor can no longer withstand the negative pressure inside the trap causing it to fire. The value of the critical negative pressure in this species was -0.26 ± 0.01 bar, comparable with the highest values measured in species from the section *Utricularia.* This study aims to measure the trap firing and resetting rates as a result of both mechanically stimulated and spontaneous firing in *U. humboldtii* so as to compare the efficiency of its trap firing and resetting with other species from the section *Utricularia* (Adamec 2011b). The aerobic RD was also measured in its traps as an indicator of the trap metabolism intensity.

MATERIALS AND METHODS

Plant cultivation. *Utricularia humboldtii* (origin from N Brazil) was grown in an indoor 30-l, naturally lit aquarium situated near a window in small plastic pots on a mixture of brown filamentous peat and perlite. The pots with terrestrial shoots stood in ca. 2.5 cm deep dystrophic, brownish water where the aquatic shoots with traps grew (Fig. 1). They were 15–18 cm long.

Measurement of trap thickness. As a measure of water flow and changes in trap volume during the processes of trap firing and resetting, the trap thickness was measured electronically in isolated traps of *U. humboldtii*. Mature traps 3.0–4.2 (mostly 3.2–3.7) mm long were excised directly underwater in the aquarium and trap firing was induced by shaking the traps to prevent the suction of bubbles into the trap during handling. After washing in tap water, the traps (without air bubbles or macroscopic prey) were immediately transferred to a 10 ml Perspex chamber containing a solution of 0.1 mM KCl, 0.05 mM $CaCl_2$ and 0.2 mM $NaHCO_3$ with a pH of 7.3–7.4 (Adamec 2011a, b). The fired traps were carefully inserted laterally into the holder of an electronic position sensor to measure any changes in trap thickness (for all technical details, see Adamec 2011a). The position sensor had a 1 µm resolution and applied a force not exceeding the weight of 0.4 g against the fixed trap. Trap thickness was monitored at 30 s intervals. The water temperature was within the range of 24.2 to 25.5°C for all the measurements, with a temperature change of < 1°C for individual measurements, and the traps were exposed to natural dim daylight. The experimental scheme was as follows: 3 h after the initial trap insertion, using a loupe, the fixed reset traps were firstly mechanically stimulated to fire using a very fine brush which gently touched the sensitive trigger hairs (Adamec 2011a, b). The stimulated firing was repeated twice with an interval of 3 h between each stimulation. The measurement then continued for a further 10–12 h to record spontaneous trap firings or the maximal resetting range. All firings were considered spontaneous if they occurred without any mechanical stimulation.

Table 1. Trap firing and resetting rates in the excised aquatic *Utricularia humboldtii* traps. The data for the first, second and third firings within the individual traps were pooled together; for the mechanically stimulated traps, n = 17–25; for the spontaneous firing, n = 5. Note the distinct lag period of resetting immediately after firings. The maximal resetting shows an apparently stable resetting after 4-8 h. The values in parentheses show the range of values. The different letters denote a significant difference (p < 0.05) between two types of firing. Means ± SE are shown.

Type of firing	Firing (μm)	Resetting (μm) after			Max. resetting range (μm)
		10 min	30 min	60 min	
Mechanically stimulated	69.4 ± 6.6[a] (23–146)	1.43 ± 0.43[a] (0–6)	8.35 ± 1.48[a] (0–21)	21.8 ± 2.6[a] (5–44)	112.8 ± 17.2 (31–273)
Spontaneous	62.2 ± 10.6[a] (24–89)	0.40 ± 0.40[b] (0–2)	3.60 ± 1.44[b] (0–8)	13.4 ± 4.2[a] (3–28)	--

Fig. 1. The stolons with aquatic traps of *Utricularia humboldtii* used for the study. Ticks indicate 1 mm. Photo by L. Adamec.

Measurement of trap respiration. The aerobic RD rate of isolated aquatic *U. humboldtii* traps was measured using a fine Clark-type oxygen sensor, an electrochemical multimeter and a chart recorder (for technical details, see ADAMEC 1997). In brief, the RD of seven freshly collected, mature and partly sliced traps ranging from 2.2–3.5 mm long (FW 11–18 mg; dry matter content 5.71%) was measured in the same solution as previously described in a 1.58 ml thermostatted, magnetically stirred chamber at 25.0 ± 0.1°C in the dark for 15 min. As the traps were partly sliced their inner structures were in contact with the ambient solution, so the RD of the inner struc-

tures was also included ('total' RD; see ADAMEC 2011b). The RD measurements were repeated six times. The RD rate is expressed in mmol $kg^{-1} h^{-1}$ per unit FW.

Statistical treatment. The following parameters were evaluated (after ADAMEC 2011b): changes in trap thickness due to stimulated or spontaneous firings as a measure of total water flow, resetting rates after both types of firing over 10, 30 and 60 min as a measure of the efficiency of the traps to pump water out of the traps, and the maximal resetting (usually after 4–8 h of resetting) when no changes were obvious. In total, 11 traps were

used for all measurements. There were no indications that the first, second and third trap firings differed from each other and, thus, all the data were pooled together (n = 17–25; n = 5 for the spontaneous firings). The results obtained on unresponsive traps were discarded. One-way ANOVA (Statistica v. 5) was used to identify any significant differences in the firing and resetting rates over 10, 30 and 60 min after firing between both types of trap firing. Means ± SE are shown.

RESULTS AND DISCUSSION

Trap respiration. The total RD rate of the sliced *U. humboldtii* traps was 4.04 ± 0.52 mmol kg^{-1} h^{-1} per unit FW (data not tabulated). Compared to the RD rates of intact or sliced traps in seven aquatic species from the section *Utricularia* (cf. Adamec 2006, 2007, 2011b, 2014, 2018), the DR rate of *U. humboldtii* traps was approximately 1.5–2.5 times lower. Accordingly, the physiological activity of these aquatic traps is apparently markedly lower than that of the highly efficient traps from the section *Utricularia*.

Trap firing and resetting rate. In the *U. humboldtii* traps, the mean values of the mechanically stimulated and spontaneous firings were similar (69.4 *vs.* 62.2 μm) and did not differ significantly (p > 0.05) from each other (Table 1). However, the resetting rates after the stimulated firings were approximately 1.7–3.5 times higher than those following the spontaneous firings, and for the 10- and 30-min periods after firing, the differences were significant. After both types of firing, the trap resetting rates were markedly non-linear in time. The resetting rates after the first 10 min were very low and suggest a marked lag-period. Between 10 and 30 min, the resetting rate increased, reaching its maximum between 30 and 60 min (Table 1). The traps were able to reset for at least 4–8 h and the maximal trap resetting value (mean 113 μm) exceeded that reached after 60 min by about 5.2 times. This further confirms the view that *U. humboldtii* traps are – on an absolute scale – physiologically rather inefficient in terms of firing and resetting rates.

Adamec (2011b) measured mechanically stimulated firing values ranging from 92 to 282 μm (mean 216 μm) in the traps of 13 aquatic *Utricularia* species with trap sizes comparable to those in the present study. This means that the volume capacity of aquatic *U. humboldtii* traps to fire is about three times lower than that in 12 species from the section *Utricularia*. Ecologically, this suggests that its traps are rather specialised for fine prey in bromeliad water tanks as catching large prey would require greater firing volumes such as those observed in species from the section *Utricularia*. In this context, the resetting rate of *U. humboldtii* traps, reflecting water pumping out of the traps and potential efficiency to repeatedly catch prey, is at least one order of magnitude

lower compared to those found in 12 species from the section *Utricularia* (cf. Adamec 2011b). Moreover, in all these species with very active traps, their very high resetting rate ensures that their traps can fire again within only 30 min, with their 30-min resetting rate usually equalling 50–90% of their firing rate. In the *U. humboldtii* traps, however, after 30 min of resetting, only about 12% of the firing rate is achieved, and after 60 min, it may reach only about 30%. Nevertheless, it is necessary to add that due to the irregular geometry of *Utricularia* traps (Poppinga *et al.* 2016; Westermeier *et al.* 2017), the water flow rate is most presumably not linearly related to changes in trap thickness.

Based on the exact linear kinetics of the resetting rate estimated immediately after stimulated firings, Adamec (2011b) suggested that water is continuously pumped out of the trap, without any lag-period after firing. However, a very distinct lag-period in the resetting rate occurred after firing in the *U. humboldtii* traps (Table 1). The lag-period included at least the first 10 min, with the rate rising between 10–30 min, with a considerable rise also observed between 30–60 min. A similar lag-period was also observed in traps of populations of Australian aquatic *U. dichotoma* from the evolutionary basal section *Pleiochasia* (Płachno *et al.* 2015). However, their lag-period lasted only 3–5 min with the resetting rate reaching its maximum shortly afterwards, while in *U. humboldtii* in the present study, the maximum rate occurred at least after 30 min. A short lag-period < 10 min was also observed in *U. volubilis* traps from the same basal section *Pleiochasia* (Adamec 2011b). The presence of such a lag-period may imply that pumping water out of the trap is regulated and at the reset state, the rate of pumping is strongly reduced and may even be zero, requiring several minutes or tens of minutes to fully operate after each firing. In general, in terms of its low values of trap firing and resetting rates, *U. humboldtii* traps are reminiscent of those in aquatic species of the section *Pleiochasia* (*U. dichotoma, U. volubilis*), despite their different habitats and ecology (Taylor 1989).

Field-grown *U. humboldtii* can produce large aquatic traps up to 12 mm in size (Taylor 1989) but the traps in our culture were only ca. 4 mm long (Fig. 1). It is not known which ecological factors determine the size of *U. humboldtii* traps or whether their size is regulated in the same way as in other aquatic species from the *Utricularia* section: i.e. positively by high CO_2 availability and low N or P tissue nutrient content (Adamec 2018).

CONCLUSION

The relatively low firing and resetting rates found in the aquatic traps of epiphytic *U. humboldtii* together with their low aerobic respiration rate indicate that these less efficient aquatic traps are specialised for catching fine prey items in bromeliad water tanks. In this species,

spontaneous trap firings were also observed and their magnitude was the same as that in mechanically stimulated firings. This implies that spontaneous firings occur consistently in all aquatic *Utricularia* species tested so far. However, although related to the continuous water pumping out of the traps, their physiological importance remains unclear.

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Fiziološka karakterizacija vodenih zamki epifitske karnivorne vrste *Utricularia humboldtii*

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Usisne zamke vodenih vrsta *Utricularia* su diskoidnog oblika veličine 1–6 mm i njihov lumen je izolovan pokretnim poklopcem od ambijentalnog medijuma. Voda se ispumpava iz zamki da bi se održao negativan pritisak u zamkama za resetovanje. Kada životinjski plen dodirne senzorne dlake na kapiji koja se otvara, okolna voda sa plenom se usisava unutra i vrata se ponovo zatvaraju. *Utricularia humboldtii* je robusna, semiakvatično-epifitska ili kopnena vrsta iz sekcije *Orchidioides* iz Južne Amerike. Stope pokretanja zamke i resetovanja kao promene debljine zamke usled mehanički stimulisanih i spontanih paljenja merene su u njenim vodenim zamkama korišćenjem elektronskog senzora kao mere efikasnosti njegovih zamki. Relativno niske stope paljenja i resetovanja pronađene su u zamkama *U. humboldtii*. Ove vrednosti su 2 do 20 puta niže od onih u drugim vodenim vrstama *Utricularia* navedenim u literaturi. Ovi rezultati zajedno sa niskom stopom aerobnog disanja isečenih zamki ukazuju na to da su manje efikasne vodene zamke kod *U. humboldtii* specijalizovane za hvatanje finog plena u rezervoarima za vodu bromelija. Utvrđeno je da se tokom prvih 30 minuta nakon pucanja javlja značajan period kašnjenja resetovanja zamke, što je u suprotnosti sa prihvaćenim konceptom kontinuiranog pumpanja vode. Uočena su i spontana paljenja zamke i njihova veličina je bila ista kao kod stimulisanih ispaljivanja. To implicira da se kod svih do sada testiranih vodenih vrsta *Utricularia* dosledno javlja spontano ispaljivanje. Međutim, njihov fiziološki značaj nije u potpunosti razjašnjen iako se odnose na kontinuirano ispumpavanje vode iz zamki.

Ključne reči: aktiviranje zamke, brzina pumpanja vode, debljina zamke, brzina aerobnog disanja

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