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Note
Light as a factor affecting the concentration of simple organics in the traps of aquatic carnivorous *Utricularia* species

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With 1 table and 1 appendix

Abstract: Rootless aquatic carnivorous *Utricularia* plants exude significant amounts of photosynthates into the trap fluid, where they have been shown to support complex microbial commensal communities. Using ion chromatography, the composition of four groups of easily metabolised, carbon-rich organic compounds (sugars, sugar alcohols, amino acids and organic acids) was investigated in trap fluid collected from three aquatic *Utricularia* species, *U. vulgaris, U. reflexa* and *U. stygia*, for different trap ages, irradiance levels during plant growth and for exposure to periods of darkness. The total sum of the concentrations of the four groups of organic compounds in the trap fluid ranged within 14–42 mg l^{-1} in greenhouse-grown *U. vulgaris*, compared to 9.0–14 mg l^{-1} in *U. reflexa*. The concentrations of organic compounds were significantly higher in younger traps than in the older traps of *U. vulgaris* grown at high irradiance. Within the same trap age categories in *U. vulgaris*, the group concentrations of sugars, organic acids, and total sums of analysed compounds were significantly higher in plants growing at high irradiance when compared with those grown in the shade. Dark exposure of cut traps for 1–2 d significantly decreased the concentrations of sugars and organic acids in the fluid. The total sum of organic compounds in traps of *U. stygia* grown outdoors (78.3 ± 19.2 mg l^{-1}) was much higher than that in 'middle aged' traps of *U. vulgaris* (49.2 ± 4.2 mg l^{-1}), grown under the same conditions. It may be concluded that the concentrations of organic compounds in the trap fluid of aquatic *Utricularia* are species specific, subject to rapid turnover and depend significantly on various endogenous (trap age) or exogenous factors (water chemistry, irradiance).

Key words: *Utricularia vulgaris, U. reflexa, U. stygia,* trap fluid analysis, ion chromatography, organic compounds, turnover, irradiance, trap age.

Introduction

The rootless carnivorous plant genus *Utricularia* L. (bladderwort, Lentibulariaceae) includes about 220 species, of which around 50 are aquatic or amphibious (Taylor 1989). They usually grow in standing, nutrient-poor humic waters and growth can often be limited by a shortage of N and P, but also of K (Adamec 1997, 2011a, Ellison 2006, Guisande et al. 2007). All necessary nutrients are taken up through the shoots, directly from the ambient water or from prey. Small aquatic animals such as crustaceans, mites, nematodes, rotifers and protozoa, as well as algae are captured by foliar traps on the plants (Mette et al. 2000, Richards

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2001, Gordon & Pacheco 2007, Peroutka et al. 2008, Alkhalaf et al. 2009). These discoid traps are hollow bladders, usually 1–5 mm long with a wall thickness of two cells, 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 & Findlay 1975, Sasago & Sibaoka 1985, Juniper et al. 1989). In a set state, prepared for firing, a negative pressure of ~ –16 kPa relative to the ambient water is maintained inside the trap (Sydenham & Findlay 1973, Sasago & Sibaoka 1985, Singh et al. 2011). When trigger hairs situated on the trap door are touched by a prey species the door opens, the prey is aspirated into the trap lumen and the watertight door closes again. As shown recently by a high-speed camera, this process is complete within 5 ms and is caused by reversibly buckling the door associated with a convex/concave door inversion (Joeyux et al. 2011, Singh et al. 2011, Vincent et al. 2011). The negative pressure is restored by removal of ca. 40 % of the water from the trap lumen during a 25–30 min period, after which the trap is ready to fire again (Sydenham & Findlay 1973, Sasago & Sibaoka 1985).

It has been widely accepted that *Utricularia* benefit from the enhanced uptake of mineral nutrients from prey captured by traps (Adamec 1997). Yet the prey availability and trapping rates reported from natural humic waters are highly variable and limited importance of prey-derived nutrients for the growth of aquatic *Utricularia* has often been reported, especially in highly oligotrophic waters (Richards 2001, Englund & Harms 2003, Adamec 2008a,b, Peroutka et al. 2008, Alkhalaf et al. 2009, 2011, Adamec et al. 2010). Aquatic *Utricularia* species are usually inhabited by diverse communities of microorganisms (mainly bacteria, algae, ciliates and rotifers) as commensals (Mette et al. 2000, Richards 2001, Peroutka et al. 2008, Alkhalaf et al. 2009, Sirová et al. 2009). The latter authors have recently evaluated the microbial community inside traps of two *Utricularia* species and have found viable components of a complete microbial food web in the trap fluid, predominantly including gramnegative bacteria. Considering the role of these commensal communities, Richards (2001) suggested that these commensal-trap interactions in *Utricularia* species growing in highly oligotrophic waters with low prey availability may be of greater nutritional importance to the plants than limited prey capture. The potential nutritional benefit of the commensal community for the plant could include facilitated digestion of animal prey, phytoplanktonic algae or detritus, i.e. the transformation of organic matter inside the traps (Sirová et al. 2009). Furthermore, high concentrations of nutrients were found in the unfiltered trap fluid free from animal prey of two Belizean *Utricularia* species. Specifically, large concentrations of total N (20–80 mg l^{-1}), total P (0.9–4.2 mg l^{-1}) and particularly total C (400–1570 mg l^{-1}) were measured; older traps always contained larger concentrations of these nutrients than younger traps. A significant proportion of these nutrients was present in dissolved form (Sirová et al. 2009).

Furthermore, Sirová et al. (2010) proved that about 20–25 % of primary production in two *Utricularia* species was exuded into the trap fluid by the traps themselves. Again, the proportion of C allocated to the trap fluid and to shoot tissues increased markedly with increasing trap age. Sirová et al. (2011) have recently shown that C exudates fuel microbial respiration within the traps of three aquatic *Utricularia* species. Up to 30 % of the total dissolved organic C found in the trap fluid in oligotrophic conditions were easily metabolised compounds (mainly glucose, fructose and lactate). It is widely accepted that exudation of organic compounds in plants is affected by multiple factors such as light intensity, nutritional status and temperature etc., with photosynthetic performance being one of the main factors affecting the amount of photosynthates exuded (Neumann & Römheld 2007). Sirová et al. (2011) have shown that the proportion of exuded compounds, as well as their microbial utilisation, decreased with increasing mineral nutrient supply (N, P) and trap age. There was, however, no information on how the exudation of organic compounds into the trap fluid is regulated by the plants under various light conditions. On the basis of our previous studies (Sirová et al. 2010, 2011), we hypothesise that the quantity of the exudation depends markedly on photosynthetic conditions and trap age, that the highest concentration of organics is found in more irradiated plants and younger traps and that the exuded compounds are the subject of rapid turnover. To test this hypothesis, we analysed the dependence of the concentration of organic compounds – sugars, sugar alcohols, amino acids and organic acids – in the trap fluid of three aquatic *Utricularia* species on irradiance during their growth, on the length of dark exposure of excised traps, and also on trap age.

Material and methods

Plant material and cultivation

Adult stock plants of *Utricularia vulgaris* L. and *U. stygia* Thor (syn. *U. ochroleuca* Hartm. sensu lato; both collected from the

Czech Republic) were cultivated outdoors in a 2.5 m^2 plastic container which approximately simulated natural conditions (for details see Adamec 2008b, Sirová et al. 2003, 2009). The pH of the cultivation medium was 6.96–7.28, total alkalinity 1.01 meq l^{-1}, free [CO_2] 0.12–0.25 mM, and electrical conductivity 19.1–20.0 mS m^{-1} at the time of the experiment. Based on the nutrient concentration, the water was considered oligotrophic and slightly humic. Adult stock plants of *U. reflexa* Oliver (from Botswana) were cultivated outdoors in a 30-l aquarium using the same substrate; pH was approximately 6.8. These three species were selected both for their comparatively large traps (up to 5 mm in the former two species and up to 6 mm in the latter one) and for their previous use in our studies (see Adamec 2011a,c). *U. vulgaris* and *U. reflexa* with their monomorphic shoots are free-floating species, while dimorphic *U. stygia* usually grows affixed to the sediment by pale, trap-bearing (carnivorous) shoots (Taylor 1989).

Shoots of *U. vulgaris* and *U. reflexa* were pre-cultivated in a 0.8 m^2 plastic container, which stood in a naturally lit greenhouse with open lateral walls for cooling (Adamec et al. 2010), for three weeks. The experimental container (volume 280 l, water depth 36 cm) contained 120 g dry weight of *Carex acuta* litter as substrate and its water chemistry was similar to that in the stock culture. Tap water was used as the source of water. The irradiance was reduced to ca. 40 % of that in the open. Small zooplankton (ostracods, *Cyclops* sp.) were added to the container to support plant growth.

Twelve shoots of each of *U. vulgaris* (35–55 cm) and *U. reflexa* (15–25 cm) from the pre-cultivation were selected and divided randomly into two variants, each consisting of 6 plants. The different size of shoots in both species is based on quite different size of adult shoots: ca. 100–200 cm in *U. vulgaris*, while only 30–40 cm in *U. reflexa*. At the water surface, the experimental container used for the pre-cultivation was partitioned into two halves using a floating plastic band. Due to large water volume in the container and the relatively low number of plant shoots, we assume that the interspecific competition was not important. One plant variant of each species grew at a higher PAR irradiance of 42 % of that in the open (white nylon mesh, light variant), while the other at only 7.2 % (green gardener's foil combined with white nylon mesh, shaded variant). A submersible temperature data logger (Minikin T, EMS Brno, Czech Rep.) monitored water temperature in the container at plant level. During the course of the whole experiment (7^{th}–22^{nd} July), the mean water temperature at plant level was 21.1 °C (daily maxima 17.9–28.3 °C, night minima 15.8–23.8 °C, diurnal oscillations within 2–5 °C). In both variants, the internode between the 2^{nd} and 3^{rd} adult leaf node was tagged by a short piece of fine thread for estimating the apical shoot growth rate (Richards 2001, Adamec 2008b, Adamec et al. 2010). The apical shoot growth rate was estimated after 12 days. During the whole experimental cultivation period of 15 days, pH of the water was within 7.12–7.30 (no pH gradients were found in the container), total alkalinity 0.99–1.05 meq l^{-1}, [CO_2] 0.12–0.17 mM, [O_2] 3–6 mg l^{-1}, and electrical conductivity 30.7–33.7 mS m^{-1}. The water was rather poor in main mineral nutrients (0 µg l^{-1} NO_3^--N; 7.5 µg l^{-1} NH_4^+-N; 17 µg l^{-1} PO_4-P).

Trap fluid collection and experimental procedures

Trap fluid was collected from experimental plants grown either in the greenhouse (*U. vulgaris, U. reflexa*) or outdoors in the stock container (*U. vulgaris, U. stygia*) during the 12^{th}–15^{th} day. The trap fluid from traps (usually > 2 mm) without any macroscopic prey was collected by a glass capillary connected to a peristaltic pump (Sirová et al. 2003, 2009, 2011). In the *U. vulgaris* plants grown in the greenhouse both in the light and the shade, the fluid was collected from larger traps on the 9^{th}–10^{th} ('young' traps), 12^{th}–13^{th} ('middle aged' traps), and 15^{th}–16^{th} ('old' traps) adult leaf nodes, while from the 3^{rd}–5^{th} ('young traps') and 6^{th}–8^{th} ('old traps') adult leaf nodes of *U. reflexa*. The different position of leaf nodes in both species was due to different growth patterns and maturation of traps: traps mature much earlier in *U. reflexa* than *U. vulgaris*. Another 12 large traps from the 6^{th}–8^{th} leaf nodes of *U. reflexa* (light variant) were cut and stored in a plastic vial in the filtered cultivation water from the outdoor container at 25 ± 1 °C in darkness for 24 h, until trap fluid collection. Similarly, cut 15^{th}–16^{th} leaf nodes with traps of *U. vulgaris* (light variant) were stored under the same dark conditions for 2 days. These experiments on cut traps or leaves with traps tested whether a 1–2 day dark period can decrease the concentration of organic solutes in the fluid. For comparison, trap fluid was also collected from medium-old traps of outdoor grown *U. vulgaris* and medium-old traps in pale carnivorous shoots of *U. stygia*. The plants were grown in the same container. Due to its dimorphic and attached shoots, the latter species could not be grown in the experimental container in the greenhouse (cf. Sirová et al. (2011). The filtered cultivation water from both containers was also analysed. We are aware of that the plant material raised in only one experimental container represents pseudoreplicates. However, the use of *Carex* litter as non-standard substrate in true replicates would cause a substantial variability of water chemistry in single containers.

From dozens of traps of each of the experimental variants described above (10–20 traps in *U. reflexa*, 30–50 traps in *U. vulgaris,* 30–40 traps in *U. stygia*), about 40–50 µl of the trap fluid was collected and forced into plastic 1.5 ml Eppendorf filtration vials (0.2 µm; Hamburg, Germany) placed on ice. The fluid was centrifuged at 4500–5000 rpm for 12–15 min. The filtrate (25–40 µl) was stored in a refrigerator at 2 °C for 3–10 h before the analysis of the solutes using ion chromatography. All analyses were performed with four parallel samples from different plant material.

In addition, trap fluid from each of the experimental variants was analysed for the presence of bacteria and other commensals, which could have an effect on the organic carbon turnover within the traps. Total bacterial and protist numbers were assessed using epifluorescence microscopy, as described in Sirová et al. (2009, 2011). For protozoa, only the dominating *Euglena* sp. and *Paramecium bursaria* cells were considered for counting.

Trap fluid analysis

Filtered fresh samples for the analysis of trap fluid and cultivation water composition were analysed on the dual channel ion chromatograph ICS 3000 (Dionex, CA, USA). The volume injected was 200 µl for the cultivation water and 5 µl for each trap fluid sample, per single channel. Mono and disaccharides, sugar alcohols, and amino acids were analysed using amperometric detection with separation on an AminoPack PA10 analytical column. Organic acids and inorganic ions were separated using an AS11-HC column and detected on a conductivity detector. Results were expressed as mg l^{-1}.

Due to relatively large number of analytes (46 different organic compounds), the data on the concentration of organic compounds in trap fluids have been collected into five groups (see Sirová et al. 2011): sugars, sugar alcohols, amino acids, organic acids and the total sum of organic compounds; only these data are presented in the text. See Appendix 1 for the complete analyses. The significance of differences in the group concentrations of compounds between the trap age categories within each species and light regime, as well as between the light and dark variants within the same age category within each species, were evaluated by one-way ANOVA. The normality of data was tested and confirmed by the Kolmogorov-Smirnov test. Means with standard error are shown, n = 4.

Results

During the 12-d growth period preceding the trap fluid collection, the apical shoot growth rate of *U. vulgaris* plants grown at high irradiance was 2.88 ± 0.11 leaf nodes d^{-1} and differed significantly at $p < 0.01$ from that of plants in the shade (*t*-test; 2.23 ± 0.08 nodes d^{-1}; data not shown), while the apical growth rate in *U. reflexa* (1.02 ± 0.02 *vs.* 0.94 ± 0.03 nodes d^{-1}, respectively) was not significantly different ($p > 0.05$) between both treatments. The difference between both species might reflect the fact that *U. reflexa* is much more shade tolerant than *U. vulgaris*.

Generally, the most abundant sugars in the trap fluid were glucose and sucrose, the most abundant sugar alcohols were mannitol, glycol and arabitol; cysteine, glycine, asparagine, valine and tyrosine were the most common amino acids, and acetic, formic and lactic acids were the most abundant organic acids (see Appendix 1). The total sum of the concentrations of the four groups of organic compounds in the trap fluid ranged within 14–42 mg l^{-1} in greenhouse-grown *U. vulgaris*, compared to only 9.0–14 mg l^{-1} in *U. reflexa* (Table 1). Although the concentrations of all four groups of compounds were always highest in 'young' traps and the lowest in 'old' traps of *U. vulgaris* grown at high irradiance, due to relatively high statistical variance of the data, however, only sugars and total sums of compounds differed significantly at $p < 0.05$ between the 'young' and 'old' traps. This concentration gradient along the trap age was not distinct in *U. vulgaris* grown in shade. Within the same trap age categories in *U. vulgaris*, only the group concentrations of sugars, organic acids and total sums of compounds were significantly higher in plants growing at high irradiance as compared with those in shade.

In *U. reflexa*, no significant difference in the concentrations of any group of organic compounds was

Table 1. Total concentrations of major groups of organic substances and their total sums (in mg l^{-1}) determined in the trap fluid of *Utricularia.* *, different batch of *U. reflexa* plants. The plants were grown in a greenhouse at either higher irradiance or in the shade. Means ± SE are shown; n = 4. The different letters on the right side of the columns within the same species and the same light regime denote statistically significant difference at $p < 0.05$, as dependent on trap age. The asterisk on the left side of the columns denotes statistically significant difference at $p < 0.05$ between the light regimes within the same species and trap age category, or dark exposure of cut *U. reflexa* traps for one day, or cut *U. vulgaris* leaves with traps for two days, and their respective controls.

Species	*Utricularia vulgaris*						*Utricularia reflexa*						*Utricularia vulgaris*	
Growth conditions	Greenhouse						Greenhouse						Outdoors	
Light growth conditions	Light			Shade			Light			Shade			Light	
Trap age (nodes)	9–10	12–13	15–16	9–10	12–13	15–16	3–5	6–8	6–8*	3–5	6–8	6–8	12–13	
Experimental conditions												Cut traps in dark 1d	Cut traps in dark 2d	
Sugars	*19.9 ± 4.4^a	9.6 ± 1.5^{ab}	8.2 ± 0.9^b	7.5 ± 1.6^a	7.6 ± 1.8^a	4.4 ± 1.3^a	3.6 ± 0.6^a	2.9 ± 0.4^a	2.1 ± 0.4	2.4 ± 0.2^a	2.0 ± 0.3^a	*1.2 ± 0.1	*3.5 ± 0.3	13.8 ± 2.0
Sugar alcohols	6.8 ± 1.4^a	4.2 ± 0.8^a	3.9 ± 0.5^a	5.5 ± 0.3^a	5.8 ± 1.4^a	3.8 ± 1.1^a	1.0 ± 0.2^a	1.0 ± 0.2^a	0.8 ± 0.2	0.9 ± 0.1^a	0.8 ± 0.1^a	1.5 ± 0.1	2.8 ± 0.1	4.4 ± 1.0
Amino acids	4.3 ± 0.8^a	2.3 ± 0.2^a	2.3 ± 0.5^a	2.1 ± 0.5^a	2.0 ± 0.3^a	2.1 ± 0.6^a	2.5 ± 0.5^a	2.5 ± 0.4^a	2.0 ± 0.7	3.1 ± 0.7^a	1.6 ± 0.6^a	2.4 ± 0.4	1.3 ± 0.8	2.9 ± 1.3
Organic acids	11.4 ± 4.9^a	*11.5 ± 3.0^a	*9.0 ± 1.0^a	2.7 ± 0.8^a	1.5 ± 0.2^a	3.9 ± 1.1^a	6.0 ± 1.7^a	7.8 ± 4.0^a	4.1 ± 1.4	3.3 ± 1.0^a	7.6 ± 2.3^a	6.7 ± 1.2	*4.8 ± 1.0	28.1 ± 7.8
Total concentration	*42.4 ± 5.3^a	*27.6 ± 2.6^{ab}	23.3 ± 2.6^b	17.8 ± 1.4^a	16.8 ± 3.5^a	14.2 ± 3.3^a	13.0 ± 2.5^a	14.3 ± 3.8^a	9.0 ± 0.4	9.7 ± 1.2^a	12.0 ± 2.9^a	11.8 ± 1.3	*12.4 ± 1.1	*49.2 ± 4.2

found between 'young' and 'old' traps within the same irradiance or between the different irradiances within the same trap age category (Table 1). Thus, no gradient of the concentration of organic compounds was evident in *U. reflexa* traps of different age. The sum concentration of organic compounds in *U. vulgaris* 'middle aged' traps grown outdoors (49.2 ± 4.2 mg l^{-1}) differed significantly ($p < 0.005$) from that in greenhouse-grown plants and the value in traps of *U. stygia*, grown under the same outdoor conditions, was even much higher (78.3 ± 19.2 mg l^{-1}; this difference from *U. vulgaris* was non-significant; data not shown). The statistically significant effect of the dark exposure on reducing the concentration of organic compounds in the trap fluid was confirmed only for sugars in cut 'old' traps of *U. reflexa* (1 d darkness) and for sugars, organic acids and total sum in cut leaves with 'old' traps of *U. vulgaris* (2 d darkness). In the latter case, the group concentrations of the organic compounds were approximately half of those in the control traps. Only trace concentrations of all groups of organic compounds were usually found in both cultivation waters (see Appendix 1).

Bacteria and protozoa were present in traps of all the experimental treatments – their numbers were in the order of $10–89 \times 10^7$ ml^{-1} and $8–10 \times 10^3$ ml^{-1} for bacteria and the two protozoan genera, respectively (data not shown), which is in agreement with previously published results (Sirová et al. 2009).

Discussion

It has been well documented in previous studies using rooted terrestrial plants that low irradiance is associated with both low root exudation and low concentration of soluble carbohydrates in the roots (e.g. Přikryl & Vančura 1980, Crapo & Ketellapper 1981, Graham et al. 1982, Johnson et al. 1982). Similar data have been entirely lacking for rootless aquatic *Utricularia* and the only study so far dealing with exudation in these species was conducted by Sirová et al. (2010).

It follows from our data that the results exhibited great variability which impaired the statistical evaluation of the data. The variation coefficient was usually within 10–80 % (Table 1), which is close to the variability found during analyses of organic compounds in *Utricularia* trap fluid reported recently (Sirová et al. 2011). A possible explanation may lie in the fact that the traps were allowed to fire and aspirate in the cultivation medium repeatedly during trap fluid collection. Other reasons might include the physiological variability of different plant individuals, and also diurnal changes in the exudation rates as well as exudate composition (Dilkes et al. 2004). The variability of the analytical measurements within the same samples did not exceed ± 0.2 mg l^{-1} and, thus, was negligible.

Generally, the concentrations of the four groups of organic substances (sugars, sugar alcohols, amino acids, organic acids) and their sum totals in the trap fluid in this study (Table 1) are comparable with or somewhat lower than those reported in traps of *U. reflexa, U. australis* and *U. stygia* (cf. Sirová et al. 2011). The total concentration of 46 organic compounds in the trap fluid in three *Utricularia* species in the present study ranged between 9–78 mg l^{-1}, compared to 1.2–8.7 mM of organic C (roughly 30–150 mg l^{-1}) shown in the latter paper. Similarly to Sirová et al. (2011), sugars and organic acids had the highest concentrations of organic compounds found in the traps. In both this and the latter study, glucose, fructose, sucrose, mannitol, xylitol, glycol, alanine, glycine, lactic and acetic/glycolic acids were present in the highest concentrations out of the assessed analytes (cf. Appendix 1). These compounds are very common in plant tissues and, with the exception of some sugar alcohols, are very easily metabolised. Sirová et al. (2011) reported a correlation between the concentrations of all these four groups of compounds and values of biodegradability (or basal respiration rate) in the filtered trap fluid in *U. reflexa* traps of increasing age. This confirms that the four groups of compounds are easily utilised and enter the energetic metabolism within the commensal community in the trap fluid.

Despite the great variability in the data from both studies, some conclusions can be drawn. Firstly, the concentration of organic compounds in the trap fluid is species specific. The concentrations of the four groups were several times lower in *U. reflexa* traps than in *U. vulgaris, U. australis* and *U. stygia* traps of comparable age and were somewhat lower in *U. stygia* than in *U. australis* growing together in the same aquaria (Table 1; Sirová et al. 2011). In our study, a much greater total sum of organic compounds was found in *U. stygia* trap fluid than that in *U. vulgaris* grown outdoors in the same container. Secondly, the suggestion made by Sirová et al. (2011) that the concentrations of organic compounds in the fluid of the same trap age within the same species depend on cultivation conditions and water chemistry, has been supported by the experiment. In our study, the total sum of analyte concentrations in 'middle aged' *U. vulgaris* traps grown outdoors was significantly higher than that in the light variant of the greenhouse-grown plants. Sirová et al.

(2011) reported for *U. stygia* that trap fluid concentrations of sugars, sugar alcohols, organic acids and total sums of the concentrations were significantly lower in plants fertilized by N or P addition to the medium. As the fertilized *U. stygia* shoots also had significantly higher tissue N or P content than the controls, it is possible to hypothesise that shoot N and P content somehow regulates trap exudation of organic compounds into the trap fluid through a negative feedback mechanism, similarly, as trap production is regulated as investment in carnivory (see Adamec 2008a).

Thirdly, we have demonstrated that the concentration of organic compounds in the trap fluid depends on trap age, although this relationship may not be unidirectional (Table 1; Sirová et al. 2011). The latter authors found the lowest concentrations of the four groups of organic compounds in the trap fluid of *U. reflexa* traps in the 5^{th}–6^{th} leaf nodes, while the concentrations rose in both younger and older shoot segments. In the present study, a significant gradient of the sugar concentration and the total sum of concentrations was found in the light variant of *U. vulgaris*, but not in the shaded variant. However, both the present study and that by Sirová et al. (2011) support the view that the highest concentration of organic compounds in the fluid occurs in young traps. This is because the largest proportion of fresh photosynthates (up to 20–25 %) is exuded just into young traps (Sirová et al. 2010) which also have the lowest commensal biomass (Sirová et al. 2009). Moreover, we showed a positive influence of high-irradiance growth conditions on the increase of organic compound concentrations inside the traps of *U. vulgaris* (significant for sugars, organic acids and total sums). We also confirmed a statistically significant decrease in organic compounds in cut off *U. reflexa* traps stored in darkness for 1 d (sugars) and in *U. vulgaris* traps on cut leaves stored in darkness for 2 d (sugars, organic acids, total sums). We assume that exudation of photosynthates into the traps completely stops in darkness. As the greatest decline in these dark treatments occurred within the total sugars and organic acid group of compounds, i.e., the groups with high biodegradability and utilisation in microbial respiratory metabolism, it is probable that these compounds were consumed by dark respiration of the commensal communities (Adamec 2007, 2011b, Sirová et al. 2011). In the oxygenated trap fluid collected from 'very old' *U. vulgaris* traps (30^{th}–31^{st} nodes), Adamec (unpubl.) measured a respiration rate as $[O_2]$ decline of 54 μM h^{-1}. Assuming that the commensal community in the 'very old' traps could be 5 times more concentrated than that in the 'old' traps in the present study, the respiration rate in the latter traps is theoretically equal to 15.6 mg l^{-1} of glucose consumption over 2 d. This value is comparable with the decrease in the total sum of organic compounds (10.9 mg l^{-1}) found in darkness after 2 d (Table 1).

In any case, study of respiratory and photosynthetic activity of trap commensal communities in the (almost) anoxic trap fluid (Adamec 2007) will be crucial for understanding the role of the community-plant interrelationships as well as the turnover of organic metabolites in the fluid. Such a study should include also 'very old' traps in which the process of nutrient uptake by the plants presumably dominates over plant enzyme secretion (Sirová et al. 2009, 2011). Moreover, the character of aerobic respiration of the inner trap structures (glands), in association with their demanding functions in the anoxic trap fluid, still remains mysterious. The inner trap structures evidently compete for the traces of oxygen with the commensals. As suggested by Sirová et al. (2011) the concentrations of different metabolites occurring in the trap fluid 'represent a certain dynamic equilibrium that reflects the rate of utilisation by either the microbial community or the trap itself and the rate of exudation by traps'.

It may be concluded that the concentration of organic compounds in the trap fluid of aquatic *Utricularia* is partly species specific and dependent on various endogenous (e.g., trap age) or exogenous factors (water chemistry, irradiance); and that the simple organic compounds are subject to rapid turnover, most probably by the active microbial communities in the trap fluid.

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Appendix 1. Concentrations of 46 organic analytes in mg.l^{-1} determined in the trap fluid of *Utricularia*. *, different batch of *Utricularia reflexa*. The plants were grown either in a greenhouse (*U. vulgaris*, *U. reflexa*) or outdoors (*U. vulgaris*, *U. stygia*), at either higher irradiance or in the shade. Trap age is expressed as mature leaf nodes; *U. stygia* traps were middle aged. Different groups of analytes are separated by a dotted line. Means ± SE are shown; n = 4.

Species	*Utricularia vulgaris*						*Utricularia reflexa*							*U. vulgaris, U. stygia*	
Growth conditions	Greenhouse						Greenhouse							Outdoor	
Light growth conditions	Light			Shade			Light			Shade		Light		Light	
Trap age (nodes)	9–10	12–13	15–16	9–10	12–13	15–16	3–5	6–8	6–8*	3–5	6–8	6–8	15–16	12–13	Med.
Experimental conditions												Cut traps in dark 1d	Cut traps in dark 2d		
Analytes															
Total sugar alcohols	6.8±1.4	4.2±0.8	3.9±0.5	5.5±0.3	5.8±1.4	3.8±1.1	1.0±0.2	1.0±0.2	0.8±0.2	0.9±0.1	0.8±0.1	1.5±0.1	2.8±0.1	4.4±1.0	7.3±2.3
Glycol/myo-Inositol/Arginine	2.7±0.7	1.8±0.4	1.5±0.6	1.3±0.6	1.5±0.5	1.3±0.8	0.5±0.1	0.4±0.1	0.3±0.2	0.4±0.1	0.3±0.1	0.6±0.1	0.9±0.2	2.0±0.6	2.9±1.7
Xylitol	0.9±1.1	0.8±1.0	0.8±0.9	0.8±0.6	1.2±0.4	0.3±0.3	0.3±0.4	0.5±0.4	0.4±0.2	0.0±0.0	0.2±0.3	0.4±0.5	0.9±0.2	0.5±0.2	0.9±0.6
Arabitol	1.3±1.8	0.5±0.9	0.7±0.8	0.5±0.6	0.1±0.2	0.8±0.9	0.2±0.3	0.0±0.0	0.0±0.0	0.5±0.1	0.2±0.3	0.4±0.5	0.0±0.0	0.0±0.0	0.3±0.6
Sorbitol/Adonitol/Dulcitol	0.1±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0
Mannitol	1.9±1.0	1.0±0.3	0.9±0.3	2.9±1.2	2.9±2.3	1.4±1.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.0±0.3	1.9±1.4	3.2±2.3
Total sugars	19.9±4.4	9.6±1.5	8.2±0.9	7.5±1.6	7.6±1.8	4.4±1.3	3.6±0.6	2.9±0.4	2.1±0.4	2.4±0.2	2.0±0.3	1.2±0.1	3.5±0.3	13.8±2.0	36.4±16.0
Fucose	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Rhamnose	0.1±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Arabinose	0.2±0.1	0.1±0.0	0.1±0.0	0.0±0.1	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.2±0.1
Galactose	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	8.1±10.5
Glucose	12.5±7.7	4.3±1.8	4.3±1.3	5.0±3.3	4.7±2.8	2.5±1.8	2.1±0.9	1.5±0.5	0.8±0.5	1.2±0.3	0.8±0.4	0.5±0.2	2.4±0.4	9.3±2.7	11.9±8.4
Mannose	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1
Xylose	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.3
Saccharose	5.4±2.2	4.4±1.2	3.0±0.4	1.8±0.6	2.3±0.5	1.5±0.8	0.9±0.2	0.9±0.4	0.6±0.3	0.9±0.1	0.8±0.3	0.4±0.1	0.4±0.3	3.4±1.2	6.1±3.7
Glutamine/Fructose	1.1±0.7	0.3±0.1	0.2±0.0	0.2±0.1	0.2±0.1	0.1±0.1	0.2±0.0	0.2±0.2	0.1±0.1	0.2±0.0	0.2±0.1	0.1±0.0	0.0±0.0	0.3±0.3	9.1±9.3
Malose	0.6±0.4	0.5±0.2	0.5±0.1	0.4±0.2	0.2±0.2	0.2±0.1	0.3±0.2	0.2±0.1	0.6±0.1	0.1±0.0	0.1±0.1	0.2±0.0	0.6±0.3	0.5±0.4	0.4±0.0
Total amino acids	4.3±0.8	2.3±0.2	2.3±0.5	2.1±0.5	2.0±0.3	2.1±0.6	2.5±0.5	2.5±0.4	2.0±0.7	3.1±0.7	1.6±0.6	2.4±0.4	1.3±0.8	2.9±1.3	19.7±3.8
Asparagine	0.2±0.1	0.2±0.1	0.1±0.1	0.3±0.2	0.2±0.1	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.7±1.3	0.1±0.1	1.6±1.5
Alanine	0.7±0.4	0.4±0.0	0.4±0.3	0.3±0.1	0.3±0.1	0.4±0.2	0.5±0.2	0.5±0.3	0.4±0.2	0.5±0.3	0.3±0.4	0.3±0.2	0.1±0.1	0.5±0.5	4.7±6.3
Tryptophane	0.1±0.1	0.1±0.0	0.0±0.1	0.1±0.0	0.0±0.0	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.0	0.1±0.1	1.0±0.6
Glycine	0.5±0.3	0.2±0.1	0.3±0.2	0.4±0.2	0.3±0.1	0.4±0.3	0.2±0.1	0.2±0.1	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.0	0.0±0.0	0.2±0.1	1.2±0.8
Valine	0.2±0.3	0.1±0.1	0.1±0.1	0.2±0.1	0.1±0.1	0.2±0.2	0.3±0.3	0.2±0.2	0.5±0.7	0.7±0.3	0.3±0.3	0.3±0.4	0.2±0.2	0.4±0.4	3.5±1.8
OH-Proline/Lactose	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Proline/Serine	0.7±0.6	0.3±0.1	0.4±0.3	0.5±0.2	0.4±0.2	0.5±0.3	0.3±0.3	0.4±0.1	0.2±0.2	0.4±0.2	0.2±0.2	0.3±0.1	0.1±0.0	0.5±0.2	1.5±0.7
Isoleucine	0.2±0.2	0.1±0.1	0.1±0.0	0.0±0.0	0.1±0.3	0.1±0.1	0.2±0.1	0.3±0.2	0.2±0.1	0.3±0.2	0.2±0.3	0.4±0.1	0.1±0.1	0.2±0.2	2.1±1.3
Leucine	0.7±0.2	0.4±0.2	0.4±0.2	0.1±0.1	0.1±0.0	0.1±0.1	0.3±0.1	0.4±0.2	0.3±0.2	0.2±0.2	0.1±0.1	0.4±0.3	0.0±0.0	0.5±0.4	0.4±0.9
Methionine	0.1±0.2	0.1±0.0	0.1±0.0	0.0±0.1	0.0±0.0	0.0±0.0	0.0±0.1	0.0±0.0	0.0±0.0	0.2±0.1	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.1	3.0±1.4
Histidine	0.3±0.2	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.1±0.2	0.2±0.1	0.1±0.1	0.0±0.0	0.1±0.1	0.1±0.2	0.4±0.9
Phenylalanine	0.5±0.1	0.3±0.1	0.3±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.3±0.1	0.3±0.1	0.0±0.0	0.3±0.2	0.2±0.2	0.3±0.2	0.0±0.0	0.3±0.6	0.0±0.0
Total organic acids	11.4±4.9	11.5±3.0	9.0±1.0	2.7±0.8	1.5±0.2	3.9±1.1	6.0±1.7	7.8±4.0	4.1±1.4	3.3±1.0	7.6±2.3	6.7±1.2	4.8±1.0	28.1±7.8	15.0±5.1
Lactic/Gluconic ac.	4.3±5.3	5.4±2.4	4.4±0.9	0.4±0.4	0.2±0.1	0.5±0.4	0.5±0.3	1.0±0.7	0.5±0.2	0.5±0.1	1.0±0.4	1.8±0.6	1.1±0.7	3.8±5.0	3.0±1.1
Acetic/Glycolic ac.	1.8±0.8	1.7±1.6	1.4±0.3	0.5±0.3	0.1±0.0	0.4±0.4	1.1±0.5	1.7±1.7	1.0±0.8	0.6±0.6	1.4±0.8	1.5±0.4	0.4±0.5	5.3±3.0	1.7±0.6
Propionic ac.	2.2±1.9	0.1±0.1	0.4±0.3	0.1±0.1	0.2±0.2	0.4±0.5	1.2±0.6	0.7±0.7	0.5±0.3	0.2±0.2	0.7±0.8	0.4±0.3	0.0±0.0	1.4±1.5	2.7±4.8
Formic ac.	1.1±1.4	3.0±2.6	1.1±0.6	0.9±0.8	0.3±0.2	0.8±0.9	1.5±1.2	3.1±4.5	1.0±1.4	0.7±0.6	2.8±2.1	1.2±0.8	1.7±0.9	11.4±8.9	3.7±4.2
Butyric ac.	0.2±0.3	0.0±0.1	0.1±0.1	0.0±0.0	0.0±0.1	0.1±0.1	0.2±0.1	0.2±0.5	0.1±0.1	0.1±0.1	0.0±0.1	0.0±0.0	0.0±0.0	0.4±0.4	0.2±0.4
Pyruvic ac.	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.0±0.0	0.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	3.7±7.4	0.1±0.1
Adipic ac.	0.2±0.1	0.1±0.1	0.1±0.1	0.2±0.2	0.2±0.1	0.2±0.1	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.1	0.2±0.1	0.1±0.1	0.3±0.1	0.2±0.0	0.1±0.1
Malic/Succinic ac.	0.3±0.3	0.3±0.3	0.4±0.1	0.1±0.1	0.1±0.0	0.3±0.1	0.2±0.2	0.2±0.0	0.2±0.1	0.2±0.1	0.2±0.1	0.3±0.2	0.2±0.1	0.5±0.4	1.2±1.9
Malonic ac.	0.1±0.0	0.2±0.1	0.2±0.1	0.1±0.1	0.1±0.0	0.2±0.0	0.4±0.3	0.2±0.2	0.1±0.1	0.2±0.1	0.3±0.2	0.3±0.1	0.2±0.1	0.2±0.1	0.2±0.2
Maleic ac.	0.0±0.1	0.1±0.0	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.1	0.1±0.0	0.1±0.1
Oxalic ac.	0.9±0.3	0.5±0.3	0.7±0.3	0.3±0.4	0.3±0.1	0.8±0.5	0.6±0.6	0.4±0.2	0.3±0.3	0.7±0.4	0.7±0.4	0.6±0.5	0.6±0.3	0.5±0.3	1.2±1.0
Citric ac.	0.0±0.1	0.1±0.2	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.1	0.1±0.0	0.3±0.2	0.1±0.2	0.0±0.0	0.5±0.1
Isocitric ac.	0.2±0.3	0.1±0.1	0.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.2	0.0±0.1	0.7±0.5	0.5±1.0