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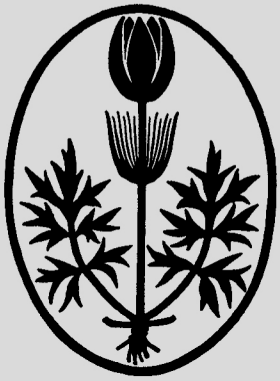

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Regulation of the Investment in Carnivory in Three Aquatic *Utricularia* Species: CO_2 or Prey Availability?

By

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Summary

Adamec L. 2015. Regulation of the investment in carnivory in three aquatic *Utricularia* species: CO_2 or prey availability? – Phyton (Horn, Austria) 55 (1): 131 – 148.

The structural investment in carnivory (IIC) as a relative proportion of trap biomass (DW) was investigated in three aquatic *Utricularia* species (*U. vulgaris*, *U. australis* and *U. reflexa*) in a 12–14 d greenhouse growth experiment. The two-factorial experiment included the presence or absence of prey (zooplankton) for a high (0.30-0.58 mM) or low (0.024-0.062 mM) CO_2 concentration in the culture water. Various plant growth parameters, including traps and foliar N and P contents in young shoot segments, were estimated. All species with either CO_2 or prey addition had significantly more mature leaf nodes on the main shoots, were more branched and their apical shoot growth was more rapid than the -CO_2 or -Prey variants. The mean trap DW increased greatly (2.7-249 times) due to CO_2 addition, while the effect of prey addition was much less and rather ambiguous. Both CO_2 and prey addition significantly influenced the trap number per mg leaf node only in *U. vulgaris*. Thus, trap DW rather than the number of traps per leaf DW, is the basis for an ecological regulation of the IIC in aquatic *Utricularia*. CO_2 addition markedly increased the IIC in all species, while the effect of prey addition was much less. The

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IIC in all species correlated significantly and negatively with shoot N and P contents but highly significantly and positively with the mean trap DW. Generally, under a surplus CO_2 and favourable light conditions, the trap production as the IIC in aquatic *Utricularia* is supported by prey capture more (positive feedback) than the apical shoot growth, but the IIC apparently does not depend on the very low shoot N or P content. At medium CO_2 concentration, shoot N and P contents are very variable and regulate the IIC by negative feedback ("nutrient" regulation). Under poor photosynthetic conditions, however, the trap production is blocked by a shortage of photosynthates, which are allocated preferentially to shoot apices and branching, but probably also by the very high shoot N and P content. The regulation of trap production in *Utricularia* therefore includes two components. High CO_2 concentration as the crucial prerequisite for high photosynthetic rate ("photosynthetic" regulation) is superior to the negative feedback regulation by tissue N or P content in young shoot segments.

Zusammenfassung

Adamec L. 2015. Regulation of the investment in carnivory in three aquatic *Utricularia* species: CO_2 or prey availability? [Wie wird die Investition in das Fleischfressen von drei *Utricularia* Arten reguliert: ist es CO_2 oder das Vorhandensein der Beute?]. – Phyton (Horn, Austria) 55 (1): 131–148.

In einem 12 bis 14 tägigen Wachstumsexperiment im Gewächshaus wurde anhand von drei *Utricularia* Arten (*U. vulgaris*, *U. australis* and *U. reflexa*) untersucht, wie viel die Pflanzen in die strukturelle Anlage für das Fleischfressen investieren (IIC). Als relatives Maß diente die Fallenbiomasse (DW). Zwei Variablen wurden im Experiment getestet: die An- oder Abwesenheit von Beute (Zooplankton) und hohe (0,30–0,58 mM) oder geringe (0,024–0,062 mM) CO_2 Konzentrationen im Kulturwasser. Von jungen Sprosssegmenten wurden verschiedene Wachstumsparameter und die N und P Gehalte der Fallen und der Blätter analysiert. Bei alle Arten zeigte sich, dass die Versuche mit entweder CO_2 oder Beutezugabe signifikant mehr Blattknoten am Haupttrieb und mehr Verzweigungen hatten. Ebenso war das Sprossspitzenwachstum schneller als bei den Varianten, denen weder CO_2 noch Beute zugegeben wurde. Mit CO_2 Zugabe stieg das durchschnittliche Fallen DW stark an (2,7- bis 249fach), während die Beutezugabe zu wesentlich geringeren und nicht eindeutigen Ergebnissen führte. Nur bei *U. vulgaris* beeinflussten sowohl CO_2 als auch Beutezugabe die Anzahl der Fallen pro mg Blattknoten signifikant. Somit ist das Fallen DW mehr als die Anzahl der Fallen pro Blatt DW für eine ökologische Regulation des IIC in der Wasserpflanze *Utricularia* verantwortlich. In allen Arten steigerte die Zugabe von CO_2 merklich die IIC, währen die der Effekt von Beutezugabe gering war. Die IIC aller Arten korrelierte signifikant und negativ mit dem Spross N und P Gehalten, aber hochsignifikant und positiv mit dem mittleren Fallen DW. Allgemein unterstützt ein Überschuss an CO_2 bei günstigen Lichtbedingungen die Fallen Produktion durch Beutefang (positive Rückkoppelung) mehr, als das Wachstum der Apikalspitze, aber die IIC hängt augenscheinlich nicht von einem sehr niedrigen Spross N oder P Gehalt ab. Bei mittlerer CO_2 Konzentration waren die N und P Gehalte des Sprosses variabel und regulierten den IIC durch negative Rückkoppelung („Ernährungs"-Regulation). Unter schlechten fotosynthetischen Bedingungen hingegen wurde die Fallenproduktion durch einen Mangel an Fotosyntheseprodukt-

ten, welche bevorzugt auf den Sprossspitzen und Verzweigungen lokalisiert sind, eingestellt. Aber eine Ursache könnte auch der sehr hohe N und P Gehalt der Sprosse sein. Die Regulation der Produktion von Fallen bei *Utricularia* hat daher zwei Komponenten: eine hohe CO_2 Konzentration als die entscheidende Voraussetzung für eine hohe Fotosyntheserate („fotosynthetische" Regulation). Diese ist wichtiger als die negative Rückkoppelunsregulierung durch das Gewebe N oder P in den jungen Sprosssegmenten.

Introduction

Within carnivorous plants, about 50 species of the genus *Utricularia* L. (bladderwort, Lentibulariaceae) are submerged aquatic or amphibious plants (Juniper & al. 1989, Taylor 1989, Guisande & al. 2007). Aquatic *Utricularia* species usually grow in shallow, standing dystrophic (humic) waters which are usually poor in inorganic N and P, and often also in K (for the review see Adamec 1997, 2011a). They are rootless and take up all necessary nutrients through their linear shoots (with filamentous leaves) either directly from the water or by traps from prey. The very rapid growth of most species of aquatic *Utricularia*, due to both rapid apical growth and frequent branching, is the typical ecophysiological trait of this functional group. Their biomass doubling time can be only 5 to 20 days with their apical shoot growth rate (ASGR) ranging from 1 to 4.2 new nodes with leaves day^{-1} (Friday 1989, Adamec & Kovářová 2006, Adamec 2009, 2010a, 2011a,b, Adamec & al. 2010); their senescent, basal shoot segments decay at the same rate. This very rapid growth of aquatic *Utricularia* in nutrient-poor habitats requires ecophysiological adaptations that enable the plants to gain limiting mineral nutrients. These adaptations include carnivory, efficient nutrient re-utilization (recycling) from senescent shoots and very a high affinity for mineral nutrients during their uptake from the water (Kosiba 1992a,b, Englund & Harms 2003, Adamec 2008a,b, 2009, 2011a). Moreover, the very high net photosynthetic rate of aquatic *Utricularia* species, which is usually higher than that of aquatic non-carnivorous plants, is another prerequisite for the very rapid growth (Adamec 2006, 2011c, 2013) as this growth pattern is associated with a significant loss of organic carbon in senescent shoot segments. As all aquatic *Utricularia* species tested so far use only free CO_2 for photosynthesis (Moeller 1978, Adamec & Kovářová 2006, Adamec 2009, Adamec & Pásek 2009), it is therefore very important for their growth rate that CO_2 concentration is commonly high in their habitats, usually >0.1 mM (see Adamec 1997, 2011a).

Suction traps of aquatic *Utricularia* are fluid filled hollow bladders, usually 1–5 mm long and mostly two cells thick. Their main function is to capture and digest small aquatic animals (Juniper & al. 1989, Englund & Harms 2003) but any mechanical irritation and/or spontaneous firing (Adamec 2011d, Vincent & al. 2011), causes detritus or organic particles, including bacteria and algae, to be frequently trapped too (Richards 2001, Peroutka & al. 2008, Sirová & al. 2009). Additionally, all mature traps con-

tain living communities of microorganisms, most probably originating in *Utricularia*-associated periphyton., and it is generally accepted that they help to digest animal prey (e.g. Płachno & al. 2006). Recently, viable components of a complete microbial food web were found in the trap fluid, with a dominating biomass of bacteria and fungi (Sirová & al. 2009) and traps have been found to exude ecologically significant amounts of organic substances (mainly glucose, fructose and lactate) to the trap fluid to support the microbial communities (Sirová & al. 2010, 2011, Borovec & al. 2012). The ecological significance of the trap communities in traps without animal prey still however remains under debate: are they digestive mutualists (commensals) or parasites (cf. Richards 2001, Sirová & al. 2009, 2010, 2011, Adamec 2011e)? On the other hand, prey capture (usually fine zooplankton) has always been known to significantly enhance the growth of aquatic *Utricularia* but from different studies, the extent of this enhancement was rather variable under experimental conditions (Kosiba 1992a,b, Englund & Harms 2003, Adamec 2008b, Adamec & al. 2010).

In aquatic *Utricularia* species, the structural, energetic and also mineral costs of traps are considerable and the plants change the proportion biomass invested in traps (and hence carnivory; IIC) according to habitat factors including prey availability (Friday 1992, Knight 1992, Bern 1997, Guisande & al. 2000, 2004, Richards 2001, Englund & Harms 2003, Manjarrés-Hernández & al. 2006, Adamec 2006, 2007, 2008a, 2009, 2010b, Kibriya & Jones 2007). In aquatic *Utricularia* species with homogeneous shoots, about 10-55% of the total plant biomass is formed by traps (e.g. Friday 1992, Porembski & al. 2006, Kibriya & Jones 2007, Adamec 2008a,). There are several pieces of evidence showing that the ecophysiological regulation of the structural IIC in aquatic *Utricularia* is species specific. Kibriya & Jones 2007 found that in *U. vulgaris*, the phosphate concentration in the ambient water and/or the shoot P content was inversely proportional to the amount of biomass allocated to traps, while for *U. australis*, the trap biomass allocation was inversely proportional to the shoot N content (Adamec 2008a; see also Sirová & al. 2011). On the other hand, the IIC in *U. foliosa* was significantly inversely proportional to both shoot N and P content (Bern 1997). As the shoot N content in *U. australis* correlated highly significantly with the abundance of trapped prey, the trap biomass allocation was inversely proportional to trapped prey. However, the IIC also showed a strong and positive dependence on CO_2 concentration in the water, which greatly influenced the shoot N and P content in an inversely proportional way (Adamec 2008a). This negative feedback of CO_2 counteracts the effect of prey capture on shoot N and P content and also helps to stabilize the level of other mineral nutrients in the shoots. Thus, in *U. australis*, the "nutrient" regulation of the IIC by the shoot N content as a key endogenous regulatory factor is also subject to "photosynthetic" regulation (Bern 1997, Englund & Harms 2003, Adamec 2008a), the role of which seems to be superior.

In this study, the structural investment in carnivory was investigated in *U. vulgaris*, *U. australis* and *U. reflexa* during a 12–14 d greenhouse growth experiment. The two-factorial experimental design included the presence or absence of animal prey (zooplankton) and high or low CO_2 concentration in the culture water. Besides various growth parameters, special emphasis was placed on maximum trap size and mean trap dry weight (DW), the percentage of traps with captured prey and the structural investment in carnivory (as the proportion of trap DW to the shoot segment DW). Foliar N and P contents were also estimated in young shoot segments. Statistical analyses were conducted to reveal to which extent the foliar N and P contents and the IIC depended on either the prey addition or high CO_2 concentration. The hypothesis that the regulatory effect of CO_2 concentration ("photosynthetic") on the investment in carnivory is superior to the feedback mechanism ("nutrient") mediated by the foliar N or P content was verified.

Materials and Methods

Plant Material

Three aquatic *Utricularia* species from the generic *Utricularia* section (Taylor 1989) with homogeneous, monomorphic shoots were used for the experiment. *Utricularia vulgaris* L. (collected in S Moravia, Czech Rep.) and *U. australis* R.Br. (collected in the Třeboň region, Czech Rep.) were pre-cultivated outdoors in two 2.5 m^2 plastic containers simulating their natural conditions (see Adamec 2008b), while *U. reflexa* Oliver (collected in the Okavango Delta, Botswana) was grown indoors under natural light in a 3 l aquarium (Adamec 2011d,e). The plants in these cultures were grown in tap water and a litter of robust *Carex* species was used as a substrate. The pH of the cultivation media was 6.5–7.5, total alkalinity ranged from 0.5 to 0.9 $meq.l^{-1}$ and the free CO_2 concentration was 0.08–0.30 mM (for details see Adamec 2008b). The water in these cultures was considered oligotrophic and humic. The three species were selected as they have relatively large traps (to 4–6 mm) with variable size, they can grow together in a greenhouse under summer conditions and they have been used in many ecophysiological experiments which also addressed growth parameters and investment in carnivory (see Adamec 2011a).

Growth Experiment

The growth experiment on the three species proceeded in two 0.8 m^2 white plastic containers, which stood in a naturally lit greenhouse with open lateral walls for cooling. The containers (40 cm high) each contained 280 l of tap water and 120 g (dry weight; DW) of *Carex elata* litter as substrate (Adamec & al. 2010, Adamec 2011f). Between adding the pre-soaked substrate and the plants, the containers were allowed to stabilise for nine days to allow the water conditions to mimic those of a mesotrophic and slightly dystrophic environment. On 14 June 2012, 24 relatively homogeneous sub-adult plants of each species (but adult plants of *U. reflexa*) were shortened to a constant number of mature apical leaf nodes, all visible branches were excised, and the shortened apical shoot segments were used for the experiment. *U. vulgaris* and *U. australis* were shortened to 15 mature apical leaf nodes

(mean shoot length was 86 mm, range 72–102 mm for the former species and 113 mm, range 91–150 mm for the latter one) and *U. reflexa* to 10 mature leaf nodes (mean shoot length 126 mm, range 112–147 mm). For all plants, the internode between the second and third mature leaf nodes was tagged by a short piece of fine thread for measuring the ASGR (see Adamec 2008b, 2011f). To study the effect of prey, two floating plastic frames were used to isolate some of the plants. These measured 0.3 m × 0.3 m, were ~6 cm deep, and were lined at the base by a 150 μm pore size mesh to exclude zooplankton but allow water exchange (Adamec 2008b, 2011f).

In each container, three randomly selected tagged shoots of each species were placed into each of the two parallel floating frames, 6 shoots in total without access to prey. Another 6 tagged shoots of each species were out with the frames and therefore had free access to prey. Thus, although all shoots within a floating frame or a container were pseudoreplicates, they grew under uniform conditions of prey availability and CO_2 concentration. The three shoots of each species (9 shoots in total) grown in each floating frame probably represented the maximum number which would not affect the shoots by interspecific competition. Every two days, the mesh on the floating frames was cleaned by tap water and fine zooplankton (copepods, ostracods, size 0.6–1.5 mm) was added repeatedly to the container to feed the control plants. The water in one container was very gently bubbled with CO_2 from a cylinder to keep the free CO_2 concentration about one order of magnitude higher ($+CO_2$ variant) than that in the other container ($–CO_2$ variant). To equalise the water chemistry and temperature within each container and the floating frames, the water in each container was gently mixed using a submersible aquarium pump. The containers were covered with a neutral-density nylon filter (one or two layers according to the weather) and the irradiance (PAR) at plant level was about 26–42% of that in the open area, which could be an optimum level for all species (Adamec & Kovářová 2006, Adamec 2011f). A submersible temperature data logger (Minikin T, EMS, Brno, Czech Rep.) was placed in each container at plant level. During the experimental growth period (14–28 June) in the $+CO_2$ container, the mean daily maximum water temperature at plant level was 28.7 °C and the mean night minimum was 24.8 °C (total range 21.4 to 32.1 °C), the temperature in the $–CO_2$ container was slightly lower (mean daily maximum 27.3 °C, mean night minimum 24.2 °C, range 20.7 to 30.1 °C).

Basic water chemistry parameters were estimated in the cultivation water at the beginning and end of the experiment (for the methods see Adamec 2000). The pH in the $+CO_2$ container was within 6.60–6.87 which corresponded to a free CO_2 concentration of 0.30–0.58 mM. The pH in the $–CO_2$ container was, however, between 7.55–7.96 corresponding to only 0.024–0.062 mM CO_2. Thus, the CO_2 concentration in the CO_2-enriched variant was 10–15 times higher. Free CO_2 concentration was calculated from total alkalinity and pH according to Helder 1988. For other parameters, the water chemistry was almost identical in both containers: the total alkalinity was 0.94–1.02 meq.l^{-1} and electrical conductivity was 25.4–31.4 mS.m^{-1} during the experiment. Due to the relatively high density of zooplankton prey added, the water was relatively rich in the main mineral nutrients and was considered mesotrophic in both containers (0 μg.l^{-1} NO_3^--N; 42–118 μg.l^{-1} NH_4^+-N; 65–74 μg.l^{-1} PO_4-P). No significant difference in pH or temperature was measured between the frames and the ambient water in each container. Tap water replaced evaporative water losses.

After 12 d (*U. vulgaris*), 13 d (*U. australis*) or 14 d (*U. reflexa*), the main shoot length, number of mature leaf nodes, shoot branching, the number of traps and the percentage of traps bearing captured macroscopic prey in the 11th–12th mature leaf nodes (in 2nd–4th nodes in *U. reflexa*) were estimated in all plants (Adamec 2008b, 2009, 2011b,f). In the same shoot segments, the structural investment in carnivory (proportion of trap biomass to the total leaf node biomass) was estimated (Adamec 2009). All traps from this material were counted, separated using fine forceps, and the traps and trap-free segments were dried (80 °C; DW). We assumed that DW of prey was negligible. Mean DW of one trap and the number of traps per mg of leaf node DW (without trap DW; see Friday 1992, Manjarrés-Hernández & al. 2006) were calculated to specify exactly the biomass and the number of traps on the leaf nodes. The number of traps per mg of leaf node DW is also equal to IIC / [Mean trap DW (1 – IIC)]. It should be added that the trap number per leaf in an *Utricularia* species can vary within one to two orders of magnitude (Knight & Frost 1991, Friday 1992, Guisande & al. 2000, 2004) but the size and DW of *Utricularia* leaves within a species are also highly variable (e.g. Friday 1992). Thus, the expression of trap number and/or biomass "per leaf" is vague and both parameters must be specified per leaf node DW (Friday 1992). Maximum trap size (to the nearest 0.5 mm) measured using a ruler (Adamec 2009) was estimated for the 12 youngest, newly produced leaf nodes (in 4 nodes in *U. reflexa*). The apical shoot growth rate was measured in all plants as the position of the tag after 12–14 days at the end of the experiment.

Tissue Nutrient Content and Statistical Treatment

Tissue N and P content was estimated after acid mineralisation of the 2nd–5th mature leaf nodes in each of the 6 parallel plants in all variants (for all analytical details, see Adamec 2002). Due to highly different tissue nutrient contents in traps and leaves (Adamec 2008a, 2010b) and a different proportion of trap biomass between the variants, these leaf nodes were deprived of all traps before the analyses.

The data from both floating frames within the same variant were pooled together and throughout this paper, the mean with standard error is shown wherever possible. The parameters such as the number of branches and the percentage of traps with prey evidently have non-normal distribution and, thus, only means and ranges of values are shown. Differences in growth parameters within each species were tested by two-way ANOVA (CO_2 and Prey as fixed effects) and those in the investment in carnivory, shoot N and P content and their ratio by three-way ANOVA (Species as random effect). Multiple comparisons within each species were tested by one-way ANOVA (HSD Tukey test). Linear regression models were used to look for statistically significant and meaningful correlations between pairs of dependent variables within each species. Five significant and most important regression models were identified for each species and they are included in the results. Due to interrelated factors, Bonferroni correction was used and, thus, $P < 0.01$ is the critical probability level in these models.

Results

At the end of the growth experiment, traps of all three species of the -Prey variant contained virtually no prey, while the control plants with prey

had on average 0-69% of traps with captured prey (Table 1). Great differences were found among single plants within each variant. In *U. vulgaris* and *U. reflexa*, the greatly reduced prey capture in the $-CO_2$ +Prey variant was due to very small traps. Plants of all species with either CO_2 or prey addition were usually statistically significantly longer, had more mature leaf nodes on the main shoots and more branched than the $-CO_2$ or -Prey variant. Similarly, the ASGR reflected both CO_2 and prey addition. The ASGR values were comparable in *U. vulgaris* and *U. australis*, while those of *U. reflexa* were 3–4 times lower. In all species, the largest traps were consistently and significantly formed as a result of CO_2 addition, while the effect of prey addition was much lesser and rather ambiguous: in $+CO_2$ variants, prey addition increased significantly the trap size but rather decreased it in $-CO_2$ variants. The effect of both factors on mean trap DW was much more marked than that on the maximum trap size (Table 1). In all species, and regardless of the presence of prey, the CO_2 addition increased greatly (2.7–249 times) the mean trap DW. Moreover, in all $+CO_2$ variants, feeding on prey also significantly increased the trap DW (1.5–2.3 times) but this effect was non-significant and ambiguous in $-CO_2$ variants. Both CO_2 and prey addition significantly influenced the trap number per mg leaf node in *U. vulgaris,* but not in the other two species where the numbers were fairly constant. Two-way ANOVA confirmed a highly significant effect ($P < 0.01$) of CO_2 on all growth parameters in all species (Table 2), while the prey effect was significant only in *U. vulgaris* and *U. australis* and, on the ASGR and mean trap DW, also in *U. reflexa*. However, the $CO_2 \times$ Prey interaction was significant only in *U. vulgaris* and *U. reflexa* but not in *U. australis*.

CO_2 addition increased very markedly the investment in carnivory in all species, while the effect of prey addition was much less (Table 3). In all species, the highest values of the investment in carnivory (48-59%) were attained significantly in the $+CO_2$ +Prey variants but the prey absence led to a significant decrease in the investment. On the other hand, the effect of prey addition was ambiguous and non-significant in the $-CO_2$ variants but all these values were relatively low (0.7–18%). CO_2 addition had a crucial effect on shoot N and P contents in all species and markedly decreased the contents regardless of prey addition. On analysis, significant (negative) linear correlations ($P < 0.022$) were found between the ASGR and the shoot P content within each species and, in *U. vulgaris*, also for the N content (data not shown). However, in the $-CO_2$ variants in all species, prey addition led to a significant increase in shoot N content and in *U. australis* also in P content. The N:P ratio was always highest in the $+CO_2$ variants (8.7–10.4) but this increase was not significant in *U. reflexa* (Table 3). Three-way ANOVA revealed a highly significant effect ($P < 0.001$-0.01) of Species and a less significant effect ($P < 0.05$–0.01) of CO_2 on the investment in carnivory, shoot N and P content and their N:P ratio, while the effect of Prey was non-significant (Table 4). The investment in carnivory was influenced highly signifi-

Table 1. Results of the greenhouse growth experiment (12-14 d) on three aquatic *Utricularia* species. The variants +CO_2 were permanently enriched in CO_2 (cca. 0.3–0.6 mM), while the –CO_2 variants had about 10–15 times lower the CO_2 concentration. +Prey, variants enriched by prey addition; – Prey, absence of prey. ASGR, apical shoot growth rate as production of new nodes a day. The mean trap DW, the percentage of traps with prey and the trap number per mg leaf node DW were estimated in 11^{th}–12^{th} leaf nodes in *U. vulgaris* and *U. australis* and in 2^{nd}–4^{th} nodes in *U. reflexa*. The maximum trap size was estimated within the 12 youngest, newly produced leaf nodes in *U. vulgaris* and *U. australis* and 4 nodes in *U. reflexa*. The different letters denote the statistically significant difference between the variants within single species at $P < 0.05$ (one-way ANOVA, HSD Tukey test). Means ± SE are shown; n = 6. In other cases, a range of values is shown instead.

Treatments		Shoot length (cm)	Shoot nodes	Branches per plant	ASGR (nodes/d)	Traps with prey (%)	Max. trap size (mm)	Mean trap DW (µg)	Trap No. per mg leaf DW
CO_2	Prey								
Utricularia vulgaris									
+CO_2	+Prey	28.6^{a} ±1.0	56.0^{a} ±1.2	0.67 (0–3)	3.56^{a} ±0.06	9.4 (0-27.3)	2.75^{a} ±0.11	33.3^{a} ±1.5	28.5^{a} ±1.5
+CO_2	–Prey	22.1^{b} ±1.1	47.8^{b} ±1.5	0	2.76^{b} ±0.16	0	2.00^{a} ±0.00	14.4^{b} ±1.8	9.06^{b} ±2.3
–CO_2	+Prey	19.8^{bc} ±1.5	39.7^{c} ±2.4	0.17 (0–1)	2.00^{c} ±0.18	1.5 (0-8.7)	0.88^{b} ±0.24	2.70^{c} ±2.00	14.4 (n = 2)
–CO_2	–Prey	16.2^{d} ±0.7	33.0^{d} ±0.7	0	1.50^{c} ±0.06	0	1.13^{b} ±0.29	5.69^{c} ±2.23	27.7^{c} ±2.6
Utricularia australis									
+CO_2	+Prey	74.1^{a} ±5.0	72.0^{a} ±1.2	3.33 (2–4)	4.42^{a} ±0.10	11.0 (2.6–31)	2.05^{a} ±0.05	16.1^{a} ±0.87	69.8^{a} ±5.9
+CO_2	–Prey	50.7^{b} ±2.8	65.6^{b} ±1.0	1.67 (1–3)	3.97^{b} ±0.10	0	1.57^{b} ±0.04	10.5^{b} ±1.4	66.3^{a} ±8.4
–CO_2	+Prey	53.5^{b} ±4.2	62.5^{b} ±2.8	1.00 (0-2)	3.61^{b} ±0.19	8.6 (0–32)	1.32^{bc} ±0.12	5.52^{c} ±1.03	39.7^{a} ±2.5
–CO_2	–Prey	37.5^{b} ±2.9	49.8^{c} ±1.2	0.50 (0–1)	2.57^{c} ±0.07	0	1.07^{c} ±0.24	3.94^{c} ±1.27	65.4^{a} ±11.7
Utricularia reflexa									
+CO_2	+Prey	30.4^{a} ±3.0	25.0^{a} ±2.5	1.67 (0-3)	1.19^{a} ±0.19	69.0 (40–89)	5.83^{a} ±0.28	217^{a} ±15.9	6.80^{a} ±0.56
+CO_2	-Prey	26.3^{ab} ±1.8	22.8^{ab} ±0.25	0.33 (0–1)	0.91^{ac} ±0.02	0	4.70^{b} ±0.16	111^{b} ±15.7	6.26^{a} ±1.21
–CO_2	+Prey	22.0^{ab} ±0.66	20.0^{ab} 0.58	1.00 (0–2)	0.71^{bc} ±0.04	0	0.90^{c} ±0.10	0.87^{c} ±0.35	7.53^{a} ±0.78
–CO_2	–Prey	19.6^{b} ±1.1	15.7^{b} ±0.33	0.17 (0–1)	0.41^{b} ±0.02	0	1.80^{c} ±0.37	4.15^{c} ±2.97	7.09^{a} ±0.85

cantly by the Species × CO_2 as well as the Species × CO_2 × Prey interaction. Shoot N and P contents were influenced significantly by the interactions of Species × CO_2, Species × Prey, and Species × CO_2 × Prey. As shown by linear regression models, shoot N content correlated very closely with shoot P content in all species (Table 5). Moreover, the investment in carnivory in all species correlated significantly and negatively with shoot N and P contents but highly significantly and positively with the trap size and trap DW. Thus, these relationships confirm that trap size and/or trap DW, rather than the number or traps per leaf, are the basis for the ecological regulation of the investment in carnivory in aquatic *Utricularia*.

Table 2. Results of the growth experiment on three aquatic *Utricularia* species evaluated by two-way ANOVA (CO_2 and Prey as factors). For the details see Table 1. Significance (within each species): *** – P < 0.001; ** – P < 0.01; * – P < 0.05; ns – 0.1 > P > 0.05; nss – P > 0.1.

Factor of ANOVA	Shoot length	Shoot nodes	ASGR	Max. trap size	Mean trap DW	Trap No. per mg leaf DW
Utricularia vulgaris						
CO_2	***	***	***	***	***	–
Prey	***	***	***	nss	***	–
CO_2 × Prey	nss	nss	nss	*	***	–
Utricularia australis						
CO_2	***	***	***	***	***	ns
Prey	***	***	***	***	**	nss
CO_2 × Prey	nss	ns	*	nss	ns	ns
Utricularia reflexa						
CO_2	**	**	***	***	***	nss
Prey	nss	ns	*	nss	***	nss
CO_2 × Prey	nss	nss	nss	***	***	nss

Discussion

In the present study, the *Utricularia* plants were grown in both containers under nearly-natural conditions of light, prey and CO_2 availability, summer water temperature and chemistry (cf. Adamec 2007, 2008a, 2009, 2010a, Peroutka & al. 2008). The slight differences in temperature between both containers (ca. 1 °C) should not influence the results. The great difference in the CO_2 concentration maintained between both containers covers a good deal of a common ecological range within temperate shallow dystrophic *Utricularia* sites and the concentrations may be considered suboptimal *vs.* optimal. Although the prey capture by traps estimated within a relatively narrow fraction of mature leaf nodes was very variable, both within each

Table 3. Physiological characteristics of three aquatic *Utricularia* species at the end of the growth experiment. For all details see Table 1. IIC, investment in carnivory, the proportion (in%) of trap DW to the total node biomass in 11th–12th leaf nodes in *U. vulgaris* and *U. australis* and in 2nd–4th nodes in *U. reflexa*. Tissue N and P content was estimated in 2nd–5th leaf nodes without traps in all species. The different letters denote the statistically significant difference between the variants within single species at $P < 0.05$ (one-way ANOVA, HSD Tukey test). Means ± SE are shown, n = 6.

Treatments		IIC	Shoot content		N:P
CO_2	Prey	(% DW)	N (% DW)	P (% DW)	
Utricularia vulgaris					
$+CO_2$	+Prey	48.3^{a} ±1.6	1.80^{a} ±0.06	0.160^{a} ±0.005	11.3^{a} ±0.56
$+CO_2$	–Prey	11.5^{b} ±3.4	1.72^{a} ±0.03	0.174^{a} ±0.011	10.1^{a} ±0.69
$–CO_2$	+Prey	4.00^{b} ±2.83	3.57^{b} ±0.16	0.453^{b} ±0.024	7.90^{b} ±0.19
$–CO_2$	–Prey	12.1^{b} ±4.0	2.82^{c} ±0.10	0.402^{b} ±0.020	7.08^{b} ±0.34
Utricularia australis					
$+CO_2$	+Prey	52.3^{a} ±0.89	1.45^{a} ±0.11	0.165^{a} ±0.011	8.84^{a} ±0.44
$+CO_2$	–Prey	38.9^{b} ±0.83	1.15^{a} ±0.05	0.112^{a} ±0.007	10.4^{a} ±0.48
$–CO_2$	+Prey	17.4^{c} ±2.6	4.04^{b} ±0.11	0.620^{b} ±0.029	6.59^{b} ±0.38
$–CO_2$	–Prey	17.8^{c} ±3.6	2.41^{c} ±0.09	0.375^{c} ±0.016	6.50^{b} ±0.33
Utricularia reflexa					
$+CO_2$	+Prey	58.9^{a} ±1.6	1.06^{a} ±0.11	0.127^{a} ±0.017	8.88^{a} ±1.04
$+CO_2$	–Prey	38.4^{b} ±4.5	0.759^{a} ±0.041	0.092^{a} ±0.011	8.65^{a} ±0.75
$–CO_2$	+Prey	0.72^{c} ±0.26	2.79^{b} ±0.21	0.383^{b} ±0.017	7.41^{a} ±0.74
$–CO_2$	–Prey	3.38^{c} ±2.61	1.96^{c} ±0.14	0.327^{b} ±0.026	6.13^{a} ±0.51

variant and between the containers (Table 1), prey were also captured by traps on other leaf nodes. Thus, in all $-CO_2$ +Prey variants with small traps on the measured leaf nodes, much prey was captured by older, larger traps.

The ASGR of all three *Utricularia* species was markedly enhanced by prey capture and the extent of this increase was similar at both CO_2 concentration levels (Table 1). However, the ASGR in all species was increased by

the CO_2 addition much more (by 22–94%) than by the prey addition (by 11–73%). Prey capture also very markedly stimulated the branching of the main shoot and the extent of the stimulation was comparable with the effect of the CO_2 concentration increase. Thus, for three aquatic *Utricularia* species, it has been confirmed that two main dynamic growth characteristics, which crucially influence the plant's relative growth rate (ASGR and branching), are increased approximately to the same extent by prey or CO_2 addition and that their combination has an additive effect (cf. McDermott & Darnowski 2002, Pagano & Titus 2004, 2007). Considering also the shoot N and P contents (Table 3), this marked additive growth effect suggests that the positive effect of the high photosynthetic rate is combined with the rapid growth uptake of N and P from prey provided that other growth factors (temperature, light) are optimal. Thus, both effects (CO_2, prey) act independently of each other. This also explains why aquatic *Utricularia* species grow preferentially in CO_2-rich waters (Adamec 2007, 2011a). Furthermore, the results show that in growth experiments with aquatic carnivorous plants, it is always necessary to also regulate CO_2 concentration; the *Utricularia* growth enhancement (ASGR) due to prey addition in this study (i.e., by 11–73%) would otherwise have been comparable with that in other studies (Adamec 1997, 2008b, 2011f, Kosiba 1992a,b, Englund & Harms 2003, Adamec & al. 2010).

Table 4. Physiological characteristics of three aquatic *Utricularia* species at the end of the growth experiment evaluated by three-way ANOVA (Species, CO_2 and Prey as factors). IIC, investment in carnivory. For the details see Tables 1 and 3. Significance (within each species): *** – $P < 0.001$; ** – $P < 0.01$; * – $P < 0.05$; ns – $0.1 > P > 0.05$; nss – $P > 0.1$.

Factor of 3-way ANOVA	IIC	Shoot content		N:P
		N	P	
Species	***	***	***	**
CO_2	*	**	*	*
Prey	ns	ns	nss	nss
Species × CO_2	***	**	***	nss
Species × Prey	nss	**	***	ns
CO_2 × Prey	ns	ns	nss	nss
Species × CO_2 × Prey	***	*	**	nss

For the ecological regulation of the IIC in aquatic *Utricularia*, the crucial question is what is regulated: the number of traps or their size? Our present data (Tables 1, 2) show that the trap number per leaf node DW significantly differed among variants only in *U. vulgaris* (about 3 ×) but was rather conservative in the other two species, without any relationship to the mean trap DW in all three species. Moreover, the trap number

Table 5. Statistically significant and ecologically important linear regression models of important parameters; n = 24. As a result of Bonferroni correction, only values of P < 0.01 represent significant correlation. For units and explanation of variables, see Tables 1 and 3. r^2, coefficient of determination.

No.	Linear regression model	r^2	P
Utricularia vulgaris			
1	N = 0.837 + 5.52 P	0.903	< 0.0001
2	IIC = 52.4 – 13.5 N	0.336	0.0031
3	IIC = 44.7 – 86.6 P	0.410	0.0008
4	IIC = –11.3 + 18.0 Trap Size	0.694	< 0.0001
5	IIC = –0.453 + 1.39 Trap DW	0.897	< 0.0001
Utricularia australis			
6	N = 0.540 + 5.42 P	0.935	< 0.0001
7	IIC = 54.5 – 10.1 N	0.543	< 0.0001
8	IIC = 50.9 – 60.7 P	0.626	< 0.0001
9	IIC = –21.4 + 35.2 Trap Size	0.801	< 0.0001
10	IIC = 8.31 + 2.58 Trap DW	0.797	< 0.0001
Utricularia reflexa			
11	N = 0.308 + 5.76 P	0.778	< 0.0001
12	IIC = 62.9 – 22.3 N	0.599	< 0.0001
13	IIC = 62.3 – 158 P	0.692	< 0.0001
14	IIC = –12.1 + 11.4 Trap Size	0.906	< 0.0001
15	IIC = 4.39 + 0.254 Trap DW	0.889	< 0.0001

per leaf node DW also correlated closely (r^2 = 0.52; P < 0.0007) with the IIC only in *U. vulgaris*, while it did not correlate at all in the other two species (data not shown). Friday 1992 found that in field-grown *U. vulgaris*, the mean IIC was fairly constant during its seasonal growth (49.7 ± 1.1%) and the trap number correlated with leaf length. Yet in four parallel plants with exactly the same IIC collected in the middle of the season, a great variation of the trap number per leaf node DW (3.3–14.4 mg^{-1}) and the mean trap DW (93–365 µg) was found. These data confirm that in this species as well as in *U. foliosa* (Guisande & al. 2000, 2004, Manjarrés-Hernández & al. 2006), the trap number per leaf node DW is highly variable and takes part in the regulation of the IIC. In *U. purpurea*, both the trap number per leaf node DW and the mean trap DW vary little (only 1.6 times) and contribute to IIC changes in a similar way (Richards 2001). Unlike the rather conservative values of the trap number per leaf node DW in *U. australis* in the present data (means 40–70; Table 1), Adamec 2009 found a conspicuous range between 9–67 (mean 26.4 ± 3.8) in a field study at 17 very variable sites. In the present study, a very strong correlation was found between the IIC and the mean trap DW in all three species (Table

5). Thus, the pattern of IIC regulation in aquatic *Utricularia* can be species specific but it can be inferred from all data that the regulation through the trap size (biomass) is universal and decisive.

It is well known that aquatic *Utricularia* species regulate their structural IIC to optimise their cost-benefit relationships, mainly according to some ecological habitat factors which relate to plant mineral nutrition. These include: water chemistry (mainly mineral N and P concentration), prey capture and irradiance and CO_2 concentration as ecological factors which directly influence photosynthesis and growth rate (Knight & Frost 1991, Friday 1992, Bern, 1997, Guisande & al. 2000, 2004, Richards 2001, McDermott & Darnowski 2002, Englund & Harms 2003, Adamec & Kovářová 2006, Manjarrés-Hernández & al. 2006, Kibriya & Jones 2007, Adamec 2007, 2008a, 2009, Adamec & al. 2010, Sirová & al. 2011). Generally, in the majority of these studies, conditions with a greater mineral nutrient availability (i.e., prey capture, higher concentrations of NH_4^+, NO_3^-, PO_4–P in the ambient water) led to a decrease in the IIC and vice versa by a negative feedback mechanism. Tissue N or P content in young shoot segments was found to perform a key physiological regulatory role in trap production (Bern 1997, Kibriya & Jones 2007). All nutritional influences which changed (indirectly) shoot N content in *U. australis* also influenced the IIC in the opposite direction (Adamec 2008a).

However, several pieces of evidence confirm that the regulation of the investment in carnivory in aquatic *Utricularia* also includes an above-threshold level of photosynthetic rate and/or irradiance and CO_2 concentration (Bern 1997, McDermott & Darnowski 2002, Englund & Harms 2003, Adamec 2008a, this study). *Utricularia foliosa* grown in an oligotrophic wetland did not produce any traps in shade (only 10% of the incident irradiance in the open; Bern 1997) and neither did strongly shaded *U. vulgaris* grown in filtered lake water when fed on prey (Englund & Harms 2003). In all three *Utricularia* species, the IIC and shoot N and P content depended very markedly both on CO_2 and prey addition but the influence of CO_2 addition on all three parameters was much stronger (Tables 3, 4). The non-significant interactions between CO_2 and prey addition show that both factors act on the parameters independently on each other. Linear regressions within each species confirmed significant but negative correlations between the IIC and shoot N or P contents (Table 5): higher N or P content correlated with the lower IIC by a negative feedback mechanism as reported also by Bern 1997, Kibriya & Jones 2007 and Adamec 2008a. However, in spite of these (highly) significant correlations for all data (n = 24) within each species, a detailed comparison of both +CO_2 variants (+ or –Prey) suggests that these correlations evidently do not hold for these variants: the significantly higher IIC values in the +Prey variants are associated with the same or even slightly higher N and P contents (see Table 3). Under the conditions of surplus CO_2 concentration and optimal light and temperature (i.e., high photosynthetic

rate and ASGR), the ASGR is thus limited by low shoot N and P content. Prey capture then leads to a great increase of the IIC, although it also may slightly increase the shoot N and P content (but cf. Adamec 2008b, 2011f, Adamec & al. 2010). Thus, this indicates that the negative feedback "nutrient" regulation of the IIC by shoot N and/or P nutrient content does not apply to such surplus CO_2 conditions when the trap production is stimulated more by an excess of photosynthates than by a shortage of N or P (i.e., "photosynthetic" regulation). Moreover, a positive effect of a direct uptake of organic carbon from prey on the IIC increase cannot be excluded. On the contrary, at very low CO_2 concentration (< ca. 0.02–0.03 mM; Adamec 2008a, 2009) and deep shade conditions, the resulting low photosynthetic rate and ASGR, in addition to very high shoot N and P content, prevent trap production. The IIC is very low, and any prey capture even rather decreases the IIC due to increasing the shoot N and P content, but simultaneously enhances the ASGR (Tables 1, 3, Englund & Harms 2003; "photosynthetic" regulation again). At low to medium CO_2 concentration (ca. 0.03–0.2 mM, $-CO_2$ variant in this study), the "nutrient" regulation of the IIC by variable shoot N and/or P nutrient content prevails.

It may be concluded that under the surplus CO_2 and light conditions, the trap production as the IIC in aquatic *Utricularia* is supported by prey capture more (positive feedback) than the (maximum) apical shoot growth (Tables 1, 3) but the IIC apparently does not depend on shoot N or P content. Favourable photosynthetic conditions are thus the crucial prerequisite for high trap production. Under the most common conditions of medium CO_2 concentration, shoot N and P contents are very variable and regulate the IIC by the negative feedback ("nutrient" regulation; Adamec 2008a). Under poor photosynthetic conditions (low light or CO_2 concentration) and thus minimal ASGR, however, the trap production is blocked by a shortage of photosynthates, which are allocated preferentially to shoot apices and branching, but probably also by very high shoot N and P content. In general, the regulation of trap production in *Utricularia* includes two components; high CO_2 concentration as a prerequisite for high photosynthetic rate (positive feedback) is superior to the negative feedback regulation by tissue N or P content in young shoot segments. This negative feedback also helps to stabilize the tissue contents of mineral nutrients as a result of mineral nutrient uptake from prey. As prey capture also supports plant growth (ASGR, branching), which in turn decreases the tissue N and P content, the growth rate itself is obviously also a component of this endogenous regulatory system of the IIC. To further elucidate the IIC regulation mechanisms, a combination of methodical approaches such as the use of photosynthetic inhibitors, different light intensities, replacement of prey capture by a mineral nutrient addition to the ambient water or a modulation of the shoot N and P contents by a mineral deficiency of other nutrients (especially K and Mg) could be beneficial.

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References

Adamec L. 1997. Mineral nutrition of carnivorous plants: A review. – Bot. Rev. 63: 273-299.

Adamec L. 2000. Rootless aquatic plant *Aldrovanda vesiculosa*: physiological polarity, mineral nutrition, and importance of carnivory. – Biol. Plant. 43: 113–119.

Adamec L. 2002. Leaf absorption of mineral nutrients in carnivorous plants stimulates root nutrient uptake. – New Phytol. 155: 89–100.

Adamec L. 2006. Respiration and photosynthesis of bladders and leaves of aquatic *Utricularia* species. – Plant Biol. 8: 765–769.

Adamec L. 2007. Investment in carnivory in *Utricularia stygia* and *U. intermedia* with dimorphic shoots. – Preslia 79: 127–139.

Adamec L. 2008a. Mineral nutrient relations in the aquatic carnivorous plant *Utricularia australis* and its investment in carnivory. – Fundam. Appl. Limnol. 171: 175–183.

Adamec L. 2008b. The influence of prey capture on photosynthetic rate in two aquatic carnivorous plant species. – Aquat. Bot. 89: 66–70.

Adamec L. 2009. Photosynthetic CO_2 affinity of the aquatic carnivorous plant *Utricularia australis* (Lentibulariaceae) and its investment in carnivory. – Ecol. Res. 24: 327–333.

Adamec L. 2010a. Field growth analysis of *Utricularia stygia* and *U. intermedia* – two aquatic carnivorous plants with dimorphic shoots. – Phyton 49: 241–251.

Adamec L. 2010b. Mineral cost of carnivory in aquatic carnivorous plants. – Flora 205: 618–621.

Adamec L. 2011a. Ecophysiological look at plant carnivory: Why are plants carnivorous? – In: Seckbach J. & Dubinski Z. (eds.), All flesh is grass. Plant-animal interrelationships. Cellular origin, life in extreme habitats and astrobiology Vol. 16, pp. 455–489. – Springer Science + Business Media B. V., Dordrecht, Heidelberg, London, New York.

Adamec L. 2011b. Shoot branching of the aquatic carnivorous plant *Utricularia australis* as the key process of plant growth. – Phyton 51: 133–148.

Adamec L. 2011c. Dark respiration and photosynthesis of dormant and sprouting turions of aquatic plants. Fundam. – Appl. Limnol. 179: 151–158.

Adamec L. 2011d. The comparison of mechanically stimulated and spontaneous firings in traps of aquatic carnivorous *Utricularia* species. – Aquat. Bot. 94: 44–49.

Adamec L. 2011e. Functional characteristics of traps of aquatic carnivorous *Utricularia* species. – Aquat. Bot. 95: 226–233.

Adamec L. 2011f. By which mechanism does prey capture enhance plant growth in aquatic carnivorous plants: Stimulation of shoot apex? – Fundam. Appl. Limnol. 178: 171–176.

Adamec L. 2013. A comparison of photosynthetic and respiration rates in six aquatic carnivorous *Utricularia* species differing in morphology. – Aquat. Bot. 111: 89–94.

Adamec L. & Kovářová M. 2006. Field growth characteristics of two aquatic carnivorous plants, *Aldrovanda vesiculosa* and *Utricularia australis*. – Folia Geobot. 41: 395–406.

Adamec L. & Pásek K. 2009. Photosynthetic CO_2 affinity of aquatic carnivorous plants growing under nearly-natural conditions and in vitro. – Carniv. Plant Newslett. 38: 107–113.

Adamec L., Sirová D. & Vrba J. 2010. Contrasting growth effects of prey capture in two aquatic carnivorous plant species. – Fundam. Appl. Limnol. 176: 153–160.

Bern A. L. 1997. Studies on nitrogen and phosphorus uptake by the carnivorous bladderwort *Utricularia foliosa* L. in South Florida wetlands. – MSc-thesis, Florida Int. University, Miami.

Borovec J., Sirová D. & Adamec L. 2012. Light as a factor affecting the concentration of simple organics in the traps of aquatic carnivorous *Utricularia* species. – Fundam. Appl. Limnol. 181: 159–166.

Englund G. & Harms S. 2003. Effects of light and microcrustacean prey on growth and investment in carnivory in *Utricularia vulgaris*. – Freshwat. Biol. 48: 786–794.

Friday L. E. 1989. Rapid turnover of traps in *Utricularia vulgaris* L. – Oecologia 80: 272-277.

Friday L. E. 1992. Measuring investment in carnivory: seasonal and individual variation in trap number and biomass in *Utricularia vulgaris* L. – New Phytol. 121: 439–445.

Guisande C., Granado-Lorencio C., Andrade-Sossa C. & Duque S. R. 2007. Bladderworts. – Funct. Plant Sci. Biotechnol. 1: 58–68.

Guisande C., Andrade C., Granado-Lorencio C., Duque S. R. & Núñez-Avellaneda M. 2000. Effects of zooplankton and conductivity on tropical *Utricularia foliosa* investment in carnivory. – Aquat. Ecol. 34: 137–142.

Guisande C., Aranguren N., Andrade-Sossa C., Prat N., Granado-Lorencio C., Barrios M. L., Bolivar A., Núñez-Avellaneda M. & Duque S. R. 2004. Relative balance of the cost and benefit associated with carnivory in the tropical *Utricularia foliosa*. – Aquat. Bot. 80: 271–282.

Helder R. J. 1988. A quantitative approach to the inorganic carbon system in aqueous media used in biological research: dilute solutions isolated from the atmosphere. – Plant Cell Environ. 11: 211–230.

Juniper B. E., Robins R. J. & Joel D. M. 1989. The carnivorous plants. – Academic Press Ltd, London.

Kibriya S. & Jones J. I. 2007. Nutrient availability and the carnivorous habit in *Utricularia vulgaris*. – Freshwater Biol. 52: 500–509.

Knight S. E. 1992. Costs of carnivory in the common bladderwort, *Utricularia macrorhiza*. – Oecologia 89: 348–355.

Knight S. E. & Frost T. M. 1991. Bladder control in *Utricularia macrorhiza*: lake-specific variation in plant investment in carnivory. – Ecology 72: 728–734.

Kosiba P. 1992a. Studies on the ecology of *Utricularia vulgaris* L. I. Ecological differentiation of *Utricularia vulgaris* L. population affected by chemical factors of the habitat. – Ekol. Pol. 40: 147–192.

Kosiba P. 1992b. Studies on the ecology of *Utricularia vulgaris* L. II. Physical, chemical and biotic factors and the growth of *Utricularia vulgaris* L. in cultures in vitro. – Ekol. Pol. 40: 193–212.

Manjarrés-Hernández A., Guisande C., Torres N. N., Valoyes-Valois V., González-Bermúdez A., Díaz-Olarte J., Sanabria-Aranda L. & Duque S. R. 2006. Temporal and spatial change of the investment in carnivory of the tropical *Utricularia foliosa*. – Aquat. Bot. 85: 212–218.

McDermott M. & Darnowski D. W. 2002. Ecology of bladderworts in a unique site on the Eastern shore of Maryland. – Carniv. Plant Newslett. 31: 67–74.

Moeller R. E. 1978. Carbon-uptake by the submerged hydrophyte *Utricularia purpurea*. – Aquat. Bot. 5: 209–216.

Pagano A. M. & Titus J. E. 2004. Submersed macrophyte growth at low pH: contrasting responses of three species to dissolved inorganic carbon enrichment and sediment type. –Aquat. Bot. 79: 65–74.

Pagano A. M. & Titus J. E. 2007. Submersed macrophyte growth at low pH: carbon source influences response to dissolved inorganic carbon enrichment. – Freshwat. Biol. 52: 2412–2420.

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.

Płachno B. J., Adamec L., Lichtscheidl I. K., Peroutka M., Adlassnig W. & Vrba J. 2006. Fluorescence labelling of phosphatase activity in digestive glands of carnivorous plants. – Plant Biol. 8: 813–820.

Porembski S., Theisen I. & Barthlott W. 2006. Biomass allocation patterns in terrestrial, epiphytic and aquatic species of *Utricularia* (Lentibulariaceae). – Flora 201: 477–482.

Richards J. H. 2001. Bladder function in *Utricularia purpurea* (Lentibulariaceae): is carnivory important? – Am. J. Bot. 88: 170–176.

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.

Sirová D., Borovec J., Šantrůčková H., Šantrůček J., Vrba J. & Adamec L. 2010. *Utricularia* carnivory revisited: Plants supply photosynthetic carbon to traps. – J. Exp. Bot. 61: 99–103.

Sirová D., Borovec J., Picek T., Adamec L., Nedbalová L. & Vrba J. 2011. Ecological implications of organic carbon dynamics in the traps of aquatic carnivorous *Utricularia* plants. – Funct. Plant Biol. 38: 583–593.

Taylor P. 1989. The genus *Utricularia*: A taxonomic monograph. – Kew Bulletin, Additional Series, XIV.

Vincent O., Weisskopf C., Poppinga S., Masselter T., Speck T., Joyeux M., Quilliet C. & Marmottant P. 2011. Ultra-fast underwater suction traps. – Proc. R. Soc. B 278: 2909–2914.