Shoot Branching of the Aquatic Carnivorous Plant *Utricularia australis* as the Key Process of Plant Growth

By

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With 4 Tables

Summary

ADAMEC L. 2011. Shoot branching of the aquatic carnivorous plant *Utricularia australis* as the key process of plant growth. – Phyton (Horn, Austria) 51 (1): 133–148.

Utricularia australis is a submerged rootless aquatic carnivorous plant with homogeneous shoots, exhibiting a great morphological and ecological plasticity and propagating only vegetatively by shoot branching. Detailed branching and morphometric characteristics of two *U. australis* populations were compared at two sites in the Třeboň basin, Czech Republic. The sites, mesotrophic fishpond Ruda and oligotrophic sand-pit Cep, differed greatly in their trophic status and prey availability, and thus in plant size, fitness, and flowering. Flowering plants at Ruda were robust, highly branched, and produced on average totally 52 branches of various orders forming about 52.9 % dry weight of the total vegetative plant dry weight. All plants collected were on average relatively short, 12.5 % flowered, and 90 % contained a branch >3 cm long with functional traps. In comparison, plants at Cep were much shorter, their internodes were strongly shortened, only 5 % of plants flowered, and only 60 % of plants bore distinguishable branches (on average only 1.1 branches a plant and 1.4 % of total plant dry weight). Shoot branching was recognized as one of the most important reasons for high plasticity of *U. australis* and for attaining high

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relative growth rate. Branching intensity of main shoots in *U. australis* might reflect discrete ecological conditions of each micropopulation. To understand the dynamic growth traits of aquatic carnivorous plants, a combination of branching characteristics and mathematical modelling is essential.

Zusammenfassung

ADAMEC L. 2011. Shoot branching of the aquatic carnivorous plant *Utricularia australis* as the key process of plant growth. [Die Verzweigung des Sprosses der fleischfressenden Pflanze *Utricularia australis* als Schlüsselprozess des Pflanzenwachstums]. – Phyton (Horn, Austria) 51 (1): 133–148.

Utricularia australis ist eine submerse, wurzellose, pflanzenfressende Wasserpflanze mit homogenen Sprossen. Sie zeigt große morphologische und ökologische Anpassungsfähigkeit und vermehrt sich ausschließlich vegetativ durch Sprossverzweigungen. Einzelheiten der Verzweigung und morphometrische Besonderheiten von zwei *U. australis*-Populationen wurden an zwei Standorten im Třeboň Becken in der Tschechischen Republik verglichen. Die Standorte (ein mesotropher Fischteich: Ruda und eine oligotrophe Sandgrube: Cep) variieren sehr stark in ihrem trophischen Status und in der Verfügbarkeit von Beute. Daher waren Pflanzengröße, Vitalität und Blühverhalten unterschiedlich. Die blühenden Pflanzen von Ruda waren robust, stark verzweigt und hatten insgesamt 52 Verzweigungen verschiedener Ordnung, die in etwa 52,9 % des Gesamttrockengewichtes der vegetativen Pflanzenteile ausmachten. Alle Pflanzen waren im Mittel relativ kurz, 12,5 % blühten und 90 % hatten eine mehr als 3 cm lange Verzweigung mit funktionierenden Fallen. Zum Vergleich dazu waren die Pflanzen von Cep noch wesentlich kürzer, die Internodien waren stark verkürzt, nur 5 % blühten und bloß 60 % der Pflanzen hatten erkennbare Verzweigungen (im Durchschnitt 1,1 Verzweigungen pro Pflanze mit 1,4 % des Gesamttrockengewichts der Pflanzen). Die Verzweigungen von *U. australis* werden als einer der wichtigsten Gründe für die Anpassungsfähigkeit und für ihre relativ hohe Wachstumsrate angesehen. Der Grad der Verzweigungen der Hauptsprosse von *U. australis* spiegeln möglicherweise die jeweiligen ökologischen Bedingungen für jede Mikropopulation wider. Um die Charaktereigenschaften der Wachstumsdynamik fleischfressender Wasserpflanzen zu verstehen, muss eine Kombination aus der Verzweigungscharakteristik und mathematisches Modellieren herangezogen werden.

Introduction

The rootless carnivorous plant genus *Utricularia* L. (bladderwort, *Lentibulariaceae*) includes about 220 species. Around 50 species are aquatic or amphibious plants growing usually in standing, nutrient-poor, and humic waters and their growth can often be limited by a shortage of N, P, and also of K in these waters (TAYLOR 1989, GUISANDE & al. 2007). The plants use their rootless shoots to take up all necessary nutrients, either from the water or from captured prey. The plants are able to capture fine animal prey, typically aquatic crustaceans, mites, nematodes, rotifers, and protozoa, by their traps of foliar origin (e.g., HARMS 1999, RICHARDS 2001). Most aquatic *Utricularia* species exhibit very rapid apical shoot growth (1–4 leaf node d^{-1}) and a high relative growth rate (RGR; doubling time of

biomass, T_2, 6.6-33 d; FRIDAY 1989, PAGANO & TITUS 2004, ADAMEC & KO-VÁŘOVÁ 2006, ADAMEC 2008a, 2009, 2010). This very rapid growth in nutrient-poor habitats requires several ecophysiological adaptations, including a very high net photosynthetic rate of shoots, carnivory, activity of commensals inside traps, efficient nutrient re-utilization (recycling) from senescent shoots, and a very high affinity for mineral nutrient uptake from ambient water (KOSIBA 1992a,b, RICHARDS 2001, ENGLUND & HARMS 2003, ADAMEC 2006, 2008a,b, SIROVÁ & al. 2009).

Most species of aquatic *Utricularia* have a linear, modular and fairly regular shoot structure, consisting of nodes with dissected leaves and thin cylindrical internodes (FRIDAY 1989, TAYLOR 1989, SATTLER & RUTISHAUSER 1990, RUTISHAUSER 1993). However, morphologically and developmentally, the distinction between stems, branches, air shoots, and leaves is not clear and great discussions have arisen concerning the distinction and/or homology of these organs (for the review of concepts see SATTLER & RU-TISHAUSER 1990, RUTISHAUSER 1993). It may be concluded that all these organs might be considered homologous and that greatly overlap with each other, exhibiting a great morphological and functional plasticity. In this paper in the context of aquatic *Utricularia* species, I shall use the term "leaf" for the flat, dissected organ with the prevailing photosynthetic function, growing in leaf nodes (or untrue whorls) in a perpendicular plane to the "stem" and usually bearing traps (sensu SATTLER & RUTISHAUSER 1990, RUTISHAUSER 1993). "Stem" is denoted as a thin linear, cylindrical organ which regularly forms leaf nodes and branches. "Shoot" is denoted as a combination of "stem", "leaves", and "branches". "Branch" is denoted as a daughter shoot that separates from the main shoot and forms a separate plant.

Aquatic *Utricularia* species propagate mostly vegetatively by branching shoots. Therefore, the number of branches per shoot represents one of the main growth characteristics of these plants as high branching rate indicates favourable growth conditions and is a prerequisite for a high RGR (KOSIBA 1992a,b, ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2008a, 2009, 2010, ADAMEC & al. 2010). Exactly the same relationship also holds for the ecologically and morphologically similar aquatic carnivorous plant *Aldrovanda vesiculosa* (Droseraceae; KAMIŃSKI 1987, ADAMEC 1999, 2000, 2008a, ADAMEC & al. 2010). Moreover, branching rate (i.e., the number of new branches on a shoot or whole plant produced per unit time) together with apical shoot growth rate in aquatic *Utricularia* species are the growth processes which are most markedly stimulated by prey capture (KOSIBA 1992b, ENGLUND & HARMS 2003, ADAMEC 2008a, ADAMEC & al. 2010). However, mild competition occurs between the rate of branching and apical shoot growth (ADAMEC & KOVÁŘOVÁ 2006). As shown for some aquatic *Utricularia* species (SATTLER & RUTISHAUSER 1990, RUTISHAUSER 1993), shoot branches are initiated in the shoot apex and sprout in the axillary

position of leaf nodes. It has been found in some species that their branching intensity (i.e., the number of internodes between successive branches) is remarkably regular as dependent on species or ecological conditions (ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2007, 2009, 2010).

Vegetatively reproducing aquatic *Utricularia* species might comply with a concept on the differentiation of meristem allocation pattern, as a partial criterion of plant fitness, which may change with environmental conditions (BONSER & AARSSEN 1996). Accordingly, the developmental fate of shoot meristems enters three main categories: growth, reproductive, and inactive meristems. Growth meristems produce a new shoot or branch and reproductive meristems produce a flower or inflorescence, while inactive meristems remain dormant. It holds generally that all lateral meristems are in the axils of leaves and that each leaf axil bears a single axillary meristem (BELL 1991). Hence, the number of growth meristems is equal to the number of branches, the number of reproductive meristems is equal to the number of flowers or inflorescences, and the number of inactive meristems is equal to the number of leaf axils with no flowers or branches plus the number of main shoot apices (BONSER & AARSSEN 1996). Different strategies of meristem allocation have been proposed for terrestrial plants as dependent on availability of soil nutrient resources and light. However, this concept has never been applied to rootless aquatic plants with linear shoots.

Utricularia australis R.Br. (southern bladderwort) is a free floating, submerged aquatic carnivorous plant with homogeneous shoots bearing thousands of traps (TAYLOR 1989). Like other aquatic *Utricularia* species, *U. australis* exhibits continuous, rapid apical shoot growth during the growing season (2.6–4.2 new leaf nodes a day), while progressively ageing and decomposing at the base (ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2009, ADAMEC & al. 2010). Under optimal summer conditions, it can propagate rapidly by branching. *U. australis* is considered a eurytopic species and shows a very wide ecological tolerance of water chemistry (KOSIBA & SAROSIEK 1993, HOFMANN 2001, KOSIBA 2004, NAVRÁTILOVÁ & NAVRÁTIL 2005, ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2008b, 2009). In this study, *U. australis* has been chosen as a model aquatic carnivorous plant for its widespread distribution in different habitats in the Třeboň basin, Czech Republic, and for its great ecological plasticity.

The aim of the study was to compare detailed branching and morphometric characteristics of two *U. australis* populations at two sites in the Třeboň basin, Czech Republic, differing greatly in their trophic status and prey capture and, thus, in plant size, fitness, and flowering. Although the data obtained in this study are static and descriptive, the aim is to consider how the branching parameters function in a dynamic manner for attaining high RGR and rapid vegetative propagation of this species. In this way, the study closely follows up on former ones on the growth of *U.*

australis under outdoor of field conditions, dealing with different prey capture (ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2008a,b, 2009, ADAMEC & al. 2010). A mathematical model explaining plant growth parameters on the basis of branching characteristics in aquatic *Utricularia* is presented.

Material and Methods

Study Sites

The Třeboň Basin Biosphere Reserve and Protected Landscape Area in Southern Bohemia (approx. 49° N, 14° 45' E) is one of the centres of *U. australis* distribution in the Czech Republic. Here, it is widespread at hundreds of sites in different habitats (see ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2008b, 2009). The collection of plant material from two sites differing greatly in water chemistry and, also, plant size and fitness was conducted on 12 August 2008, during the height of the summer season. Plants were collected from the inlet of the Ruda fishpond and shallow sand-pit Cep. The former site is a meso-eutrophic, highly dystrophic water with highly organic bottom with a sufficient prey availability and represents an ecological optimum for *U. australis*. A dense, flowering population of this species was found here regularly in previous years. Sand-pit Cep is a shallow (ca. 80 cm) oligotrophic, very soft oligohumic water with sandy-clayish bottom and a very poor prey availability for *U. australis* (see ADAMEC 2009). Here, the plants were relatively small and weak and flowered only exceptionally.

Plant Sampling

In the Ruda fishpond, two different types of plant sampling were conducted. In the typical and relatively homogeneous aquatic vegetation at the water depth of 25–30 cm, dominated by *Glyceria fluitans* (ca. 40 % coverage) and *U. australis* (ca. 80 % coverage), a 6 m transect after each 2 m was set out. All *U. australis* plants reaching the transect points within 20 cm by their shoots were carefully collected, put in plastic bags in humid air, and stored in a refrigerator at 2–3 °C until processing. Totally, 40 plants of highly variable size were collected to characterize the population structure. Along another transect travelling 1 m in parallel, 15 flowering *U. australis* plants were carefully collected, each plant was put in a plastic bag, and stored as above to characterize the cohort of large, highly branching flowering plants. In sand-pit Cep, all *U. australis* plants (totally 40 plants) were collected along 8 m of the shoreline, 0.5–3 m from the shore at the water depth of 10–40 cm, and stored as above. The sampling zone, dominated by *Juncus bulbosus* (ca. 20 % coverage) and *Eleocharis acicularis* (ca. 10 % coverage), represented the densest stand of *U. australis* in the sand-pit, though the plants flowered only exceptionally, lay on the bottom, and their coverage was < 5 %. Due to the fact that the plants flowered rarely in sand-pit Cep flowering plants were not selectively collected from this site.

On the day of plant collection, pH and electrical conductivity in the stand water were measured directly in the field, while total alkalinity and concentrations of humic acids + tannins and of main nutrients were measured in the filtered water (for the methods see ADAMEC 1999, 2000). Concentration of free CO_2 was calculated from total alkalinity and pH according to HELDER 1988.

Plant Processing

The following morphometric parameters were estimated in each of the 15 flowering plants from the Ruda fishpond using a ruler (to the nearest 1 mm; see ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2008a, 2009, 2010): main shoot length, adult leaf nodes of the main shoot, number of all distinguishable branches of the main shoot (i.e., 1st-order branches) and of the 1st-order branches (i.e., 2nd- and 3rd-order branches), length of all 1st-order branches in the term of cm and number of adult leaf nodes, position of all 1st-order branches on the main shoot as the sequence of the adult leaf node from which it sprouts, number of internodes between successive branches on the main shoot and 1st-order branches, number of flower stems, and total length of flower stems. In 5 randomly selected plants, the percentage of traps with any macroscopic prey (using a binocular loupe), structural investment in carnivory (IIC; in % of the dry weight /DW/ of traps to the total DW of the given shoot segments), and mean DW of one trap were estimated in 11th to 12th adult leaf nodes (ADAMEC 2008b, 2009, ADAMEC & al. 2010). The total biomass of each of 15 plants was separated into DW (dried at 80 °C) of main shoot, all branches, and reproductive organs (rhizoids on the base of flower stems were added to the DW of main shoot). The same analyses were also conducted in each of 40 plants collected from sand-pit Cep and the same processing of traps was also conducted in 5 selected plants however in 11th-14th adult leaf nodes. To get basic information on the population structure of *U. australis* in the Ruda fishpond and in comparison with that at Cep, main shoot length, adult leaf nodes of the main shoot, and the percentage of flowering and branching plants were estimated in each of 40 collected plants at Ruda. For this data set, a plant was only counted as branched if a 1st order branch was longer than 3.0 cm and bore traps longer that 1.0 mm. This criterion was accepted to discriminate between growing and non-growing branches. Thus, short, non-growing branches with inactive meristem bearing no functional traps were not counted as branches.

Statistical Treatment

On the basis of differences in collected material at both sites, the data sets (flowering plants at Ruda vs. all plants at Cep) cannot be compared directly between sites. Only the differences between all 40 collected plants between both sites were tested for a statistical significance by a two-tailed t-test. Linear regression models were used to determine statistical significance of meaningful relationships between important dependent variables. Totally, 29 regression models were identified for three data sets (17; 1; 11 items) and were included in the results. Taking into account Bonferroni correction to minimize interrelated factors, regressions of flowering plants at Ruda were significant at $P < 0.0029$, while those of all plants at Cep at $P < 0.0045$. To determine the relationship of the apical growth rate of branches (in terms of new leaf nodes d^{-1}) and that of the main shoot (see ADAMEC 1999 for *A. vesiculosa*), the number of adult leaf nodes of 1st order branches was plotted against the position of these branches on the main shoot and analysed. As many short, non-growing 1st-order branches occurred commonly also in older segments of main shoots, the linear regression between these two parameters was conducted only for the longest, growing branches, which followed the growth of the main shoot since their apical initiation.

Results

The ambient water at the Ruda fishpond had an electrical conductivity 61 $\mu S.cm^{-1}$, pH 5.40, total alkalinity 0.15 meq.l^{-1}, free CO_2 concentration 1.40 mM, sum of humic acids + tannins 20.2 mg.l^{-1}, 0.0 $\mu g.l^{-1}$ NO_3^--N, 12.6 $\mu g.l^{-1}$ NH_4^+-N, and 21.2 $\mu g.l^{-1}$ PO_4-P, while the water at sand-pit Cep a conductivity 81 $\mu S.cm^{-1}$, pH 4.97, total alkalinity 0.46 $\mu eq.l^{-1}$, free CO_2 concentration 0.012 mM, sum of humic acids + tannins 1.24 mg.l^{-1}, 7.1 $\mu g.l^{-1}$ NO_3^--N, 0.0 $\mu g.l^{-1}$ NH_4^+-N, and 12.6 $\mu g.l^{-1}$ PO_4-P.

Flowering *U. australis* plants at Ruda were robust and highly branched (Table 1A): their mean length was 101 cm with 93 adult leaf nodes of the main shoot, they produced on average 18.6 1st-order branches and 33.5 2nd- and 3rd-order branches (the most branched plant had totally 80 apices on all branches), while all plants collected at Ruda (Table 1B) were much shorter (on average 55.5 cm and 57.4 leaf nodes), only 12.5 % of them flowered, and 90 % of them contained a branch >3 cm long with functional traps. On the other hand, the plants at Cep were much shorter (Table 1C; on average only 29.0 cm), their internodes were strongly shortened, only 5 % of plants flowered, and only 60 % of plants born distinguishable branches (on average only 1.1 branches a plant). In flowering plants at Ruda, the mean number of internodes between two successive 1st-order

Table 1. Morphometric parameters of *U. australis* plants at a meso-eutrophic site in the Ruda fishpond (A, only flowering plants; B, all collected plants) and an oligotrophic site in sand-pit Cep (C, all collected plants). Branching within A and C includes the presence of any 1st-order branch, while within B, a plant is only counted as branched if a 1st-order branch is longer than 3.0 cm and bears traps longer than 1.0 mm. Means $\pm$ SE are shown where possible. Statistically significant difference between sites B and C, * – P < 0.001; NS – P > 0.05.

Main shoot length		Flower.	Flowers per plant	Flower stem length	Plant branch	Number of branches per plant		Internodes between two branches	
(cm)	(nodes)	(% plants)		(cm)	(% plants)	1st order	$2^{nd}+3^{rd}$ order	1st order	$2^{nd}+3^{rd}$ order
A. Ruda fishpond – flowering plants (n = 15)									
100.8 $\pm$ 5.1	93.1 $\pm$ 3.3	100	2.07 (1–3)	9.6 $\pm$ 1.0	100	18.6 $\pm$ 1.4	33.5 $\pm$ 4.5	5.94 $\pm$ 0.25	6.56 $\pm$ 0.13
B. Ruda fishpond – all plants (n = 40)									
55.5* $\pm$3.8	57.4^{NS} $\pm$2.6	12.5	–	–	90	–	–	–	–
C. Cep sand-pit – all plants (n = 40)									
29.0 $\pm$ 1.9	63.7 $\pm$ 2.4	5.0	–	17.7 (n = 2)	60	1.10 $\pm$ 0.17	0.0	22.6 $\pm$ 1.06	–

Table 2. Evaluation of variability of 1st-order branches in the term of length and number of adult nodes in the Ruda fishpond and sand-pit Cep. Mean $\pm$ SE and lower and upper quartiles are shown.

Site	Length (cm)				Adult nodes			
	Mean	Median	Quartiles	Range	Mean	Median	Quartiles	Range
Ruda – flowering plants (n = 271)	12.6 $\pm$ 0.91	7.0	1.4; 18.1	0.2–76.5	15.9 $\pm$ 0.93	12	3; 24	0–86
Cep – all plants (n = 43)	1.75 $\pm$ 0.41	0.50	0.2; 2.2	0.1-12.1	3.91 $\pm$ 1.04	0	0; 6	0–26

Table 3. Dry weights of *U. australis* plants at a meso-eutrophic site in the Ruda fishpond (n = 15) and an oligotrophic site in sand-pit Cep (n = 40). DW of branches is expressed in % of the sum of main shoots + branches. Traps with captured a macroscopic prey, investment in carnivory (IIC; in % of the DW in the given shoot segments), and DW of one trap were estimated in 11th + 12th adult leaf nodes at Ruda, while at 11th to 14th nodes at Cep, always in 5 plants. Means $\pm$ SE are shown where possible.

DW of main shoots	DW of branches	DW of reprod	Total plant DW	DW of reprod	DW of branches	Traps with prey	IIC (trap DW)	DW of one trap
(mg)				(% tot.)	(% shoots)	(%)	(% of total)	(μg)
Ruda fishpond – flowering plants								
226.8 $\pm$ 13.9	277.2 $\pm$ 35.6	28.2 $\pm$ 3.1	532.2 $\pm$ 46.5	5.27 $\pm$ 0.43	52.9 $\pm$ 2.5	28.4 $\pm$ 4.6	41.1 $\pm$ 2.1	21.6 $\pm$ 3.3
Cep sand-pit – all plants								
24.4 $\pm$ 2.5	0.76 $\pm$ 0.33	0.34	25.5 $\pm$ 2.8	0.53	1.44 $\pm$ 0.59	2.63 $\pm$ 0.79	34.0 $\pm$ 0.9	5.62 $\pm$ 0.24

branches (5.94 $\pm$ 0.25; median 5) differed significantly (P < 0.05) from that between 2nd- and 3rd-order branches (6.56 $\pm$ 0.13; median 6), while highly significantly (P < 0.0001) from that at Cep (22.6 $\pm$ 1.06).

A distribution diagram for flowering plants at Ruda revealed a dominance of very short 1st-order branches (Table 2): median 12.0 nodes, but 10.0 % of all 1st-order branches had no adult leaf node with traps and 24.0 % of them born only 1–5 leaf nodes. In the term of length, the median was 7.0 cm, but 31.0% of these 1st-order branches were only 0–2.0 cm long and 14.8 % of them 2.1–5.0 cm long. Thus, 45.8 % of the branches were only 0–5 cm long. Within all plants at Cep, the dominance of very short 1st-order branches was even more striking (Table 2): mean 3.91 $\pm$ 1.04 nodes, median 0 nodes, and 67.4 % of all branches had only 0–2 nodes (n = 43). In the term of length, mean length was only 1.75 $\pm$ 0.41 cm, median 0.5 cm, and 74.4 % of all branches were shorter than 2.0 cm.

Table 4. List of linear regression models with biometric (cm) and biomass data (mg DW) on *U. australis* plants collected either at a meso-eutrophic site in the Ruda fishpond or in oligotrophic sand-pit Cep. Due to Bonferroni correction, regressions for flowering plants at Ruda (A) were significant at P < 0.0029, while those for all plants at Cep (C) at P < 0.0045 (labelled by asterisk); n, number of plants; r^2, coefficient of determination.

No.	Linear regression models	r^2	P
A. Ruda fishpond – flowering plants (n = 15)			
1	Main shoot length = 76.3 + 0.108 main shoot DW	0.087	0.285
2	Main shoot length = -24.5 + 1.35 main shoot nodes	0.738	<0.0001*
3	Main shoot length = 44.8 + 3.01 1st-order branches	0.658	0.0003*
4	Main shoot length = 71.3 + 0.879 2nd- +3rd-order branches	0.346	0.0009*
5	Main shoot length = 71.5 + 0.106 DW of all branches	0.543	0.0017*
6	Main shoot DW = 168 + 0.633 main shoot nodes	0.022	0.598
7	Main shoot DW = 172 + 0.200 DW of all branches	0.260	0.052
8	Main shoot DW = 194 + 1.78 1st-order branches	0.031	0.532
9	Main shoot nodes = 74.8 + 0.066 DW of all branches	0.523	0.0023*
10	Main shoot nodes = 59.5 + 1.81 1st-order branches	0.581	0.0010*
11	Main shoot nodes = 76.1 + 0.507 2nd- +3rd-order branches	0.230	0.0042
12	1st-order branches = 11.2 + 0.220 2nd- +3rd-order branches	0.508	0.0028*
13	DW of all branches = 21.5 + 13.7 1st-order branches	0.281	0.042
14	DW of all branches = 60.5 + 6.46 2nd- +3rd-order branches	0.654	0.0003*
15	DW of all branches = 13.3 + 5.06 all branches	0.616	0.0005*
16	Length of 1st-order branches = –1.54 + 0.894 nodes of 1st-order branches (n = 271)	0.841	<0.0001*
17	Nodes of 1st-order branches = -0.853 + 0.341 position of 1st-order branches in nodes of main shoots (n = 271)	0.269	<0.0001*
B. Ruda fishpond – all plants (n = 40)			
18	Main shoot length = –23.7 + 1.38 main shoot nodes	0.828	<0.0001*
C. Cep sand-pit – all plants (n = 40)			
19	Main shoot length = 11.3 + 0.727 main shoot DW	0.931	<0.0001*
20	Main shoot length = -15.7 + 0.703 main shoot nodes	0.828	<0.0001*
21	Main shoot length = 20.1 + 8.08 1st-order branches	0.552	<0.0001*
22	Main shoot length = 26.9 + 2.84 DW of all branches	0.251	0.0010*
23	Main shoot DW = –29.2 + 0.842 main shoot nodes	0.674	<0.0001*
24	Main shoot DW = 21.2 + 4.26 DW of all branches	0.323	0.0001*
25	Main shoot nodes = 53.2 + 9.53 1st-order branches	0.458	<0.0001*
26	Main shoot nodes = 61.3 + 3.05 DW of all branches	0.174	0.0074
27	Length of 1st-order branches = 0.228 + 0.390 nodes of 1st-order branches (n = 43)	0.960	<0.0001*
28	DW of all branches = –0.384 + 1.03 all branches	0.291	0.0003*
29	Nodes of 1st-order branches = –1.51 + 0.123 position of 1st-order branches in nodes of main shoots (n = 43)	0.082	0.063

Mean total plant DW of flowering plants at Ruda was 532 mg (Table 3) and consisted of 5.3 ± 0.4 % reproductive biomass; all branches formed 52.9 ± 2.5 % DW of the total vegetative plant DW. At sand-pit Cep, mean total plant DW was only 25.5 ± 2.8 mg, the proportion of reproductive biomass to the total one was negligible (0.53%), and the same held for the proportion of all branches (1.44 ± 0.59%). Based on the data in Tables 1 and 2, mean DW of a 1st-order branch was 14.9 ± 3.1 mg in flowering plants at Ruda, while only 0.69 ± 0.42 mg in all plants at Cep. Around ten times more mature traps captured any macroscopic prey at Ruda than at Cep (28.4 ± 4.6 vs. 2.63 ± 0.79 %; $P < 0.0001$). The investment in carnivory (as the relative trap DW) was slightly but significantly greater ($P < 0.02$) at Ruda, while the mean trap DW was 3.8 times higher at Ruda ($P < 0.0001$).

The linear regression models for the flowering plants at Ruda showed that main shoot length was not correlated significantly with main shoot DW (Table 4A, No. 1) but only with main shoot nodes, number of 1st-order branches, number of 2nd- and 3rd-order branches, and also with DW of all branches (Nos. 2–5). However, main shoot DW correlated significantly neither with main shoots nodes, DW of all branches, nor the number of 1st-order branches (Nos. 6–8). Main shoot nodes correlated significantly with DW of all branches and the number of 1st-order branches, but not with the number of 2nd- and 3rd-order branches (Nos. 9–11). The numbers of the latter two groups of branches correlated significantly with each other (No. 12). DW of all branches correlated significantly with the number of 2nd- and 3rd-order branches as well as of all branches, but not with 1st-order branches (Nos. 13–15). Nodes of 1st-order branches correlated highly significantly both with length of 1st-order branches and their position in the main shoots (Nos. 16, 17). Within all plants at Ruda, main shoot length correlated highly significantly with main shoot nodes (Table 4B, No. 18). Within all plants at sand-pit Cep, main shoot length correlated highly significantly both with main shoot DW, main shoot nodes, the number of 1st-order branches, and DW of all branches (Table 4C, Nos. 19–22). Main shoot DW correlated highly significantly both with main shoot nodes and DW of all branches (Nos. 23, 24). Main shoot nodes correlated significantly with the number of 1st-order branches, but not with DW of all branches (Nos. 25–26). The length of 1st-order branches correlated highly significantly with nodes of 1st-order branches and DW of all branches did with the number of all branches (Nos. 27, 28). However, nodes of 1st-order branches did not correlate with the position of the branches on the main shoot (No. 29).

Discussion

The selected *U. australis* sites differed markedly in water chemistry. While mesotrophic water chemistry at the Ruda fishpond can be con-

sidered optimum for this species (good N, P, and prey availability, very high CO_2 concentration; see Results), very oligotrophic conditions and very low CO_2 concentration together with low prey availability enabled only plant survival at sand-pit Cep (cf. ADAMEC 2008b, 2009). Due to very high concentration of humic acids + tannins and a good prey availability at Ruda, it is evident that a good deal of N for the plants could be covered from this pool of humic acids as well as from prey.

One of the aims of this paper was to determine the great morphological plasticity of *U. australis* associated with branching characteristics, as a prerequisite for rapid plant's propagation, at an optimum site with robust and prolificaly flowering plants (fishpond Ruda) as opposed to a very barren site (sand-pit Cep) with rather small and almost non-flowering plants. Although the evaluation of all collected plants at Ruda included only very basic and simple characteristics (Table 1B) it enabled a comparison of both populations. Even within the set of all collected plants, those at Ruda were significantly longer than those at Cep (mean 55.5 cm, maximum 113.5 cm vs. mean 29.0 cm, maximum 54.5 cm). Yet the number of main shoot nodes was similar at both sites (mean 57.4, maximum 108 vs. mean 63.7, maximum 97) which shows that shoot internodes were around twice shortened at Cep. Also, the plants at Ruda flowered more frequently (12.5 %) than those at Cep (only 5.0 %) and more branched but these differences could not be tested statistically. The plants at Cep were more or less adult though they were small and rarely flowered, while the most plants collected at Ruda represented growing branches, which had been separated earlier from the main shoots. They were gradually maturating and started flowering. Only plants longer than about 70 cm with at least 61 adult leaf nodes could flower at Ruda, while longer than 40.5 cm with at least 61 nodes and at least two branches could at Cep.

The cohort of flowering plants collected at Ruda represented clearly the longest and, also, most robust plants with a very frequent shoot branching (Table 1, 3). While the proportion of reproductive biomass to the total plant one was rather low (5.3 %; cf. POREMBSKI & al. 2006: 16.9 %), the mean proportion of all branches to the total shoot DW was very high (53 %). Moreover, although the majority of 1st-order branches were very short and probably not growing, initiated at axils on older shoot segments (Table 2), a good deal represented long and growing branches. This feature, associated with dynamic plant growth and propagation, represented the main difference between the plants from Ruda and Cep and generally, between the plants from an optimal and a very barren site (ADAMEC & KOVÁŘOVÁ 2006, ADAMEC 2009). This difference in the initiation and growth of branches was also manifested by very prolific 2nd- and 3rd-order branching in flowering plants at Ruda which did not occur at all at Cep (Table 1). However, as it follows from distribution diagrames (data not

shown) a distinct difference in the pattern of branching intensity (i.e., the number of internodes between successive branches) occurred between main shoots and 1st- and 2nd-order branches in flowering plants at Ruda (see also Table 1). While branching intensity of main shoots was very high and on average 15.5 % of these branches were separated only by 1–2 internodes (they were mainly the non-growing, short branches without traps), branching of 1st- and 2nd-order branches was much more regular and only 3.1 % of these branches were separated by 1–2 internodes. It is probable that this very intensive branching was a result of optimum ecological conditions at Ruda. Flowering plants at Ruda could form the first new branches (of the 2nd-order) on their 1st-order branches as early as at the stage of four adult leaf nodes.

To look for relationships between main shoots and branches in flowering plants at Ruda, the number of 1st- or 2nd- and 3rd-order branches and DW of all branches correlated best with main shoot length but weaker with main shoot DW or main shoot nodes (Table 4). Furthermore, the 2nd- and 3rd-order branches contributed to formation of the DW of all branches much more than the 1st-order branches. In all plants collected at Cep, all parameters associated with branching correlated highly significantly with main shoot length and its DW.

Although the RGR was measured at neither site, due to the relatively high proportion of growing branches at Ruda, it is possible to assume that the RGR of flowering plants at Ruda was very high. While the RGR in all plants at Cep, due to their very low proportion of branches (1.44 % of DW), could be very low or almost zero and the RGR of adult flowering plants virtually zero (see ADAMEC 2009). The aim of the present paper was also to estimate the apical growth rate of branches as compared to that of main shoot. In ecologically similar *A. vesiculosa*, a biphasic relationship between branch and main shoot growth was found (ADAMEC 1999): the apical growth rate of young branches (below 6 adult nodes) was only about 67 % of that of main shoot, and was similar at maturity. The analysis of branching data on flowering *U. australis* plants at Ruda revealed a monophasic relationship. The maximum apical growth rate of branches in terms of production of new leaf nodes was about 85 % of that of main shoot at Ruda and only about 65 % in all plants at Cep. Thus, branches grow markedly slower than main shoots.

Growth Models for *Utricularia* and *Aldrovanda* based on Shoot Branching

It is evident that aquatic carnivorous plants with linear shoots can grow and propagate in an exponential manner for a sufficiently long growth period, similar to duckweeds (Lemnaceae; REJMÁNKOVÁ & al. 1990). This has mainly been demonstrated for *A. vesiculosa* growing under field conditions (ADAMEC 1999, ADAMEC & LEV 1999). These studies have shown

that the total number of distinguishable shoot apices (i.e., main shoots + all branches), which can be estimated simply and in a non-destructive way, is a suitable and reliable growth parameter for the determination of RGR, which is definitorily based on DW-derived data. As almost all studies on RGR determination in aquatic carnivorous plants consist in growing markedly shortened, young non-branching apical shoot segments (ADAMEC 2000, 2010, PAGANO & TITUS 2004, ADAMEC & KOVÁŘOVÁ 2006, ADAMEC & al. 2010), the estimated RGR values denote mainly the elongation growth of the main shoot rather than branching intensity. However, in the case of adult, prolificaly branched shoots, the main shoot length is stable and the RGR would entirely be based on branching rate and the apical growth rate of branches – both attached and separated.

The exponential increase of DW of aquatic carnivorous plants at time can be expressed by the following exponential equation (REJMÁNKOVÁ & al. 1990)

$$DW_t = DW_o \cdot 2^{\wedge}(t/T_2) \tag{1}$$

where DW_o and DW_t denote initial and final biomass at time t, respectively, T_2 is doubling time of biomass. With a simplification, working with adult plants and if the proportion of main shoots to all branches is relatively low, the same equation may also be used for total number of shoot apices (on main shoots and all branches; ADAMEC 1999). However, if the number of apices on main shoots (N_{ms}) is not negligible as compared to that on all branches (N_{br}), then it is necessary to consider and count separately both main shoots and all branches. It is evident that this state commonly reflects natural populations of aquatic carnivorous plants (e.g., KAMIŃSKI 1987, KOSIBA & SAROSIEK 1993, ADAMEC 1999, 2009, ADAMEC & KOVÁŘOVÁ 2006). It may be assumed that both the initial number of main shoots (N_{ms-o}) and all branches (N_{br-o}) shall increase exponentially, by the same T_2, to attain the final numbers N_{ms-t} and N_{br-t} at time t

$$N_{ms-t} = N_{ms-o} \cdot 2^{\wedge}(t/T_2) \tag{2}$$

$$N_{br-t} = N_{br-o} \cdot 2^{\wedge}(t/T_2) \tag{3}$$

It is apparent that such a simple quantitative approach might be used conveniently for determination of RGR (or T_2) of adult branched plants under natural conditions. Yet certain correction factors might be applied to subtract the contribution of short, non-growing branches (see above).

If adult main shoots in themselves do not grow anymore and only allocate a new biomass to flowering (negligible) and branching (important), respecting the exponential increase of the number of main shoots (2) and branches (3), it is possible to assume that the RGR (or T_2) is dependent on the proportion of the total branch DW (DW_{br}) to the total plant DW (DW_{to}). If this proportion is very low (small number of short or non-growing branches) and for a shorter time t, the DW of main shoots at time t

(DW$_{ms-t}$) stays nearly constant and is equal to the initial value (DW$_{ms-o}$). The equation (1) can then be changed to

$$DW_{to-t} = DW_{ms-o} + DW_{br-o} \cdot 2^{\wedge}(t/T_2) \tag{4}$$

where DW$_{to-t}$ is the final total plant DW at time t and DW$_{br-o}$ the initial DW of all branches. However, if the proportion of branch DW is great, the equation (4) may come to the simpler equation (1). Evidently, for practical reasons, it is much simpler to determine RGR (or T$_2$) of adult branched plants under natural conditions using non-destructive methods of counting branches using the equations (2) and (3) than to estimate destructively plant DW. However, the interpretation of such an exponential plant growth should always be conducted cautiously due to limited carrying capacity of the habitat and mortality (see REJMÁNKOVÁ & al. 1990, KOSIBA & SAROSIEK 1993).

In conclusion, great morphological plasticity of *U. australis* shoots was confirmed at a couple of sites differing greatly in their trophic status. Shoot branching was recognized as one out of most important reasons for this plasticity, and for subsequent high RGR. Branching intensity of main shoots in *U. australis* might reflect discrete ecological conditions of each micropopulation (cf. ADAMEC 1999, 2009, ADAMEC & KOVÁŘOVÁ 2006). To understand the dynamic growth traits of aquatic carnivorous plants, a combination of detailed knowledge of dynamic branching characteristics and mathematical modelling is essential.

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