

Capture of algae promotes growth and propagation in aquatic *Utricularia*

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- **Background and Aims** Some carnivorous plants trap not only small animals but also algae and pollen grains. However, it remains unclear if these trapped particles are useless bycatch or whether they provide nutrients for the plant. The present study examines this question in *Utricularia*, which forms the largest and most widely spread genus of carnivorous plants, and which captures prey by means of sophisticated suction traps.
- **Methods** *Utricularia* plants of three different species (*U. australis*, *U. vulgaris* and *U. minor*) were collected in eight different water bodies including peat bogs, lakes and artificial ponds in three regions of Austria. The prey spectrum of each population was analysed qualitatively and quantitatively, and correlated with data on growth and propagation, C/N ratio and $\delta^{15}\text{N}$.
- **Key Results** More than 50 % of the prey of the *Utricularia* populations investigated consisted of algae and pollen, and *U. vulgaris* in particular was found to capture large amounts of gymnosperm pollen. The capture of algae and pollen grains was strongly correlated with most growth parameters, including weight, length, budding and elongation of internodes. The C/N ratio, however, was less well correlated. Other prey, such as moss leaflets, fungal hyphae and mineral particles, were negatively correlated with most growth parameters. $\delta^{15}\text{N}$ was positively correlated with prey capture, but in situations where algae were the main prey objects it was found that the standard formula for calculation of prey-derived N was no longer applicable.
- **Conclusions** The mass capture of immotile particles confirms the ecological importance of autonomous firing of the traps. Although the C/N ratio was little influenced by algae, they clearly provide other nutrients, possibly including phosphorus and trace elements. By contrast, mosses, fungi and mineral particles appear to be useless bycatch. Correlations with chemical parameters indicate that *Utricularia* benefits from nutrient-rich waters by uptake of inorganic nutrients from the water, by the production of more traps per unit of shoot length, and by the capture of more prey particles per trap, as nutrient-rich waters harbour more prey organisms.

Key words: Algae, aquatic plant, bladderwort, carnivorous plant, $\delta^{15}\text{N}$, nutrient uptake, prey spectrum, suction trap, *Utricularia australis*, *U. minor*, *U. vulgaris*.

INTRODUCTION

Carnivorous plants trap, digest and utilize small animals. Nitrogen, phosphorus and other elements absorbed from the prey lead to increased growth (Büsgen, 1883; Chandler and Anderson, 1976; Thum, 1988), increased formation of flowers, fruits and offshoots (Thum, 1988; Krafft and Handel, 1991) or increased mineral concentrations in the tissue (Oudman, 1935; Christensen, 1976). At least 724 species of vascular plants use prey capture to improve their mineral nutrition; with about 228 species, *Utricularia* (bladderworts, Lentibulariaceae) forms the largest and most widely spread genus of carnivorous plants (McPherson, 2010). In *Utricularia*, prey is captured by means of sophisticated suction traps (Lloyd, 1942; Sasago and Sibaoka, 1985a, b; Adamec, 2011a; Vincent and Marmottant, 2011; Vincent *et al.*, 2011a, b).

In spite of their ability to trap animals, carnivorous plants such as *Utricularia* are by no means independent of the inorganic nutrient supply from their environment. All carnivorous plants are able to absorb at least limited amounts of nutrients via their roots or, in aquatic species, their shoots (Adamec,

1992, 2002; Adlassnig *et al.*, 2005). Nitrogen absorbed from the prey was originally derived from the non-carnivorous plants on which prey animals had been feeding. Thus, carnivorous plants use their prey animals to accumulate nitrogen. Nutrient-rich conditions increase the abundance of prey animals and therefore the benefit gained from prey (Ulanowicz, 1995). In aquatic *Utricularia*, epiphytic algae attract prey animals towards the traps; a good supply of inorganic nutrients may lead to enhanced growth of algae and therefore to increased prey attraction (Ulanowicz, 1995).

Despite these benefits, most carnivorous plants are restricted to oligotrophic habitats. Leaves transformed to traps contribute only little to CO₂ assimilation (Adamec, 2006; Sirová *et al.*, 2010). The investment into traps leads to reduced photosynthesis and growth compared with non-carnivorous plants (Kostytschew, 1923; Ellison and Gotelli, 2001). Carnivorous plants therefore require full sunlight, especially for trap formation, as shown in *Utricularia vulgaris* (Maier, 1973; Englund and Harms, 2003) or *Pinguicula vallisneriifolia* (Zamora *et al.*, 1998). A general model of carnivorous plant ecology, developed by Benzing (1987) and Givnish (1989), predicts a net

benefit for plant carnivory only for a combination of high irradiation, sufficient water supply and oligotrophic substrate. Indeed, this model is able to give a satisfying explanation for the distribution and niching of carnivorous plants. Some additional effects limit the growth of carnivorous plants under nutrient-rich conditions as well. In *U. vulgaris*, extremely high prey concentrations cannot be utilized (Englund and Harms, 2001). Furthermore, dense growth of epiphytic algae inhibits plant growth by shadowing, although it may attract additional prey animals (Ulanowicz, 1995).

Although these basic correlations are roughly understood, the problem becomes more complex by the capture of other objects than animals. In *Utricularia*, algae may form the most abundant group of prey objects in some water bodies (Garbini, 1898; Schumacher, 1960; Gordon and Pacheco, 2007; Peroutka et al., 2008), although the benefit for the plant is not yet clear (Jobson et al., 2000). It is not clear whether algae are digested in the traps or if they survive and even propagate (Peroutka et al., 2008; Alkhalaf et al., 2011; Płachno et al., 2012). *Pinguicula* and other terrestrial plants frequently trap pollen grains and seem to gain benefit (Harder and Zemplin, 1968). The capture of protozoa seems to be crucial in *Genlisea* (Barthlott et al., 1998) and in part of *Utricularia* (Hegner, 1926; Seine et al., 2002). Other objects such as fungal spores and hyphae, moss leaflets or mineral particles are trapped at least occasionally by various species (our own observations).

This study compares eight populations of aquatic *Utricularia* with regard to the chemistry of their home water, various growth parameters, $\delta^{15}\text{N}$, and their trapping success in order to answer the following questions:

1. Are prey-derived nutrients able to compensate for a lack of minerals from the water? Is the mineral content and size of *Utricularia* therefore the same in eutrophic and oligotrophic habitats?
2. Is the investment into carnivory – i.e. the number of traps – proportional to the benefit gained from the prey? In oligotrophic waters, the plant desperately needs additional mineral supply. However, in nutrient-poor waters prey animals are less frequent as well; therefore, additional trap formation might gain little net benefit.
3. Is there evidence for a beneficial effect of prey objects other than animals, such as algae or pollen?
4. Are $\delta^{15}\text{N}$ measurements a useful tool to study prey benefit in aquatic *Utricularia*?

MATERIAL AND METHODS

Sampling and measuring of plants

Utricularia plants of three different species (*U. australis*, *U. vulgaris* and *U. minor*) were collected in eight different water bodies including peat bogs, lakes and artificial ponds in three regions of Austria, i.e. the Lungau/Salzburg, Waldviertel/Lower Austria and Vienna. Details on the sampling sites are given in Supplementary Data Table 1. Sampling took place in late spring (May–June) 2009.

For determination of prey-derived nitrogen in carnivorous plants, samples of prey animals and non-carnivorous water plants were required (Schulze et al., 1991). Only plant species floating such as *Utricularia* were used as control plants. Due to

the low biodiversity in all habitats, only one suitable species was available in each habitat; the collected species are shown in Supplementary Data Table 2. Prey animals were collected by sieving of the water as well as by collecting small animals manually. For both control plants and animals, all samples from each water body were pooled, dried at 70 °C and homogenized before analysis.

In *Utricularia* plants, fresh weight, shoot length, the number of buds and the numbers of traps and leaves within the first 10 cm of the main shoot were determined. In each plant individual, the prey was identified and quantified microscopically in 20 fully grown traps that were randomly selected from the first 10 cm of the main shoot. Older traps were excluded as strong evidence exists that the content of the traps differs significantly between young and old, inactive traps, especially with regard to algae (Płachno et al., 2012). The size of the traps was determined microscopically, although it has been shown to be irrelevant for the capture of algae (Alkhalaf et al., 2011). The rest of the plants were dried at 70 °C, and dry weight was determined. The dried material was used for chemical and isotope measurements.

pH and water conductivity were determined at the natural habitat, using a Voltcraft PH-100ATC for pH and a Lutron Pure Water Tester WA300 for conductivity. Water hardness was measured in the laboratory, using Aquamerck Total Hardness Test no. 1-08048-0001 and Aquamerck Carbonate Hardness Test no. 1-0804-0001. The concentrations of macronutrients were determined by photometry. (Detection limits, as stated by the manufacturer, are given in parentheses.) Spectroquant Cell Test no. 1-14739-0001 was used for ammonium (0.01 mg L⁻¹), no. 1-14542-0001 for nitrate (2.2 mg L⁻¹), no. 1-14543-0001 for phosphate (0.05 mg L⁻¹) and no. 1-14562-0001 for potassium (5.0 mg L⁻¹). Due to the very low concentrations of inorganic nutrients next to the limit of detection, these data were not included into statistical evaluation. All measurements were repeated twice.

To clarify the fate of algae within the traps, *U. australis* collected at Postteich was added to growing cultures of the colonial green alga *Scenedesmus* sp. (Scenedesmaceae). After 1 week, the vitality of algae inside and outside of the traps was checked microscopically.

Element analysis and isotope measurements

The dry plants were homogenized; carbon and nitrogen content were determined using an elemental analyser (EA 1110 by CE Instruments, Milan, Italy) interfaced via a ConFlo III device (Thermo Finnigan, Bremen, Germany) to a continuous-flow stable isotope ratio mass spectrometer (DeltaPLUS, Thermo Finnigan).

For determination of prey-derived nitrogen, $\delta^{15}\text{N}$ was used. Since Shearer and Kohl (1988), the phenomenon of ^{15}N discrimination has been used in ecological research: autotrophic plants exhibit a low $\delta^{15}\text{N}$, whereas herbivorous animals a higher $\delta^{15}\text{N}$. In predators and top predators, the highest $\delta^{15}\text{N}$ values are achieved. Therefore, if the $\delta^{15}\text{N}$ of an organism is known, its position within the food chain can be determined. The same method can be applied to carnivorous plants, which get part of their nitrogen from prey and part from their substrate. Thus, they exhibit a higher $\delta^{15}\text{N}$ than

non-carnivorous plants. If the $\delta^{15}\text{N}$ values of the accompanying, non-carnivorous flora and of typical prey animals are known, the percentage of prey-derived nitrogen can be estimated (Schulze *et al.*, 1991). The percentage of animal-derived nitrogen (N_{Prey}) in *Utricularia* was calculated according to the equation

$$N_{\text{Prey}} = \frac{\delta^{15}\text{N}_{\text{CP}} - \delta^{15}\text{N}_{\text{RP}}}{\delta^{15}\text{N}_{\text{Z}} - \delta^{15}\text{N}_{\text{RP}}} \times 100 \% \quad (1)$$

where N_{Prey} is the percentage of prey-derived nitrogen, $\delta^{15}\text{N}_{\text{RP}}$ is the ^{15}N in the reference plant, $\delta^{15}\text{N}_{\text{CP}}$ is the ^{15}N in the carnivorous plant and $\delta^{15}\text{N}_{\text{Z}}$ is the ^{15}N in the zooplankton.

Statistical analysis

PASW Statistics 18.0 was used for statistical analysis. As there was little evidence for a normal distribution of the data, the Mann–Whitney and the Kruskal–Wallis tests were used to test for differences, and the Pearson rank correlation test for correlations. In some tests, partial correlation was used as well. Correlations are shown as R^2 , with plus or minus signs indicating positive or negative correlations. For factor analysis, Varimax rotation and normalization to -1 to $+1$ were used.

Statistical tests were carried out twice: once for all samples, i.e. after pooling the data for all three species of *Utricularia*, and once for *U. australis*, the most widespread of the investigated species.

RESULTS

The investigated water bodies and their *Utricularia* species

All investigated water bodies proved to be rather poor in nutrients (highly oligotrophic to mesotrophic). The most oligotrophic conditions were found in the undisturbed mire lake Seetaler Lake and the two investigated ponds. Higher nutrient concentrations were measured in the three disturbed peat bogs, Schrems, Brand and Hochstand, as well as in the lagg of the Seetal mire. pH was very low in the fens of Waldviertel but circumneutral in the other habitats. Conductivity and hardness were low in the fen habitats but high in the calcareous Postteich. Details on water chemistry are given in [Supplementary Data Table 2](#).

Both *U. minor* and *U. australis* showed differences in most growth parameters between the various water bodies (Mann–Whitney Test): *U. minor* from Seetaler Lake had a larger fresh weight compared with *U. minor* from the Lagg of the same fen ($P < 0.01$), together with fewer buds ($P < 0.01$), and a lower leaf and a higher trap density ($P < 0.01$). The C/N ratio was lower in Seetaler Lake ($P < 0.01$). Similarly, differences between the five investigated populations of *U. australis* were found (Kruskal–Wallis test) regarding fresh weight, dry weight, shoot length, number of buds, leaf and trap density, traps per leaf and C/N ratio (all $P < 0.01$). Within each population, the variation was much higher for shoot length and dry weight than for C/N ratio or leaf and trap density, which were rather uniform. Details on the growth characteristics of all

populations are given in [Table 1](#). The size of the traps did not differ significantly between the habitats (*U. vulgaris*: $1640 \pm 413 \mu\text{m} \times 1310 \pm 367 \mu\text{m}$; *U. australis*: $1627 \pm 366 \mu\text{m} \times 1316 \pm 300 \mu\text{m}$; *U. minor*: $1341 \pm 363 \mu\text{m} \times 934 \pm 248 \mu\text{m}$) but the traps of *U. minor* were smaller than those of *U. vulgaris* and *U. australis* ($P < 0.01$).

In *U. minor* (Seetaler Lake) and all populations of *U. australis* the C/N ratio was lower than in the non-carnivorous control plants ($P < 0.01$). In *U. vulgaris* (Seetaler Lake), the C/N ratio did not differ significantly from the non-carnivorous control plants. The same was true for *U. minor* from the Seetaler Lagg.

Prey composition

In all populations, the prey consisted of animals (mainly microcrustaceans and insect larvae but also rotifers, tardigrads and Acari), pollen grains (mainly *Pinus sylvestris* and *Picea abies*, the dominant species in the area surrounding most sites), algae and other objects (protozoa, fungal hyphae or fungal spores). Moss leaflets and other plant particles (≤ 0.2 % of the total prey) and protozoa (≤ 0.5 % of the total prey) were of negligible abundance, with the exception of *U. australis* from Postteich containing 1.5 % plant particles and 1.8 % protozoa. Concerning algae, the same genera were observed as reported in a previous study (Peroutka *et al.*, 2008). *Ankistrodesmus*, *Batrachospermum*, *Bulbochaete*, *Microthamnion*, *Sphaerocystis*, *Plectonema* and *Monoraphidium* are newly added to the list of algae trapped by *Utricularia*. In all habitats, algal prey was dominated by filamentous Chlorobionta, mainly Zygnemataceae, followed by coccale Chlorobionta. Other taxonomic groups such as Bacillariophyta, Rhodophyta and Cyanobacteria did occur but were of negligible importance. The Kruskal–Wallis test gave evidence that all categories of prey objects (animals, plants, algae, pollen grains and other objects) were distributed unevenly between the populations ($P < 0.01$). [Table 2](#) shows the prey composition in the different populations.

In only one water body (Seetaler Lake), two species of *Utricularia* (*U. vulgaris* and *U. minor*) grew simultaneously. In these two species, the composition of prey showed remarkable differences: *U. vulgaris* caught more total prey ($P < 0.01$), more pollen grains ($P < 0.01$) and more animals ($P < 0.01$) per trap, but fewer algae and other objects ($P < 0.01$). The most important difference was pollen grains, which counted for 90.2 % of the total prey in *U. vulgaris* but only 12.6 % in *U. minor*. However, this dominance of pollen is possibly restricted to the pollen-producing season of Gymnosperm trees.

Most algae trapped by *Utricularia* were dead and showed signs of decay ([Fig. 1](#)). Under controlled conditions, colonies of *Scenedesmus* within the traps were dead as well whereas the colonies outside the traps of *Utricularia* were still alive ([Fig. 2](#)).

Isotope measurements

In the investigated *Utricularia* populations, $\delta^{15}\text{N}$ values between $+0.1$ ‰ (*U. australis* from the Peat Bog Schrems) to $+6.0$ ‰ (*U. australis* from Hochstand) were found. In both *U. minor* and *U. australis*, the differences between the various

TABLE 1. Growth characteristics of the investigated *Utricularia* populations

Water body	Dry weight (mg)	Total length (cm)	No. of buds	Leaf density (dm ⁻¹)	Trap density (dm ⁻¹)	Traps per leaf	N content (%)	C/N ratio (<i>Utricularia</i>)	C/N ratio (control plant)
Seetaler Lake (<i>U. vulgaris</i>)	216 ± 89	50.2 ± 15.0	2.9 ± 1.5	11.7 ± 1.9	439.0 ± 99.3	37.9 ± 7.7	2.4 ± 0.5	17.9 ± 3.8	19.9
Seetaler Lake (<i>U. minor</i>)	21 ± 20	40.7 ± 15.9	2.8 ± 1.7	11.5 ± 1.6	138.6 ± 51.3	12.8 ± 6.2	2.4 ± 0.2	16.3 ± 1.2	
Seetaler Lagg (<i>U. minor</i>)	10 ± 5	35.9 ± 12.7	6.1 ± 3.0	22.0 ± 3.4	52.8 ± 21.3	2.5 ± 1.0	2.1 ± 0.4	19.6 ± 5.3	18.2
Peat Bog Schrems (<i>U. australis</i>)	72 ± 43	72 ± 42	8.3 ± 6.1	19.9 ± 3.4	163.6 ± 80.2	8.9 ± 5.5	2.7 ± 0.8	16.4 ± 3.8	14.3
Winkelaer Pond (<i>U. australis</i>)	2 ± 2	8.2 ± 3.2	1.3 ± 0.7	28.3 ± 7.7	63.3 ± 24.4	2.4 ± 1.1	5.2 ± 0.7	8.2 ± 0.9	24.8
Peat Bog Hochstand (<i>U. australis</i>)	59 ± 60	42.5 ± 23.7	3.2 ± 1.3	15.4 ± 2.7	367.6 ± 180.0	25.6 ± 15.6	2.0 ± 0.3	20.5 ± 2.9	25.3
Peat Bog Brand (<i>U. australis</i>)	38 ± 25	46.6 ± 28.4	4.0 ± 1.7	12.6 ± 2.5	202.6 ± 54.4	17.1 ± 4.4	4.2 ± 0.4	9.4 ± 0.7	21.0
Postteich (<i>U. australis</i>)	35 ± 25	49.4 ± 24.7	5.7 ± 3.7	23.0 ± 3.7	156.4 ± 51.1	7.2 ± 3.1	1.1 ± 0.1	32.6 ± 3.4	35.3

$n = 8-12$; data are shown as arithmetic mean ± s.d.

TABLE 2. Trapping success of the investigated *Utricularia* populations; all values are objects per trap

Population	Total objects	Plant particles	Algae	Pollen grains	Animals	Other objects
Seetaler Lake (<i>U. vulgaris</i>)	31.1 ± 32.9	0.0 ± 0.1 (≈ 0.1 %)	1.9 ± 3.8 (≈ 10.2 %)	28.0 ± 32.1 (≈ 80.2 %)	0.6 ± 0.8 (≈ 4.9 %)	0.6 ± 0.7 (≈ 4.7 %)
Seetaler Lake (<i>U. minor</i>)	13.9 ± 9.7	0.0 ± 0.1 (≈ 0.0 %)	10.8 ± 8.2 (≈ 74.7 %)	1.8 ± 3.8 (≈ 9.7 %)	0.4 ± 1.1 (≈ 4.0 %)	0.9 ± 1.0 (≈ 11.7 %)
Seetaler Lagg (<i>U. minor</i>)	5.6 ± 7.5	0.0 ± 0.1 (≈ 0.2 %)	2.0 ± 5.2 (≈ 35.6 %)	2.6 ± 5.3 (≈ 34.1 %)	0.4 ± 0.8 (≈ 10.4 %)	0.6 ± 0.8 (≈ 19.9 %)
Peat Bog Schrems (<i>U. australis</i>)	20.3 ± 20.8	0.0 ± 0.1 (≈ 0.0 %)	17.7 ± 20.2 (≈ 74.6 %)	0.6 ± 1.6 (≈ 3.2 %)	1.1 ± 1.6 (≈ 9.8 %)	0.9 ± 0.6 (≈ 12.4 %)
Winkelaer Pond (<i>U. australis</i>)	4.2 ± 6.1	0.0 ± 0.1 (≈ 0.1 %)	2.5 ± 5.1 (≈ 37.4 %)	0.2 ± 1.3 (≈ 4.0 %)	0.8 ± 1.5 (≈ 19.7 %)	0.8 ± 1.1 (≈ 38.9 %)
Peat Bog Hochstand (<i>U. australis</i>)	6.0 ± 4.9	0.0 ± 0.1 (≈ 0.1 %)	2.3 ± 2.5 (≈ 34.1 %)	2.2 ± 2.6 (≈ 26.9 %)	0.6 ± 1.0 (≈ 12.4 %)	0.8 ± 0.6 (≈ 26.6 %)
Peat Bog Brand (<i>U. australis</i>)	7.6 ± 5.9	0.0 ± 0.1 (≈ 0.1 %)	2.5 ± 3.9 (≈ 25.6 %)	0.4 ± 1.0 (≈ 5.5 %)	4.4 ± 4.0 (≈ 59.1 %)	0.4 ± 0.6 (≈ 9.8 %)
Postteich (<i>U. australis</i>)	1.7 ± 1.5	0.0 ± 0.2 (≈ 1.5 %)	0.4 ± 0.9 (≈ 12.7 %)	0.0 ± 0.2 (≈ 1.1 %)	0.1 ± 0.4 (≈ 4.5 %)	1.2 ± 1.1 (≈ 81.7 %)

$n \geq 180$ traps per population. 'Other objects' include detritus, protozoa, fungal hyphae and spores, Crustacean eggs, and unidentified particles (data are shown as arithmetic mean ± s.d. and as the percentage of the total prey).

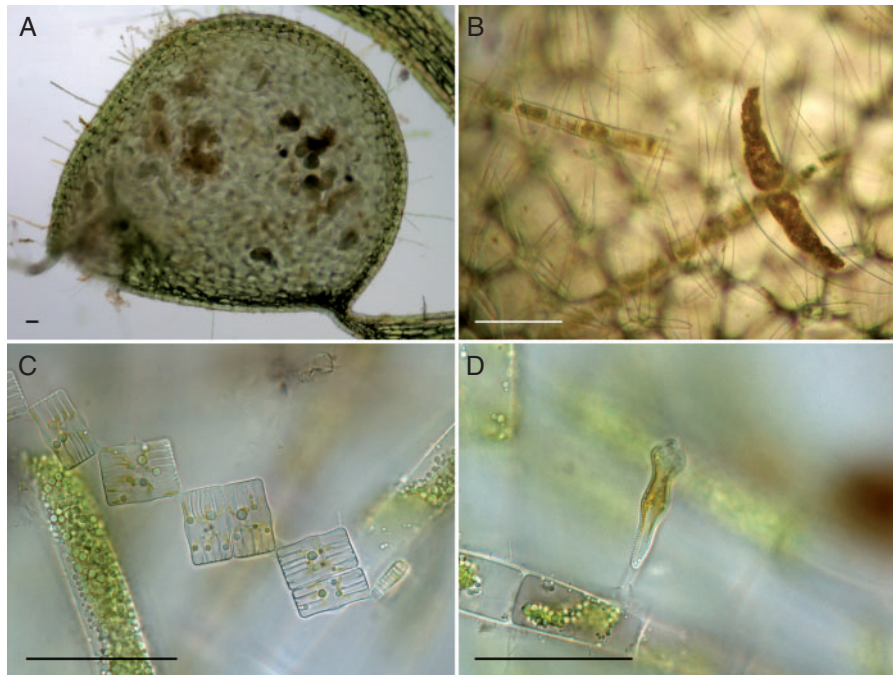


FIG. 1. Algae trapped by *Utricularia*. (A) Trap of *U. vulgaris* from Seetaler See, containing algae and pollen grains. (B) Dead cells of *Closterium* sp. and *Spirogyra* sp. within a trap. (C) Dead *Tabellaria flocculosa* and an undetermined filamentous alga from a trap. (D) Dead *Gomphonema constrictum* growing on *Spirogyra* sp. from a trap. Bright field; scale bars ≈ 50 μm.

habitats were highly significant. The differences between the three species were not significant, even for *U. minor* and *U. vulgaris* which show extremely different growth characteristics.

The proportion of N derived from prey calculated from eqn (1) gave mean values of 71 ± 18 % for *U. vulgaris*, 68 ± 26 % for *U. minor* and 120 ± 86 % for *U. australis*. In *U. australis*, many unrealistically high values (≥ 100 %) were calculated, up to 383 %. Some values ≥ 100 % were also found in *U. minor*. Detailed results of the isotope measurements are given in Table 3.

Correlations between growth parameters, prey capture and $\delta^{15}\text{N}$

Most growth characteristics as well as $\delta^{15}\text{N}$ data showed strong correlations both with water chemistry and with trapping success. All data were analysed both for *U. australis*, the most widespread species used in this study, and for all samples, to investigate the factors determining the growth of *Utricularia* in general. Figure 3 provides a visualization of these correlations.

Among the total prey, the respective numbers of animals, algae and pollen grains were weakly correlated ($R^2 \leq 0.15$, $P < 0.05$). By contrast, the number of plant particles was correlated with the number of other objects ($R^2 = 0.17$, $P < 0.01$). The same correlations were found in *U. australis*, with an $R^2 \leq 0.21$ ($P < 0.01$) for algae, animals and pollen grains, and $R^2 = 0.20$ ($P < 0.01$) between plant particles and other objects.

In both test series, the number of total prey objects per trap correlated with the dry weight and explained it to more than

0.30 ($P < 0.01$). The same was true for shoot length, with a slightly lower R^2 of 0.22 ($P < 0.01$) in both tests. The correlations with the numbers of animal prey were much weaker; in *U. australis*, in particular, filamentous algae were more strongly correlated with dry weight or shoot length ($P < 0.01$) than animal prey. The number of trapped pollen grains also correlated with both shoot length and dry weight ($P < 0.01$). The number of other objects seemed to have little influence.

The number of growing buds, i.e. the branching factor, was not correlated with the capture of animals or pollen grains but with the number of trapped algae in both test series ($P < 0.01$). In *U. australis*, the capture of Desmidiaceae explained 0.43 ($P < 0.01$) of the branching. Leaf density, i.e. the length of internodia, was negatively correlated with the total trapping success ($P < 0.01$). In this case, the correlation with the number of trapped animals was equal to or stronger than with trapped algae ($P < 0.01$). Trap density correlated positively with total trapping success ($P < 0.01$); the strongest correlation was found for the number of pollen grains, followed by the number of animals. Among algae, only the number of filamentous algae correlated with trap density in both test series ($P < 0.01$). The C/N ratio correlated negatively with the total prey ($P < 0.01$) and, much more strongly, with the number of trapped animals, especially Crustaceae ($P < 0.01$). Algae and C/N ratio showed a positive but much weaker correlation ($P < 0.01$). The number of pollen grains did not correlate with the C/N ratio, whereas other objects showed a positive correlation ($P < 0.01$). Details on the correlations between growth parameters and trapping success are given in Supplementary Data Table 3.

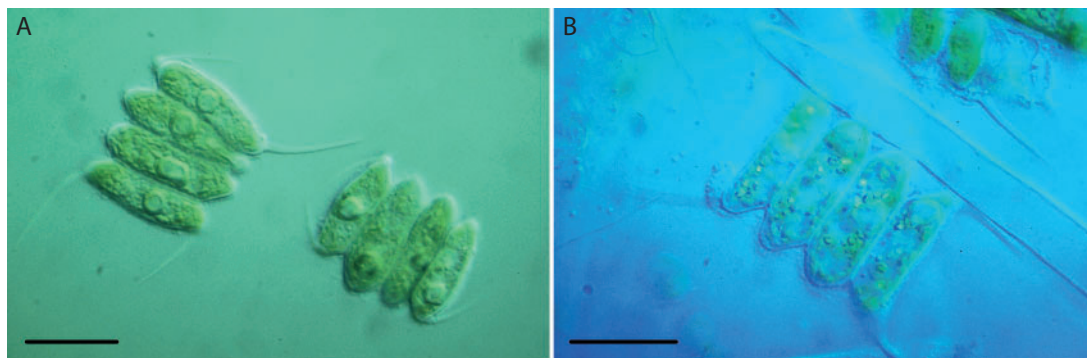


FIG. 2. Colonies of *Scenedesmus* sp., cultivated together with *Utricularia australis*. (A) Living colony from the medium; (B) decaying colony next to a digestive gland within a trap. Differential interference contrast; scale bars ≈ 10 μm .

TABLE 3. Results of the $\delta^{15}\text{N}$ measurements

Water body	$\delta^{15}\text{N}_{Utricularia}$ (‰)	$\delta^{15}\text{N}_{Control plants}$ (‰)	$\delta^{15}\text{N}_{Zooplankton}$ (‰)	Calculated N_{Prey} (%)
Seetaler Lake (<i>U. vulgaris</i>)	2.6 ± 0.8	-0.6	3.8	71 ± 18
Seetaler Lake (<i>U. minor</i>)	1.5 ± 0.3	-0.6	3.8	48 ± 7
Seetaler Lagg (<i>U. minor</i>)	3.1 ± 0.7	-0.1	3.5	89 ± 21
Peat Bog Schrems (<i>U. australis</i>)	5.1 ± 0.5	6.6	5.5	134 ± 46
Winkelauer Pond (<i>U. australis</i>)	3.7 ± 0.4	1.3	2.1	303 ± 45
Peat Bog Hochstand (<i>U. australis</i>)	0.3 ± 0.1	-2.4	2.8	51 ± 2
Peat Bog Brand (<i>U. australis</i>)	1.3 ± 0.2	-0.2	4.3	33 ± 4
Postteich (<i>U. australis</i>)	2.3 ± 0.4	4.3	2.9	1.4 ± 3

$n = 8-12$; data are shown as arithmetic mean \pm s.d.

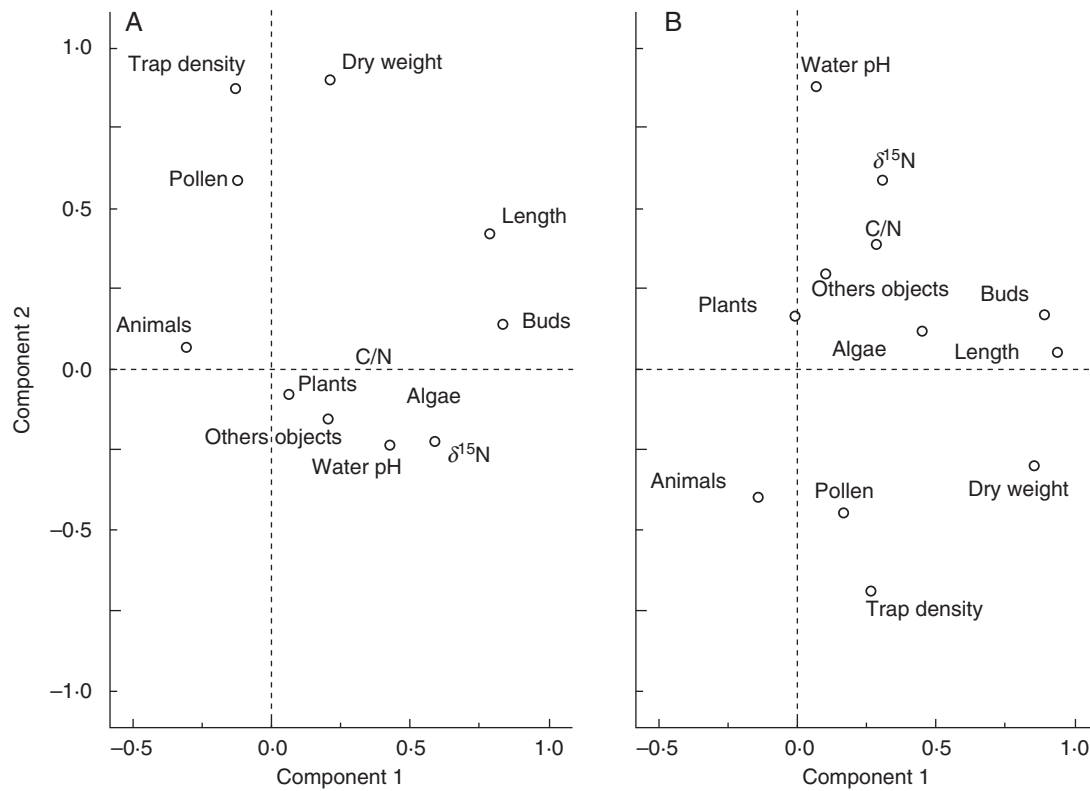


FIG. 3. Main component analyses (Varimax) of various growth parameters and trapping success; (A) all investigated *Utricularia* samples; (B) *U. australis*.

Water chemistry and plant growth proved to be interlinked as well. In both test series, leaf density was positively correlated with pH, conductivity, carbonate and total hardness (all $P < 0.01$). Trap density was negatively correlated with pH, conductivity, carbonate and total hardness (all $P < 0.01$). The C/N ratio was correlated with pH ($P < 0.01$ for all *Utricularia* species/ $P < 0.05$ for *U. australis* only), conductivity, carbonate and total hardness (all $P < 0.01$) in both test series. Details on correlations between growth parameters and water chemistry are given in [Supplementary Data Table 4](#). Our preliminary data on inorganic nutrients suggest also positive correlations between plant growth and NO_3^- , PO_4^{3-} and K^+ (data not shown) but require validation by more sensitive analytical techniques. Trapping success also correlated with water chemistry. In both test series, total prey, animals and algae correlated negatively with pH, conductivity, carbonate and total hardness (all $P < 0.01$). The other prey objects showed the opposite trend. Details on correlations between trapping success and water chemistry are also given in [Supplementary Data Table 4](#).

Particular interest was paid to a possible growth promotion by the capture of algae and pollen grains. However, the number of trapped algae was correlated with the number of trapped animals, and thus any correlation between growth and the capture of algae could be a side effect of successful N uptake from animal prey. Therefore, we tested for partial correlations between plant growth and the capture of algae, using animal prey as the control variable ([Table 4](#)). The number of pollen grains was positively correlated with dry weight in both test series ($P < 0.01$) but the effect was weaker in *U. australis*.

TABLE 4. Partial correlation between the trapping of algae and pollen with plant growth, corrected for animal prey

	All samples		<i>Utricularia australis</i>	
	Algae	Pollen	Algae	Pollen
Dry weight	–	0.48**	0.22**	0.18**
Total length	0.29**	–	0.32**	–
Number of buds	0.26**	–0.12**	0.32**	–
Leaf density	–	–0.31**	–	–0.19**
Trap density	–0.10**	0.34**	–0.07*	0.28**
C/N ratio	–0.10**	–0.11**	–0.10**	–

A dash indicates non-significant correlations. * $P < 0.05$, ** $P < 0.01$.

In *U. australis*, dry weight was also correlated with the number of trapped algae ($P < 0.05$). Total length and the number of buds correlated with the capture of algae ($P < 0.01$) but not with the capture of pollen. Trap density was positively correlated with the capture of pollen ($P < 0.01$) but negatively with the capture of algae ($P < 0.05$ for all *Utricularia* species/ $P < 0.01$ for *U. australis* only).

A comparison of isotope measurements with trapping success showed that $\delta^{15}\text{N}$ correlated strongly with the total prey, but even more with the number of algae. The correlation with the number of trapped animals was very weak and sometimes even negative; the same was true for the number of pollen grains. Algae explained 0.37 ($P < 0.01$) of the $\delta^{15}\text{N}$ in *U. australis*. Little or no correlation was found between $\delta^{15}\text{N}$ and dry weight, shoot length or C/N ratio ([Table 5](#)).

TABLE 5. Correlations between $\delta^{15}\text{N}$ and selected growth parameters and the trapping success

	All samples	<i>Utricularia australis</i>
Total prey	0.05*	0.22**
Animal prey	–	–0.10**
Algal prey	–0.09**	0.37**
Pollen prey	–0.09**	–0.18**
Other objects	0.05*	0.14**
Dry weight	–	–
Total length	–	–
Number of buds	0.29**	0.28*
Leaf density	0.51**	0.51**
Trap density	–0.38**	–0.52**
C/N ratio	–	–

A dash indicates non-significant correlations. * $P < 0.05$, ** $P < 0.01$.

DISCUSSION

Algae and pollen grains are the most abundant prey objects

Although *Utricularia* is commonly regarded as a carnivorous plant, very different kinds of small objects are collected by the traps. In 1844 traps, a total of 20 856 prey objects were found, i.e. 11.3 objects per trap. Among these, only 1912 (9.2 %, i.e. 1.0 per trap) were multicellular animals. Among metazoa, the most important taxa were Crustacea (1640; 7.9 %) and insect larvae (193; 0.9 %). Acari, Nematoda, Tardigrada and Rotifera were rarely found; the same was true for eggs of Crustacea (<50; 0.2 %). Kurbatova and Yershov (2009) confirm the negligible importance of Rotifera but suggest that their fast digestion by the traps may form a bias in quantification.

With 10 483 (50.3 %) individuals, algae formed the most abundant group of prey objects. Coccale algae (7353; 35.3 %) were dominant, followed by filamentous algae (2968; 14.2 %). Flagellates were rare (163; 0.7 %), and capsale and rhizopodiale species were lacking completely. These results are in accordance with a previous study which used partly the same water bodies (Peroutka et al., 2008). Pollen formed another important group of prey objects with 7018 (33.6 %) grains. As all studied habitats except Postteich are surrounded by large coniferous forests, pollen grains are seasonally abundant on the surface of all water bodies. However, not all species of *Utricularia* are equally efficient in trapping floating pollen grains. In Seetaler See, *U. vulgaris* contained 28.0 ± 32.1 (maximum 123) pollen grains per trap, whereas in *U. minor* in the same water body only 1.8 ± 3.8 (maximum 38) grains per trap were found. The position of the traps of *U. vulgaris* just beneath the water surface may facilitate the capture of pollen floating on the surface. By contrast, *U. minor* in the same water body trapped highly significantly more algae, especially Desmidiaceae and other coccale algae, as well as more other objects. The position of the leaves and traps within the water body, i.e. in the surface microlayer in *U. vulgaris* or further down in *U. minor*, seems to have a significant influence on the prey spectrum and may be a strategy to reduce competition.

In all investigated traps, only 21 other plant-derived particles were found, exclusively moss leaflets. All other organisms were trapped in negligible amounts as well: 35 protozoa, seven fungal hyphae and 13 fungal spores.

The ratio between the various groups of prey objects differed highly significantly between the different water bodies. The key result of a previous study (Peroutka et al., 2008) was confirmed: the percentage of algae among the total prey correlated negatively with the conductivity of the water [$R^2 = -0.19$ ($P < 0.01$)] for all three species, $R^2 = -0.13$ ($P < 0.01$) for *U. australis*. Thus, algae are trapped especially in nutrient-poor waters. The composition of algal prey from the samples from the Alps was different from those from the Bohemian massif; the same was true for northern Germany, Canada, south-eastern USA or West Africa as described by Alkhalaf et al. (2009, 2011) and Wołkowski et al. (2011). For example, Desmidiaceae such as *Micrasterias*, *Penium* or *Staurodesmus* were rare in northern Germany, whereas the diversity of Bacillariophyta was higher. Differences in the prey of *Utricularia* seem to reflect differences in the regional algal diversity.

The low number of protozoa contradicts the results of Płachno et al. (2012) who found a large portion of *Paramecium bursaria* in *U. reflexa*. These authors, however, used plants cultivated in plastic tanks, growing in the greenhouse under reduced light conditions and at a higher pH than in the water bodies used in our study. As *Utricularia* seems to trap small objects from the ambient water unselectively (Ellison and Gotelli, 2001), these conditions may have caused an increase in the abundance of protozoa both in the medium and in the traps.

Algae and pollen grains contribute to the nutrition of Utricularia

As animals play only a limited role within the total prey, the question arises if algae and pollen grains are only useless by-catch which cannot be avoided by the unselective suction traps, or if they contribute to the nutrition of the plant. Concerning algae, Jobson et al. (2000) found that feeding with exclusively *Euglena* caused a negative effect on the growth of *Utricularia*. Gordon and Pacheco (2007) suggest that *Utricularia* would achieve the maximum benefit of a balanced diet consisting of both animals and algae but do not provide experimental evidence for this. Alkhalaf et al. (2009) refer to the uptake of N from algae artificially labelled with ^{15}N , and further suggest (Alkhalaf et al., 2011) that algae may form an important source of N. Concerning pollen grains, the situation is similar: *Pinguicula lusitanica* was shown to benefit from feeding with pollen grains (Harder and Zedler, 1968), and the same may be true for some species of *Drosera* (Lavarack, 1979).

Among the parameters measured in this study, long shoots and a heavy dry weight can be regarded as indicators of good growth. Intensive branching, i.e. the formation of numerous buds, seems to be of specific importance as branching has been shown to correlate with successive sexual reproduction in *Utricularia* (Adamec, 2011b). Stunted growth, i.e. reduced length of internodia and a high leaf density, indicates low vitality according to Adamec et al. (2010). Similarly, a high C/N ratio indicates poor N supply. A high number of traps was regarded not only as an indicator of good growth but also of high investment into carnivory. Indeed, the measured dry weight correlated positively with total length and the number of buds and traps but negatively with leaf density.

The number of trapped animals correlates strongly negatively and highly significantly with the C/N ratio and strongly and

highly significantly with dry weight, but much less strongly with other growth parameters such as shoot length or the number of buds. These factors show a stronger correlation with the capture of algae and pollen grains which contribute little to a low C/N ratio. Pollen of evergreen trees usually exhibits a C/N ratio of 20–45 (Descolas-Gros and Schölzel, 2007). In spite of the increased $\delta^{15}\text{N}$ in pollen grains (Descolas-Gros and Schölzel, 2007), the number of trapped pollen shows only a very weak correlation with $\delta^{15}\text{N}$, thus confirming the limited relevance of pollen for N uptake.

Clearly, animals serve as the most important source of N, possibly besides N-fixing bacteria (Sirová et al., 2014), but this is not the only limiting factor for *Utricularia*. Algae and pollen grains seem to contribute other essential compounds, possibly phosphorus, trace elements or organic growth factors. The high importance of phosphorus for aquatic *Utricularia* was stressed by Sirová (2012).

The benefit gained from algae correlates with their size: the numbers of multicellular filamentous algae correlate much better with growth parameters than the small, unicellular Desmidiaceae. Unlike algae and pollen grains, prey particles summarized as ‘other objects’ (protozoa, fungi, moss leaflets and bacterial colonies) appear to be useless bycatch. The number of these objects correlated only weakly or even negatively with the various growth parameters; leaf density and C/N ratio were increased by the capture of this kind of prey.

Trap formation correlated well with other growth parameters; thus, abundant trap formation is only found in healthy, vigorously growing plants. The number of traps was higher in artificial peat bogs that contain more inorganic nutrients than natural fen waters (Loub et al., 1954). A similar correlation between substrate nutrient supply and trap formation was also described by Knight and Frost (1991). Correlation was also found between trap formation and trapping success: plants with many traps caught significantly more animals, filamentous algae and pollen grains, both in total and per trap. Thus, *Utricularia* seems to benefit simultaneously in three ways from nutrient-rich water: (1) improved nutrient supply from the water enables (2) increased formation of traps which (3) catch more prey objects per trap as nutrient-rich waters are more productive. It remains to be investigated if the dissolved nutrients in the water are the stimulus for trap formation, or if trap formation is promoted by the high trapping success of existing bladders.

It remains of debate whether algae are killed within the traps (Mette et al., 2000; Richards, 2001; Peroutka et al., 2008) or survive for an elongated period and support *Utricularia* possibly as mutualistic symbionts (Schumacher, 1960; Botta, 1976; Płachno et al., 2012). Our observations, both from the field and from the lab, yielded evidence for death and decay of the majority of algae, regardless of their taxonomic affiliation. More resistant species of algae may be able to survive within the traps but such species seem to be rare in the clean and undisturbed water bodies used in this study. Ongoing studies should resolve this question.

Autonomous firing of traps is confirmed

According to conventional opinion, the suction process in *Utricularia* is triggered by mechanical stimulation of sensitive hairs next to the trap door (e.g. Lloyd, 1942; Sydenham and Findlay, 1973; Barthlott et al., 2004). Thus, algae and pollen

grains could be trapped only as bycatch after stimulation of the trap by animals, and thus a strong correlation between prey animals and other prey objects could be predicted. A first statistical analysis (Peroutka et al., 2008), however, gave little evidence for a correlation between the numbers of trapped algae and animals. In this study, with a much larger sample size, a significant but poor correlation ($R^2 = 0.15$, $P < 0.01$) between trapped algae and animals was found. The correlation between animals and pollen grains was even weaker ($R^2 = 0.05$, $P < 0.05$) and the correlation between animals and other objects was negative ($R^2 = -0.11$, $P < 0.01$). Furthermore, 33.4 % of the traps (616 of 1844) contained algae but no animals. In 954 traps (51.4 %), only immotile or extremely small prey including algae, moss leaflets, pollen grains, protozoa or bacterial colonies were found. Similar results have been described by Mette et al. (2000) and Jobson et al. (2000). Thus, mechanical stimulation of a trap is not a prerequisite for successful prey capture.

Recent studies by Adamec (2011a) and Vincent et al. (2011a, b) provided evidence that the suction traps of *Utricularia* are capable of autonomous firing under laboratory conditions. After an elongated time without stimulation, the trap door opens spontaneously and sucks in the surrounding water, possibly containing small organisms. The results presented here indicate that this behaviour is of great ecophysiological relevance and significantly contributes to the nutrition of *Utricularia*.

*$\delta^{15}\text{N}$ data are of limited use in aquatic *Utricularia**

Some of the $\delta^{15}\text{N}$ data shown in this study allow for easy interpretation, e.g. the strong correlation between total prey and $\delta^{15}\text{N}$. The calculation of prey-derived N, however, leads to serious problems: the established formula which provides excellent results for terrestrial carnivorous plants (Schulze et al., 1991, 2001; Brearley, 2011) is not applicable for aquatic *Utricularia* because it gave unrealistically high values (≥ 100 % prey-derived N) for several test populations. Possible reasons include that only one species of floating non-carnivorous plants was available at each site. These control plants were not submerged vascular plants absorbing nutrients via the whole shoot surface like *Utricularia* but either algae or floating Lemnaceae absorbing nutrient via the root. Thus, ^{15}N uptake from the water is not necessarily comparable between these species.

Besides the evidence discussed previously, the $\delta^{15}\text{N}$ results further point to the absorption of N from trapped algae. Algae may exhibit very different $\delta^{15}\text{N}$ compared with vascular plants (Cole et al., 2004). Pollen of evergreen trees also exhibits an increased $\delta^{15}\text{N}$ (Descolas-Gros and Schölzel, 2007) compared with the control plants used in this study. Thus, the unrealistically high calculated proportion of prey-derived N seems to be caused by the mass capture of algae and pollen grains. Unfortunately, this explanation implies that the established formula for the calculation of animal-derived N is of limited value in systems where other types of captured objects play a major role.

CONCLUSIONS

Prey capture does not compensate completely for an oligotrophic substrate. On the contrary, *Utricularia* benefits both from

high nutrient concentrations in the water and from successful prey capture. Abundant traps are formed only by well-nourished plants growing in waters rather rich in nutrients. In such water bodies, prey objects of all kinds are more abundant as well. Thus, the benefit gained from prey is much higher in mesotrophic than in oligotrophic waters. The contribution of pollen grains and algae to the nutrition of the plant is comparable with the benefit gained from prey animals. Whereas animals are the main source of N, other nutrients or growth factors are derived from algae and pollen. Due to the aberrant $\delta^{15}\text{N}$ in some algae, $\delta^{15}\text{N}$ measurements are of limited use for the quantification of prey benefit in *Utricularia* populations trapping significant amounts of algae.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. **Table S1:** Overview of the water bodies sampled. **Table S2:** *Utricularia* species and non-carnivorous plants occurring in the studied water bodies and their major chemical characteristics. **Table S3:** Correlations between selected growth parameters and trapping success. **Table S4:** Correlations between water chemistry, growth parameters and trapping success.

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