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Oxygen Concentrations Inside the Traps of the Carnivorous Plants *Utricularia* and *Genlisea* (Lentibulariaceae)

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• *Background and Aims* Species of *Utricularia* and *Genlisea* (Lentibulariaceae) are carnivorous, capturing small prey in traps which are physiologically very active, with abundant quadrifid and bifid glands. Traps of *Utricularia* have walls composed of two cell layers, and are filled with water. Diverse communities of commensal microorganisms often live inside the traps. *Genlisea* forms long, hollow subterranean traps of foliar origin, growing in anoxic wet substrate. Knowledge of the O_2 concentrations inside *Utricularia* and *Genlisea* traps is vital for understanding their physiological functioning and conditions for the life of commensals. To test the hypothesis that prey are killed by anoxia inside the traps, and to measure respiration of traps, $[O_2]$ was measured in the fluid in mature traps of these species.
• *Methods* Oxygen concentration and electrical redox potential were measured using a small Clark-type oxygen sensor and a miniature platinum electrode, respectively, in the fluid of excised and intact traps of six aquatic *Utricularia* species and in *Genlisea hispidula* traps.
• *Key Results* Steady-state $[O_2]$ in the traps of both genera always approached zero (median 0·0–4·7 μM). The $[O_2]$ decreased after electrodes were inserted into *Utricularia* traps at a rate which ranged from 0·09 to 1·23 mM h^{-1} and was lower in traps of irradiated and intact shoots with higher $[O_2]$ in shoot tissues. Redox potential ranged from −24 to −105 mV in the traps, confirming the very small or zero $[O_2]$.
• *Conclusions* Very small or zero $[O_2]$, effectively anoxia, is demonstrated in *Utricularia* and *Genlisea* traps. This is probably below the critical $[O_2]$ for prey survival, and causes captured prey to die of suffocation. Internal trap glands and trap commensals are considered to be adapted to facultative anoxia interrupted by limited periods of higher $[O_2]$ after firings.

Key words: Aquatic *Utricularia* species, *Genlisea hispidula*, intact traps, trap fluid, dissolved oxygen concentration, oxygen minisensor, anoxia, trap respiration, electrical redox potential.

INTRODUCTION

The Lentibulariaceae, the largest family of carnivorous plants, comprises the genera *Utricularia* (bladderworts), *Genlisea* (corkscrew plant) and *Pinguicula* (butterworts) (Juniper *et al.*, 1989). About 50 *Utricularia* species are rootless aquatic or amphibious plants which grow in stagnant, nutrient-poor wetlands and water with a large humic acid concentration (Juniper *et al.*, 1989). *Utricularia* species supplement photoautotrophic nutrition by trapping and utilizing aquatic prey, such as small crustaceans, mites, rotifers and protozoa (Harms, 1999; Jobson and Morris, 2001; Richards, 2001). The trap is a water-filled utricle 1–4 mm long with a wall generally two cell layers thick and a variety of glands and trichomes on both the inner and outer surfaces. This is the most complicated trap, both functionally and morphologically, among carnivorous plants (Juniper *et al.*, 1989). The function of the glands is unresolved. If an organism outside the trap irritates trigger hairs situated close to the trap door, it is sucked in as a result of under-pressure maintained inside the trap (Sydenham and Findlay, 1973, 1975). Immediately after firing, the pressure within the trap is that of the external solution, but is decreased by rapid removal of approx. 40 % of the water from the lumen, thus restoring the original compressed shape of the utricle. This process lasts about 30 min, when the trap is ready to fire again (Sydenham and Findlay, 1975). Traps in the field can be fired by any mechanical stimulus (e.g. wind, water movement). As a consequence of firing, the trap is partly re-filled with the ambient water together with all the solutes it contains (gases – including oxygen, mineral ions, organic substances and exoenzymes) and microorganisms. Resting electrical potential difference measured by electrodes between the lumen of *Utricularia* traps and the ambient solution is about +135 mV (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985).

Little is known about the mechanisms of digestion of prey in *Utricularia* traps. Several groups of hydrolytic enzymes were detected cytochemically in the quadrifid digestive glands (for a review, see Juniper *et al.*, 1989). However, Sirová *et al.* (2003) investigated activities of five enzymes in the trap fluid from four aquatic *Utricularia* species. The activities of all the enzymes, except phosphatase, were greater in the ambient culture solution than in the empty traps (i.e. without prey). Thus, the enzymes could have entered the trap from the ambient solution, rather than have been secreted within the traps. In four aquatic *Utricularia* species, the pH of the fluid within the trap was between 4·9 and 5·1, a narrow range which may be an optimum for exoenzyme functioning (Sirová *et al.*, 2003).

Diverse communities of microorganisms, mainly bacteria, algae, protozoa and rotifers, live inside traps (Skutch, 1928;

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Jobson and Morris, 2001; Richards, 2001; Sirová *et al.*, 2003). This has led to the hypothesis that *Utricularia* plants with low trapping success, due to low prey availability, may benefit more from by-products of these commensals than from carnivory itself (Richards, 2001). As suggested by D. Sirová *et al.* (University of České Budějovice, Czech Republic, unpubl. res.), these commensals create a food chain (bacteria ↔ bacterivorous protists), similar to that found in *Sarracenia purpurea* pitcher fluid (Gray *et al.*, 2006), that could play an important ecological role for N and P uptake obtained from either the ambient medium or prey. Traps in aquatic *Utricularia* species are often short lived, which may be associated with very rapid apical shoot growth (Friday, 1992). In *U. vulgaris*, most traps complete their life cycle in 30 d or less, with traps active for only 10–19 d (Friday, 1989). Trap age is therefore a vital consideration in any ecophysiological study of *Utricularia* traps (Friday, 1989; Sirová *et al.*, 2003).

In aquatic *Utricularia* species, about 10–55 % of the total biomass consists of traps (Friday, 1992; Knight, 1992; Richards, 2001; Englund and Harms, 2003; Porembski *et al.*, 2006; Adamec, 2007). In six aquatic species, the respiration rate (per unit of fresh weight) of traps was 75–200 % greater than of the leaves or shoots of the plant, while the photosynthetic rate of traps was 7–10 times smaller than of leaves/shoots (Adamec, 2006; see also Knight, 1992). Aquatic *Utricularia* species commonly grow in low oxygen concentrations (Guisande *et al.*, 2000, 2004) or even under complete anoxia (Adamec, 2007). However, the relationship between [O_2], trap functions and the ecology of commensal communities within traps is unclear.

Genlisea grows in anoxic wet substrates and forms short stems with a rosette of small green leaves and tubular subterranean traps of foliar origin (Juniper *et al.*, 1989; Reut, 1993). The inverted Y-shaped traps are 3–12 cm long and about 1 mm in diameter. The two arms of the trap have a fluid-filled central cavity (diameter 0·2–0·5 mm) connecting a hollow tubular neck (inner diameter 0·2–0·5 mm), which ends in a slightly dilated vesicle (or bulb). The whole central cavity of the arms and neck is filled with water and is lined with forward-facing hairs that direct prey towards the vesicle. As such, these traps function as ‘eel traps’. Two types of glands are abundant in the neck and vesicular cavity, and digestive enzymes are secreted by the bulb-shaped glands (Juniper *et al.*, 1989; Płachno *et al.*, 2006). Organisms found within these traps consist of bacteria, algae, protozoans, nematodes, rotifers, annelids, crustaceans and mites (Barthlott *et al.*, 1998; Płachno *et al.*, 2005). Some of these organisms might grow inside the traps as commensals (Studnička, 1996). Unlike *Utricularia* traps, those of *Genlisea* are passive. No water flow was detected in a study using two *Genlisea* species (Adamec, 2003). The respiration rate (per unit fresh mass) of traps was very low despite the high rate of radial oxygen loss from them to the anoxic medium (Adamec, 2005). The high rate of radial oxygen loss is due to the occurrence of large air spaces in the trap walls and a permeability of the trap epidermis to oxygen.

It follows from the above studies that knowledge of the oxygen regime inside *Utricularia* and *Genlisea* traps is vital for understanding both their physiological functioning and ecological conditions for the life of commensal communities. As the traps of both genera contain abundant internal glands and their trap fluid is completely (*Utricularia*) or partly (*Genlisea*) closed and isolated, it is possible to assume that the [O_2] inside the traps is very low. Thus, it may be hypothesized that prey organisms captured will be killed by anoxia, whereas other organisms adapted to anoxia will be able to survive inside the traps and behave as commensals. The aim of this study was to measure [O_2], using a minisensor, in the fluid of large, mature traps of six aquatic *Utricularia* species. The [O_2] in the fluid within traps attached to shoot segments or intact plants was compared with that in excised traps (removed from plants but intact), as the traps attached to shoot segments or intact plants under light could be supplied with oxygen from photosynthetic tissues. The same measurements were also performed in *G. hispidula* traps. In both genera, the values of [O_2] measured in the trap fluid were compared with those measured in air spaces in the adjacent shoots or leaves (*Utricularia*) or trap walls (*Genlisea*). To confirm further and determine the oxygen and redox status of the fluid medium inside the traps, electrical redox potential was also measured in traps of both genera. To estimate the extent of isolation of the internal trap fluid from the ambient medium and to assess dark respiration rate of internal trap structures (glands), dark respiration of intact, excised traps of *U. reflexa* was compared with that of sliced traps.

MATERIALS AND METHODS

Plant material

Oxygen concentration was measured inside larger mature traps of adult plants of six aquatic *Utricularia* species. *Utricularia vulgaris* L., *U. ochroleuca* R.Hartm. (determined *sensu stricto* as *Utricularia stygia* Thor) and *U. intermedia* Hayne were collected in the Třeboň region, Czech Republic, and grown outdoors in plastic containers (area 2 m^2, 750 L for the former two species, area 0·8 m^2, 220 L for the latter species; Adamec, 1997*a*,*b*; Sirová *et al.*, 2003). Water depth was 25–35 cm in both cultures and was maintained by addition of soft tap water. *Utricularia reflexa* Oliver (collected in the Okavango Delta, Botswana), *U. foliosa* L. (northern Florida, USA) and *U. volubilis* R.Br. (south-western Australia) were grown indoors in 3 L aquaria in water approx. 20 cm deep (Sirová *et al.*, 2003). In all of the above cultures, litter of robust sedges was used as a substrate to simulate natural conditions. From the concentrations of nutrients and humic substances (humic acids + tannins), the water in these cultures was considered oligotrophic and humic (Adamec, 1997*b*; Sirová *et al.*, 2003). The pH of the cultivation media ranged from 6·6 to 7·6, dissolved oxygen concentration from 0·9 to 0·35 mM, and free CO_2 concentration from 0·05 to 0·15 mM. To promote plant growth, fine

zooplankton (a mixture of crustaceans including ostracods, body size 0·6–1·5 mm) were added weekly to the cultures.

Adult plants of *Genlisea hispidula* Stapf (from South Africa) were grown in a greenhouse under natural light in plastic 10 × 10 × 10 cm pots using a completely anoxic medium consisting of fibrous peat (pH approx. 4·2) with approx. 10 % (v/v) of perlite (for details, see Adamec, 2002, 2005).

Measurements of oxygen concentration in traps

Oxygen concentration was measured in mature *Utricularia* traps, 3–3·5 mm in diameter in *U. vulgaris* and *U. ochroleuca*, 2–3 mm in *U. intermedia*, 4–6 mm in *U. reflexa*, 2–2·5 mm in *U. foliosa*, and 4–5 mm in *U. volubilis*. Traps contained no visible prey. Oxygen concentration in the trap fluid and in the air spaces of adjacent shoots or leaves was measured using a miniaturized polarographic Clark-type oxygen sensor OX100 (Unisense A/S, Aarhus, Denmark). The guard cathode supplied was not used, as its polarization did not improve the signal. The tip of the sensor was placed in a glass capillary (outer tip diameter 120 μm) which could be inserted into softer plant tissues. The sensor was connected to a digital picoammeter (MEM 102, Inst. Botany, Třeboň, Czech Republic) and recorded, with 1 pA sensitivity, on a chart recorder. The sensor produced electrical current between 190 and 400 pA in air-saturated water at 25 °C, and a residual current of 13–20 pA at zero $[O_2]$ (in Na_2SO_3 solution). It had a 2 s reaction half-time, sensitivity to stirring <0·5 % of the signal, temperature stability of about 2 % of the signal K^{-1}, and time stability better than ±1 % signal h^{-1}. The O_2 consumption by the minisensor itself was calculated and shown to be negligible even in small traps. However, the minisensor had a very low resolution close to zero $[O_2]$. Concentrations below approx. 1·3 μM could not be distinguished from zero.

Oxygen concentration was measured either in the fluid of excised traps of the six *Utricularia* species under shaded conditions, in traps in intact shoot segments in four of the species under stirred conditions in light or in traps of intact *U. reflexa* plants growing in an aquarium under unstirred conditions in light. The latter approach simulated natural conditions. Excised leaves with traps of *Utricularia* spp. are relatively autonomous organs and are able to survive in ambient water for several weeks, with their traps functioning for at least 4 d (see Sirová *et al.*, 2003). As reported by Sydenham and Findlay (1973, 1975) and Sasago and Sibaoka (1985) the 2 h exposure of excised traps does not reduce their physiological reaction. Generally, traps of *U. vulgaris* were either from fifth to sixth adult leaf whorls (rose coloured, denoted as ‘young’) or from 30th to 31st leaf whorls as the oldest functioning traps (dark pink coloured, ‘old’). Light-rose or greenish coloured ‘young’ traps of *U. reflexa* were from third to six leaf whorls, and pink ‘old’ traps were from 25th to 31st leaf whorls. Greenish ‘young’ *U. foliosa* traps were cut from third leaf whorls of photosynthetic shoots; dark-pink ‘old’ *U. volubilis* traps represented old functioning traps, and were usually covered by periphytic algae. Pale ‘medium-old’ traps of *U. ochroleuca* and *U. intermedia* were from the carnivorous shoots.

Selected empty traps of *Utricularia* were stimulated gently with a pair of forceps to promote firing and were excised under water. They were then washed in water (filtered through a 44 μm mesh) obtained from the outdoor culture, and kept in the cultivation water in dim daylight (approx. 10–20 μmol m^{-2} s^{-1} PAR) before examination (5–15 min). Each trap was gently placed under water on a miniature holder made of a 2 cm piece of translucent soft silicon tubing (inner diameter 1–1·5 mm, outer diameter 3–3·5 mm) the bevelled end of which was cut longitudinally. The holder with attached trap was then transferred to a 90 mL thermostatted perspex chamber (25·0 ± 0·2 °C) and, while under water, was impaled, by hand, longitudinally with the tip of the fixed minisensor. The impaled trap was then released from the holder. The position of the sensing tip was checked under a magnifying hand lens and was moved into the middle of the trap. Great attention was paid to prevent air bubbles forming inside the trap. The chamber, filled with standard solution (1 mM $NaHCO_3$ + 0·5 mM KCl + 0·1 mM $CaCl_2$, pH 7·8), was gently stirred and aerated so that $[O_2]$ was constant (air saturation, 256 μM) and was then used as a standard value for minisensor calibration. During insertion of the electrode into the trap (1–2 min), the chamber was illuminated by a halogen light (approx. 180 μmol m^{-2} s^{-1} PAR). Afterwards, the chamber was partly shaded (<6 μmol m^{-2} s^{-1} PAR). The $[O_2]$ in the trap usually fell within 1–2 min of insertion of the minisensor to values between 40 and 80 μM, and stabilized close to zero within 5–40 min. In other cases, stabilization of the signal close to zero was achieved after deeper tip penetration. Unstable records were discarded. Before and after a set of measurements, residual current was measured at zero $[O_2]$. Its value was found to be very stable and was subtracted from all measured values. The recorded decline of trap $[O_2]$ was usually linear between 10 and 50 μM, and the linear part of the curve was used to calculate the rate of ‘internal’ trap respiration.

Oxygen concentration was also measured in empty traps of *Utricularia* shoot segments in a 0·5 L thermostatted glass chamber at 25·0 ± 0·2 °C. The gently stirred and aerated chamber ($[O_2]$ 256 μM) was continuously illuminated using a halogen light (180 ± 20 μmol m^{-2} s^{-1} PAR). Oxygen concentration was measured in ‘young’ traps in shoot segments of *U. vulgaris* comprising fifth and sixth adult leaf whorls, in ‘young’ traps (third to sixth leaf whorls) in 20 cm long apical shoot segments of *U. reflexa*, and in ‘medium-old’ traps in carnivorous shoots of *U. ochroleuca* and *U. intermedia* (segments consisted of one photosynthetic shoot and 1–2 carnivorous shoots). Before measurements, the washed segments were kept in the filtered water for 5–15 min. After each measurement inside the trap, the minisensor tip was inserted into an adjacent shoot (or leaf base, approx. 1–2 cm from the base of the trap) at an angle of 45 ° to estimate the internal $[O_2]$ of air spaces in organs bearing the measured trap. The signal stabilized within 2–4 min. Although it was not determined whether the sensing tip recorded $[O_2]$ strictly from

the gaseous or aqueous phase in the air spaces of the impaled tissues, it was assumed that $[O_2]$ was in equilibrium with that of the gaseous phase (i.e. air space). Oxygen concentration was also measured in 'young' (third to sixth leaf whorls) and 'old' traps (25th to 31st leaf whorls) of intact *U. reflexa* plants growing in a 3 L aquarium. Measurements of $[O_2]$ in empty traps and adjacent shoots in the unstirred aquarium water were conducted at 25·4–28·0 °C, at $[O_2]$ in the water ranging from 234 to 400 μM, under constant illumination (120–180 μmol m^{-2} s^{-1} PAR).

Oxygen concentration was measured in mature, 5–8 cm long isolated traps of *G. hispidula* plants in the 0·5 L stirred and aerated chamber, in the above solution, at 25·0 ± 0·2 °C under dim daylight (<6 μmol m^{-2} s^{-1} PAR). The minisensor tip was manually inserted into the traps at an angle of approx. 45 °. The sensing tip was localized in the following positions: arms 10 mm below the branching, tubular neck both 10 and 2 mm below the vesicle, in the vesicle, and in the apical stalk 10 mm above the vesicle. At these positions, $[O_2]$ was measured both in the fluid of the central cavity and in air spaces of trap wall tissues and the apical stalk. The $[O_2]$ stabilized within 4–10 min.

Measurements of electrical redox potential and trap respiration

To determine the reduction state of the fluid, and confirm previous $[O_2]$ measurements, electrical redox potential (Eh) was measured in large excised traps of *U. vulgaris*, *U. reflexa*, *U. ochroleuca* and *G. hispidula* using a miniature platinum electrode. The Pt electrode was produced by sealing a 40 μm Pt wire in a thick-walled glass capillary (outer diameter approx. 200 μm), finely bevelled at the tip for better penetration. Eh inside traps was measured in the 90 mL thermostatted chamber (25·0 ± 0·2 °C) in dim light (<6 μmol m^{-2} s^{-1} PAR) and the solution (see above) was gently stirred and aerated ($[O_2]$ 256 μM). The method of inserting the electrode tip under water into *Utricularia* traps and into the central cavity in *G. hispidula* vesicles was similar to that for $[O_2]$ measurements, and used the same recording instruments. An Ag/AgCl electrode with a porous ceramic tip was used as reference electrode. Its electrical potential (+218 mV) was subtracted from the measured value of the electrical potential. Before impalement of each trap, the tip of the Pt electrode was gently cleaned with fine abrasive paper and rinsed in distilled water. Eh stabilized within 20–150 min after trap impalement. Owing to the relative robustness of the electrode, the resting potential difference of about 135 mV (Sydenham and Findlay, 1973) can be short-circuited to almost zero. In *U. reflexa*, Eh was measured in 'young' and 'old' empty traps, and in 'young' traps having been fed on 1 mm large ostracods for 43–70 h. These traps each contained 1–2 ostracods.

To compare the 'external' dark respiration of intact, excised traps of *Utricularia* with that of the halved traps (i.e. the 'total' respiration rate with an integration of the 'internal' respiration of trap glands), dark respiration rate was measured in six large 'young' traps of *U. reflexa* (batch II; fresh weight 35–48 mg) at 25·0 ± 0·1 °C using an oxygen sensor (for all technical details, see Adamec, 1997*a*, 2005). The respiration rate of intact traps had been measured for 15 min in the dark, and traps were then partly sliced with a razor blade to allow an inflow of the stirred solution into the trap lumen. Measurements of dark respiration were then repeated. Six independent measurements were performed on different traps. It was estimated, by weighing, that the large traps (diameter 5·5–6·0 mm) of this species after firing contained 23·8 ± 0·6 μL of trap fluid, and fresh weight (without luminal fluid) was 8·6 ± 0·2 mg ($n = 10$).

Statistical treatment

At least eight measurements of $[O_2]$ and Eh were performed on traps of each of the different species of *Utricularia* and *G. hispidula*. As the values of $[O_2]$ in *Utricularia* traps usually had a non-normal distribution and were mostly below the resolution near zero, only the mean, median and range of values are shown. Values of the linear rate of $[O_2]$ decline in traps of different species for the same type of measurement, for 'young' *U. reflexa* traps across different type of measurements, and Eh values across different traps of *U. reflexa* were evaluated by one-way analysis of variance (ANOVA; Tukey HSD test for multiple comparisons). Where possible, all paired values were evaluated by a two-tailed *t*-test ($P < 0·05$).

RESULTS

Oxygen concentration in the traps of six *Utricularia* species was consistently very small (range 0·0–4·7 μM; Tables 1 and 2) and usually approached zero, regardless of the experimental conditions (light, stirring, intact or excised traps) or the age of the traps. Mean values were usually below the resolution of the oxygen minisensor (approx. 1·3 μM) and were considered to be around zero. Low $[O_2]$ inside traps was independent of the position of the sensing tip. Small differences between two batches of *U. reflexa* (Table 1) may be partly attributed to the low resolution of the minisensor and differences in growth conditions between the batches. Generally, a steady state for the minimum $[O_2]$ was achieved most rapidly in shaded, excised traps (within 5–40 min), and more slowly in illuminated, intact traps (10–60 min), especially those of intact *U. reflexa* plants in an unstirred medium (20–100 min). The linear rate of $[O_2]$ decline after trap impalement is a measure of the respiration rates of internal trap structures and commensal organisms offset by oxygen influx from trap walls. The decrease in $[O_2]$ in excised traps in the shade was somewhat faster than in intact traps in the light (compare Tables 1 and 2). The linear rate of $[O_2]$ decrease was 0·27–1·23 mM h^{-1} in excised traps of the six *Utricularia* species exposed in shade, 0·16–0·86 mM h^{-1} for intact traps from stirred medium across four of the species in light, and 0·09–0·13 mM h^{-1} in *U. reflexa* traps in the unstirred medium. However, only excised 'young' and 'old' *U. reflexa* traps

TABLE 1. *Steady state, minimum oxygen concentration in the fluid in excised empty traps of aquatic* Utricularia *species in an aerated, gently stirred solution*

Species	Trap age	Oxygen concentration (µM)			Rate of $[O_2]$ decline (mM h^{-1})
		Mean	Median	Range	
U. vulgaris	Young	4·7	4·7	2·8–6·6	1·19 ± 0·11[ac]
U. vulgaris	Old	0·7	0·5	0·0–2·2	1·23 ± 0·17[a]
U. reflexa	Young	0·1	0·0	0·0–1·0	0·47 ± 0·03[bde]
U. reflexa II.	Young	1·8	1·4	1·3–2·5	0·27 ± 0·03[bd]
U. reflexa	Old	0·3	0·0	0·0–1·0	0·43 ± 0·05[bde]
U. ochroleuca	Medium	1·4	0·5	0·0–3·4	0·65 ± 0·06[bde]
U. intermedia	Medium	0·7	1·1	0·0–1·2	0·73 ± 0·18[ce]
U. foliosa	Young	0·0	0·0	–	–
U. volubilis	Old	0·5	0·0	0·0–2·6	–

Oxygen concentration in the trap fluid was measured at 25·0 ± 0·2 °C in an aerated, gently stirred solution in dim daylight (<6 µmol m^{-2} s^{-1} PAR); $n = 8$. Two batches of plants were used for *U. reflexa*. The rate of linear decline of trap $[O_2]$, recorded usually between 10 and 50 µM trap $[O_2]$, is shown as mean ± s.e.; $n = 5–10$.

Different supersript letters denote a statistically significant difference ($P < 0·05$; one-way ANOVA).

differed significantly from intact traps ($P < 0·001$) under illumination, in both stirred and unstirred conditions. *Utricularia reflexa*, with larger traps, had a slower decrease of $[O_2]$ in comparison with species with smaller traps. In contrast to the almost zero $[O_2]$ inside *Utricularia* traps, $[O_2]$ in homogeneous, non-differentiated illuminated shoots of *U. vulgaris* and *U. reflexa* bearing traps was slightly over-saturated (268–272 µM), and less so (191–249 µM) for pale, carnivorous shoots in *U. ochroleuca* and *U. intermedia* (Table 2). The $[O_2]$ (378–400 µM) in shoots of *U. reflexa* growing under illumination in an unstirred aquarium was much greater than that in the ambient solution. Thus, the ambient, oxygen-saturated and stirred medium had a weaker influence on $[O_2]$ regime inside *Utricularia* traps than photosynthesis of intact shoots.

Oxygen concentrations in the central cavities of excised traps of *G. hispidula* varied greatly according to position within the trap. Steady state $[O_2]$ in the trap walls was nearly constant (232–238 µM; Table 3) and slightly below the saturated $[O_2]$ of the medium. Oxygen concentration of the fluid in the central cavity of the open trap arms reached about 84 % (215 µM) of air saturation, even though the trap was immersed in a slowly stirred, air-saturated ambient medium. However, $[O_2]$ was significanly less (155 ± 14 µM) in the central cavity of the tubular neck, 10 mm below the trap vesicle. Oxygen concentration in the central cavity declined steeply towards the vesicle and was zero 2 mm below and in the middle of the vesicle. In the apical stalk above the vesicle, the oxygen concentration was relatively large and close to air saturation (245–248 µM) both in central air spaces and in the trap walls (Table 3).

Mean Eh values of between −24 and −102 mV were measured in excised traps of three *Utricularia* species, and −105 mV in *G. hispidula* (Table 4). Mean Eh values in empty 'young' traps of *U. reflexa* (−24 ± 9 mV) were statistically significantly greater than those in empty 'old' traps (−75 ± 20 mV) or in 'young' traps with prey (−102 ± 10 mV). For *U. reflexa*, the 'external' dark respiration rate of intact traps (3·72 ± 0·24 mmol kg^{-1} f. wt h^{-1})

TABLE 2. *Steady-state, mimimum oxygen concentration in the fluid in empty traps of intact shoots or shoot segments of aquatic* Utricularia *species. (A) In light in a stirred, constant temperature chamber. (B) In light in an unstirred aquarium in which the plants were growing*

Species	Trap age	$[O_2]$ in trap fluid (µm)			Rate of $[O_2]$ decline (mM h^{-1})	$[O_2]$ in shoots (µM)
		Mean	Median	Range		
A. Aerated thermostatted chamber						
U. vulgaris	Young	0·2	0·0	0·0–1·5	0·86 ± 0·13[a]	268 ± 2
U. reflexa	Young	0·2	0·0	0·0–1·4	0·16 ± 0·02[b]	272 ± 2
U. ochroleuca	Medium	0·5	0·0	0·0–2·7	0·51 ± 0·07[ab]	191 ± 16
U. intermedia	Medium	0·3	0·0	0·0–2·7	0·57 ± 0·16[ab]	249 ± 3
B. Unstirred aquarium						
U. reflexa	Young	0·6	0·0	0·0–3·8	0·09 ± 0·02[a]	378 ± 23
U. reflexa	Old	0·2	0·0	0·0–0·6	0·13 ± 0·02[a]	400 ± 8

(A) Measured at 25·0 ± 0·2 °C, 256 µM O_2, 180 ± 20 µmol m^{-2} s^{-1} PAR; $n = 8$. (B) Measured at 25·4–28·0 °C and 120–180 µmol m^{-2} s^{-1} PAR; $n = 8$. The rate of linear decline of trap $[O_2]$, recorded usually between 10 and 50 µM trap $[O_2]$, is shown as mean ± s.e.; $n = 5–10$.

Different letters within each section denote a statistically significant difference ($P < 0·05$; one-way ANOVA). Oxygen concentration in shoots (stems or petioles) bearing measured traps is also shown; $n = 4–8$.

TABLE 3. *Steady state oxygen concentration in different parts of mature traps of* Genlisea hispidula

Trap part	Oxygen concentration (μM)	
	Trap walls	Central cavity
Arms 10 mm below branching	232 ± 4[a]	215 ± 20[a]
Tubular neck 10 mm below vesicle	238 ± 3[a]	155 ± 14[b]
Tubular neck 2 mm below vesicle	235 ± 2[a]	0·4 ± 0·3[b]
Vesicle	234 ± 5[a]	0·0 ± 0·0[b]
Apical stalk 10 mm above vesicle	248 ± 3[a]	245 ± 2[a]

Measured in a stirred, constant temperature chamber (25·0 ± 0·2 °C) in an aerated mineral solution (256 μM O_2). The central part of apical stalks contained air spaces instead of fluid. Means ± s.e. are shown; $n = 8–9$.

Different superscript letters in individual lines denote a statistically significant difference at $P < 0·01$ (*t*-test).

was significantly lower ($P < 0·05$, *t*-test) than the 'total' respiration rate for traps that were sliced into two halves (4·73 ± 0·38 mmol kg^{-1} f. wt h^{-1}; data not shown).

DISCUSSION

The steady state [O_2] measured in functional traps of six aquatic *Utricularia* species, and *G. hispidula* without prey, was consistently close to zero, or zero, under all experimental conditions (Tables 1–3). These results were not affected by the position of the sensor tip inside the traps, and it is therefore likely that [O_2] is very close to zero at all positions inside traps. The [O_2] was also close to zero, or zero, in traps of *Utricularia* spp. which had been perforated by inserting the oxygen minisensor 2–3 times. Also, [O_2] was close to zero, or zero, in traps with a fine internal air bubble. Thus, the physiological impact of trap impalement by the minisensor on trap functioning is minimal. Despite rapid oxygen influx into traps after firing, the very high respiration rate of internal trap structures (glands) was apparently able to reduce [O_2] to zero. Zero [O_2] inside both *Utricularia* and *Genlisea* traps was also confirmed by measuring Eh values between −24 and −105 mV (Table 4), attributed to an anoxic medium, but with a relatively weak reducing power (Brix and Sorrel, 1996).

TABLE 4. *Electrical redox potential (Eh) in traps of aquatic* Utricularia *species and in vesicles of* Genlisea hispidula *traps*

Species	Trap age	Eh (mV)
U. vulgaris	Young	−50·1 ± 13·9
U. reflexa	Young	−23·8 ± 9·2[a]
U. reflexa with prey	Young	−102·4 ± 10·3[b]
U. reflexa	Old	−75·2 ± 20·4[ab]
U. ochroleuca	Medium	−87·0 ± 14·7
G. hispidula	Medium	−104·5 ± 4·5

Measured at 25·0 ± 0·2 °C in an aerated, gently stirred mineral solution (256 μM O_2) in dim daylight (<6 μmol m^{-2} s^{-1} PAR). Means ± s.e. are given; $n = 8–10$.

For *U. reflexa*, different supersript letters denote a statistically significant difference ($P < 0·05$; one-way ANOVA).

Measurements of [O_2] for intact *Utricularia* traps after firing show a similar time-course to that measured for intact traps under natural field conditions in fired traps. As a result of the suction of air-saturated ambient water into the lumen, [O_2] increased to about 40–130 μM after 1–2 min, and then gradually declined to about zero within 20–100 min. Thus, under natural conditions, long periods of anoxia inside *Utricularia* traps can be interrupted by short periods of higher [O_2] after accidental trap firings. One of the aims of this study was to measure oxygen fluxes occurring between the trap fluid and the ambient water (see Sasago and Sibaoka, 1985; Adamec, 1995, 2006). In 'young' *U. reflexa* traps, the measured differences in dark respiration rates between halved ('total' respiration including the 'internal' respiration) and intact traps ('external' respiration) was 1·01 mmol kg^{-1} f. wt h^{-1}, i.e. about 21 % of the 'total' respiration. This difference can be attributed mainly to the high metabolic rate of bifid and quadrifid glands due to the presence of transfer cells (Sasago and Sibaoka, 1985; Płachno and Jankun, 2004). As internal trap glands form only about 3–5 % of total trap fresh weight, their potential aerobic respiration rate per unit of fresh weight may be about one order of magnitude greater than that of the whole trap. The 'internal' respiration rate can also be derived from the linear rate of [O_2] decline in the traps of this species (0·27 mM h^{-1}; Table 1). If we assume the mean trap volume for *U. reflexa* is 24 μL and the trap fresh weight is 8·6 mg, then this [O_2] decline is equal to an 'internal' trap respiration rate of 6·5 nmol $trap^{-1}$ h^{-1} or 0·75 mmol kg^{-1} f. wt h^{-1}. Both values of the 'internal' respiration rate, estimated in different ways (i.e. 1·01 and 0·75 mmol kg^{-1} f. wt h^{-1}), are comparable even though the latter value of 0·75 mmol kg^{-1} f. wt h^{-1} could have decreased due to oxygen leakage after trap perforation or diffusion from trap walls. Thus, the estimated difference between the 'internal' respiration rates of 2·2 nmol $trap^{-1}$ h^{-1} and/or 0·26 mmol kg^{-1} f. wt h^{-1} might be equal to permanent oxygen influx into the impaled traps amounting to about 35 % of the rate of [O_2] decline. If we presume that halved traps have a greater respiration rate than isolated intact traps, that the presence of dead or living prey in traps does not increase the 'external' trap respiration rate (trap fluid is very isolated; Adamec, 1995, 2006) and that there is permanent anoxia inside the traps, then it is highly probable that aerobic respiration inside the traps is very low. Furthermore, oxygen from the ambient oxygenated water diffuses to the traps so slowly that its internal concentration approaches zero. Thus, the [O_2] inside the traps is not increased by oxygen diffusion from the ambient medium or shoot. As shown in Tables 1 and 2, the ambient, oxygen-saturated medium has less of an influence on [O_2] decline rates inside *Utricularia* traps than photosynthetically evolved oxygen in air spaces and intercellular spaces of the shoots.

The above considerations suggest that internal glands of *Utricularia* traps have potentially a very high aerobic dark respiration rate. However, it is unclear whether these glands maintain this high rate inside the traps under anoxic conditions. The only exception is the period of 20–100 min after a trap has fired, when all the oxygen

sucked into the trap is probably consumed by internal glands. This period is comparable with the 30 min required to reset traps (Sydenham and Findlay, 1975). It is likely that this is the only period in which internal glands respire aerobically (Adamec, 1995, 2006). Therefore, when [O_2] is low in the ambient water in which a trap is fired (Guisande *et al.*, 2000, 2004; Adamec, 2007), the period of aerobic respiration could be markedly shorter than the resetting time. As a consequence of near anaerobic conditions in traps, it is probable that the internal glands, in spite of their very efficient aerobic respiration, obtain their metabolic energy for prey digestion and nutrient absorption from anaerobic fermentation. This conclusion does not contradict the hypothesis and findings of Jobson *et al.* (2004), who suggest a link between faster reaction kinetics of *Utricularia* bladders and mutations occurring in the mitochondrial respiratory chain enzyme cytochrome *c* oxidase. Laakkonen *et al.* (2006) further hypothesize decoupling of mitochondrial proton pumping from electron transfer, which could be a rich source of ATP energy after trap firing. Such decoupling would permit *Utricularia* traps to optimize power output during times of need, albeit with a 20 % decrease in overall energy efficiency of the respiratory chain.

Water flow in vesicles of *Genlisea* has not been found (Adamec, 2003), suggesting permanent internal anoxia (Table 3). Although traps in this genus are open to the environment, anoxia of the vesicle fluid and adjacent parts of the tubular neck is possibly maintained by the high respiration rate of abundant digestive glands lining the central cavity (Płachno *et al.*, 2006). Under natural anoxic soil conditions (Adamec, 2005), the whole central cavity in the tubular neck should also be anoxic.

Near zero [O_2] in traps of aquatic *Utricularia* species probably determines the type of organisms that can live inside traps. First, those that cannot tolerate [O_2] below approx. 1 μM will die, and are thus potential prey. Many species of zooplankton (e.g. crustaceans, ostracods) do not tolerate [O_2] below 6–40 μM (e.g. Newrkla, 1985; Weider and Lampert, 1985). Prey can only survive in intact *Utricularia* traps for 1–10 h (Skutch, 1928). Since activities of digestive enzymes (except for phosphatases) in trap fluid are relatively small and are comparable with those occurring in the ambient water (Sirová *et al.*, 2003), most captured organisms probably die of oxygen deprivation. It is concluded that maintaining zero [O_2] in the trap fluid is the mechanism for killing captured prey. Other organisms living in *Utricularia* traps (bacteria, algae, protozoa and rotifers) are more tolerant to long-lasting anoxia, interrupted by short periods of hypoxia. They could be facultatively anaerobic and can be considered commensals (Richards, 2001; Sirová *et al.*, 2003). Moreover, in the absence of proteases in the trap fluid (Sirová *et al.*, 2003), autolysis of prey tissues could be important. High rates of bacterial dinitrogen fixation occurred in the pitcher fluid of *Sarracenia purpurea* (Prankevicius and Cameron, 1991) and on the external trap surface of an aquatic species *U. inflexa* (Wagner and Mshigeni, 1986). Owing to anoxia and the presence of organic substances in the *Utricularia* trap fluid (D. Sirová *et al.*, University of České Budějovice, Czech Republic, unpubl. res.), high rates of dinitrogen fixation can also be expected to occur in empty *Utricularia* or *Genlisea* traps.

CONCLUSIONS

Traps of *Utricularia* and *Genlisea* are completely or partly isolated organs, and their internal fluids contain abundant, highly active metabolic glands, in addition to commensal communities of microorganisms. In excised or intact empty traps of both genera, almost zero [O_2] was found in the trap fluid under all experimental conditions. It is concluded that maintainance of zero [O_2] in the trap fluid is the mechanism for killing captured prey. Measurements of the rate at which [O_2] decreases in the fluid after insertion of the sensor into *Utricularia* traps indicates that stirring of the ambient oxygenated medium has less influence on trap [O_2] than photosynthesis of adjacent shoots under unstirred conditions. Under natural growth conditions, long periods (hours to dozens of hours) of anoxia inside *Utricularia* traps are interrupted by short periods (20–100 min) of higher [O_2] after accidental firings. In *Genlisea* traps, anoxia is probably permanent. Therefore, captured organisms in both plant genera either die of oxygen deprivation within several hours and are prey, or are able to tolerate anoxia and are commensals. This study demonstrates the great respiratory activity of *Utricularia* traps and their glands immediately after firing. However, it remains unclear how traps (glands) respire under anoxic conditions.

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