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Ecophysiological characteristics of aquatic carnivorous plants

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The aim of the Dissertation was to summarize all recent substantial pieces of knowledge covering all aspects and topics of the ecophysiological study of aquatic carnivorous plants (ACPs) as a special ecological grouping, to which the dissertant (L. Adamec) has contributed significantly within his research activity. About 60 species of the genera *Aldrovanda* (monotypic *A. vesiculosa,* waterwheel plant, Droseraceae) and *Utricularia* (bladderwort, Lentibulariaceae) are submerged aquatic or amphibious carnivorous plants (Taylor 1989; **Adamec 1997a, 2018a,b**; Guisande *et al*. 2007). All ACPs (including also amphibious) are strictly rootless and take up mineral nutrients for their growth only via their trap-bearing shoots from the ambient water (or loose sediment) and captured prey. However, they fulfil all principal functional criteria generally placed on carnivorous plants (CPs; Juniper *et al.* 1989; **Adamec 1997a; Ellison and Adamec 2011, 2018**). Moreover, traps of ACPs are known to exhibit rapid movements, which are among the fastest within the plant kingdom, and represent fascinating objects for biological studies (Juniper *et al*. 1989; Vincent *et al*. 2011a; **Masi *et al*. 2016; Westermeier *et al*. 2017**; Poppinga *et al*. 2018). However, when the excellent and voluminous monograph on carnivorous plant biology (Juniper *et al.* 1989) appeared the ecophysiological study of ACPs was still in its infancy as ACPs generally have always been understudied relative to terrestrial CPs due to methodological problems or ignorance (Juniper *et al*. 1989; **Adamec 1997a, 2011a, 2018a; Ellison and Adamec 2011**). Therefore, Juniper *et al.* (1989) dealt only very marginally with ACP ecophysiology and the dominant majority of ecophysiological studies on ACPs post-date Juniper *et al*. (1989).

The dissertant has been working on the ecophysiological studies on ACPs since 1995 up to the present, he (or with collaborators) has elaborated many pioneering studies, which have become fundamental in this field, and several times has comprehensively reviewed this topic. Ecophysiology of ACPs was thoroughly reviewed first by **Adamec (1997a)**, who separately analyzed traits and characteristics of both terrestrial CPs and ACPs. Guisande *et al*. (2007) published a detailed review on *Utricularia* that includes information on ecophysiological traits and cost-benefit relationships. **Adamec (2011a)** reviewed comprehensively all ecophysiological aspects of ACP biology, including those of *Utricularia* traps. **Ellison and Adamec (2011)** reviewed functional differences between terrestrial and ACPs with an emphasis on growth rate, photosynthesis, shoot mineral content, and cost-benefit relationships. **Adamec (2011b)** reviewed thoroughly the ecophysiology of aquatic *Utricularia* traps, with an emphasis on the role played by microbial trap commensals as well as biophysical aspects. The present review follows up tighly on those by **Adamec (2018a,c)** and **Ellison and Adamec (2011)**. The ecology of habitats of ACPs and characteristics of their growth traits, photosynthesis, mineral nutrition, regulation of the investment in carnivory in *Utricularia,* peculiarities of *Utricularia* traps, and turion ecophysiology are included in this review. The ecophysiological differences between terrestrial and ACPs and those between ACPs and aquatic rooted non-CPs are also discussed here and inspirations for future research are suggested. Experimental ecophysiological studies by the dissertant were based both on field research and cold-greenhouse experiments. Moreover, they often used plants kept in the

world-largest collection of ACPs in the Institute of Botany at Třeboň (guided by the dissertant), recently counting 21 populations of *Aldrovanda vesiculosa* and about 30 aquatic *Utricularia* species.

ACPs usually grow in shallow standing or slowly running humic (dystrophic), nutrient-poor waters and their main trait is high free-CO_2 concentration, usually >0.1 mM (**Adamec 1997a,b, 2007a, 2011a, 2012a, 2018a,b,c**). Evidently, pH itself is not important for their growth but high $[CO_2]$ is. A high $[CO_2]$ >0.15 mM is considered the primary factor supporting vigorous growth and propagation of ACPs. The main ecophysiological trait of ACPs with linear, modular shoots exhibiting a distinct growth polarity is their very rapid growth, which differentiates them markedly from terrestrial CPs (**Adamec 2011a, 2018a,c; Ellison and Adamec, 2011**). Under optimal conditions, both very rapid apical shoot growth and high branching rate and frequency underlie the high relative growth rate of ACPs (Friday 1989; **Adamec 2000, 2008b, 2009, 2010a, 2015; Adamec and Kovářová 2006; Adamec *et al*. 2010a**). In conclusion, very rapid growth of rootless ACPs requires ecophysiological adaptations that enable the plants to access the highly limited supplies of mineral nutrients from the water and also very high photosynthesis. These adaptations include carnivory, efficient nutrient reutilization from senescing shoots, and a very efficient nutrient uptake from water (Kamiński 1987; Friday and Quarmby 1994; **Adamec 2000, 2008a,b, 2014**; Englund and Harms 2003).

Very high maximum net photosynthetic rate (P_{max}) occurs along whole linear shoots of ACPs with rapid growth but is also a prerequisite both for this rapid growth (**Adamec 2006, 2014**), as the rapid, permanent decay of senescent shoot segments causes a great loss of structural and non-structural carbohydrates (**Adamec 2000**), and for covering high maintenance costs of traps (**Adamec 2006, 2013, 2018a,c**). Traps of aquatic *Utricularia* species are physiologically very active organs and have high dark respiration rates (RD). Simultaneously, they incur great photosynthetic (metabolic, energetic) costs due to very low P_{max}. In seven aquatic *Utricularia* species, RD of traps per unit FW was 1.7-3.0 times higher than that in leaves, while P_{max} in photosynthetic leaves exceeded that in the traps 7-10 times (**Adamec 2006, 2013**). Such a very high RD:P_{max} ratio (50-140%) in *Utricularia* traps, unlike that in leaves (3.6-8.2%), indicates high maintenance and photosynthetic costs of traps. The influence of prey capture on P_{max} and RD in ACPs is still unclear due to shortage of data (**Adamec, 2008b**) and raises the question which proportion of organic carbon can be covered in ACPs by carnivory from prey.

The composition of mineral nutrients in rootless ACP shoots is similar to rooted aquatic non-CPs, even at low prey availability (**Adamec 1997a, 2008a,b, 2014, 2018a; Ellison and Adamec 2011**). A very efficient N and P reutilization (but zero for K) from senescing shoot segments was found in rapidly growing aquatic species (Friday and Quarmby 1994; **Adamec 2000, 2008a, 2014, 2016**). In conclusion, ACPs permanently lose only a relatively small part of their N and P in their senescent shoots but almost all K, Ca, and Mg. However, their N and P reutilization efficiency is on average 10-30 percentage points lower than that in terrestrial CPs. Zero K reutilization in ACPs is probably common for all submersed aquatic plants but the reasons for are unknown (**Adamec 2014**). The influence of prey capture on the rate of mineral nutrients from the ambient water still remains ambiguous due to shortage of data (**Adamec 2016**). Moreover, it is not clear by which mechanism prey capture supports growth of ACPs: hypothesis that N and P absorbed from prey preferentially supports essential growth processes associated with cell division in shoot apices (**Adamec 2008b**) was not generally

confirmed (**Adamec 2011e**). Yet capture of prey in ACPs is one of the decisive factors for their rapid growth and especially for their propagation (**Adamec 2011a, 2018a,c**).

Utricularia suction traps are hermetically closed bladders 1-5 mm long functioning on the basis of negative pressure (Sydenham and Findlay 1973; Juniper *et al*. 1989; Guisande *et al*. 2007; **Adamec 2011b, 2018a,c**; Vincent *et al*. 2011a). The unique use of a high-speed camera and a linear position sensor represented a breakthrough in the knowledge of the biophysical functioning of *Utricularia* traps (**Adamec 2011f,g,** Vincent *et al*. 2011a,b; Poppinga *et al*. 2016). These studies specified the complete process of trap firing to last only 2.5-5 ms, characterized the trap-door movement during trap firing as buckling and unbuckling, proved spontaneous trap firing (without mechanical stimulation) and suggested a permanent water pumping out from the traps. Moreover, using an electronic pressure sensor, **Adamec and Poppinga (2016)** estimated the critical negative pressure inside cut off aquatic *Utricularia* traps at which the traps (located in air) fire and aspirate an air bubble (as in spontaneous firings). In summary, the biophysical diversification of *Utricularia* traps and different trap sizes could contribute to a diversification of prey capture in environments with various prey availability. Moreover, spontaneous firings and new findings indirectly support the mechanical (not electrophysiological) concept of *Utricularia* trap triggering (**Adamec 2012b**).

Using microfluorimetric methods, only the activity of acid phosphatases was determined as significant in prey-free trap fluid in aquatic *Utricularia* species and was independent of prey capture; other enzymes were sucked from the ambient water (**Sirová *et al*. 2003**). The activity of all enzymes was independent of prey capture and was not inducible by prey or loading of N and P salts into the traps. Furthermore, it is not clear which proportion of any enzyme activity in the filtered trap fluid is produced by trap glands alone or various trap commensals. In prey-free traps, a miniature microbial food web may develop (**Sirová *et al*. 2009, 2011**). Its main components are bacteria, euglens, Dinophyta, ciliates, and rotifers acting as digestive mutualists (e.g., Richards 2001; Peroutka *et al*. 2008; **Sirová *et al*. 2018**). High amount of organic substances is exuded by the traps to the trap fluid to support the microbial commensals (**Sirová *et al*. 2010, 2011; Borovec et al. 2012**). Yet the nutritional role of these commensals to provide N and P for the plants is unknown but model calculations show that the commensals in prey-free traps might behave as parasites (**Adamec 2011g, 2018a,c**). A novel transcriptomic study (**Sirová *et al*. 2019**) revealed a great analogy of microbial commensals between *Utricularia* trap and herbivore (rumen) intestine. Very rapid turnover of organic substances was found in *U. reflexa* traps as a result of a specific occurrence of a specialized, newly described bacterivorous ciliate *Tetrahymena utriculariae* (**Pitsch *et al*. 2017; Šimek *et al*. 2017**).

Zero O_2 concentration was consistently measured in the fluid of excised and intact prey-free traps of aquatic *Utricularia* species bathed in an oxygenated medium (**Adamec 2007b**). Thus, the aerobic respiration of the inner glands and trap walls is so high that all O_2 is exhausted within 10-40 min after each firing. The extremely low $[O_2]$ in the trap fluid is a result of a functional compromise (**Adamec 2018a**): it must be very low to reliably kill the captured prey (requiring ca. >15-30 μM) but higher than a threshold for an effective aerobic respiration of the glands (ca. >0.4 μM). Anoxia causes captured prey to die of suffocation in traps, while all trap commensals are adapted to facultative anoxia (**Adamec 2007b**).

As an investment in carnivory, traps in ACP species incur costs in structure (organic biomass), photosynthesis (decrease of P_{max}), energy (consumption for trap operation,

metabolism, and maintenance), and minerals (total amount of minerals in the traps or that lost in senescent traps; **Adamec 2010b**). In aquatic *Utricularia* species, the proportion of traps to the total plant biomass is usually 10-65%, but this structural cost is regulated flexibly to minimize all costs according to habitat factors, especially water chemistry, prey capture, and irradiance (Friday 1989; Guisande *et al.* 2007; **Adamec 2008a, 2011a,b, 2015, 2018a,c**). Increased mineral nutrient availability usually led to decreased investment in carnivory in terms of trap number per leaf, mean trap size (weight), or proportion of trap biomass. Generally, all external nutrient factors, which decrease tissue N content in young shoots (poor prey capture, low $[NH_4^+]$, high $[CO_2]$, etc.), increase trap production in young shoots and *vice versa*, but photosynthesis is superior to the 'nutrient' regulation (**Adamec 2015**).

Turions are dormant vegetative winter buds formed by perennial aquatic plants as a response to unfavorable ecological conditions – mainly decreased temperature, daylength or nutrient availability (Bartley and Spence 1987; **Adamec 2018a,d**). The majority of temperate ACP species form turions. Turions of ACPs as modified shoot apices are formed by extreme condensation of short modified trap-free leaves at the end of the growing season. They are partly frost resistant and protect fragile plant shoots from freezing. ACP turions can be hardened by weak frosts and their frost hardiness is based on the shift from frost avoidance in non-hardened turions to frost tolerance (**Adamec and Kučerová 2013**). Two dormancy states (innate and imposed) and their hormonal patterns were described for ACP turions (Winston and Gorham 1979a,b; **Adamec 2003**). Turions of ACPs are storage organs and in autumn, they accumulate starch, free sugars, reserve proteins and lipids (Winston and Gorham 1979a; **Adamec 2000, 2003, 2018d; Płachno *et al.* 2014**). They also represent storage organs for mineral nutrients (N, P) though this storage function is presumably weaker (**Adamec 2010c, 2011d**). Their RD was 30-75% lower per unit FW and 80-85% lower per unit DW than adult shoots (leaves) of the same species (**Adamec 2018c**). In summary, ecophysiological traits of turions of ACPs are the same as those of turions of other aquatic non-CPs (**Adamec, 2018d**). Very high P_{max} of sprouting turions of rootless ACPs, together with the storage functions of turions for N, P, S, and Mg, contributes to rapid growth of sprouting turions and rapid production of standing biomass at the beginning of the growing season and represents a great competitive advantage (**Adamec 2011d, 2018a,d**). Unlike the seed bank, turions of ACPs can only survive from one season to another, not longer (**Adamec 2018d**).

To obtain further insight into the ecophysiology of ACPs, the following questions could profitably be addressed (see also **Ellison and Adamec 2011** and **Adamec 2018a**):

1. Which physiological processes are most important for growth enhancement of ACPs? Which are primary and which are secondary? Is the positive growth effect caused by stimulation of cell divisions in shoot apices? What is the role of tissue N and P content in this growth effect?
2. What is the uptake affinity of shoots for mineral nutrients from water? Is shoot nutrient uptake from the ambient water stimulated by prey capture (analogously with terrestrial species)? What is the efficiency of mineral and organic nutrient uptake from prey?
3. Unlike terrestrial CPs but similarly to all submerged plants, ACPs do not reutilize K^+ in senescent shoots. What are the K^+ uptake characteristics of ACP shoots (localization of uptake, affinity, uptake rates) and are they similar to those in aquatic non-CPs?
4. In *Utricularia*, what is the role of the commensal community in the nutrient interactions within the plant having traps with or without prey? How is the trap exudation of C, N, and P into the trap fluid regulated and what is the role of the commensals for this regulation? The importance of phytoplankton, bacteria, and detritus as a potential nutrient source (N, P, K, Mg) for *Utricularia* in barren waters could be modelled and tested by analyzing trap fluid.

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Summary

About 60 species of the genera *Aldrovanda* (monotypic *A. vesiculosa,* waterwheel plant, Droseraceae) and *Utricularia* (bladderwort, Lentibulariaceae) are submerged aquatic or amphibious carnivorous plants. All aquatic carnivorous plants (ACPs; including also amphibious) are strictly rootless and take up mineral nutrients for their growth only via their trap-bearing shoots from the ambient water (or loose sediment) and captured prey. However, they fulfil all principal functional criteria generally placed on carnivorous plants (CPs). Traps of ACPs exhibit rapid movements, which are among the fastest within the plant kingdom, and represent fascinating objects for biological studies. Relative to terrestrial CPs, ACPs have always been understudied because of methodological problems or ignorance. Moreover, most ecophysiological studies on ACPs post-date the Juniper's *et al.* monograph of 1989. In this dissertation, ACPs are reviewed separately from their terrestrial counterparts. The ecology of habitats of ACPs and characteristics of their growth traits, photosynthesis, mineral nutrition, regulation of the investment in carnivory in *Utricularia,* peculiarities of *Utricularia* traps, and turion ecophysiology are reviewed here.

ACPs usually grow in shallow standing or slowly running humic (dystrophic) waters, commonly with a partly decomposed, nutrient-poor litter of reeds and sedges or *Sphagnum*-based peat on the bottom. The slowly decomposing litter gradually releases mineral nutrients, humic acids, tannins, and CO_2. The nutrient-poor waters can be usually rather rich in free $[CO_2]$ (0.1-1 mM), which is considered the primary factor supporting vigorous growth and propagation of ACPs.

Most ACPs have a linear, modular shoot structure consisting of nodes with filamentous leaves and tubular, fragile internodes. ACPs show very rapid apical, polar shoot growth but their basal shoot segments age and die at about the same rate ('conveyer-belt' shoot growth system). The new biomass is allocated only into branching or flowering. Under favorable conditions, the apical shoot growth rate of ACPs with linear shoots was 1.0-1.2 whorls/d in *Aldrovanda*, 1.2-4.2 nodes/d in field- or greenhouse-grown (0.9-4.4 nodes/d) *Utricularia* species. As 'Ruderal- or Competitive-strategists', their mean doubling time of biomass (ln 2/RGR) in *Aldrovanda* was only 8.5-28.7 d and similarly 4.9-40.5 d in six *Utricularia* species. Very rapid growth of ACPs, based on rapid apical shoot growth and frequent shoot branching in nutrient-poor but CO_2-rich habitats, requires ecophysiological adaptations that enable the plants to access the highly limited supplies of mineral nutrients from the water, and also very high photosynthesis. These adaptations include carnivory, efficient nutrient reutilization from senescing shoots, and a very efficient nutrient uptake from water.

Unlike their terrestrial counterparts, the maximum net photosynthetic rate (P_{max}) per unit dry (DW) or fresh weight (FW) in ACP species with linear shoots (40-160 mmol $O_2 \cdot kg^{-1}_{FW} \cdot h^{-1}$) is comparable to or even higher than the highest values found in submerged aquatic non-CPs. Surprisingly, relatively high values were found even in old leaves of *U. australis* and *U. vulgaris* without functional traps. Traps of aquatic *Utricularia* species are physiologically very active organs and have high dark respiration rates (RD). Simultaneously, they incur great photosynthetic (metabolic, energetic) costs due to very low P_{max} so that the RD:P_{max} ratio in *Utricularia* traps is very high (50-140 %).

Shoot mineral nutrient content in ACPs is similar to that in rooted aquatic non-CPs. A very efficient N and P reutilization from senescing shoot segments was found in rapidly growing ACP species. However, very low or negative K reutilization in senescent shoots of ACPs greatly contrasts with that in terrestrial CPs. Thus, ACPs permanently lose only a relatively small part of their N and P in their senescent shoots but almost all K, Ca, and Mg. Zero K reutilization in ACPs is probably common for all submerged aquatic plants but the reasons for are unknown. As shown in various feeding experiments, capture of prey in ACPs is one of the decisive factors for their rapid growth and propagation.

Utricularia suction traps are hermetically closed bladders 1-5 mm long functioning on the basis of negative pressure. Four types of glands occur inside or outside the traps and large internal quadrifid and bifid glands are crucial for trap physiology. The complete process of trap firing was found to last only 2.5-5 ms in several species. Recently, spontaneous trap firing without any mechanical stimulation has been discovered in many *Utricularia* species. Spontaneous firings indirectly support the physical (not electrophysiological) concept of *Utricularia* trap triggering. Moreover, water is pumped out from the traps permanently. Highly significant differences in firing and resetting rates of traps were found between various species. The biophysical diversification of *Utricularia* traps into three types of spontaneous firing and different trap sizes could contribute to a diversification of prey capture in environments with various prey availability.

Using microfluorimetric methods, only the activity of acid phosphatases was determined as significant in prey-free trap fluid in aquatic *Utricularia* species and was independent of prey capture; other enzymes were sucked from the ambient water. Furthermore, it is not clear which proportion of any enzyme activity in the filtered trap fluid is produced by trap glands alone or various trap commensals. In prey-free traps, a miniature microbial food web may develop. Its main components are bacteria, euglens, Dinophyta, ciliates, and rotifers acting as digestive mutualists. High amount of organic substances is exuded by the traps to the trap fluid to support the microbial commensals. Yet the nutritional role of these commensals to provide N and P for the plants is unknown but model calculations show that the commensals in prey-free traps might behave as parasites.

Zero O_2 concentration was consistently measured in the fluid of excised and intact prey-free traps of aquatic *Utricularia* species bathed in an oxygenated medium. Thus, the aerobic respiration of the inner glands and trap walls is so high that all O_2 is exhausted within 10-40 min after each firing. The extremely low $[O_2]$ in the trap fluid is a result of a functional compromise: it must be very low to reliably kill the captured prey (requiring ca. >15-30 μM) but higher than a threshold for an effective aerobic respiration of the glands (ca. >0.4 μM).

As an investment in carnivory, traps in ACP species incur costs in structure (organic biomass), photosynthesis (decrease of P_{max}), energy (consumption for trap operation, metabolism, and maintenance), and minerals (total amount of minerals in the traps or that lost in senescent traps). In aquatic *Utricularia* species, the proportion of traps to the total plant biomass is usually 10-65 %, but this structural cost is regulated flexibly to minimize all costs according to habitat factors, especially water chemistry, prey capture, and irradiance. Increased mineral nutrient availability usually led to decreased investment in carnivory in terms of trap number per leaf, mean trap size (weight), or proportion of trap biomass. Generally, all external nutrient factors, which decrease tissue N content in young shoots (poor prey capture, low $[NH_4^+]$, high $[CO_2]$, etc.), increase trap production in young shoots and *vice versa*, but photosynthesis is superior to the 'nutrient' regulation.

Turions are dormant vegetative winter buds formed by perennial aquatic plants as a response to unfavorable ecological conditions – mainly decreased temperature, daylength or nutrient availability. The majority of temperate ACP species form turions. Turions of ACPs as modified shoot apices are tough and sturdy green organs 1-25 mm large, formed by extreme

condensation of short modified trap-free leaves at the end of the growing season. They are partly frost resistant and protect fragile plant shoots from freezing. ACP turions can be hardened by weak frosts and their frost hardiness is based on the shift from frost avoidance in non-hardened turions to frost tolerance. By early spring, turions separate from dead mother shoots and rise to the water surface where they sprout. Two dormancy states (innate and imposed) and their hormonal patterns were described for ACP turions. Turions of ACPs are storage organs and in autumn, they accumulate starch, free sugars, reserve proteins and lipids. They also represent storage organs for mineral nutrients (N, P) though their storage function is presumably weaker. Their RD was 30-75 % lower per unit FW and 80-85 % lower per unit DW than adult shoots (leaves) of the same species. Ecophysiological traits of turions of ACPs are the same as those of turions of other aquatic non-CPs.

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