

Review

Ecophysiological characteristics of turions of aquatic plants: A review

Lubomír Adamec



Institute of Botany of the Czech Academy of Sciences, Section of Plant Ecology, Dukelská 135, CZ-379 82 Třeboň, Czech Republic

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ABSTRACT

Turions or winter/overwintering buds are vegetative, dormant storage organs formed by perennial aquatic plants. They are formed in subtropical to polar zones in response to unfavourable ecological conditions, usually at the beginning of autumn. In this way, fragile shoots of aquatic plants avoid freezing and damage. Turions are formed in at least 14 genera of aquatic vascular plants from nine plant families, mainly in submerged (or amphibious) and free-floating species. Turions are propagules formed by extreme condensation of apical stems (internodes) with modified attached leaves or scales which are dissimilar to summer leaves; they germinate and sprout after they separate from the mother plant. As opposed to pale subterranean rhizome apices, turions contain chlorophyll and sprouting turions are able to photosynthesize, which gives them an ecological advantage. Here I review various aspects and particulars of turion biology – structure, ecological significance, costs and benefits, turion formation, dormancy, germination, sprouting, metabolism and frost tolerance – from an ecophysiological point of view with an emphasis on ecological and biochemical developmental signals of turion formation and germination or sprouting. All turion forming genera and species are included except *Spirodela polyrhiza* because of the high ecophysiological specificity of this species. Unpublished data are also included. Most of the *Potamogeton crispus* turions have an inverted environmental regulation of their formation and sprouting as they sustain over the warm summer period in a dormant stage and sprout afterwards (aestivation).

1. Introduction

1.1. Aims and specifications of the review

Turions (Latin *turio* = shoot) or winter/overwintering buds are vegetative, dormant organs formed by perennial aquatic plants (Fig. 1). They are formed in subtropical to polar zones in response to unfavourable ecological conditions, usually at the beginning of autumn, and the fragile shoots of aquatic plants cannot be damaged by freezing (Glück, 1906; Sculthorpe, 1967; Weber and Noodén, 1976a; Sastroutomo, 1981; Bartley and Spence, 1987; Netherland, 1997; Adamec, 1999a; Appenroth, 2002). Turions are formed in at least 14 genera of aquatic vascular plants: *Ceratophyllum* (Ceratophyllaceae), *Brasenia* (Cabombaceae), *Aldrovanda* (Droseraceae), *Utricularia* (Lentibulariaceae), *Myriophyllum* (Haloragaceae), *Potamogeton* (Potamogetonaceae), *Caldesia* (Alismataceae), *Hydrocharis*, *Elodea*, *Hydrilla*, *Spirotes* (Hydrocharitaceae), *Spirodela*, *Lemna*, *Wolffia* (Araceae) from nine plant families, both in basal Angiosperms, Monocots and Eudicots (APG, 2016), mainly in submerged (or amphibious) and free-floating species (Glück, 1906; Sculthorpe, 1967). Thus, the origin of turions is typically polyphyletic, representing functional convergencies. Various

aspects of turion biology have been studied and published for many genera. These have mainly considered turion morphology and anatomy, their ecological significance, the ecophysiology of their formation, dormancy stages, breaking dormancy, germination and sprouting, as well as turion metabolism, biochemistry and management practices. This work has spanned the last 70 years, with a boom over the last 25 years. Nevertheless, the only general review on turion biology by Sculthorpe (1967) and on turion dormancy and ecophysiology by Bartley and Spence (1987) are evidently out of date, while Adamec (1999a) only partly reviewed the ecophysiology of the aquatic carnivorous plant turions of the genera *Aldrovanda* and *Utricularia*.

The aim of this paper is to review various aspects and particulars of turion biology – structure, ecological significance, costs and benefits, turion formation, dormancy, germination, sprouting and metabolism – from an ecophysiological point of view with an emphasis on the ecological and biochemical developmental signals of turion formation and germination or sprouting. Unpublished data are also included. As *Spirodela polyrhiza* (great duckweed) turions are physiologically very different from those of the other turion-forming genera, this species (together with other lemnids) has been omitted from this review and is only briefly mentioned in more general and comparative aspects

Abbreviations: ABA, abscisic acid; BA, benzyladenine; GA₃, gibberellic acid; IIA, indole-3-acetic acid; DMC, dry matter content; DW, dry weight; FW, fresh weight; P_N, net photosynthetic rate; RD, dark respiration rate; RH, relative air humidity

E-mail address: lubomir.adamec@ibot.cas.cz.

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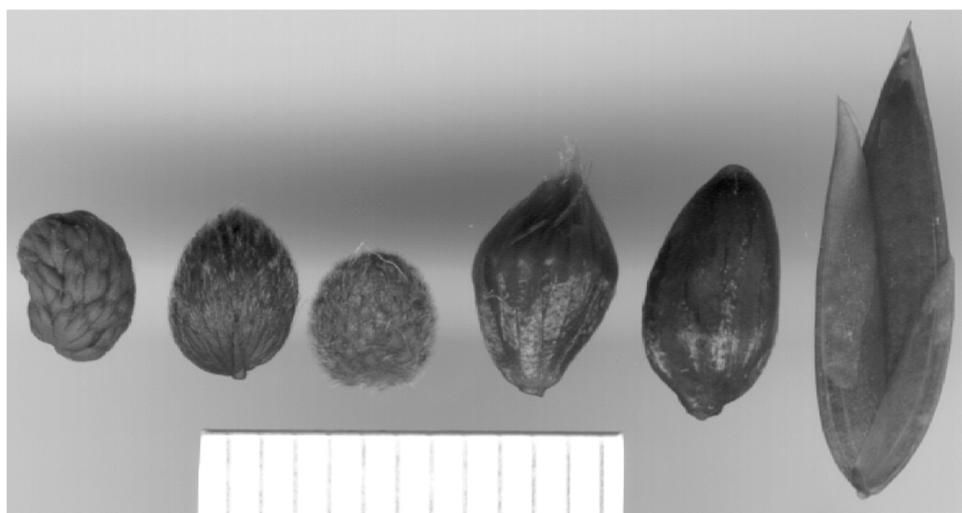


Fig. 1. Ripe dormant turions of several aquatic plants. From the left: *Utricularia bremii*, *U. australis*, *U. ochroleuca*, *Aldrovanda vesiculosa*, *Hydrocharis morsus-ranae* and *Caldesia parnassifolia*. Ticks indicate 1 mm.

Original: Adamec (2008a). Published with a permission of Springer.

(Section 3.1). In this species, turion formation strongly depends on mineral deficiency in the water and there is a strict dependence of turion germination and sprouting on the presence of mineral nitrogen in the medium. Methodologically, its biology has mostly been studied in sterile *in-vitro* cultures and comprehensive studies have been conducted over the last 30 years (see Malek and Cossins, 1983; Appenroth, 1993, 2002; Appenroth and Nickel, 2010). On the other hand, most of *Potamogeton crispus* turions have an inverted environmental regulation of their formation and sprouting as they sustain over the warm summer period in a dormant stage and sprout afterwards (aestivation; Sastroutomo, 1980, 1981; Kunii, 1982; Heuschele and Gleason, 2014). That is why they are dealt with in a separate section (Section 6).

1.2. Definition and specification of turions and their functions

Turions were first named and defined by Glück (1906) as vegetative buds functioning as overwintering organs (and also propagules) in many aquatic vascular plants from temperate to polar zones. Moreover, he defined three structural traits of turions: i) a short stem (internodes) with attached leaves or scales; ii) separation from the mother plant; iii) germination only in that turion that is separated from the mother plant. Later, Sculthorpe (1967, p. 346) emphasized that turions have modified leaves "which are specialised in form and quite unlike the normal foliage leaves". Comparing the dark respiration rate (RD) of true dormant turions and non-dormant winter apices of *Aldrovanda* populations (Fig. 2) and *Utricularia* species, Adamec (2008a, p. 519) emphasized that "the term *turion* should only be reserved for morphologically distinct organs which can enter the true dormancy". Yet, the decision about whether dormant shoot apices are true turions may not be clear and unambiguous as there is a continuum between less specialised types with dense clusters of little-modified apical leaves (e.g. *Ceratophyllum demersum*, *Elodea canadensis*) and typical, strongly condensed turions (see the discussion in Sculthorpe, 1967). The criteria for defining turions are thus partly arbitrary. Moreover, some authors unnecessarily described as turions the pale, apoplastic, dormant overwintering subterranean tubers of many aquatic plants (e.g. *Sagittaria sagittifolia*, *Potamogeton pectinatus*, *Hydrilla verticillata*) or pale, dormant, subterranean banana-shaped rhizome apices of many broad-leaved *Potamogeton* species; the latter are firmly connected to the parent plant by creeping rhizomes (see Sculthorpe, 1967; Spencer and Ksander, 1996; Wolfer et al., 2006). The latter authors described the overwintering organs of *Potamogeton perfoliatus* as "dormant, pale rhizome apices firmly connected with the mother plant, buried in the sediment".

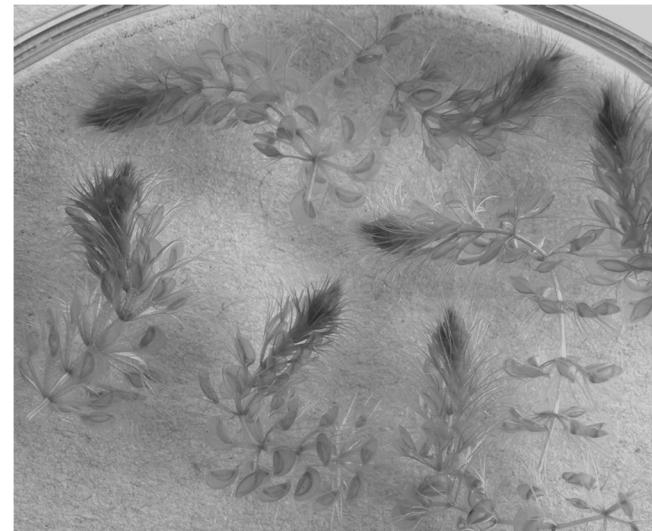


Fig. 2. Non-dormant winter apices of a subtropical population of *Aldrovanda vesiculosa* from SW Australia grown in an outdoor aquarium on 18 November. Note long and marked foliar bristles in the apex which are typical for growing summer leaves.

Therefore, the term "turion" should be used only for modified above-ground, detachable and green aquatic shoots (consisting of leaves and stems) capable of photosynthesis (Adamec, 2011). In addition to other characteristics, turions are also storage organs for carbohydrates (e.g. Winston and Gorham, 1979a; Ley et al., 1997; Adamec, 2003) and also for some mineral nutrients (Adamec, 2010, 2011).

The spring development of turions is associated with their germination and sprouting. However, both terms are commonly used quite ambiguously in the available literature: several authors (e.g. Sculthorpe, 1967; Weber and Noodén, 1976b) use the term "germination" *sensu lato* for the whole process of turion development including also the production of new organs as a phenomenon of new growth, while most authors (e.g. Bartley and Spence, 1987; Ley et al., 1997; Adamec, 2003, 2011) discriminate both terms *sensu stricto*. In this review, "germination" is understood as the initial developmental stage when turions only reflect their leaves or scales and elongate their internodes slightly, while "sprouting" denotes the subsequent stage of the resumption of growth of new organs (leaves, shoots or roots) combined with marked



Fig. 3. Ripening turion of *Potamogeton crispus* collected from a fishpond in the Czech Republic on 15 June. Turion length is ca. 3 cm.

internode enlargement (see Adamec, 2003, 2011).

2. Turion structure, functioning and ecology

2.1. Turion structure

Turions are always modified shoot apices and consist of modified, short leaves condensed on extremely shortened stems (Glück, 1906; Sculthorpe, 1967; Weber and Noodén, 1976b; Adamec, 1999a, 2003, 2008a, 2011). *Spirodela polyrhiza* and *Lemna turionifera* turions are flat and rounded (Appenroth, 1993). Turions are ca. 1–50 mm long, tough and sturdy organs being spherical, rhomboid, cone-shaped, or flattened and elongated in shape (Fig. 1). *Potamogeton crispus* turions are propeller-shaped (Fig. 3). In most species, turions originate in the apices of main shoots but commonly also in stem axils and branches (Sculthorpe, 1967). In *Stratiotes aloides*, turions are formed as offsets on short stolons, while in *Hydrocharis morsus-ranae*, they are formed as condensed organs at the apex of slender stolons and, similarly in *Caldesia parnassifolia*, they are formed on special turion-forming shoots or also on flower stems (Glück, 1906; Sculthorpe, 1967). In their cross section, turions have radial (*Ceratophyllum*, *Aldrovanda*, *Utricularia*, *Myriophyllum*, *Elodea*, *Hydrocharis*; Fig. 4) or bilateral (*Caldesia*, *Potamogeton*) symmetry and consist of rosettes of concentric short leaflets (or phylloclades in *Utricularia*) or bands of ribbon-like leaves (Glück, 1906; Sculthorpe, 1967; Adamec, 2003, 2011; Plachno et al., 2014). In aquatic carnivorous plants (*Aldrovanda*, *Utricularia* spp.), the turion leaflets contain only tiny, non-functional rudiments of traps (Adamec, 2011). At least in *Aldrovanda*, *Utricularia*, *Caldesia*, *Hydrocharis* and *Potamogeton*, turion leaflets (in some genera also turion stems) contain voluminous air spaces which can be arranged in air canals. In *Aldrovanda* (Fig. 5) and *Utricularia*, the turion leaflets contain one cell layered septae which divide the leaflet lamina into large, regular lacunae containing a fluid or air bubbles (Glück, 1906; Adamec, 2003; Plachno et al., 2014). In *Aldrovanda*, these lacunae are filled with a slimy reticulum stainable with Alcian blue with an unknown function (Adamec, 2003). In *Aldrovanda*, *Utricularia vulgaris* and *U. stygia* turion cells, the nucleus is the dominant organelle. The cytoplasm contains starch grains, many protein storage vacuoles ca. 1.5–8 µm large at various degrees of degradation, and numerous lipid bodies, while small numbers of mitochondria, rough ER and Golgi bodies (Plachno et al., 2014) indicate that turions are storage organs with low metabolism intensity.

2.2. Strategies of turion formation, sinking and floating

Turions of all aquatic plants usually overwinter and break their innate dormancy in warmer water at the bottom of aquatic habitats, mostly under anoxic conditions in darkness, to escape from being frozen and embedded in ice. Various aquatic plant species, however, differ greatly in their ecological strategy of turion overwintering and two principal strategies of formation (ripening) and germination/sprouting may be distinguished (Sculthorpe, 1967; Bartley and Spence, 1987; Adamec, 2008a, 2011). Turions of free-floating and submerged rootless species (e.g. *Aldrovanda*, *Utricularia*, *Lemna*, *Spirodela*, *Wolffia*, *Hydrocharis*) form, physiologically ripen, and usually also germinate and sprout at the water surface. In contrast, turions of submerged (amphibious) rooted species (e.g. *Myriophyllum*, *Caldesia*, *Potamogeton*, *Elodea*, *Hydrilla*) usually form at depth and also germinate, sprout, and root at the bottom.

Moreover, two different strategies of autumnal sinking and spring rising (floating up, buoyancy) of turions of free-floating and submerged rootless species can be distinguished (Sculthorpe, 1967; Adamec, 1999a, 2008a, 2011). Turions of *Aldrovanda* and *Hydrocharis* possess an active physiological mechanism of sinking and rising. Their ripe turions usually break from the dying mother shoots in autumn (an abscission layer is developed; Sculthorpe, 1967), float at the water surface a few days, and then sink gradually to the bottom. In spring as the water warms, after breaking the imposed dormancy, they rise to the water surface within a few days (Adamec, 1999a, 2003). The sinking and rising of turions in both species is caused partly by different starch contents in autumn vs. spring, but mainly by variable gas volumes in the large gas lacunae in turion leaflets. In dormant *Aldrovanda* turions overwintered at ca. 3 °C in a refrigerator, the proportion of gas volume in the lacunae was only < 10% (Fig. 5), while after breaking imposed dormancy (20 °C in darkness for 2 d), the proportion rose to 20–100%. Presumably, the source of this gas (CO₂) originates from leaf respiration or fermentation in CO₂-rich water (Adamec, 2003). The release of the gas in *Aldrovanda* turions is so conspicuous that originally dark-green turions change their colour to light silver-green (Adamec, unpubl. data). Active turion sinking and rising occurs also in *Spirodela polyrhiza*, but during the breaking of the imposed dormancy in light or darkness, an external air bubble forms on the “waxy” turion surface and pulls the turion to the surface (Newton et al., 1978). The gas in the bubble can contain a variable proportion of O₂ and CO₂ (Appenroth and Adamec, unpubl. res.).

In contrast, turions of temperate *Utricularia* species are usually less dense than water, are firmly connected to the mother shoots and dragged to the bottom as these gradually decompose (Sculthorpe, 1967; Adamec, 1999a, 2008a). By early spring, the stem connecting the turion with the mother shoot is usually decayed and the turions separate and rise to the surface. However, a significant proportion of ripe turions of *U. australis* and *U. minor* may rise to the surface as early as October or November and overwinter there (Adamec, unpubl. data). In *Utricularia minor* and *U. bremii*, antler-like branched leaves form a basket (1–2 cm in diameter) which encloses the developing turion. In autumn, after the mother shoot dies, it drags the turion to the bottom. Later, the turions break free but remain entangled in the apical baskets until these decompose (Adamec, 1999a). As the majority of turion-forming temperate aquatic plant species can also grow or survive as amphibious, semi-terrestrial plants on wet organic soils (Sculthorpe, 1967), their turions can also ripen and overwinter in a terrestrial ecophase. Therefore, turions of all temperate species occasionally face the stressful conditions of frost and drought during their overwintering and are partly adapted to both factors (Glück, 1906; Adamec, 1999a,b, 2008b; Adamec and Kučerová, 2013; Section 5).

However, there are common exceptions to the above strategies. Germinating *Myriophyllum verticillatum* turions can float up to the water surface in the spring (Weber and Noodén, 2005). This sometimes also happens with the turions of various *Potamogeton* species or *Caldesia*

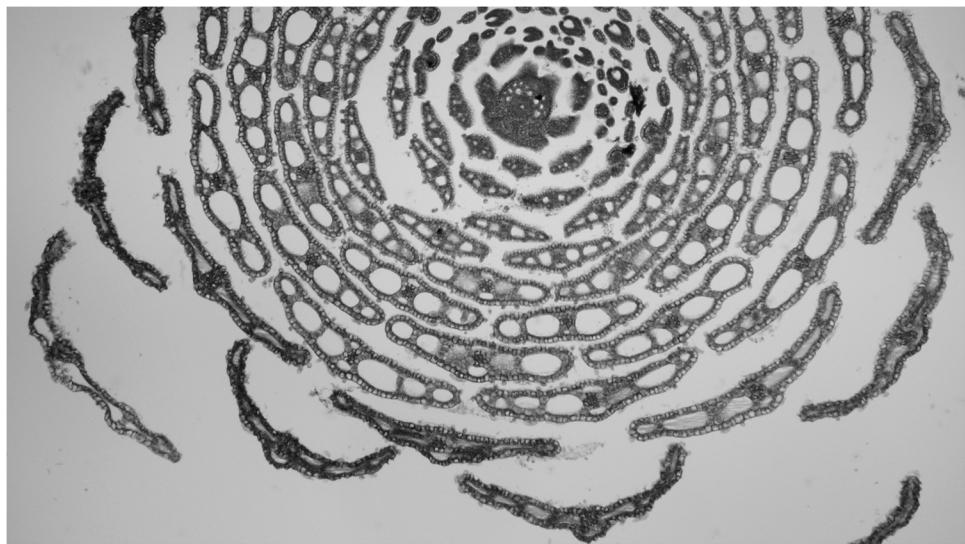


Fig. 4. Cross-section through an *Aldrovanda vesiculosa* turion, which overwintered in a refrigerator, at the end of March. Turion diameter is ca. 4 mm.

parnassifolia in outdoor cultures (Adamec, unpubl. data; Section 6.2). The ecological function of this behaviour is probably to spread the turions as propagules within the site. Another physiological anomaly can be found in some ripe *Aldrovanda* turions which do not sink to the bottom in outdoor cultures in autumn (see Sculthorpe, 1967; Adamec, unpubl. data) or do not rise after breaking their imposed dormancy (Adamec, 2003) or even not after germination or sprouting (Adamec, unpubl. data). As opposed to the general rule, a small proportion of ripe, separated *Utricularia* turions (especially *U. intermedia*, *U. ochroleuca* s.s., *U. stygia*) sink to the bottom in outdoor cultures in autumn or turions germinating at the surface give rise to sprouting plants which sink to the bottom transiently in the spring (especially *U. vulgaris*, *U. macrorhiza*; Adamec, unpubl. data). In line with Weber and Noodén (2005), it is possible to explain all these physiological anomalies of turions at any developmental stage as consequences of changed starch accumulation or gas evolution in air lacunae in turion leaves, which happens due to suboptimal ecological conditions occurring in semi-natural outdoor cultures (e.g. higher temperature, poor photosynthetic conditions of low light or CO₂) or during overwintering in a refrigerator.

2.3. Ecological costs and benefits of turion overwintering

Firstly, it is necessary to stress that the formation of partly frost-resistant turions is only one of several possible ecological overwintering strategies of aquatic (mostly submerged and free-floating) plants in temperate to polar zones. Other strategies include annuality with seed formation (e.g. *Najas*, *Zannichellia*, *Trapa*), the sinking of non-dormant rootless shoots to the bottom of water bodies in the autumn (*Utricularia inflata*, *U. purpurea*, *Lemna trisulca*), permanent growth at the bottom (isoëtids), the presence of dormant shoot buds near the bottom (e.g. *Myriophyllum spicatum*, *Persicaria amphibia*), a thick frost-resistant rhizome (e.g. nymphaeids, *Menyanthes trifoliata*, *Calla palustris*), or the production of dormant subterranean tubers or rhizome apices (*Potamogeton pectinatus*, *P. distinctus*, *P. lucens*, *Sagittaria sagittifolia*, *Hydrilla verticillata*; Sculthorpe, 1967; Moeller, 1980; Bartley and Spence, 1987). Of the aquatic plant species forming turions within the world flora, species with fragile, weak shoots without extensive mechanical tissues (*Utricularia* spp., Hydrocharitaceae), represent the greatest proportion of species number within the given temperate genus or family. For example, within the functional group of aquatic rootless

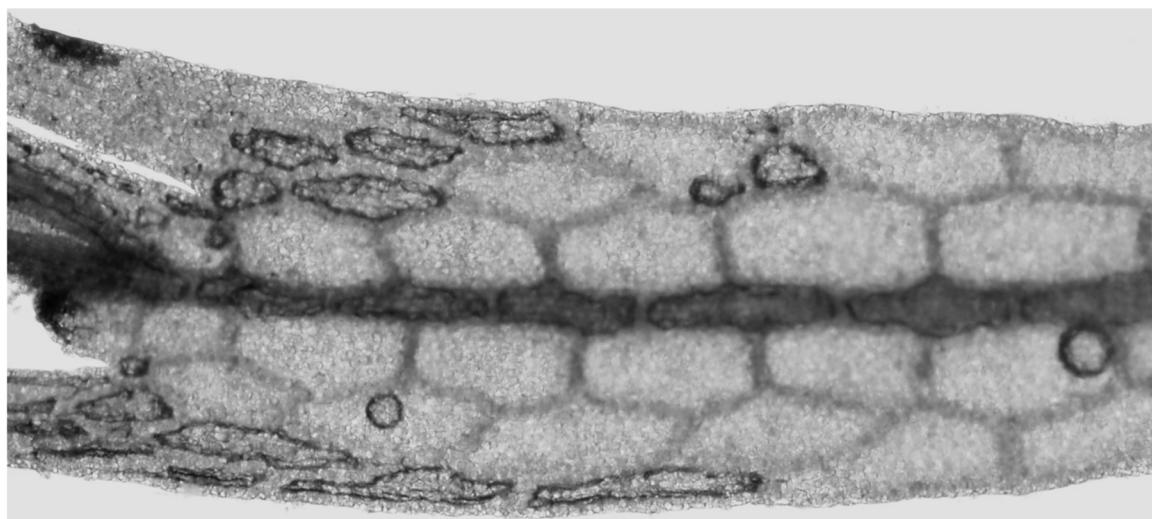


Fig. 5. Lateral view on the intact leaf of the 6th leaf whorl (from the turion base) of an *Aldrovanda vesiculosa* turion in mid-January. The turion was kept in a refrigerator at ca. 3 °C for two months. Small volume of gas bubbles (< 10% of lateral projection) occurs in leaf lacunae. Leaf width is ca. 1 mm. Note the rudimentary trap.

carnivorous plants (genera *Aldrovanda* and *Utricularia*) comprising a total of 17 temperate species (Taylor, 1989), 12 species (71%) form turions. However, species exhibiting huge distribution areas across several climatic zones may not form turions in their (sub)tropical populations. *Aldrovanda* forms dormant turions in its whole temperate Eurasian range but only some (sub)tropical Australian populations are able to form dormant turions; African plants from Botswana do not form turions (Adamec, 2008a; Elansary et al., 2010; Cross, 2013).

The ecological benefit gained by preventing turions from being embedded in ice at the surface by escaping to warmer water at the bottom is offset by unfavourable ecological conditions here. These conditions include hypoxia or anoxia (e.g. Adamec, 1999b, 2007) and very reduced irradiance or darkness as turions are often weakly covered by organic sediments (Adamec, 1999a,b). Ecophysiological adaptations to these unfavourable conditions and the negative impact of such overwintering on population density may represent ecological costs. A field study on *Aldrovanda* growing in nylon bottomless and open enclosures at experimental sites in the Czech Republic revealed that only 0–70% of autumnal ripening turions (mean ca. 20–30%) survived and sprouted in the next season (Adamec, 1999b). At some sites, many turions were grazed by ducks before sinking. When turions overwintered on the top of wet soils they were partly grazed by small rodents so that turion survival was much higher (35–100%) when they overwintered under water at the same microsites. I have repeatedly observed at several artificial *Aldrovanda* sites in the Czech Republic that herbivorous water birds do not graze adult *Aldrovanda* plants but their grazing is focused on reserve-rich turions (Adamec, unpubl. data). In preliminary experiments in closed nylon cages at natural *Aldrovanda* sites in Eastern Poland, only 0–20% of *Aldrovanda* turions overwintered (Kamiński, unpubl. data). Additionally, germinating and sprouting turions of *Aldrovanda* and other species with floating turions can become entangled at the bottom and die (Adamec, 1999b, unpubl. data).

In conclusion, turion overwintering may be the most critical and ecologically vulnerable phase of the seasonal growing cycle of many aquatic plants. To compensate for these overwintering losses, perennial aquatic plants have commonly adopted the growth strategy of rapid vegetative growth and propagation (by branching) in order to produce as many turions as possible, which simultaneously serve as propagules for the colonization of new sites mainly by water birds and hydrochory (Sculthorpe, 1967; Adamec, 1999b; Santamaría, 2002). A considerable ecological benefit of turions is also based on the fact that turions contain chlorophyll and sprouting turions exhibit high photosynthetic rates under favourable conditions (Adamec, 2011; Section 4.2). This trait contributes to the rapid growth of sprouting turions and the production of high standing biomass at the onset of the growing season in aquatic habitats. This mainly applies in warmer water at the surface, and can represent a great phenological competitive advantage over other aquatic plants sharing other overwintering strategies. Judging from the huge distribution of several temperate but sterile aquatic plant species forming detachable turions (e.g. *Utricularia australis*, *Myriophyllum verticillatum*, *Hydrocharis morsus-ranae*, *Elodea* spp., *Spirodela polyrhiza*), such an overwintering strategy is ecologically successful and its ecological benefit exceeds the costs. Moreover, as found in *U. vulgaris*, larger turions give rise to larger adult plants (Otto, 1999). In the Czech Republic the common and sterile *U. australis* has been transferred to newly-formed sites over a distance of a few km during a period of only a few years, probably as small turions carried by water birds (Adamec, unpubl.).

Several studies have compared the ecological characteristics of subterranean tubers and true turions among different aquatic species or in *Hydrilla verticillata* as this species forms both types of overwintering organ. Spencer and Ksander (1991) compared the growth characteristics of plants arising from subterranean tubers and axillary turions of *H. verticillata* in culture. After 8–12 weeks growth under similar conditions, plants grown from tubers weighed 1.7–2 times more and produced more new propagules (by number and weight) and roots per

plant than those grown from turions. Similarly, greenhouse-grown monoecious *H. verticillata* plants arising from turions were weaker competitors than those raised from tubers, when grown together with the widely distributed *Potamogeton pectinatus* (Spencer and Rejmánek, 1989). Nevertheless, the results of outdoor-growth experiments revealed that *H. verticillata* plants from turions were able to grow successfully in a macrophyte bed overgrown by *P. pectinatus* or *P. gramineus*. Thus, during the early stages of *H. verticillata* colonisation of native macrophyte beds, existing plants may co-exist with *H. verticillata*. However, after the abundance of subterranean *H. verticillata* tubers has increased at a given site, the native plants may be outcompeted.

2.4. Turion longevity

Turion longevity and hypothetical functioning as a propagule bank for several seasons are important ecological aspects of turion functioning at natural sites. Although all turions are dormant storage organs (e.g. Winston and Gorham, 1979a; Ley et al., 1997; Adamec, 2003) and overwinter at low temperatures at very low dark respiration rates (RD; Adamec, 2003, 2008a), they gradually exhaust their carbohydrate reserves (Sections 4.1 and 4.2) and their longevity is thus limited. The exhaustion of turion reserves might be accelerated by high summer temperatures at the water-sediment interface or turion survival might be impaired by sediment anoxia or a low redox potential during the summer. In an invaded river habitat in Ontario, Canada, overwintering *Stratiotes* turions could not survive in the sediment for longer than 8–9 months (Weissflog and Sager, 2016). In natural Czech sites, Adamec (unpubl. res.) has never found live turions of aquatic plants formed during the previous season at the end of the present season (August, September) – they had either sprouted or decayed. This finding suggests that unlike seeds, turions under natural conditions can only survive from one season to the next and not longer. Under constant and optimal overwintering conditions in a refrigerator (ca. $3 \pm 1^\circ\text{C}$, darkness, hypoxic medium), ripe culture- or field-raised turions of *Aldrovanda*, *Hydrocharis*, *U. minor* and *U. intermedia* were usually able to survive only 7–8 months and germinated; those of *Caldesia*, *U. vulgaris*, *U. macrorhiza*, *U. ochroleuca* and *U. stygia* up to 8–12 months, while those of *U. tenuicaulis* at least 16–18 months and *U. australis* even 23 months (Adamec, 2008b; unpubl. res.). The longevity of overwintered (“brown”) *Potamogeton crispus* turions under different conditions can be 12 months to five years (Sastroutomo, 1981; Heuschele and Gleason, 2014). As opposed to the limited longevity of true turions, Netherland (1997) reports that subterranean tubers of *Hydrilla* can remain quiescent in undisturbed sediments for up to four years.

3. Developmental ecophysiology of turions

3.1. Ecological and experimental conditions of turion formation

In spite of extensive knowledge on turion biology, the exact ecological conditions for turion formation for most of the turion-forming species (except for *Spirodela polyrhiza* and *Potamogeton crispus*) are as yet unknown (cf. Bartley and Spence, 1987). In *S. polyrhiza*, unlike all other aquatic species, turion formation depends strictly (in addition to climatic factors) on the shortage of key mineral nutrients in the media, namely sulphate, nitrate (ammonium) or phosphate. Other turion-inducing factors include low temperature, high irradiance of PAR and exogenously applied sugars or abscisic acid (Malek and Cossins, 1983; Bartley and Spence, 1987; Appenroth, 1993, 2002; Appenroth and Nickel, 2010). High irradiance plays no photomorphogenic role in the turion induction, but acts through abundant photosynthates. However, turion formation was not dependent on the N or P contents alone in mother fronds or newly developing turions at the onset of turion formation as an endogenous inducing factor, either (Appenroth and Adamec, 2015). Thus, in *S. polyrhiza*, turion formation is mainly induced by low external phosphate concentrations, which are highly

clone dependent partly within the latitudinal gradient.

Turion induction in most species of temperate aquatic plants is commonly associated with shortening days and decreasing water temperature (Weber and Noodén, 1976a; Winston and Gorham, 1979a; Bartley and Spence, 1987; Adamec, 1999a). The relative importance of these two turion-inducing factors probably differs in different species. From the many ecological studies of turion induction under natural conditions (e.g. Winston and Gorham, 1979a; Adamec, 1999a), it is still not possible to determine whether turions are formed in response to low temperatures, short days, or an interaction. Laboratory studies separating both factors are thus necessary to answer this question. In *Myriophyllum verticillatum*, turions were induced to form only at 15 °C or lower during short days (8 and 12 h light), but not during long days (16 h light) nor at 20 °C (Weber and Noodén, 1976a). In this species, turion formation is thus induced by the combination of temperature and day length. Treatment of plants with abscisic acid (ABA; 10 µM) enhanced turion formation under weakly inductive conditions but could not induce it during long days. The cytokinin benzyladenine (BA; 10 µM) completely blocked turion formation, while gibberellic acid (GA₃; 10 µM) only slowed down turion formation; indole-3-acetic acid (IAA; 10 µM) retarded turion formation but also caused plant deterioration and decay. As determined through bioassays, the endogenous level of ABA markedly increased during turion development while cytokinin levels fluctuated. The influence of short days on the formation of turions was also proven in *Hydrocharis morsus-ranae* (Vegis, 1953), *Hydrilla verticillata* (Klaine and Ward, 1984) and *U. macrorhiza* (Winston and Gorham, 1979a). In three populations of *Hydrilla*, turion formation occurred both during short (10 h) and long days (16 h), but was more efficient at short days (Steward, 2000). Turion formation thus appears to be mainly regulated by low temperature, but an interaction with day length may also be important.

Additional knowledge of environmental turion-inducing factors has been deduced from studying the overwintering of the aquatic carnivorous genera *Aldrovanda* and *Utricularia* in the outdoor collection at the Institute of Botany CAS at Třeboň, Czech Republic (Adamec, unpubl. res.). Temperate accessions of *Aldrovanda* grown outdoors under similar conditions in 3 L miniaquaria differ distinctly in the time of turion induction. The most northern accessions (from N and NW Russia and N Lithuania) regularly start turion formation about 3–5 weeks earlier (beginning of August) than the more southern accessions (e.g. Poland, Germany, Romania, Japan), and fully ripen about one month earlier (Elansary et al., 2010). A similar trait has been confirmed for northern accessions of *Utricularia intermedia*. The timing of turion induction in *Aldrovanda* is thus under genetic control and is, at least partly, latitudinally (climatically) dependent. It has been observed during a period of over 20 years that a discrete accession begins turion formation in single seasons sooner or later, within ca. a 4–5 week period. This means that turion induction in *Aldrovanda* depends on discrete climatic conditions (water temperature and its fluctuations, irradiance) much more than on day length alone. In October, Eastern Polish *Aldrovanda* also formed turions in a heated greenhouse where the water temperature never fell below 18 °C. Turions of this accession were induced in colder dystrophic (humic) shallow waters in the Třeboň region about three weeks earlier (late August–early September; Adamec, 1999a,b) than in plants growing in the relatively warm outdoor culture (Adamec, unpubl.). When *Aldrovanda* was growing from turions on a window ledge in a heated room, normal turions were also repeatedly formed in March–May after a marked cooling combined with cloudy weather or after a transfer of the plants outdoors to cold water, even when the day length was increasing. In summary, turions in *Aldrovanda* and temperate *Utricularia* spp. are induced after a long-term (ca. one week), marked relative temperature fall (e.g. from 28 to 20 °C) rather than after reaching some discrete low threshold temperature. This type of regulation would explain why turion induction is stimulated by high temperatures preceding the induction, which then occurs at lower temperatures (Weber and Noodén, 1976a; Bartley and Spence,

1987). Apparently, the simultaneous decrease in irradiance (or daily sum of irradiance) during colder and cloudy days amplifies the effect of temperature fall on turion induction in aquatic carnivorous plants.

The induction of turions in aquatic carnivorous plants can occur at relatively high water temperatures of 18–22 °C under long-day conditions as early as late July or early August (Winston and Gorham, 1979a). Yet, full physiological turion ripening (maturation) requires continuously low temperatures of 8–10 °C or lower, low irradiance and possibly also short days (Adamec, 1999a). Morphologically, turion ripening results in increasing the size of turions, markedly shortening the internodes, thus making the turion structure very condensed and compact (Glück, 1906; Sculthorpe, 1967; Adamec, 2003). Simultaneously, the mother shoots gradually acropetally senesce and decay so that their length is reduced. In *Aldrovanda* and *Utricularia* spp., ripe turion size relates to the size and robustness of the growing shoot apex: larger shoot apices give rise to larger turions (Adamec, unpubl.). Physiologically, turion ripening is associated with the massive and effective translocation (reutilization) of the majority of N and P (> 90% in *Aldrovanda*) and storage of organic substances (carbohydrates) from the senescing mother shoots (Winston and Gorham, 1979a; Adamec, 2000, 2003). Apparently, effective nutrient reutilization is induced by low temperatures of < 8 °C. As turions ripen they become more compact, contain more storage substances and increase their dry weight (DW) to fresh weight (FW) ratio (dry matter content, DMC; Section 4.1).

In several aquatic species (e.g. *Aldrovanda*, *Utricularia* spp., *Myriophyllum*, *Hydrocharis*, *Caldesia*, *Potamogeton* spp.) an abscission (dehiscence) layer is developed on the base of ripe turions (Sculthorpe, 1967), while the connection between turion and mother shoot is rather firm in other species and is broken only after decay of the stem over winter. When induced turions of *Aldrovanda* ripen at relatively high temperatures (> 15 °C) their shoots do not decay over the winter season and stay firmly connected to turions; the abscission layer is not developed (Adamec, unpubl.; cf. also Sculthorpe, 1967). All temperate *Aldrovanda* accessions are able to form abscission layers under suitable conditions but Australian (sub)tropical accessions forming turions are not. When *Aldrovanda* with induced turions grows under conditions of low CO₂, their ripening turions are slender due to the low storage of photosynthates (Adamec, unpubl.); full turion ripening thus requires high net photosynthetic rates (P_n) in induced plants. The full ripening of turions of aquatic carnivorous plants, which commonly grow in dystrophic waters, is also partly controlled by humic acids. In *Aldrovanda* shoots, humic acids in the water stimulate ripening, abscission and sinking of turions (Kamiński, 1987; see also Adamec, 1999b) and a similar effect was also described for *U. vulgaris* (Kosiba, 1992).

3.2. Dormancy stages

True dormancy is defined as “a state in which growth or normal growth cannot be resumed whatever the external conditions may be” (Vegis, 1964). However, some overwintering temperate aquatic plants are not truly dormant (e.g. *Elatine canadensis*, *Utricularia radiata*, *U. purpurea*), but quiescent: their winter vegetative growth is inhibited only by unfavourable environmental factors such as low temperature, short days and low irradiance (Bartley and Spence, 1987; Adamec, 2008a). These species usually do not form morphologically distinct turions but only little-modified “non-dormant winter apices” which are not detached from the mother shoots (Adamec, 2008a). Yet the boundary between both types may be unclear and continuous.

Using bioassays, two main dormancy stages and their hormonal regulation were thoroughly described for turions of Canadian *Utricularia macrorhiza* (Winston and Gorham, 1979a,b). At the end of the summer season, ripening turions enter a stage of innate dormancy when their growth is blocked by endogenous factors in the turions. In a stage of innate dormancy, they cannot germinate even at high temperature. Their innate dormancy lasts ca. two months. High endogenous levels of ABA induce turion formation and in the stage of innate

dormancy, are connected with low levels of free gibberellins, auxin and cytokinins (Winston and Gorham, 1979b). Innate dormancy could only be broken by the combination of low temperatures and short days at the end of October. Then, the turions entered a stage of imposed dormancy: turion germination depends only on higher temperature (Winston and Gorham, 1979a). Imposed dormancy is associated with a decreasing level of ABA and increasing levels of gibberellins, auxin and cytokinins. Low levels of ABA and high levels of gibberellins, auxin and cytokinins were found during the later phase of breaking the imposed dormancy when the first turions germinated (Winston and Gorham, 1979b). In the following stage, germinated turions start sprouting new shoots and this occurs under conditions of sufficient temperature and light. These two dormancy stages were also discovered in turions of rootless *Aldrovanda*: innate dormancy of ripe turions was broken after at least two months of cold treatment at 4 °C (Adamec, 2003). Similar patterns and actions of phytohormones with the central role of ABA accumulation were also confirmed in overwintering dormant turions of *Myriophyllum verticillatum* (Weber and Noodén, 1976a). In *M. oguraense* turions collected from the field in December, innate dormancy was completely broken after 6 weeks of cold treatment at 3 to 5 °C in darkness (Kadono, 1988). Field-raised turions of *Ceratophyllum demersum* entered their innate dormancy at the end of October and 4–8 weeks of cold treatment under short days were necessary to break it (Best, 1979; Best and Meulemans, 1979). In the turions collected from the field at the beginning of the innate dormancy, the turion IAA content was very low and reached only ca. 12–25% of the summer values, while the ABA content increased 4–8 times simultaneously (Best, 1979). The contents of both IAA and ABA strongly correlated (IAA positively, ABA negatively) with the elongation growth rate of shoots. The addition of GA₃ (0.1 mM) to innately dormant turions fully broke their dormancy in terms of stem elongation.

Simura et al. (2016) compared different cytokinin and auxin types in shoot segments and ripe autumnal turions (innate dormancy) of *Aldrovanda* and *U. australis*. In *Aldrovanda* turions, the proportion of the molar contents per DW of four cytokinin types (*trans*-zeatin, *cis*-zeatin, dihydrozeatin, isopentenyladenine) was very similar to that found in growing shoot apices, with *trans*-zeatin and dihydrozeatin as the prevailing types. This proportion was quite different in *U. australis* turions, in which isopentenyladenine strongly dominated over the other three cytokinin types and the turion cytokinin pattern was very similar to that in medium-aged and old shoot segments. The cytokinin metabolites ribosides, ribotides and O-glucosides dominated in *Aldrovanda* turions, while only 9-glucosides (as a biologically inactive form) dominated markedly (> 95% of all glucosides) in *U. australis*. The content of biosynthetic cytokinin precursors (ribotides) in turions was 1–2 orders of magnitude higher in *Aldrovanda* than in *U. australis*. The molar content of IAA metabolites in turions was several times higher in *U. australis* than *Aldrovanda*. The content of IAA metabolites in turions of *Aldrovanda* was several times lower than that in shoot segments, while their turion content in *U. australis* was comparable with that in shoot segments. The marked difference in phytohormone profiles in ripe turions between both species could partly reflect the differences in physiological shoot polarity of both species and the great taxonomic difference (Nepenthales vs. Lamiales).

3.3. Breaking imposed dormancy and turion germination

Turion germination represents the first morphologically and physiologically distinguishable developmental process following the break of imposed dormancy and it precedes turion sprouting as the resumption of vegetative growth (Bartley and Spence, 1987; Adamec, 2003, 2011; see Section 1.2). Under natural ecological conditions in the spring, the breaking of imposed dormancy is associated with a combination of increasing water temperature and day length. However, in experimental studies, the breaking of imposed dormancy is usually conducted by transferring turions from cold and dark conditions to a

higher temperature at a certain photoperiod or in darkness (Weber and Noodén, 1976b; Winston and Gorham, 1979a; Bartley and Spence, 1987; Kadono, 1988; Appenroth, 1993; Adamec, 2003). Judging from some laboratory studies in growth chambers, the process of breaking imposed dormancy is relatively rapid: the first symptoms of turion germination or even sprouting can occur as early as two days from the onset of favourable conditions. As overwintering turions are usually gently covered by a layer of organic sediment at the bottom, light may not play a strong role for breaking imposed dormancy, while the effect of temperature increase is crucial (Newton et al., 1978; Winston and Gorham, 1979a; Kadono, 1988; Adamec, 1999a). Ecophysiological and phenological studies (e.g. Winston and Gorham, 1979a; Adamec, 1999a,b, 2003) indicate that in various temperate aquatic species, the critical temperature for breaking imposed dormancy lies between 10 to 15 °C and is about 12 °C in *Aldrovanda* turions. Low levels of ABA and high levels of the other three stimulatory phytohormones (auxins, gibberellins, cytokinins) correspond to this phase in *U. macrorhiza* (Winston and Gorham, 1979b).

In species with active regulation of autumnal turion sinking and spring rising (*Aldrovanda*, *Hydrocharis*, *Spirodela*), turion rising represents an important physiological marker of breaking imposed dormancy but is not considered a strict physiological prerequisite for turion germination (Adamec, 2003; Section 2.2). Under natural conditions, *Aldrovanda* turions almost always rise before they germinate and sprout; turions entangled on the bottom under unfavourable conditions would rot and die (Adamec, 1999a,b). Turion rising is thus of crucial ecological importance.

The exact character of the physiological processes occurring in turions during the breaking of imposed dormancy is still a mystery (cf. e.g. Winston and Gorham, 1979a,b; Bartley and Spence, 1987; Ley et al., 1997; Adamec, 1999a, 2003, 2008a). In a limited number of physiological studies, no expected significant changes either in energy metabolism (aerobic dark respiration rate, RD) or free sugar content were described (Adamec, 2003, 2008a) as attributes of this phase. Therefore, the only changes found during this phase are phytohormone changes (Winston and Gorham, 1979b; see above) leading both to complete abolition of growth inhibition and the subsequent initiation of the sprouting of new organs under favourable conditions. When *Aldrovanda* turions were breaking the imposed dormancy, no change in turion RD or anaerobic fermentation rate (in N₂) was measured during the first 48 h after their transfer to media at 20 °C in darkness, although turions started rising after 24 h (Adamec, 2003). During imposed dormancy, turion RD was only 13–22% higher than that in innate dormancy but anaerobic fermentation rates in innate dormancy were around two times higher (Section 4.2). The necessity of aerobic energy metabolism for turion rising and germination was however proven, as an aerobic respiration inhibitor (NaN₃) or N₂ atmosphere completely inhibited both processes at 20 °C in darkness. In continuous light, however, both processes were unchanged. Moreover, the content of saccharides (starch, glucose, fructose, sucrose) in *Aldrovanda* and *U. vulgaris* was remarkably constant during the first 48 h of breaking the imposed dormancy (Adamec, 2003). It may be concluded that no specific metabolic changes occur during the breaking period as a metabolic signal. The observed physiological changes in turions (evolution of a gas into internal gas spaces in *Aldrovanda*; Adamec, 2003; Section 2.2) are attributable only to markedly increased temperature and gently increased temperature quotient (Q₁₀) of RD (see also Weber and Noodén, 2005). The rapid decrease of starch content and metabolism increase preceding germination and sprouting in *Spirodela* turions (Ley et al., 1997) cannot be interpreted as primary regulatory steps but as a facilitation of the availability of soluble sugars for subsequent turion sprouting.

3.4. Turion sprouting

Under favourable conditions, germinated turions continue to resume their vegetative growth and sprout. As shown in many studies for

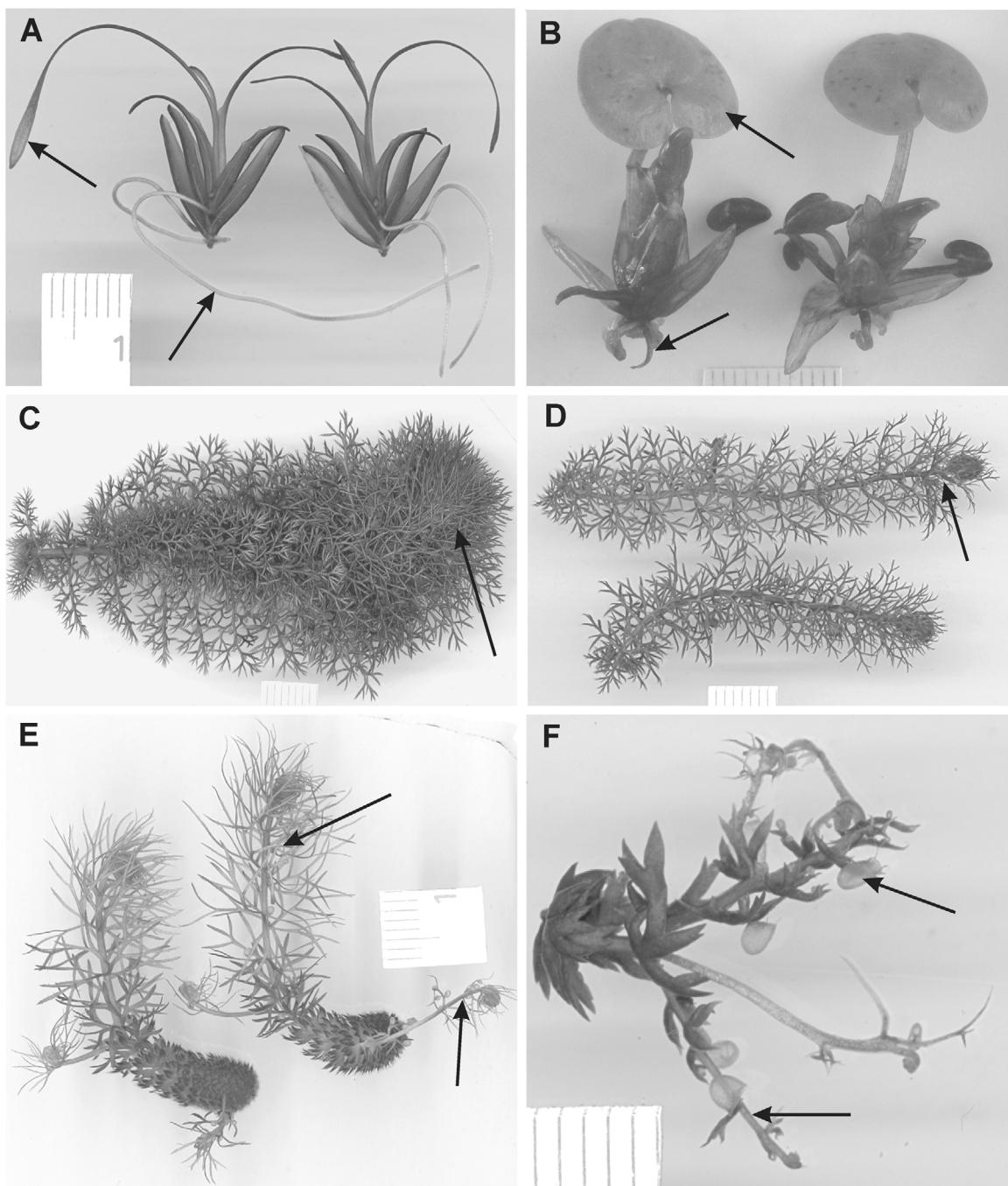


Fig. 6. Turions sprouting at 20 °C at 14 h photoperiod in a N- and P-free medium. A, *Caldesia parnassifolia* (20 d), B, *Hydrocharis morsus-ranae* (9 d), C, *Utricularia vulgaris* (10 d), D, *U. australis* (8 d), E, *U. stygia* (10 d), F, *U. bremii* (15 d). Arrows indicate the newly sprouting organs. Ticks in the scale, 1 mm. Original: Adamec (2011). Published with a permission of Schweizerbart Science Publishers.

several species, the first step of turion sprouting is associated with a marked elongation of very short internodes. This results in the turions becoming much less compact and their leaves are then capable of better gas and solute exchange with the ambient water and better light interception (e.g. Glück, 1906; Sculthorpe, 1967; Weber and Noodén, 1976b, 2005; Bartley and Spence, 1987; Kamiński, 1987; Kadono, 1988; Adamec, 1999a, 2011). *Aldrovanda*, *M. verticillatum* and *M. oguraense* turions sprout acropetally, while those of *Utricularia* spp., *Caldesia* and *Hydrocharis* basipetally (cf. Weber and Noodén, 1976b; Kadono, 1988; Adamec, 1999a, 2011). The next stage in turion sprouting is the formation of new organs – leaves and stems – in the apical meristems (Fig. 6). Newly-formed leaves possess the summer character and those of *Aldrovanda* and *Utricularia* spp. contain small

functional traps (Sculthorpe, 1967; Adamec, 2011). Increased temperature of ca. > 12 °C and light are the key ecological requirements for turion sprouting in several species. Although turion sprouting, at least in several species, is photoperiodically regulated and stimulated during long days, this regulation is obviously not strict but is rather of a quantitative nature (Weber and Noodén, 1976b; Bartley and Spence, 1987). Temperature increase thus appears to be the major factor under natural conditions.

Generally, great differences in light and temperature requirements for turion sprouting exist between various temperate species. Turions of *Aldrovanda*, *U. australis* and *M. oguraense* sprout strictly at higher temperatures and in light only, while those of *U. vulgaris*, *U. macrorhiza*, *U. minor*, *U. bremii*, *U. intermedia*, *U. ochroleuca* and *M. verticillatum* start

sprouting even in the refrigerator at ca. 4 °C in darkness (Weber and Noodén, 1976b; Kadono, 1988; Adamec, 1999a). In *M. oguraense* turions, however, adventitious root formation did not require a cold pre-treatment to break the innate dormancy (unlike leaf reflexing and the opening of leaf segments), and the roots were formed at 20 °C both in darkness and light. Thus, sprouting of these turions involves differently regulated steps. In monoecious *Hydrilla verticillata* in a Californian lake, Spencer and Ksander (2001) determined accumulated degree-day based equations which predicted the dependence of sprouting of axillary turions and subterranean tubers on measured water and sediment temperatures.

Turion sprouting is endogenously regulated by phytohormones. Externally applied GA₃ (10 µM) or cytokinins (e.g. 1 µM BA) to non-dormant *M. verticillatum* turions under weak sprouting conditions markedly stimulated turion stem elongation and led to normal sprouting, while ABA addition (10 µM) partly retarded the elongation of control and GA₃-treated turions (Weber and Noodén, 1976b). IAA addition (10 µM) had no effect. The activity of endogenous ABA-like substances (by bioassay) was about the same in innately dormant and sprouting turions but the activity of an unidentified acidic inhibitor greatly decreased in sprouting turions. A short application (4 h) of BA, GA₃ or naphthalacetic acid (at 0.15–0.75 mM) to dormant turions of *Potamogeton crispus* rapidly broke turion dormancy and promoted starch degradation (Zhu et al., 2015). Out of these substances, benzyladenine most strongly stimulated the subsequent turion sprouting.

Turion sprouting is also regulated by humic acids. In *Aldrovanda* turions under imposed dormancy, humic acids mildly inhibit sunken turions from rising by changing their density but also enhance the growth of already sprouting turions (Kamiński, 1987). Humic acids (and maybe also tannins) in waters thus play a role as exogenous growth substances and optimise overwintering of *Aldrovanda* turions. High chlorophyll content in dormant turions (Adamec, 2000) contributes to high P_N values in sprouting turions under favourable conditions without the need of *de novo* synthesis (Adamec, 2011; Section 4.2) and is a prerequisite for the high growth rate of sprouting turions (Section 2.3). Turions of many species sprout and root strictly at the bottom in colder water and shade (e.g. *Caldesia*, *Elodea* spp., *Potamogeton* spp.). These conditions unfavourable for growth are offset by the ability to take up mineral nutrients by developing roots. In turions of *U. vulgaris*, no significant changes occurred in the glycolipid classes during sprouting but the ratio of unsaturated to saturated fatty acids in some glycolipid classes was increasing (Simola et al., 1985).

The sprouting of turions of rooted species and their emergence from sediment is also influenced by turion size and ecological factors such as burial depth and water depth, which can act through water temperature and light penetration. In an outdoor study on *Myriophyllum oguraense* turions of different sizes buried in lake sediments of different depths and at different water depths, the percentage at emergence, shoot height and total plant biomass strongly correlated with turion size (Zhou and Wang, 2012). The emergence time was significantly shorter in larger turions but longer at increasing water depths. Large turions and shallow water thus favor the early establishment and survival of the immature plants. In a similar study (Zhan et al., 2010), the percentage at emergence was strongly affected by both burial (0–6 cm) and water depth (0–60 cm): turion sprouting was highest at zero burial depth and decreased with increasing burial depth at each water depth. In deeper water, plant height, total biomass and root:shoot biomass ratio decreased. Sediment accumulation and water-level fluctuations might thus disturb the natural turion sprouting of *M. oguraense*, which in turn could influence its abundance and population dynamics (cf. Section 6.2).

4. Turion metabolism and storage substances

4.1. Storage substances

Turions are overwintering storage organs and when they ripen they accumulate starch, free sugars, reserve proteins and lipids (Winston and Gorham, 1979a; Vintéjoux, 1984; Ley et al., 1997; Adamec, 2003; Weber and Noodén, 2005; Płachno et al., 2014). Starch is always the main storage substance and in ripe, innately dormant turions, its content can be ca. 16–70% of DW or 5–8.4% of FW. Positive correlation between starch content and the dry matter content (DMC; DW/FW ratio) or turion density was found in overwintering turions (Winston and Gorham, 1979a; Adamec, 2003; Weber and Noodén, 2005). The DMC in ripe turions is ca. 2.5–4 times higher when compared to that in growing summer shoots or newly sprouting turions of the same species (thus, their water content decreased ca. 1.5 times; cf. Winston and Gorham, 1979a; Adamec, 2003, 2008a, 2011). As a result of turion energy metabolism – aerobic respiration and/or anaerobic fermentation – in cold water, both in the field and in a refrigerator during overwintering, the starch content in turions gradually declines and can amount to only ca. 4.1–37% of DW at the end of the imposed dormancy (i.e., ca. 16–52% of the initial starch content; Winston and Gorham, 1979a; Ley et al., 1997; Adamec, 2003; Weber and Noodén, 2005; but cf. Heuschele and Gleason, 2014; Section 6.1). However, the total content of three main free sugars (glucose, fructose, sucrose) in *Aldrovanda* turions in the innate dormancy (ca. 14% DW) was the same both in the imposed dormancy and/or in mature summer shoot segments of this species (cf. Adamec, 2000, 2003). Surprisingly, a very low content of storage substances was found in *Elodea canadensis* turions collected from an Austrian site in late January (Janauer, 1981), with starch only 0.73% DW, free sugars 0.42% DW and organic acids as another pool of storage substances (aconitate 0.84% DW, tartarate 0.23%).

Turions can markedly differ in the content of organic storage substances other than starch or sugars. Unlike *Aldrovanda* turions (imposed dormancy), those of *U. vulgaris* and *U. stygia* were rich in protein storage vacuoles, and storage proteins were also accumulated as crystalline inclusions in the nuclei (Płachno et al., 2014). In innately dormant *Hydrocharis* turions, a high arginine content of 325 mmol kg⁻¹ DW created the main storage pool of N and represented in itself a N turion content of 1.82% of DW (Villanueva et al., 1985). In sprouting turions, this pool was even 518 mmol kg⁻¹ DW, equal to 2.90% N of total DW. The turion content of lysine was around 100 times lower. The content of the polyamines cadaverine, putrescine and spermidine in *Hydrocharis* and *U. intermedia* turions was too low (0.01–2.4 mmol kg⁻¹ DW) to act as N-storage; these metabolites could rather act as growth substances (Villanueva et al., 1985).

Turions are also storage organs for several mineral nutrients (N, P, S, Mg) though their storage function may be less distinct than that for carbohydrates (Adamec, 2000, 2010, 2011). In innately dormant turions of 12 European aquatic species across a total of 16 populations, the mean turion nutrient contents (in % DW ± SE) were as follows (Adamec, 2010; Table 1): N, 1.87 ± 0.14; P, 0.26 ± 0.02; K, 0.65 ± 0.09; Ca, 0.15 ± 0.02; Mg, 0.19 ± 0.01; mean DW:FW ratio was 30.3 ± 1.4%. For all plants (rooted and rootless) and sites, nutrient content in turions did not depend on nutrient concentrations found in the ambient water at the time of turion induction. When compared with compiled literature data on leaf/shoot nutrient contents in growing plants of the same species (mainly Dykyjová, 1979), the N and P content in turions is moderately, but for K, Ca and Mg greatly lower than that in growing plants. However, taking into account that i) the nutrient contents found in ripe turions are considerably “underestimated” due to extensive accumulation of storage carbohydrates in turions and ii) the critical N content in submerged plant shoots is only ca. 1.3% DW and 0.13% DW for P (Gerloff and Krombholz, 1966), around 30% of the total turion's N and 50% of P might be used to

Table 1

Tissue nutrient content and molar N:P ratio in innately dormant turions of 12 species of aquatic plants collected either from the field or the outdoor collection of the Institute of Botany (modified after Adamec, 2010).

Species	DMC (% FW)	N (% DW)	P	K	Ca	Mg	N:P
<i>Caldesia pannassifolia</i>	37.1	2.03	0.387	0.852	0.049	0.164	11.6
<i>Hydrocharis morsus-ranae</i>	31.4	2.75	0.291	1.69	0.193	0.335	20.9
<i>Potamogeton acutifolius</i>	33.8	1.26	0.158	0.893	0.221	0.268	17.6
<i>Potamogeton crispus</i>	32.3	0.561	0.205	1.04	0.364	0.108	6.04
<i>Potamogeton obtusifolius</i>	35.2	1.19	0.193	0.705	0.213	0.167	13.6
<i>Utricularia australis</i> ^a	35.4	1.80	0.154	0.271	0.089	0.161	25.9
<i>Utricularia australis</i> ^b	18.3	2.02	0.375	0.839	0.117	0.226	11.9
<i>Utricularia vulgaris</i>	21.1	2.07	0.252	0.674	0.169	0.244	18.2
<i>Utricularia minor</i>	29.5	2.80	0.405	0.585	0.107	0.154	15.3
<i>Utricularia bremii</i>	31.6	1.57	0.223	0.398	0.147	0.156	15.6
<i>Utricularia stygia</i>	33.7	1.96	0.298	0.498	0.120	0.178	14.5
<i>Utricularia intermedia</i>	27.0	2.18	0.314	0.435	0.116	0.166	15.3
<i>Utricularia macrorhiza</i> ^c	—	3.05	0.301	0.709	0.161	0.193	22.4
<i>Aldrovanda vesiculosa</i>	25.2	1.84	0.232	0.574	0.110	0.135	17.5

Means are shown for five parallel samples. The DMC (DW:FW proportion) is also shown. Data for *U. australis* populations from oligotrophic (a) and eutrophic site (b) are shown; (c), data from Knight (1988) for *U. macrorhiza* in Wisconsin (USA) lakes, means of three lakes shown.

support the newly sprouting organs in turions (Adamec, 2010). Turions of six European aquatic species sprouted and distinctly formed new shoots, leaves or roots in a diluted mineral solution deprived of several macronutrients (Adamec, 2011). Thus, at least N, P, S, Mg and Fe were allocated to new organs from the turions. Presumably, modified turion leaves cannot take up mineral nutrients from the water. This nutrient allocation supports the growth of new organs in relatively cold spring water, which represents a competition benefit.

4.2. Dark respiration and photosynthesis

The aerobic RD of innately dormant turions of six aquatic plants (*Aldrovanda*, *Utricularia* spp., *Hydrocharis*, *Caldesia*) at 20 °C was very low when compared to that of growing shoots or leaves of the same or similar species (Adamec, 2008a, 2011). It was around 30–75% lower per unit FW (1.3–5.2 mmol kg⁻¹ h⁻¹) and 80–85 % lower per unit DW (3.6–15.8 mmol kg⁻¹ h⁻¹). FW-based RD increased not at all or by up to 114% at the end of the imposed dormancy in April (Adamec, 2008a). Thus, the temperature quotient Q₁₀ of RD (FW-based, between 4 and 20 °C) increased in the turions of six species from 1.94 to 2.56 in innate dormancy to 2.26–3.39 at the end of imposed dormancy (cf. Adamec, 2003). For turions in innate dormancy as typical storage organs, a high proportion of cyanide-resistant respiration on the total RD (22–90%) indicated a low energy demand. In *Aldrovanda* turions in both innate and imposed dormancy, the anaerobic fermentation rate in N₂ at 20 °C was only 1.5–7% of RD (0.08–0.25 mmol kg⁻¹ h⁻¹ per FW) and did not increase during the two days of breaking the imposed dormancy (Adamec, 2003). A question is which energy consumption rate truly occurs during overwintering at ca. 4 °C in the field or a refrigerator. A model designed for *Aldrovanda* turions overwintering in a refrigerator at 4 °C shows that at measured aerobic RD rates, all respiratory carbohydrate reserves could be consumed after only 90 days, i.e., 2–3 times faster than the true turion longevity is (Adamec, 2008a; cf. Section 2.4). Therefore, the low values of anaerobic fermentation found seem more realistic under hypo- or anoxic conditions. Big differences in turion RD found among species apparently do not reflect ecological features but are rather species-specific (Adamec, 2008a, 2011); yet very low RD values found in *U. australis* turions at 4 °C could underlie their high longevity (Adamec, 2008b). As compared to true, imposed dormant turions, RD values of non-dormant winter apices (Australian *Aldrovanda* strains, *U. radiata*, *U. purpurea*) are on average 2.1–3.4 times higher (7.6–11.2 mmol kg⁻¹ h⁻¹ per FW) and are similar to those of

sprouting turions (Adamec, 2008a, 2011). The intensity of metabolism (RD) in overwintering organs might be a criterion of dormancy.

Unlike the very low RD in dormant turions, FW-based RD values of “old”, original segments of sprouting turions of six aquatic species at 20 °C ranged from 5.2 to 14 mmol kg⁻¹ h⁻¹ and were around 1.9–13 times higher than those in dormant turions (Adamec, 2011). In two *Utricularia* species, RD values in “old” segments were the same as those in newly sprouted segments. Generally, RD values of sprouting turions are comparable with those commonly estimated in growing shoots/leaves of submerged plants (cf. Adamec, 2006, 2013). Turions contain chlorophyll but even under optimum conditions, the P_N of imposed dormant turions of seven aquatic species was mostly negative or very low (Adamec, 2003, 2011; cf. Beer, 1985). In innately dormant *Ceratophyllum demersum* turions, P_N values of 6.3–7.2 mmol C kg⁻¹ h⁻¹ per DW at 20 °C reached only 2.1–6.3% of those in summer plants (Best and Meulemans, 1979). However, the FW-based P_N of sprouting turions of five submerged species (*Aldrovanda*, *Utricularia* spp.) ranged from 30 to 94 mmol kg⁻¹ h⁻¹ and were comparable with high values commonly reported for growing shoots of these or similar aquatic species, with “old” turion segments similar to newly formed ones (Adamec, 2011; cf. 2006, 2013). P_N values were much lower (8.8–17.6 mmol kg⁻¹ h⁻¹) in sprouting turions of *Hydrocharis* and *Caldesia* and probably reflect the ecology of these species (free-floating or amphibious habit) relying mostly on photosynthetic uptake of aerial CO₂. Photosynthesis is obviously negligible for dormant turions containing stored carbohydrates, but high P_N is important for sprouting turions, which rapidly produce new organs. This offers the sprouting turions a great competitive advantage over annual species or subterranean tubers at the beginning of the growing season.

5. Frost and drought resistance

Although one of the ecological functions of overwintering turions is to escape ice inclusion, turions of different species also overwinter in very shallow water or above the water surface on wet substrates where they experience frost and occasional drought (Adamec, 1999a,b). It has been known for a long time that ripe turions exhibit a certain degree of frost and drought resistance (Glück, 1906; Sculthorpe, 1967; Maier, 1973a,b; Winston and Gorham, 1979a; Adamec, 1999a,b; Adamec and Kučerová, 2013). Glück (1906) experimentally found that turions of *U. vulgaris*, *M. verticillatum* and *Potamogeton trichoides* frozen in ice blocks at -6 to -10 °C for 3–10 days could survive and sprout, while *Hydrocharis* turions could not. Increasing the length of freezing reduced the turion survival rate. Turions of *U. macrorhiza* included in ice were able to fully survive -8 °C, but not at all -12 °C (Winston and Gorham, 1979a). Similarly, *Aldrovanda* turions were able to survive in ice in a refrigerator at -4 °C for months, but died at -10 to -12 °C (Adamec, 1999a). However, when gradually adapted to frosts, lying on wet natural substrate either in the field or outdoor culture for the whole winter season, they fully survived temperatures down to around -15 to -20 °C at the turion level (Adamec, 1999a,b). Turion frost resistance thus depends on whether turions are included in ice or lie freely on the wet substrate and whether the frost is sudden or turions can gradually harden-off by mild frosts over several weeks.

Using mini-thermocouples, Adamec and Kučerová (2013) found that imposed dormant, non-hardened turions of nine aquatic plants stored in a refrigerator at 2.5 ± 1 °C, exhibited extracellular freezing within a narrow temperature range of -7.0 to -10.2 °C (only *Hydrocharis* froze at -3.6 °C), but the survival rate after freezing down to -9 to -10 °C was very low (0–38%). The freezing temperature was unchanged after turions of four species had been allowed to break their dormancy at 20 °C for 1–6 days and even had started germinating. In contrast, the mean freezing temperature of outdoor-hardened turions of six species (*Aldrovanda*, *Utricularia* spp., *Caldesia*) was 4–7 °C higher than that for the control ones kept in the refrigerator and was within a very narrow range of -2.8 to -3.3 °C. Yet the hardened turions

survived mild winter frosts down to ca. -4.2°C at 76–100% (median 100%) and also their survival rate after freezing measurements was on average 71% (median 100%). Moreover, the water content of the hardened turions of seven species overwintered outdoors on wet substrate decreased by 1.7–16 percentage points when compared to non-hardened turions stored in the refrigerator. In summary, turions can be hardened by weak frosts, and their frost hardiness leads to the shift from frost avoidance in non-hardened turions to frost tolerance.

A relatively large body of knowledge on the drought resistance of turions is available for aquatic carnivorous plants (Adamec, 2008b, 2015). Turions of *U. vulgaris* dried at 33% relative humidity (RH) at 23°C for 1–123 days, were able to sprout (Maier, 1973a). However, the longer the turions were stored in a refrigerator before drying, the less they tolerated the drying: those stored for five months survived very poorly. Moreover, innately dormant turions of *U. australis*, *U. vulgaris*, *U. intermedia* and *U. minor* tolerated drying (33% RH, $24 \pm 3^{\circ}\text{C}$) for 5–19 days and the drying markedly shortened the innate dormancy stage: after rehydration at 24°C , dried turions sprouted after only 5–7 days, but control ones after 12–48 days (Maier, 1973b). Adamec (2008b) found that the survival of dried turions in imposed dormancy was markedly species-specific. *Utricularia australis* and *U. ochroleuca* fully survived drying at 3°C for five days, but *U. bremii* and *Aldrovanda* did not. *Utricularia australis* proved to be very resistant and had a survival rate of 89–100% even at a long drought of 375 days, but *U. ochroleuca* did not. Moreover, unlike other species, dried turions of *U. australis* fully survived freezing at -11°C for five days. This indicates the possibility for long turion survival in a dry state. However, out of four *Utricularia* species, only *U. australis* turions (innate dormancy) dried at 3°C were able to fully survive the storage at -12°C for 17 months (Adamec, 2015). The extreme resistance of *U. australis* turions to drought and frost could partly account for the huge spread of this sterile species over all continents of the Old World (see Taylor, 1989).

6. The biology of *Potamogeton crispus* turions

6.1. Turion formation and sprouting: effect of temperature, light and phytohormones

Unlike most temperate aquatic species forming turions at the end of the growing season (Sculthorpe, 1967; Bartley and Spence, 1987), most *Potamogeton crispus* turions have an inverted environmental regulation of formation and sprouting. As a result, they are innately dormant over the warm summer period, sprout afterwards in autumn and grow during the winter (aestivation, called “green” turions; Sastroutomo, 1980, 1981; Kunii, 1982; Heuschele and Gleason, 2014). However, a small fraction of turions (15–40%) called “overwintered” or “brown” after their orange-brown colour due to tannin accumulation, remain innately dormant and the turions overwinter and mainly sprout in the following spring. In this species, turions are formed both in shoot apices and axils and ripen from May to August (Fig. 3). Their development is complete within two weeks. It is generally true for the whole northern temperate range (e.g. Scotland; Japan; Minnesota; USA) turion induction during the end of spring (April–June) depends on three important ecological factors: high water temperatures, long days and high irradiance (Sastroutomo, 1980, 1981; Kunii, 1982; Chambers et al., 1985; Bartley and Spence, 1987; Heuschele and Gleason, 2014). Chambers et al. (1985) found that for Scottish plants grown in a growth chamber, turions were formed during long days (18 h) only at $> 16^{\circ}\text{C}$ (maximum 30°C) and at 30°C only for long days ($> 16\text{ h}$). These results are fully consistent with the finding that turions were formed in a Scottish loch at water temperatures $> 15^{\circ}\text{C}$ during long days of 16–18 h in the mid-June to mid-August period. Turion formation is clearly phytochrome regulated as high red:far-red light ratios or red night break strongly induced turion formation. During long days, the increasing irradiance stimulated turion formation. The turion formation is stimulated because of increased P_N , but likely is not triggered photoperiodically. In

summary, *P. crispus* turion formation depends on the combination of high temperatures and long days at high irradiance in early summer.

In Japan, “green” *P. crispus* turions start sprouting in mid-October (Sastroutomo, 1981). Cold and dark pretreatment of innately dormant turions at 5°C for 1–2 weeks markedly stimulated the sprouting rate and shoot growth at 22°C , but a longer pretreatment of 3–4 weeks significantly reduced their sprouting rate and growth. At 22°C , the cold-pretreated turions could also sprout slowly in darkness but the sprouting in light was faster. Warm pretreatment of turions at 35°C for 1–4 weeks was found to be insufficient to break the innate dormancy (Sastroutomo, 1981). Both IAA and GA₃ at 1 or 10 μM added to imposed dormant turions markedly enhanced the elongation rate of sprouting turions, but only GA₃ enhanced their sprouting rate. Sprouted turions stop their growth over winter but remain viable under ice cover. They resume their growth at higher temperatures in the spring, before most aquatic plants have begun to grow (Heuschele and Gleason, 2014). These authors compared the sprouting regulation in overwintered, imposed dormant (“brown”) turions collected from a Minnesota lake in May, and in innately dormant ones collected in June (“green”) at 23°C for five weeks. The “brown” turions sprouted at 63–68% both under short or long days and even in darkness (38%), while only less than 10% of the “green” ones sprouted under these regimes. Cold pretreatment of the “green” turions at 4°C in dim light for seven weeks markedly increased the sprouting rate to 25–70%. Thus, the dormant turions need a short period in cold water to break the innate dormancy, before they start sprouting under short-day autumnal conditions. Generally, the different overwintering and sprouting strategies of both turion subpopulations contribute to a better long-term population survival as the “brown” turions function as a seed bank and limit efficient herbicide use to reduce the stand biomass.

Both “green” and “overwintered” *P. crispus* turions contain chlorophyll (ca. 0.5–1.0 g chl. $a\text{ kg}^{-1}$ DW) and the content is stable during turion development (Heuschele and Gleason, 2014). Nevertheless, this is ca. one order of magnitude lower than that in submerged plant leaves (Adamec, 2013). Starch content in freshly formed innately dormant turions was surprisingly low at 1.5% DW and glucose at only 0.25% DW (Heuschele and Gleason, 2014). In contrast, Wang et al. (2012, 2013) state starch contents of 8–58% DW in turions at the same phase). However, the starch content doubled to 3% DW in the turions naturally exposed for ca. three months and collected in September (Heuschele and Gleason, 2014). In similar experiments on innately dormant turions, the starch content doubled to 3% DW at 23°C in short or long days after five weeks, but declined to a half in darkness. The same starch content of 3% DW was also estimated in overwintered turions. These data show that innately dormant *P. crispus* turions are metabolically very active and double their starch reserves by photosynthesis during the aestivation phase, later the content is very stable. In line with this, “green” turions also exhibited a very high chlorophyll-a-based P_N at 23°C ($170\text{ mmol O}_2\text{ g}^{-1}\text{ h}^{-1}$) in light and also a high RD. Per unit chlorophyll a , this P_N is comparable with that in leaves in aquatic plants (Adamec, 2013), while the turion RD rate expressed per unit DW even exceeded that of dormant turions of other species by 3–8 times (Adamec, 2008a, 2011). On the contrary, both P_N and RD rates in overwintered turions were zero, indicating very low metabolism as a basis for their extreme longevity (Section 2.4).

Repeated short-term application of GA₃ or BA (at 2.5 or 25 mg L⁻¹) to small, overwintered plants of *P. crispus* in a greenhouse strongly decreased the formation of apical and axillary turions but low GA₃ application stimulated the formation of axillary turions (Wang et al., 2012). BA strongly inhibited the induction of turion formation and had a low effect on the growth of induced turions, while GA₃ had the opposite effect. In weakly dormant turions, a short application of BA or GA₃ or naphthalacetic acid (at 0.15–0.75 mM for 4 h) doubled the sprouting rate both for apical and axillary turions during seven days and promoted starch degradation and a rise in the soluble sugar content (Zhu et al., 2015). Metyrapone, an inhibitor of zeatin-type cytokinin

biosynthesis, completely inhibited turion sprouting. This suggests that zeatin-type cytokinins may play a regulatory role in turion sprouting.

6.2. Environmental influences on turion formation and sprouting

Under natural conditions, the formation and sprouting of *P. crispus* turions depend on ecological conditions such as water depth and transparency, sediment type and the extent of turion burial and water and sediment nutrient levels (Section 3.4). Xie and Yu (2011) were growing small plants of *P. crispus* in outdoor tanks in a nutrient-rich or nutrient-poor lake sediment and proved that the nutrient level greatly influenced turion production. Turion number was slightly lower in nutrient-poor sediment but both the turion biomass fraction and mean turion DW were by 51–87% higher when compared to nutrient-rich sediment. Turions grown in nutrient-rich sediment almost doubled their tissue N and P content, their starch content was significantly reduced but their soluble sugar content was unchanged. Thus, soil-derived mineral nutrients can markedly reduce turion size and biomass fraction, though turion number may be increased. In line with this, high nutrient levels in the water can similarly influence the size and number of formed turions. In a nine month (November to August) outdoor growth experiment, the turion DW was highest in weakly eutrophic lake water (controls) and was comparable in N-enriched water, while strong P enrichment of the water (from 0.04 to 0.20 mg L⁻¹ of total P) reduced turion DW to about half though their number was maximal (Qian et al., 2014). High plant density associated with higher pH in the water also markedly reduced turion DW, indicating that a low P_N underlies this effect. In an outdoor growth experiment in a nutrient solution, increased P concentrations (> 0.25 mg P L⁻¹) in a nutrient solution greatly reduced turion DW and size, while the turion starch and sugar contents per unit DW stayed almost unchanged (Wang et al., 2013). Thus, high P level both in the sediment and the water seems to be the major external nutritional factor, which reduces the mean DW and size of *P. crispus* turions. Yet simultaneously, through a larger stand plant biomass, this may lead to the production of a maximal number of turions.

The autumnal sprouting time of turions in Chinese lakes increased on average by one extra day per 0.05 m with increasing water depth between 3–5.5 m (Jian et al., 2003). Darkening reduced the sprouting rate by only 10–20 percentage points but substrate type had no effect. Removal of sprouted turion buds promoted the sprouting of other dormant buds, which indicates an internal control of optimal plant density and a high tolerance of grazing by herbivorous fish. Similarly, the sprouting rate of weakly innate dormant turions in a small lake was markedly delayed by an increasing burial depth in rice-field soil from 1 to 5 cm (Wang et al., 2016). Moreover, weakly dormant turions placed on the top of a lake sediment in aquaria sprouted 68–73% less due to sediment anoxia and low redox potential produced by sucrose addition, but the effect of shading was ambiguous (Wu et al., 2009).

Similar to *Myriophyllum verticillatum* turions (Weber and Noodén, 2005), a fraction of ripe dormant turions of *P. crispus* also float at the water surface, which contributes to better turion dispersal within a site (Xie et al., 2014). Field-collected floating dormant turions sprouted in a growth chamber at 55%, while parallel sunken turions sprouted only at 30% (Xie et al., 2014). Turion density was inversely proportional to turion N and P content and to turion leaf porosity (*i.e.*, proportion of gas spaces), but not to starch content. Moreover, the content of free amino acids and soluble carbohydrates was also higher in floating turions, which suggests that floating turions are metabolically more active than sunken ones. Floating of the turions thus contributes to partial differentiation of populations at sites.

7. Conclusions

In line with the prevailing literature terminology, it is reasonable to define turions of aquatic plants as dormant, overwintering, chlorophyll-

containing storage and propagation organs with highly modified leaves dissimilar to the summer growing ones and with extremely shortened internodes. Ripe turions are partly frost resistant and tolerant of anoxia. They can be hardened by weak frosts, and their frost hardiness leads to the shift from frost avoidance to frost tolerance. By the end of the overwintering period, when the mother shoots are decayed, turions separate. Then, their innate dormancy is broken, turions can germinate and sprout, and are able to photosynthesize. True turion dormancy is regulated by phytohormones and is strictly associated with low dark respiration and nearly zero net photosynthetic rates. The high photosynthetic rates of sprouting turions, together with the storage functions of turions for N, P, S and Mg, contributes to the rapid growth of sprouting turions and a rapid production of standing biomass at the beginning of the growing season.

During overwintering, the total content of non-structural carbohydrates in turions is significantly reduced due to a much decreased starch content. The longevity of turions (except for *P. crispus*) is limited to their safe survival only from one season to the next. The ecological success of one generation is transferred to the next one *via* turions: larger plants give rise to larger turions from which larger plants sprout. All mineral nutrients allocated for the next season's plant growth are transferred by turions as these propagules are the only part of the plant that survives over winter. Ecophysiological traits of turions of rootless aquatic carnivorous plants are comparable to those of other aquatic (rooted) non-carnivorous plants. Most turions of *P. crispus* have an inverted environmental regulation of their formation and sprouting, as they aestivate and grow over winter, giving them a phenological benefit.

8. Perspectives for further research

To gain further insight into the ecophysiology of turions, the following suggestions could be considered. Determining simple morphological criteria associated with the start of turion formation soon after turion induction in various species could refine our understanding of the ecological effects on turion formation. The physiological nature of breaking imposed dormancy in relation to the simultaneous regulation of gas content inside turion leaves in *Aldrovanda* and *Hydrocharis* is still partly unresolved. Is only a mild increase of turion dark respiration rate sufficient to evolve a gas into turion gas spaces filled with water? The limiting minimal content of starch and free sugars should be estimated in turions of different species overwintered under standard conditions in a refrigerator or in the field for a long period, to determine a threshold for the ability to germinate and sprout. It should be investigated what is the role of photosynthesis in ripening turions. Is it to provide sufficient amounts of reserve carbohydrates or do these turions rather rely on reutilization of carbohydrates from senescing mother shoots? Novel transcriptomic approaches should be used to reveal which genes are expressed during different stages of dormant turion overwintering as opposed to non-dormant winter apices.

Anearobic metabolism, which better reflects the natural hypo- or anoxic overwintering conditions, should be studied in overwintering turions. Can we assume marked biochemical and physiological differences between turions of non-rooted, free-floating species (*Aldrovanda*, *Utricularia*, *Hydrocharis*) that usually germinate and sprout at the water surface in warmer water and with high irradiance and those of rooted species (*Potamogeton* spp., *Caldesia*, *Elodea*), which germinate and sprout on the bottom in colder and shaded water. Is the ecological regulation of turion formation and their sprouting similar to that of apical dormant rhizome apices of several *Potamogeton* species? To what extent does turion induction in other species depend on the deficiency of mineral nutrients (N, P, S) in the ambient soil and water, as has been found in *Spirodela polyrhiza*? Do turions possess any ecophysiological adaptations to optimise their dispersal by water birds?

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