Int. J. Lim. 2025, 61, 1
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https://doi.org/10.1051/limn/2024025

Available online at:
www.limnology-journal.org

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Water or dry land – that is not a question for amphibious plant species

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Received: 13 June 2024; Accepted: 11 December 2024

Abstract – Amphibious plants attract much attention due to their unique ability to live in aquatic and terrestrial environments and sustain abrupt changes of water regime in the habitat. They may colonise habitats with pronounced water level fluctuations and water /dry land interface areas. Water and air differ in many aspects, like density, buoyancy, light and thermal conditions, as well as humidity and diffusion of gases, including oxygen and carbon dioxide, that all affect plant growth and development. The evolution of amphibious plant species resulted in high phenotypic plasticity that is manifested as a variety of ecophenes performing optimally in contrasting conditions. The most important adaptations are heterophylly and/or different life forms that differ at morphological, anatomical, biochemical, and physiological levels. These adaptations comprise the wide span of traits that are found in submerged and terrestrial plant species. The plasticity of amphibious plant species offers a potential to colonise water bodies where hydrology is affected by climate changes and present a unique model system where different scientific aspects of plants may be studied in genetically identical specimens.

Keywords: Amphibious plants / physiology / morphology / ecophene / heterophylly

1 Introduction

Amphibious plants can successfully grow and photosynthesize in two physically and chemically contrasting environments, namely in water or dry land. They may occupy different habitats along land water interface and face and overcome pronounced changes of water level in their habitat during their vegetation period. This flexibility regarding the water regime in their habitat is enabled by numerous adaptations regarding their habitus, morphological, biochemical, and physiological traits, and reproductive strategies (Braendle and Crawford, 1999). They are mainly perennial plants that commonly reproduce *via* tubers and rhizomes with storages which present a safety factor and enable them to adjust their life cycle to the periods when water levels are favourable (Sosnová *et al.*, 2010). Many may develop adventitious roots with multiple roles (Rich *et al.*, 2011). Amphibious plants have high phenotypic plasticity, as plants with the same genotype may produce a variety of ecophenes (phenotypes) in different environments (Robe and Griffiths, 2000). These ecophenes possess a wide span of traits characteristic of different aquatic and terrestrial plant species. Scientists have long been interested in this puzzling group, especially in mechanisms that allow amphibious plant species to colonise and sustain in environments where growth conditions may change abruptly during their developmental cycle (Pedersen *et al.*, 2013; van Veen and Sasidharan, 2021).

Currently, aquatic environments are exposed to many human threats (*e.g.*, habitat loss, pollution, and the introduction of alien species) and to climate change accompanied by extreme hydrological changes. These threats endanger various ecological groups of aquatic plants differently. In this context, the high adaptability and resilience of amphibious plants present a competitive advantage compared to other groups and thus, a potential to colonise frequently disturbed areas. In addition, these plants present a unique model system (Koga *et al.*, 2021) where different aspects of plants, including ecology and physiology, may be studied in genetically identical specimens with significantly different traits, which may be used in biotechnology studies.

To present the advantages and ecological importance of this outstanding group of plant species, we analysed numerous references and discussed the following questions: (1) What are the main differences between aquatic and terrestrial environments? (2) What kind of adaptations enable plant survival in terrestrial and aquatic environments? (3) What are the main adaptations of amphibious plants at morphological, anatomical,

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Table 1. Environmental differences between aquatic and terrestrial habitats and related plant adaptations. CAM – crassulacean acid metabolism, C3, C4–photosynthesis types.

Parameter	Aquatic environment characteristics	Adaptations of submerged plants	Terrestrial environment characteristics	Adaptations of terrestrial plants
Media density	999.1 kg/m^3 (water at 15°C)	flexible shoots, well developed aerenchyma, ribbon–like or dissected leaves	1.22 kg/m^3 (air at 15°C, at sea level)	stiff shoots with little sclerenchyma
Radiation	quality and quantity decrease with depth, R/FR* increases with water depth	high specific leaf area, in some cases chloroplasts present in the epidermal layer, more photosynthetic pigments per DM	from full sunlight to deep shade, R/FR decreases as passing through plant stand	thicker leaves and cuticle, trichomes, less photosynthetic pigments per DM
Temperature	high water specific heat capacity, cca. 4184 J/kg K (at 20°C), higher thermal stability	no specific adaptations	low air specific heat capacity cca. 1 J/kg K at (20°C), thermal instability of environment	thick cell walls, cuticle, trichomes, transpiration, succuence, heat shock proteins, CAM,....
Metabolic gases availability	0.03% CO_2, 0.66% O_2**	aerenchyma, high area/volume ratio aerial leaves CCMs, HCO_3^- as a C source	0.04% CO_2, 21% O_2,	CO_2 as a carbon source O_2 present in excess uptake of gases *via* active stomata regulation C3, C4 or CAM photosynthesis
Gas diffusion velocity	1	hydrosoil CO_2 use for photosynthesis, photosynthetic O_2 use for respiration	10.000	
Nutrient availability	from water column and from water in sediment	from soil through roots, in some cases from water *via* the whole plant surface, minor support of mycorrhizal fungi, active mass transport through plant, acropetal transport	from soil water	from soil water *via* roots, significant support of mycorrhizal fungi
Water availability	no limitations	acropetal transport of water	variable, could be limited	from the soil *via* roots, significant support of mycorrhizal fungi, controlled release of water through transpiration *via* stomata and passive release *via* the cuticle and periderm

biochemical, and physiological levels? and (4) What is a future perspective of amphibious plant species?

2 The characteristics of aquatic and terrestrial environments and related plants' adaptations

Water and air differ significantly in their physical properties, like density, light and thermal capacity, diffusion of gases (including metabolic gases), and humidity, which may limit plant growth and development (Tab. 1). These differences present a variety of challenges and thus, requirements for plants living in changing air/water interfaces. Terrestrial plants have easy access to gases, a favourable radiation environment, while often being exposed to water limitations. Thus, compared to aquatic plants, the leaves of terrestrial plants are thicker, have fewer intercellular spaces, and more sclerenchyma (Boeger and Poulson, 2003). Plants in terrestrial environments possess a well-developed vascular system which is used for uptaking water and inorganic nutrients from the rhizosphere. This comprises the presence of a xylem for transporting water and a phloem for transporting assimilates to all plant parts, including the storage organs in roots (Larcher, 2003). These transport systems connect the roots in the rhizosphere to the leaves, with active stomata. Active stomata support one of the most important trade-offs in terrestrial plant life history since they enable efficient carbon dioxide (CO_2) exchange to maintain high photosynthetic activity while remaining plants sufficiently hydrated (Woudenberg *et al.*, 2022). This trade-off optimises water use and thus enabling plant survival during drought, which may impact the whole ecosystem (Henry *et al.*, 2019). Beside active stomata, plants in terrestrial environment possess structures such as cuticular waxes and trichomes that also prevent excessive water loss.

In aquatic environments, the diffusion of gases is much slower, and together with light limitation, may negatively affect photosynthesis of aquatic plants (Tab. 1).

Plants living in water have less developed mechanical and vascular tissues and thus more flexible shoots that are resistant to water movement, thin cuticle, leaves without stomata, and abundant aerial tissue called aerenchyma. Aerenchyma with large intercellular spaces develops in different plant organs and enables the diffusion of gases through the whole plant (Hutchinson, 1975; Maberly and Madsen, 2002). Aerenchyma increases buoyancy and maintains aquatic plants in the upright position within the water column. Therefore, it reduces the need for well-developed mechanical tissue and, thus, the specific weight of plant organs (Raven, 1996). Gas diffusion is about 10,000 times slower in water than in the air. Thus, aquatic plants may face the problem of low gas availability (van Veen and Sasidharan, 2021). In addition, in deep waters, pressure is high and light intensity lower, since part of photosynthetically active radiation (PAR) is absorbed by water

(Jin *et al.*, 2020) (Tab. 1). On the other hand life in water also offers some advantages in comparison to terrestrial life. Plants dwelling in aquatic habitat may expand into the different parts of the water column, restricting competition. Water can also protect plants during frosty winters (Haslam, 1987). When plants are thriving in deep water, the temperature is stable (Jin *et al.*, 2020). Flowing water enables the dispersal of their propagules downstream and colonisation of new habitats (Sarneel, 2013).

Shortage of CO_2 in water resulted in the development of carbon-concentrating mechanisms (CCMs), the use of bicarbonate (HCO_3^-), soil (from roots) and respiratory CO_2 as carbon source and C4 or CAM types of photosynthesis (Yin *et al.*, 2017). Different HCO_3^- uptake strategies and CCMs have been evidenced in cyanobacteria, algae, seagrasses, and also higher plants (Poschenrieder *et al.*, 2018). There are three mechanisms known in angiosperms (van Veen and Sasidharan, 2021). The first is the conversion of HCO_3^- to CO_2 by apoplastic carbonic anhydrases. The second is acidification of the apoplast through H^+-ATPase and diffusive boundary layer, which moves the CO_2/HCO_3^- equilibrium towards CO_2. The last is a symporter-mediated cotransport of HCO_3^-/H^+, and subsequent HCO_3^- dehydration to CO_2 *via* cytosolic carbonic anhydrases. At high CO_2 concentrations and low light, when carbon is not limited, the use of CCM is suppressed, presumably to reduce investment in CCM and save energy (Madsen and Sand-Jensen, 1991).

CO_2 from soil and plant respiration are transferred from roots to leaves *via* aerenchyma, facilitating photosynthesis, while in the opposite direction, O_2 that derives from photosynthesis is transferred to roots, enabling their respiration in anoxic media (Jackson and Armstrong, 1999).

Submerged macrophytes transport nutrients to different organs without transpiration. Transport of nutrients is enabled by acropetal transport, an active mass transport that moves water solution throughout the plant from roots to leaves. In some submerged plants, it was shown that they have water guttation from leaf hydathodes and impermeable barriers around the vascular tissues to avoid solution loss (Rascio, 2002). The major function of the hydathodes that are present in all vascular plants is to function as a pressure protection valve (Tena, 2023).

3 Growth forms and heterophylly in amphibious plant species

Amphibious plant species exhibit their amphibious character in different ways. They can live in water, at water/air interface and on dry land. Some amphibious plant species develop only one leaf type (homophylly) while the others, mainly taller species, develop different leaf types (heterophylly) or/and growth forms specifically adapted to either aquatic or terrestrial environments (Braendle and Crawford, 1999). The term of homophylly thus refers to plants that have morphologically similar leaves, however these leaves can differ in their anatomy, biochemistry, and functionality (Germ and Gaberščik, 2003; Šraj Kržič and Gaberščik, 2005; Šraj-Kržič *et al.*, 2009). The term heterophylly refers to a single plant with two or more different leaf types that usually occur in habitats with variable environmental conditions (*e.g.* water, light) and optimize plant's use of energy and other resources. These leaf types may be submerged, natant, and aerial, with a variety of transitional types. They are exposed to different radiation and humidity environments. Submerged leaves often form dense stands in water and may be subjected to limited light availability; natant leaves are floating at the water surface and are thus exposed to high light intensity (Klančnik *et al.*, 2014), while in aerial leaves, the amount of received light depends on their orientation (Larcher, 2003). Highly variable and specialized assimilation areas enable amphibious plants to take advantage of the positive aspects of both water and terrestrial environment (van Veen and Sasidharan, 2021) and sufficient availability of metabolic gases in these contrasting conditions (Björn *et al.*, 2022). The main sources of gases are air (in natant/aerial leaves), gases dissolved in water, dissolved bicarbonate (source of carbon) in submerged shoots, soil and respiratory CO_2 and O_2 released from photosynthesis to some extent in various growth forms of wetland species.

The development of heterophylly is triggered by different environmental factors, namely light intensity and quality (blue light and red light, far-red light ratio – R/FR), photoperiod, osmotic potential, mechanical forces and CO_2 concentration (Li *et al.*, 2019). For example, *Myriophyllum brasiliense* (Cambess) grown on a solid substrate in the atmosphere with 5% CO_2 produced submerged leaves, terrestrial specimens grown in the atmosphere with 0.03% CO_2 developed the aerial leaf type, while submerged specimens developed an intermediate leaf type (Bristow, 1969). Bodkin *et al.* (1980) evidenced that a red (wavelength 660 nm)/far red (wavelength 730 nm) ratio induced aerial leaves in *Hippuris vulgaris* L. (Fig. 1). In the same species, osmotic stress also induced aerial leaves (Goliber and Feldman, 1990). Submerged *Callitriche heterophylla* Pursh exposed to high temperature developed the aerial leaf type (Deschamp and Cooke, 1984).

The process of new leaf development is regulated by hormones (Kawa, 2021). In many species, gibberellic acid and ethylene stimulate the development of submerged leaves, while abscisic acid (ABA) promotes the development of aerial leaves (Koga *et al.*, 2021). The same authors discovered that gibberellin, ethylene, and abscisic acid regulate the formation of submerged leaves in *Callitriche palustris* L. Authors evidenced in their comprehensive study that the molecular mechanism that regulates heterophylly in *C. palustris* relates to changes in hormones and different transcription factor gene expression profiles. The study of *H. vulgaris*, a plant species with a pronounced heterophylly, revealed a regulatory role for endogenous ABA, mediated by emergence-induced water stress, in the role of aerial leaf development (Kane and Albert, 1987). Kim *et al.* (2018), who studied the molecular basis for heterophylly in *R. trichophyllus* found out that heterophylly in this species is controlled by ABA and ethylene, which control both terrestrial and aquatic leaf development.

4 Structural traits of amphibious plant species organs

Ecophenes of amphibious plant species thriving in different habitats are very variable, not only regarding their growth forms but also regarding leaf shapes and structure of

Fig. 1. Aerial shoots of two amphibious plant species *Hippuris vulgaris* (left) and *Myriophyllum verticillatum* L. (right), which developed as the water level decreased (Photo: Alenka Gaberščik).

other organs. This variability enables optimal function in specific environments.

In some species ecophenes are so diverse that it is hard to recognize that they belong to the same plant species (*e.g. Sium latifolium* L., Fig. 2 or *Sagittaria sagittifolia* L., Fig. 3).

Extreme plasticity of different plant organs is found in *Polygonum amphibium* L. Aquatic form of this species has long stems and natant leaves, with stomata at the upper leaf surface. The transitional form is usually creeping on the partly flooded ground, and has different leaf types that are predominantly amphistomatic, while the terrestrial form is emergent with hairy hypostomatic leaves (Fig. 4). Due to this, morphological adaptations plants' function was not disturbed as environmental conditions change (Gaberščik and Martinčič, 1992; Gaberščik, 1993). However, the study of Blom and Voesenek (1996) shows that *P. amphibium* produces higher shoot biomass when growing in flooded and reduced biomass when growing in drained soils. The development of new leaves requires time. One week after the water level decreases, *P. amphibium* with epistomatic natant leaves rises in the upper parts of the shoots above the water surface. At the same time, roots and new leaves develop; however, they still have all the properties of a natant leaf. After two weeks, only the first amphistomatic leaf appeared. The same timing and pattern were observed when the water drained completely (personal observations).

Amphibious plant species growing in water have various morphological adaptations to overcome low diffusion rates of gases. As in submerged plant species, aquatic leaves are thin (*i.e. Veronica anagallis-aquatica*) or finely dissected (*i.e. Sium latifolium*, *R. trichopyllus*), have thin cuticles, and fewer or no stomata. In addition aquatic leaves may be very narrow and finely dissected, as is a case in *Ranunculus aquatilis* L. (Fig. 5), filamentous or ribbon-like as in *Alisma plantago-aquatica* L., *Sparganium emersum* Rehmann and *Sagittaria sagittifolia* (Fig. 3). Narrower leaves have a thinner boundary layer, that improves gas exchange with the environment. Therefore, submerged amphibious plants grow well at high CO_2 concentrations in early spring. As water level decreases, submerged leaves approach the water surface, which induces aerial leaf growth that enables efficient reproduction in summer (Sand-Jensen *et al.*, 2022).

Aerenchyma is crucial for life in water-saturated soils, which may be anoxic (Björn *et al.*, 2022) as also holds true for amphibious plant species. Aerenchyma enables the transfer of gases throughout plants, including the aeration of the rhizosphere. In some species, aerenchyma is constitutive, while in others, it is induced by flooding (Visser and Bögemann, 2006). Aerenchyma is formed *via* one of two basic ways: lysigenous (by programmed cell death that results in gas space) and schizogenous (by the increase of intercellular spaces) (Evans, 2004). Different plant organs of the same plant may have aerenchyma of lysigenous or schizogenous origin. In *Sagittaria lancifolia* L., aerenchyma in roots shows lysigeny but schizogeny in its shoots (Jung *et al.*, 2008). The same authors claimed that aerenchyma within polyphyletic groups may be the product of the convergence process. The two ways of forming aerenchyma can be found in different organs of an aquatic plant or even in the same organ, since plants can react in a specific way to endogenous or environmental signals (Rascio, 2002). Aerenchyma develops schizogenously in the long ribbon-shaped leaves of plants such as *Sparganium.* Schizogenous aerenchyma morphology is well known, while the genetic basis for its formation needs further research (Evans, 2004). In the genus *Nasturtium* and *Ranunculus,* a hollow tissue is formed by lysogeny, the autolysis of the medullary cells (Rascio, 2002). Gases, such as O_2 and CO_2, can be transferred in emergent plants by molecular diffusion, pressurized gas flow, and Venturi-induced convection (Jackson and Armstrong, 1999). Aerenchyma is also an important pathway for releasing methane (CH_4 greenhouse gas) from the rhizosphere of plants growing in flooded soils to the atmosphere (Evans, 2004). Aerenchyma can be present in high proportion in different plant organs (Fig. 6). Photosynthetic O_2 is transported to roots and to the rhizosphere, where it enables respiration, aerates the rhizosphere and accelerates

Fig. 2. Extreme heterophylly is commonly found in the amphibious species *Sium latifolium*; submerged leaf form (left) and some of various forms of aerial leaves (middle and right) (Photo: Alenka Gaberščik).

Fig. 3. *Sagittaria sagittifolia* may produce a variety of different leaf forms. Ribbon like submerged (and partly natant) leaves (left), young terrestrial (middle) and fully developed terrestrial form of plant (right) (Photo: Mateja Germ & Alenka Gaberščik).

mineralization, while soil CO_2 from roots is transported towards leaves and it can be used for photosynthesis (Björn *et al.*, 2022).

A plant trait that increases the availability of metabolic gases is also the elongation of stems and petioles (Ridge, 1987). Elongated shoots that emerge from water function as a 'snorkel' that provide access to air and thus essential metabolic gases (Voesenek and Blom, 1999). In genus *Oryza,* shoot elongation during submergence positively affects post-submergence photosynthetic rate and the PSII maximum efficiency (Sakagami *et al.*, 2013). Shoot elongation was also found in many other plants, like in different species of *Polygonum* (Carter and Grace, 1990)*, Rorippa amphibia* (L.) Besser and *Ranunculus lingua* L. (personal observation at intermittent Lake Cerknica) (Fig. 5). A similar role of a "snorkel" also contributes to floating or aerial leaves in predominately submerged plants as in *Ranunculus aquatilis* (Fig. 5).

5 Functional traits of amphibious plant species

The most important functional aspects in plants are photosynthesis and light harvesting, water management and nutrient acquisition. In water, photosynthesis may be hampered by low light and slow diffusion of gases, however on dry land, light and gases may be in excess (Maberly and Madsen, 2002). Morphological adaptations of specific ecophenes enable the efficient use resources including the use of energy in specific environment. When comparing the leaf optical properties (light reflectance and transmittance) of plants from the same habitat type across different species, they appear to be much more similar than the different leaf types within the same species (Klančnik *et al.*, 2014). This shows the importance of specific leaf traits for undisturbed plant function in specific environment.

Fig. 4. Two extreme forms of highly plastic *Polygonum amphibium* f. *natans*, with shiny floating leaves and long hollow stems (left) end f. *terrestris* with hairy leaves (right) (Photo: Alenka Gaberščik).

Fig. 5. Terrestrial shoots of *Ranunculus lingua* that emerge from long decumbent stems that developed in water (left); heterophylly in *Ranunculus aquatilis* L., with finely dissected submerged and more compact aerial leaves, and aerial palmately lobed leaves (that are floating at the water surface if water is deeper) (right) (Photo: Alenka Gaberščik).

Most amphibious plants depend exclusively on CO_2 as a carbon source for photosynthesis. In water some amphibious plants possess carbon-concentrating mechanisms (CCMs) and/or have the ability to uptake bicarbonate (HCO_3^-) as a carbon source to have more efficient underwater photosynthesis (van Veen and Sasidharan, 2021), however only a few have a C4 or CAM strategy (Bristow, 1969; Maberly and Madsen, 2002; Murphy *et al.*, 2007). This is also agreed by previous studies of Maberly and Spence (1989), who claim that the inability to use HCO_3^- is characteristic of homophilic amphibious species that have occasional access to CO_2 in the atmosphere. Nielsen and Sand-Jensen (Nielsen and Sand-Jensen, 1991) hypothesized that homophilic amphibious species (amphibious plants with the same leaf shape) cannot utilize HCO_3^- because the leaf surface is inadequate for active transport. Sand-Jensen and coworkers (Sand-Jensen *et al.*, 1992) report that submerged leaves of heterophyllous amphibious species can utilize HCO_3^-, while aerial leaves can use only CO_2 as a carbon source. For example, *Stratiotes aloides* (Fig. 7) can utilize HCO_3^- with submerged but not aerial leaves (Prins and de Guia, 1986). In their latter study of Danish streams (Sand-Jensen *et al.*, 2022) found that terrestrial and most amphibious species can only use CO_2 for photosynthesis, while submerged species can satisfy their inorganic carbon demand with bicarbonate.

Another potential HCO_3^- user is *Myosotis scorpioides* agg. which has a very low compensation point, which may be attributed to CCM (Nielsen, 1993). However, this is not necessarily true since high internal CO_2 concentrations in aquatic specimens may contribute to lower photorespiration and, thus, lower compensation point (CO_2 concentrations at which photosynthetic CO_2 uptake equals respiration CO_2 release, therefore net CO_2 concentration ratio is zero) as proposed by Gaberščik (1993). Genus *Isoetes* has a CAM strategy that is similar to those in terrestrial plants. It contains 'bacterial type' of enzyme phosphoenol carbohydrase (PEPC)

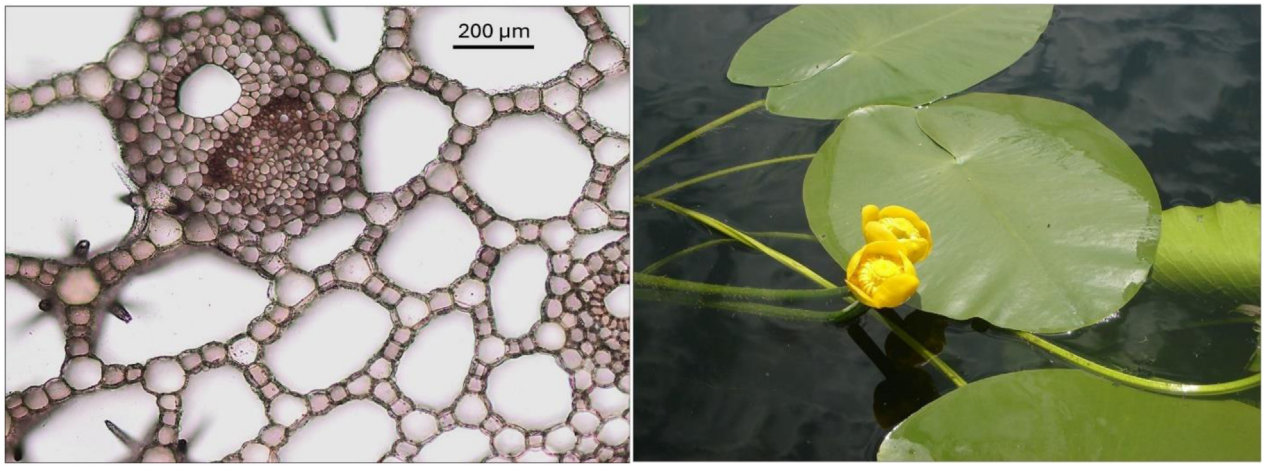

Fig. 6. Aerenchyma in leaf petiole of *Nuphar lutea* (L.) Sibth. & Sm. (left) and flowering plants with floating leaves (right) (Photo: Matej Holcar & Alenka Gaberščik).

Fig. 7. *Stratiotes aloides* is a loosely rooted aquatic species with emergent and submerged leaves (Photo: Alenka Gaberščik).

along with PEPC used in terrestrial CAM and C4 plants. Genetic differences of *Isoetes* compared to terrestrial plants suggest different evolutionary paths to CAM (Wickell *et al.*, 2021).

Increased concentrations of CO_2 that we face today affect terrestrial plants with C3 photosynthesis in different ways; by affecting the stomata openings and lowering photorespiration rate, due to decreasing CO_2/O_2 ratio (Obermeier *et al.*, 2017). However rising atmospheric CO_2 will not benefit the photosynthesis of aquatic plants in streams due to their ability to use bicarbonate and due to CO_2 supersaturation in most streams (Sand-Jensen *et al.*, 2022). The same may hold true for different plant groups including aquatic leaves/forms of amphibious plants using CCM in standing waters.

Some amphibious plant species may also produce aquatic adventitious roots from stems (Rich *et al.*, 2011). This is also a case in *Cotula coronopifolia* and *Meionectes brownii* where adventitious roots contain chlorophyll and perform photosynthesis (Rich *et al.*, 2012)

The research of roots and shoots of *R. trichophyllus* revealed the presence of acropetal transport, which was stronger in the roots, showing the importance of the roots for the throughflow of water (Pedersen and Sand-Jensen, 1993). Besides water, acropetal transport also moves nutrients and hormones. It has been reported that the estimated root pressure may be too low to drive water flow acropetally and that this transport depends on energy conversion in the roots (Pedersen, 1993).

The study of Manolaki *et al.* (2020) showed that nutrient uptake differed among submerged and amphibious species. The presence of both plant groups in Danish streams extended the length of vegetative period and nutrient uptake and positively affected nutrient cycling. The molecular characterization of ion transporters in aquatic plants would further clarify

Fig. 8. Amphibious species *Myosotis scorpioides* agg., *Ranunculus trichophyllus* and *Veronica anagallis-aquatica* L. in their natural habitat (Photo: Alenka Gaberščik).

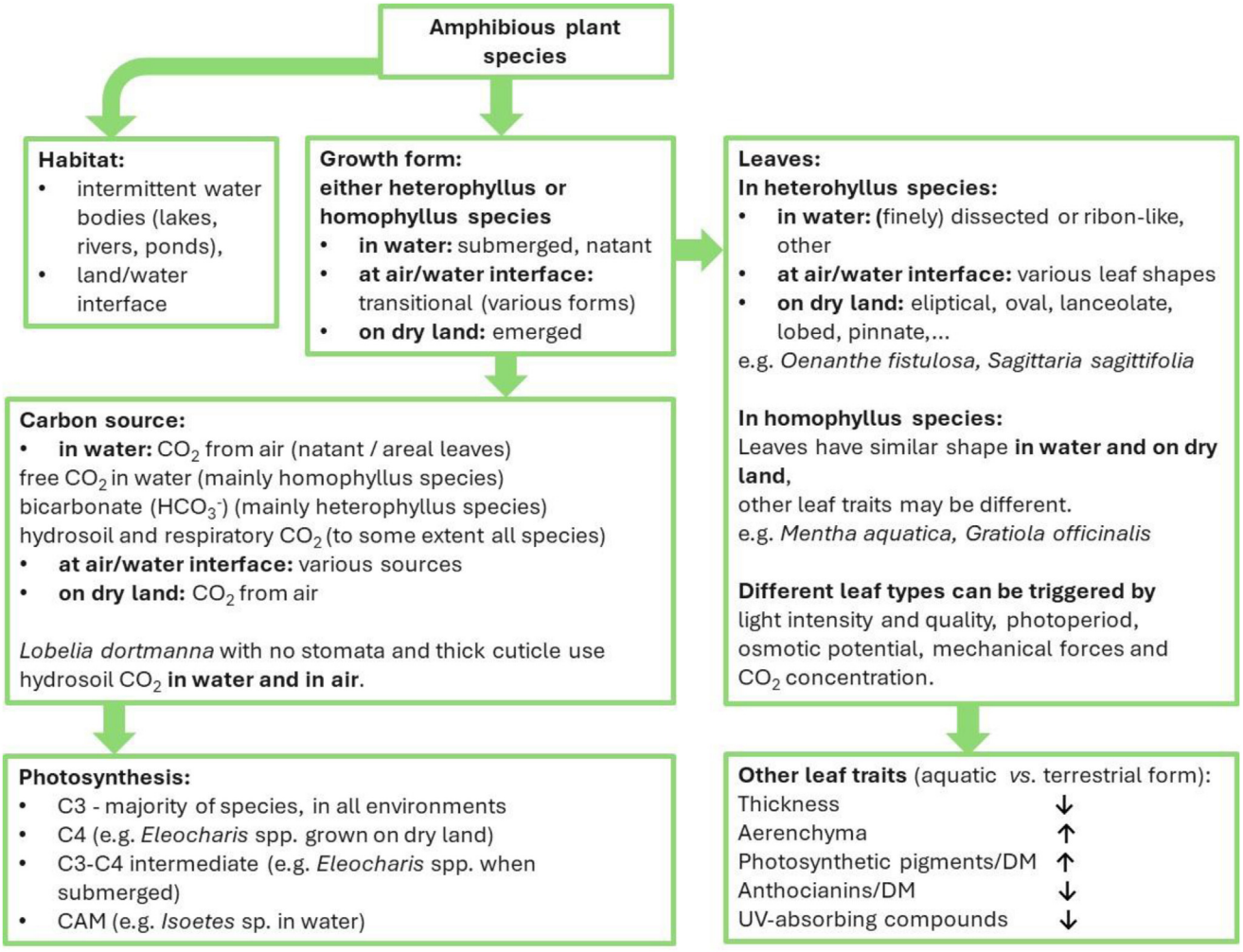

Fig. 9. A summary diagram showing the main differences between growth /leaf forms of amphibious plant species from habitats along gradient water/ dry land. CAM – crassulacean acid metabolism, C3, C4–photosynthesis types, DM – dry mass.

this process. Only two transporters were described to date: an aquaporin and the SV channel (Babourina and Rengel, 2010).

6 The expression of different traits is species specific

The expression of different traits in ecophenes of amphibious species is species specific as seen from many studies mentioned above. Germ and Gaberščik (2003) made comparative study of the characteristics of aerial and aquatic leaves in two amphibious species, *Myosotis scorpioides* agg. L. and *Ranunculus trichophyllus* Chaix (Fig. 8) under the same habitat conditions. These species responses revealed different life strategies to survive in the habitat where water level fluctuates frequently and unpredictably (intermittent Lake Cerknica, Slovenia). *M. scorpioides* agg. exhibited more terrestrial character in comparison to *R. trichophyllus*. This was evidenced by some of its traits, such as the high content of UV-B absorbing substances that assure protection against UV-radiation, the presence of stomata and the mesophyll differentiated to palisade and spongy tissue, even in aquatic leaves. In *M. scorpioides* agg. the ratio between palisade and spongy tissue thickness was 0.67 ± 0.16 in water and 0.76 ± 0.19 on dry land (unpublished data). The unfavourable conditions for *M. scorpioides* agg. in water also manifested in the greater respiratory potential activity (terminal electron transport system activity) in aquatic specimens compared to terrestrial ones. Respiratory potential increases as a response to unfavourable environment which was related to increased need for energy (Bartoli *et al.*, 2005). A higher respiratory potential provided more energy, allowing the plant to establish protective and repair mechanisms, increasing its resistance to adverse, stressful conditions (Germ *et al.*, 2006). On the other hand, the aquatic form of *R. trichophyllus* revealed all the properties of submerged plant species, such as the absence of stomata, the ability to use HCO_3^- and a low content of UV-B absorbing substances. Respiratory potential activity that was greater in aerial leaves than in aquatic ones, suggested that the aquatic environment was more favourable for this species than the terrestrial one (Germ and Gaberščik, 2003).

7 Overview of amphibious plant species complexity

The complexity of amphibious plant species is summarised in Figure 9. This group of perennial plants exhibit outstanding phenotypic plasticity, enabling them to have high resilience and undisturbed function in variable environments of intermittent water bodies and at land/water interface, where they face different light, humidity and availability of nutrients and gasses. The plasticity of single plant species may comprise an expression of a variety of morphological, anatomical, biochemical and physiological traits found in different submerged and terrestrial plant species. It manifests in different plant forms and plant organs, especially in leaves and stems.

8 Conclusions

Global environmental changes, including climate changes, affect the complexity and resilience of different water bodies. Hydrological changes are becoming more and more intensive, which will probably continue in the future. Aquatic and terrestrial plants with narrow ecological valence will suffer due to rapid changes of environmental conditions, while plants with amphibious character will be favourised since they are able to develop growth form and organs adapted to current conditions when growing either in water or on dry land. Because of their great ecological importance the knowledge on the ecological basis of amphibious plant adaptations is thus crucial, as they are possible candidates for colonizing disturbed littoral and riparian areas (Šraj-Kržič *et al.*, 2009).

Acknowledgements

This research was funded by the Slovenian Research and Innovation Agency, the programs P1-0212 “Biology of Plants” and by the Commission of the European Communities through the project Life Watch and the infrastructure project eLTER.

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Cite this article as: Germ M, Gaberščik A. 2025. Water or dry land – that is not a question for amphibious plant species. *Int. J. Lim.* 61: 1.