PHOTOSYNTHETICA 41 (1): 91-96, 2003

Comparison of aerial and submerged leaves in two amphibious species, *Myosotis scorpioides* and *Ranunculus trichophyllus*

M. GERM* and A. GABERŠČIK*,**

*National Institute of Biology, Večna pot 111, Ljubljana, Slovenia**
*Department of Biology, Biotechnical Faculty, Večna pot 111, Ljubljana, Slovenia***

Abstract

Both amphibious species, *Myosotis scorpioides* and *Ranunculus trichophyllus*, thrive in a stressful environment (alternated flooding and drying), which is variable regarding water and radiation regimes. Plants from the field and plants grown under controlled water table maintained at 40 cm were analysed for content of chlorophyll (Chl) and UV-B screening compounds, and the efficiencies of PS2 and electron transport systems. We detected no significant differences in contents of Chl and UV-B screening compounds between submerged and aerial leaves. The measurements of respiratory potential and photochemical efficiency revealed the presence of permanent stress in *M. scorpioides* in the natural environment. Differences in physiological responses of submerged and aerial leaves indicated that the terrestrial environment was more favourable for *M. scorpioides* than for *R. trichophyllus*. Characteristics of both species suggested that *R. trichophyllus* might be a phylogenetically older aquatic plant than *M. scorpioides*.

Additional key words: chlorophylls; electron transport system activity; fluctuating water level; fluorescence induction; leaf length; photochemical efficiency of photosystem 2; stomata number; UV-B screening compounds.

Introduction

Aquatic and terrestrial environments differ in many parameters essential for plant survival. Terrestrial plants have evolved structures such as cuticle and stomata, which on one hand reduce the loss of water, but on the other hand limit the uptake of carbon dioxide from the air. The main factors limiting growth and development of aquatic plants are variable irradiance, slow diffusion of CO_2 (Frost-Christensen and Sand-Jensen 1992, Vadstrup and Madsen 1996, Clevering *et al.* 1996), reduced supply of oxygen (Bucher *et al.* 1996), absence of transpiration as a driving force for water and nutrients (Madsen and Breinholt 1995), wave action (Clevering *et al.* 1996), and non-stable sediment, particularly in streams (Sand-Jensen *et al.* 1992, Barrat-Segretain and Amoros 1996).

The present paper deals with two amphibious freshwater dicotyledons – *Ranunculus trichophyllus*, exhibiting aquatic and terrestrial forms of leaves, and *Myosotis scorpioides* that can survive in both media with slight morphological changes. In a natural environment *R. trichophyllus* usually prevails in the beds of streams, while *M. scorpioides* is more or less equally distributed in the bed and on the shore. Some data on biochemical and physiological characteristics in *M. scorpioides* (Nielsen 1993, Albrecht *et al.* 1998, Albrecht and Biemelt 1998) and biochemical and morphological characteristics of *R. trichophyllus* (Webster 1991, Gluchoff-Fiasson *et al.* 1997, Dalla Vecchia *et al.* 1999, Rascio *et al.* 1999) are available, but there have been no coherent studies of the physiological aspects of amphibious character in these species. To get an insight into the survival strategy of *R. trichophyllus* and *M. scorpioides* in a stressful environment we monitored some biochemical, morphological,

---

Received 16 December 2002, *accepted* 5 February 2003.

Fax: 386 1423 50 38, e-mail: mateja.germ@nib.si

Abbreviations: Chl – chlorophyll; ETS – electron transport system; F_0 – minimum fluorescence in the dark adapted leaf (all reaction centres are open); F_m – maximum fluorescence in the dark adapted leaf (all reaction centres are closed); F_v – variable fluorescence ($F_v = F_m - F_0$); F_m', F_v' – the same for the light-adapted leaves; F_v/F_m – optimal quantum yield of PS2; INT – iodo-nitro-tetrazolium-chloride; PPFD – photosynthetic photon flux density; PS – photosystem.

Acknowledgements: This research was a part of the project "The role of UV-B radiation on aquatic and terrestrial ecosystems: an experimental and functional analysis of the evolution of protective and adaptive mechanisms in plants. Environment and Climate" (PL 970637) supported by the EU (DGXII, Environmental Programme, ENV4-CT97-0580). It was also a part of SLO Alpe2 (3311-01-2183388) financed by Ministry of Education, Science and Sport of Republic Slovenia. The financial support is gratefully acknowledged. Authors are grateful to Dr. Roger H. Pain for correction of the paper.

and physiological characteristics. Different authors have pointed out the increased need for energy during stress (Amthor 1995). Therefore special attention was paid to energy demand and efficiency of energy conversion in photosystem 2 (PS2) in different leaves. To estimate respiration potential we applied measurements of mitochondrial electron transport system (ETS) activity, which express energy availability. On the other hand, measurements of photochemical efficiency of PS2 have been recognised as a stress indicator (Schreiber *et al.* 1995).

Materials and methods

Plants: M. scorpioides L. [M. palustris (L.) Hill] belongs to the Boraginaceae family. It is perennial with creeping rhizomes. M. scorpioides flowers from May to August (Martinčič et al. 1999) and grows on river and lake shores. R. trichophyllus Chaix [Batrachium trichophyllum (Chaix) van den Bosch] has branched stems with leaves finely divided into tiny cylindrical segments. It flowers from May to July (Martinčič et al. 1999) and thrives in perennial or intermittent water bodies, ditches, ponds, streams, and rivers. Semi-terrestrial and aquatic genera of the Ranunculaceae family exhibit an extreme degree of phenotypic plasticity in many morphological characteristics (Dahlgren and Svensson 1994, Fiasson et al. 1997). Hennion et al. (1994) reported that the plasticity of the individual specimens depends on the degree and frequency of submergence. R. trichophyllus has short living generation, which renews very fast (Dahlgren and Cronberg 1996).

Two groups of plants were used for analyses. One group was sampled directly from natural environment. Our sampling site was the riverbed and the shore of the stream Lipsenjščica (45°45'N, 14°20'E) in Notranjska, Slovenia. Lipsenjščica is moderately loaded with nutrients. It is a tributary of the intermittent lake Cerkniško jezero, where the water level fluctuates frequently. Plants are subjected to both flooded and dry conditions during the year. Another group was cultured under controlled water table conditions maintained at 40 cm, and in regularly watered soil. Plants from the natural environment were transplanted into sediment from Lipsenjščica and cultured in the Botanical Garden of the University of Ljubljana (46°35'N, 14°55'E), Slovenia, one season prior to the experiment. Aquatic specimens were put into perforated polyethylene pots, which were evenly distributed in plastic containers of 30×75 cm, 45 cm high, filled with water. The water table was maintained at the same level during the experiment. Terrestrial specimens were planted in clay pots, which were buried at ground level to minimise soil temperature variation and desiccation (Sullivan and Teramura 1992). They were watered regularly. Plants were sampled every 2-4 weeks.

Morphological analyses: The counting and measuring of trichomes, stomata, and cross sections of the leaves were made with the computer program *AnalySIS 3.0* (*Soft Imaging System*, Münster, Germany).

Chl: A weighed leaf was homogenised in 8 cm^3 of 90 % (v/v) acetone and centrifuged (19 000×*g*, 3 min, 4 °C) in a top refrigerated ultracentrifuge (*2K15*, *Sigma*, Osterode, Germany). Absorbance was measured at 647, 664, and 750 nm with a UV/VIS Spectrometer System *Lambda 12* (*Perkin-Elmer*, Norwalk, CT, USA). The amounts of Chl were calculated per sample dry mass (DM), using absorption values as described by Jeffrey and Humphrey (1975).

Methanol soluble UV-B screening compounds: UV-B screening substances were extracted from fresh homogenised plant material with methanol : distilled water : HCl = 79 : 20 : 1 (v/v/v) according to Caldwell (1968). After 20 min of incubation the samples were centrifuged in a top refrigerated ultracentrifuge (1 600×*g*, 10 min, 10 °C). In samples from natural habitats, absorbance of the supernatants was measured at 300 nm. The supernatants of the samples from outdoor conditions were scanned in the range of 280-320 nm at intervals of 1 nm with the UV/VIS Spectrometer System. The absorbance values were integrated and calculated per dry mass (DM) of the sample.

Fluorescence measurements: The photochemical efficiency of PS2 was measured using a fluorometer *OS-500* (*Opti-Sciences*, Tyngsboro, MA, USA). The optimal quantum yield, which is a measure of the efficiency of energy conversion in PS2, was expressed as F_v/F_m. For dark adaptation, plants were kept in cuvettes for 15 min before measurement at ambient temperature. Fluorescence was excited with a saturating beam of "white light" (PPFD = 8 000 μmol m^{-2} s^{-1}, 0.8 s). The effective quantum yield of PS2 (yield) estimated the actual efficiency of energy conversion in PS2. It was measured under saturating irradiance (1 500-2 000 μmol m^{-2} s^{-1}) at the prevailing ambient temperature by providing a saturating pulse of "white light" (PPFD = 9 000 μmol m^{-2} s^{-1}, 0.8 s) using a standard 60° angle clip. The effective quantum yield of PS2 was defined as $Y = (F_m' - F_0')/F_m'$ (Schreiber *et al.* 1995).

Electron transport system (ETS) in mitochondria: Respiratory potential was estimated *via* potential electron transport system activity of mitochondria (ETS) as reported in detail by Packard (1971) and modified by Kenner and Ahmed (1975). Determination of ETS activity is based on reduction of the artificial electron acceptor iodo-nitro-tetrazolium-chloride (INT) and on the spectrophotometric measurement of formazan production rate, which is directly related to oxygen consumption of the investigated tissue. Weighed plant material was homogenised in ice-cold homogenisation buffer and sonicated in ultrasound homogeniser (2-3 min; 40 W, model *4710*, *Cole-Parmer*, Vernon Hills, IL, USA). The homogenate was then centrifuged (8 500×g, 4 min, 0 °C) in a top refrigerated ultracentrifuge. Substrate solution and INT were added to triplicates of the supernatant and incubated at 20 °C for 40 min. After stopping the reaction with stopping solution (formaldehyde and phosphoric acid, 1 : 1), the formazan absorption at 490 nm was determined. ETS activity was measured as a rate of INT reduction, which was converted to the amount of oxygen (Kenner and Ahmed 1975) utilised per DM unit of leaves per s.

All analyses were made on the middle part of the first fully developed leaf as proposed also by Tosserams and Rozema (1996).

Statistical analyses: Measurements were carried out on 3 to 5 parallel samples. The significance of differences was tested with F-test and two-way Student's *t*-test.

Results

No significant difference in the thickness of the leaves of *M. scorpioides* in the two forms was obtained (Table 1). The size and number of trichomes and size of stomata were similar in the submerged and aerial leaves regardless of the side of the leaf (data not shown). The numbers of stomata in the aerial and submerged leaves were not significantly different. In *R. trichophyllus*, submerged leaves were significantly longer than the aerial ones. Stomata were found in terrestrial form on the adaxial side of the leaves only (Table 1).

There were no significant differences in chlorophyll (Chl) *a*+*b* contents between submerged and aerial leaves of *M. scorpioides* and *R. trichophyllus* (Table 2). The comparison of these species revealed lower Chl *a*+*b* contents in the leaves of *M. scorpioides* than in *R. trichophyllus*, regardless of the leaf form and the cultivation. The amount of UV-B screening compounds did not differ between submerged and aerial leaves in either species. Comparison of the species revealed more UV-B screening compounds in *M. scorpioides* than in *R. trichophyllus*, regardless of the form (Table 2).

ETS activity was lowest in the winter period in *M. scorpioides*, while seasonal dynamics were absent in *R. trichophyllus* (Fig. 1). Comparison of submerged and aerial leaves in *M. scorpioides* showed higher ETS activity in the former. The opposite was shown for *R. trichophyllus* where higher demand for energy was found in the aerial leaves in comparison to submerged ones. ETS activity was higher in specimens of *M. scorpioides* grown under variable natural conditions than in plants from water-controlled conditions where the water table was maintained constant during the experiment. In *R. trichophyllus* the values of ETS activity were similar for the plants from the two locations.

The values of F_v/F_m and fluorescence yield were lower in natural environment than in water table-controlled conditions, except F_v/F_m in both forms of *R. trichophyllus* (Fig. 2). Values of F_v/F_m and yield did not differ between the studied species.

Table 1. Morphological characteristics of *M. scorpioides* and *R. trichophyllus* growing in natural environment. *statistically significant difference ($p \leq 0.05$). Means ± SD.

Species	Parameter	Side of leaf	*f. aquatica*	*f. terrestris*
M. scorpioides	no. of stomata [mm^{-2}]	adaxial	65 ± 29	86 ± 21
		abaxial	57 ± 30	109 ± 58
	leaf thickness [μm]		214 ± 32	255 ± 42
R. trichophyllus	no. of stomata [mm^{-2}]	adaxial	0	152 ± 16
		abaxial	0	0
	leaf length [mm]		25.4 ± 4.5	8.1 ± 2.0*

Discussion

The evolution of amphibious plants at the land/water interface has resulted in outstanding morphological features. These adaptations, in addition to biochemical ones, enable undisturbed physiological functioning. Both studied species differed regarding survival strategy in a varying environment. Morphological differences between submerged and aerial leaves in *M. scorpioides* were slight (Table 1), similar as reported for some other amphibious species (Pedersen and Sand Jensen 1992, Madsen and Breinholt 1995, Nielsen and Sand Jensen 1997). In *R. trichophyllus* submerged leaves are without stomata, and their uniform epidermis is rich with chloroplasts (Dalla Vecchia *et al.* 1999). Aerial leaves are thicker and mesophyll is differentiated (Tratnik 1977). Arial leaves were also shorter, having stomata on the adaxial side (Table 1). Stems of aerial shoots are more rigid. Consequently they experience less self-shading and support themselves when the water level decreases. The phenotypic plasticity of *R. trichophyllus* probably acts as some kind of environmental buffer, enabling this species to occupy a range of habitats as reported for *R. nanus* (Menadue and Crowden 1990).

Table 2. Contents of chlorophyll (Chl) *a+b* [g kg^{-1}(DM)] and UV-B screening compounds[relative]. SD is standard deviation. Months indicates the time of the minimum or maximum value (number of samplings was 7-13, the number of samplings in *M. scorpioides* f. *aquatica* was 2. num-ber of samples for each sampling was 3-5). min – minimum, max – maximum.

	Species/form	Chl *a+b* min (month)	mean±SD	max (month)	UV-B min (month)	mean±SD	max (month)
Controlled water table	*M. scorpioides* f. *aquatica*	-	14.50±1.32	-	-	954 ±158	-
	M. scorpioides f. *terrestris*	6.59 (V)	8.71±1.44	10.38 (IV)	914 (IV)	1023±128	1155 (VIII)
	R. trichophyllus f. *aquatica*	7.64 (IV)	13.40±3.99	17.09 (VIII)	375 (VII)	552±113	788 (VI)
	R. trichophyllus f. *terrestris*	8.88 (VIII)	11.42±5.19	18.90 (VIII)	427 (VIII)	535±166	878 (VI)
Natural environment	*M. scorpioides* f. *aquatica*	7.04 (XI)	11.79±3.31	18.08 (X)	36.6 (XII)	58.3±15.6	86.5 (VII)
	M. scorpioides f. *terrestris*	7.33 (II)	10.51±1.84	13.25 (IV)	33.6 (X)	51.7±16.8	78.1 (VII)
	R. trichophyllus f. *aquatica*	8.06 (VII)	15.97±4.32	23.11 (X)	20.0 (XI)	31.4±6.6	41.4 (IV)
	R. trichophyllus f. *terrestris*	9.05 (VII)	17.27±6.15	25.08 (X)	28.7 (VI)	35.0±5.0	40.5 (VII)

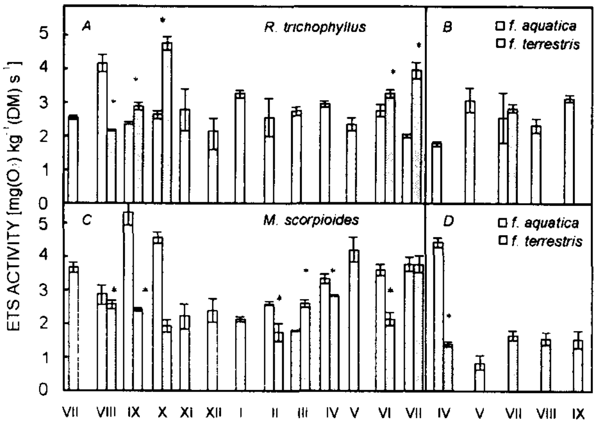

Fig. 1. Terminal electron transport system (ETS) activity in *M. scorpioides* and *R. trichophyllus* in natural environment (*A*, *C*) and under controlled water table (*B*, *D*). For differences between leaf forms, $^{*}p \leq 0.05$, $n = 3$-5.

In general, amphibious species exhibit great variability in Chl content. On the one hand, Ueno (1996) reported more Chl *a* and *b* in submerged than in aerial leaves. On the other hand, some authors found less Chl in submerged leaves or the same Chl content in submerged and aerial leaves (Pedersen and Sand-Jensen 1992, Nielsen and Sand-Jensen 1993, 1997). In our study submerged and aerial leaves of *M. scorpioides* and *R. trichophyllus* did not show differences in the amount of Chl *a+b* per DM (Table 2) which was also reported by Nielsen (1993) for the former species. *M. scorpioides* and *R. trichophyllus* usually grow in shallow water, where irradiance is similar to that on the shore (Madsen and Breinholt 1995). These two species may lack sun/shade adaptations found for some other amphibious plants (Osmond *et al.* 1981). UV-B screening compounds are very important because they constitute an UV-B selective filter, which protects vulnerable targets in the leaf (Björn 1999). The relative amount of UV-B screening compounds (per DM) in *R. trichophyllus* was almost 50 % lower than in *M. scorpioides* (Table 2). The low amount of UV-B screening compounds in the leaves of *R. trichophyllus* may show that it is a phylogenetically older aquatic plant than *M. scorpioides*. Webster (1991) found that a reduction in the variety and quantity of flavonoids accompanied a reduction in morphological complexity, and appeared to be associated with an aquatic habitat. The reduced production of UV-B screening compounds may reflect a saving of metabolic energy in a variable radiation environment. In *R. trichophyllus* contents of the same range had been already measured in specimens growing in alpine lakes (Mazej and Gaberščik 1999), where the UV-B irradiance is usually greater (Blumthaler *et al.* 1992). Absence of seasonal dynamics and low content indicated possibly saturated amounts of UV-B screening compounds (Germ *et al.* 2002). The production is probably genetically fixed and less dependent on environment. A similar phenomenon was found in *Potamogeton nodosus* (Les and Sheridan 1990). In both species examined we detected no difference in content of UV-B screening compounds between aquatic and terrestrial leaves. The reason could be the quickly changing radiation environment in amphibious habitats. Irradiation in shallow waters was temporarily high and did not much differ from those on the shore. Plants under stress need more energy, what can be estimated with ETS activity. *M. scorpioides* exhibited higher ETS activity in water than in air, which might indicate its more terrestrial character. The opposite was observed for *R. trichophyllus* (Fig. 1). In aerial leaves we found higher respiratory potential that might indicate unfavourable conditions. ETS activity in the two species was higher than in some other aquatic plants (Mazej and Gaberščik 1999). Respiratory potential was about twice as high in *M. scorpioides* growing in a natural environment than in specimens growing under water table-controlled conditions. Plants in the field were permanently exposed to fluctuations of water level, which induced adaptations to new media. On the other hand, terrestrial plants in outdoor experiments were never submerged and this kind of pressure was absent. Measurements of photochemical efficiency of PS2 supported the results of respiratory potential measurements. Deviations from the optimum value (0.80-0.85) indicated stress (Schreiber *et al.* 1995, Critchley1998), which was more pronounced in the natural environment, especially in *M. scorpioides* (Fig. 2). The diffe-rences in fluorescence between natural environment and water table-controlled conditions were even more pro-nounced when effective quantum yield was compared. Not only excess radiation, but also water stress and vari-able temperature may result in depressing the effective yield, which can be lower than the optimal quantum effi-ciency of PS2 (Adams *et al.* 1990).

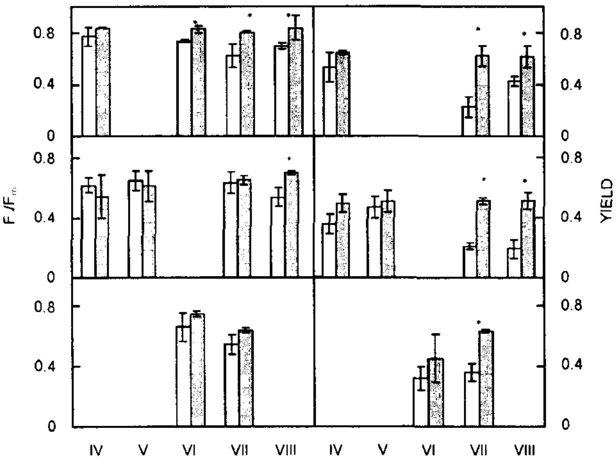

Fig. 2. Photochemical efficiency (F_v/F_m) and fluorescence yield in *M. scorpioides* (*upper two graphs*) and *R. trichophyllus* (*lower four graphs*) in natural environment (*white columns*) and under controlled water table (*grey columns*). * $p \le 0.05$, n = 5-8. In submerged leaves of *M. scorpioides* growing under controlled water table the measurements were made in April only. The mean values were 0.62 and 0.41, respectively.

The other mentioned parameters showed the permanent presence of stress in the natural environment that was not a case in water table-controlled environment. Stress was more pronounced in *M. scorpioides*. Differences in the responses of submerged and aerial leaves revealed that the terrestrial environment was more favourable for *M. scorpioides* than for *R. trichophyllus*. Specific properties of *M. scorpioides*, *i.e.* the presence of stomata and trichomes, high amount of UV-B screening compounds in both leaf forms, higher ETS activity in submerged specimens, and the fact that only the terrestrial form produced flowers, indicated a more terrestrial character. On the contrary, physiological and morphological characteristics of *R. trichophyllus* revealed that this species was better adapted to an aquatic environment. Submerged leaves can take up HCO_3^- as a source of inorganic carbon (Adamec 1992, Rascio *et al.* 1999) which is undoubtedly the advantage of submerged *R. trichophyllus* over *M. scorpioides*. Low amounts of UV-B screening compounds in both forms of *R. trichophyllus*, higher respiratory potential in terrestrial specimens, and morphological differences between submerged and aerial leaves indicate that it was possibly a phylogenetically older aquatic plant than *M. scorpioides*.

References

Adamec, L.: Interrelationship between uptake of mineral ions and photosynthesis in submerged aquatic plants. – Biol. Plant. **34**: 501, 1992.

Adams, W.W., III, Demmig-Adams, B., Winter, K., Schreiber, U.: The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measured in leaves at ambient temperature and at 77 K, as an indicator of the photon yield of photosynthesis. – Planta **180**: 166-174, 1990.

Albrecht, G., Biemelt, S.: A comparative study on carbohydrate reserve and ethanolic fermentation in the roots of two wetland and non-wetland species after commencement of hypoxia. – Physiol. Plant. **104**: 81-86, 1998.

Albrecht, G., Biemelt, S., Baumgartner, S.: Accumulation of fructans following oxygen deficiency stress in related plant species with different flooding tolerances. – New Phytol. **136**: 137-144, 1998.

Amthor, J.S.: Higher plant respiration and its relationship to photosynthesis. – In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 71-101. Springer-Verlag, Berlin – Heidelberg – New York 1995.

Barrat-Segretain, M.H., Amoros, C.: Recovery of riverine vegetation after experimental disturbance; a field test of the patch dynamic concept. – Hydrobiologia 321: 53-68, 1996.

Björn, L.O.: Effects of ozone depletion and increased ultraviolet-B radiation on terrestrial plants. – In: Baumstark-Khan, C. (ed.): Fundamentals for the Assessment of Risks from Environmental Radiation. Pp. 463-470. Kluwer Academic Publishers, Dordrecht 1999.

Blumthaler, M., Ambach, W., Rehwald, W.: Solar UV-A and UV-B radiation fluxes at two alpine stations at different altitudes. – Theor. appl. Climatol. **46**: 39-44, 1992.

Bucher, M., Brandle, R., Kuhlemeier, C.: Glycolytic gene expression in amphibious *Acorus calamus* L. under natural conditions. – Plant Soil **178**: 75-82 1996.

Caldwell, M.M.: Solar ultraviolet radiation as an ecological factor for alpine plants. – Ecol. Monogr. **38**: 243-268, 1968.

Clevering, O.A., Blom, C.W.P.M., Van Vierssen, W.: Growth and morphology of *Scirpus lacustris* and *S. maritimus* seedlings as affected by water level and light availability. – Funct. Ecol. **10**: 286-296, 1996.

Critchley, C.: Photoinhibition. – In: Raghevendra, A.S. (ed.): Photosynthesis. A Comprehensive Treatise. Pp. 264-272. Cambridge University Press, Cambridge 1998.

Dahlgren, G., Cronberg, N.: Species differentiation and relationship in *Ranunculus* subgenus *Batrachium* (Ranunculaceae) elucidated by isozyme electrophoresis. – Symb. Bot. Ups. **31**

(3): 91-104, 1996.

Dahlgren, G., Svensson, L.: Variation in leaves and petals of *Ranunculus* subgenus *Batrachium* on the Aegean Islands, analysed by multivariate analysis. – Bot. J. Linn. Soc. **14**: 253-270, 1994.

Dalla Vecchia, G., Cuccato, F., La Rocca, N., Larcher, W., Rascio, N.: Endodermis-like sheats in the submerged freshwater macrophyte *Ranunculus trichophyllus* Chaix. – Ann. Bot. **83**: 93-97, 1999.

Fiasson, J.L., Gluchoff-Fiasson, K., Dahlgren, G.: Flavonoid patterns in European *Ranunculus* L. subgenus *Batrachium* (Ranunculaceae). – Biochem. Syst. Ecol. **25**: 327-333, 1997.

Frost-Christensen, H., Sand-Jensen, K.: The quantum efficiency of photosynthesis in macroalgae and submerged angiosperms. – Oecologia **91**: 377-384, 1992.

Germ, M., Mazej, Z., Gaberščik, A., Häder, D.P.: The influence of enhanced UV-B radiation on *Batrachium trichophyllum* and *Potamogeton alpinus* – aquatic macrophytes with amphibious character. – J. Photochem. Photobiol. B **66**: 37-46, 2002.

Gluchoff-Fiasson, K., Fiasson, J.L., Waton, H.: Quercetin glycosides from European aquatic *Ranunculus* species of subgenus *Batrachium*. – Phytochemistry **45**: 1063-1067, 1997.

Hennion, F., Fiasson, J.L., Gluchoff-Fiasson, K.: Morphological and phytochemical relationships between *Ranunculus* species from Iles Kerguelen. – Biochem. Syst. Ecol. **22**: 533-542, 1994.

Jeffrey, S.W., Humphrey, G.F.: New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1 and c_2 in higher plants, algae and natural phytoplankton. – Biochem. Physiol. Pflanzen **167**: 191-194, 1975.

Kenner, R.A., Ahmed, S.I.: Measurements of electron transport activities in marine phytoplankton. – Mar. Biol. **33**: 119-127, 1975.

Les, D.H., Sheridan, D.J.: Biochemical heterophylly and flavonoid evolution in North American potamogeton (*Potamogetonaceae)*. – Amer. J. Bot. **4**: 453-465, 1990.

Madsen, T.V., Breinholt, M.: Effect of air contact on growth, inorganic carbon sources, and nitrogen uptake by an amphibious freshwater macrophyte. – Plant Physiol. 107: 149-154, 1995.

Martinčič, A., Wraber, T., Jogan, N., Ravnik, V., Podobnik, A., Turk, B., Vreš, B.: [Flora of Slovenia.] – Tehniška založba Slovenije, Ljubljana 1999. [In Slovenian.]

Mazej, Z., Gaberščik, A.: ETS-activity as a measure of vitality of different macrophyte species. – Phyton **39**: 181-185, 1999.

Menadue, Y., Crowden, R.K.: Leaf polymorphism in *Ranunculus nanus* Hook. (Ranunculaceae). – New Phytol. **114**: 265-274, 1990.

Nielsen, S.L.: A comparison of aerial and submerged photosynthesis in some Danish amphibious plants. – Aquat. Bot. **45**: 27-40, 1993.

Nielsen, S.L., Sand-Jensen, K.: Photosynthetic implications of heterophylly in *Batrachium peltatum* (Schrank) Presl. – Aquat. Bot. **44**: 361-371, 1993.

Nielsen, S.L., Sand-Jensen, K.: Growth rates and morphological adaptations of aquatic and terrestrial forms of amphibious *Littorella uniflora* (L.). Aschers. – Plant Ecol. **129**: 135-140, 1997.

Osmond, C.B., Valaane, N., Haslam, S.M., Votila, P., Roksandic, Z.: Comparison of δ^{13} values in leaves of aquatic macrophytes from different habitats in Britain and Finland; some implications for photosynthetic processes in aquatic plants. – Oecologia **50**: 117-124, 1981.

Packard, T.T.: The measurement of respiratory electron transport activity in marine phytoplankton. – J. mar. Res. **29**: 235-243, 1971.

Pedersen, O., Sand-Jensen, K.: Adaptation of submerged *Lobelia dortmanna* to aerial life form: morphology, carbon sources and oxygen dynamics. – Oikos **65**: 89-96, 1992.

Rascio, N., Cuccato, F., Dalla Vecchia, F., La Rocca, N., Larcher, W.: Structural and functional features of the leaves of *Ranunculus trichophyllus* Chaix., a freshwater submerged macrophyte. – Plant Cell Environ. **22**: 205-212, 1999.

Sand-Jensen, K., Pedersen, M.F., Nielsen, S.L.: Photosynthetic use of inorganic carbon among primary and secondary water plants in streams. – Freshwater Biol. **27**: 283-293, 1992.

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. – In: Schulze, E.-D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 49-61. Springer-Verlag, Berlin – Heidelberg – New York 1995.

Sullivan, J.H., Teramura, A.H.: The effects of UV-B radiation on loblolly pine. 2. Growth of field-grown seedlings. – Trees Struct. Funct. **6**: 115-120, 1992.

Tosserams, M., Rozema, J.: Effects of ultraviolet-B radiation (UV-B) on growth and physiology of the dune grassland species *Calamagrostis epigeios*. – Environ. Pollut. **89**: 209-214, 1996.

Tratnik, I.: [Genus *Ranunculus* subg. *batrachium* in Slovenia.] – Graduation Thesis. Dep. Biology, Biotechnical Faculty, Ljubljana 1977. [In Slovenian.]

Ueno, O.: Structural characterization of photosynthetic cells in an amphibious sedge, *Eleocharis vivipara*, in relation to C_3 and C_4 metabolism. – Planta **199**: 382-393, 1996.

Vadstrup, M., Madsen, T.V.: Growth limitation of submerged aquatic macrophytes by inorganic carbon. – Freshwater Biol. **34**: 411-419, 1996.

Webster, S.D.: A chromatographic investigation of the flavonoids of *Ranunculus* L. subgenus *Batrachium* (DC.) A. Gray (water buttercups) and selected species in subgenus *Ranunculus*. – Aquat. Bot. **40**: 11-26, 1991.