

Wastewater Treatment, Plant Dynamics and Management in Constructed and Natural Wetlands

Jan Vymazal
Editor

Wastewater Treatment, Plant Dynamics and Management in Constructed and Natural Wetlands

 Springer

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Preface

At present, constructed wetlands for wastewater treatment are a widely used technology for treatment of various types of wastewaters. The International Water Association (then International Association on Water Pollution Research and Control) recognized wetlands as useful tools for wastewater treatment and established the series of biennial conferences on the use of wetland systems for water pollution control in 1988. In about 1993, we decided to organize a workshop on nutrient cycling in natural and constructed wetlands with the major idea to bring together researchers working on constructed and also natural wetlands. It was not our intention to compete with IWA conferences, but the workshop should rather complement the series on treatment wetlands by IWA. We believed that the exchange of information obtained from natural and constructed wetlands would be beneficial for all participants. And the time showed that we were correct.

The first workshop took place in 1995 at Třeboň in South Bohemia and most of the papers dealt with constructed wetlands. Over the years we extended the topics on natural wetlands (such as role of wetlands in the landscape or wetland restoration and creation) and during the 6th workshop held at Třeboň from May 30 to June 3, 2006, nearly half of 38 papers presented during the workshop dealt with natural wetlands. This workshop was attended by 39 participants from 19 countries from Europe, Asia, North and South Americas and Australia. The volume contains 29 peer-reviewed papers out of 38 papers which were presented during the workshop.

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Praha
August 2007

Jan Vymazal

In Memoriam for Olga Urbanc-Berčič



Olga Urbanc-Berčič (1951–2007)

Olga Urbanc-Berčič was a biologist in the real sense of the word. She regarded her profession as a vocation which influenced her whole life. In 1975, after her diploma she got a post on the National Institute of Biology in Ljubljana in the laboratory for electronic microscopy. Some years later she joined the group researching freshwater and terrestrial ecosystems in the same institution. In 1988 she finished her Master's with a thesis titled "The use of *Eichhornia crassipes* and *Lemna minor* for wastewater treatment". In 2003 she successfully defended her Ph.D. thesis titled "The availability of nutrients in the rhizosphere of reed stands (*Phragmites australis*) in relation to water regime in the intermittent Lake Cerknica". Her service to her professional interests was totally unselfish. She was involved in many different projects, but most of all she liked the research dedicated to wetlands and aquatic plants. We were a perfect team for many years. I will never forget the fruitful time we spent in the field sampling and researching. The results of her research are

summarised in numerous scientific and professional publications. Her studies of the role of water-level fluctuations in nutrient cycling led to a wider understanding of wetland functions. Her work additionally clarified the importance of macrophytes in aquatic systems. She was active in different non-governmental organisations, being the president of the Slovenian Ecological Society for many years. As a warm-hearted, generous, enthusiastic and positively oriented person she was a link among people and an efficient advocate of nature. On a cold, grey Wednesday in February, we accompanied her to her last home. Her death was a great loss for family, friends, colleagues and the community. We will miss her, but her work and her spirit will live with us forever.

Selected Bibliography

Olga Urbanc-Berčič authored more than 100 contributions in international and Slovenian research and popular journals, monographs and conference proceedings. The following list contains only a short selection of her publications.

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Contents

Preface	v
In Memoriam for Olga Urbanc-Berčič	vii
Contributors	xv
1 Reed Stand Conditions at Selected Wetlands in Slovenia and Hungary	1
Mária Dinka, Edit Ágoston-Szabó, Olga Urbanc-Berčič, Mateja Germ, Nina Šraj-Kržič, and Alenka Gaberščik	
2 Water Quality and Macrophyte Community Changes in the Komarnik Accumulation Lake (Slovenia)	13
Brigita Horvat, Olga Urbanc Berčič, and Alenka Gaberščik	
3 Latitudinal Trends in Organic Carbon Accumulation in Temperate Freshwater Peatlands	23
Christopher Craft, Chad Washburn, and Amanda Parker	
4 Buffering Performance in a Papyrus-Dominated Wetland System of the Kenyan Portion of the Lake Victoria Basin	33
Herbert John Bavor and Michael Thomas Waters	
5 Changes in Concentrations of Dissolved Solids in Precipitation and Discharged Water from Drained Pasture, Natural Wetland and Spruce Forest During 1999–2006 in Šumava Mountains, Czech Republic	39
Jan Procházka, Jakub Brom, Libor Pechar, Jana Štířchová, and Jan Pokorný	
6 Dynamics of Litterfall and Decomposition in Peatland Forests: Towards Reliable Carbon Balance Estimation?	53
Raija Laiho, Kari Minkkinen, Jani Anttila, Petra Vávřová, and Timo Penttilä	

7	Near Infrared Reflectance Spectroscopy for Characterization of Plant Litter Quality: Towards a Simpler Way of Predicting Carbon Turnover in Peatlands?	65
	Petra Vávřová, Bo Stenberg, Marjut Karsisto, Veikko Kitunen, Tarja Tapanila, and Raija Laiho	
8	Leachate Treatment in Newly Built Peat Filters: A Pilot-Scale Study	89
	Pille Kängsepp, Margit Kõiv, Mait Kriipsalu, and Ülo Mander	
9	Monthly Evapotranspiration Coefficients of Large Reed Bed Habitats in the United Kingdom.	99
	Katy E. Read, Peter D. Hedges, and Phil M. Fermor	
10	The Hydrological Sustainability of Constructed Wetlands for Wastewater Treatment.	111
	Peter D. Hedges, Phil M. Fermor, and Jiří Dušek	
11	Factors Affecting Metal Accumulation, Mobility and Availability in Intertidal Wetlands of the Scheldt Estuary (Belgium)	121
	Gijs Du Laing, Annelies Van de Moortel, Els Lesage, Filip M.G. Tack, and Marc G. Verloo	
12	Reed Bed Sewage Treatment and Community Development/Participation	135
	Sean O’Hogain	
13	The Constructed Wetland Association’s Database of Constructed Wetland Systems in the UK.	149
	Paul Cooper	
14	Nitrogen Removal by a Combined Subsurface Vertical Down-Flow and Up-Flow Constructed Wetland System	161
	Suwasa Kantawanichkul, Kiattisak Pingkul, and Hiroyuki Araki	
15	Statistical Analysis of Treatment Performance in Aerated and Nonaerated Subsurface Flow Constructed Wetlands	171
	Scott Wallace, Jaime Nivala, and Troy Meyers	
16	Constructed Wetland Břehov: Three Years of Monitoring	181
	Lenka Kröpfelová	

17 Factors Affecting the Longevity of Subsurface Horizontal flow Systems Operating as Tertiary Treatment for Sewage Effluent 191
 David Cooper, Paul Griffin, and Paul Cooper

18 Investigations on Nitrogen Removal in a Two-Stage Subsurface Vertical Flow Constructed Wetland 199
 Günter Langergraber, Christoph Prandtstetten, Alexander Pressl, Kirsten Sleytr, Klaus Leroch, Roland Rohrhofer, and Raimund Haberl

19 Removal of Heavy Metals from Industrial Effluents by the Submerged Aquatic Plant *Myriophyllum spicatum* L. 211
 Els Lesage, Charity Mundia, Diederik P.L. Rousseau, Anelies M.K. van de Moortel, Gijs du Laing, Filip M.G. Tack, Niels De Pauw, and Marc G. Verloo

20 Cold Season Nitrogen Removal in a High Loaded Free Water Surface Wetland with Emergent Vegetation 223
 Christer Svedin, Sofia Kallner Bastviken, and Karin S. Tonderski

21 The Role of Vegetation in Phosphorus Removal by Cold Climate Constructed Wetland: The Effects of Aeration and Growing Season 237
 Aleksandra Drizo, Eric Seitz, Eamon Twohig, David Weber, Simon Bird, and Donald Ross

22 Performance of Reed Beds Supplied with Municipal Landfill Leachate 251
 Ewa Wojciechowska and Hanna Obarska-Pempkowiak

23 Enhanced Denitrification by a Hybrid HF-FWS Constructed Wetland in a Large-Scale Wastewater Treatment Plant 267
 Fabio Masi

24 Growth Dynamics of *Pistia stratiotes* in Temperate Climate 277
 Silvana Perdomo, Masanori Fujita, Michihiko Ike, and Masafumi Tateda

25 Fractionation, Biodegradability and Particle-Size Distribution of Organic Matter in Horizontal Subsurface-Flow Constructed Wetlands 289
 Jaume Puigagut, Aracelly Caselles-Osorio, Nuria Vaello, and Joan García

**26 Wastewater-fed Aquaculture, Otelfingen, Switzerland:
Influence of System Design and Operation Parameters on the
Efficiency of Nutrient Incorporation into Plant Biomass. 299**
Andreas Graber and Ranka Junge-Berberović

**27 Is Concentration of Dissolved Oxygen a Good Indicator
of Processes in Filtration Beds of Horizontal-Flow
Constructed Wetlands? 311**
Jan Vymazal and Lenka Kröpfelová

**28 Pollutant Transformation Performance and Model
Development in African Wetland Systems: Large
Catchment Extrapolation 319**
Herbert John Bavor and Michael Thomas Waters

29 Sulfur Cycling in Constructed Wetlands 329
Paul J. Sturman, Otto R. Stein, Jan Vymazal, and Lenka Kröpfelová

Index 345

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Chapter 1

Reed Stand Conditions at Selected Wetlands in Slovenia and Hungary

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Abstract We determined the characteristics of reed stands at an intermittent lake in Slovenia and degraded and vital reed stands in Hungary. The disturbance in reed performance was measured through growth analysis, amino acid analysis in basal culm internodes, and photochemical efficiency of photosystem II (PSII) in leaves. Morphological parameters indicated higher disturbance in the development of degraded and intermittent reed stands in comparison to vital reed stands. Similarly, total free amino acid contents in basal culm internodes reflected temporary stress response in degraded and intermittent reed stands. On the other hand, potential photochemical efficiency showed undisturbed energy harvesting of all reed stands, even though actual photochemical efficiency revealed temporary disturbance of PSII. The most unfavourable condition for reed development seems to be degraded reed stand of Kis-Balaton wetland and littoral reed stand of intermittent Lake Cerknica.

Keywords Free amino acids, reed biometry, photochemical efficiency of PSII, *Phragmites australis*

1.1 Introduction

Phragmites australis (Cav.) Trin. ex Steud. (common reed) is the most widely distributed angiosperm, characteristic species of the ecotone between terrestrial and aquatic environments in freshwater to brackish ecosystems (van der Putten, 1997;

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Cronk & Fennessy, 2001; Mauchamp & Méthy, 2004). *P. australis* may be temporarily exposed to complete submersion or to drought ranging from few days to several months (Mauchamp & Méthy, 2004). It acclimatises to deep water and water deficit with phenotypic plasticity (Vretare *et al.*, 2001; Pagter *et al.*, 2005). Deep water may affect the performance of *P. australis* by constraining oxygen supply to the below-ground parts of the plant (White & Ganf, 2002). Under such conditions, reed allocates more assimilates to stem weight, and produces fewer but taller stems, maintaining positive carbon balance (Dinka & Szeglet, 1999) and effective gas exchange between emerged and below-ground parts (Vretare *et al.*, 2001).

Despite high functional plasticity of *P. australis*, reed stands throughout Europe experienced severe decline in last decades (Ostendorp, 1989). Previous studies have shown that different environmental factors may contribute to the decreasing vitality of the reed stands (Ostendorp, 1989; van der Putten, 1997): changes in water level (Dienst *et al.*, 2004), reduced oxygen supply to roots and rhizomes (Armstrong & Armstrong, 1990; Brix *et al.*, 1992), internal eutrophication (e.g. high ammonium concentration), etc. These stress factors affect metabolic pool of whole plant, which may be reflected by changes in amino acid patterns in basal culm internodes (Haldemann & Brändle, 1988; Kohl *et al.*, 1998; Rolletschek *et al.*, 1999; Koppitz, 2004). Plants subjected to stress often show accumulation of specific free amino acids and/or reduced protein synthesis (Marschner, 1995; Rabe, 1990; Smolders *et al.*, 2000; Koppitz, 2004), and decreased photochemical efficiency of PSII due to photo-inhibition (Schrieber *et al.*, 1995).

The aim of this study was to determine the characteristics of selected reed stands in Slovenia and Hungary. Localities differ in vitality of reed stands and to a great extent in water regimes. We hypothesised that different reed stands will experience different levels of disturbance, as measured through growth analysis, amino acid analysis, and photochemical efficiency. We assumed that reed stands of the intermittent lake in Slovenia and degraded reed stands in Hungary will be more disturbed in comparison to vital reed stands in Hungary.

1.2 Methods

1.2.1 Area Description

The survey of reed stand conditions was performed at selected wetlands of Slovenia (Lake Cerknica) and Hungary (Lake Fertő and Kis-Balaton wetland of Lake Balaton) in growth periods 2004 and 2005.

Lake Cerknica is *locus typicus* for intermittent lakes, appearing at the bottom of the karstic valley of Cerkniško polje (38 km²). Due to floods in spring and autumn, the valley changes into a lake (20–25 km²). Floods last on average 260 days a year and the dry period usually starts in late spring (Krajnc, 2002). The lake was designated for the Ramsar List in 2006.

Table 1.1 Reed stands characteristics at Lake Cerknica (Slovenia), and Lake Fertő and Kis-Balaton wetland (Hungary), surveyed in 2004 and 2005

Lake	Location	Characteristics	
Cerknica, SLO	Zadnji Kraj 1	CE 1	Littoral reed stands, nutrient-poor, variable water regime (0–2.5 m throughout a year)
45°45'N, 14°20'E	Zadnji Kraj 2 Gorenje jezero	CE 2	Ecotonal reed, variable water regime, but efficient water supply
		CE 3	
Fertő, HU	Fertőrákos	FE 1	Homogeneous, vital reed stand in shallow water (0–0.3 m)
(Neusiedler See)	Nádas 3	FE 3	Clumped distribution, loose, degraded reed stand (0.3–0.5 m)
47°42'N, 16°46'E	Herlakni 5	FE 5	Homogeneous, loose, vital reed stand in deep water (0.8–1.2 m)
Kis-Balaton, HU 46°50'N 17°44'E	Ingói berek 1	KB 1	Vital reed stand in deep water (0.5–0.8 m)
	Ingói berek 2	KB 2	Degraded reed stand in shallow water (0.3–0.5 m)

Lake Fertő (Neusiedler See) is the largest sodic lake in Europe (309 km²), declared as a biosphere reserve by UNESCO in 1977/79. It is a eutrophic steppe lake, situated on the Hungarian–Austrian border (Löfler, 1979). The water is permanent, but extremely shallow (mean depth 1.1 m, maximal depth 1.8 m), with regulated outflow. As a consequence of shallowness, 54% of the whole lake and 85% of the Hungarian part is covered by reed.

Kis-Balaton is 81 km² large wetland, located SW of Lake Balaton (594 km²). Large parts were drained due to agriculture in the beginning of the 20th century. Later the re-establishment of the Kis-Balaton wetland was implemented. The extended area was given the classification of Landscape Protected Area, and was designated for the Ramsar List in 1989.

All three wetlands are dominated by reed stands. Different sampling sites were selected with respect to nutrient conditions, water regime, and reed vitality (Table 1.1). Hungarian locations were nutrient-rich and with permanent water (Dinka, 1993; Pomogyi, 1993; Tátrai *et al.*, 2000; Dinka *et al.*, 2004), while Slovenian locations were nutrient-poor and with variable water regime (Šraj-Kržič *et al.*, 2006). Growth seasons 2004 and 2005 differed with regard to precipitation pattern and consequently water regime (Fig. 1.1).

1.2.2 Growth Analyses

Shoot density was measured within four squares (0.25 m²). Randomly harvested shoots ($n = 8–12$) were used for measurements of shoot height, shoot diameter, shoot dry mass, and specific leaf area (Dykyjová *et al.*, 1973; Květ, 1971). The dry weight of samples was estimated after 24 h of drying at 105°C (Sterimatic ST-11, Instrumentaria, Zagreb). The leaf area was measured using area meter (Delta-T

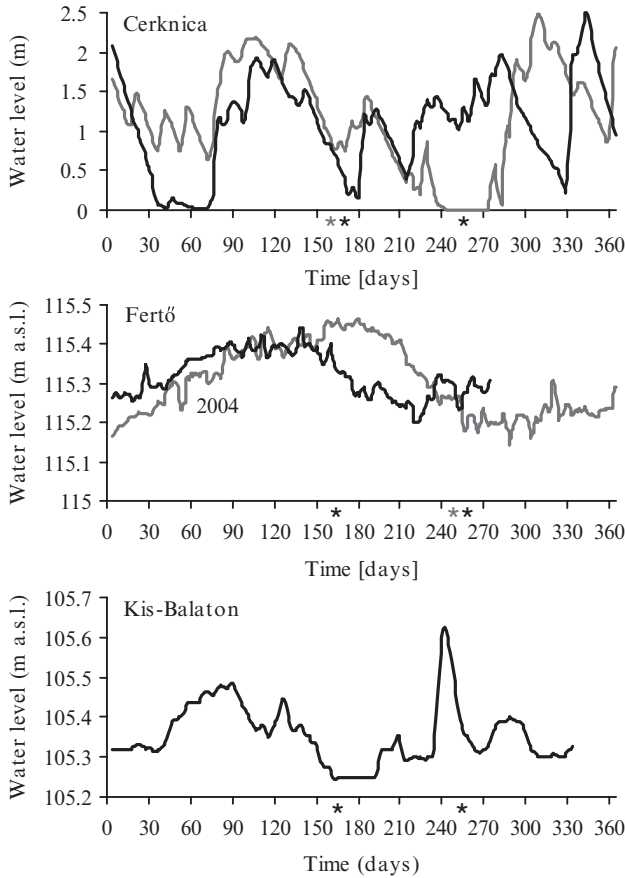


Fig. 1.1 Water level fluctuations at Lake Cerknica, Lake Fertő, and Kis-Balaton wetland in 2004 (—) and 2005 (—). Asterisks indicate sampling time in 2004 (*) and 2005 (**)

Devices Ltd., Cambridge, England). Specific leaf area was calculated as the ratio between leaf area and leaf dry weight ($\text{cm}^2 \text{g}^{-1}$).

1.2.3 Analysis of Amino Acids

For the analysis of amino acids in basal culm internodes of randomly harvested primary culms ($n = 3-6$) we followed the method of Koppitz (2004). Samples were frozen in liquid N_2 , transferred to the laboratory, stored (-20°C), pulverised under liquid N_2 , and divided into two subsamples. Powdered samples (250 mg) were extracted three times with 3 ml of ethanol (80% v/v) at room temperature. Combined fractions were sonicated in an ultrasound bath (10 min), evaporated under liquid N_2 ,

and the remaining moisture eliminated by freeze-drying. Dry samples were dissolved in 1 ml of ethanol (80% v/v). Amino acids were derivatised with 9-fluorenylmethoxycarbonyl chloride/1-aminoadamantane (FMOC/ADAM), detected using high performance liquid chromatography (HPLC) (thermo Separation P200 as pump, gradient elution, GromSil 250 × 4 mm column) and UV150 detector at 263 nm, and separated with Na-acetate buffer and acetonitrile/water. Standard mixture of 20 amino acids was used for identification and quantification of samples. The contents of amino acids were calculated per dry weight ($\mu\text{mol/g}$).

1.2.4 Measurements of Photochemical Efficiency

Chlorophyll *a* (Chl *a*) fluorescence of PSII is an indicator of photosynthetic electron transport in intact leaves and therefore reflects changes in primary processes of photosynthesis (Schrieber *et al.*, 1995). To estimate the disturbance to the light harvesting of PSII we monitored Chl *a* fluorescence (modulated fluorometer OS-500, OPTISCIENCES, Tyngsboro, MA, USA). Measurements were carried out on fully developed leaves ($n = 5\text{--}12$) on clear days at noontime, when photosynthetic photon flux density (PPFD) exceeded $1,200\mu\text{mol m}^{-2}\text{ s}^{-1}$. The potential photochemical efficiency (F_v/F_m) was determined after dark-acclimation (15 min) using saturating pulses of white light (PPFD $\approx 8,000\mu\text{mol m}^{-2}\text{ s}^{-1}$, duration 0.8 s). Actual photochemical efficiency (Y) was measured under ambient light using saturating pulses of white light (PPFD $\approx 9,000\mu\text{mol m}^{-2}\text{ s}^{-1}$, duration 0.8 s). It gives the information on energy conversion in PSII (Björkman & Demmig-Adams, 1995; Schrieber *et al.*, 1995).

1.2.5 Statistical Analyses

The significance of differences between sampling sites and sampling times was tested using the analysis of variance (one-way ANOVA) for parametrical data, and Mann–Whitney U test for non-parametrical data. Relationships between two parameters were tested using Spearman's rank-order correlation. Statistical analyses were preformed using SPSS for Windows 13.0.

1.3 Results

1.3.1 Growth Parameters

Low reed density was determined at Lake Cerknica (in average 60 reeds m^{-2}), degraded reed stand FE3 and deepwater, vital reed stand of FE5 at Lake Fertő (in average 20 and 85 shoots m^{-2} , respectively). Temporal changes in reed density

were not determined. On the contrary, high density of vital reed stand FE1 declined significantly from 110 shoots m^{-2} in 2004 to 51 shoots m^{-2} in 2005. The density of degraded reed stand KB2 (ranging from 35 to 90 shoots m^{-2}) differed significantly from vital reed stand KB1 at Kis-Balaton wetland (ranging from 150 to 200 shoots m^{-2}). Reed stands of Lake Cerknica and degraded reed stands FE3 and KB2 had the lowest basal diameter (ranging from 3 to 7 mm), followed by Lake Fertő and Kis-Balaton vital reed stands (ranging from 7 to 11 mm).

Table 1.2 shows shoot height and dry mass and specific leaf area of reeds from Lake Cerknica, Lake Fertő, and Kis-Balaton wetland, measured in June and September 2005. Significantly smaller reeds with lower dry mass were characteristic of degraded reed stands of FE3 and KB2 compared to vital reed stands of

Table 1.2 Shoot height and dry mass and specific leaf area of reed stands at Lake Cerknica, Lake Fertő, and Kis-Balaton wetland, measured in 2005. Data represent arithmetic mean \pm SD, $n = 8-12$. One-way ANOVA; letters indicate differences between sampling sites ($p \leq 0.05$), and asterisks indicate differences between sampling time

	June 2005				September 2005				
Height (cm)									
CE1	196	\pm	15	<i>b</i>	194	\pm	33	<i>b</i>	ns
CE2	224	\pm	14	<i>a</i>	238	\pm	51	<i>a</i>	ns
CE3	179	\pm	8	<i>c</i>	195	\pm	10	<i>b</i>	*
FE1	238	\pm	15	<i>a</i>	270	\pm	27	<i>a</i>	**
FE3	152	\pm	15	<i>b</i>	159	\pm	11	<i>b</i>	ns
FE5	256	\pm	39	<i>a</i>	235	\pm	25	<i>a</i>	ns
KB1	335	\pm	48	<i>a</i>	228	\pm	26	<i>a</i>	***
KB2	137	\pm	23	<i>b</i>	158	\pm	20	<i>b</i>	ns
Shoot dry mass (g)									
CE1	12	\pm	4	<i>B</i>	10	\pm	5	<i>b</i>	ns
CE2	19	\pm	6	<i>A</i>	18	\pm	6	<i>a</i>	ns
CE3	13	\pm	2	<i>B</i>	13	\pm	3	<i>a</i>	ns
FE1	30	\pm	7	<i>A</i>	51	\pm	15	<i>a</i>	***
FE3	8	\pm	2	<i>C</i>	11	\pm	2	<i>c</i>	**
FE5	22	\pm	7	<i>B</i>	23	\pm	8	<i>b</i>	ns
KB1	63	\pm	23	<i>A</i>	16	\pm	8	<i>a</i>	***
KB2	9	\pm	4	<i>B</i>	11	\pm	4	<i>b</i>	ns
Specific leaf area ($cm^2 g^{-1}$)									
CE1	615	\pm	42	<i>A</i>	1,056	\pm	137	<i>a</i>	***
CE2	508	\pm	64	<i>B</i>	1,007	\pm	109	<i>a</i>	***
CE3	409	\pm	33	<i>C</i>	767	\pm	55	<i>b</i>	***
FE1	72	\pm	7	<i>B</i>	85	\pm	12	<i>b</i>	**
FE3	75	\pm	9	<i>B</i>	111	\pm	7	<i>a</i>	**
FE5	108	\pm	10	<i>a</i>	77	\pm	14	<i>b</i>	***
KB1	107	\pm	5		112	\pm	24		ns
KB2	121	\pm	21		104	\pm	16		ns

ns 'not significant', * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Lake Fertő and Kis-Balaton wetland. Reeds from Lake Cerknica were of intermediate height and dry mass, which did not differ significantly between June and September. Specific leaf area of reeds from Lake Cerknica and Lake Fertő increased in time significantly.

1.3.2 Free Amino Acid Content

The highest content of total amino acids in basal culm internodes (Fig. 1.2) was detected at CE1 and degraded reed stand KB2 ($17\text{--}22\ \mu\text{mol g}^{-1}$), followed by other sampling sites ($3.5\text{--}12\ \mu\text{mol g}^{-1}$). Principal amino acids at all sampling sites were alanine (Ala), arginine (Arg), asparagine (Asn), γ -amino-butyric acid (Gaba), glutamine (Gln), and serine (Ser). The remaining 14 amino acids were presented as “other amino acids”. The accumulation of Ala+Gaba+Ser ranged between 22% and 47% in reeds of Lake Cerknica and Lake Fertő. The percentage increased significantly from June to September in reeds of Kis-Balaton wetland (increase from 14% to 38%) and Lake Cerknica (increase from 31% to 43%). Additionally, high accumulation of Arg+Asn+Gln was detected at all sampling sites. The percentage declined significantly from spring to autumn in reeds of Lake Cerknica (decline from 25–50% to 12–27%) and Kis-Balaton wetland (decline from 57% to 25%), while relatively constant values were characteristic of reed stands at Lake Fertő (23–50%).

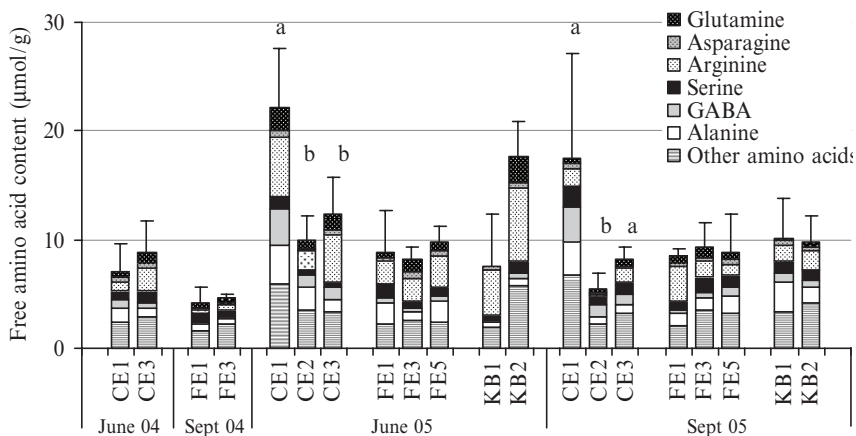


Fig. 1.2 Free amino acids in basal culm internodes in reeds at Lake Cerknica (CE), Lake Fertő (FE), and Kis-Balaton wetland (KB), sampled in 2004 and 2005. Data represent arithmetic mean \pm SD, $n = 3\text{--}6$. Mann–Whitney U test; letters indicate differences between sampling sites ($p \leq 0.05$)

1.3.3 Photochemical Efficiency

The potential (F_v/F_m) and actual (Y) photochemical efficiency of PSII of reed stands of Lake Cerknica, Lake Fertő, and Kis-Balaton wetland are presented in Fig. 1.3. F_v/F_m was close to the value 0.8 in reeds of all the lakes. Significant changes in F_v/F_m in time were calculated ($p \leq 0.05$), with the highest values in June 2005. Y ranged between 0.3 and 0.5 throughout both seasons 2004 and 2005. There were no major differences between locations in the Lake Fertő, while locations at Lake Cerknica and Kis-Balaton wetland differed significantly. Reed stands of Lake Cerknica and degraded reed stands FE3 and KB2 showed notable decline in Y from June to September ($p \leq 0.05$). Spearman's rank-order correlation did not show significant relationship between photochemical efficiency and total amino acid content.

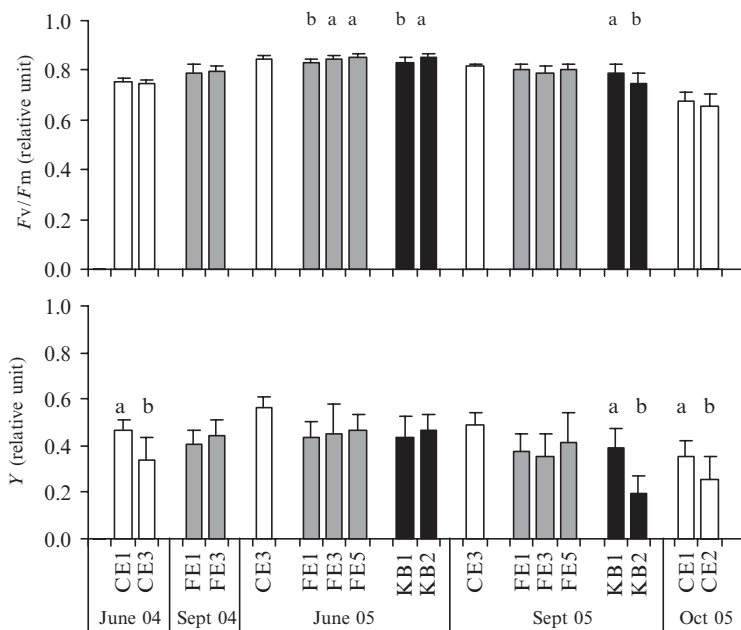


Fig. 1.3 Potential (F_v/F_m) and actual photochemical efficiency of photosystem II (Y) of reeds at Lake Cerknica (CE), Lake Fertő (FE), and Kis-Balaton wetland (KB), measured in 2004 and 2005. Data represent arithmetic mean \pm SD, $n = 5-12$. One-way ANOVA; letters indicate differences between sampling sites ($p \leq 0.05$)

1.4 Discussion

Our study revealed some characteristics of vital and degraded reed stands of wetlands with permanent water regime (Hungary) and reed stands of intermittent wetlands (Slovenia).

Reed stands differed in morphological characteristics (Table 1.2), which might be attributed to the differences in environmental conditions (Dienst *et al.*, 2004; Brix *et al.*, 1992). Vital reed stands of Lake Fertő (FE1) and Kis-Balaton wetland (KB1) were denser, with better developed shoots than degraded, as already reported in the case of reeds from Lake Fertő (Dinka & Szeglet, 2001). The density of reed stands at Lake Fertő was decreasing, as also evident from the long-term database (Dinka, 2006). At vital reed stands of Lake Fertő, shoots were well developed, while at the degraded site shoot height, dry mass, and basal diameter revealed weaker reeds. We presume that plants were affected by low water level due to increased dissolved solids (conductivity up to $4,000\mu\text{S cm}^{-1}$). Reed stands from intermittent Lake Cerknica showed intermediate growth characteristics. Despite low density, shoots were relatively well developed, which reveals great functional plasticity of *P. australis* under variable water regime (Vretare *et al.*, 2001; White & Ganf, 2002; Gaberščik *et al.*, 2003).

Similarly the analysis of free amino acids in basal culm internodes (Fig. 1.2) revealed the presence of disturbance in some reed stands (CE1 and KB2). It is widely accepted that stress induces the production of free amino acids (Gzik, 1996; Šircelj *et al.*, 1999; Hartzendorf & Rolletschek, 2001; Koppitz, 2004), which reflect the conditions during the growth period. In the intermittent Lake Cerknica the growth period in 2005 was outstanding, since water level was relatively high during the whole summer. Consequently, plants revealed significantly higher total amino acid content, which could be the result of the oxygen shortage in the soil (Koppitz, 2004). In basal culm internodes at littoral reed CE1 we determined the highest content of total free amino acids due to large fractions of Ala+Gaba+Ser, which were also recorded at other sampling sites. Ala, Gaba, and Ser are reported as indicators of hypoxia and anaerobic metabolism (Haldemann & Brändle, 1988; Kohl *et al.*, 1998; Rolletschek *et al.*, 1999; Sánchez *et al.*, 1998; Koppitz *et al.*, 2004). In reeds of all sampling sites also relatively high fractions of Arg+Asn+Gln were detected, which indicated high NH_4^+ concentration in the environment, resulting in inhibition of protein synthesis (Smolders *et al.*, 2000). Asn is the main storage and transport compound of the intermediate N metabolism in *P. australis*. Therefore, the synthesis of specific N-efficient soluble amino acids like Asn and Arg prevents the accumulation of toxic free ammonium in the cells (Haldemann & Brändle, 1988; Rolletschek *et al.*, 1999).

Besides the content of free amino acids in basal culm internodes, the photochemical efficiency of PSII in leaves also gives an insight in plant performance under stress. The potential photochemical efficiency of PSII (F_v/F_m) of unstressed leaves of many species and ecotypes ranges from 0.80 to 0.83 (Schrieber *et al.*, 1995). The measurements of F_v/F_m of reed showed that the majority of plants exhibited normal energy harvesting, with F_v/F_m close to the optimal value. That indicated

good physiological status of the reeds, which was also found by Mészáros *et al.* (2003). Decreases in F_v/F_m in intermittent Lake Cerknica in October 2005 indicated that PSII reaction centres had been damaged. This could be due to high water level through the season which suppresses oxidative processes in the reed roots (White & Ganf, 2002), or late season, when the senescence starts. Mauchamp & Méthy (2004) reported that F_v/F_m of reed was affected by submergence and exhibited varying recovery levels depending on duration and degree of submergence. Actual photochemical efficiency of PSII (Y) was generally lower than F_v/F_m , which was due to temporary stress during the midday depression. The effects of short-term photoinhibition were found to be reversible (Mauchamp & Méthy, 2004; Šraj-Kržič & Gaberščik, 2005). F_v/F_m and Y of degraded reed stands of Lake Fertő and Kis-Balaton wetland declined significantly from June to September, which reflected the temporary disturbance in the functioning of PSII. Similarly, Mészáros *et al.* (2003) reported decline in F_v/F_m in degraded reed stands of Lake Fertő.

1.5 Conclusions

This study revealed some functional characteristics of different reed stands (Lake Cerknica, Lake Fertő, and Kis-Balaton wetland). Biometric parameters indicated that degraded (FE1 and KB2) and intermittent reed stands (CE) were more disturbed in their development than vital reed stands. Similarly, total free amino acid contents reflected temporary stress response in some sampling sites (CE1 and KB2). Photochemical efficiency showed normal energy harvesting of all reed stands throughout the season. The most unfavourable condition for reed development seems to be reed stands of intermittent Lake Cerknica (littoral reed stand CE1) and Kis-Balaton wetland (degraded reed stand KB2).

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