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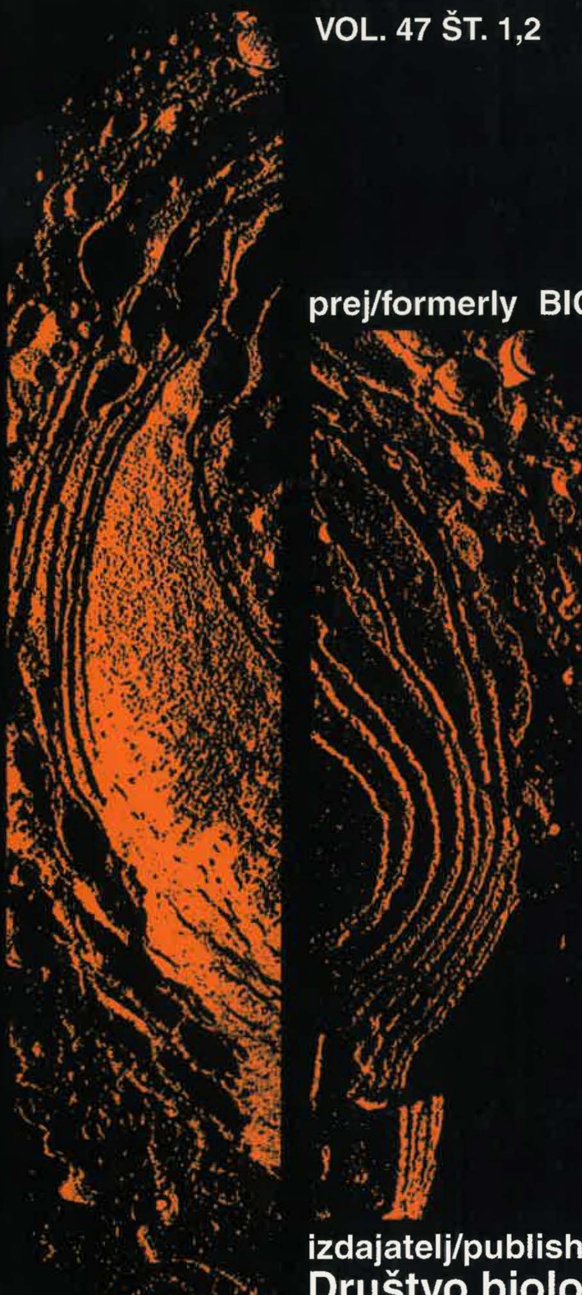

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# Coenological and synphysiological investigations on loess grassland vegetation *(Salvio-Festucetum rupicolae)* close to Gödöllő Hills (Hungary)

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**Abstract.** Parallel coenological and synphysiological examinations were carried out on three typical xerophilous loess grassland stands of *Salvio-Festucetum* rupicolae community in the Gödöllő Hills, near Isaszeg village. Three stand types, a *Carex humilis*, a *Chamaecytisus austriacus* and a *Stipa dasiphylla* dominated ones were investigated at the same spatial scale (mesoscale). In our sample area apparent dominance by three species (*Festuca rupicola, Stipa dasiphylla, and Carex humilis*) suggested that these stands of this subassociation have been emerged from three types of differing species composition. According to these analyses three groups dominated by *Carex humilis, Chamaecytisus austriacus* and *Stipa dasiphylla of festucetosum rupicolae* subassociation of a stand of *Salvio-Festucetum rupicolae* association were separated. Results of the synphysiological measurements show, that water shortage and senescence in autumn are responsible for the different physiological performances of the three types. In the well-watered summer period we found significant difference only between the physiological activity of the *Carex*- and *Chamaecytisus*- dominated types, however this difference is due to the significant LAI-difference and after relating photosynthesis to 1 $m^2$ leaf area, the difference is non-significant.

**Keywords:** coefficient of variation, coenology, grassland, loess stands, multivariate analysis, *Salvio-Festucetum rupicolae* Zólyomi 1958, synphysiology.

## Introduction

Temperate grasslands cover large areas of the Earth's vegetation (COUPLAND 1992), and they are locate in one of the regions where the impact of global climate change is predicted to be high (MITCHELL et al. 1990). The temperate grassland surface has large and increasing areas with arid climate. Even in the middle of Europe, Hungary has areas where the relatively low and unevenly distributed yearly precipitation results in semi-arid grasslands like loess grasslands. Loess grassland stands in the Carpathian Basin are among the richest in species considering the plant communities of the Pannonian vegetation. These stands are at the western edge of the continental-Eurasian forest steppe-steppe zone. Unfortunately, intact stands exist only as small patches today. The *Salvio-Festucetum rupicolae* association has several subassociations differing in their physiognomy.

These stands were dominated by *Festuca rupicola*, but differed in physiognomy, texture, spatial pattern, vegetation dynamical traits and physiological activity from each other. (VIRÁGH, BARTHA 1998). This may partly be caused by the constantly changing long term dynamism of spots of species (therophytes, hemitherophytes, stoloniferous species). The long term processes are frequently modified in rates or in directions of small scale disturbances. These rapid changes are expressed by degradation, decreased diversity of stands or invasion by advancing weeds or disturbance tolerant species.

Coenological and synphysiological examinations were carried out on three typical xerophilous loess grassland stands (*Carex humilis, Chamaecytisus austriacus* and *Stipa dasiphylla dominated*) of *Salvio-Festucetum rupicolae* Zólyomi 1958 community in the Gödöllõ Hills, near Gödöllõ (Isaszeg village). The synphysiological measurements were started in three apparently different and arbitrarily chosen types.

We were interested whether these types could be detected also by multivariate analysis from many coenological samples of the area. We also tried to reveal what kind of synphysiological traits are characterising the physiognomically distinct, adjacent, mosaic-like types of the *festucetosum sulcatae* subassociation.

## Materials and methods

### Study area, coenological survey

Sample plots were chosen between villages Isaszeg and Kerepes (230 m a. s. l.) in typical and continuous stands of the *Salvio-Festucetum rupicolae* association (ZÓLYOMI 1958). In this area the steppe grassland stands occupy marginal positions in the vicinity of Gödöllő Hills. The annual precipitation: 601 mm, the average temperature: 9,1°C, the medium daily maximum: 14,1°C, the medium daily minimum: 4,1°C. (KAKAS 1969). This flora spectrum is poor in characteristic species and contains several steppe species typical of the Central Range. Hungarian loess vegetation strictly related to eastern European steppe vegetation. This flora composition of western steppe fragments similar to e. g. Ukrainian steppe vegetation (ZÓLYOMI & FEKETE 1994), so these sample plots of this area could represent adequately the East European loess vegetation.

We tried to set up plots representative to this association, rich in species, diverse in physiognomy, far from plantations (*Populus* forest, hedges, etc.).

The synphysiological study was carried out in June (summer aspect) and in September (autumn aspect) 2000. During June we made physiological measurements both under stressed and under non-stressed conditions in the vegetation's fully developed phase, while in September measurements were made under wet conditions, but in the vegetation's senescent phase. The coenological survey was carried out on 3rd July 2001. Seventy five quadrats of 2 x 2 m were chosen. The species list and cover values are detected. The species composition consists of 62 species. Dominant species with decreasing cover values are the following: *Festuca rupicola, Carex humilis, Dorycnium germanicum, Cytisus austriacus, Chrysopogon gryllus, Stipa dasiphylla.* There are several subdominant species,

e. g.: *Phleum phleoides, Seseli osseum, Teucrium chamaedrys, Galium verum,Helictotrichon pubescens, Filipendula vulgaris, Euphorbia pannonica, Asperula cynanchica.* Characteristic and accessorial species the next: *Salvia nemoralis, Isatis tinctoria* and *Agropyron repens, Agropyron caninum, Centaurea sadleriana, Hypericum perforatum, Falcaria vulgaris, Dactylis glomerata, Anthericum ramosum, Adonis vernalis.* For statistical evaluation several ecological characters of species were taken from Hungarian Database 1.2 (HORVÁTH et al. 1995). The ecological indicator values of species (BORHIDI 1995) are given in percentage pro rata. These characters are: the relative temperature requirement of species (TB), soil acidity (RB), humidity (WB), nitrate supply (NB), relative light intensity (LB), continentality (KB) and the types of social behaviour (SOC). The nature conservation ranks (NCR) (Simon 1988) are given in 10 categories separated in two groups. Proportion of the first group (U-unique, KV-strictly protected, V-protected, E-native species, K-accessorial species, TP-nature pioneers) reflects natural conditions, proportion of the second group indicates the degree of degradation (TZ-disturbance tolerant native species, A-adventives, G-cultivated species, GY-weeds) compared to the Hungarian average. The categories of simplified flora element spectrum of Hungary (FL) contain the main groups completing with relationship of Pannonic viewpoint. This kind of simplification is suitable for the analysis. The coenosystematical groups (COENOS) are established for categories of the hierarchical system. Multivariate analysis on cover values of species was carried out by using the SYN-TAX program (PODANI 1993, 1997). For the cluster analysis the Czekanowsi-index was used (PODANI 1993, 1997).

### Synphysiological methods

$CO_2$-exchange, transpiration, air-temperature, relative humidity and vapour pressure, and stomatal conductance were measured by using a portable closed-loop IRGA gas exchange system (LI-COR 6200) sampling the air in a plexi chamber of 60 cm diameter (ground area of the chamber is 2826 $cm^2$) and 70 cm height with three replicate measurements in each plot (BALOGH et al. 2002). PPFD values were recorded and LAI was estimated using sunfleck ceptometers (Decagon). Canopy-surface temperature was measured with an infrared thermometer (MX4, Raytek). Soil water content was measured by a TDR reflectometer (ML2, Delta-T Devices) in three replications at 5, 10, 20 cm soil depths. Forty plots were measured in summer and 30 in autumn in each stand types.

## Results and discussion

### Coenological pattern

The dendrogram of the sample plots can be seen on Figure 1. Using the Czekanowski-index there are 7 well organized groups at 0.58 similarity value. The first group (1-37. sample plots) is characterized by the dominance of *Carex humilis, Chrysopogon gryllus, Chamaecytisus austriacus, Dorycnium germanicum, Teucrium chamaedrys* and the presence of *Phleum phleoides, Filipendula vulgaris (Carex* dominated type). This fragment has three levels: the tall grasses (50-70 cm), the hemikryptophyte and chamaephyte species (30-50 cm) and th therophytes (5-15 cm). The levels have compact stand.

The *Chrysopogon gryllus* is almost absent from the second group (2-50. samples), and there is less *Chamaecytisus austriacus, Phleum phleoides, Filipendula vulgaris, Dorycnium germanicum, Teucrium chamaedrys* too, however the dominance of *Festuca rupicola* is more pronounced, and the

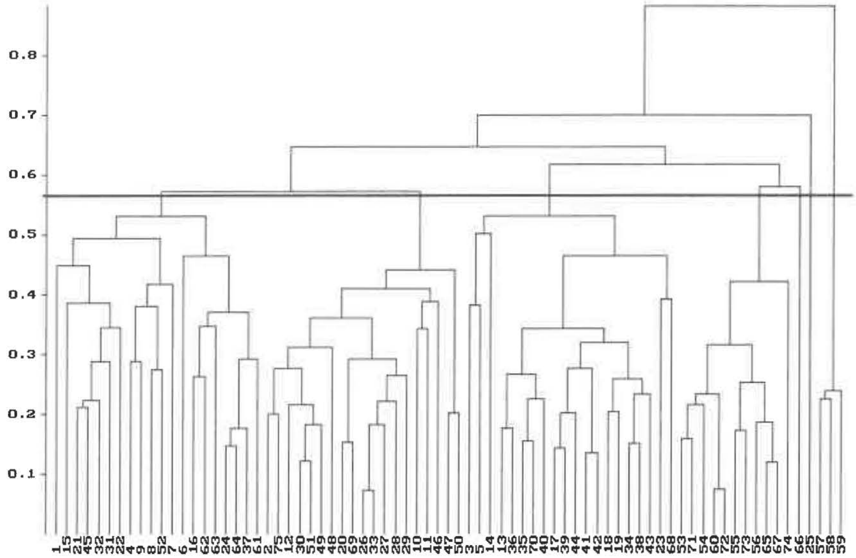


Figure 1. Dendrogram of sample plots of *Festucetum rupicolae* stand.

denominative species of this association, *Salvia nemorosa* is also present (*Cytisus* dominated type). This fragment is compact too, but smaller (50 cm) than the *Carex* type.

The third group (3-68.) (*Stipa* dominated type) is characterized by lower cover values of *Stipa dasiphylla, Chrysopogon gryllus, Chamaecytisus austriacus, Dactylis glomerata, Festuca rupicola, Filipendula vulgaris, Phleum phleoides, Teucrium chamaedrys, Helictotrichon pubescens.* The four other groups are different from each other in species composition and cover values, but are coupled by the presence of *Stipa dasiphylla.* This fragment is not so compact, it comprised loose and tall grass tufts and spots (50-70 cm) and smaller other hemikryptophyte and chamaephyte species. There are sevaral gaps between the grass tufts (30-40 cm).

Result of principal coordinates analysis (PCoa) shows 3 loose groups of sample plots (data are not shown), identical with the three types found by cluster analysis. The rest of sample plots contains rare species making them diverse and mosaic-like, not typical to festucetosum rupicolae subassosiation.

Analysing several standard coenological attributes the investigated area and the three grassland types can be characterized as follows. The distribution of temperature indicator values (TB) shows two peaks, similarly to the continentality diagram's two maximum values. The proportion of mesophyl and xerophyte species is more than 55 %. The high frequency of these species is characteristic for both open or closed grassland communities.

The distribution of relative humidity indicator values (WB) shows a normal bell-shaped form (Table 1.). Its maximum value is due to the high frequency of xerotolerant plants. The diagram is asymmetric in the case of xeromorph habitats.

The distribution of soil acidity values (RB) sign the high frequency of basiphylous species in the area's three types. 10 % of them are lime indicator species and do not occur on acidic soil (*Carex humilis, Chamaecytisus austriacus, Thalictrum pseudominus*).

Table 1. Percentage (%) distribution of relative humidity indicator values (WB) of the species in the investigated temperate loess grassland.

| categories | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| **WB values of Carex-type** | 0,15 | 3,75 | 20,6 | 5,86 | 1,2 | 1,65 | 0,15 |
| **WB values of Cytisus-type** | 0,18 | 4,42 | 19,3 | 6,63 | 1,29 | 1,47 | |
| **WB values of Stipa-type** | 0,18 | 4,59 | 19,6 | 6,53 | 0,53 | 1,94 | |

Distribution of relative nitrogen indicator values (NB) can be seen in Table 2. Half of the species are characteristic of habitats very poor in nitrogen, 10 % of them live in places extremely poor in nitrogen or in dry places (*Seseli osseum, Minuartia verna, Phleum phleoides*).

Table 2. Percentage (%) distribution of relative nitrogen indicator values (NB) of the species in the investigated temperate loess grassland.

| categories | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| **NB values of Carex-type** | 8,11 | 15,6 | 4,35 | 1,8 | 0,6 | 1,65 | 1,35 |
| **NB values of Cytisus-type** | 8,29 | 15,3 | 3,13 | 1,47 | 1,47 | 1,66 | 2,03 |
| **NB values of Stipa-type** | 8,11 | 18,2 | 3,17 | 1,06 | 0,18 | 1,94 | 0,71 |

In terms of temperature (TB), water (WB) and soil (RB) requirements the distribution and the maximum of values are similar in the three types. The tendency of the distribution of nitrogen requirement (NB) is similar to the others, but the rate of Stipa type is higher at the maximum value (9%), that at the Chamaecytisus type.

Distribution of relative light indicator values (LB) shows high ratio of light plants (45%), full light plants of open habitats (20%) and shadow tolerant ones (32%). There are only few typical shadow plants (Table 3.).

Table 3. Percentage (%) distribuiton of relative light indicator values (LB) of the species in the investigated temperate loess grassland.

| categories | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|
| **LB values of Carex-type** | 0,3 | 0,45 | 11,6 | 15 | 5,86 |
| **LB values of Cytisus-type** | 0,74 | 0,18 | 12,3 | 14 | 6,08 |
| **LB values of Stipa-type** | 0,35 | 0,71 | 10,9 | 12,5 | 8,82 |

The distribution of plants according to degree of continentality shows two peaks (Table 4.). The first maximum corresponds to the suboceanic species, mainly central European but expanding to East (*Bromus sterilis, Teucrium chamaedrys, Galium verum, Agrimonia eupatoria*), the second max-

Table 4. Percentage (%) distribuiton of species according to degree of continentality of the species in the investigated temperate loess grassland.

| categories | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|
| **KB values of Carex-type** | 1,35 | 9,46 | 5,56 | 4,05 | 9,61 | 3,15 |
| **KB values of Cytisus-type** | 1,1 | 10,3 | 4,79 | 3,31 | 12 | 1,84 |
| **KB values of Stipa-type** | 1,23 | 8,99 | 5,64 | 5,82 | 8,11 | 3,53 |

imum shows the continental-subcontinental species spread in East Europe (*Festuca rupicola, Isatis tinctoria, Silene otites*). The difference between the maximum values of light requirement (LB) and nitrogen requirement (NB) of the *Stipa* and *Chamaecytisus* type is nearly the same (8%). The values of continentality (KB) are markedly different being 10 % higher in the *Chamaecytisus* type, than in the *Stipa* type. This *Stipa* dominated group of plots is the most uniformly distributed out of the three types.

The distribution of categories of nature conservation values (SIMON 1988) shows native species are the most frequent (60%), native accessorial species are less abundant (20%). The proportion of disturbance tolerant native species is low (10 %), as the proportion of weeds, cultivated plants and adventitious species too (less, than 4%). There is however considerable quantity of strictly protected and protected species of Hungary (8 %), like *Stipa dasiphylla, Centaurea sadleriana, Adonis vernalis, Thalictrum pseudominus.*

According to distribution of floral elements (FL) the share by the Eurasian elements is the highest (27%), the submediterranean and the Pontic-Pannonian elements (e. g. *Chamaecytisus austriacus, Isatis tinctoria*) are present in considerable quantity (together 28 %) and there is a smaller quantity (10%) of the Pannonian (*Dianthus pontederae*) and the Pannonian-Balkan elements (*Euphorbia pannonica*). The proportion of cosmopolitan species is less than 5% (*Koeleria cristata*), and the rate of adventive species is low (1,5%, e. g. *Erigeron canadensis, Onobrychis viciifolia*). This shows that this area is more or less intact. The distribution of the floral elements shows that the *Stipa* type has the highest quantity of Eurasian, continental and submediterranean elements, in contrast to the *Carex* type that almost have the lowest levels of these. The *Chamaecytisus* group has the highest quantity of European and Pontic-Pannonian elements.

Table 5 shows the distribution of social behaviour types (SOC). Generalists (G) and the competitors (C) give half of the species. The cover by natural pioneers (NP)(*Myosotis stricta*) and specialists (S)(*Adonis vernalis, Thalictrum pseudominus* ) is remarkable, too (together 26 %). There are few (15 %) disturbance tolerant (DT) species (*Achillea collina, Agrimonia eupatoria, Galium verum*). The quantity of weeds (W), alien competitors (AC) and ruderal competitors (RC) is insignificant (*Viola arvensis, Descurainia sophia*), less than 4,5 %.

Table 5. Percentage (%) distribution of social behaviour types (SOC) of the species in the investigated temperate loess grassland.

| **categories** | c | s | g | np | dt | w | rc | ac |
|---|---|---|---|---|---|---|---|---|
| **soc. behav. typ. Carex-type** | 6,16 | 1,95 | 17,9 | 0,15 | 5,41 | 0,6 | 0,9 | 0,15 |
| **soc. behav. typ. Cytisus-type** | 5,16 | 1,66 | 18,2 | 0,37 | 5,16 | 1,29 | 1,47 | |
| **soc. behav. typ. Stipa-type** | 7,41 | 1,06 | 18,7 | | 5,29 | 0,18 | 0,53 | 0,18 |

The distribution according to the coenosystematic categories shows the group of indifferent species is comprised of several xerophilous species (*Hypericum perforatum, Melandrium album*) and species occurring due to anthropogenic disturbance (*Erigeron canadensis*), with a share of 14 %. Presence of forest elements shows the bushy character of this area. The rest are the elements of grassland associations (80 % of all). The distributions of the social behaviour type (SOC) and of the nature conservation rank categories (NCR) are similar in the three types.

The rate of monocotyledons/dicotyledons/Fabaceae shows (Table 6.) that the quantity of Fabaceae species is double in the *Stipa* type as compared to the two other groups (*Carex*, *Chamaecytisus*). Effects of a warmer and drier climate may favor the spread of species from the *Chamaecytisus* and the *Stipa* types.

Table 6. The percentage (%) rate of monocotyledons/dicotyledons/Fabaceae of the species in the investigated temperate loess grassland.

| categories | monocotyl | dicotyl | fabaceae |
|---|---|---|---|
| Carex-type | 8,69 | 24,63 | 2,17 |
| Cytisus-type | 10,52 | 22,8 | 1,75 |
| Stipa-type | 10,71 | 22,61 | 4,76 |

## Synphysiological characteristics

Synphysiological measurements were started in three apparently, physiognomically different types of the community. The above mentioned coenological considerations prove that there's many differences in the three types' composition, constraints by abiotic conditions, etc., but the physiological performance is not necessarily different.

Considering the whole dataset of summer (120 plots) and autumn (91 plots) aspect physiological measurements (temporal variability) it is obvious, that the average values and coefficient of variation of photosynthesis are higher in summer (Table 7.). Decreasing values of these variables till autumn is partly caused by senescence. The last two columns show that comparing summer water-stressed (56 plots) and non-stressed (64 plots) periods separately, CV values are much smaller in the latter case.

Table 7. Some data of the photosynthetical performance of a temperate loess grassland in summer and autumn 2000.

| | Summer aspect | Autumn aspect | Non-stressed summer period | Water-stressed summer period | Carex-type, non-stressed summer period | Carex-type, water-stressed summer period |
|---|---|---|---|---|---|---|
| Mean of LAI ($m^2 . m^{-2}$) | 3,09 | 3,84 | 2,91 | 3,30 | 1,64 | 3,66 |
| SD of LAI | 1,25 | 0,97 | 1,44 | 0,96 | 0,49 | 0,84 |
| CV of LAI (%) | 40,35% | 25,20% | 49,34% | 29,13% | 30,05% | 23% |

The estimated average value of LAI (Table 8.) was higher in autumn than in summer, but the method used for LAI-estimation does not distinguish photosynthetically active and fully senescent leaves. Nor did we pursue the interannual change of LAI, the measurements in summer may have not been at the highest LAIas it can be inferred from increasing average LAI with decreasing CV in June (non-stressed period: 2000. 06.08., 13., 15., 16.; water-stressed period: 2000. 06. 21., 22.).

Table 8. Some data of the estimated leaf area index.

| | Summer aspect | Autumn aspect | Non-stressed summer period | Water-stressed summer period | Carex-type, non-stressed summer period | Carex-type, water-stressed summer period |
|---|---|---|---|---|---|---|
| Mean of LAI ($m^2 . m^{-2}$) | 3,09 | 3,84 | 2,91 | 3,30 | 1,64 | 3,66 |
| SD of LAI | 1,25 | 0,97 | 1,44 | 0,96 | 0,49 | 0,84 |
| CV of LAI (%) | 40,35% | 25,20% | 49,34% | 29,13% | 30,05% | 23% |

Consideration of the three different types shows, that water shortage and senescence in autumn are responsible for the different physiological performances. In the well-watered summer period (soil water content: 12,38% in the *Carex*-type, 10 plots; 13,54% in the *Chamaecytisus*-type, 12 plots and 11,86% in the *Stipa*-type, 40 plots) we found significant difference only between the physiological activity of the *Carex*- and *Chamaecytisus*- dominated types, however this difference is due to the significant LAI-difference and after relating photosynthesis to 1 m2 leaf area, the difference is non-significant.

In the case of water-stressed summer period (soil water content: 6,55% in the *Carex*-type, 28 plots; 7,54% in the *Chamaecytisus*-type, 28 plots) photosynthesis were significantly different between the types and the same was found for the autumn period (soil water content: 7,02 and 11,33% in the Carex-type on the two days of investigation, 10,89% in the *Chamaecytisus*-type) (Table 9.).

Table 9. Some data of the photosynthetical performance of different types of the investigated grassland in different conditions. ( , ** means significant difference when referred to 1 $m^2$ leaf area)

| | Non-stressed summer period | | | Stressed /water deficient/ summer period | | Autumn aspect | | |
|---|---|---|---|---|---|---|---|---|
| | Carex-type | Cytisus-type | Stipa-type | Carex-type | Cytisus-type | Carex-type | Cytisus-type | Stipa-type |
| Mean of A ($\mu mol \cdot m^{-2} \cdot s^{-1}$) | 17,89 | 29,15 | 22,55 | 2,53(**) | 3,29(**) | 3,24(**) | 4,55(*,**) | 2,87(*) |
| SD of A | 8,00 | 13,72 | 10,35 | 1,53 | 1,32 | 1,83 | 1,60 | 1,38 |
| CV of A (%) | 44,71% | 47,07% | 45,9% | 60,45% | 39,97% | 56,39% | 35,23% | 47,9% |

These results contain both abiotic and biotic variability rates. At higher light intensities (PPFD>1000 $\mu mol.m^{-2}.s^{-1}$) the average $CO_2$ exchange rates were higher and CVs were lower (Table 10.) except for the case of the stressed period measurements.

There is a clear trend of increase of the value of photosynthesis and decrease of its CV (number of plots are: summer: 74, autumn: 44, water-stressed period: 24, non-stressed period: 50).

Table 10. Data of photosynthetical performance at saturating light conditions.

| | Summer aspect | Autumn aspect | Non-stressed summer period | Stressed /water deficient/ summer period |
|---|---|---|---|---|
| Mean of A ($\mu mol \cdot m^{-2} \cdot s^{-1}$) | 16,63 | 3,62 | 23,36 | 2,59 |
| SD of A | 12,69 | 1,66 | 9,80 | 1,60 |
| CV of A (%) | 76,34% | 45,72% | 41,94% | 61,79% |

## Conclusions

In a typical stand we tried to set up plots, which represents this *Salvio-Festucetum rupicolae* association. Statistical evaluation of several ecological characters of species was carried out together with a hierarchical cluster analysis. According to these analyses three groups dominated by *Carex humilis, Chamaecytisus austriacus* and *Stipa dasiphylla* of *festucetosum rupicolae* subassociation of a stand of *Salvio-Festucetum rupicolae* association were separated. Results of the synphysiological measurements show that water shortage and senescence in autumn are responsible for the different physiological performances of the three types. In the well-watered summer period we found significant difference only between the physiological activity of the *Carex*- and *Chamaecytisus*- dominated types, however this difference is due to the significant LAI-difference and after relating photosynthesis to 1 $m^2$ leaf area, the difference is non-significant.

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# Seasonal and daily pattern, temporal and spatial variability of ecosystem $CO_2$-exchange in a temperate Pannonian loess grassland

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**Abstract.** In the photosynthetically most active spring, summer and autumn vegetation period the investigated grassland did maintain a relatively strong daytime carbon gain. During winter the grassland displayed a slight daytime carbon loss. These data suggest that the grassland was a weak sink for carbon in the investigated period. $CO_2$-exchange variability during the day seemed to be independent from that of the daily photosynthetic radiation. Thus other factors like soil respiration, soil moisture content and temperature and their interactions could be responsible for the high daily variability of grassland $CO_2$-exchange. The considerable temporal (daily and seasonal) variability of the grassland $CO_2$-exchange can be considered as a characteristic feature of the grassland $CO_2$-exchange. In the investigated loess grassland vegetation the variability of $CO_2$-exchange showed clear dependence on measuring area, which is obvious in the CV of NEE. We hypothetised that the spatial scale with the lowest variability is the characteristic area of the grassland ecosystem's $CO_2$-exchange ($CO_2$-exchange physiological unit). In general decreased variability indicates a more regulated state.

**Keywords:** carbon-balance, temperate grassland, chamber technique, net ecosystem $CO_2$-exchange, carbon gain

**Abbreviations:** Net Ecosystem Exchange (NEE), Photosynthetic Photon Flux Density (PPFD), air temperature (Ta), $CO_2$-concentration (Ca), Coefficient of Variation (CV), Net $CO_2$-uptake (A), leaf area index (LAI)

## Introduction

Detailed leaf-, individual- and macroscale $CO_2$-exchange studies have long been conducted on grasslands (Breymeyer et al. 1996). On the other hand there are relatively few information available on the micro-scale level, more specifically in temperate grasslands including loess grasslands.

Out of the physiological processes, the carbon cycle based on photosynthesis-respiration balances of ecosystems is of primary importance. Grasslands are characterised by the fact that large part of their organic matter (C-content) is contained in the belowground living plant parts and in the soil (RICE AND GARCIA 1994) and this feature is important also in terms of their C-cycle. The temporal pattern of the C-balance of a grassland is also influenced by soil respiration and microbial activity (DÖRR AND MÜNNICH 1987). Investigation of the $CO_2$-exchange rates of these grasslands is necessary to estimate their significance in the global carbon cycle and global climate change.

Temporal dynamics of the photosynthetical activity and its relation to climate have been intensively studied up to the present date. There are less information available on the relationships between the climate and phenology (leaf structure phenology) and the temporal variability of the $CO_2$-exchange. Phenology should definitely be considered in a study conducted on a yearly temporal scale. Net $CO_2$-exchange is primarily affected by senescence in the autumn period and not by the abrupt weather changes (HAM AND KNAPP 1998).

Moreover, patches of a grass stand are not alike. Considerable structural variability can be perceived even in intensively managed grasslands with profound consequences on their primary functioning. This also implies the necessity of carrying out scale-dependent stand level physiological studies when spatial variability is concerned. Closed chamber techniques are suitable tools for studying small-scale spatial variability and dynamics of $CO_2$ gas-exchange (ANGELL ET AL. 2001).

The temperate Salvio-Festucetum rupicolae loess grassland (steppe) has also been investigated in structural, dynamical and conservation terms for decades resulting in a considerable amount of information in these subject (ZÓLYOMI AND FEKETE 1994, VIRÁGH AND FEKETE 1984). At the same time, studies conducted on the spatial organization of these grasslands have revealed that the spatial scale of the most important processes (species exchanges, coexistence patterns, and diversity) is in the order of a few $dm^2$ to $m^2$ (MUCINA AND BARTHA 1999).

The aims of the present work are: i) to explore the daily and seasonal courses of $CO_2$ gas-exchange at a fixed spatial scale, ii) to describe the temporal (daily and seasonal) and spatial variability (heterogeneity) of the photosynthetical activity and iii) to study the spatial scale dependence of $CO_2$ gas-exchange in a Pannonian loess grassland (steppe) ecosystem.

## Materials and Methods

### Study site

The measurements were conducted in the years 2000-2001 on the loess grassland situated at the village Kerepes (Gödöllő-Monor-Irsa Hills, 170 m a.s.l., 25 km south-east from Budapest). The climate is a temperate continental with hot dry summers and cold winters; mean annual precipitation 550-600 mm or less; annual mean temperature of 11 °C; and large annual amplitude of temperature changes (22 °C).

### The investigated loess grassland

The vegetation is a xeric temperate loess steppe (*Salvio-Festucetum rupicolae pannonicum* Zólyomi). The community is dominated by *Festuca rupicola, Chrysopogon gryllus, Stipa dasyphylla, Cytisus austriacus,* and *Carex humilis.*

The parent rock is sandy loess with thick humus- and nutrient-rich A layer. The original grassland is made up of more than 90 species. It is a perennial and overwintering, vertically well-structured (60-80 cm height) grassland, with many broad-leaved dicotyledonous species. The loess grassland can be considered as the representative of the European temperate xeric grassland (steppe) vegetation.

### Measurements of the net ecosystem $CO_2$-exchange rates

Net ecosystem $CO_2$-exchange rates (parallel with transpiration, air temperature, relative humidity, vapour pressure and stomatal conductance) were measured by using a portable closed-loop IRGA (LI-COR 6200, operated in absolute mode) sampling the air in a cylinder-shaped plexi-chamber of 60 cm diameter and 70 cm height, with three replicates in five plots. Mixing of the air in the chamber was achieved by operation of mixing fans. Ambient conditions (Ta and Ca) at the beginning of each measurement have been re-established by lifting the chamber while the fans were running. The duration of a measurement was 10 to 25 seconds, therefore the changes in the Ta and Ca in the chamber were small. PPFD values and canopy surface temperatures were recorded using ceptometers (Decagon) and an infra red thermometer (Raytek MX4). $CO_2$ gas-exchange values from five plots were used to calculate average net photosynthesis values and daytime C-balances. Coefficient of variation was also calculated for each average. Daily courses of gas-exchange have been measured from sunrise to sunset (1.5-2 hours intervals) seasonally, (04/10/2000, 20/03/2001, 23/05/2001, 03/07/2001) to consider phenological effects, too.

### Set-up for measuring spatial scale-dependence of the ecosystem $CO_2$-exchange

The set-up for estimating spatial dependence of $CO_2$ gas-exchange on measuring area consisted of six chambers with different diameters. Ground areas of the six gas exchange chambers follow a logarithmic scale with the diameter of the chambers doubling from 7.5 cm to 240 cm. The height of each chamber is 70 cm. The cylinder-jacket of the chambers has been arched from UV-B resistant water clean plexiglass. The air motion within the chambers is supplied by outer fan except for the two largest chambers, where the ventilation systems are within the chamber. The chambers are suited for measurements in closed system. The measurements have been carried out on 13th June 2001, in nine patches with three replications at each chamber size.

## Results and Discussion

### Daily courses of net $CO_2$-exchange in the four seasons

Daily courses of net ecosystem $CO_2$-exchange in the four seasons (including soil and root respiration in addition to photosynthesis), air temperature and PPFD average values from the five measured patches are presented (Fig. 1.). The aim was to describe the seasonal features of NEE, as based on these measurements.

### Autumn (04/10/2000)

Air temperature was higher than expected at this date and ranged between 16.2-32.9 °C. Cloudiness caused 20-30 % CV considering PPFD. There was a strong correlation between NEE and PPFD until midday. After midday, stomatal limitation of photosynthesis due to water shortage caused this correlation to become weaker at high Ta values. NEE reached its maximum (5.37 $\mu mol\ CO_2 m^{-2} s^{-1}$) in the early morning hours, this value was far below those measured in spring or summer, which is the consequence of the decreased photosynthetically active LAI due to the autumn senescence of many species.

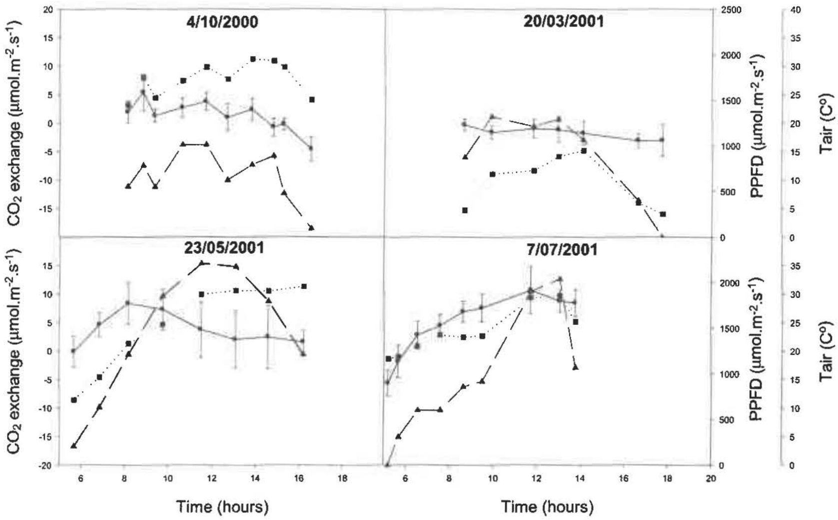


Figure 1.: Daily course of loess grassland $CO_2$ exchange (dots with error bars, solid line) measured on 60 cm diameter stand plots, of the photosynthetic photon flux density (PPFD, triangles, dashed line) and of air temperature (Tair, squares, dotted line) on four seasonally different days. One symbol represents the average value of five stand plots.

### Late Winter – Early spring (20/03/2001)

Soil respiration increased at Ta values above 15°C, balancing the $CO_2$-uptake. As a result NEE was negative throughout the day caused also by dawn frosts, small LAI values and below optimal Ta values for photosynthesis while PPFD was high.

### Spring (23/05/2001)

Growth is most intensive in loess grasslands in this period of the year. NEE followed the rapidly increasing Ta and PPFD values during the morning with the maximum of 9.51 µmol $CO_2m^{-2}s^{-1}$. From midday Ta was above 30°C and NEE declined with low soil water content (12% by volume) and high vapor pressure deficit of the air.

### Summer (03/07/2001)

NEE followed the rapidly increasing Ta and PPFD values during the morning with the maximum of 10.68 µmol $CO_2m^{-2}s^{-1}$. Ta range was narrower than that measured in May. Increasing cloud cover from early afternoon caused decreasing PPFD and hence NEE values, with the break of the measurements due to afternoon rains.

The observed large seasonal fluctuation of the daily $CO_2$-exchange rates also indicates that this pattern is subjected to large variation season by season and year by year due to the fluctuation of the climatic factors. Among others this inter-seasonal variability underlines the necessity of the continuous long-term measurements.

**Temporal variability of grassland $CO_2$-exchange**

Daily course of variability of NEE in representative, still rather variable, randomly selected patches of the loess grassland was investigated. PPFD data (average of the three replicates) and CV of NEE (of the three replicates) are presented for each patch (Fig. 2.). Large CV values in the early morning hours in May and July can be explained by the light intensity values around the compensation point (large part of the canopy is self-shaded), resulting in either positive or negative NEE values and therefore high standard deviation. CV values decreased later in the morning with the majority of values between 10-30%. Increase of CV (NEE) in the afternoon suggest water shortage induced, species and leaf specific, variable stomatal closure as the cause of high variation in NEE.

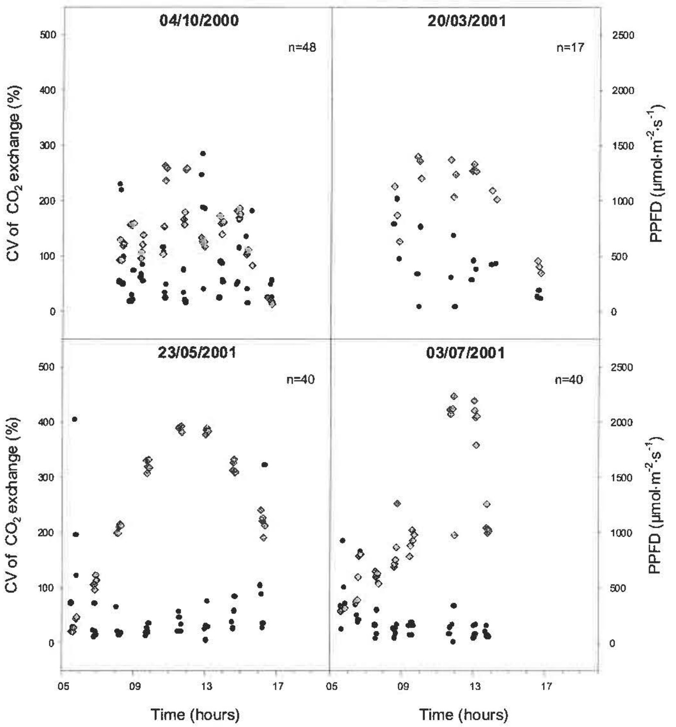


Figure 2.: Daily courses of the coefficient of variation of loess grassland $CO_2$ exchange measured on 60 cm diameter single stand plots (CV, dots) and of the photosynthetic photon flux density (PPFD, diamonds) on four seasonally different various days. (One symbol represents the CV value of three independent measurements on the same plots, n=number of measurements.)

Spatial variability of the litter decomposition and soil respiration rates are the probable causes of the highly varying NEE at the time of the spring measurement. High CVs of NEE in October are most probably caused by highly varying PPFD during the day as opposed to the situation experienced in May, when CV(NEE) was much smaller due to the steadily changing light conditions. Temporal variabilities of NEE in the patches and the average variability considering all the patches are presented in Tab. 1.

Concerning the daily temporal variability of grassland $CO_2$-exchange, one of the most remarkable observation was that after sunrise the $CO_2$-exchange variability during the day seems to be rather independent from the considerable changes of the daily photosynthetic radiation. Thus other factors like soil respiration, soil moisture content and temperature and their interactions can be responsible for the high daily variability of grassland $CO_2$-exchange. The considerable temporal (daily and seasonal) variability of the grassland $CO_2$-exchange can be considered as the characteristic feature of the grassland $CO_2$-exchange. This reflects the necessity of the high number and continuous measurements during the days and as much possible during the seasons.

Table1: Daily temporal and spatial variability of $CO_2$ exchance rates on five different plots with 60 cm diameter (plot 1- plot 5) of temperate loess grassland (04/10/2001). Variability is expressed as % value of variation coefficient (CV%).

| Periods of measurements | Plot 1 | Plot 2 | Plot 3 | Plot 4 | Plot 5 | Period's average |
|---|---|---|---|---|---|---|
| 08:07 - 08:24 | 54,0 | 229,0 | 219,0 | 49,0 | 98,0 | 98,1 |
| 08:44 - 08:58 | 18,0 | 16,0 | 29,0 | 21,0 | 73,0 | 58,5 |
| 09:17 - 09:30 | 991,0 | 330,0 | 66,0 | 84,0 | 54,0 | 92,5 |
| 10:34 - 10:49 | 115,0 | 108,0 | 34,0 | 24,0 | 48,0 | 61,9 |
| 11:43 - 11:55 | 34,2 | 74,6 | 21,3 | 18,5 | 14,2 | 41,0 |
| 12:43 - 12:56 | 245,8 | 187,9 | 283,9 | 39,5 | 185,4 | 246,9 |
| 13:46 - 13:59 | 23,9 | 89,8 | 85,9 | 56,7 | 52,8 | 80,9 |
| 14:43 - 14:56 | 48,7 | 52,5 | 114,6 | 164,0 | 167,3 | 212,5 |
| 15:15 - 15:36 | 135,0 | 39,7 | 15,4 | 513,3 | 180,8 | 568,9 |
| 16:27 - 16:43 | 24,4 | 22,5 | 47,6 | 55,6 | 24,9 | 46,0 |

### Spatial variability/spatial heterogeneity of grassland $CO_2$-exchange

The spatial variability of grassland $CO_2$-exchange rate can be seen as the sign of the spatial heterogeneity of the ecosystem $CO_2$-exchange. CVs of NEE (Tab. 1.) also demonstrate the spatially different behaviour of the five measured patches.

Coenological studies have proved that a few dm$^2$ to m$^2$ sampling unit size is suitable for finding the highest variability in species composition and combinations in this grassland (Mucina and Bartha 1999, Bartha et al.1997). Consequently, variability of LAI is also high at micro-scale (CV over 30% at 60cm) explaining in part the background of the spatial variability of $CO_2$-exchange.

From this one can conclude that parallel measurements of many unevenly distributed grassland plots with the same diameter are required.

### Daily maximum values of grassland $CO_2$-exchange and daytime carbon gains

Daily maximum values of NEE (Fig. 3.) are in good agreement with both the daytime carbon gain values (528, -864, 2624 and 2171 mgCm$^{-2}$ on the four representative days, respectively) and with the phenological stages of the vegetation. The maximum of NEE and hence the carbon balance are negative in March, the autumn NEE values are considerably lower than the ones in the summer. Consequently, in the photosynthetically most active spring, summer and autumn vegetation period the investigated grassland did presumably maintain a relatively strong daytime carbon gain, while during winter the grassland displayed a slight daytime carbon loss. The above data indicate that in the year of 2000-2001 the investigated overwintering grassland vegetation was very probably acting as a carbon sink.

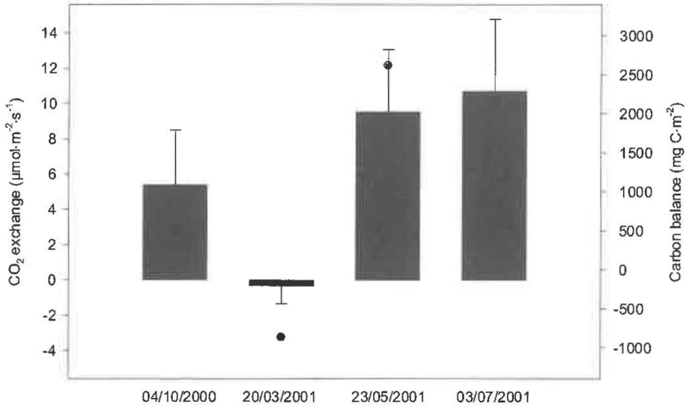


Figure 3.: Daily maximum values of $CO_2$ exchange rates (bars) and the carbon balance values of the investigated days (dots) in the temperate loess grassland.

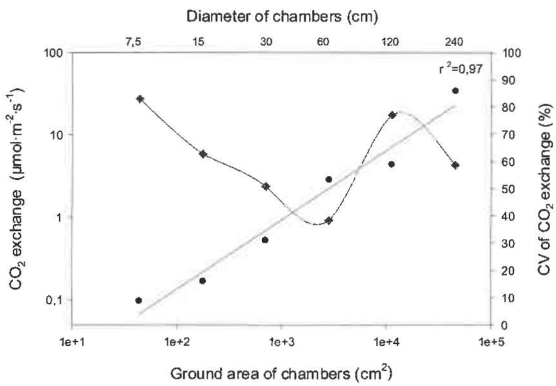


Figure 4.: Spatial scale dependence of $CO_2$ exchange (dots) and its coefficient of variation (CV, diamonds) in temperate loess grassland. (One symbol refers to nine plots measured in three separate replicates on 13/06/2001, the $CO_2$ exchange and the ground area values are plotted on a logarithmic scale.)

## Spatial scale-dependence of grassland $CO_2$-exchange

Spatial scale-dependence of NEE was investigated with different chamber sizes (different diameters, 70cm height). Logarithmic values are shown in Fig. 4. The regression $CO_2$-uptake values vs. chamber size shows good fit ($p<<0.01$). However CV of NEE shows scale-dependence with minimum of variability at 60 cm patch diameter, suggesting this scale to be characteristic unit of this grassland, where the suparindividual regulation is the most pronounced.

In the investigated loess grassland vegetation the variability of $CO_2$-exchange showed clear spatial scale-dependence. The most probable factors which are candidates for causes of variability pattern along the investigated space series are: the ratio of covered and uncovered soil surfaces, the spatial heterogeneity of soil moisture, soil temperature and litter deposition, the changes of species composition (e.g. dicots/monocots ratio, plant density), the height and the physiognomical and micrometeorological structure of the canopy in the relation to the changes of the botanical composition. Presumably the spatial scale with the lowest variability can be considered as the characteristic scale of the $CO_2$-exchange ($CO_2$-exchange physiological unit) of the grassland ecosystem. But this aspect and relationship between coenological (botanical composition) and physiological scale-dependence should be a matter of future detailed analysis. In general decreased variability indicates a more regulated state. Thus it is probably that the spatial scale with the lowest variability represents the supraindividually most regulated physiological – $CO_2$–exchange- units of the grassland.

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# Pollution of garden soils and vegetables in the Šalek valley

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**Abstract.** Soil pollution and accumulation of heavy metals in the most common vegetables were investigated in the Šalek Valley, where the largest Slovenian thermal power plant of Šoštanj (ŠTPP) is located. Methods were chosen according to international and Slovenian standards. Heavy metal content in soils and plants was determined by ICP-MS, ETAAS and hydride technique after appropriate digestion of samples. Results revealed that garden soils in the Šalek Valley are not polluted in general with heavy metals. On the contrary, some plant species exceeded permitted levels of Cd. Our results showed that the most sensitive group of plants are still affected, although emissions of heavy metals markedly decreased after the desulfurisation device was built at the ŠTPP.

**Key words:** Vegetables, Pollution, Heavy metal accumulation, Cadmium, Lead, Mercury, Arsenic.

## Introduction

High quantities of heavy metals are released into the environment by anthropogenic activities, such as mining, combustion of fuels and waste materials as well as other industrial processes. The fate and transformation of metals in a soil – plant system depends very much on soil type and prevailing soil conditions (Ross 1996). In general availability of metal decreases as the pH level rises and the contents of clay and humus increase (Miller & Donahue 1990). Besides uptake from soils, plants can also derive significant amounts of some elements through foliar absorption. It depends on the plant species, its nutritional status, the thickness of its cuticle, the age of the leaf, the presence of stomatal guard cells, the humidity at the leaf surface and the nature of the solutes (Alloway 1990). Heavy metals enter the biological cycle and are enriched in various plant organs (Bergman 1992). Plants in which roots or leaves are used for human or animal consumption are especially critical concerning the accumulation of heavy metals.

The main objective of the study was to assess the quality of soil and commonly grown vegetables which have been exposed to electricity production in the Šalek Valley.

## Material and methods

The field experiment with lettuce (*Lactuca sativa*), endive (*Cichorium endiviae*) and carrot (*Daucus carota* – Nantes) as test plants was carried out. 28 locations in distances within 10 km from the ŠTPP where included in the experiment. Altitudes of experimental plots ranged between 312 and 778 m above sea level. Seedlings of lettuce and endive were grown in the greenhouse; afterwards, 15 plantlets were transplanted to each location. Carrot was sown directly on the experimental sites. Each species occuped approximately 3 $m^2$ at each site. For each test species 12 – 15 plants were sampled. Individual parts of plants were separated as washed roots of carrot, washed and unwashed leaves of endive and lettuce. Plant samples were dried at 36°C and ground in an agate mortar. 250-500 mg DW plant samples were treated with 65 % $HNO_3$ acid. Heavy metal concentrations were measured in three replicates. The electrothermal technique was used for the determination of Cd, As and Pb (Perkin Elmer SIMAA 6000), flame atomic absorption spectrometry for the determination of Zn content (Analyst 100) and hydride technique for the determination of Hg content in plant after the acid dissolution technique with microwave heating (CEM MSP 1000 – Varian). Quality control was performed by comparison of results with the standard reference material NIST SRM 1515 Apple Leaves.

Soil samples were taken from the topsoil (0 – 20 cm). An average soil sample from each sampling site was prepared as a composite of 25 sub-samples taken from an area 250 $m^2$ in size (ISO 10381-1 1996). Soil samples were homogenized and ground in a ceramic grinder, then passed through a 2 mm plastic sieve before soil analysis (ISO 11464 1994). Pedologic soil parameters were determined as follows: pH in 0.1 M KCl solution, the content of organic matter by the Walkley-Black method, soil texture by pipette method, respectively (Janitzky 1986). For the analysis of metals content, the samples were ground further in an agate mill for 10 minutes then passed through 150 μm sieve. Heavy metal (Pb, Cd, As, Zn) content in soils was determined using flame and electrothermal atomic absorption spectrometric methods, while the hydride technique (Perkin Elmer SIMAA 6000) was used for the determination of Hg. A standard reference material »Montana Soil« was used for analytical quality control.

## Results and disscusion

A wide range of Pb, As, Cd, Zn and Hg content was determined in the garden soil of the Šalek Valley. Levels of heavy metal samples from the surface soil layer at the depth 0 – 20 cm are presented (Table 1).

Table 1: Heavy metals content of the surface soil (n = 28) in the Šalek Valley.

| | n | Cd $mgkg^{-1}DW$ | Pb $mgkg^{-1}DW$ | Zn $mgkg^{-1}DW$ | As $mgkg^{-1}DW$ | Hg $mgkg^{-1}DW$ |
|---|---|---|---|---|---|---|
| min. – max. | 28 | 0.3 – 1.3 | 23.6 – 77.9 | 92.0 – 348.0 | 4.8 – 138.0 | 0.07 – 0.48 |
| average | 28 | 0.76 | 45.65 | 181.08 | 16.39 | 0.18 |
| stdev | 28 | 0.25 | 12.18 | 59.24 | 24.17 | 0.09 |
| KV % | 28 | 33.41 | 26.69 | 32.71 | 147.49 | 52.35 |

The critical value for As (55 mg kg-1; Off. Gaz. Rep. Slov. 68/96) was exceeded twofold in the southern hilly margin of the Valley (Mali Vrh), thereofore this site has to be defined as polluted considering As. Levels of Cd, Pb, Hg and Zn do not exceed even warning values (Off. Gaz. Rep. Slov. 68/96), which were 2 mg kg-1, 100 mg kg-1, 2 mg kg-1 and 300 mg kg-1, respectively, thus soils are not treated as polluted considering Cd, Pb, Hg and Zn. Additionally, the pedological results suggest that soil characteristics do not induce high heavy metal accumulation in plants. pH levels ranged between 4.8 and 7.1 with an arithmetic mean of 6.5, content of organic matter ranged between 3.6 and 1.3 % with an arithmetic mean of 8.5, while the content of clay ranged between 5.4 and 18.4 % with an arithmetic mean of 8.2.

Most of metals in plants originate from root uptake, but foliar uptake of some metals (especially Cd) may be relatively high as well (KABATA PENDIAŞ & PENDIAS 1984). Leafy vegetables are especially sensitive to foliar heavy metal absorption (ZUPAN & al. 1995). Results of our study revealed that the highest content of heavy metals was observed in edible green parts of lettuce (Table 2). Statistical analysis confirmed a significantly higher concentration of Cd in lettuce in comparison with other vegetables (MannWhitney U test: $U = 3.5023$, $p < 0.001$).

Table 2: Ranges of heavy metals in vegetables from garden soils in the Šalek Valley.

| Vegetable | | | n | Cd mgkg$^{-1}$DW | Pb mgkg$^{-1}$DW | As mgkg$^{-1}$DW | Hg mgkg$^{-1}$DW |
|---|---|---|---|---|---|---|---|
| endive leaves | unwashed | min. – max. | 28 | 0.14 – 1.78 | 0.08 – 3.64 | 0.05 – 1.46 | 0.01 – 0.06 |
| (*Cichorium endivae*) | | average | | 0.40 | 0.83 | 0.31 | 0.01 |
| | | stdev | | 0.33 | 0.78 | 0.33 | 0.005 |
| | | KV % | | 84.34 | 93.90 | 107.88 | 30.79 |
| | washed | min. – max. | 28 | 0.12 – 2.2 | 0.08 – 2.0 | 0.04 – 1.0 | 0.01 – 0.05 |
| | | average | | 0.42 | 0.36 | 0.11 | 0.03 |
| | | stdev | | 0.44 | 0.45 | 0.10 | 0.04 |
| | | KV % | | 105.68 | 124.83 | 94.65 | 175.05 |
| lettuce leaves | unwashed | min. – max. | 23 | 0.3 – 4.4 | 0.53 – 5.9 | <1.0 – 5.86 | 0.04 – 0.08 |
| (*Lactuca sativa*) | | average | | 0.88 | 1.61 | / | 0.06 |
| | | stdev | | 0.88 | 1.19 | / | 0.01 |
| | | KV % | | 99.94 | 73.60 | / | 23.78 |
| | washed | min. – max. | 28 | 0.2 – 4.0 | 0.33 – 3.37 | <1.0 – 1.53 | 0.05 – 0.08 |
| | | average | | 0.96 | 0.97 | / | 0.07 |
| | | stdev | | 1.21 | 0.77 | / | 0.01 |
| | | KV % | | 125.60 | 79.57 | / | 14.85 |
| carrot roots | washed | min. – max. | 28 | 0.19 – 1.4 | 0.41 – 1.03 | < 0.1 | < 0.05 |
| (*Daucus carota*) | | average | | 0.48 | 0.57 | / | / |
| | | stdev | | 0.30 | 0.12 | / | / |
| | | KV % | | 61.95 | 21.71 | / | / |

Over 95 % of washed lettuce samples exceeded the prescribed value for Cd in vegetables, which is 0.3 mgkg-1 DW (Off. Gaz. Soc. Fed. Rep. Yug. 59/83). Differences between Cd content in washed and unwashed edible green parts of vegetable were trivial, which suggest that a small fraction of Cd penetrates through the leaf cuticle (Figure 1A).

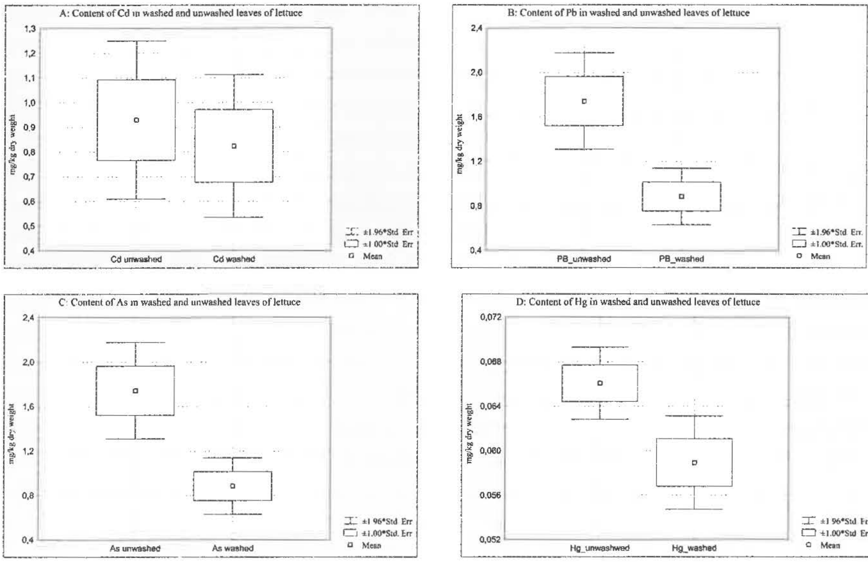


Figure 1(A – D): Differences between Cd, Pb, As and Hg content in washed and unwashed edible green parts of lettuce (*Lactuca sativa*).

On the contrary, Pb, As and Hg content significantly differed between washed and unwashed leaves and roots of carrot (Figure 1 B – D). Results also revealed that vegetable is not polluted in general with Pb, As and Hg, although on some plots (Topolšica, Mali Vrh) even washed lettuce leaves exceeded prescribed values, which are 3 $mgkg^{-1}$ and 1 $mgkg^{-1}$ DW for Pb and As, respectively (Off. Gaz. Soc. Fed. Rep. Yug. 59/83).

## Conclusions

It was estimated that ŠTPP had emitted 0,2 t of Cd, 22,1 t of Pb, 4,5 t of As, 0,3 t of Hg and 298 t of Zn in the period 1981 – 2001 (Pokorny 2003). After the desulfurisation device was built in 1995 the annual dust emission decreased from 8121 t in 1994 to 1077 t in 1999 (Sevšek 2000). Although exact data on heavy metal emissions are not known, our results revealed that atmospherical inputs, enriched with Cd, still affect the most sensitive group of plants. A high content of Cd in test plants is consistent with the data, which confirmed a significantly higher content of Cd in the surface layer of soil (0 – 5 cm) in comparison with deeper layers (5 – 20 cm, 20 – 30 cm) (Kugonič & Stropnik 2001).

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# Harmful impact of exceptional cold air outbreak in april 1997 on silver fir in Croatia

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**Abstract.** After a severe outbreak of cold air in mid and late April in 1997, locally occurring damages on twigs and needles of silver fir were recorded in some forests in Croatia. Field examinations in the first half of July 1997 revealed the damages on one-year and older needles while fully developed twigs of current year growth were left intact. Southern and western slopes with fir growing forests represented typically affected sites. Further laboratory analyses confirmed the field observation that no harmful insect or pathogens were the cause of these damages. Spread of symptoms, expressed more heavily at lower elevations, indicated a probable connection with recent exceptionally cold air outbreak in mid-April. Physiological disturbances caused by potassium deficiency are discussed in the light of specific occurrences of damage symptoms on silver fir trees.

**Key words:** Abies alba, cold air damages, potassium.

## Introduction

Abiotic damages on forest trees are well documented and known in forest science and practice for a long time (EDLIN & NIMMO 1956). Among the commonest are injuries caused by cold air and late or early frost (REIF & PAPP 1995). Very often, such damages occur on tree species not fully adapted to their artificial habitat (such as plantations) or plants growing in the border zones of their natural area of spread. In the case of silver fir (*Abies alba* Mill.) cold air damages are not common but are documented and known in the literature (HARTMANN & al. 1988). This paper deals with the rare event of atypical damages on silver fir in natural forest stands of central Croatia in the spring of 1997. Change of needle colour and dieback of twigs and branches was recorded in a relatively isolated area of roughly 40 ha with no visible cues to known biotic agents. At the same time, it was the year of extraordinary atmospheric events and cold air outbreak which occurred during mid and late April and hit the whole country, especially the maritime and mountainous region (BRZOVIĆ 1999). As with the other tree species, the nature of cold hardiness of silver fir is strongly correlated with potassium physiology as potassium is known to increase frost resistance. Potassium deficient plants are more prone to winter damage (BAULE & FRICKER 1970, MENGEL & KIRKBY 1987, BERGMANN 1992).

Based on the data of Diagnostic and Prognostic Service of Forest Research Institute Jastrebarsko, on reported atypical symptoms on silver fir trees in May 1997, in the north Vinodol area (NW Croatia), this research was initiated to find out the possible causes. Impacts of phenological, climatic and physiological factors were analysed in relation with spatially and temporally occurring damages in the research area.

## Materials and methods

Samples were collected and trees were analysed on July 10, 1997, at two locations Treskavac and Kalić in the north Vinodol area. Both sites are located at altitudes between 900 and 960 m, with inclination from 0 and 10 °. The site Treskavac covers an area of 17.11 ha, with various expositions, and the site Kalić covers an area of 19.92 ha, with SW exposition. Sampled fir trees were randomly selected, felled and debarked to find out the possible presence of defoliators, suckers or xylophages (expectedly, bark beetles) and pathogenic fungi (wood decay and root rot fungi).

Samples of needles, twigs and branches, with observed visible symptoms, were collected from sampled trees and randomly selected young firs. Standard laboratory analyses were carried out to examine the presence of possible harmful insect fauna or pathogenic fungi.

In the year 2000, the second study was conducted to determine the possible influence of nutrient dynamics on cold hardiness of silver fir, 5 plots (each 100 x 100 m) were chosen in the region of Gorski kotar: Belevine – at altitude of 800 m, SW exposition; Kupjački vrh – at altitude of 1000 m, SW exposition; Leska S – at altitude of 697 m, NW exposition; Leska D – at altitude of 708 m, NW exposition. Aerial distances between these plots and sites Treskavac and Kalić are approximately 20 km. The 5th plot Sljeme was chosen on Medvednica (near Zagreb), at altitude of 954 m, S exposition. Current and 1-year old needles from a total of 141 trees were sampled monthly from February to November. Samples (24-36 trees per location, representing a 30 % size sample of all dominant and co-dominant trees) were pooled by location into a composite sample. Samples were dried at 105 °C and ground. After wet digestion ($H_2SO_4$ / $HClO_4$) potassium was determined through flame photometer Eppendorf (AOAC 1996).

Climatic data for 1996 and 1997 were obtained from the State Hydrometeorological Institute Zagreb.

## Results

Field examinations of affected silver firs revealed irregularly distributed dead needles and twigs resulting with reddish tint of some parts of the crowns or whole trees. In adult trees some branches, randomly positioned in the crowns, showed the abovementioned symptoms. A definitive pattern of damage symptoms was observed on smaller trees and fir saplings: significantly highest proportion of one-year old needles was affected (those developed a year before). In most cases there was also an inexistence of current growth on these shoots. All of these symptoms were recorded with greater intensity at lower elevations in the research area.

Examination of sampled trees in the field obtained no visible attack of sap suckers, defoliators, secondary xylophages nor wood decay or root rot fungi. Laboratory analyses of samples confirmed the field results. There were no pathogenic fungi, neither insect pests as possible causal agents of described symptoms in sites Treskavac and Kalić.

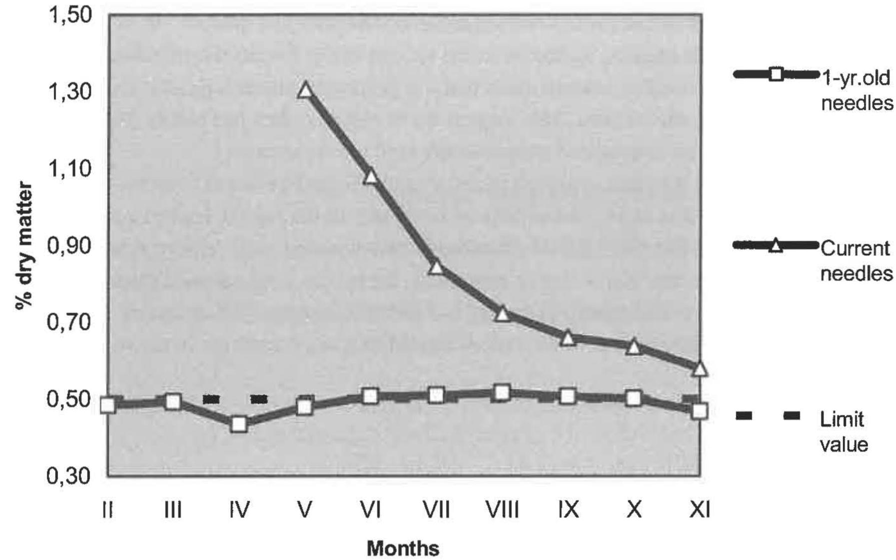


Figure 1: Seasonal dynamics of potassium concentrations in silver fir needles in the year 2000 (averaged values from five locations). Limit value (0.50 %) after BERGMANN (1992).

Dynamics of potassium concentrations in needles revealed a drop of $K^+$ concentration in older needles due to its redistribution into younger tissues (Figure 1). Translocation of $K^+$ from one-year old needles (developed in 1999) towards buds is apparent just before the budburst in April, resulting in strong potassium deficiency and high $K^+$ concentrations in current-year needles.

## Discussion

The hypothesis on biotic complex as causal agents for the new case of silver fir dieback, based on the aetiology of already recorded damages in the area, was rejected. In spite of the large amount of wind-felled trees and woody debris, detailed field analysis of newly affected trees and laboratory check-up could not confirm the presence of any significant biotic agent. Spatial distribution of damaged trees, as well as their age-class structure, suggest that some abiotic factor must have had been involved in this event.

A period of exceptionally low temperatures in mid and late April 1997 (both distinct minima, April 16th and 21th, were lower than two standard deviations of the 30-year average) served as a basis for case study by which BRZOVIĆ (1999) explained the mechanisms of local modifications of larger scale atmospheric processes in the area of Northern Mediterranean.

What we consider highly relevant for this study is (I) the fact that the outbreak of cold air in April was one of the strongest ever recorded and (II) the affected area is considered a border zone of naturally growing fir because of the milder climate due to the strong maritime influence from the west. This conforms to the conclusions by FORSTER & al. (1988) and FORDERER & al. (1990) in the case of similar large scale damages recorded in Switzerland after the winter 1986/87.

According to BERGMANN (1992) potassium deficient plants are more prone to winter damage. Older leaves (needles) first reveal symptoms of potassium deficiency (ANIĆ 1973). The dynamics of potassium in the silver fir needles, as shown in the second study, reveals the translocation of potassium from one-year old needles towards buds that are becoming physiologically active, leading to potassium deficiency in older tissues. This drop of $K^+$ in older needles just before budburst in April coincided in 1997 with the outbreak of exceptionally cold air in the area.

Synthesis of all available data, gathered in the area of affected trees and from the separate study analyzing the nutrient status and cold hardines of silver tree in the region, lead to a conclusion that it must have been a 1997 exceptional cold air outbreak that initiated such damage symptoms. This is further backed up by the fact that at higher elevations, hit by the same climatic phenomenon, were no damages recorded. It is reasonable to deduct that the phenological differences of fir trees in different altitudes acted as key factors in the distribution of damage symptoms in the wider area.

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# Algae of specific environments in Slovenia

## Alge posebnih okolij v Sloveniji

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**Abstract.** Algae studies were carried out in some specific biotopes of Slovenia: eutrophic lakes (Koseški bajer, Sotelsko jezero), peat bogs (Lovrenška jezera, Šijec), waterfalls (Savica, Krka falls, small waterfall on Pohorje), springs (springs at Medvedje Brdo and Pohorje, hot spring at Terme Čatež, mineral spring at Rimski vrelec), brackish waters (Fiesa lake, Dragonja estuary), hypereutrophic biotopes (manure water, constructed wetlands), aerial biotopes (concrete wall, stony wall, lime tree trunk (*Tilia platyphyllos*), limestone rock, Krška jama cave) and the Dragonja River.

Samples were taken seasonally at 26 sampling sites in 1998, 1999, 2000 and 2001. Similarity in species structure and abundance of algae were determined with the Bray-Curtis coefficient of similarity.

Altogether, 537 species and subspecies of algae (of nine classes) were determined and most of them belonged to Bacillariophyceae. 146 species and subspecies were identified for the first time in Slovenia; of these 107 belonged to Bacillariophyceae, 28 to Cyanophyceae, six to Chlorophyceae, four to Zygnematophyceae and one to Xanthophyceae.

The appearance of almost half of all species and subspecies was limited to a single sampling site. Eight species (*Achnanthes minutissima, Cymbella affinis, Cymbella silesiaca, Gomphonema angustum, Navicula veneta, Navicula* sp., *Phormidium* sp. and *Trentepohlia aurea*) were present in more than half of all specific biotopes.

**Key words:** algae, eutrophic lakes, waterfalls, springs, peat bogs, aerial biotopes, constructed wetlands, caves, brackish waters, manure waters

**Izvleček.** V prispevku je predstavljena raziskava alg v nekaterih posebnih biotopih v Sloveniji: eutrofnih jezerih (Koseški bajer, Sotelsko jezero), visokih barjih (Lovrenška jezera, Šijec), slapovih (Savica, slapovi na Krki, slapič na Pohorju), izvirih (izvir na Medvedjem Brdu in Pohorju, termalni izvir v Termah Čatež, mineralni izvir Rimski vrelec), brakičnih vodah (jezero v Fiesi, izliv Dragonje), hiperevtrofnih biotopih (gnojevka, rastlinskih čistilnih napravah), kopenskih biotopih (betonski zid, kamniti zid, deblo lipe (*Tilia platyphyllos*), apnenčasta skala, Krška jama) in reki Dragonji.

Vzorci so bili odvzeti na 26 vzorčnih mestih v letih 1998, 1999, 2000 in 2001. Z Bray – Curtisovim koeficientom podobnosti je bila ugotovljena podobnost v vrstni strukturi in abundanci alg.

Skupno je bilo določenih 537 vrst in podvrst alg iz devetih razredov, po številu določenih vrst so prevladovale Bacillariophyceae. 146 vrst in podvrst je bilo v Sloveniji prvič zabeleženih, od tega jih 107 pripada razredu Bacillariophyceae, 28 razredu Cyanophyceae, šest razredu Chlorophyceae, štiri razredu Zygnematophyceae in ena razredu Xanthophyceae.

Pojavljanje skoraj polovice vseh določenih vrst in podvrst je bilo omejeno na posamezna vzorčna mesta. Osem vrst (*Achnanthes minutissima, Cymbella affinis, Cymbella silesiaca, Gomphonema angustum, Navicula veneta, Navicula* sp., *Phormidium* sp. in *Trentepohlia aurea*) je bilo prisotnih v več kot polovici vseh posebnih biotopov.

**Ključne besede:** alge, eutrofna jezera, slapovi, izviri, visoka barja, kopenski biotopi, rastlinske čistilne naprave, jame, brakične vode, gnojevka

## Introduction

Algae are present in sea water, brackish water, freshwater and in aerial biotopes. Same of them exist also in specific environments like snow, hot springs, caves, peat bogs, etc. In Slovenia, the number of specific environments is very large because of its geographic diversity and a relatively low degree of pollution. Algae in such environments have been investigated only partly, despite the exceptional species diversity.

Because most algal species are spread worldwide, they are not considered to be an endangered group of organisms. It is also difficult to consider a single algal species to be endangered. Changing or even disappearing of some ecosystems (desiccation of swamps, regulation of rivers, building of artificial lakes, polluted rivers, etc.) causes quality and quantity changes in algal associations. In most cases, the species diversity lowers and consequently, the numerousness of species, which are tolerant to changes in ecosystems, increases. In the case of a permanent change of ecosystems (desiccated swamps and peat bogs, etc.), the algal species in such areas could be lost forever (Kosi & Vrhovšek 1996).

We investigated algae in some specific biotopes of Slovenia, i.e. the biotopes with low pH, high temperature, high or low conductivity, low light, high velocity of water flow, low humidity, the environments rich in food, etc. Our purpose was to establish the species structure and the abundance of species and subspecies in those biotopes. With regard to special ecological conditions, particular attention was given to the species and subspecies present only in specific biotopes and to those existing in various biotopes. The study was carried out in biotopes of Slovenia that have been investigated only partly or never before: eutrophic lakes (Koseški bajer, Sotelsko jezero), peat bogs (Šijec, Lovrenška jezera), springs (spring at Medvedje Brdo, spring on Pohorje, hot spring at Terme Čatež, mineral spring at Rimski vrelec), waterfalls (Savica, Krka falls, small waterfall on Pohorje), aerial biotopes (concrete wall, stony wall, lime tree trunk (*Tilia platyphyllos*), limestone rock, Krška jama cave), the Dragonja River, brackish lake at Fiesa, manure water and two constructed wetlands (Barje, Dragonja). Altogether, 26 sampling sites were investigated. For description of sampling sites, see KRIVOGRAD KLEMENČIČ (2001).

## Materials and Methods

Samples were taken seasonally in 1998, 1999, 2000 and 2001. For the dates of sampling, see KRIVOGRAD KLEMENČIČ (2001). In lakes, peat bogs, springs, waterfalls and in the Dragonja River, the samples of periphyton for qualitative analysis were brushed from the surface of stones, rocks, wood, macrophytes and other sunk objects like bottles, plastic and iron objects, etc. In peat bogs, the Krka falls, the spring at Medvedje Brdo and the small waterfall on Pohorje, the samples of periphyton for qualitative analysis were also squeezed out of water mosses. In the mineral spring at Rimski vrelec and the hot spring at Terme Čatež, the samples of periphyton were brushed from the bottom and the walls of the fountains.

Terrestrial algae were brushed from a limestone rock, a concrete wall, a stony wall and a lime tree trunk. In the Krška jama cave, algae were brushed from the rock bellow the light, which was most distant from the cave entrance. The samples of manure water were taken on the grassland above the manure pit. The samples of the Barje constructed wetland were taken from the collecting pool. The samples of phytoplankton were taken also in lakes (Koseški Bajer, Sotelsko jezero, Fiesa lake), peat bogs (Lovrenška jezera, Šijec) and in the Dragonja estuary.

The samples were immediately bottled and preserved in a four per cent solution of formaldehyde, except the samples of manure water and the samples from both constructed wetlands, which were first examined and then preserved. All samples were treated by concentrated $HNO_3$ to determine the species from the Bacillariophyceae class.

We determined the species and subspecies of algae using a light microscope and according to the following determination keys: LAZAR (1960), BOURRELLY (1966, 1968, 1970), STARMACH (1966, 1968, 1972, 1974, 1977, 1980, 1983), GOLUBIĆ (1967), KRAMMER & LANGE-BERTALOT (1986, 1988, 1991a, 1991b), POPOVSKY & PFIESTER (1990), HINDAK (1978, 1996), CVIJAN & BLAŽENČIĆ (1996), LENZENWEGER (1996, 1997). Abundance was estimated by numbers from 1 to 5 (1-single, 2-rare, 3-customary, 4-frequent, 5-dominant). Similarity in species structure and the abundance of algae were determined by the Bray-Curtis coefficient of similarity.

## Results and Discussion

Altogether, 537 species and subspecies of algae (of nine classes) were determined. Most of them (295) belonged to Bacillariophyceae, 116 belonged to Cyanophyceae, 58 to Chlorophyceae, 44 to Zygnematophyceae, eight to Xanthophyceae, six to Dinophyceae, five to Euglenophyceae, three to Chrysophyceae and two to Florideophyceae. For algal species lists with the estimation of abundance for individual sampling sites, see KRIVOGRAD KLEMENČIČ (2001).

Figure 1 shows the algal structure by classes at all 26 sampling sites. In the eutrophic lakes of Koseški bajer and Sotelsko jezero, the Savica waterfall, the Krka falls, the small waterfall on Pohorje, the spring at Medvedje Brdo, the mineral spring at Rimski vrelec, the peat bog at Lovrenška jezera, the Krška jama cave, the Fiesa brackish lake and the Dragonja River, most species and subspecies belonged to Bacillariophyceae, while in the Šijec peat bog those belonging to

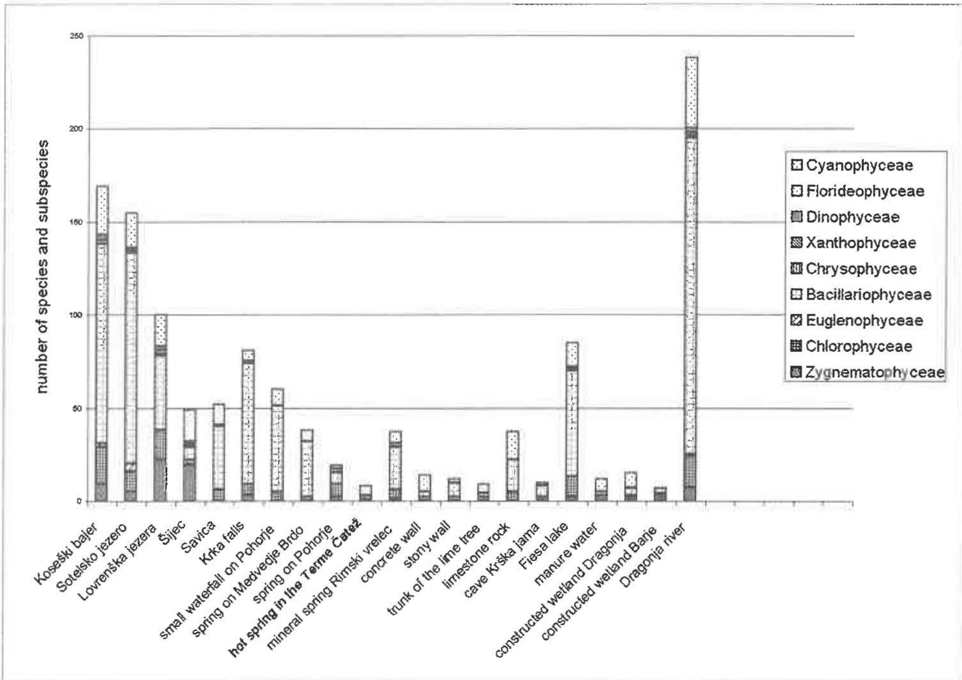


Figure 1: Algal structure by classis at all sampling sites
Slika 1: Sestava alg po razredih na vseh vzorčnih mestih

Zygnematophyceae were predominant. In the spring on Pohorje, most species and subspecies belonged to Chlorophyceae and in the hot spring to Cyanophyceae. Cyanophyceae was predominant also in manure water, the Dragonja constructed wetland and in aerial biotopes (concrete wall, trunk of the lime tree). In the other two aerial biotopes (stony wall, limestone rock), most species and subspecies belonged to Bacillariophyceae.

146 new species and subspecies in Slovenia were determined, of these 107 belonged to Bacillariophyceae, 28 to Cyanophyceae, six to Chlorophyceae, four to Zygnematophyceae and one to Xanthophyceae (Table 1).

Table 1: List of species and subspecies new to Slovenia
Tabela 1: Seznam vrst in podvrst novih za Slovenijo

| taxon |
|---|
| **CYANOPHYTA** |
| CYANOPHYCEAE |
| *Anabaena affinis* Lemm. |
| *Borzia trilocularis* Cohn |
| *Calothrix thermalis* (Schwabe) Hansg. |
| *Gloeocapsa bituminosa* (Bory) Kuetz. |
| *Lyngbya cryptovaginata* Schkorbatoff |
| *Lyngbya hieronymusii* Lemm. |
| *Lyngbya perelegans* Lemm. |
| *Microcystis hansgirgiana* (Hansgirg) Elenkin |
| *Microcystis viridis* (Braun) Lemm. |
| *Nostoc spongiaeforme* Agardh |
| *Oscillatoria laetevirens* (Crouan) Gomont |
| *Phormidium angustissimum* W. & G.S. West |
| *Phormidium dimorphum* Lemm. |
| *Phormidium henningsii* Lemm. |
| *Phormidium lignicola* Fremy |
| *Phormidium rotheanum* Itzig. |
| *Phormidium setchelianum* Gomont |
| *Phormidium valderiae* (Delp.) Geitler |
| *Plectonema terebrans* Bornet & Flahault |
| *Pseudanabaena papillaterminata* (Kisselev) Kukk |
| *Pseudospirulina amoena* Pankow & Jahnke |
| *Schizothrix friesii* (Agardh) Gomont |
| *Spirulina flavovirens* Wislouch |
| *Spirulina gomontiana* (Setchell) Geitler |
| *Spirulina meneghiniana* Zanar. |
| *Spirulina tenuissima* Kuetz. |
| *Synechocystis septentrionalis* Skuja |
| *Tolypothrix cucullata* Jaag |
| |
| **HETEROKONTOPHYTA** |
| XANTHOPHYCEAE |
| *Characiopsis minima* Pasch. |
| |
| BACILLARIOPHYCEAE |
| *Achnanthes amoena* Hust. |
| *Achnanthes catenata* Bily in Marvan |
| *Achnanthes lanceolata* ssp. *dubia* (Grun.) Lan.-Bert. |
| *Achnanthes lanceolata* ssp. *frequentissima* Lan.-Bert. |
| *Achnanthes oblongella* Oestrup |
| *Achnanthes septata* A. Cleve |
| *Amphora angusta* (Greg.) Cleve |
| *Bacillaria paradoxa* Gnalin |
| *Caloneis molaris* (Grun.) Kramm. |
| *Cymatopleura solea* var. *apiculata* (W.Smith) Ralfs |
| *Cymbella caespitosa* (Kuetz.) Brun |
| *Cymbella cuspidata* Kuetz. |
| *Cymbella descripta* (Hust.) Kramm. & Lan.-B. |
| *Cymbella gaeumannii* Meister |
| *Cymbella pusilla* Grun. |
| *Cymbella tumidula* var. *lancettula* Kramm. |

*Denticula kuetzingii* Grun.
*Denticula subtilis* Grun.
*Diatoma ehrenbergii* Kuetz.
*Diatoma moniliformis* Kuetz.
*Epithemia turgida* var. *granulata* (Ehr.) Brun
*Eunotia circumborealis* Noerpel in Lan.-Bert.
*Eunotia denticulata* (Breb.) Raben.
*Eunotia microcephala* Krass.
*Eunotia paludosa* Grun.
*Fragilaria biceps* (Kuetz.) Lan.-Bert.
*Fragilaria capucina* var.*mesolepta* (Rab.) Rab
*Fragilaria montana* (Krass.) Lan.-Bert.
*Fragilaria parasitica* var. subconstricta Grun.
*Frustulia spicula* Amosse
*Gomphonema amoenum* Lan.-Bert.
*Gomphonema clevei* Hust.
*Gyrosigma nodiferum* (Grun.) Reimer
*Gyrosigma tenuissimum* (W.Smith) Cleve
*Gyrosigma wansbeckii* (Dankin) Cleve
*Melosira moniliformis* (Muell.) Agardh
*Melosira nummuloides* (Dillwyn) Agardh
*Navicula accomoda* Hust.
*Navicula aerophila* Krass.
*Navicula angusta* Grun.
*Navicula bryophila* Pet.
*Navicula capitata* var. *capitata* Ehren.
*Navicula cincta* (Ehren.) Ralfs & Prit.
*Navicula duerrenbergiana* Hust.
*Navicula erifuga* Lan.-Bert.
*Navicula goeppertiana* (Bleisch) H.L. Smith
*Navicula gregaria* Donkin
*Navicula harderii* Hustedt
*Navicula heufleriana* (Grun.) Cleve
*Navicula incertata* Lan.-Bert.
*Navicula integra* (W.Smith) Ralfs
*Navicula libonensis* Schoeman
*Navicula margalithii* Lan.-Bert.
*Navicula menisculus* var. *upsaliensis* Grun.
*Navicula mutica* var. *ventricosa* Cleve & Grun.
*Navicula nivalis* Ehren.
*Navicula oppugnata* Hust.
*Navicula pseudokotschyi* Lan.-Bert.
*Navicula recens* Lan.-Bert.
*Navicula salinarum* Grun.
*Navicula schroeterii* Meister
*Navicula subhamulata* Grun. & Van Heurck
*Navicula suecorum* var. *dismutica* (Hust.) Lan.-Bert
*Navicula viridula* var. *linearis* Hust.
*Navicula viridula* var. *rostellata* (Kuetz.) Cleve
*Navicula vitiosa* Schiman.
*Neidium bisulcatum* (Lager.) Cleve
*Neidium ladogensis* (Cleve) Foged
*Nitzschia angustatula* Lan.-Bert.
*Nitzschia calida* Grun.

*Nitzschia capitellata* Hust.
*Nitzschia commutatoides* Lan.-Bert.
*Nitzschia compressa* var. *compressa* (Bailey) Boyer
*Nitzschia constricta* (Kuetz.) Ralfs
*Nitzschia dissipata* var. *media* (Hant.) Grun.
*Nitzschia filiformis* var. *conferta* (Rich.) Lan.-Bert.
*Nitzschia flexa* Schum.
*Nitzschia granulata* Grun.
*Nitzschia levidensis* var. *salinarum* Grun.
*Nitzschia linearis* var. *subtilis* (Grun.) Hust.
*Nitzschia linearis* var. *tenuis* (W.Smith) Grun.
*Nitzschia littoralis* Grun.
*Nitzschia longissima* var. *genuina* A.Cleve
*Nitzschia lorenziana* Grun.
*Nitzschia navicularis* (Breb.) Grun.
*Nitzschia perspicua* Choln.
*Nitzschia recta* var. *robusta* Hust.
*Nitzschia scalpelliformis* Grun.
*Nitzschia sigma* (Kuetz.) W.Smith
*Nitzschia sinuata* var. *delognei* (Grun.) Lan.-Bert.
*Nitzschia sinuata* var. *tabellaria* (Grun.) Grun.
*Nitzschia sociabilis* Hust.
*Nitzschia vermicularis* (Kuetz.) Hant.
*Nitzschia wuellerstorffii* Lan.-Bert.
*Pinnularia divergens* W. Smith
*Pinnularia microstauron* var. *brebissonii* (Kuetz.) May.
*Pinnularia rupestris* Hant.
*Pinnularia subcapitata* var. *hilseana* ( Jan.) Muell.
*Pinnularia sudetica* (Hilse) Peragallo
*Pleurosigma salinarum* Grun.
*Pleurosigma strigosum* W.Smith
*Rhizosolenia eriensis* H.L.Smith
*Rhopalodia brebissonii* Krammer
*Rhopalodia constricta* (W.Smith) Kramm.
*Surirella brebissonii* Kramm. & Lan.-Bert.
*Surirella constricta* W.Smith
*Surirella striatula* Turpin

**CHLOROPHYTA**
CHLOROPHYCEAE
*Elakatothrix biplex* (Nyg.) Hindak
*Elakatothrix spirochroma* (Rev.) Hindak
*Gloeobotrys monochloron* Ettl.
*Koliella crassa* Hindak
*Koliella variabilis* (Nyg.) Hindak
*Scenedesmus velitaris* Kom.
ZYGNEMATOPHYCEAE
*Cosmarium pseudamoenum* Wille
*Spondylosium pulchellum* Arch.
*Spondylosium tetragonum* W. West
*Staurastrum chaetoceras* (Schr.) Smith

The appearance of almost half of all determined species and subspecies (248) was limited to a single sampling point. Eight species were determined in more than a half of all specific biotopes (Table 2). The *Phormidium* sp. species was most frequent, present in eutrophic lakes, waterfalls, springs, brackish waters, the Dragonja River, aerial biotopes and hypereutrophic biotopes. The *Achnanthes minutissima* species was present in eutrophic lakes, peat bogs, waterfalls, springs, hot spring, brackish waters, the Dragonja River and in aerial biotopes. The *Navicula veneta* species was present in eutrophic lakes, waterfalls, springs, brackish waters, the Dragonja River, aerial biotopes and hypereutrophic biotopes. The *Trentepohlia aurea* species was present in peat bogs, waterfalls, springs, a hot spring, the Dragonja River, aerial biotopes, Krška jama cave and hypereutrophic biotopes. The *Cymbella silesiaca* species was present in eutrophic lakes, peat bogs, waterfalls, springs, brackish waters, the Dragonja River and hypereutrophic biotopes. The *Gomphonema angustum* species was present in eutrophic lakes, peat bogs, waterfalls, springs, brackish waters, the Dragonja River and aerial biotopes. The *Navicula* sp. species was present in eutrophic lakes, waterfalls, springs, brackish waters, the Dragonja River, aerial biotopes and hipereutrophic biotopes. The *Cymbella affinis* species was present in eutrophic lakes, peat bogs, falls, springs, brackish waters, the Dragonja River and aerial biotopes.

Table 2: List of algal species determined in more than half of all specific biotopes
Tabela 2: Seznam vrst prisotnih v več kot polovici vseh posebnih biotopov

| taxon | sampling sites |
|---|---|
| *Phormidium* sp. | Koseški bajer, Sotelsko jezero, waterfall Savica, Krka falls, small waterfall on Pohorje, spring on Medvedje Brdo, spring on Pohorje, mineral spring Rimski vrelec, brackish lake Fiesa, river Dragonja, limestone rock, manure water, constructed wetland Dragonja |
| *Achnanthes minutissima* | Koseški bajer, Sotelsko jezero, peat bog Lovrenška jezera, waterfall Savica, Krka falls, small waterfall on Pohorje, spring on Medvedje Brdo, hot spring, mineral spring Rimski vrelec, brackish lake Fiesa, river Dragonja, limestone rock |
| *Navicula veneta* | Koseški bajer, Sotelsko jezero, waterfall Savica, Krka falls, small waterfall on Pohorje, spring on Medvedje Brdo, mineral spring Rimski vrelec, brackish lake Fiesa, river Dragonja, limestone rock, constructed wetland Dragonja |
| *Trentepohlia aurea* | peat bog Lovrenška jezera, waterfall Savica, small waterfall on Pohorje, spring on Medvedje Brdo, spring on Pohorje, hot spring, mineral spring Rimski vrelec, river Dragonja, stony wall, limestone rock, cave Krška jama, manure water, constructed wetland Dragonja |
| *Cymbella silesiaca* | Koseški bajer, Sotelsko jezero, peat bog Lovrenška jezera, waterfall Savica, Krka falls, small waterfall on Pohorje, spring on Medvedje Brdo, brackish lake Fiesa, river Dragonja, constructed wetland Barje |
| *Gomphonema angustum* | Koseški bajer, Sotelsko jezero, peat bog Lovrenška jezera, waterfall Savica, Krka falls, small waterfall on Pohorje, spring on Medvedje Brdo, brackish lake Fiesa, river Dragonja, limestone rock |
| *Navicula* sp. | Koseški bajer, Sotelsko jezero, waterfall Savica, Krka falls, small waterfall on Pohorje, spring on Medvedje Brdo, brackish lake Fiesa, river Dragonja, concrete wall, stony wall, manure water, constructed wetland Barje |
| *Cymbella affinis* | Koseški bajer, Lovrenška jezera, waterfall Savica, small waterfall on Pohorje, mineral spring Rimski vrelec, brackish lake Fiesa, river Dragonja, stony wall |

### Bray-Curtis Coefficient of Similarity

The Bray-Curtis coefficient showed no significant similarity in sampling sites with high water velocity (Fig. 2). The Krka falls were more similar to the Sotelsko jezero and to the Koseški bajer than to the Savica waterfall. In the Dragonja River the differences depended more on the season than on the water velocity. Therefore, the velocity does not significantly influence the species structure and the numerousness of algal association. Regarding the eutrophic waters, there is a similarity between the Sotelsko jezero, the Koseški bajer and the Krka River, while there is a very low similarity between them and the extreme eutrophic sampling sites (manure water and constructed wetlands), as only two common species were found there, i.e. *Cymbella silesiaca* and *Navicula veneta.* Similarity was determined between the brackish sampling points: the Fiesa lake and the Dragonja estuary. High influence of geological structure was also determined. All Dragonja River sampling sites (except the Dragonja estuary) were similar. Because of the limestone ground there was also a similarity between the Medvedje Brdo spring and the Savica waterfall. A higher similarity was expected between the spring and the small waterfall on Pohorje, but in the spring samples some terrestrial species (*Navicula contenta, Klebsormidium flaccidum, Trentepholia aurea*) were found. Thus, the results showed that the spring on Pohorje was more similar to the terrestrial sampling sites, like the limestone rock and the Krška jama cave. There was a high similarity between the terrestrial sampling sites: the lime tree trunk, concrete wall and stony wall where limitation factors were the absence of food and humidity. High water temperature was the major limiting factor for species structure and the numerousness of algal association in the Terme Čatež hot spring.

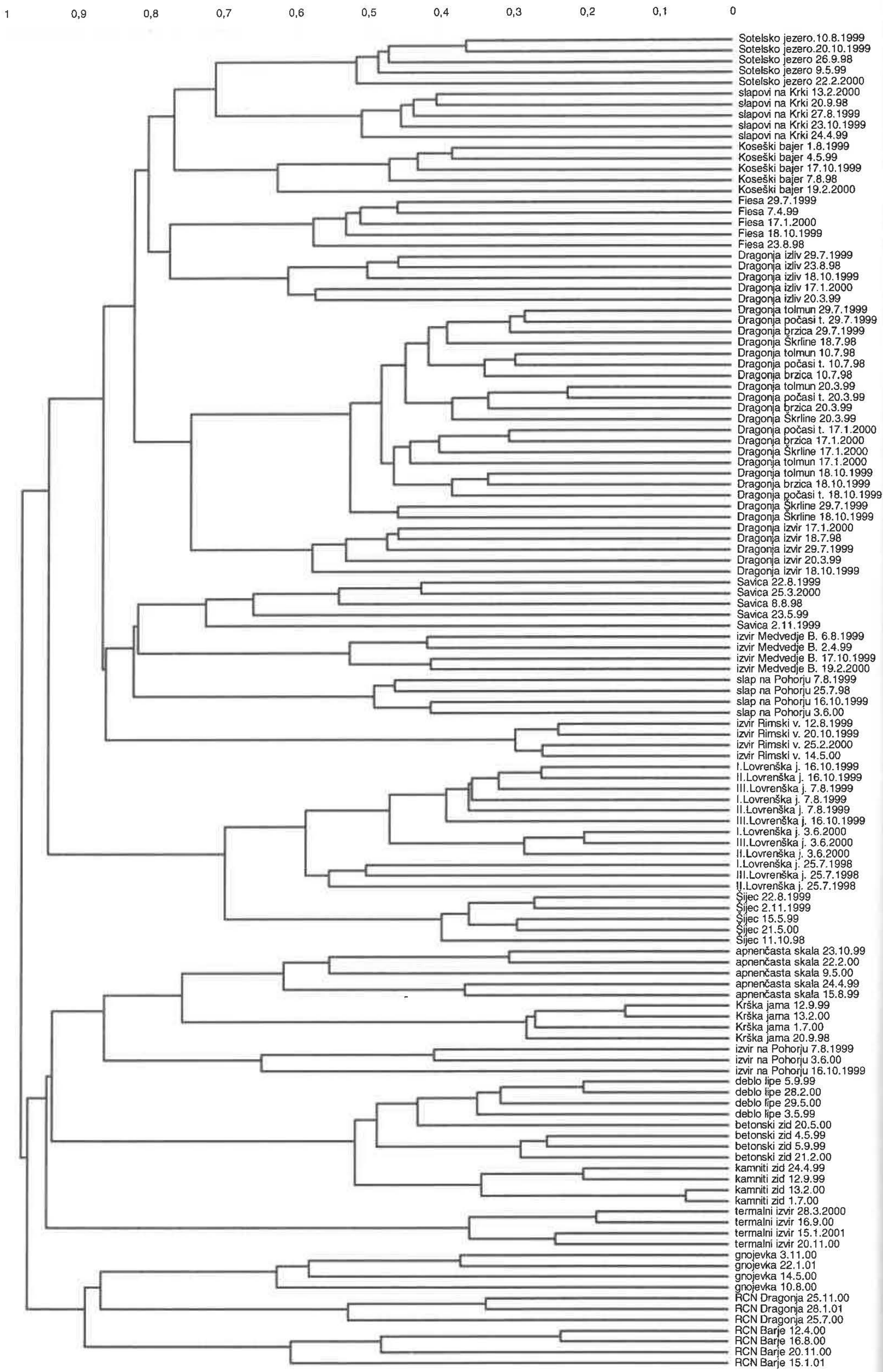


Figure 2: Dendrogram: Bray-Curtis coefficient of similarity for all sampling sites
Slika 2: Dendrogram: Bray-Curtisov koeficient podobnosti za vsa vzorčna mesta

## Povzetek

V nalogi smo raziskovali alge nekaterih posebnih biotopov Slovenije v različnih letnih časih od leta 1998 do 2001 z namenom ugotoviti kvalitativno vrstno sestavo algnih združb in pogostost pojavljanja posameznih vrst in podvrst alg v določenih biotopih s posebnimi okoljskimi dejavniki (nizek pH, visoka temperatura, visoka ali nizka elektroprevodnost, pomanjkanje svetlobe, visoka hitrost vodnega toka, pomanjkanje vlage, visoka vsebnost hranil itd.). Zanimale so nas predvsem vrste, ki so prisotne samo v posameznih posebnih biotopih in vrste, ki glede na posebne ekološke pogoje lahko nastopajo v večih posebnih biotopih hkrati. Omejili smo se na sladkovodne, brakične in kopenske biotope, ki dosedaj še niso bili ali pa so bili le delno raziskani. Alge smo določevali v evtrofnih jezerih (Koseški bajer, Sotelsko jezero), visokih barjih (barje Šijec, Lovrenška jezera), izvirih (kraški izvir na Medvedjem Brdu, izvir na Pohorju, termalni izvir v Termah Čatež, mineralni izvir Rimski vrelec), slapovih (lehnjakovi slapovi na reki Krki, slapič na Pohorju, slap Savica), raziskovali smo kopenske alge na apnenčasti skali, betonskem zidu, deblu lipe (*Tilia platyphyllos*) in v Krški jami ter alge v flišni reki Dragonji, brakičnem jezeru v Fiesi in hiperevtrofnih biotopih (gnojevki in rastlinskih čistilnih napravah na deponijah Barje in Dragonja). Z Bray – Curtisovim koeficientom podobnosti smo ugotavljali podobnost v vrstni sestavi in pogostosti pojavljanja alg med posameznimi vzorčnimi mesti.

Skupno smo na šestindvajsetih vzorčnih mestih določili 537 različnih vrst in podvrst alg iz devetih razredov. 295 vrst in podvrst pripada razredu Bacillariophyceae, 116 razredu Cyanophyceae, 58 razredu Chlorophyceae, 44 razredu Zygnematophyceae, 8 razredu Xanthophyceae, 6 razredu Dinophyceae, 5 razredu Euglenophyceae, 3 razredu Chrysophyceae in 2 razredu Florideophyceae.

146 določenih vrst in podvrst je novih za Slovenijo, od tega pripada 107 vrst in podvrst razredu kremenastih alg, 28 razredu Cyanophyceae, 6 razredu Chlorophyceae, 4 razredu Zygnematophyceae in 1 razredu Xanthophyceae.

Pojavljanje skoraj polovice vseh določenih vrst in podvrst (248) je bilo omejeno na posamezna vzorčna mesta. Vrste *Achnanthes minutissima, Cymbella affinis, Cymbella silesiaca, Gomphonema angustum, Navicula veneta, Navicula* sp., *Phormidium* sp. in *Trentepohlia aurea* so splošno razširjene vrste, prisotne so bile v večini posebnih biotopov.

Primerjava vrstnih sestavov algnih združb in pogostosti pojavljanja posameznih vrst in podvrst med vzorčnimi mesti z Bray – Curtisovim koeficientom podobnosti je pokazala, da si vzorčna mesta, kjer je bila hitrost vodnega toka velika, med seboj niso bila preveč podobna. Lehnjakovi slapovi na reki Krki so bili bolj podobni Sotelskemu jezeru in Koseškemu bajerju kot slapu Savici in slapiču na Pohorju. Bray – Curtisov koeficient je pokazal med vzorčnimi mesti na reki Dragonji, ki se v glavnem razlikujejo le v hitrosti vodnega toka, večjo sezonsko kot krajevno odvisnost. Iz zgoraj naštetega lahko sklepamo, da hitrost vodnega toka ni bistveno vplivala na vrstno sestavo in številčnost algnih združb. Evtrofne vode: Sotelsko jezero, Koseški bajer in reka Krka so si bile med seboj podobne, medtem ko je bila njihova podobnost z ekstremno evtrofnimi vodami (gnojevko in RČN) izredno majhna, saj med njimi razen dveh (*Cymbella silesiaca* in *Navicula veneta*) ni bilo skupnih vrst. Med seboj sta si bili podobni tudi obe brakični vodi: priobalno jezero v Fiesi in izliv reke Dragonje. Na vrstno sestavo in številčnost algnih združb ima velik vpliv geološka podlaga. Vsa vzorčna mesta na flišni reki Dragonji (razen izliva) so si bila med seboj podobna. Podobna sta si bila tudi izvir na Medvedjem Brdu in slap Savica, oba ležita na apnenčasti podlagi. Pričakovali bi večjo podobnost med izvirom in slapičem na Pohorju, vendar smo v izviru na Pohorju določili nekaj kopenskih vrst alg (*Navicula contenta, Klebsormidium flaccidum, Trentepohlia aurea*), zaradi česar je bil izvir na Pohorju bolj podoben kopenskima vzorčnima mestoma: apnenčasti skali in Krški jami. Precejšnja podobnost je bila med kopenskimi vzorčnimi mesti – deblo lipe, kamniti in betonski zid, kjer sta verjetno glavna omejujoča dejavnika za uspevanje alg pomanjkanje vlage in hranilnih snovi. V termalnem izviru v Termah Čatež pa na vrstni sestav in številčnost alg v prvi vrsti vpliva zelo visoka temperatura izvirne vode.

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# Tissue culture of pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Schultz Bip.)

Tkivna kultura bolhača (Tanacetum cinerariifolium (Trevir.) Schultz Bip.)

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**Abstract.** Pyrethrum (*Tanacetum cinerariifolium*), is plant species with the highest amount of natural insecticides – pyrethrins. An in-house information system for the development of different plant tissue cultures and marketing of their products was designed. The application of the relational information system in setting up a research hypothesis of high probability is discussed. By processing the information system, plant tissue cultures' parameters were identified, selected and modified. They were tested on the plant tissue culture of *Tanacetum cinerariifolium*. The influence of jasmonic acid on axillary shoot differentiation was studied. An inhibitory effect of jasmonic acid on shoot tissue culture differentiation was proven (100 μM, 10 μM), and a prediction method for determination of the variable optimal concentration interval was presented (between 0,5 and 4,5 μM). In addition a HPLC method was introduced for pyrethrins determination.

**Key words:** *Tanacetum cinerariifolium*, pyrethrum, plant tissue culture, shoot tissue culture, axillary shoot, natural insecticides, pyrethrins, jasmonic acid, information system

**Izvleček.** Bolhač (*Tanacetum cinerariifolium*) je rastlinska vrsta z največjo vsebnostjo naravnih insekticidov – piretrinov. Postavili smo informacijski sistem za razvoj različnih sistemov tkivnih kultur in trženje njihovih proizvodov.

Preučevali smo uporabo relacijskega informacijskega sistema za vpeljavo znanstvene hipoteze z visoko verjetnostjo. Z njegovo uporabo smo prepoznali, izbrali in priredili parametre sistema tkivnih kultur. Kot poskusni sistem smo uporabili tkivno kulturo bolhača in ugotavljali vpliv jasmonske kisline (JA) na tvorbo zalistnih poganjkov v tkivni kulturi. Dokazan je bil zaviralni učinek JA na diferencijo kulture poganjkov (100 µM, 10 µM) in predstavljena metoda za napoved intervala spreminjanja optimalne koncentracije JA (med 0,5 in 4,5 µM). Uvedli smo metodo HPLC za ugotavljanje vsebnosti piretrinov.

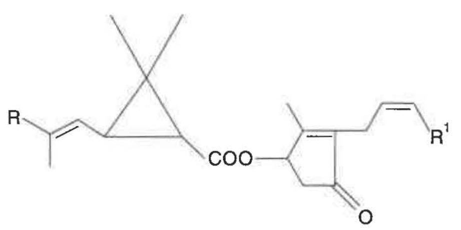
**Ključne besede:** *Tanacetum cinerariifolium,* bolhač, rastlinska tkivna kultura, tkivna kultura poganjkov, zalistni poganjki, naravni insekticidi, piretrini, jasmonska kislina, informacijski sistem

**Abbreviations:** MS = Murashige and Skoog growth medium; JA = jasmonic acid; HPLC = high pressure liquid chromatography; IAA = indole-3-acetic acid; 2,4 D = 2,4-diclorophenoxy acetic acid; NAA = naphthalene acetic acid; BAP = 6-benzylamino purine; SD = standard deviation; R = correlation index; EPA = Environmental Protection Agency

## Introduction

About 10 000 million insect species cause foodstuff and wood damage. Some species are even fatal, especially for people (ELLIOT 1995). But an extensive usage of synthetic persistent insecticides in the last fifty years has led to their dangerous accumulation in the environment and rapid insect resistance development (COSHRAN 1995). One possible way to minimize the effect of insecticides on biological diversity, and at the same time to ensure food quality, is the application of biodegradable insecticides with low toxicity for Mammals. One of the most promising group of such insecticides are pyrethrins (CASIDA & QUISTAD 1995).

Pyrethrins are natural stereoisomer mixtures of six monoterpenes esters (Fig.1). The basic components of pyrethrins are rethrolone (pyrethrolone, cinerolone and jasmolone) alcohols esterified with chrysanthemic monocarboxylic (pyrethrins I) or dicarboxylic/pyrethric (pyrethrins II) acid (TOMLIN 1994, CROMBIE 1995). Pyrethrins precursor chrysanthemic acid is a volatile monoterpene formed from mevalonic acid of isoprenoid metabolism. Chrysanthemyl alcohol with its typical cyclopropane derives from two molecules of isopentenyl pyrophosphate (IPP). Oxidation of chrysanthemyl alcohol yields chrysanthemic acid. The both have been identified from explant derived callus of pyrethrum. Chrysanthemic acid is found not only as the precursor of chrysanthemic dicarboxylic/pyrethric acid and pyrethrins I/II but also the important precursor in squalene synthesis (KESKITALO 1999).




| | | | | |
|---|---|---|---|---|
| - pyrethrin I | R | CH3, | R1 | CH=CH2 |
| - jasmolin I | R | CH3, | R1 | CH2CH3 |
| - cinerin I | R | CH3, | R1 | CH3 |
| - pyrethrin II | R | CO2CH3, | R1 | CH=CH2 |
| - jasmolin II | R | CO2CH3, | R1 | CH2CH3 |
| - cinerin II | R | CO2CH3, | R1 | CH3 |

Figure 1: Monoterpenes esters of pyrethrins.
Slika 1: Monoterpenski estri piretrinov.

The detailed synthesis of rethrolones still remains unclear. However the rethrolones, charecterized by the cyclopentene, are derived most properly from fatty acid metabolism. The composition of the crude extracts of pyrethrum flowers is as follows: one third is the active constituents pyrethrins and two thirds are linoleic and linolenic acids (40 %), alkanes and terpenoides (CROMBIE 1995, KESKITALO 1999). The origin of jasmolone presumably derives from linoleic acid via 12–oxophytodienoic acid (12–oxo PDA). Experimentally is firmly established that 12 oxo–PDA undergoes hydrogenations and ß–oxidations to form jasmonic acid (CROMBIE 1995).

A natural means for controlling a wide range of insects and rapid insecticidal action by pyrethrins is found in the composition of esters mixture. It was also found that a moderate use might cause a slow insect resistance development (Coshran 1995). Pyrethrins were found in some species, e.g. T. coccineum, but more potent is pyrethrum (*T. cinerariifolium*). From the middle of 19th century to World War I, Dalmatia dominated in the pyrethrum production and trade. After the war the domination was replaced by Japan, and after World War II by Kenya, Tanzania and Australia (WAINAINA 1995, GULLICKSON 1995). There were always constraints on ensuring a sufficient supply because of the laborious work, demand for low labour costs, and weather dependency of plant production. In recent years, the efficacy of pyrethrins and the unstable supply, which can no longer meet the world demand (JOVETIĆ & DE GOOIJER 1995, ROYAL SOCIETY OF CHEMISTRY 1988, 1996), stimulated interest for their biotechnological production (HITMI & al. 2000). Analysis of patents concerning pyrethrins in the last thirty-five years has shown a distinctive increasing research interest during the last decade (Fig. 2).

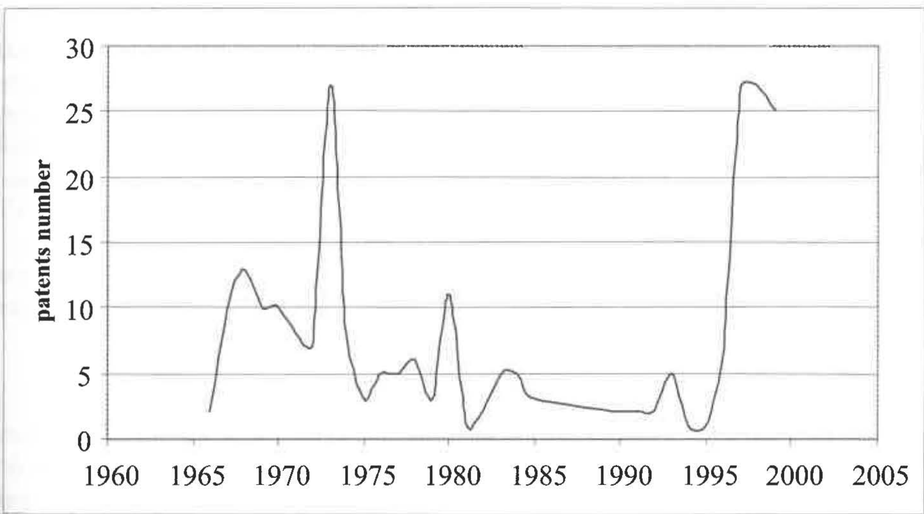



Figure 2: Oscillating pyrethrins research trend in course of time and increasing interest in the last decade (CAplus, march 2000).
Slika 2: Nihanje zanimanja za raziskovanje piretrinov v preteklih letih in naraščanje zanimanja za piretrine v zadnjem desetletju (CAplus, marec 2000).

An alternative production of pyrethrins could be in vitro production by plant tissue cultures, the main advantage of which is flexible control of biotechnological processes. Therefore plant tissue cultures are becoming a tool for producing many important products: e.g. quality food, wood, phytotherapy compounds, biopesticides etc (SASSON 1992, WALTON & al. 1999, RAVNIKAR & ŽEL 1992). The application of the tissue culture technique for the pyrethrum plant has developed also from the self-incompatibility of the plant (PAL & DHAR 1985). For the successful commercialisation of a biotechnological process the most important criterion must be fulfilled – less expensive in vitro in relation to in vivo pyrethrins production (JOVETIĆ & DE GOOIJER 1995).

The basic research problem which we tried to solve was, how to optimise tissue culture cultivation of a commercially potent plant to achieve quality products, e.g. pyrethrins. The problem was structured into several subproblems: (ELLIOT 1995) careful selection of a substance and its source – plant, (COSHRAN 1995) selection of economic cultivating conditions, and (CASIDA & QUISTAD 1995) target-oriented development of cost-effective products. Fragmented information on the defined sub problems is dispersed among several bibliographic and factual sources, while the technological parameters are most often considered proprietary information and are therefore publicly available (HUMPRIES & al. 1991, GULLICKSON 1995). To increase the information content of the accessible and relevant data and information, a relational information system for plant tissue cultures development was designed. The system was tested on the experiment where the effect of JA on shoot production of pyrethrum was studied.

## Methods

### Tissue culture

Plants of *Tanacetum cinerariifolium* were obtained from the island of Cres, Croatia. Their seeds germinated in vivo and two months old vegetative plantlets of 8 cm height were used in spring for establishment of the shoot tissue culture on MS medium (MURASHIGE & SKOOG 1962) supplemented with 3 % sucrose, 1 % agar, 3 µM IAA and 2 µM BAP The pH was adjusted to 5.7 and the medium was sterilized at 121°C for 20 min. The induced axillary buds had been regulary subcultures, every 4 to 5 weeks, for a year. The tissue cultures were grown in a growth chamber at 23 +/- 1°C under 100 – 110 µM $m^{-2}s^{-1}$ daylight illumination with a 16 h photoperiod.

Axillary buds were separated and cultivated in 50 ml test-tubes on modified MS medium with different concentrations of JA and control medium without JA. In two experiments three JA concentrations were tested: 1 µM, 10 µM and 100 µM JA.

### Growth and differentiation measurement

Two and four weeks after subculture (first experiment) the height of the central shoot and shoots number in fifteen test-tubes were determined, and the results were statistically evaluated by Student's t-test: * $P < 0{,}05$, ** $P < 0{,}01$, *** $P < 0{,}001$. In the second experiment the procedure was repeated after three and five weeks.

### Differentiation prediction

To fulfil the remaining information gap a prediction method was introduced which will have to be tested in further research. On the basis of all and the selected average shoots number (data inside +/- 2 SD), measured after five weeks of cultivation, the curve with the highest correlation index (R) was determined: at concentration intervals between 0 to 10 µM JA and between 0 to 1 µM JA.

### Isolation and quantification of pyrethrins

Approximately 1 g of in vitro grown shoots was crushed in a mortar with a silicic sand and ¼ anhydrous sodium sulfate to obtain a homogenous powder. Pyrethrins were extracted two to three times with 5 ml/g FW petroleum ether (40-60°C). The clear separated extract was evaporated to dryness with rotated evaporator, redissolved in 3 ml $CH_3CN$, filtered through 0,22 µm mesh filter and analysed.

Pyrethrins were analyzed by Waters HPLC system with a diode array (PDA) detector.

Separations were performed on Nova Pack C18 column (Waters, 150 x 3.9 mm) using a gradient of $CH_3CN$ (solvent A) and Milli Q $H_2O$ (solvent B): 40 % solvent A gradually increased to 80 % during 25 min, followed by a further 10 min at 80 %. Flow rate was 1.4 ml/min. Absorbance was monitored at 225 nm. Pyrethrins in the sample were identified on the basis of retention times and characteristic absorption spectra. Standards used were cinerin I and II, pyrethrin I and II and jasmolin I and II (Pyrethrins technical mixture, PESTANAL, Riedel-de-Haën).

## Results and discussion

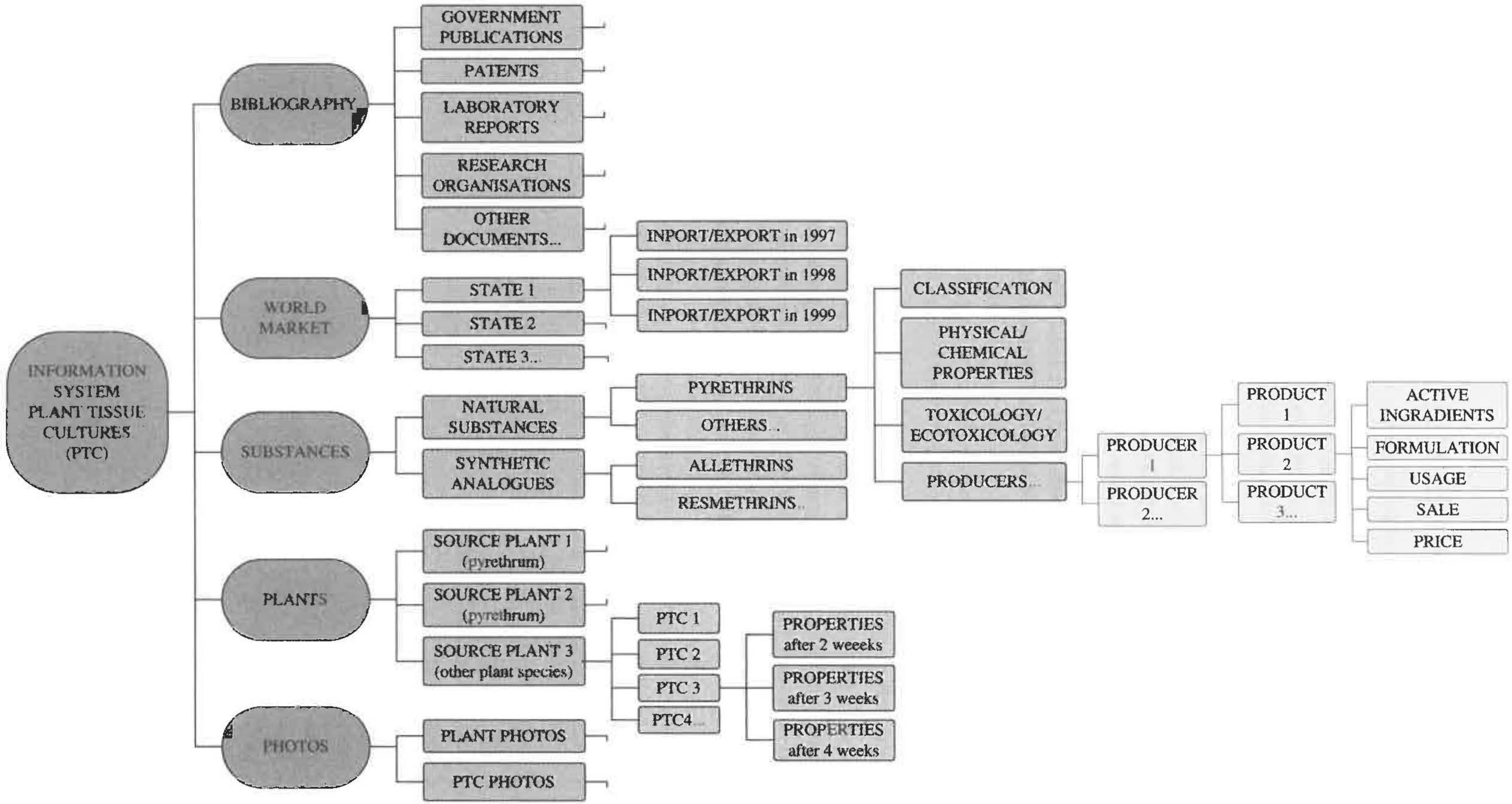
### Relational information system

The in-house information system supports problem solving through establishing relationships among five main modules: (a) bibliography, (b) substances (properties, structures, producers, products), (c) world market, (d) plants and (e) photos (Fig. 3). Processing the relational information system for identifying the most effective parameters for biotechnological production of the biosynthetic high-yielding pyrethrum tissue culture clone gave the following result (Table 1):

Table 1: Information densities of breeding parameters' values of pyrethrum plant tissue cultures.
Preglednica 1: Informacijska gostota parametrov vzgoje bolhača v rastlinskih tkivnih kulturah.

| Plant tissue culture cultivating parameters | Information densities (number of defined values of specific parameter in documents) |
|---|---|
| medium modification | 38 |
| inoculum | 14 |
| basic medium | 14 |
| temperature | 14 |
| irradiation | 13 |
| medium pH | 10 |
| plant no. | 9 |
| photoperiod | 9 |
| light quality | 4 |
| inoculum modification | 1 |
| storage name | 1 |
| storage temperature | 1 |

Figure 3: The structure of five main modules (oval boxes) of the relational information system on plant tissue culture development.
Slika 3: Struktura petih glavnih modulov (ovalna polja) relacijskega informacijskega sistema za razvoj rastlinskih tkivnih kultur.



Based on the information density (Table 1), the basic parameter which promotes plant production is medium modification. This result led to setting up a high probability hypothesis which assumed that the target-oriented medium modification would have the strongest impact on the growth, differentiation and biosynthetic activity of the pyrethrum tissue culture. The information gap on medium modification possibilities was bridged by additional processing of the Bibliographic module of the Relational information system, which gave examples of medium modifications with pyrethrin biosynthesis stimulation effect (Table 2).

In the literature the following pyrethrum tissue culture's stimulation medium modifications were recognised (Table 2): auxins 2,4 D, NAA; cytokinins BA, BAP, kinetin; vitamin ascorbic acid, sucrose, diluted basic medium MS and nutrient stress. It is important to stress that, regarding the bibliographic search in Chemical Abstracts, jasmonic acid (JA) was not reported as a pyrethrum plant growth regulator although it is quite widespread among plants. It is gradually synthesised from linoleic acid, as is suggested for pyrethrins rethrolones (Crombie 1995).

Table 2: Medium modifications with pyrethrins biosynthesis stimulative effect obtained from bibliographic module of the information system.
Preglednica 2: Gojišča, ki pospešijo biosintezo piretrinov. Podatki iz bibliografskega modula informacijskega sistema.

| Reference | Medium modification |
|---|---|
| Hitmi & al., 1998 | 2 µM BAP; 21,5 µM NAA |
| Hitmi & al., 1997 | 2 µM BAP; 21,5 µM NAA; ½ MS;1,8 g/l sucrose |
| Dhar & Pal, 1993 | 2,3 µM 2,4 D; 2,2 µM BA |
| Rajasekaran & al., 1991 | 0,9 µM 2,4 D; 23,2 µM kinetin; MS without agar and nitrates |
| Ravishankar & al., 1989 | 0,57 mM ascorbic acid; 0,9 µM 2,4 D; 2,3 µM kinetin |
| Rajasekaran & al., 1990 | 0,9 µM 2,4 D; 23,2 µM kinetin |

## Statistically proven inhibitory effect of jasmonic acid

After two and four weeks an inhibitory effect of 100 µM JA on pyrethrum tissue culture growth and differentiation was observed and statistically proven (Figs. 4, 5). JA clearly inhibited number of

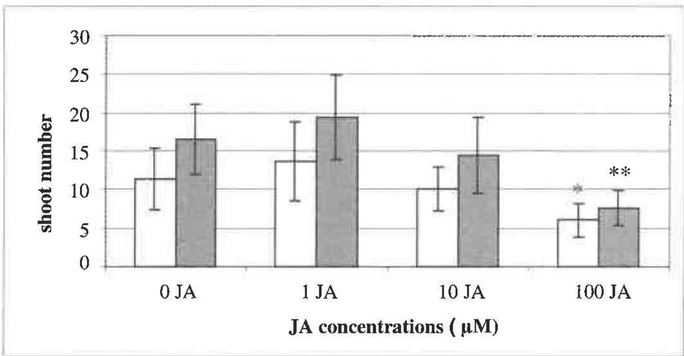



Figure 4: The effect of JA on number of shoots after two (light bar) and four (darker bar) weeks in tissue culture (first experiment). Average shoot number (n>10), standard deviation (SD), and statistical significant differences (t-test) between control media without JA and with different concentrations of JA are shown.
Slika 4: Vpliv JA na število poganjkov po dveh (svetel stolpec) in štirih (temen stolpec) tednih tkivne kulture (prvi poskus). Prikazana so povprečna števila poganjkov (n>10), standardne deviacije (SD) in statistično značilne razlike (t-test) med kontrolnim gojiščem brez JA in z različnimi koncentracijami JA.

shoots, especially on the highest JA concentration (Fig. 4), and the same trend was observed in second experiment (data not shown). JA also inhibited the early lengthening of shoots. After two weeks of culture were shoots on the highest JA concentration significantly smaller then shoots on control media without JA (Fig. 5). Differences were not observed after four weeks of culture.

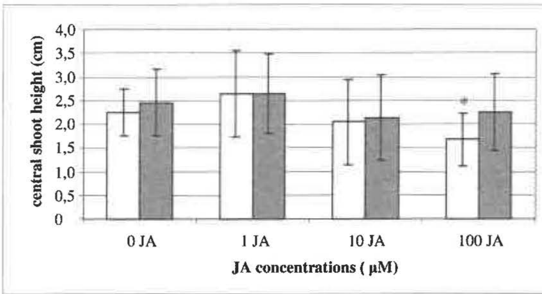



Figure 5: The effect of JA on central shoot length after two (light bar) and four (darker bar) weeks in tissue culture (first experiment). Average shoot number (n>10), standard deviation (SD), and statistical significant differences (t-test) between control media without JA and with different concentrations of JA are shown.
Slika 5: Vpliv JA na velikost poganjkov po dveh (svetel stolpec) in štirih (temen stolpec) tednih tkivne kulture (prvi poskus). Prikazana so povprečna števila poganjkov (n>10), standardne deviacije (SD) in statistično značilne razlike (t-test) med kontrolnim gojiščem brez JA in z različnimi koncentracijami JA.

### Prediction of Jasmonic acid stimulative effect

Based on the results (Figs. 4, 5) it was assumed that a stimulatory JA concentration on pyrethrum tissue culture differentiation may lie at the interval between 0 to 10 µM. At intervals between 0 to 10 µM JA and 0 to 1 µM JA the curve that mostly fit both the experimental raw data measured after five weeks of cultivation (second experiment) as well as selected data (+/- 2SD) was found (Figs. 6 and 7). According to the curves' peaks, which indicate the most copious pyrethrum tissue culture differentiation, the inter-

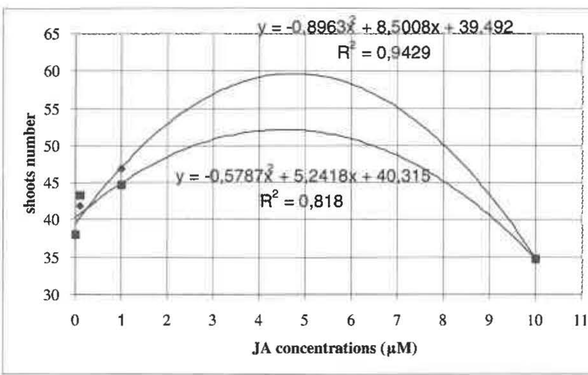



Figure 6: Curves of average values from all (♦) and selected (▪) data show at the interval between 0 and 10 µM JA a high dependency ($R^2 = 0,9429$; $R^2 = 0,818$) between specific JA concentration and pyrethrum tissue culture differentiation.
Slika 6: Krivulje povprečnih vrednosti vseh (♦) in izbranih (▪) podatkov kažejo v intervalu med 0 in 10 µM JA veliko odvisnost ($R^2 = 0,9429$; $R^2 = 0,818$) med koncentracijo JA in diferenciacijo bolhača v tkivni kulturi.

val of optimal JA concentrations was determined. The same approach could be used in further research for prediction of other plant tissue cultures properties: e.g. biomass, growth index, pyrethrins content.

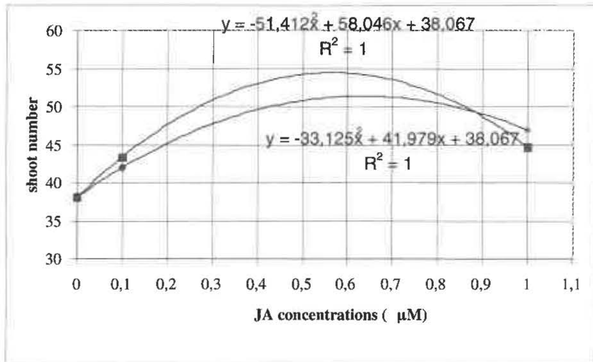



Figure 7: Average values from all (♦) and selected (■) data show at the interval between 0 and 1 μM JA complete dependency ($R^2 = 1$) between specific JA concentration and pyrethrum tissue culture differentiation.
Slika 7: Povprečne vrednosti vseh (♦) in izbranih (■) podatkov kažejo v intervalu med 0 in 1 μM JA popolno odvisnost ($R^2 = 1$) med koncentracijo JA in diferenciacijo bolhača v tkivni kulturi.

## Effect of jasmonic acid on pyrethrins content

In all tested cultures six pyrethrins were detected: cinerin I and II, pyrethrin I and II and jasmolin I and II (Fig. 8). Among them pyrethrin I predominated. The total amounts of pyrethrins in the con-

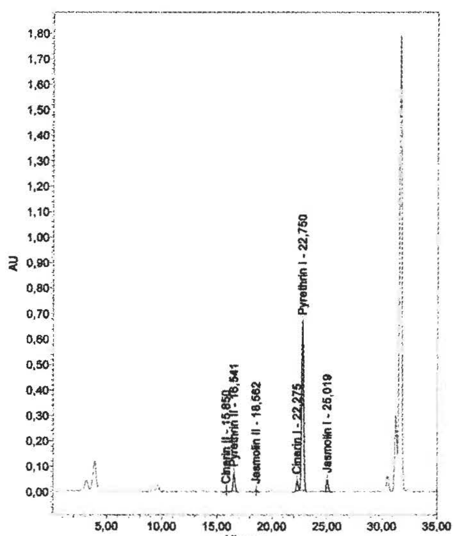



Figure 8: A representative chromatogram: Content of pyrethrins in pyrethrum shoots tissue culture grown four weeks on control medium without JA.
Slika 8: Reprezentativni kromatogram: Vsebnost piretrinov v poganjkih bolhača po štirih tednih tkivne kulture na kontrolnem gojišču brez JA.

trol and JA treated cultures never exceeded 0.44 % of plant dry weight. These were much lower amounts then in flower heads, which contained 0.8 % to 2 % of pyrethrins per dry weight (RAJASEKARAN & al. 1990). The pyrethrins I\II ratio, especially important for insecticide activity, was 0,1 or lower. However in flower heads this ratio is between 0.5 and 3.5 (RAJASEKARAN & al. 1990). In preliminary detections of pyrethrin content in shoots tissue cultures of pyrethrum, jasmoinc acid did not effect pyrethrin contents.

The total amount was lower in tissue culture then in flower heads, grown in vivo. Further work by different inducers is needed to increase the synthesis of pyrethrins in pyrethrum tissue culture.

## Conclusions

A systematic selection of economically important pyrethrins produced in tissue culture conditions was outlined. Target-oriented research supported by the in-house information system is a time-effective contribution to the less expensive in vitro pyrethrin production.

The specific inhibitory effect of the chosen entry variable jasmonic acid on *T. cinerariifolium* tissue culture´s differentiation was proven. A method for variable optimal value prediction was presented and applications for further research were indicated.

## Povzetek

Bolhač (*Tanacetum cinerariifolium*) je rastlinska vrsta z največjo vsebnostjo naravnih insekticidov – piretrinov, ki imajo vrsto prednosti pred ostalimi insekticidi, kot sta hitra razgradljivost in majhna toksičnost za sesalce (JOVETIĆ & DE GOOIJER 1995). Danes klasična proizvodnja zaostaja za svetovnimi potrebami (GULLICKSON 1995, JOVETIĆ & DE GOOIJER 1995), zato se je obnovilo zanimanje za biotehnološko pridobivanje piretrinov.

V našem delu smo postavili informacijski sistem za razvoj različnih sistemov tkivnih kultur in trženje njihovih proizvodov. Preučevali smo o uporabi relacijskega informacijskega sistema za vpeljavo znanstvene hipoteze z visoko verjetnostjo. Z uporabo informacijskega sistema smo prepoznali, izbrali in priredili parametre sistema tkivnih kulturi.

Ker do sedaj še ni bilo poročil o vplivu jasmonske kisline (JA) na tkivno kulturo bolhača, smo sistem preizkusili na preučevanju vpliva JA na tkivno kulturo poganjkov bolhača. Vzpostavili smo tkivno kulturo bolhača in ugotavljali vpliv JA na tvorbo zalistnih poganjkov v tkivni kulturi. Dokazan je bil zaviralni učinek JA na diferencijo kulture poganjkov (100 µM, 10 µM) in predstavljena metoda za napoved intervala spreminjanja optimalne koncentracije JA (med 0,5 in 4,5 µM).

Uvedli smo tudi metodo HPLC za ugotavljanje vsebnosti piretrinov. V tkivni kulturi poganjkov smo določili vseh šest piretrinov: cinerin I in II, piretrin I in II in jasmolin I in II. Izmed piretrinov najbolj prevladuje piretrin I. Vsebnost piretrinov v poganjkih na kontrolnem gojišču in na gojišču z JA nikoli ne presega 0,44 % suhe mase, kar je veliko manj, kot je piretrinov v cvetnih glavicah, ki vsebujejo od 0,8 % do 2 % piretrinov v suhi masi.

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# UV-B radiation screen and respiratory potential in phytoplankton in mountain lakes

## Zaščita pred UV-B sevanjem in dihalni potencial fitoplanktona iz visokogorskih jezer

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**Abstract.** UV-B radiation screen and the respiratory potential (terminal electron transport system – ETS activity) were investigated in phytoplankton from five mountain lakes located on an elevation gradient from 1383 m to 2150 m a.s.l.. The amount of UV absorbing compounds in phytoplankton was generally higher on the lakes from higher elevation. The increased ETS activity of phytoplankton in higher lakes was suggested to reflect the energetic cost of generating the internal mechanisms for photoprotection.

**Key words:** mountain lakes, UV-B radiation, chlorophyll *a*, UV absorbing compounds, ETS activity, nutrient status

**Abbreviations:** DOC – dissolved organic matter, ETS – electron transport system, LK – Lake Krn, LKIn. – Lake Kriško Inferior, LKSup. – Lake Kriško Superior, LL – Lake Ledvica, LP – Lake Planina, UV – ultraviolet, UV AC – UV absorbing compounds.

**Izvleček.** Ugotavljali smo sposobnost za izgrajevanje UV zaščitnih snovi ter dihalni potencial (aktivnost terminalnega elektronskega transportnega sistema – ETS) fitoplanktona na petih visokogorskih jezerih, ki ležijo na različnih nadmorskih višinah, od 1383 do 2150 m. Vsebnost UV zaščitnih snovi je bila večja v fitoplanktonu iz višje ležečih jezer. Večja aktivnost ETS fitoplanktona iz višje ležečih jezer odraža potrebo po energiji, ki jo organizmi rabijo za mehanizme, s pomočjo katerih se zaščitijo pred sevanjem.

**Ključne besede:** visokogorska jezera, UV-B sevanje, klorofil *a*, UV absorbirajoče snovi, aktivnost ETS, obremenjenost s hranili

**Okrajšave:** DOC – raztopljena organska snov, ETS – elektronski transportni sistem, LK – Krnsko jezero, LKIn. – Spodnje Kriško jezero, LKSup. – Zgornje Kriško jezero, LL – Jezero v Ledvicah, LP – Jezero na Planini pri Jezeru, UV – ultravijolično, UV AC – UV absorbirajoče snovi.

## Introduction

The increase of UV radiation at the Earth's surface, due to the gradual depletion of ozone in the atmosphere (WILLIAMSON 1995), has caused great concern about the consequent effects on terrestrial (BJÖRN 1999, GABERŠČIK & al. 2001, GABERŠČIK & al. 2002a) and aquatic ecosystems (HÄDER & al. 1998, GERM & al. 2002a,b). Mountain lakes are potentially vulnerable to climate changes due to their high elevation and, usually, their small size. Data on the dependence of intensity of UV-B on elevation differ. Increases of UV-B radiation (280-320 nm) are reported to range from 6-8% (CALDWELL & al. 1980) to 20% (BLUMTHALER & al. 1993) per 1,000 m of elevation. Higher lakes usually contain lower concentrations of DOC which is considered to be the important factor controlling the penetration of UV into water (HUOVINEN & al. 2003). Organisms in higher and cleaner lakes are therefore exposed to higher UV-B doses, usually without any refuge from damaging solar radiation (WILLIAMSON 1995). UV-B radiation affects many biological and physiological processes in primary producers (HÄDER & al. 1998, ROZEMA & al. 1997). Experimental studies show that species and populations originating from naturally high UV-B locations i.e. from high elevations or low latitudes are less sensitive to UV-B radiation than those from low UV-B locations (SULLIVAN & al. 1992, VILLAFAÑE & AL. 1999). Protection against direct and indirect influences of UV-B is most important in photosynthetic organisms that depend on solar radiation as the primary source of energy in their natural environment (HESSEN & al. 1995). One of the important responses of phytoplankton to UV is the synthesis of UV absorbing compounds, such as mycosporine-like amino acids (MAAs), which protect the cells by preventing UV radiation from reaching and damaging vital molecules such as nucleic acids, especially DNA (HÄDER & al. 1998, BJÖRN 1999, GABERŠČIK & al. 2002a, GERM & al. 2002a). There is an increased need for energy during stress (AMTHOR 1995, GERM & GABERŠČIK 2003) since the establishment of protective mechanisms i.e. synthesis of component materials demand additional supplies of energy from the respiratory process (GULMON & MOONEY 1986).

In this study we have estimated the respiratory potential, and the ability of phytoplankton from mountain lakes located on an elevation gradient, to produce UV screening substances.

## Materials and methods

The study was carried out during summer 2001 and 2002 in the Julian Alps in NW Slovenia. The lakes differ in elevation, trophic status, dimensions and type of activities in the watershed. Their characteristics are listed in Table 1.

**Chlorophyll (Chl.) a:** samples of water were taken with a Van Dorn sampling device (Wildco Ltd., USA) from the boat. 0.5 to 3 litres of water at 2.5 m intervals from the lake surface to the bottom at the deepest point of the lakes were collected around noon and immediately filtered through Whatman GF/F filters and frozen. The samples were homogenised in 2 ml of extraction solution (90% (v/v) acetone) and centrifuged at 8500 g, 4°C, for 4 min in a top refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany) and the absorbance of the supernatant measured. The equation of JEFFREY & HUMPHREY (1975) was used to calculate the concentration of chl. *a*.

**UV absorbing compounds:** the basic procedure used for extracting UV screening substances follows the method of CALDWELL (1968). The frozen samples (see above) were homogenised in 5 ml extraction solution containing methanol : distilled water : HCl (37% v/v) (79:20:1 v/v/v), incubated for 20 minutes and centrifuged (1600 g, 10°C, 10 min) in a top refrigerated ultracentrifuge. The absorbance of the supernatants was measured from 280 to 400 nm at intervals of 1 nm. The relative amounts of UV absorbing compounds were determined by integrating the values of absorbance and normalised to the concentration of chl. a.

Table 1. Key characteristics of studied lakes. Geographical characteristics are listed according to DOBRAVEC AND ŠIŠKO (2002).
Tabela 1. Osnovne značilnosti preučevanih jezer. Geografske značilnosti so povezete po DOBRAVEC IN ŠIŠKO (2002).

| Lake | Kriško Sup. | Kriško In. | Ledvica | Planina | Krn |
|---|---|---|---|---|---|
| Geogr. position | N 46°24'32'' | N 46°23'59'' | N 46°20'25'' | N 46°18'40'' | N 46°17'09'' |
| | E 13°48'34'' | E 13°48'24'' | E 13°47'12'' | E 13°49'56'' | E 13°41'08'' |
| Altitude | 2150 | 1880 | 1830 | 1430 | 1383 |
| Surface | 0.662 | 0.862 | 2.187 | 1.562 | 4.534 |
| Depth | 9 | 9 | 15 | 11 | 18 |
| Total P | 14.07 | 11.38 | 21.24 | 111.66 | 19.4 |
| Total N | 1.75 | 1.83 | 2.03 | 2.65 | 1.86 |
| chl. *a* | 0.85 | 0.57 | 0.37 | 14.31 | 6.36 |
| Temperature | 7.5 | 9.8 | 6.6 | 9.0 | 10.8 |
| Secchi disc | bottom | bottom | bottom | bottom | bottom |
| Max penet.UV-B | 9 | 8 | 7 | 1.5 | 5 |
| Trop. state | oligotrophic | oligotrophic | oligotrophic | hypereutrophic | eutrophic |

Legend: Geogr. position (geographical position), altitude (m), surface (ha), depth (m), total P (total phosphorus, µg/l), total N (total nitrogen, mg/l), chl. *a* (chlorophyll *a*, µg/l), temperature (°C), Secchi disc (m), max. penet. UV-B (maximum penetration of UV-B, m), trop. state (trophic state).
Legenda: Geogr. position - geografska lega, altitude - nadmorska višina (m), surface - površina (ha), depth - globina (m), total P - celoten fosfor (µg/l), total N - celoten dušik (mg/l), chl. *a* - klorofil *a* (µg/l), temperature - temperatura (°C), Secchi disc - Sekijeva plošča (m), max. penet. UV-B - maksimalna globina prodiranja UV-B (m), trop. state - trofično stanje.

**Terminal electron transport system activity:** the terminal electron transport system (ETS) activity of mitochondria was measured as described by PACKARD (1971). Samples of water taken from the vertical profile, were filtered through 100 µm mesh size to remove zooplankton, and then through Whatman GF/F filters. Samples were homogenised in 4 ml of ice-cold buffer in a mortar followed by an ultrasound homogenizer (4710; Cole-Parmer, Vernon Hills, IL, USA) at 40W and centrifuged (8500 g, 2°C, 4 min) in a top refrigerated ultracentrifuge. The 0.5 ml of supernatant was mixed with 1.5 ml of substrate solution and 0.5 ml of iodo-nitro-tetrazolium-chloride (INT) solution and incubated for 40 minutes at room temperature. During incubation the INT was reduced to formazan. The absorption of formazan was measured at 490 nm. ETS activity was calculated from the rate of INT reduction, which was converted to the equivalent amount of oxygen (Kenner & Ahmed 1975), and normalised to the concentration of chl. *a*.

**Chemical analyses:** samples of water were taken as stated above. The contents of total phosphorus (Valderrama 1981) and total nitrogen (A.P.H.A. 1998) were measured spectrophotometrically.

## Results and discussion

The trophic levels of the lakes varied with the elevation; the lakes above 1800 m were oligotrophic while those at lower altitudes were eutrophic to hypereutrophic. Average of total phosphorus, total nitrogen and concentration of chl. *a*, as well as temperature in water column were measured in year 2001 and 2002 (Tab. 1). The correlation between ETS activity and concentration of phosphorus was $r=0.98$ ($p<0.05$), in agreement with *del Giorgio* (1992). Factors like latitude, altitude, land use, vegetation cover and geological composition of watershed can strongly influence the level of incident radiation and the optical properties of the water column (Karentz & al. 1994). Clean water ecosystems in high elevation mountains, may receive high doses of UV-B light at significant depths (Hessen & al. 1995). The maximum penetration depth of UV-B was significantly negatively correlated to the content of chl. *a* ($r=-0.94$, $p<0.05$). The maximum penetration depth of UV-B radiation was highest in Lake Kriško Sup., and lowest in Lake Planina. Depth of maximum penetration reflects the DOC concentrations and the presence of planktonic organisms (Nielsen 1996). Hodoki & Watanabe (1998) also proved, that attenuation coefficient for UV-A (320-400 nm) and UV-B (280-320 nm) was highly correlated with the concentration of chl. *a*. The amount of chl. *a* in lake water was highest in the hypereutrophic Lake Planina (Tab. 1). The highest concentration of chl. *a* was observed to be close to the bottom in most of the lakes. This has been reported for other high-mountain lakes (Sommaruga & Garcia-Pichel 1999). Tôth & al. (1995) suggested that the photosynthetically most active zone had been forced deeper in the lake due to UV-B.

Sommaruga & Garcia-Pichel (1999) reported about the maximum amount of UV absorbing compounds (normalised to the concentration of chl. *a*) close to the bottom. That was not the case in all studied lakes (Fig. 1). The bioaccumulation of UV absorbing compounds in copepods has been suggested to be the reason for the observed gradient (Sommaruga & Garcia-Pichel 1999). Villafañe & al. (1999) also observed a high rate of bioaccumulation of these compounds in the food web. The possible reason for the concentration of UV absorbing compounds reaching a maximum near the bottom was probably the sedimentation of phytoplankton, coupled with the fact that UV absorbing compounds were chemically more stable than chl. *a*. Phytoplankton from transparent, high elevation and oligotrophic lakes generally contained more UV absorbing compounds than specimens from lower elevation lakes (Fig. 1). These results showed that the production of UV absorbing compounds in primary producers in the high elevation lakes was likely a response to the higher flux of UV-B. The frequently observed correlation between UV-B radiation and the concentration of UV absorbing compounds in many primary producers shows that these substances protect vulnerable targets in organisms (Sommaruga & Garcia-Pichel 1999, Gaberščik & al. 2002a, Germ & al. 2002a, Winkel-Shirley 2002).

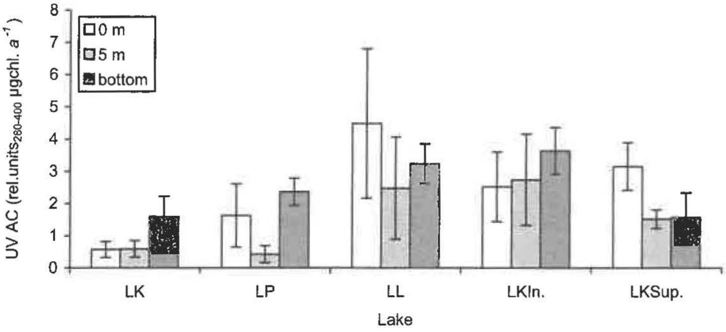


Fig. 1. Amount of UV absorbing compounds (normalised) in phytoplankton on the lake surface (white bars), 5 m deep (light grey bars), and bottom (dark grey bars) (n=3, mean±SD).
Sl. 1. Vsebnost UV absorbirajočih snovi (normirano) v fitoplanktonu na površini (beli stolpci), na 5 metrih (svetlo sivi stolpci) in na dnu (temno sivi stolpci) (n=3, povprečje±SD).

The synthesis of secondary substances is energetically costly and they are produced if the damage due to UV-B is bigger than metabolic costs for production (GABERŠČIK & al. 2002b). ETS activity is a measure of the metabolic potential of organisms. ETS activity per volume of water was higher in lower, eutrophic lakes (Fig. 2), which had high concentration of chl. *a* (Tab. 1). Several investigations (CHO & AZAM 1990, DEL GIORGIO 1992) showed a significant correlation between ETS and chl. *a* in a wide range of lakes. On the other hand, ETS activity (normalised to the concentration of chl. *a*) was higher in the pytoplankton from more oligotrophic lakes receiving higher UV-B doses (Fig. 3). It has already been reported, that ETS activity of pytoplankton increased under enhanced UV-B radiation (FERREYRA & al. 1997, GABERŠČIK & al. 2002a, GERM & al. 2002a). This can be explained by the increased need for energy, for mechanisms involved in photoprotection, i.e. production of UV absorbing compounds.

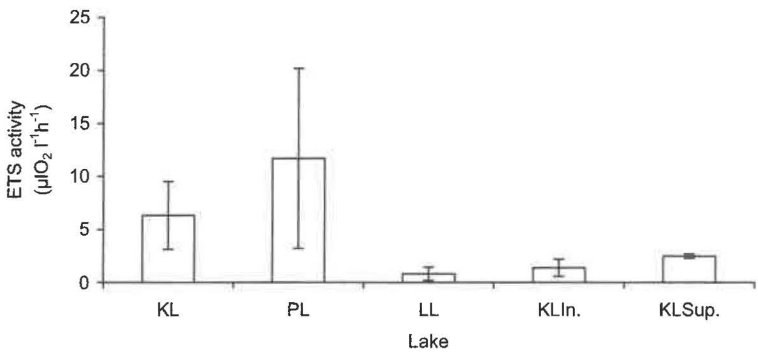


Fig. 2. ETS activity (μl$O_2$/l/h) in phytoplankton in vertical profile (n=6-9, mean±SD).
Sl. 2. Aktivnost ETS (μl$O_2$/l/h) v fitoplanktonu na vertikalnem profilu (n=6-9, povprečje±SD).

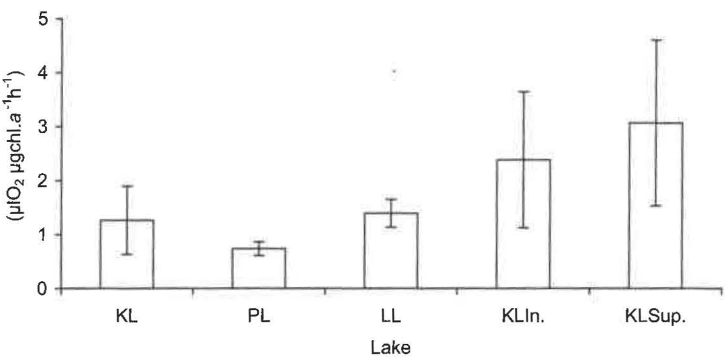


Fig. 3. ETS activity (normalised) in phytoplankton in vertical profile (n=6-9, mean±SD).
Sl. 3. Aktivnost ETS (normirano) v fitoplanktonu na vertikalnem profilu (n=6-9, povprečje ±SD).

In conclusions, the present study reveals, that phytoplankton from high mountain and olygotrophic lakes protected their vulnerable targets in the cells by synthesis of UV screening substances. This process required additional energy, derived from respiratory potential.

## Povzetek

Naraščanje UV sevanja na Zemeljski površini, ki je posledica tanjšanja ozonske plasti v atmosferi, je predmet mnogih raziskav. Čeprav je delež UV-B sevanja v sončnem sevanju majhen, je njegov vpliv na primarne proizvajalce velik. Visokogorska jezera so še posebej občutljivi sistemi na klimatske spremembe. Po nekaterih podatkih se UV-B sevanje (280-320 nm) na vsakih 1000 m nadmorske višine poveča za 6-8%, oz. za 20%.

Visokogorska jezera vsebujejo navadno nizke koncentracije raztopljenih organskih snovi, ki v veliki meri vplivajo na prodiranje UV sevanja v vodo. Organizmi v visokogorskih in čistih jezerih so tako izpostavljeni višjim odmerkom UV-B sevanja brez možnega zatočišča pred škodljivimi žarki. UV-B sevanje vpliva na mnoge biološke in fiziološke procese pri primarnih proizvajalcih.

Ugotavljali smo sposobnost fitoplanktona za izgrajevanje UV zaščitnih snovi ter dihalni potencial (aktivnost terminalnega elektronskega transportnega sistema – ETS) na Jezeru na Planini pri Jezeru, Krnskem jezeru, Jezeru v Ledvicah ter Spodnjem in Zgornjem Kriškemu jezeru, ki ležijo na različnih nadmorskih višinah, od 1383 do 2150 m. Vsebnost UV zaščitnih snovi je bila večja v fitoplanktonu iz višje ležečih jezer. Aktivnost ETS (normalizirana) je bila večja v Jezeru v Ledvicah ter obeh Kriških jezerih. Večja ETS aktivnost fitoplanktona iz višje ležečih jezer odraža potrebo po energiji, ki jo organizmi rabijo za mehanizme, s pomočjo katerih se zaščitijo pred sevanjem.

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# The phenotypic plasticity of *Glyceria fluitans* growing over the water/land gradient

## Fenotipska plastičnost vrste *Glyceria fluitans* na prehodu voda/kopno

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**Abstract.** The amphibious species *Glyceria fluitans* successfully thrives in an intermittent ecosystem Lake Cerknica, where floods alternate with dry periods. The species grows over the environmental gradient from open water to dry land. The plant's phenotypic plasticity was studied analysing anatomical, morphological, biochemical and physiological characteristics in specimens from different locations. Floating leaves were thicker, having abundant aerenchyma, lower chlorophyll a+b contents and higher chlorophyll a/b ratio. The relative amounts of total UV-B and UV-A screening compounds per leaf area were high and similar in all forms that indicated possibly saturated amounts. The measurement of fluorescence parameters revealed no disturbance in energy harvesting since the values of potential and effective photochemical efficiencies, photochemical and non-photochemical quenching were similar over the gradient.

**Key words:** *Glyceria fluitans*, land/water gradient, amphibious character

**Izvleček.** Amfibijska vrsta *Glyceria fluitans* se pojavlja v presihajočem ekosistemu Cerkniškega jezera, kjer se poplave izmenjujejo s sušnimi obdobji. Vrsta porašča širok pas na prehodu iz vode na kopno. Na podlagi nekaterih anatomskih, morfoloških, biokemijskih in fizioloških analiz rastlin na prehodu smo ugotavljali fenotipsko plastičnost vrste. Plavajoči listi so bili debelejši, z obsežnejšim aerenhimom, nižjo vsebnostjo klorofila a+b in višjim razmerjem med klorofiloma a in b. Relativna vsebnost UV-B in UV-A zaščitnih snovi na listno površino se med proučevanimi oblikami ni bistveno razlikovala kar kaže na saturirane vrednosti. Izmerjeni parametri fluorescence so odražali nemoten privzem energije, saj so bile vrednosti potencialne in dejanske fotokemične učinkovitosti ter fotokemičnega in nefotokemičnega dušenja podobne na celotnem prehodu iz vode na kopno.

**Ključne besede:** Glyceria fluitans, prehod med kopnim in vodo, amfibijske značilnosti

## Introduction

Macrophytes are limited to the macroscopic flora including aquatic spermatophytes, pteridophytes and bryophytes. Their growth form usually classifies them in four-group system, which is widely accepted: emergent macrophytes (i.e. *Phragmites australis*), floating-leaved macrophytes (i.e. Nuphar luteum), free-floating macrophytes (i.e. *Eichhornia crassipes*) and submerged macrophytes (i.e. *Myriophyllum spicatum*). Some plants are not restricted to one category only due to their amphibious character (Fox 1992). Plants/species with an amphibious character plants are found among all major groups of plants, including mosses, ferns and angiosperms (Hutchinson 1975).

In the intermittent water bodies, such as Lake Cerknica, water depth and water retention play a major role in the occurrence of plant species (Fernández-Aláez & al. 1999). The water level raising drowns emergent plants, while lowering exposes submerged ones to drying, freezing or heat (Cronk & Fennessy 2001). Plants/species with an amphibious character plants possess either distinct aerial and aquatic leaves (heterophylly) either different shoots or growth forms (Hutchinson 1975, Fox 1992). The phenotypic plasticity enables the species to colonize a range of habitats. It is an expression of flexibility in evolutionary strategy of the species (Menadue & Crowden 1990).

Amphibious character gives them the ability to photosynthesize under contrasting conditions in aquatic and terrestrial environment (Germ 2002). Beside morphological adaptations, amphibious plant species may also respond to the fluctuations of water by changing the reproduction strategy (Boulton & Broc 2001).

Scarce information on *Glyceria fluitans* is available in the literature (Jogan 1996). We hypothesised that the species possessing temporal or spatial segregated submerged, floating and emergent shoots might have a competitive advantage over less tolerant submerged or emergent macrophytes in habitats characterised by water fluctuations, such as Lake Cerknica. To get an insight into survival strategy of *G. fluitans*, growing over environmental gradient in an intermittent Lake Cerknica, we studied some anatomical, morphological, biochemical and physiological characteristics. The latter included the measurements of fluorescence parameters that give us useful information about the ability of plants to tolerate the water level fluctuations.

## Materials and methods

### Area description

The study was carried out in Lake Cerknica (45°45'N, 14°20'E) during extremely dry vegetation season in June 2003. The closed depression of Cerkniško polje extends on an area of 38 $km^2$ and usually the area of 25 $km^2$ is flooded when the polje changes into a lake. The Lake Cerknica is locus typicus for intermittent lakes and karst poljes. The water reaches its normal level of 550 m a.s.l. in spring and late autumn to early winter (Kranjc 2002a, Kranjc 2002b). Usually in summers the lake becomes dry.

### Material studied

*Glyceria fluitans L.* belongs to Poaceae family (Martinčič & al. 1999). It colonizes more or less permanent, shallow still or slowly running water. It is widespread to scattered in lowland and lower montane area rarely exceeding 600 m a.s.l. (Jogan 1996). We examined different growth forms of *G. fluitans* growing over environmental gradient from open water to dry land (1 – open water; 2 – water-land interface, 3 – 2 m from water body, 4 – dry land).

### Morphological and anatomical analyses

In samples collected at different locations we measured the total stem length and counted the number of leaves per stem. We determined the thickness of leaves, spongy tissue and palisade tissue and calculated the palisade/spongy tissue ratio. Dry weights were obtained after drying the plant material at 105°C for 24 hours.

### Biochemical analyses

For determination of chlorophylls (chl a+b and chl a/b ratio) we followed the equations developed by JEFFREY & HUMPHREY (1975). The chlorophyll extracts were made using 90% (v/v) acetone solution. The chlorophyll contents were calculated considering absorption values at 647, 664 and 750 nm measured with UV/VIS Spectrometer System (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). The extracts of UV-A and UV-B screening compounds we made using the methanol solution Šmethanol : distilled water : HCl (37% (v/v)) = 79:20:1Ć. We followed the method proposed by CALDWELL (1968). The extinctions of the samples were measured in the UV-B spectral range 280-320 nm and UV-A spectral range 320-400 nm (Lambda 12, Perkin-Elmer, Norwalk, CT, USA). The extinctions were integrated for UV-A and UV-B range and expressed in relative units per dry mass and per area.

### Measurements of fluorescence parameters

Measurements of chlorophyll a fluorescence were carried out on clear days during dry period in summer 2003. Photosynthetic photon flux density (PPFD) was more than 1100 mmol/m2s, relative humidity around 40% and temperature (air and water) was in range between 20°C and 25°C. The measurements of chlorophyll fluorescence were made using a modulated fluorometer (OS-500 fluorometer; OPTI-SCIENCES, Tyngsboro, MA, USA). The actual (yield) and potential (Fv/Fm) photochemical efficiencies were measured to estimate the disturbance to light harvesting in different plant forms from different locations. Values of Fv/Fm were obtained after dark-adaptation by using dark-leaf clips for 10 minutes. Fv stands for variable fluorescence. This is the difference between maximal (Fm) and minimal (Fo) fluorescence in dark-adapted leaves excited with a saturating beam of white light. The yield of illuminated sample was defined as (F´m-F´o)/F´m (F´m is maximal and F´o is minimal fluorescence of light-adapted leaves) (CAMPBELL & al. 2003). The photochemical (qP) and non-photochemical (qN) quenching was traced using a series of saturating pulses of white light (PPFD=8000 mmol/m2s; 0.8s). Sample leaves for kinetics determination were dark adapted for 30 minutes.

### Statistical analysis

The measurements were carried out on 5 parallel samples. Standard deviations (SD) were calculated. The analysis of variance (one factor ANOVA) was performed to estimate the differences among locations over environmental gradient.

## Results and discussion

The intermittent Lake Cerknica is characterised by extreme water level fluctuations. The flora of Cerkniško polje is very diverse, due to great diversity of biotopes and specific water regime (MARTINČIČ 2002). The life histories of plants in the lake are intimately coupled to the periodicity of the water regime (GABERŠČIK & al. 2004). The water level fluctuations are a limiting factor for the plant growth, development and reproduction (GABERŠČIK & URBANC-BERČIČ 2002); therefore the growth of plants/species with an amphibious character is favoured (GABERŠČIK & MARTINČIČ 1992, MARTINČIČ 2002, MARTINČIČ & LESKOVAR 2002).

*G. fluitans* thrives at the locations were the water is present also in dry period. At that time this species occupies the locations from open water to dry land, exhibiting different growth forms in contrasting environment. The analysis of the habitus of plants revealed that plants in open water developed more leaves than terrestrial ones, but the length of the plants was the highest in both extreme locations. The leaves became thinner with the increasing distance from the water body, due to less developed spongy tissue (Tab. 1). Looser tissue, well developed in floating specimens, might improve the floating ability of leaves. Abundant aerenchyma contributes to lower specific leaf weight of floating leaves, even though they are much thicker. Thicker floating leaves were also found in a case of *Polygonum amphibium* (GABERŠČIK & MARTINČIČ 1992). *P. amphibium* exhibited only slight differences in palisade/spongy tissue ratio. It seems that thicker floating leaves are an attribute of many plants/species with an amphibious character (HUTCHINSON 1975, NIELSEN 1993). On a contrary, the floating leaves in *Batrachium peltatum* were thinner than aerial ones (NIELSEN & SAND-JENSEN 1993).

Tab. 1. Morphological, anatomical and biochemical characteristics of *Glyceria fluitans* growing over the environmental gradient (1 - open water; 2 - water-land interface, 3 - 2 m from water body, 4 - dry land) in Lake Cerknica.
Tab. 1. Morfološke, anatomske in biokemijske značilnosti vrste *Glyceria fluitans* rastoče v različni oddaljenosti od proste vodne površine (1 - odprta vodna površina, 2 - prehod med vodo in kopnim, 3 - 2 m od vodnega telesa, 4 - kopno) na Cerkniškem jezeru.

| **Characteristic** | **Location** | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **4** |
| Total steam length [cm] | 89 ± 7 | 48 ± 3 | 66 ± 4 | 87 ± 10 |
| No. leaves per steam | 7.8 ± 0.4 | 6. 7 ± 0.5 | 6.6 ± 0.9 | 4.9 ± 0.9 |
| Leaf thickness [μm] | 539 ± 120 | 372 ± 74 | 256 ± 38 | 180 ± 22 |
| Palisade/spongy tissue | 0.28 ± 0.08 | 0.44 ± 0.06 | 0.80 ± 0.22 | 2.15 ± 0.47 |
| Spec. leaf weight [$g/dm^2$] | 33.8 ± 6.9 | 35.2 ± 9.0 | 39.1 ± 2.8 | 40.7 ± 1.6 |
| Spec. leaf area [$dm^2/g$] | 0.031 ± 0.008 | 0.028 ± 0.001 | 0.025 ± 0.002 | 0.024 ± 0.001 |
| Chl *a+b* [mg/gDM] | 7.19 ± 1.34 | 14.87 ± 2.45 | 10.05 ± 2.09 | 8.49 ± 2.17 |
| Chl *a/b* | 7.19 ± 2.95 | 3.95 ± 0.31 | 3.90 ± 0.48 | 5.35 ± 3.17 |

Amphibious species exhibit great variability in chlorophyll content (GERM & GABERŠČIK 2003). The floating leaves of *G. fluitans* had lower chlorophyll a+b content (mg g-1 dry mass) than the emergent leaves (Tab. 1). The investigations of *Callitriche copocharpa* and *Batrachium peltatum* are in line with our results (NIELSEN 1993, NIELSEN & SAND-JENSEN 1993), but this was not the case in *Batrachium aquatile* (NIELSEN 1993). We determined the highest values of chlorophyll a/b ratio in floating leaves. This might be a consequence of the fact that a lot floating leaves were more exposed to irradiation, while aerial leaves were erect and therefore they avoided high irradiance flux during midday.

The ability of plants to protect themselves from UV radiation plays the important role in open habitats. The majority of primary producers is able to produce UV screening compounds that present an UV-B selective filter (BJÖRN 1999). This appears to be an important protective mechanism because they could effectively reduce detrimental effect of UV-B radiation in leaves (MIDDELTON & TERAMURA 1993). In *G. fluitans* the relative amount of total UV-B and UV-A screening compounds per unit of dry mass (Fig. 1) was the highest in floating leaves. The investigations reveal that UV absorbing compounds are mainly synthesised in epidermis of the leaves that prevent UV penetration into the mesophyll (Day & al. 1996). For that reason we also compared the amounts of total UV-B and UV-A screening compounds per unit of leaf area. The values showed no significant difference among different forms. These results are in agreement with other researches of plants/species with an amphibious character (Germ & al. 2002). The comparison of the amounts determined in *G. fluitans* with other amphibious and terrestrial plants revealed relatively high values in the former. That indicated possibly saturated amounts of UV-B and UV-A screening compounds. The production is probably genetically fixed and less dependent on environmental factors. A similar phenomenon was found in *Potamogeton nodosus* (Les & Sheridan 1990).

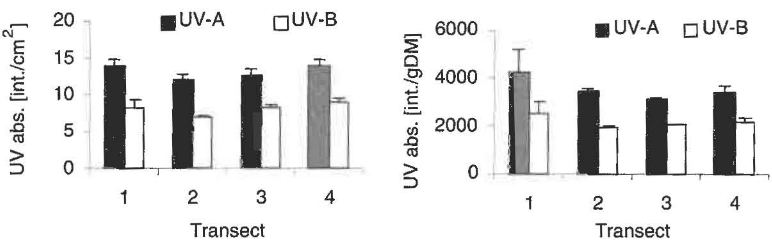



Fig. 1. The amount of UV absorbing compounds of *Glyceria fluitans* growing over the environmental gradient (1 - open water; 2 - water-land interface, 3 - 2 m from water body, 4 - dry land) in Lake Cerknica.
Sl. 1: Količina UV absorbirajočih snovi pri vrsti *Glyceria fluitans* rastoče v različni oddaljenosti od proste vodne površine (1 - odprta vodna površina, 2 - prehod med vodo in kopnim, 3 - 2 m od vodnega telesa, 4 - kopno) na Cerkniškem jezeru.

The water-logging should not cause a physiological stress in macrophytes, although photochemical efficiency might be disturbed due to other stresses which are the consequence of water level fluctuation; i.e. drought (Colom & Vazana 2003), photoinhibition (Gaberščik & Mazej 1995, Ralph & Burchett 1995, Beer & Björk 2000, Mouget & Tremblin 2002, Campbell & al. 2003) and UV-B radiation (Conde-Alvarez 2002, Gaberščik & al. 2002, Germ et al. 2002). Our results showed no significant differences in potential (Fv/Fm) and actual (yield) photochemical efficiencies of PS II in *G. fluitans* growing over the environmental gradient (Fig. 2, Tab. 2). Fv/Fm was close to the opti-

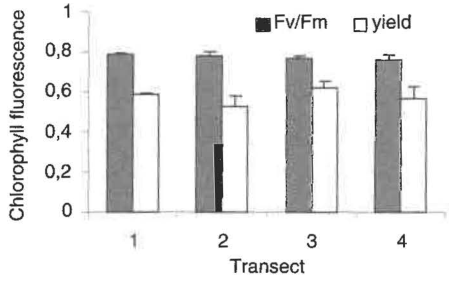
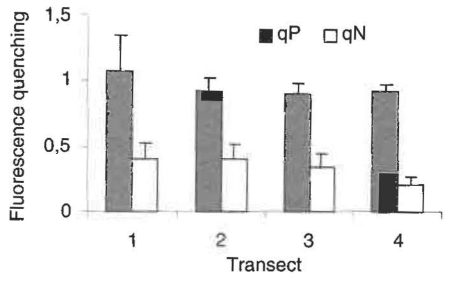



Fig. 2. Photochemical efficiency of *Glyceria fluitans* growing over the environmental gradient (1 - open water; 2 - water-land interface, 3 - 2 m from water body, 4 - dry land) in Lake Cerknica.
Sl. 2: Fotokemična učinkovitost vrste *Glyceria fluitans* rastoče v različni oddaljenosti od proste vodne površine (1 - odprta vodna površina, 2 - prehod med vodo in kopnim, 3 - 2 m od vodnega telesa, 4 - kopno) na Cerkniškem jezeru.

mal values (0.83) at all locations as an indication of normal function of PS II. Lower values of yield indicated the presence of stress with no respect to the location and form. WALDHOFF & al. (2002) also found no correlation between the changing water levels and the Fv/Fm ratios of the tree species *Symmeria paniculata.* The leaves fully retained the potential to photosynthesize during the aerial and aquatic phases. Fv/Fm ratio in *Genipe americana* also remains constant despite flooding conditions (MIELKE & al. 2003). Our study revealed no differences in photochemical (qP) and nonphotochemical (qN) quenching in *G. fluitans* appearing in different growth forms over the gradient water-land (Fig. 3). Photochemical quenching had greater values and affected the actual photochemical efficiency more than non-photochemical quenching. Values of qN in *Genipe americana* increase by flooding, while qP remains constant (MIELKE & al. 2003).

Fig. 3. Photochemical and non-photochemical fluorescence quenching in *Glyceria fluitans* growing over the environmental gradient (1 - open water; 2 - water-land interface, 3 - 2 m from water body, 4 - dry land) in Lake Cerknica.
Sl. 3. Fotokemično in nefotokemično dušenje fluorescence pri vrsti *Glyceria fluitans* rastoče v različni oddaljenosti od proste vodne površine (1 - odprta vodna površina, 2 - prehod med vodo in kopnim, 3 - 2 m od vodnega telesa, 4 - kopno) na Cerkniškem jezeru.




Tab. 2. The significance of differences of investigated plant characteristics among 4 locations over environmental gradient water/land (ANOVA) (1 - open water; 2 - water-land interface, 3 - 2 m from water body, 4 - dry land) in Lake Cerknica.
Tab. 2. Satistična značilnost razlik pri proučevanih značilnostih rastline med 4 lokacijami na okoljskem gradientu voda/kopno (ANOVA) (1 - vodno telo, 2 - prehod med vodo in kopnim, 3 - 2 m od vodnega telesa, 4 - kopno) na Cerkniškem jezeru.

| **Characteristic** | **Significance** | **Characteristic** | **Significance** |
|---|---|---|---|
| Total steam length [cm] | *** | UV-A [int./gDM] | NS |
| No. leaves per steam | *** | UV-B [int./gDM] | * |
| Leaf thickness [μm] | *** | UV-A [int./cm$^2$] | NS |
| Palisade/spongy tissue | *** | UV-B [int./cm$^2$] | ** |
| Spec. leaf weight [g/dm$^2$] | * | Fv/Fm | NS |
| Spec. leaf area [dm$^2$/g] | NS | Yield | * |
| Chl *a+b* [mg/gDM] | *** | Photchemical quenching | *** |
| Chl *a/b* | NS | Non-photchemical quenching | *** |

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; non significant (NS) $p > 0.05$.

## Conclusions

The floating shoots of *G. fluitans* develop thicker leaves than emergent ones. They have abundant aerenchyma that contributes to lower specific leaf weight and their floating ability.

The floating leaves had higher chlorophyll a/b ratio than the emergent ones, which might be due to higher irradiance of direct sunrays. The relative amount of total UV-B and UV-A screening compounds showed no significant difference among diverse forms of *G. fluitans*.

No significant differences in potential and effective photochemical efficiencies as well as in photochemical and non-photochemical quenching were found over the environmental gradient. These fluorescence characteristics indicate normal functioning of PS II.

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## Povzetek

*Glyceria fluitans* je vrsta z amfibijskim značajem in raste v presihajočem ekosistemu Cerkniškega jezera, ki ga označujejo izmenjave poplav in sušnih obdobij. V sušnem delu leta, ko nivo vodne gladine upade, porašča širok pas na prehodu iz vode na kopno. *G. fluitans* v odvisnosti od vodnih razmer, ki so pogojene prostorsko ali časovno, tvori morfološko različe poganjke. V vodi razvije poganjke, ki so potopljeni ali plavajoči, na kopnem pa zračne poganjke. V raziskavi smo ugotavljali fenotipsko plastičnost vrste na prehodu iz vode na kopno. Spremljali smo nekatere anatomske, morfološke, biokemijske in fiziološke parametre posameznega tipa poganjkov. V naravnem okolju smo s pomočjo parametrov fluorescence ugotavljali uspešnost prestrezanja energije. Rezultati so pokazali, da so plavajoči listi *G. fluitans* debelejši, z obsežnejšim aerenhimom, ki zmanjšuje specifično listno težo in prispeva k večji plovnosti. Nižja vsebnost klorofila a+b in višje razmerje med klorofiloma a in b pri plavajočih listih pa sta odraz večje jakosti sevanja zaradi direktnih sončnih žarkov. Realtivna vsebnost UV-B in UV-A zaščitnih snovi na listno površino je bila v vseh proučevanih oblikah podobna in je nakazovala saturirane vrednosti. Izmerjeni parametri fluorescence klorofila a v FS II so odražali normalno fotosintezno aktivnost vrste na celotnem prehodu iz vode na kopno. Vrednosti potencialne fotokemične učinkovitosti (Fv/Fm) so bile na celotnem prehodu blizu optimalni vrednosti 0,83. Dejanska fotokemična učinkovitost (yield) je bila nižja od potencialne, kar je rezultat rahlega stresa, vendar pa razlik na gradientu nismo opazili. Tudi vrednosti fotokemičnega in nefotokemičnega dušenja so bile podobne na celotnem prehodu iz vode na kopno.

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# Pollen grain bioassay for environmental contamination biomonitoring

## Biomonitoring polucije okolja z analizo poškodovanosti pelodnih zrn

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**Abstract.** Pollen grain bioassay near some highways was performed in 45 plant species for identification of environmental genotoxic impact on naturally growing plants due to environmental traffic contaminants. A relationship between the duration of heavy traffic on particular road sections and the degree of developmental and morphological changes of pollen grains was indicated. Increased frequency of pollen deformation after more than 20 years of traffic influence was established, indicating the applicability of pollen grain bioassay for genotoxic risk assessment of chronic low level contamination impact in natural habitats.

**Key Words:** naturally growing bioindicator plants, traffic contaminants, pollen grain deformation

**Izvleček.** V biomonitoringu onesnaženosti okolja zaradi prometa smo analizirali stopnjo poškodovanosti pelodnih zrn pri 45 vrstah rastlin z naravnih rastišč ob naših avtocestah. Ugotovili smo, da je stopnja poškodovanosti peloda na poskusnih lokalitetah povezana s trajanjem prometa na posameznih avtocestnih odsekih, povečan obseg poškodovanosti peloda je bil pri biondikatorskih rastlinah na rastiščih, obremenjenih z vplivi prometa več kot 20 let. Ti rezultati kažejo, da je analiza poškodovanosti pelodnih zrn uporabna za sledenje kroničnih vplivov nizkih nivojev zračne polucije z genotoksičnimi agensi in oceno ogroženosti rastlin na naravnih rastiščih.

**Ključne besede:** samonikle bioindikatorske rastline, onesnaževanje z izpušnimi plini, poškodovanost pelodnih zrn.

## Introduction

Environmental contamination caused by highway traffic has been identified as a serious ecological problem. Air contaminants produced by motor traffic and released along the highways and main road connections are particularly hazardous to public health and the environment. Low concentrations of many environmental pollutants are considered as harmless, but for the genotoxic contaminants there is no safe dose or concentration, and environmental hazard identification is required. Naturally growing plants are exposed to a changing mixture of environmental contaminants, and when used as bioindicators of long lasting exposure to complex pollution impact they have a key role in genotoxic risk evaluation of low contamination levels in natural habitats.

Our previous observation in plant natural habitats with elevated contamination load (PARADIŽ & LOVKA 1998, 1999a, 1999b, 2000) and literature data (KORMUTAK 1996, MIČIETA & MURIN 1996, MULCAHY 1981, MURIN 1995) suggest that with many different species, plant bioassay results could give a more complex information about the pollution threats in the environment. Using 45 species of naturally growing plants in pollen grain bioassay near some highways in Slovenia, we have performed screening of genotoxicity for risk identification of environmental impact of heavy traffic in natural habitats.

## Material and Methods

Experimental sites near some highways in Slovenia, concerning the duration of traffic on the specific road sections were chosen for biomonitoring with pollen grain bioassay. At 8 localities (Bizovik, Arja vas, Vransko, Čebulovica, Divača, Dolenja vas, Zajčica, and Sela) plants in their habitats had been exposed to traffic influence for the period up to 10 years, at 2 localities (Povodje and Brdo) for 10 to 20 years, and at 5 localities (Gabrje, Ivanje selo, Ravnik, Trebnje and Žiče) for 20 to 30 years. Localities at Komen, Predmeja, Pugled, and Škofja Loka with no obvious traffic pollution sources were used for the comparison.

For pollen grain bioassay flower buds were sampled in 45 species of plants growing naturally at experimental and control sites (Tab. 1) on $18^{th}$, $24^{th}$, and $31^{st}$ of March, $7^{th}$ of April, $19^{th}$ and $26^{th}$ of May, $2^{nd}$, $9^{th}$, $17^{th}$ and $23^{rd}$ of June 1999. The samples were fixed in ethanol acetic acid mixture (3:1), then kept at $-20^{o}C$ in a refrigerator.

Microscopic slides of fully developed anthers were made by staining in aceto–carmine. Generally, five slides from five different flowers per bioindicator species at the site were examined. In at least 300 pollen grains per slide, stained (viable) and empty (sterile) grains, as well as pollen size and shape deformations were observed under an optical microscope. Deformation frequencies of pollen grains, as well as portions of morphological changes in relation to the duration of highway traffic for up to 10 years, for 10-20 years and for 20-30 years are presented.

## Results

In naturally growing bioindicator plants at the control sites, over 95% of stained (fertile) pollen grains were observed. In plants at experimental sites near highways, deformation frequency of pollen grains increased with the duration of traffic influence. The frequency of empty (sterile) grains increased up to 10% after 30 years of traffic influence (Fig. 1). Smaller size (for about 1/3 of the normal one) as well as shape deformations were less frequent than empty not stained grains, however, their portion increased with the duration of highway traffic as well (Tab. 2).

Table 1. List of bioindicator species for pollen grain bioassay within sampling periods from March to June at experimental sites near highways and the control sites. Duration of traffic influence for particular road sections in brackets is stated.
Tabela 1. Seznam rastlinskih vrst vključenih v analizo poškodovanosti pelodnih zrn od marca do junija na poskusnih lokalitetah ob odsekih avtocest in kontrolnih lokalitetah. V oklepajih je navedeno trajanje prometa na posameznih odsekih avtocest.

| Sampling | Bioindicator plants | Experimental and control sites |
|---|---|---|
| March | *Anemone nemorosa* L. | Bizovik (1), Arja vas (2) , Trebnje (30) |
| | *Capsella bursa-pastoris* (L.) Med. | Arja vas (2) |
| | *Cornus mas* L. | Komen, Žiče (23) |
| | *Corydalis cava* (L.) Schweigger & Koerte | Komen |
| | *Corylus avellana* L. | Povodje (14) |
| | *Crocus albiflorus* Kit. ex Schult. | Dolenja vas (4), Gabrje (25) |
| | *Crocus napolitanus* Mord. & Loisel. | Bizovik (1), Povodje (14), Brdo (18), Trebnje (30) |
| | *Crocus variegatus* Hoppe & Hornsch. | Komen |
| | *Daphne mezereum* L. | Trebnje (30) |
| | *Erythronium dens-canis* L. | Brdo (18) |
| | *Galanthus nivalis* L. | Komen |
| | *Hacquetia epipactis* (Scop.) DC. | Ravnik (27) |
| | *Helleborus niger* L. | Pugled |
| | *Helleborus odorus* W. & K. ex Willd. | Pugled, Dolenja vas (4) |
| | *Lamium purpureum* L. | Arja vas (2) |
| | *Leucojum vernum* L. | Povodje (14) |
| | *Omphalodes verna* Moench | Ravnik (27) |
| | *Oxalis acetosella* L. | Arja vas (2) |
| | *Polygala chamaebuxus* L. | Pugled |
| | *Potentilla erecta* (L.) Räuschel | Čebulovica (4) |
| | *Pulmonaria officinalis* L. | Povodje (14), Žiče (23), Ivanje selo (27) |
| | *Symphytum officinale* L. | Žiče (23) |
| | *Tussilago farfara* L. | Vransko (2), Divača (4) |
| | *Vinca minor* L. | Vransko (2) |
| April/May | *Campanula rapunculus* L. | Sela (7) |
| | *Crocus napolitanus* Mord. & Loisel. | Trebnje (30) |
| | *Erythronium dens-canis* L. | Sela (7), Povodje (14), Brdo (18) |
| | *Lamium orvala* L. | Povodje (14) |
| | *Lamium purpureum* L. | Bizovik (1), Povodje (14) |
| | *Melittis melissophyllum* L. | Pugled |
| | *Myosotis sylvatica* Ehrh. ex Hoffm. | Povodje (14), Brdo (18) |
| | *Oxalis acetosella* L. | Trebnje (30) |
| | *Polygala chamaebuxus* L. | Pugled |
| | *Potentilla erecta* (L.) Räuschel | Pugled |
| | *Ranunculus acris* L. | Bizovik (1) |
| | *Ranunculus repens* L. | Sela (7), Povodje (14) |
| | *Symphytum officinale* L. | Bizovik (1) |
| | *Trifolium pratense* L. | Sela (7) |
| | *Trifolium repens* L. | Bizovik (1) |
| | *Vinca minor* L. | Sela (7) |
| June | *Calamintha grandiflora* (L.) Moench | Ravnik (27) |
| | *Campanula rapunculus* L. | Škofja Loka |
| | *Coronilla emeroides* Boiss. & Sprun. | Vransko (2) |
| | *Coronilla varia* L. | Komen |
| | *Dianthus sanguineus* Vis. | Komen |
| | *Erigeron annuus* (L.) Pers. | Škofja Loka |

| | |
|---|---|
| *Coronilla emeroides* Boiss. & Sprun. | Vransko (2) |
| *Coronilla varia* L. | Komen |
| *Dianthus sanguineus* Vis. | Komen |
| *Erigeron annuus* (L.) Pers. | Škofja Loka |
| *Filipendula vulgaris* Moench | Komen |
| *Geranium sylvaticum* L. | Ravnik (27) |
| *Lilium martagon* L. | Gabrje (25) |
| *Lysimachia nummularia* L. | Škofja Loka |
| *Muscari racemosum* (L.)Mill. em. Lam. & DC. | Komen |
| *Potentilla erecta* (L.) Räuschel | Škofja Loka |
| *Ranunculus acris* L. | Predmeja, Škofja Loka, Arja vas (2) |
| *Ranunculus bulbosus* L. | Dolenja vas (4), Zajčica (4) |
| *Ranunculus repens* L. | Gabrje (25) |
| *Sanicula europaea* L. | Predmeja |
| *Symphytum officinale* L. | Arja vas (2) |
| *Valeriana officinalis* L. | Divača (4) |

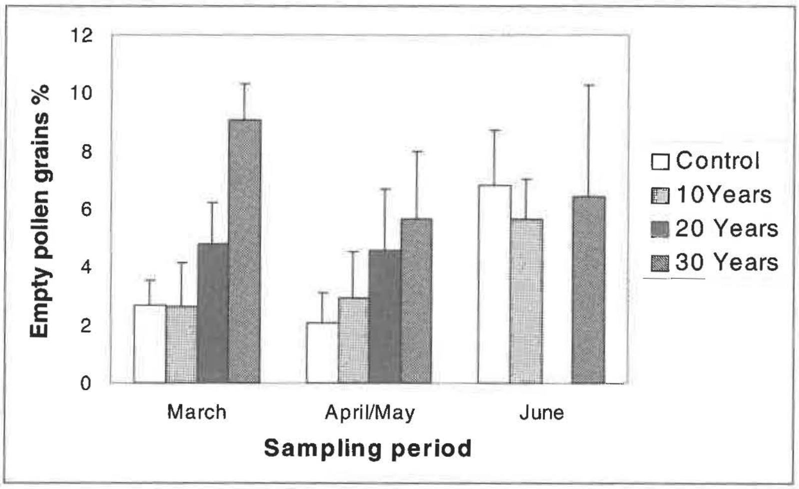


Figure 1. Pollen grain bioassay in naturally growing plants at experimental sites near highways and the control sites.
Slika 1. Poškodovanost peodnih zrn pri naravno rastočih rastlinah na poskusnih lokalitetah ob avtocestah in kontrolnih lokalitetah.

Table 2. Portion (%) of size (small) and shape deformations of pollen grains in naturally growing plants near highways and the control.
Tabela 2. Delež (%) malih in deformiranih pelodnih zrn pri naravno rastočih rastlinah ob avtocestah in kontroli.

| Traffic | March | | | April/May | | | June | | |
|---|---|---|---|---|---|---|---|---|---|
| influence | Size | Shape | Nr. | Size | Shape | Nr. | Size | Shape | Nr. |
| Control | 7,2 | 0,1 | 7 | 4,2 | 0,0 | 3 | 3,4 | 0,1 | 11 |
| 10 Years | 5,6 | 4,1 | 10 | 5,0 | 3,2 | 9 | 13,3 | 1,8 | 5 |
| 20 Years | 9,5 | 2,0 | 5 | 4,5 | 11,7 | 5 | | | |
| 30 Years | 8,7 | 11,8 | 9 | 1,5 | 1,7 | 2 | 4,6 | 6,3 | 4 |

Nr. – Number of bioindicator species used in the pollen grain bioassay

## Discussion

The genotoxic risk of environmental contamination due to increasing traffic density and enlarged highway network in our country is of concern to both public health and the environment. Plants in their habitats are exposed to a broad spectrum of ecological and pollution risks, and they as bioindicators of long lasting exposure to genotoxic effects in the environment are used in biomonitoring pollution in view of environmental risk assessment (Bessonova & al. 1996, Gichner & Veleminsky 1999, Jabeen & Abraham 1996, Kordyum & Sidorenko 1997, Monarca & al. 1999, Müller & al. 1991, Paradiž & Lovka 1998, 1999a, 1999b, 2000).

In genotoxic risk identification, reduced pollen viability is one of the serious pollution impacts on reproductive capacity of plants that might link to ecological consequences. When the amount of stained pollen grains decrease less than 10%, pollen fertility of warm season species is not seriously affected (Tedesco & al. 2002), but under normal climatic conditions pollen abortion in plants is less than 5% (Murin 1995). This is in agreement with our results in bioindicator plants; the average frequency of 3% of empty (sterile) pollen grains at the control sites was established.

At experimental sites near highways, the frequency of empty pollen grains (generally due to irregularities during meiosis) in bioindicator plants increased with the duration of traffic to 10% after 30 years of traffic influence (Fig. 1), as well as the portion of size and shape deformations (Tab. 2). These results indicate that the genotoxic impact of long lasting environmental pollution might be related with traffic contaminants.

When naturally growing plants for biomonitoring genotoxicity of pollutants are used, synergy with other environmental factors, such as meteorological conditions could not be excluded. With successive sampling times at experimental sites near highways, the influence of extreme temperature or drought in summer on the results (Fig. 1) could be verified. But generally, prolonged sampling period for pollen grain bioassay is needed because of blooming season of various plant bioindicators.

On the basis of the present results, pollen grain bioassay for continuous biomonitoring could be suggested, in addition to screening of detailed cytogenetical damage in plants, as well as the routine measurements of atmospheric contaminants for risk assessment of traffic pollution impact in the environment.

## Acknowledgements

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## Povzetek

Ugotavljali smo vpliv škodljivih onesnaževalcev prometa na stopnjo poškodovanosti pelodnih zrn pri naravno rastočih rastlinah ob avtocestah. V raziskavo smo vključili 45 rastlinskih vrst in jemali cvetne popke od marca do julija za opazovanje sprememb v razvoju in obliki pelodnih zrn. Vzorčili smo na lokalitetah: Bizovik, Arja vas, Vransko, Čebulovica, Divača, Dolenja vas, Zajčica in Sela z vplivi prometa do 10 let; Povodje in Brdo z vplivi prometa 10 do 20 let; Gabrje, Ivanje selo, Ravnik, Žiče in Trebnje z vplivi prometa 20 do 30 let. Za primerjavo rezultatov smo uporabili lokalitete, oddaljene od glavnih prometnih cest (Komen, Predmeja, Pugled in Škofja Loka).

Na poskusnih lokalitetah ob posameznih odsekih avtocest, kjer se odvija promet več kot 20 let smo ugotovili povečano stopnjo poškodovanosti pelodnih zrn pri naravno rastočih rastlinah. To kaže na uporabnost analize poškodovanosti pelodnih zrn za ugotavljanje dolgotrajnega vpliva zračne polucije na rastline v naravnem okolju. Z nadaljevanjem tovrstnih analiz bi doprinesli rezultate o škodljivih vplivih nizkih nivojev kontaminacije za oceno stopnje ogroženosti rastlin obremenjenih rastišč.

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# Respiration rate and respiratory electron transport system (ETS) activity of chironomid larvae from mountain lakes (NW Slovenia)

## Stopnja dihanja in aktivnost dihalnega elektronskega transportnega sistema (ETS) pri ličinkah trzač iz gorskih jezer (SZ Slovenija)

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**Abstract.** Respiration rate (R) and electron transport system (ETS) activity were measured in larvae of four chironomid species, *Chironomus thummi* (Kieffer), *Paratanytarsus austriacus* (Kieffer), *Procladius (Holotanypus)* spp., and *Zavrelimyia barbatipes* (Kieffer) from three mountain lakes. ETS activity and respiration rate, measured at a standard temperature of 20 °C, differed significantly between species (ANOVA: p<0.001). ETS activity ranged from 10.3 μL $O_2$ $mg^{-1}$ DW $h^{-1}$ for *Zavrelimyia* to 27.6 μL $O_2$ $mg^{-1}$DW $h^{-1}$ for *Paratanytarsus*. The lowest respiration rate was observed for *Procladius* (4.0 μL $O_2$ $mg^{-1}$DW $h^{-1}$) and the highest for *Paratanytarsus* (12.4 μLO2 $mg^{-1}$ DW $h^{-1}$). ETS activity and respiration rate of chironomids decreased with increasing size of the animals (p<0.001); b values for ETS activity and respiration rate were 0.82 and 0.66, respectively. Respiration rate correlated significantly with ETS activity for all four species investigated. This study revealed that the ETS/R ratio differed between species (ANOVA: p<0.001). A low ratio (0.98) was determined for *Zavrelimyia*, while *Procladius* showed the highest value (3.88). The differences can be explained by body size, and the ecological preference and tolerance of each chironomid species.

**Key words:** Chironomidae, Diptera, benthos, ETS/R ratio, ETS activity, respiration, metabolism

**Izvleček.** Stopnjo dihanja in aktivnost elektronskega transportnega sistema (ETS) smo določali pri ličinkah štirih različnih vrst trzač, *Chironomus thummi* (Kieffer), *Paratanytarsus austriacus* (Kieffer), *Procladius (Holotanypus)* spp., in Zavrelimyia barbatipes (Kieffer), ki smo jih nalovili v treh gorskih jezerih. Aktivnost ETS in stopnja dihanja, ki smo ju merili pri standardni temperaturi 20 °C, sta se značilno razlikovali med vrstami (ANOVA: p<0.001). Aktivnost ETS se je gibala med 10.3 μL $O_2$ $mg^{-1}$DW $h^{-1}$ pri ličinkah Zavrelimyia in 27.6 μL $O_2$ $mg^{-1}$ DW $h^{-1}$ pri ličinkah Paratanytarsus. Najnižjo stopnjo dihanja smo določili pri ličinkah *Procladius* (4.0 μL $O_2$ $mg^{-1}$DW $h^{-1}$), najvišjo pa pri ličinkah *Paratanytarsus* (12.4 μL $O_2$ $mg^{-1}$DW $h^{-1}$). Aktivnost ETS in stopnja dihanja ličink sta se zmanjševali z naraščajočo velikostjo živali ($p<0.001$); vrednosti b sta znašali 0.82 za aktivnost ETS in 0.66 za dihanje. Opazili smo pozitivno korelacijo med stopnjo dihanja in aktivnostjo ETS pri vseh štirih vrstah. Raziskava je pokazala značilne razlike v razmerju ETS/R med vrstami (ANOVA: $p<0.001$). Nizko razmerje (0.98) je bilo določeno pri ličinkah *Zavrelimyia*, medtem ko je bilo pri ličinkah *Procladius* določeno najvišje razmerje (3.88). Različna razmerja so posledica razlik v vrstno-specifični telesni velikosti ter različne ekološke preference in tolerance pri posamezni vrsti trzač.

**Ključne besede:** Chironomidae, Diptera, bentos, razmerje ETS/R, aktivnost ETS, dihanje, metabolizem

## Introduction

The family Chironomidae is widely distributed in all types of aquatic environments, where they form the major part of nearly all freshwater communities, and they occupy a variety of niches. The relatively short life cycle and large total biomass of the numerous larvae confer ecological energetic significance on this taxon, as both consumers and prey, and the partitioning of ecological resources by a large number of species presumably enhances the biologic stability of aquatic ecosystems (MERRITT & CUMMINS 1984).

As chironomid larvae play an important role in aquatic food webs, an estimation of their respiratory energy loss is essential in order to estimate the energy flow through the ecosystems. Direct determination of respiration usually involves incubating animals in a controlled environment, and determining the time-dependent change in oxygen concentration. These methods are time consuming and impractical. The development of enzymatic techniques, however, has allowed metabolic activity to be estimated from the electron transport system activity (OWENS & KING 1975, PACKARD 1985). The electron transport system (ETS)-assay, based on the reduction of tetrazolium salt (INT) in the presence of a cell-free homogenate of the organisms and excess substrates of the ETS, has proved to be useful for estimating the metabolic activity of different organisms (PACKARD 1985, DEL GIORGIO 1992, G.-TÓTH & AL. 1995). It is simple, rapid and extremely sensitive and has been used extensively with marine plankton (KENNER & AHMED 1975, BAMSTEDT 1980, VOSJAN & OLANCZUK-NEYMAN 1991) and freshwater plankton (BORGMANN 1978, JAMES 1987, DEL GIORGIO 1992), benthic organisms (CAMMEN & al. 1990, MUSKÓ & al. 1995, 1998), marine sediments (VOSJAN & OLANCZUK-NEYMAN 1977, RELEXANS 1996), and freshwater sediments (TREVORS 1984, G.-TÓTH & AL. 1994). The slow response of ETS activity to short-term variations in environmental factors makes the method superior to direct respiratory measurements on incubated animals (BAMSTEDT 1980). It is, however, necessary to determine empirically the relation between the highest potential metabolic activity and realised oxygen consumption in order to interpret the ETS data properly (DEL GIORGIO 1992). Much has been published on the respiration of chironomids (ERMAN & HELM 1970, KONSTANTINOV 1971, BAIRLEIN 1989, HAMBURGER & DALL 1990, HAMBURGER & AL. 1994), but there is no study concerning their ETS activity.

Previous investigations have shown that the chironomid larvae are the dominant group of the macrozoobenthos in mountain lakes in NW Slovenia (SIMČIČ unpubl. data). These lakes are shallow and, except the eutrophic lakes Krnsko jezero and Jezero na Planini pri Jezeru, transparent to the bottom. Zoobenthos are reported to have a greater effect on energy flux through secondary production in shallow lakes than in deeper ones (JÓNASSON & al. 1990, LINDEGAARD 1994). Therefore, chironomids should be taken into account when energy flow through the food webs in these lakes is investigated.

The purpose of this paper was to determine ETS activity and respiration rate of four different chironomid species from mountain lakes, and to calculate the ETS/R ratios of these species in order to contribute to the estimation of energy flux through secondary production of benthic organisms.

## Materials and methods

**Sampling of animals:** ETS activity and respiration rate were measured in the laboratory for *Paratanytarsus austriacus* (Kieffer) and *Procladius (Holotanypus)* spp. from the oligotrophic lake Zgornje Kriško jezero, *Zavrelimyia barbatipes* (Kieffer) from the oligotrophic lake Srednje Kriško jezero, and *Chironomus thummi* (Kieffer) from the eutrophic lake Krnsko jezero. Zoobenthos samples were taken using van Veen grab (Eijkelkamp). It was important to handle the larvae as little as possible during identification. Single animals were used for each analysis. Different sets of animals were taken to be investigated separately in the respirometric measurements and separately in ETS activity measurements in the first part of experiments. To determine relationship between ETS activity and respiration rate, the individual animal was examined firstly by microrespirometer and then by ETS-assay. Before experiments animals were rinsed with filtered water to remove bacteria adhering to the body surface, and weighed on an electrobalance. The dry mass of animals was calculated using factors determined by drying five samples of animals for 24 h at 60°C.

**Respiration measurements:** Respiration rate was estimated using a twin-flow microrespirometer (CYCLOBIOS, Innsbruck, Austria) (GNAIGER 1983). In the open-flow system the concentration of oxygen was measured before and after the chamber containing the animal. Oxygen consumption was calculated from the reduction of oxygen concentration and the flow rate of the water with a computer program Dat.Graf 2.1 Analysis.

**ETS activity measurements:** ETS activity was measured using the method proposed by PACKARD (1971), modified by OWENS & KING (1975) and improved by G.-TÓTH (1999). Each freshly weighed animal was homogenized in 4 ml of cold homogenization buffer (0.1 M sodium phosphate buffer pH = 8.4, 75 μM $MgSO_4$, 0.15% (w/v) polyvinyl pyrrolidone, 0.2% (v/v) Triton-X-100) for 2 min. The homogenate was then sonicated using an ultrasound homogenizer (Cole-Parmer) for 20 sec and centrifuged in refrigerated centrifuge (Sigma) for 4 min at 0 °C at 8500 x g. Three 0.5 ml samples from each homogenate were incubated in 1.5 ml substrate solution (0.1 M sodium phosphate buffer pH = 8.4, 1.7 mM NADH, 0.25 mM NADPH, 0.2% (v/v) Triton-X-100) with 0.5 ml of 2.5 mM 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) solution for 40 min at a standard temperature of 20 °C. The reaction was stopped by adding 0.5 ml of stopping solution (formalin(conc.):$H_3PO_4$(conc.)=1:1). Formazan production was determined spectrophotometrically from the absorbance of the sample at 490 nm against the blank (1.5 ml substrate solution and 0.5 ml INT solution were incubated as for the samples, and 0.5 ml of homogenate was added after the stopping). ETS activity was measured as the rate of tetrazolium dye reduction and converted to equivalent oxygen utilised per dry mass (DW) in a given time interval as described by KENNER & AHMED (1975).

**Analysis of data:** ETS/R ratios were calculated as the ratio of the calculated maximum oxygen consumption (ETS), as measured by *in vitro* enzymatic rates, to the rate of respiration (R) *in vivo* (OWENS & KING 1975). An analysis of variance (ANOVA) was carried out to test for differences between species. Linear regression between dry mass (DW) and the parameters investigated (ETS activity, respiration rate, ETS/R ratio), and between ETS activity and respiration rate were calculated after logarithmic transformation of data, according the power function $Y = a X^b$ ($\log Y = \log a + b \log X$) (DEL GIORGIO 1992). The statistical tests were performed using Microsoft Excel.

## Results

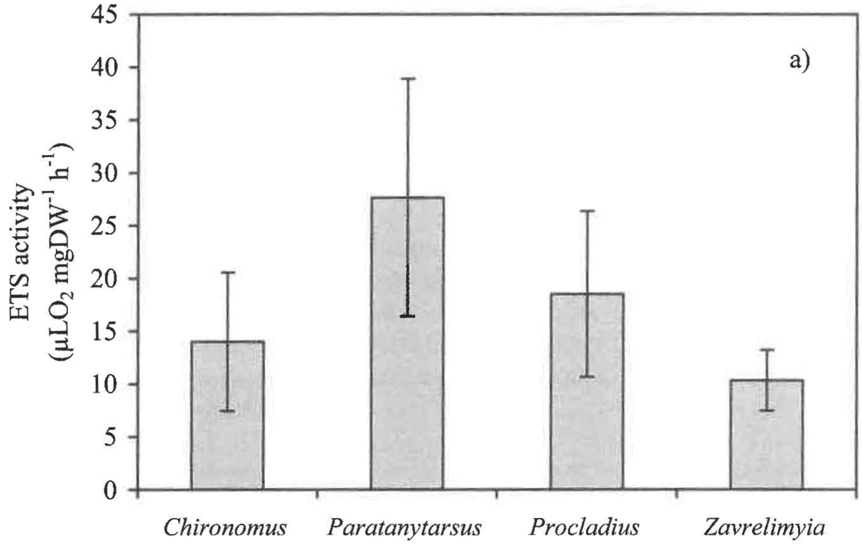
### ETS activity

ETS activity was found to differ significantly between species (Tab. 1). The highest value (27.6 $\mu L\ O_2\ mg^{-1}\ h^{-1}$) was measured in *Paratanytarsus* (Fig. 1a). Significantly lower values were observed in *Procladius* (18.5 $\mu L$ O2 $mg^{-1}\ h^{-1}$), followed by *Chironomus* (14.0 $\mu L\ O_2 mg^{-1}\ h^{-1}$) and *Zavrelimyia* (10.3 $\mu L\ O_2\ mg^{-1}\ h^{-1}$).

The relationship between ETS activity and body mass is shown in Fig. 2a. The mass-specific ETS activity of chironomids decreased with increasing size of the animals ($\log ETS = 1.06 - 0.18 \log DW$; $r=-0.53$; $n=68$; $p<0.001$). The b value was 0.82. The body mass of the animals used in the experiments differed significantly between species (Tab. 1). The mean values (± SD) were 0.07 ± 0.04 mg DW for *Paratanytarsus*, 0.08 ± 0.03 mg DW for *Zavrelimyia*, 0.35 ± 0.27 mg DW for *Procladius* and 1.06 ± 0.94 mg DW for *Chironomus*.

Table 1: Results of analysis of variance applied to ETS activity, respiration rate (R), ETS/R ratio and body mass (DW) in chironomids with species as factor; d.f. - degree of freedom.
Tabela 1: Rezultati analize variance za aktivnost ETS, stopnjo dihanja (R), razmerje ETS/R in telesno maso (DW) pri ličinkah trzač z vrsto kot faktorjem; d.f.-stopinje prostosti

| | d.f. between group | d.f. within group | F-value | P-value |
|---|---|---|---|---|
| ETS | 3 | 64 | 14.29 | <0.001 |
| R | 3 | 39 | 7.94 | <0.001 |
| ETS/R | 3 | 38 | 15.34 | <0.001 |
| DW | 3 | 75 | 17.94 | <0.001 |



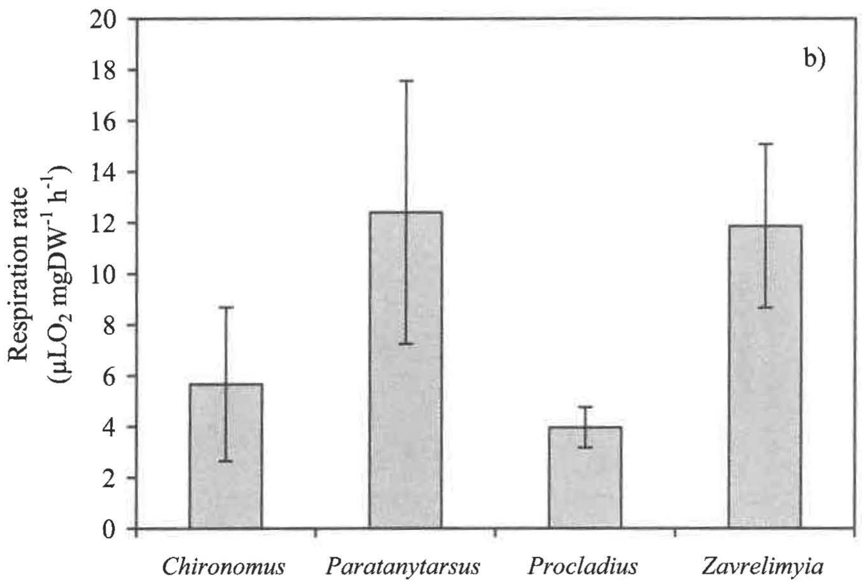




Figure 1: (a) ETS activity and (b) respiration rate of different chironomid larvae (mean ± standard deviation) at 20 °C.

Slika 1: (a) Aktivnost ETS in (b) stopnja dihanja pri ličinkah različnih vrst trzač (srednja vrednost ± standardna deviacija) pri 20 °C.

Table 2: The ratios ETS/R for different species of chironomids, measured at 20 °C.
Tabela 2: Razmerja ETS/R za različne vrste trzač, izmerjena pri 20 °C.

| | n | ETS/R ratio | Standard deviation |
|---|---|---|---|
| *Chironomus thummi* | 18 | 2.73 | 0.93 |
| *Paratanytarsus austriacus* | 9 | 2.44 | 0.86 |
| *Procladius (Holotanypus)* spp. | 8 | 3.88 | 0.75 |
| *Zavrelimyia barbatipes* | 7 | 0.98 | 0.21 |

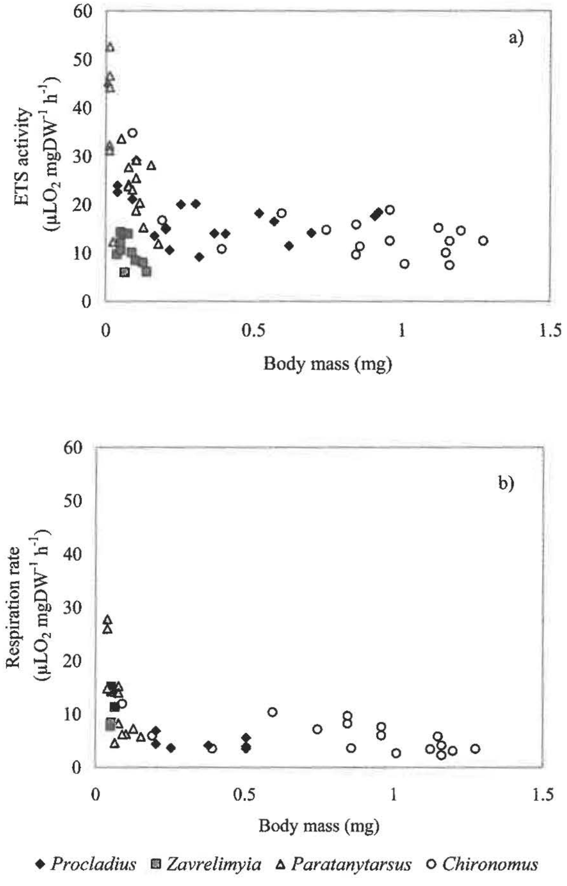



Figure 2: The relationship between (a) ETS activity and body mass (DW), and (b) respiration rate (R) and body mass of different chironomid larvae at 20 °C.
Slika 2: Razmerje med (a) aktivnostjo ETS in telesno maso (DW) ter (b) stopnjo dihanja in telesno maso pri ličinkah različnih vrst trzač pri 20 °C.

### Respiration rate

Respiration rate differed significantly between species (Tab. 1). The highest values were obtained for *Paratanytarsus* (12.4 μL $O_2$ $mg^{-1}$ $h^{-1}$) and *Zavrelimyia* (11.9 μL $O_2$ $mg^{-1}$ $h^{-1}$), while respiration rates of *Chironomus* (5.7 μL $O_2$ $mg^{-1}$ $h^{-1}$) and *Procladius* (4.0 μL $O_2$ $mg^{-1}$ $h^{-1}$) were approximately two and three times lower (Fig. 1b). The regression between respiration rate and body mass of chironomids was statistically significant ($\log R = 0.60 - 0.34 \log DW$; $r=-0.71$; $n=43$; $p<0.001$; Fig. 2b); b value was 0.66.

### The ETS/R ratio

The ratio ETS/R differed significantly between species (Tab. 1). A relatively low value, close to 1, was determined for *Zavrelimyia*, while the highest ratio of 3.88 was obtained for *Procladius* (Tab. 2). Positive correlation between ETS/R ratios and body mass was observed ($r=0.61$; $n=27$; $p<0.001$).

Respiration rate correlated strongly with ETS activity for all four species (Fig. 3). Correlation coefficients and regression constants for relationships between ETS activity and respiration rate for each species are shown in Tab. 3.

Table 3: Relationship between ETS activity and respiration rate of four chironomid species (data were log transformed).
Tabela 3: Razmerje med aktivnostjo ETS in stopnjo dihanja pri štirih vrstah trzač (podatki so bili logaritmirani).

| | n | Correlation coefficient | b (slope) | log a (intercept) | P-value |
|---|---|---|---|---|---|
| *Chironomus thummi* | 18 | 0.73 | 0.56 | 0.72 | <0.001 |
| *Paratanytarsus austriacus* | 9 | 0.82 | 1.47 | -1.02 | <0.01 |
| *Procladius (Holotanypus)* spp. | 8 | 0.71 | 0.41 | 0.09 | <0.05 |
| *Zavrelimyia barbatipes* | 7 | 0.85 | 0.74 | 0.30 | <0.05 |

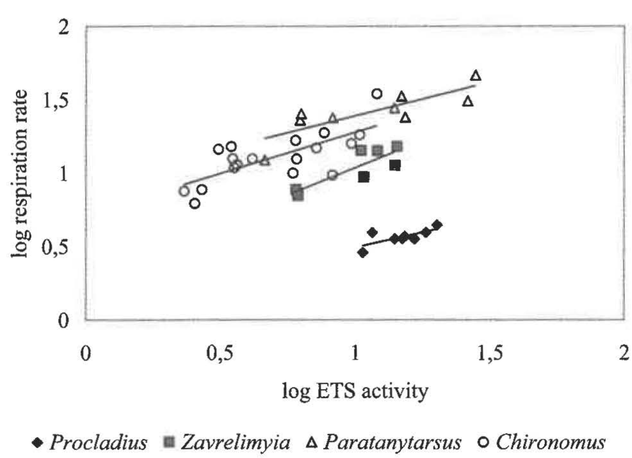



Figure 3: The relationship between ETS activity and respiration rate of different chironomid larvae. Correlation coefficients and regression constants are given in Tab. 3.
Slika 3: Razmerje med aktivnostjo ETS in stopnjo dihanja pri ličinkah različnih vrst trzač. Korelacijski in regresijski koeficienti so podani v Tab. 3.

## Discussion

Results of the present study revealed that ETS activity and respiration rate differed significantly between chironomid species (Fig. 1; Tab. 1). Chironomid species vary significantly in body size (Tab. 1) and thus characters related to body size would be expected to vary between species. KONSTANTINOV (1971) reported that the mass-specific respiration rate of chironomid larvae was inversely related to body size. An effect of size on ETS activity was also found in previous investigations, with larger species having lower ETS activities (SIMČIČ & BRANCELJ 1997, 2001). The larger average body sizes of *Chironomus* and *Procladius* were probably one of the reasons for the lower metabolic activity than in the smaller larvae of *Zavrelimyia* and *Paratanytarsus*. The reasons for higher metabolic activity in smaller animals are well discussed in PETERS (1983).

The significant correlations between body mass and ETS activity and between body mass and respiration rate confirmed the effect of body size on metabolic activity in chironomids (Fig. 2). A b value of 0.82 was found in the case of ETS activity and 0.66 for respiration rate. HAMBURGER & DALL (1990) found a value of 0.73 for respiration rate for four different species of chironomids. Similar values of ETS activity were reported for the benthic macrofaunal species *Corophium volutator*, 0.86 (CAMMEN & al. 1990), for *Nereis virens*, 0.84 (CAMMEN & al. 1990), for *Chelicorophium curvispinum*, 0.66 (MUSKÓ & al. 1995), and for Anostraca *Chirocephalus croaticus*, 0.787 (SIMČIČ & BRANCELJ 2000). In general, b values ranged from 0.58 to 0.96 (see LAMPERT 1987). However, the distribution of ETS activity of *Zavrelimyia* compared to similar sized individuals of *Paratanytarsus, Chironomus* and *Procladius* (Fig. 2a) indicated that other factors, beside body size, had influenced on ETS activity. Larvae of *Zavrelimyia* are considered as cold-stenotherms (FERRARESE 1983, FITTKAU & ROBACK 1983). Therefore, one of the reasons for low ETS activities of *Zavrelimyia* was probably respiratory enzyme systems with narrow temperature optima which caused their inactivation at temperature of 20 °C. Contrary to ETS activities, respiration rates of *Zavrelimyia* were close to those of similar-sized individuals of *Paratanytarsus.*

ETS activity correlated significantly with respiration rate in all species investigated (Fig. 3). These results are in accord with those of KING & PACKARD (1975) and SIMČIČ & BRANCELJ (2001) who also found a correlation between ETS activity and respiration rate in invertebrate species. However, ETS activity is a direct enzymatic process, depending on the concentration (BAMSTEDT 1980) and characteristics (PACKARD 1971) of the enzymes, whereas respiration is a complex physiological process. It means that respiration is also influenced by the intact intracellular environment, substrate concentrations, and structure and properties of intact lipid membranes (WITHERS 1992). As investigated species differed in body size (Tab. 1) and their ecological tolerance (FITTKAU & ROBACK 1983, PINDER & REISS 1983), the differences in the ETS activity-respiration rate relationship between them were expected.

One of the purposes of the present study was to obtain an ETS/R ratio that could be used for estimating actual metabolism in chironomids from ETS activity. As shown in Tab. 2 the values of this ratio differed significantly between chironomid species (Tab. 1). In the present experiments, the basal metabolism and expenditure on locomotion were measured. The expenditure on feeding and specific dynamic action (SDA) was minimal, because the animals were not fed just prior to or during the experiments. The low ETS/R ratio in *Zavrelimyia* means that this species exploited 100% of its metabolic potential for basal metabolism and locomotion. *Procladius* had a much higher ETS/R ratio, which means less intensive exploitation of metabolic potential for basal metabolism and activity (~25%). These ratios are similar to the values found in other studies concerning invertebrate species.

Ratios approaching 2 are characteristic of zooplankton (BAMSTEDT 1980, JAMES 1987, SIMČIČ & BRANCELJ 1997) but, in the case of benthic species, higher values were determined. MUSKÓ & al. (1995) found that the amphipod *Chelicorophium curvispinum* exhibited an ETS/R ratio of 4.07 at 20 °C. CAMMEN & al. (1990) reported an ETS/R ratio for *Corophium volutator* of 2.38, and for the polychaete *Nereis volutator* 11.11 (measured at 10 °C). The latter extremely high value was explained by *N. volutator's* low activity. ETS/R ratio was also influenced by the size of organisms. Present results are in accord with those of CAMMEN & al. (1990) who found that larger animals have higher ETS/R ratios. The reason for higher ETS/R ratios is the greater decrease in respiration relative to ETS activity with increasing body size. Previous studies have also shown that the ETS/R ratio differs between related species having different ecological tolerance and preference, and consequently they exploit their metabolic potential differently (MUSKÓ & AL. 1995, SIMČIČ & BRANCELJ 1997). FANSLOW & al. (2001) reported that, in organisms with high ratios, the capacity for elevated metabolism is maintained so that maintaining the enzyme machinery for increased metabolic activity is an advantage. Investigated chironomid species have different temperature and food preference and tolerance (FITTKAU & ROBACK 1983, PINDER & REISS 1983), and it is reasonable to assume that ETS/R ratio is influenced by these differences as well. Low ETS/R ratio of *Zavrelimyia* suggest that its energy metabolism is less adaptable to environmental changes compared with the more widely distributed *Chironomus, Paratanytarsus* and *Procladius*. However, further studies will provide additional data in order to obtain detailed conclusions related to relationship between ETS/R ratio and different ecological demands of chironomid larvae. Nevertheless, as ETS/R ratio differed significantly between chironomid species, the energy flux through macrozoobentos has to be estimated by using different conversion factors. Preliminary studies on the estimation of energy flow through respiration in mountain lakes revealed that contribution of chironomids to total respiration differed between shallow oligotrophic and eutrophic lakes.

## Conclusions

1. ETS activity and respiration rate, measured at a standard temperature of 20 °C, differed between chironomid species; the body size of species is one of the factors that affected metabolic activity; b value was 0.82 for ETS activity and 0.66 for respiration rate. This indicates the greater decrease in respiration rate relative to ETS activity with increasing body size.

2. The ETS-assay is shown to be a convenient method for estimating respiration rate in chironomid larvae.

3. ETS/R ratios differ between species, so the use of different conversion factors to calculate respiration rate from ETS activity in different chironomid species is recommended. The differences can be explained by different species-specific body size, and different ecological tolerance and preference of chironomid species.

## Povzetek

Ličinke trzač (Chironomidae) so široko razširjena skupina, ki ima pomembno vlogo pri pretoku energije skozi prehranjevalne splete, še zlasti v plitvih jezerih. Predhodne raziskave so pokazale, da ličinke trzač številčno prevladujejo v makrozoobentoški združbi gorskih jezerih SZ Slovenije, zato je pri oceni pretoka energije v jezeru potrebno upoštevati tudi njihov delež. Ker je neposredna določitev stopnje dihanja običajno zamudna in nepraktična, so razvili biokemijsko metodo, s katero merimo aktivnost dihalnega elektronskega transportnega sistema (ETS), ki predstavlja metabolni potencial organizmov. Številne raziskave so pokazale, da obstaja med aktivnostjo ETS in dihanjem pozitivna korelacija, tako da lahko na osnovi izmerjene aktivnosti ETS s pomočjo razmerja ETS/R hitro ocenimo intenzivnost dihanja organizmov. Stopnjo dihanja in aktivnost ETS smo določali pri ličinkah štirih različnih vrst trzač, ki smo jih nabrali v treh gorskih jezer: ličinke *Chironomus thummi* v Krnskem jezeru, ličinke *Paratanytarsus austriacus* in *Procladius (Holotanypus)* spp. v Zgornjem Kriškem jezeru in ličinke *Zavrelimyia barbatipes* v Srednjem Kriškem jezeru. Aktivnost ETS in stopnja dihanja, ki smo jo določali pri standardni temperaturi 20 °C, sta se značilno razlikovali med vrstami. Aktivnost ETS in stopnja dihanja trzač sta se zmanjševali z naraščajočo velikostjo živali, kar kaže na vpliv telesne velikosti na intenziteto metabolizma. Opazili smo pozitivno korelacijo med stopnjo dihanja in aktivnostjo ETS pri vseh štirih vrstah, kar pomeni, da je metoda ETS primerna za oceno dihanja tudi pri ličinkah trzač. Raziskava je pokazala značilne razlike v razmerju ETS/R med preiskovanimi vrstami trzač. Različna razmerja so posledica razlik v vrstnospecifični telesni velikosti trzač ter njihove različne ekološke preference in tolerance.

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# Diverziteta dnevnih metuljev (Lepidoptera: Rhopalocera) v Regijskem parku Škocjanske jame

## Diversity of Butterfly Fauna (Lepidoptera: Rhopalocera) in the Škocjanske jame Regional Park

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**Izvleček.** V letih 2001 in 2002 smo v Regijskem parku Škocjanske jame popisovali favno dnevnih metuljev v 9 habitatnih tipih: suhi travniki, polsuhi travniki, zaraščajoči suhi travniki, gojeni travniki, njive, gozdni robovi, gozdne poti, melišča in naselja. Zabeležili smo 90 vrst dnevnih metuljev, kar je 50 % favne dnevnih metuljev Slovenije. Prevladujejo vseevropske (33 %), južnoevropske (20 %) in južnovzhodnoevropske (17 %) vrste. Favno sestavljajo travniške (56 vrst), grmovne (16 vrst), gozdne (8 vrst) in ubikvitarne (10 vrst) vrste. Med travniškimi vrstami je 34 (39 %) mezofilnih in 22 (24 %) kserotermofilnih vrst. 18 (20 %) registriranih vrst je evropsko in nacionalno ogroženih. Sestava favne metuljev v habitatnem tipu je odvisna od vrstne sestave in strukturiranosti vegetacije v habitatnem tipu ter zgradbe krajinskega mozaika, ki vpliva na disperzijo vrst. Alfa diverziteta je največja na košenih (62 vrst) in zaraščajočih (60 vrst) suhih travnikih. Razloga za velike vrednosti beta diverzitet in visoko gama diverziteto sta prisotnost ekološko specializiranih vrst in heterogenost območja. Z vidika ohranjanja vrstne raznolikosti in ogroženih vrst dnevnih metuljev so v parku najpomembnejši naslednji habitatni tipi: suhi travniki, polsuhi travniki, zaraščajoči suhi travniki, gozdne poti in gozdni robovi ter melišča.

**Ključne besede:** regijski park Škocjanske jame, dnevni metulji (Rhopalocera), razširjenost, favnistični elementi, vrstna diverziteta, ogrožene vrste

**Abstract.** In 2001 and 2002 butterfly diversity were sampled in Škocjanske jame Regional Park, across 9 habitat types: dry grasslands, semi-dry grasslands, unmanaged dry grasslands (dry grasslands in the early successional stages), cultivated grasslands, abandoned fields, woodland margins, woodland paths, tracks and rides, screes and settlements. A total of 90 species of butterflies were recorded, which represent 50% of Slovenian butterfly fauna. All-European (33%), south-

European (20%) and southeast-European (17%) species are prevailing. The butterfly fauna consists of grassland (56 spp.), seminemoral (16 spp.), nemoral (8 spp.) and ubiquitous (10 spp.) species. Among grassland species are 34 (39%) mesophilous and 22 (24%) xerothermophilous species. A total of 18 (20%) species are threatened at a European and a national level. Species composition of habitat type depends on floristic composition and structure of vegetation in habitat type, and landscape structure, which affects dispersal of butterfly species. The highest alfa diversity was found in dry grasslands (62 spp.) and unmanaged dry grasslands (60 spp.). The large proportions of specialist species and habitat heterogeneous area result in high values of beta diversity between habitat types and high gama diversity. For preserving the high species richness and threatened species in studied area, the most important habitat types are dry grasslands, semi-dry grasslands, unmanaged dry grasslands, woodland margins, woodland paths and screes.

**Keywords:** Škocjanske jame Regional Park, butterflies (Rhopalocera), distribution, faunal elements, species diversity, threatened species

## Uvod

Regijski park Škocjanske jame je bil ustanovljen 1.10.1996 z namenom varovati izjemne geomorfološke, geološke in hidrološke znamenitosti, redke in ogrožene rastlinske in živalske vrste, paleontološka in arheološka najdišča, etnološke in arhitekturne značilnosti ter kulturno krajino (Ur.l. RS št. 57/96) na območju Škocjanskih jam, ki so od leta 1986 vpisane v seznam svetovne dediščine pri UNESCO. Park se nahaja v občini Divača, na skrajnem jugovzhodnem obrobju mezoregije Kras (Perko & Kladnik 1998). To je prehodno območje med apnencem matičnega krasa in flišnim svetom severozahodnega dela Brkinov (Park Škocjanske jame 2003). Meja zavarovanega območja parka poteka na zahodu ob magistralni cesti Divača-Kozina, na severu južno od naselij Dolnje Ležeče, Gradišče pri Divači in Brežec pri Divači, na severovzhodu po vrhnjem robu sten desnega brega reke Reke, na vzhodu južno od vasi Famlje do mostu čez Reko med Vremskim Britofom in Škofljami, na jugovzhodu po vrhnjem robu sten levega brega Reke, vključno s spodnjim delom iztočne soteske potoka Sušice, do vasi Naklo ter na jugu po obrobju kraške planote nad udornicama Sokolak in Globočak. Površina parka, ki obsega območje jam, površino nad jamami, sistem udornic in sotesko Reke do mostu pri Škofljah, je 4,13 $km^2$ (Ur.l. RS št. 57/96). Razgibano površje se nahaja na nadmorskih višinah med 300 in 450 metri. Na zavarovanem območju parka so tri manjša naselja s tipično kraško arhitekturo: Škocjan, Betanja in Matavun.

Regijski park Škocjanske jame leži na vzhodnem robu dela Slovenije, ki ima submediteransko podnebje (Ogrin 1995). Povprečna letna temperatura zraka je med 10 in 12 °C, povprečna januarska med 0 in 2 °C ter povprečna julijska temperatura med 16 in 18 °C (Marolt 2003a). Povprečna letna količina padavin je 1400-1500 mm (Marolt 2003b).

Upoštevaje zoogeografsko regionalizacijo Slovenije po Carneluttiju (1992) sodi regijski park Škocjanske jame v primorsko regijo. Wraber (1969) uvršča Škocjanske jame z okolico v submediteransko fitogeografsko območje, Zupančič & al. (1987) ter Zupančič & Žagar (1995) pa jih uvrščajo v Kraško-vipavski distrikt severnega obalnega sektorja jadranske province mediteranske regije.

Razgiban relief s sistemom udornic in vrtač (npr. Velika dolina, Mala dolina, Dol Globočak, Dol Sokolak, Dol Lisičina, Dol Jablanc, Sapendol, Lesen) in raznolik mikroklimatski režim na območju parka sta razloga za zelo raznovrstno rastlinstvo. Prevladuje kraška flora, ki jo sestavljajo termofilne ilirske, dinarske, balkanske in submediteranske vrste, manjši je delež srednjeevropskih, alpinskih in mediteranskih vrst (Martinčič 2001). Avrikelj (*Primula auricula*), skorjasti kamnokreč (*Saxifraga* *crustata*), dvocvetna vijolica (*Viola biflora*) in skalna kernerjevka (*Kernera saxatilis*), značilne alpinske vrste, katerih pojavljanje na tem območju je pogojeno s poletno temperaturno inverzijo v udornicah, so ostanki ledenodobne flore (glacialni relikti). Topli zrak, ki izhaja pozimi iz podzemlja, ustvarja specifičen zimski temperaturni režim, ki omogoča uspevanje mediteranskih vrst, kot so npr. venerini laski (*Adiantum capillus-veneris*), rdečeplodni brin (*Juniperus oxycedrus*) in ostrolistni beluš (*Asparagus acutifolius*). Te predstavljajo v Škocjanskih jamah termofilne relikte, ki so tu ostali iz borealnega obdobja holocena ali iz terciarja. (MARTINČIČ 2001)

Primarna oblika vegetacije na območju Škocjanskih jam je klimatozonalna gozdna združba črnega gabra in puhastega hrasta *Ostryo-Quercetum pubescentis* (Ht. 1950) Trinajstić 1974 (MARTINČIČ 2001). Zaradi izsekavanja gozda je v sedanjem času večinoma uničena. Kot degradacijske oblike primarne asociacije so razširjene sekundarne gozdne združbe, grmišča, travišča, obdelovalne in urbane površine.

Pregled prispevkov o flori in vegetaciji Škocjanskih jam, objavljenih do konca prejšnjega stoletja, je podal MARTINČIČ (1973, 2001). Kasneje so v okviru projekta Flora, favna in vegetacija regijskega parka Škocjanske jame floro in gozdne združbe raziskovali DAKSKOBLER & al. (2002), vegetacijo gozdnih robov in grmišč je proučeval ČARNI (2002), traviščne in meliščne združbe, vegetacijo zidov in skalnih razpok ter vodno in obvodno vegetacijo je opisal SELIŠKAR (2002).

O favni regijskega parka Škocjanske jame je razmeroma malo podatkov in objav. Na poljuden način je favno podzemeljske Reke na območju Škocjanskih jam predstavil SKET (1999). POLENEC (1965, 1968) je pisal o pajkih v okolici Divače. Inventarizacijo ptic na območju Škocjanskih jam sta napravila POLAK & TRONTELJ (neobjavljeno). Pregled raziskav in objav o mehkužcih, hroščih in metuljih na območju parka so zbrali SLAPNIK (2002), DROVENIK & PIRNAT (2002) in ČELIK (2002). O metuljih na območju Škocjanskih jam in okolice je izredno malo objavljenih virov. V preglednem delu »Verzeichnis der bisher in Krain beobachteten Grossschmeterlinge«, ki je najpopolnejša favna o metuljih Kranjske do 1. svetovne vojne, HAFNER (1909) navaja le podatke iz okolice Senožeč. POLAK (1991) v kratkem prispevku o zooloških zanimivostih iz okolice Škocjanskih jam omenja tudi eno vrsto metulja.

V prispevku predstavljamo rezultate proučevanj favne dnevnih metuljev v Regijskem parku Škocjanske jame. Glavni cilji so bili inventarizirati doslej neraziskano favno dnevnih metuljev tega zavarovanega območja, raziskati vrstno sestavo in diverziteto v različnih habitatnih tipih in opredeliti tiste, ki so najpomembnejši za ohranitev redkih in ogroženih vrst.

## Metode dela

### Terenski popis

Dnevne metulje v Regijskem parku Škocjanske jame smo proučevali v letih 2001 in 2002. Vrste smo popisovali na območjih, kjer so prisotni habitatni tipi, pomembni za prisotnost dnevnih metuljev na območju parka. Na vsakem območju smo opravili terenski popis vsaj enkrat v vsakem mesecu od aprila do oktobra. Čas, potreben za enkraten popis posameznega območja, je bil odvisen od zahtevnosti terena in raznolikosti habitatnih tipov. Vrste smo determinirali z opazovanjem odraslih osebkov, le posamezne primerke težje določljivih vrst smo ujeli z metuljnico. Po determinaciji smo jih večinoma izpustili, nekatere pa shranili za kasnejšo determinacijo v laboratoriju.

### Klasifikacija, opis in razširjenost proučevanih habitatnih tipov

Za dnevne metulje primerne življenjske prostore smo razvrstili v devet habitatnih tipov: suhi kraški travniki (SuTr), polsuhi travniki (PSuTr), zaraščajoči suhi travniki (ZSuTr), gojeni travniki

(GoTr), opuščene njive (Nj), gozdni robovi (GoR), gozdne poti (GoP), melišča (Me) in naselja (Na). Tekoče vode (reka Reka), kali in jame so za dnevne metulje neprimerni življenjski prostori.

Vegetacija suhih in polsuhih travnikov večinoma pripada redu toploljubnih submediteransko-ilirskih suhih travišč. Travnike kosijo enkrat v letu. Suhi travniki so razširjeni na plitkih tleh, predvsem na uravnavah med udornicami in vrtačami. Polsuhi travniki poraščajo globlja tla v večjih depresijah in manjših vrtačah. Zaraščajoči suhi travniki so opuščena suha travišča, ki jih ne kosijo. Zaraščajo se z grmovnimi in drevesnimi vrstami gozdnega roba ter dajejo pokrajini značilno podobo kraške gmajne. Gojene travnike gnojijo in kosijo dvakrat na leto. Razširjeni so predvsem v dnu vrtač in na rahlo nagnjenih pobočjih. Opuščene njive se pojavljajo večinoma na dnu vrtač. Zaraščajo se s plevelno in ruderalno vegetacijo. Gozdne poti na območju parka so neasfaltirane sprehajalne in kolesarske steze ter kolovozi, speljani skozi gozd in zaraščajočo kraško gmajno. Zaradi vrzelastega sklepa krošenj so poti večinoma svetle, z razgibano zgradbo (drevesna, grmovna, zeliščna vegetacija, skale, gola tla) in pestro rastlinsko sestavo. Poti na obeh straneh mejijo na svetle gozdove (izjema sestoji črnega bora in mezofilne gozdne združbe na pobočjih velikih udornic), ki jih ponekod prekinjajo zadnji sukcesijski stadiji zaraščanja nekoč suhih kraških travišč. V primerjavi s potmi so gozdni robovi ob traviščih strukturno in vrstno osiromašeni, ker travnike pokosijo popolnoma do gozda. Kjer gozd meji na melišče, je zeliščna vegetacija gozdnega roba še revnejša, saj manjkajo traviščne vrste. Melišča so razširjena na strmih pobočjih udornic. Zaradi grobe strukture tal so po floristični sestavi zelo revna. Za metulje primerne površine v naseljih so predvsem vrtovi, zidovi, groblje in druge ruderalne površine.

Habitatne tipe smo primerjali glede na prisotnost vrst dnevnih metuljev. Numerično analizo podobnosti habitatnih tipov smo izvedli s programskim paketom SYN–TAX 2000 (Podani 2001), z metodo hierarhične klasifikacije (Metoda popolnega povezovanja = Complete Linkage Clustering). Kot mero različnosti smo uporabili Sorensenov koeficient podobnosti.

### Opredelitev zoogeografske in ekološke pripadnosti vrst

Vsaki vrsti smo določili zoogeografsko pripadnost po kriterijih evropske horizontalne razširjenosti vrst (Carnelutti 1981).

Na podlagi empirično pridobljenih avtekoloških podatkov na terenu in objavljenih virov o ekoloških potrebah vrst (npr. Ebert & Rennwald 1993a, Ebert & Rennwald 1993b, Schweizerischer Bund für Naturschutz 1991, Tolman & Lewington 1997, Weidemann 1995), smo vsako vrsto uvrstili v ekološko skupino. To sestavljajo vrste, ki imajo podobne ekološke potrebe in na določenem območju poseljujejo iste habitatne tipe (Blab & Kudrna 1982; Kudrna 1986). Prednost ekološke klasifikacije vrst je predvsem v naravovarstvenem smislu, saj so v posamezni ekološki skupini združene vrste, ki so na določenem območju izpostavljene enakim antropogenim dejavnikom. Pri razvrščanju v ekološke skupine smo upoštevali naslednje ekološke potrebe vrst: dejanske in potencialne hranilne rastline gosenic in metuljev ter mesta za počivanje in termoregulacijo odraslih osebkov.

### Ogroženost vrst

Ogroženost vrst v evropskem in nacionalnem merilu smo povzeli po Rdečem seznamu evropskih dnevnih metuljev (van Sway & Warren 1998), Direktivi sveta Evropske skupnosti 92/43/E.E.C (= Habitatna direktiva) (van der Made & Wynhoff 1996), Konvenciji o varstvu prosto živečega evropskega rastlinstva in živalstva ter njunih naravnih življenjskih prostorov (= Bernska konvencija) (Ur.l. RS 17/55) ter Pravilniku o uvrstitvi ogroženih rastlinskih in živalskih vrst v rdeči seznam (Ur.l. RS 82/2002).

### Vrstna diverziteta in naravovarstveni pomen habitatnih tipov

Naravovarstveno vrednost proučevanih habitatnih tipov smo opredelili na podlagi (1) vrstne diverzitete dnevnih metuljev ter (2) števila in razporeditve ogroženih vrst po posameznih habitatnih tipih.

Vrstno diverziteto dnevnih metuljev smo opredelili na podlagi analize alfa (α), beta (β) in gama (δ) diverzitete inventariziranih vrst v letih 2001 in 2002. Alfa diverziteto smo ocenili kot število vrst v habitatnem tipu (KRYŠTUFEK 1999, MORENO & HALFFTER 2001).

Beta diverziteta je merilo raznolikosti vrstne sestave med različnimi habitatnimi tipi (MORENO & HALFFTER 2001, HARRISON & INOUYE 2002). Izračunali smo jo kot odstotek vrst, ki so prisotne le v enem od obeh habitatnih tipov, ki ju primerjamo (COLWELL & CODDINGTON 1994 cit. po MORENO & HALFFTER 2001):

$ß = (S_i+S_j-2V_{ij})/(S_i+S_j-V_{ij})*100$,

pri čemer je $S_i$ in $S_j$ število vrst v habitatnih tipih $_i$ in $_j$; $V_{ij}$ je število vrst, ki se pojavljajo v obeh habitatnih tipih. Beta diverziteta ima vrednosti med 0 (vrstna sestava v obeh habitatnih tipih je enaka) in 100 (habitatna tipa poseljujejo različne vrste) (MORENO & HALFFTER 2001).

Gama diverziteta je število vrst na raziskovanem območju (MORENO & HALFFTER 2001).

### Nomenklaturni vir

Za metulje smo nomenklaturo in sistematiko povzeli po KARSHOLT & RAZOWSKI (1996), za rastline po MARTINČIČ & al. (1999) in za vegetacijo po ZUPANČIČ (1997).

## Rezultati in razprava

V regijskem parku Škocjanske jame smo v letih 2001 in 2002 zabeležili 90 vrst dnevnih metuljev (Tab. 1), kar je 50 % vseh vrst metuljev iz skupine Rhopalocera, ki živijo v Sloveniji.

Tabela 1. Seznam vrst dnevnih metuljev, opaženih v letih 2001 in 2002 v Regijskem parku Škocjanske jame, njihova ekološka pripadnost in ogroženost. Legenda: RSS_Pravilnik o uvrstitvi ogroženih rastlinskih in živalskih vrst v rdeči seznam, Priloga 16: Rdeči seznam metuljev (Lepidoptera) (Ur. l. RS 82/2002): E - prizadeta vrsta, V - ranljiva vrsta; RSE_Rdeči seznam evropskih dnevnih metuljev (van SWAY & WARREN 1998): EN - prizadeta vrsta, VU - ranljiva vrsta; FFH_Direktiva sveta Evropske skupnosti 92/43/E.E.C (= Habitatna Direktiva) (van der MADE & WYNHOFF 1996): II - vrsta s seznama Aneksa II k Habitatni Direktivi, IV - vrsta s seznama Aneksa IV k Habitatni Direktivi; BK_Konvencija o varstvu prosto živečega evropskega rastlinstva in živalstva ter njunih naravnih življenjskih prostorov (= Bernska konvencija) (Ur. l. RS 17/55): II - vrsta s seznama Aneksa II k Bernski konvenciji. (*razlaga okrajšav: glej Ekološka pripadnost vrst)

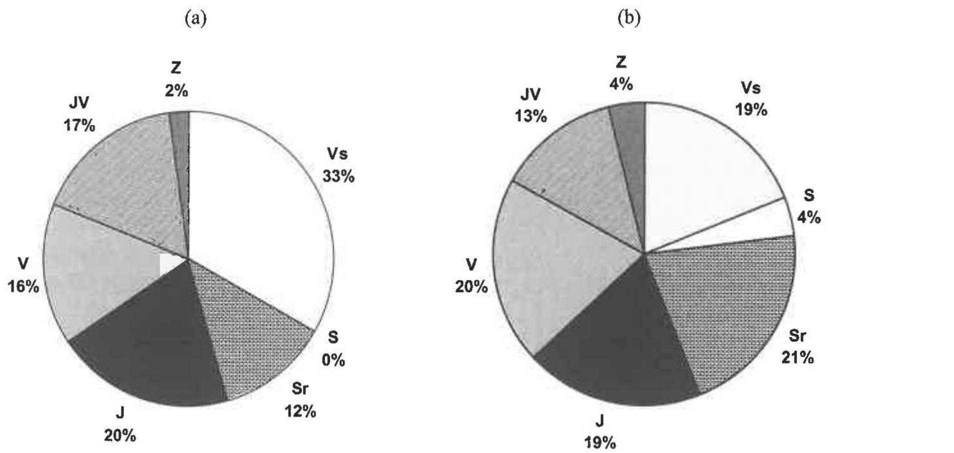
Table 1. List of butterflies found in Škocjanske jame Regional Park in 2001 and 2002, their ecological requirements and threat status. Legend: RSS_Slovenian Red List: Appendix 16: Red list of butterflies and moths (Lepidoptera) (Ur. l. RS 82/2002);: E - endangered species, V - vulnerable species; RSE_Red Data Book of European Butterflies (Rhopalocera) (van SWAY & WARREN 1998): EN - endangered species, VU - vulnerable species; FFH_Council Directive 92/43/E.E.C (= Habitats Directive) (van der MADE & WYNHOFF 1996): II - species in Annex II, IV - species in Annex IV; BK_Convention on the conservation of European wildlife and natural habitats (= Bern Convention) (Ur. l. RS 17/55): II - species in Annex II. (*for explanation see chapter Ekološka pripadnost vrst)

| Vrsta | Ekološka skupina* | RSS | RSE | FFH | BK |
|---|---|---|---|---|---|
| *Erynnis tages* (Linnaeus, 1758) | MezTr | | | | |
| *Spialia sertorius* (Hoffmannsegg, 1804) | KseTr | V | | | |
| *Pyrgus carthami* (Hübner, 1813) | KseTr | V | | | |
| *Pyrgus malvae* (Linnaeus, 1758) | MezTr | | | | |
| *Pyrgus armoricanus* (Oberthür, 1910) | KseTr | V | | | |
| *Pyrgus alveus* (Hübner, 1803) | MezTr | V | | | |
| *Heteropterus morpheus* (Pallas, 1771) | KseTr | | | | |
| *Carterocephalus palaemon* (Pallas, 1771) | MezTr | | | | |
| *Thymelicus lineola* (Ochsenheimer, 1808) | MezTr | | | | |
| *Thymelicus sylvestris* (Poda, 1761) | MezTr | | | | |
| *Hesperia comma* (Linnaeus, 1758) | MezTr | | | | |
| *Ochlodes venata* (Bremer & Grey, 1853) | MezTr | | | | |
| *Zerynthia polyxena* (Denis & Schiffermüller, 1775) | KseTr | V | | IV | II |
| *Parnassius mnemosyne* (Linnaeus, 1758) | MezTr | V | | IV | II |
| *Iphiclides podalirius* (Linnaeus, 1758) | KseGr | | | | |
| *Papilio machaon* (Linaeus, 1758) | MezTr | | | | |
| *Leptidea sinapis* (Linnaeus, 1758) | MezTr | | | | |
| *Anthocharis cardamines* (Linnaeus, 1758) | MezTr | | | | |
| *Aporia crataegi* (Linnaeus, 1758) | KseGr | | | | |
| *Pieris brassicae* (Linnaeus, 1758) | Ubikv | | | | |
| *Pieris rapae* (Linnaeus, 1758) | Ubikv | | | | |
| *Pieris napi* (Linnaeus, 1758) | MezTr | | | | |
| *Pontia edusa* (Linnaeus, 1758) | Ubikv | | | | |
| *Colias croceus* (Fourcroy, 1785) | Ubikv | | | | |
| *Colis alfacariensis* (Ribbe, 1905) | MezTr | | | | |
| *Gonepteryx rhamni* (Linnaeus, 1758) | MezGo | | | | |
| *Hamearis lucina* (Linnaeus, 1758) | MezGr | | | | |
| *Lycaena phlaeas* (Linnaeus, 1758) | MezTr | | | | |
| *Lycaena tityrus* (Poda, 1761) | MezTr | | | | |
| *Callophrys rubi* (Linnaeus, 1758) | MezGr | | | | |
| *Satyrium spini* (Denis & Schiffermüller, 1775) | MezGr | | | | |
| *Satyrium ilicis* (Esper, 1779) | MezGr | | | | |
| *Satyrium acaciae* (Fabricius, 1787) | MezGr | | | | |
| *Leptotes pirithous* (Linnaeus 1767) | Ubikv | | | | |
| *Cupido minimus* (Fuessly, 1775) | MezTr | | | | |
| *Everes argiades* (Pallas, 1771) | MezTr | | | | |
| *Everes alcetas* (Hoffmannsegg, 1804) | MezTr | | | | |
| *Celastrina argiolus* (Linnaeus, 1758) | MezGr | | | | |
| *Scolitantides orion* (Pallas, 1771) | KseTr | V | VU | | |
| *Glaucopsyche alexis* (Poda, 1761) | MezTr | | VU | | |
| *Plebeius argus* (Linnaeus, 1758) | MezTr | | | | |
| *Plebeius idas* (Linnaeus, 1761) | MezTr | V | | | |
| *Plebeius argyrognomon* (Bergsträsser, 1779) | MezTr | V | | | |
| *Aricia agestis* (Denis & Schiffermüller, 1775) | MezTr | | | | |
| *Cyaniris semiargus* (Rottemburg, 1775) | MezTr | | | | |
| *Polyomatus amandus* (Schneider, 1792) | KseTr | | | | |
| *Polyomatus thersites* (Cantener, 1835) | KseTr | E | | | |

| Vrsta | Ekološka skupina* | RSS | RSE | FFH | BK |
|---|---|---|---|---|---|
| *Polyomatus icarus* (Rottemburg, 1775) | Ubikv | | | | |
| *Meleageria bellargus* (Rottemburg, 1775) | KseTr | | | | |
| *Meleageria coridon* (Poda, 1761) | KseTr | | | | |
| *Libythea celtis* (Laicharting, 1782) | Ubikv | | | | |
| *Argynnis paphia* (Linnaeus, 1758) | MezGo | | | | |
| *Argynnis aglaja* (Linnaeus, 1758) | MezGr | | | | |
| *Argynnis adippe* (Denis &Schiffermüller) | MezGr | | | | |
| *Isoria lathonia* (Linnaeus, 1758) | MezTr | | | | |
| *Brenthis daphne* (Denis & Schiffermüller, 1775) | KseGr | | | | |
| *Brenthis hecate* (Denis & Schiffermüller, 1775) | KseTr | | | | |
| *Clossiana euphrosyne* (Lynnaeus, 1758) | MezGo | | | | |
| *Clossiana dia* (Linnaeus, 1767) | MezTr | | | | |
| *Vanessa atalanta* (Linnaeus, 1758) | Ubikv | | | | |
| *Vanessa cardui* (Linnaeus, 1758) | Ubikv | | | | |
| *Inachis io* (Linnaeus, 1758) | Ubikv | | | | |
| *Polygonia c-album* (Linnaeus, 1758) | MezGo | | | | |
| *Euphydryas aurinia* (Rottemburg, 1775) | KseTr | V | VU | II | II |
| *Melitaea cinxia* (Linnaeus, 1758) | KseTr | | | | |
| *Melitaea trivia* (Denis & Schiffermüller, 1775) | KseTr | V | | | |
| *Melitaea phoebe* (Denis & Schiffermüller, 1775) | KseTr | | | | |
| *Melitaea didyma* (Esper, 1778) | KseTr | | | | |
| *Melitaea aurelia* (Nickerl, 1850) | KseTr | V | VU | | |
| *Melitaea britomartis* (Assmann, 1847) | KseTr | V | VU | | |
| *Melitaea athalia* (Rottemburg, 1775) | MezTr | | | | |
| *Limenitis camilla* (Linnaeus, 1758) | MezGo | | | | |
| *Pararge aegeria* (Linnaeus, 1758) | MezGo | | | | |
| *Lasiommata megera* (Linnaeus, 1767) | MezTr | | | | |
| *Lasiommata maera* (Linnaeus, 1758) | MezGr | | | | |
| *Lopinga achine* (Scopoli, 1763) | MezGo | | VU | IV | II |
| *Coenonympha arcania* (Linnaeus, 1761) | MezGr | | | | |
| *Coenonympha glycerion* (Borkhausen, 1788) | MezTr | | | | |
| *Coenonympha pamphilus* (Linnaeus, 1758) | MezTr | | | | |
| *Pyronia tithonus* (Linnaeus, 1767) | MezGr | | | | |
| *Aphantopus hyperantus* (Linnaeus, 1758) | MezGr | | | | |
| *Maniola jurtina* (Linnaeus, 1758) | MezTr | | | | |
| *Erebia aethiops* (Esper, 1777) | MezGo | | | | |
| *Erebia medusa* (Dennis & Schiffermüller, 1775) | MezTr | | VU | | |
| *Melanargia galathea* (Linnaeus, 1758) | MezTr | | | | |
| *Minois dryas* (Scopoli, 1763) | KseTr | | | | |
| *Hipparchia fagi* (Scopoli, 1763) | KseGr | | | | |
| *Hyparchia semele* (Linnaeus, 1758) | KseTr | V | | | |
| *Arethusana arethusa* (Denis & Schiffermüller, 1775) | KseTr | | | | |
| *Brintesia circe* (Fabricius, 1775) | KseTr | | | | |

## Zoogeografska pripadnost vrst

Na obravnavanem območju prevladujejo vseevropsko razširjene vrste (33 %), ki jih je v Sloveniji le 19 % (Sl. 1). Geografska lega, mikroklimatske razmere in apnenčasta geološka podlaga raziskovanega območja se odražajo v večjem deležu južnoevropskih (20 %) in južnovzhodnoevropskih (17 %) vrst ter manjšem deležu vzhodnoevropskih (16 %) in srednjeevropskih vrst glede na zastopanost omenjenih skupin v Sloveniji. Odsotnost severnoevropskih vrst na območju parka je pričakovana glede na razširjenost osmih predstavnikov te skupine v Sloveniji: šest vrst živi le v Alpah ter subalpinskem in montanskem pasu predalpske in dinarske regije, dve vrsti pa sodita v skupino domnevno izumrlih vrst. Od osmih zahodnoevropskih vrst v Sloveniji, v parku Škocjanske jame živita le dve (*Colias alfacariensis, Spialia sertorius*). Vzrok je redkost preostalih šest vrst: dve živita le v osrednjem delu Julijskih Alp, ena v skrajnem jugozahodnem delu primorske regije, tri vrste pa so v Sloveniji domnevno izumrle.




Slika 1. Porazdelitev dnevnih metuljev po kriterijih evropske horizontalne razširjenosti (CARNELUTTI 1981) v Regijskem parku Škocjanske jame (a) in v Sloveniji (b) (Vs: vseevropske vrste, S: severnoevropske vrste, Sr: srednjeevropske vrste, J: južnoevropske vrste, V: vzhodnoevropske vrste, JV: južnovzhodnoevropske vrste, Z: zahodnoevropske vrste).
Figure 1. Partitioning of butterflies according to european horizontal distribution (CARNELUTTI 1981) in Škocjanske jame Regional Park (a) and Slovenia (b) (Vs: all-European species, S: north-European species, Sr: middle-European species, J: south-European species, V: east-European species, JV: southeast-European species, Z: west-European species).

## Ekološka pripadnost vrst

V Regijskem parku Škocjanske jame živijo vrste, ki pripadajo naslednjim ekološkim skupinam (Sl. 2):

• mezofilne travniške vrste (**MezTr**): poseljujejo ne preveč intenzivno obdelane travnate, bogato cvetoče odprte površine (polsuhi travniki, vrtovi, z gosto vegetacijo zarasle opuščene njive, gozdni robovi in poti). 34 vrst;

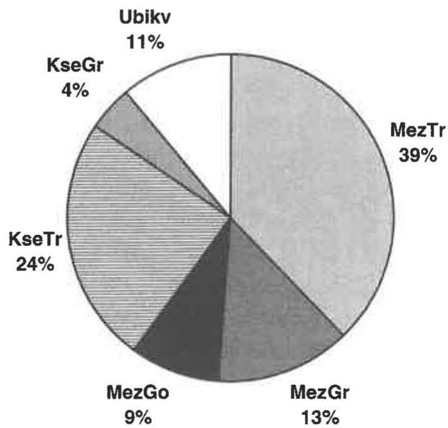
• mezofilne grmovne vrste (**MezGr**): poseljujejo bogato cvetoče gozdne robove, mejice in zaraščajoče travnike. 12 vrst;

• mezofilne gozdne vrste (**MezGo**): poseljujejo svetle gozdove, gozdne jase, robove in poti. 8 vrst;

• kserotermofilne travniške vrste (**KseTr**): poseljujejo suha in topla travišča, peščene, kamnite in skalnate predele. 22 vrst;

• kserotermofilne grmovne vrste (**KseGr**): poseljujejo suhe in tople grmiščne predele (robovi svetlih toploljubnih gozdov, zaraščajoča suha travišča, skalnati predeli in melišča). 4 vrste;

• ubikvitarne vrste (**Ubikv**): poseljujejo bogato cvetoče predele vseh življenjskih prostorov primernih za metulje. V to skupino uvrščamo tudi migratorne vrste. 10 vrst.

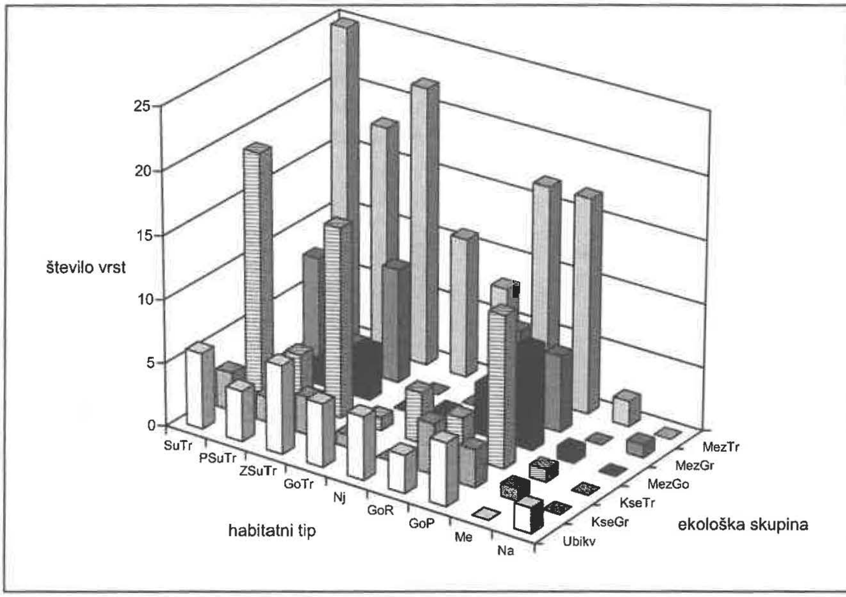



Slika 2. Porazdelitev dnevnih metuljev Regijskega parka Škocjanske jame po ekoloških skupinah (MezTr: mezofilne travniške vrste, MezGr: mezofilne grmovne vrste, MezGo: mezofilne gozdne vrste, KseTr: kserotermofilne travniške vrste, KseGr: kserotermofilne grmovne vrste, Ubikv: ubikvitarne vrste).
Figure 2. Partitioning of butterflies of Škocjanske jame Regional Park among ecological formations (MezTr: mesophilous grassland species, MezGr: mesophilous seminemoral species, MezGo: mesophilous nemoral species, KseTr: xerothermophilous grassland species, KseGr: xerothermophilous seminemoral species, Ubikv: ubiquists).

V favni dnevnih metuljev Regijskega parka Škocjanske jame prevladujejo travniške vrste (56 vrst, 62 %), mezofilnih (39 %) je več kot kserotermofilnih (24 %). Grmovnih vrst je 16 (17 %), gozdnih pa le 8 (9 %).

**Vrstna sestava in ekološke značilnosti favne metuljev v habitatnih tipih**

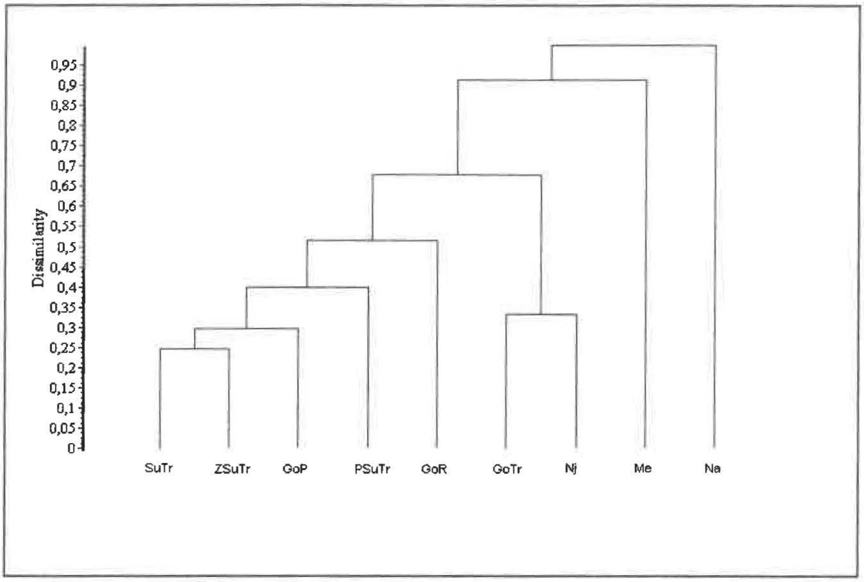
Na podlagi števila in porazdelitve vrst določene ekološke skupine v posameznih habitatnih tipih (Sl. 3) lahko opredelimo habitatni tip, ki je najpomembnejši za obstoj izbrane skupine vrst (ekološki specialisti). Kserotermofilne travniške vrste so specialisti, za njihovo preživetje na obravnavanem območju so najpomembnejši suhi travniki (19 vrst, 86 %), zaraščajoči suhi travniki (15 vrst, 68 %) in svetle gozdne poti (12 vrst, 55 %) (Sl. 3). Mezofilne travniške vrste imajo širšo ekološko valenco kot kserotermofilne travniške vrste, saj jih več kot polovica prisotnih v petih habitatnih tipih: suhi travniki (25 vrst, 74 %), zaraščajoči suhi travniki (22 vrst, 65 %), polsuhi travniki (18 vrst, 53 %), gozdni robovi (17 vrst, 50 %) in gozdne poti (17 vrst, 50 %). Gozdne poti so ključnega pomena za obstoj mezofilnih gozdnih vrst, saj se v omenjenem habitatnem tipu pojavljajo vse vrste te ekološke skupine (8 vrst, 100 %). Za mezofilne grmovne vrste imajo na obravnavanem območju največji pomen zaraščajoči suhi travniki (9 vrst, 75 %), za kserotermofilne grmovne vrste pa gozdni robovi (4 vrste, 100 %).




Slika 3. Število vrst posamezne ekološke skupine v habitatnih tipih Regijskega parka Škocjanske jame (razlaga okrajšav: glej Metode dela in Ekološka pripadnost vrst).
Figure 3. Number of species of each ecological formation in habitat types of Škocjanske jame Regional Park (for explanation see chapter Metode dela and Ekološka pripadnost vrst).

Mobilnost odraslih osebkov je razlog, da se ekološko specializirana vrsta lahko pojavlja v različnih habitatnih tipih, vendar je stopnja ekološke navezanosti vrste na posamezen habitatni tip zelo različna zaradi različnih ekoloških potreb posameznih ontogenetskih stadijev. Larvalni stadiji monofagne (gosenice se hranijo z eno rastlinsko vrsto) ali oligofagne (gosenice se hranijo le z nekaj vrstami rastlin) vrste lahko živijo le v enem habitatnem tipu, medtem ko se metulji iste vrste, če niso prehranski specialisti, lahko hranijo povsod, kjer so prisotne hranilne rastline odraslih osebkov. Vzrok za pojav stenekih vrst v različnih habitatnih tipih je tudi spreletavanje odraslih osebkov, ki iščejo partnerje ter primerna mesta za počivanje in uravnavanje telesne temperature. Na podlagi rezultatov analize podobnosti habitatnih tipov glede na prisotnost vrst dnevnih metuljev (sl. 4) sklepamo, da je favna metuljev posameznega habitatnega tipa odraz vrstne sestave in strukturiranosti vegetacije.

Po sestavi vrst sta si najbolj podobni združbi metuljev na suhih travnikih (SuTr) in zaraščajočih suhih travnikih (ZSuTr) v zgodnjih sukcesijskih stadijih (sl. 4). To je posledica podobnih ekoloških razmer v obeh habitatnih tipih, kar je razvidno iz razmerja med številom mezofilnih in kserotermofilnih travniških vrst (SuTr: 1,3; ZsuTr: 1,5). V primerjavi s košenimi suhimi travniki se na zaraščajočih pojavlja manj travniških vrst in več mezofilnih grmovnih in gozdnih vrst (sl. 3), razmerje med njimi je 4,9 (SuTr) oziroma 2,9 (ZSuTr). Favni metuljev obeh tipov suhih travnikov je najbolj podobna favna na gozdnih poteh. Mozaik svetlih gozdov, grmišč in zadnjih sukcesijskih stadijev nekdanjih




Slika 4. Dendrogram podobnosti proučevanih habitatnih tipov Regijskega parka Škocjanske jame glede na vrstno sestavo dnevnih metuljev (SuTr: suhi travniki, PSuTr: polsuhi travniki, ZSuTr: zaraščajoči suhi travniki, GoTr: gojeni travniki, Nj: opuščene njive, GoR: gozdni robovi, GoP: gozdne poti, Me: melišča, Na: naselja).
Figure 4. Dendrogram of researched habitat types of Škocjanske jame Regional Park according to the butterfly species composition (SuTr: dry grasslands, PSuTr: semi-dry grasslands, ZSuTr: unmanaged dry grasslands (dry grasslands in the early successional stages), GoTr: cultivated grasslands, Nj: abandoned fields, GoR: woodland margins, GoP: woodland paths, tracks and rides, Me: screes, Na: settlements).

suhih travišč ob poteh, bogata zeliščna vegetacija in velik delež osvetljenih golih tal so razlogi, da je razmerje med številom mezofilnih in kserotermofilnih travniških vrst podobno (1,4) kot na obeh tipih suhih travnikov. Na gozdnih poteh je število mezofilnih grmovnih in gozdnih vrst večje kot na košenih in zaraščajočih suhih travnikih, zato je razmerje med travniškimi ter mezofilnimi grmovnimi in gozdnimi vrstami nižje (2,1). Na polsuhih travnikih je razmerje med mezofilnimi in kserotermofilnimi travniškimi vrstami (4,5) trikrat večje kot na košenih in zaraščajočih suhih travnikih. Višja in gostejša zeliščna vegetacija, v kateri je več mezofilnih rastlinskih vrst kot na obeh tipih suhih travnikov, preprečuje pojavljanje nekaterih kserotermofilnih travniških vrst metuljev (*S. orion, P. thersites, M. bellargus, M. coridon, M. didyma, M. aurelia, M. dryas, A. arethusa, B. circe*). Vegetacija gozdnih robov je strukturno in vrstno revna, saj večinoma meji na travnike, ki jih pokosijo do gozda (izjema zaraščajoči suhi travniki). Zato je v združbi metuljev razmerje med travniškimi ter mezofilnimi grmovnimi in gozdnimi vrstami še nižje (1,8) kot v vseh že omenjenih habitatnih tipih. V favni metuljev gojenih travnikov in opuščenih njiv prevladujejo ubikvitarne in splošno razširjene mezofilne travniške vrste (Sl. 4). Opuščene njive so bolj ugoden habitat za kserotermofilne travniške vrste metuljev kot gnojeni in košeni travniki. Na njivah, ki niso v rabi, cvetijo ruderalne rastlinske vrste, ki so pomemben vir nektarja, neporasla osončena tla pa so primerna mesta za termoregulacijo odraslih osebkov. Zaradi majhnega števila opaženih vrst, se melišča in naselja po vrstni sestavi favne metuljev najbolj razlikujejo od ostalih habitatnih tipov.

**Naravovarstveni pomen proučevanih habitatnih tipov**

V Regijskem parku Škocjanske jame je prisotnih 18 evropsko in nacionalno ogroženih vrst dnevnih metuljev (Tab. 1), ki predstavljajo 10 % favne dnevnih metuljev Slovenije. Med njimi je 11 kserotermofilnih travniških vrst, 6 mezofilnih travniških vrst in ena mezofilna gozdna vrsta. Največ ogroženih vrst (13) živi na suhih travnikih, ki imajo tudi največjo alfa diverziteto (Tab. 2).

Tabela 2. Alfa (α) diverziteta in število ogroženih vrst dnevnih metuljev v proučevanih habitatnih tipih regijskega parka Škocjanske jame.
Table 2. Alpha (α) diversity and number of threatened butterfly species in researched habitat types of Škocjanske jame Regional Park.

| Habitatni tip | Alfa (α) diverziteta | Število ogroženih vrst |
|---|---|---|
| Suhi travniki | 62 | 13 |
| Polsuhi travniki | 32 | 4 |
| Zaraščajoči suhi travniki | 60 | 9 |
| Gojeni travniki | 18 | 1 |
| Opuščene njive | 18 | 1 |
| Gozdni robovi | 38 | 2 |
| Gozdne poti | 51 | 6 |
| Melišča | 5 | 1 |
| Naselja | 3 | 0 |

Na gojenih travnikih, opuščenih njivah, meliščih in v naseljih je število vrst najmanjše (Tab. 2). Velika razlika v številu vrst med omenjenimi in preostalimi habitatnimi tipi je razlog za visoke vrednosti beta diverzitete (nad 60 % vrst) med temi pari habitatnih tipov (Tab. 3).

Tabela 3. Beta diverziteta (zgoraj, desno) dnevnih metuljev in število skupnih vrst (spodaj, levo) med proučevanimi habitatnimi tipi regijskega parka Škocjanske jame.
Table 3. Beta diversity (upper, right) of butterfly species and the number of species in common (lower, left) between pairs of habitat types of Škocjanske jame Regional Park.

| | SuTr | PSuTr | ZSuTr | GoTr | Nj | GoR | GoP | Me | Na |
|---|---|---|---|---|---|---|---|---|---|
| SuTr | | 55,4 | 39,5 | 75,0 | 75,0 | 64,9 | 45,2 | 93,7 | 95,2 |
| PSuTr | 29 | | 54,0 | 61,1 | 75,0 | 67,9 | 56,9 | 84,4 | 100 |
| ZSuTr | 46 | 29 | | 74,2 | 76,2 | 60,0 | 45,8 | 93,4 | 95,0 |
| GoTr | 16 | 14 | 16 | | 50,0 | 75,6 | 76,8 | 95,5 | 95,0 |
| Nj | 16 | 10 | 15 | 12 | | 80,9 | 74,6 | 90,5 | 95,0 |
| GoR | 26 | 17 | 28 | 11 | 9 | | 56,5 | 89,7 | 97,5 |
| GoP | 40 | 25 | 39 | 13 | 14 | 27 | | 92,3 | 98,1 |
| Me | 4 | 5 | 4 | 1 | 2 | 4 | 4 | | 100 |
| Na | 3 | 0 | 3 | 1 | 1 | 1 | 1 | 0 | |

MORENO & HALFFTER (2001) navajata, da so strukturiranost krajine, mobilnost in ekološka specializacija vrst pomembni dejavniki, ki vplivajo na beta diverziteto. Avtorja sta mnenja, da so nizke beta diverzitete na določenem območju posledica zelo mozaične pokrajine z veliko raznolikimi habitatnimi krpami, ki delujejo kot koridorji za razširjanje vrst, ter velikega števila zelo mobilnih evriekih vrst. V parku Škocjanske jame se melišča, naselja, gojeni travniki in opuščene njive po vrstni sestavi metuljev zelo razlikujejo (visoka β diverziteta) od ostalih habitatnih tipov (Tab. 3). To je posledica velikega števila ekološko specializiranih vrst, ki živijo na suhih in polsuhih travnikih ter gozdnih robovih in poteh, ter načina gospodarjenja. Na gojenih travnikih, njivah in v naseljih se pojavlja 26 vrst, od teh je 19 vrst iz skupin mezofilnih travniških in ubikvitarnih vrst, 7 vrst iz ostalih ekoloških skupin so predstavniki bolj mobilnih vrst. Večina vrst, ki se pojavljajo na gojenih travnikih, opuščenih njivah, meliščih in v naseljih je torej bolj mobilnih ali so generalisti in živijo tudi v ostalih habitatnih tipih (Tab. 2, Tab. 3). Gojeni travniki, njive in površine v naseljih so prehranjevalni habitat odraslih osebkov teh vrst, saj imajo zaradi pogostih človekovih posegov v omenjenih habitatnih tipih vrste manj možnosti, da izpeljejo celotne življenjske cikle. Predpostavko, da ti habitatni tipi nimajo ključnega pomena za vrstno diverziteto dnevnih metuljev v parku potrjuje tudi ugotovitev, da 98 % (88) vrst živi v štirih drugih habitatnih tipih: košeni in zaraščajoči suhi travniki, gozdni robovi in poti. Vrstno bogastvo pa ni nujno najboljši pokazatelj naravovarstvene vrednosti območja: območje, v katerem živi malo vrst, ki so redke ali ogrožene ima večji naravovarstveni pomen kot območje, ki ga poseljujeje veliko število splošno razširjenih in pogostih vrst (STORK & al. 2003). Ogroženo vrsto *Parnassius mnemosyne* smo na območju parka opazovali le na meliščih in polsuhem travniku na vznožju melišč udornice Sokolak. Druga vrsta, ki je v štirih, za vrstno bogastvo parka najpomembnejših habitatnih tipih (SuTr, ZSuTr, GoP, GoR) nismo našli, je modrinček *Plebeius argyrognomon.* Vrsta je v Sloveniji redka, na raziskovanem območju smo jo zabeležili le v Dolu Jablanc, na cvetočem gojenem travniku. Ta habitatni tip za obstoj vrste ni odločilen, saj vrsta živi predvsem na suhih zagrmičenih travnikih, gozdnih robovih in jasah, redkeje v opuščenih vinogradih, kamnolomih in na pustih, ruderalnih površinah. Najpogostejši hranilni rastlini gosenic sta pisana šmarna detelja *(Coronilla varia)* in sladki grahor *(Astragalus glacyphyllos)*, včasih pa samice odlagajo jajčeca tudi na lucerno *(Medicago sativa)*, ranjak *(Anthyllis spp.)*, detelje *(Trifolium spp.)*, turške detelje *(Onobrychis spp.)* in kozjo deteljo *(Lembotropis nigricans)* (SCHWEIZERISCHER BUND FÜR NATURSCHUTZ 1991). Velika množina hranilnih rastlin gosenic na omenjenem travniku je razlog za prisotnost vrste v tem habitatnem tipu. Vse ostale ogrožene vrste (16), ki živijo v parku, najdemo v treh habitatnih tipih: na košenih in zaraščajočih suhih travnikih ter gozdnih poteh.

Gama vrstna diverziteta dnevnih metuljev na raziskovanem območju je večja (90 vrst) kot največja alfa diverziteta (SuTr: 62 vrst) (Tab. 2). To pomeni, da vrstno bogastvo metuljev na območju parka ni odvisno le od števila vrst, ki živijo v habitatnem tipu z največjo alfa diverziteto, temveč od topografske in ekološke heterogenosti krajine.

## Zaključki

Vrstna sestava in diverziteta dnevnih metuljev v regijskem parku Škocjanske jame je odraz geografske lege, relifne razgibanosti in ekološke heterogenosti območja. Prisotnost različnih habitatnih tipov je razlog za pojavljanje vrst iz šest ekoloških skupin: mezofilne travniške, mezofilne grmovne, mezofilne gozdne, kserotermofilne travniške, kserotermofilne grmovne in ubikvitarne vrste. Vrstno najbogatejši habitatni tipi so suhi travniki, zaraščajoči suhi travniki in gozdne poti. V njih se pojavlja 16 od 18 evropsko in nacionalno ogroženih vrst, ki živijo v parku. Sestava favne metuljev v habitatnem tipu je odvisna od vrstne sestave in strukturiranosti vegetacije v habitatnem tipu ter zgradbe krajinskega mozaika, ki vpliva na disperzijo vrst. Zaradi velike raznolikosti habitatnih tipov na majhnem območju (4 $km^2$), mobilnosti odraslih osebkov ter razlik v ekoloških potrebah gosenic in metuljev, se nekatere ekološko specializirane vrste pojavljajo tudi v habitatnih tipih, ki nimajo ključnega pomena za njihov obstoj (GoTr, Nj, Na, Me) in v njih večinoma (izjema melišča) ni drugih, za te habitatne tipe specifičnih vrst. Ti habitatni tipi imajo v primerjavi z ostalimi nizke alfa diverzitete, kar je vzrok za visoke bete diverzitete med omenjenimi pari habitatnih tipov. Visoka gama diverziteta je odraz heterogenosti raziskovanega območja, ki ga poseljujejo ekološke specializirane vrste, vezane na določene habitatne tipe. Ugotovitvi, da se ogrožena vrsta *P. mnemosyne* pojavlja izključno v habitatnih tipih z nizko alfa diverziteto in da je gama diverziteta večja od maksimalne alfa diverzitete, pomenita, da je pri naravovarstvenem vrednotenju raziskovanega območja in oblikovanju naravovarstvenih smernic potrebno upoštevati dva dejavnika: alfa diverziteto in razporeditev ogroženih vrst po habitatnih tipih. Z vidika ohranjanja vrstne raznolikosti in ogroženih vrst dnevnih metuljev so v parku najpomembnejši naslednji habitatni tipi: suhi travniki, polsuhi travniki, zaraščajoči suhi travniki, gozdne poti in gozdni robovi ter melišča.

## Summary


Because of their extraordinary significance for the world's natural heritage, the Škocjanske jame were included in UNESCO's World Heritage List in 1986. The Škocjanske jame Regional Park was established on October 1st 1996 with the intention of preserve exceptional geomorphological, geological and hydrological remarkablenesses, rare and threatened plant and animal species, paleontological and archeological sites, ethnological and arhitectural features, and traditional landscape (Ur.l. RS št. 57/96).

The Škocjanske jame Regional Park is situated in the south-western part of Slovenia, in the Divača municipality. It is placed on south-eastern part of the region Kras, on the passage from flysch to limestone. It extends over an area of 413 hectares and encompasses the area of the caves, the surface above the caves, the system of collapsed dolines and the Reka river gorge to the bridge in Škoflje (Ur.l. RS št. 57/96). The sub-Mediterranean climate (Ogrin 1995) with very diverse microclimatic conditions in the dolines and their surroundings account for the presence of Mediterranean thermophilous flora and Alpine species (glacial relicts) (Martinčič 2001).

In this region of Slovenia, primary vegetation is climax forest association *Ostryo-Quercetum pubescentis* (Ht. 1950) Trinajstić 1974 (Martinčič 2001). Because of forest clearing in past centuries, above all for pastures and meadows, the primary vegetation was destroyed. Abandonment of pasturing and stockbreeding, which was the phenomenon most common in the second half of the 20th century, reflects in mosaic of secondarily developed forest associations, scrub and dry grasslands in different succesional stages.

Still, despite its conservation status, this area is relatively poorly known in entomofaunistical terms, and particularly with respect to its fauna of Lepidoptera. Thus, in the present study, we investigated species composition of the area, species richness and diversity in different habitat types, and significance of habitat types for preserving threatened species.

In 2001 and 2002 butterfly diversity were sampled in Škocjanske jame Regional Park, across 9 habitat types: dry grasslands, semi-dry grasslands, unmanaged dry grasslands, cultivated meadows,

abandoned fields, woodland margins, woodland paths, tracks and rides, screes and settlements. Zoogeographical partitioning of registered butterfly species based upon European horizontal distribution according to CARNELUTTI (1981) was made. All species were sorted into ecological formations considering ecological classification of European butterflies (BLAB & KUDRNA 1982; KUDRNA 1986). Threat status of each species was determined allowing for Slovenian Red List of butterflies and moths (Lepidoptera) (Ur.l. RS 82/2002), Red Data Book of European Butterflies (Rhopalocera) (van SWAY & WARREN 1998), Council Directive 92/43/E.E.C (van der MADE & WYNHOFF 1996) and Convention on the conservation of European wildlife and natural habitats (Ur. l. RS 17/55). The similarity in the species composition of the butterfly fauna of different habitat types was estimated using the programme package SYN-TAX (PODANI 2001). Nature conservation value of each habitat type and researched area as a whole was assessed on the basis of (1) alpha, beta and gama butterfly diversity, (2) number of species of each ecological formation in each habitat type and (3) number and distribution of threatened species among habitat types.

A total of 90 species of butterflies were recorded, which represent 50% of Slovenian butterfly fauna. All-European (33%), south-European (20%) and southeast-European (17%) species are prevailing. A total of 18 (20%) species are threatened at a European and a national level. The butterfly fauna consists of mesophilous grassland species (34 spp.), mesophilous seminemoral species (12 spp.), mesophilous nemoral species (8 spp.), xerothermophilous grassland species (22 spp.), xerothermophilous seminemoral species (4 spp.) and ubiquists (10 spp.). Species composition of habitat type depends on floristic composition and structure of vegetation in habitat type, and landscape structure, which affects dispersal of butterfly species. The highest alfa diversity was found in dry grasslands (62 spp.) and unmanaged dry grasslands (60 spp.), between which the smallest difference in butterflies assemblages occurred. Both habitat types are also the most important for the existence of xerothermophilous grassland, mesophilous grassland and mesophilous seminemoral species in the researched area. Woodland paths and margins are of the greatest significance for mesophilous nemoral and xerothermophilous seminemoral species. A total of 88 (98%) registered species were found in four habitat types: dry grasslands, unmanaged dry grasslands, woodland paths and woodland margins. Two remaining species, *Parnassius mnemosyne* and *Plebeius argyrognomon,* were registered on screes, semi-dry and cultivated grasslands, each of them in only one locality. Cultivated meadows, abandoned fields and settlements not contribute to species richness of the area (gama diversity). In these habitat types the additional nectar sources and suitable sites for termoregulation are the main reasons for appearance of imagoes of generalist and more vagile specialist species. The large proportions of specialist species and heterogeneous landscape result in high values of beta diversity between habitat types, and high gama diversity. Due to the facts, that threatened species *Parnassius mnemosyne* lives only in habitat types with very low alpha diversity and that gama diversity is higher than maximum alpha diversity, conservation management must base upon two measures: alpha diversity and distribution of threatened species among habitat types. For preserving the high species richness and threatened species in Škocjanske jame Regional Park, the most important habitat types are dry grasslands, semi-dry grasslands, unmanaged dry grasslands, woodland margins, woodland paths and screes.

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# JUBILEJI

## Profesor dr. Miroslav Zei – devetdesetletnik

Kazimir TARMAN in Jože ŠTIRN

Profesor Miroslav Zei se je rodil 25. julija 1914 v Nabrežini pri Trstu. Po italijanski zasedbi Trsta se je družina Zeijevih umaknila v Slovenijo. Nov dom so našli v Mariboru. Tu je mladi Miro leta 1932 maturiral. Po maturi se je vpisal na Filozofsko fakulteto v Ljubljani. Odločil se je za študij biologije, še posebno ga je pritegnila zoologija. Že leta 1936 je diplomiral in takoj začel pripravljati doktorsko tezo. Minila so le štiri leta po diplomi in že je obranil disertacijo:*«Prispevek k morfologiji in sistemu jadranskih vrst družine Maenidae (giric)«* in bil potem promoviran za doktorja filozofskih ved.

Kot, da bi dečku rojenemu ob morju, vile sojenice že pri rojstvu naklonile moč odkrivanja skrivnosti morskih širjav in globin, se je lotil raziskovanja ribjega sveta. Sprejel je izziv morja in se zaposlil kot asistent na Oceanografskem inštitutu v Splitu. Od samega začetka mu je stal ob strani mentor, ljubljanski prof. dr. Jovan Hadži, ki je bil tudi sam raziskovalno povezan z raziskovanjem podmorskega življenja. Spoprijel se je z ihtiološko problematiko. Lotil se je proučevanja giric (rod Maena), njihove neznane biologije in ekologije. S primerjalno anatomskimi proučevanji in eksperimentalno metodo umetne oploditve jajčec ter vzgojo mladic, je dokazal obstoj treh vrst giric v Jadranskem morju.

Splitsko obdobje je bilo zelo pomembno za njegovo osebno znanstveno pot in delovanje. Hkrati tudi zelo pomembno za širšo zoološko in biološko oceanografsko stroko. Z Zeijem začenja na Jadranu nov pristop v raziskavah ribjega bogastva, zlasti uvajanje ekološko in populacijsko urejenega ribolova. Njegove tedanje raziskave in znanstvene objave o pridnenih ribjih naseljih so bile pionirsko delo v sodobno biologijo rib in praktično uporabo tega znanja v ribolovu, tako na Jadranu kot v drugih območjih Sredozemskega morja. Prav te raziskave so ga napeljale na že omenjeno odkrivanje vrst pri giricah in za tiste čase biološke neznanke – njihovega menjavanja spola. Odkritje, ki je pozneje prišlo v ihtiološke in zoofiziološke učbenike. V splitskem obdobju je vodil tečaje in praktične vaje iz biologije morja za študente tedanjih jugoslovanskih univerz. Po letu 1945 pa je postal vršilec dolžnosti direktorja Oceanografskega inštituta v Splitu.

Leta 1947 ga je povabil na ljubljansko univerzo njegov učitelj in predstojnik Zoološkega inštituta prof. dr. Jovan Hadži. Svet takratne Filozofske fakultete ga je potem imenoval za izrednega profesorja. Leta 1954 je postal že redni profesor. Po upokojitvi prof. Hadžija je leta 1956 postal predstojnik Zoološkega inštituta na tedanji Prirodoslovno-matematični fakulteti Univerze v Ljubljani.

Po prihodu na univerzo je prevzel predmet *primerjalno anatomijo vretenčarjev.* Kasneje je uvedel še predmeta *biologija strunarjev in praktični tečaj iz biologije* morja na rovinjskem morskobiološkem inštitutu JAZU, ki ga je kot direktor uspešno vodil vrsto let (1951 – 1960). S svojim ugledom in organizacijsko spretnostjo je privabil na rovinjske tečaje iz morske biologije tudi učitelje in študente avstrijskih in nemških univerz. Tu je nastajala, v letih 1960-63, z njegovim sodelovanjem odlična knjiga: *Fauna und Flora der Adria* v redakciji znanega dunajskega profesorja dr. R. Riedla.V povojnih časih, ko je primanjkovalo raziskovalne opreme in znanstvene literature, je v zameno za ponujene usluge na inštitutu izpopolnil delovno opremo laboratorijev in obogatil, po umiku Italijanov, zelo osiromašeno knjižnico.

Nekdanji učenci se spominjamo prof. Zeija kot odličnega učitelja. Dokaj težko snov iz primerjalne biologije vretenčarjev, še posebno manj privlačno osteološko materijo, je predstavljal s sproščeno besedo in sliko, zelo nazorno. Zato smo njegova predavanja poslušali z zanimanjem. Filogenetsko podajana poglavja iz primerjalne anatomije so se odvijala kot napet film. Najtesnejši stik s profesorjem pa smo imeli študenti na praktičnih vajah iz biologije morja. Vodil je laboratorijske vaje in z nami na ladjici Bios križaril po »rovinjskem morju«, ko smo zajemali planktonske vzorce in z mrežo vlačnico strgali morsko dno. Ob njegovi razlagi smo spoznavali biotsko raznolikost podmorskega življenja. V sprehodih po rovinjskem parku pa nam je približal še obalni rastlinski in živalski svet. V vsem njegovem pedagoškem delu je izžarevala sla po predajanju znanja mladim. Bil je učitelj v najžlahtnejšem pomenu besede. V spominu nam je ostala tudi pokončna drža obeh učiteljev zoologije: prof. J. Hadžija in Zeija, ko sta ostala zvesta genetiki v nevarnih časih politično podprtega prodiranja šarlatanskega lisenkizma iz Sovjetske zveze tudi na ljubljansko Univerzo. Svoj pogled v obrambo genetikov sta posredovala na petkovih seminarskih urah.

Profesorja Zeija pa poznamo njegovi sodelavci tudi kot prodornega in ustvarjalnega znanstvenika. Zeijevo raziskovalno delo zajema snov iz sistematike, biologije in ekologije rib ter mnogoščetincev, morske ekologije in problemov speciacije populacij plazilcev (Lacertidae) na izoliranih istrskih otočkih. Ukvarjal se je tudi z uvajanjem novih raziskovalnih metod in tehnik, n.pr. tedaj še s povsem pionirskim delom na podmorski televiziji. O svojih raziskovalnih problemih, največkrat povezanih z ribolovno tematiko se je z nami rad pogovarjal. Imel je izvirne zamisli o ekološko-populacijskem pristopu k izkoriščanju ribjih naselij v našem morju. Žal je pri tedanjih ustanovah, ki so se ukvarjale z morskim ribištvom naletel pogosto na gluha ušesa. Posledice tega, kot je pojav »prelova«, se kažejo pri upravljanju z ribjim bogastvom našega Jadrana še dandanes. Dela je objavljal v domačih in tujih revijah.

Po prevzemu vodstva v Zoološkem inštitutu smo se dnevno srečevali pri kavici v njegovem kabinetu. Bili so to nepozabni delovni sestanki. S širokim pogledom na razvoj inštituta in biologije v celoti je razvijal zamisli o novih področjih. Tako je spodbujal širitev inštitutskih dejavnosti od splošne zoologije, zoološke sistematike in evolucije še na limnologijo in ekologijo živali. S svojimi poznanstvi je poskrbel za dopolnilno izobraževanje sodelavcev na univerzah in raziskovalnih inštitucijah po svetu.

Klic Pozejdona iz morja širjav ga je leta 1962 pozval na afriško morje. Postal je direktor oceanografsko-ribiških projektov ZN v Gani. S taksonomskimi, ekološko-populacijskimi in ribiško-ekonomskimi raziskovanji je postavil osnovo za učinkovitejši ribolov v Gvinejskem zalivu. S podobnimi projekti se je kasneje ukvarjal še na FAO v Rimu, v Tuniziji in zahodni Afriki, od Maroka do Angole. Vodil je poslanstva Tehnične pomoči ZN v vzhodni in zahodni Afriki. Kot cenjen znanstvenik je bil član številnih mednarodnih delegacij in direktor morsko biološke šole pri FAO-ju ter UNESCO-ju v Carigradu. S svojimi raziskovalnimi izsledki in izkušnjami je prispeval k učinkovitosti morskega ribištva. Kljub znanemu načelu, da FAO od sodelavcev ne pričakuje znanstvenih objav, ampak predvsem rezultate, ki so hitro in neposredno uporabni za razvoj gospodarnega ribištva, je prof. Zei objavljal svoje strokovne in znanstvene prispevke tudi v obdobju delovanja pri tej organizaciji. Objavil je dela, ki so pomembna za območje osrednjega Atlantika in za boljše poznavanje biologije sardel ter njim sorodnih vrst. Kar 13 polnih let je deloval pri mednarodni organizaciji. S pridobljenim ugledom je potem privabil k sodelovanju pri ZN še štiri nekdanje učence, ki so potem postali nosilci pomembnih nalog pri FAO in UNESCO. Po upokojitvi pri ZN leta 1975 se je vrnil iz Rima v domovino in se naselil v Portorožu.

Leta 1969 je bila ustanovljena Morska biološka postaja ali kratko MBP pri Inštitutu za biologijo univerze v Ljubljani. S tem dejanjem se je izpolnila dolgoletna želja obeh profesorjev: Hadžija in Zeija, da bi tudi Slovenci dobili raziskovalno ustanovo na Jadranu. Po vrnitvi v domovino, je v letih 1977-1983, prof. Zei prevzel mesto honorarnega predstojnika v tej ustanovi. S svojo avtoriteto je pospešil financiranje adaptacije in gradnjo novih prostorov v obmorski stavbi na Fornačah pri Piranu. V novo zgradbo se je inštitucija preselila leta 1980. Glede na pridobljene izkušnje je poskušal uveljaviti uporabna ribiško-biološka raziskovanja za razvoj marikultur komercialnih ribjih vrst in školjk.

Tudi po vrnitvi iz Rima je ostal prof. Zei v svoji stroki zelo dejaven. Obnovil je naziv rednega univerzitetnega profesorja. Med leti 1976 – 1985 je predaval predmeta ihtiologijo z ribiško biologijo in morsko biologijo. Od leta 1980 – 1989 je bil glavni predavatelj cikličnega mednarodnega tečaja ribiške biologije in oceanografije za slušatelje afriških, azijskih in latino-ameriških držav, ki je potekal v organizaciji Zavoda SFRJ za mednarodno sodelovanje na Inštitutu za oceanografijo in ribištvo v Splitu. Po nalogu tega zavoda je opravil še misiji tehnične pomoči za razvoj morskega ribištva v Sao Tome – Principe in na Zanzibarju (1980 – 1982).

Po letu 1983 se je posvetil pisanju znanstvenih in strokovnih člankov. Zbral in uredil je slovenska imena morskih rib, s čimer je prispeval k Slovarju slovenskega jezika (SAZU, 1970 – 1991).

Prof. Zei je avtor mnogih poljudnoznanstvenih člankov in knjig. Veliko je pisal v naš Proteus. Kar 11 knjig je namenil morju, ribam in vretenčarjem. Knjiga Človek in ocean (1950) je še vedno edino slovensko delo iz oceanografije. Knjiga Življenje našega Jadrana (1947), ki jo je napisal skupaj s prijateljem- zdravnikom dr. J. Zhanělom, je bila dolga leta nepogrešljiva dijaška in študentska spremljevalka pri spoznavanju morskega življenja. Kot mojster umetniško zapisane besede se je predstavil s knjigo Obrazi morja (1987), kjer razkrije svoje globoko doživljanje in razumevanje morja, življenja in človeka. V zaporedju poglavij filozofsko povezuje znanost in resnico z umetnostjo ter mistično domišljijo. Legende o morju se prepletajo s stvarnimi doživetji. Zei je mojster besede in razmišljanja. Ker pozna in ljubi morje piše iz srca.

Na koncu mu avtorja tega zapisa želiva še veliko srečnih let, da bi nam še podarjal znanje in »zeijevsko« izpovedoval misli o morju in življenju v njem. Prepričana sva, da prav to želijo našemu jubilantu vsi, ki ga poznajo in še posebno njegovi bivši ter hvaležni učenci.

V Ljubljani in Portorožu, julija 2004

# NAVODILA AVTORJEM

## 1. Vrste prispevkov

a) ZNANSTVENI ČLANEK je celovit opis originalne raziskave in vključuje teoretični pregled tematike, podrobno predstavljene rezultate z diskusijo in sklepe ter literaturni pregled: shema IMRAD (Introduction, Methods, Results And Discussion). Dolžina članka, vključno s tabelami, grafi in slikami, na sme presegati 15 strani; razmak med vrsticami je dvojen. Recenzirata ga dva recenzenta.

b) PREGLEDNI ČLANEK objavi revija po posvetu uredniškega odbora z avtorjem. Število strani je lahko večje od 15.

c) KRATKA NOTICA je originalni prispevek z različnih bioloških področij (sistematike, biokemije, genetike, mikrobiologije, ekologije itd.), ki ne vsebuje podrobnega teoretičnega pregleda. Njen namen je seznaniti bralca s preliminarnimi ali delnimi rezultati raziskave. Dolžina na sme presegati 5 strani. Recenzira ga en recenzent.

d) KONGRESNA VEST seznanja bralce z vsebinami in sklepi pomembnih kongresov in posvetovanj doma in v tujini.

e) DRUŠTVENA VEST poroča o delovanju slovenskih bioloških društev.

## 2. Originalnost prispevka

Članek, objavljen v reviji Acta Biologica Slovenica, ne sme biti predhodno objavljen v drugih revijah ali kongresnih knjigah.

## 3. Jezik

Teksti naj bodo pisani v angleškem jeziku, izjemoma v slovenskem, če je tematika zelo lokalna. Kongresne in društvene vesti so praviloma v slovenskem jeziku.

## 4. Naslov prispevka

Naslov (v slovenskem in angleškem jeziku) mora biti kratek, informativen in razumljiv. Za naslovom sledijo imena avtorjev in njihovi polni naslovi (če je mogoče, tudi štev. faxa in e-mail).

## 5. Izvleček - Abstract

Podati mora jedrnato informacijo o namenu, uporabljenih metodah, dobljenih rezultatih in zaključkih. Primerna dolžina za znanstveni članek naj bo približno 250 besed, za kratko notico pa 100 besed.

## 6. Ključne besede - Keywords

Število naj ne presega 10 besed, predstavljati morajo področje raziskave, predstavljene v članku. Člankom v slovenskem jeziku morajo avtorji dodati ključne besede v angleškem jeziku.

## 7. Uvod

Nanašati se mora le na tematiko, ki je predstavljena v članku ali kratki notici.

## 8. Slike in tabele

Tabele in slike (grafi, dendrogrami, risbe, fotografije idr.) naj v članku ne presegajo števila 10, v članku naj bo njihovo mesto nedvoumno označeno. Ves slikovni material naj bo oddan kot fizični original (fotografija ali slika). Tabele in legende naj bodo tipkane na posebnih listih (v tabelah naj bodo le vodoravne črte). Naslove tabel pišemo nad njimi, naslove slik in fotografij pod njimi. Naslovi tabel in slik ter legenda so v slovenskem in angleškem jeziku. Pri citiranju tabel in slik v besedilu uporabljamo okrajšave (npr. Tab. 1 ali Tabs. 1-2, Fig. 1 ali Figs. 1-2; Tab. 1 in Sl. 1).

**9. Zakjučki**

Članek končamo s povzetkom glavnih ugotovitev, ki jih lahko zapišemo tudi po točkah.

**10. Povzetek – Summary**

Članek, ki je pisan v slovenskem jeziku, mora vsebovati še obširnejši angleški povzetek. Velja tudi obratno.

**11. Literatura**

Uporabljene literaturne vire citiramo med tekstom. Če citiramo enega avtorja, pišemo ALLAN (1995) ali (ALLAN 1995), če sta dva avtorja (TRINAJSTIĆ & FRANJIĆ 1994), če je več avtorjev (PULLIN & al. 1995). Kadar navajamo citat iz večih del hkrati, pišemo (HONSIG-ERLENBURG & al. 1992, WARD 1994A, ALLAN 1995, PULLIN & al. 1995). V primeru, če citiramo več del istega avtorja, objavljenih v enem letu, posamezno delo označimo s črkami a, b, c itd. (WARD 1994a,b). Če navajamo dobesedni citat, označimo dodatno še strani: TOMAN (1992: 5) ali (TOMAN 1992: 5-6). Literaturo uredimo po abecednem redu, začnemo s priimkom prvega avtorja, sledi leto izdaje in naslov članka, mednarodna kratica za revijo (časopis), volumen poudarjeno, številka v oklepaju in strani. Npr.:

HONSIG-ERLENBURG W., K. KRAINER, P. MILDNER & C. WIESER 1992: Zur Flora und Fauna des Webersees. Carinthia II **182/102** (1): 159-173.

TRINAJSTIĆ & J. FRANJIĆ 1994: Ass. *Salicetum elaeagno-daphnoides* (BR.-BL. et VOLK, 1940) M. MOOR 1958 (*Salicion elaeagni*) in the Vegetation in Croatia. Nat. Croat. **3** (2): 253-256.

WARD J. V. 1994a: Ecology of Alpine Streams. Freshwater Biology **32** (1): 10-15.

WARD J. V. 1994b: Ecology of Prealpine Streams. Freshwater Biology **32** (2): 10-15.

Knjige, poglavja iz knjig, poročila, kongresne povzetke citiramo sledeče:

ALLAN J. D. 1995: Stream Ecology. Structure and Function of Running Waters, 1st ed. Chapman & Hall, London, 388 pp.

PULLIN A. S., I. F. G. MCLEAN & M. R. WEBB 1995: Ecology and Conservation of *Lycaena dispar*: British and European Perspectives. In: PULLIN A. S. (ed.): Ecology and Conservation of Butterflies, 1st ed. Chapman & Hall, London, pp. 150-164.

TOMAN M. J. 1992: Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1-7.

**12. Format in oblika članka**

Članek naj bo poslan v obliki Word dokumenta (doc) ali kot obogateno besedilo (rtf) v pisavi "Times New Roman CE 12" z dvojnim medvrstnim razmakom in levo poravnavo ter s 3 cm robovi na A4 formatu. Odstavki naj bodo med seboj ločeni s prazno vrstico. Naslov članka in poglavij naj bodo pisani krepko in v velikosti pisave 14. Vsa latinska imena morajo biti napisana ležeče. Uporabljene nomenklaturne vire navedemo v poglavju Metode. Tabele in slike so posebej priložene tekstu. Vse strani (vključno s tabelami in slikami) morajo biti oštevilčene. Glavnemu uredniku je potrebno oddati original, dve kopiji in elektronski zapis na disketi 3,5", na CD-romu ali kot priponko elektronske pošte (slednjega odda avtor po opravljenih strokovnih in jezikovnih popravkih).

**13. Recenzije**

Vsak znanstveni članek bosta recenzirala dva recenzenta (en domači in en tuji), kratko notico pa domači recenzent. Avtor lahko v spremnem dopisu predlaga tuje recenzente. Recenziran članek, ki bo sprejet v objavo, popravi avtor. Po objavi prejme 30 brezplačnih izvodov. V primeru zavrnitve se originalne materiale vrne avtorju skupaj z negativno odločitvijo glavnega urednika.

## INSTRUCTIONS FOR AUTHORS

### 1. Types of Articles

a) SCIENTIFIC ARTICLES are comprehensive descriptions of original research and include a theoretical survey of the topic, a detailed presentation of results with discussion and conclusion, and a bibliography according to the IMRAD outline (Introduction, Methods, Results, and Discussion). The length of an article including tables, graphs, and illustrations may not exceed fifteen (15) pages; lines must be double-spaced. Scientific articles shall be subject to peer review by two experts in the field.

b) REVIEW ARTICLES will be published in the journal after consultation between the editorial board and the author. Review articles may be longer than fifteen (15) pages.

c) BRIEF NOTES are original articles from various biological fields (systematics, biochemistry, genetics, microbiology, ecology, etc.) that do not include a detailed theoretical discussion. Their aim is to acquaint readers with preliminary or partial results of research. They should not be longer than five (5) pages. Brief note articles shall be subject to peer review by one expert in the field.

d) CONGRESS NEWS acquaints readers with the content and conclusions of important congresses and seminars at home and abroad.

e) ASSOCIATION NEWS reports on the work of Slovene biology associations.

### 2. Originality of Articles

Manuscripts submitted for publication in Acta Biologica Slovenica should not contain previously published material and should not be under consideration for publication elsewhere.

### 3. Language

Articles and notes should be submitted in English, or as an exception in Slovene if the topic is very local. As a rule, congress and association news will appear in Slovene.

### 4. Titles of Articles

Titles (in Slovene and English) must be short, informative, and understandable. The title should be followed by the name and full address of the author (and if possible, fax number and e-mail address).

### 5. Abstract

The abstract must give concise information about the objective, the methods used, the results obtained, and the conclusions. The suitable length for scientific articles is approximately 250 words, and for brief note articles, 100 words.

### 6. Keywords

There should be no more than ten (10) keywords; they must reflect the field of research covered in the article. Authors must add keywords in English to articles written in Slovene.

### 7. Introduction

The introduction must refer only to topics presented in the article or brief note.

### 8. Illustrations and Tables

Articles should not contain more than ten (10) illustrations (graphs, dendrograms, pictures, photos etc.) and tables, and their positions in the article should be clearly indicated. All illustrative material should be provided as physical originals (photographs or illustrations). Tables with their legends should be submitted on separate pages (only horizontal lines should be used in tables). Titles of tables should appear above the tables, and titles of photographs and illustrations below. Titles of tables and illustrations and their legends should be in both Slovene and English. Tables and illustrations should be cited shortly in the text (Tab. 1 or Tabs. 1-2, Fig. 1 or Figs. 1-2; Tab. 1 and Sl. 1).

**9. Conclusions**

Articles shall end with a summary of the main findings which may be written in point form.

**10. Summary**

Articles written in Slovene must contain a more extensive English summary. The reverse also applies.

**11. Literature**

References shall be cited in the text. If a reference work by one author is cited, we write Allan (1995) or (Allan 1995); if a work by two authors is cited, (TrinajstiĆ & FranjiĆ 1994); if a work by three or more authors is cited, (Pullin & al. 1995); and if the reference appears in several works, (Honsig-Erlenburg & al. 1992, Ward 1994a, Allan 1995, Pullin & al. 1995). If several works by the same author published in the same year are cited, the individual works are indicated with the added letters a, b, c, etc.: (Ward 1994a,b). If direct quotations are used, the page numbers should be included: Toman (1992: 5) or (Toman 1992: 5-6).

The bibliography shall be arranged in alphabetical order beginning with the surname of the first author followed by the year of publication, the title of the article, the international abbreviation for the journal (periodical), the volume (in bold print), the number in parenthesis, and the pages. Examples:

HONSIG-ERLENBURG W., K. KRAINER, P. MILDNER & C. WIESER 1992: Zur Flora und Fauna des Webersees. Carinthia II **182/102** (1): 159-173.

TRINAJSTIĆ & J. FRANJIĆ 1994: Ass. *Salicetum elaeagno-daphnoides* (BR.-BL. et VOLK, 1940) M. MOOR 1958 (*Salicion elaeagni*) in the Vegetation in Croatia. Nat. Croat. **3** (2): 253-256.

WARD J. V. 1994a: Ecology of Alpine Streams. Freshwater Biology **32** (1): 10-15.

WARD J. V. 1994b: Ecology of Prealpine Streams. Freshwater Biology **32** (2): 10-15.

Books, chapters from books, reports, and congress anthologies use the following forms:

ALLAN J. D. 1995: Stream Ecology. Structure and Function of Running Waters, 1st ed. Chapman & Hall, London, 388 pp.

PULLIN A. S., I. F. G. MCLEAN & M. R. WEBB 1995: Ecology and Conservation of *Lycaena dispar*: British and European Perspectives. In: PULLIN A. S. (ed.): Ecology and Conservation of Butterflies, 1st ed. Chapman & Hall, London, pp. 150-164.

TOMAN M. J. 1992: Mikrobiološke značilnosti bioloških čistilnih naprav. Zbornik referatov s posvetovanja DZVS, Gozd Martuljek, pp. 1-7.

**12. Format and Form of Articles**

Articles should be send as *Word document* (doc) or Rich text format (rtf) using "Times New Roman CE 12" font with double spacing, align left and margins of 3 cm on A4 pages. Paragraphs should be separated with an empty line. The title and chapters should be written bold in font size 14. All scientific names must be properly italicized. Used nomenclature source should be cited in the Methods section. Tables and illustrations shall accompany the texts separately. All pages including tables and figures should be numbered. The original manuscript, two copies, and an electronic copy (after all corrections) on a 3.5" computer diskette, on CD-ROM or by e-mail must be given to the editor-in-chief. All articles must be proofread for professional and language errors before submission.

**13. Peer Review**

All Scientific Articles shall be subject to peer review by two experts in the field (one Slovene and one foreign) and Brief Note articles by one Slovene expert in the field. Authors may nominate a foreign reviewer in an accompanying letter. Reviewed articles accepted for publication shall be corrected by the author. Authors shall receive thirty (30) free copies of the journal upon publication. In the event an article is rejected, the original material shall be returned to the author together with the negative determination of the editor-in-chief.