Introduction

Light is a key driving factor of life in river ecosystems. Strong shadowing of river channels limits the development of aquatic plants, their abundance, and biodiversity [1]. Increased sunlight penetration generally enhances the vegetation biomass, plant cover, and biological diversity [2-3]. In the case of photophobic species, excessive sunlight can lead to limiting shoot elongation and slowing down biomass development [4]. On the other hand, a decrease of sunlight caused by emergent macrophytes can lead to a reduction in the number of phytoplanktonic organisms in lowland rivers [5]. Changes in aquatic plant cover caused by limitation of light penetration can affect spatial variability and characteristics of river channel sediments, which are modified by the presence of vascular plants [6].

Some submerged macrophytes, e.g., *Myriophyllum spicatum*, can dominate the subsurface water layer under shading conditions due to rapid elongation to sites with better light intensity [7-9], observed higher retention of nutrients (especially ammonia and nitrogen) in shaded river sections thanks to the accumulation in macrophytes biomass. Besides impact on light and nutrient conditions in waters, macrophytes have also proven to carry out allelopathic activities in relation to different groups of organisms, which simultaneously can strongly modify aquatic species structure [10-11].

Jusik and Szoszkiewicz [12] observed significant correlations between the level of morphological...
modifications and overshadow of lowland river channels. Together with the increase of modifications of watercourses, the rate of shadow was decreasing due to the changes of land use and simplification of the riparian species structure, commonly related to regulation works. In sites localised on natural or seminatural rivers (Habitat Modification Score, HMS≤8), the level of shadow varied from 45% to 70%, while on sites moderately modified (8<HMS≤44) it dropped to 20-50%. In strongly modified river sites (HMS>44), the level of shadow was low and ranged from 5% to 15%. Hachoł and Bondar-Nowakowska [13] found that the approach to riverside management, connected with the rate of human pressure, influence the macrophyte species structure. The effect of construction works on macrophytes depends on their scope [14]. During the planning of river conservation or regulation works, it is crucial to maintain proper light conditions for macrophytes due to the possible problems with both absence and limitation of aquatic plant development [15]. Excess light, which can cause even full overgrowth of the river channel, thus changes water flow conditions [16] and causes a decrease in channel permeability [17]. The phenomenon of river channel overgrowth is very intensive in lowland rivers flowing through agricultural areas [18], where the presence of strong sunlight conditions (lack of trees, removed during regulation works) and significant input of nutrients due to the surface flow are present simultaneously [17].

Macrophytes are an important group of aquatic organisms and in regard to the water framework directive (WFD) are widely used to assess water quality and the ecological state of rivers in the EU [3, 19-22]. The presence and coverage of aquatic plants in waters can be utilised as data for calculating macrophyte indices [23-24].

The main goal of these studies was to estimate the relationship between the shaded area of a river site and the diversity of aquatic vegetation. Shading is one of many factors affecting macrophytes in rivers. Therefore, when studying this issue, it is very important to eliminate the variability of other environmental factors that may be a potential source of error. In our study, all environmental variables in addition to shading were similar in all studied rivers. In addition, the study was conducted in pairs of river sections, differing only in shading and directly adjacent to each other. In this paper, the impact of shading was evaluated both for indices of ecological status and for macrophyte biodiversity. Dividing pairs of river sections into four groups was a unique statistical approach that enabled us to better evaluate the role of shading in shaping biodiversity of macrophytes under different shading conditions. The new and poorly explained impact question of shading the river channel on assessment of ecological status was studied. This is an important issue for river monitoring and for selecting the right location for field research.

**Materials and Methods**

**Field Surveys**

The field studies were carried out at the peak of the growing season (July-August) in 2003-2010. In total, 40 pairs of river sites were surveyed (Fig. 1). Analysed pairs of river sites were divided into four groups differing in shading caused by trees and bank shrubs (Figs 2, 3). Shading was defined as a vertical plan of tree canopies on the water surface (approximately the shading of river water surface at noon) and estimation accuracy of river shading was equal to 5% of each surveyed river site. The purpose of this segregation of river sites was to check whether the macrophyte response was based solely on the level of...
The studied rivers were similar in terms of water depth and width, flow type, river bottom substrate, and water quality parameters. In all sites representing small and average rivers (catchment area 40-450 km², water width 2.5-7.5 m, water depth 0.3-0.8 m) sand was a dominant bottom substrate (with silt and gravel in smaller amounts), smooth or ripple flow type.

The hydromorphological evaluation was conducted according to the river habitat survey (RHS) method [25], which gathers data from 500-m stretches of rivers. Two numerical metrics based on the RHS protocol were produced. High values of the habitat quality assessment (HQA) indicate an extensive presence of a number of natural river features and high landscape diversity along a river. High values of the habitat modification score (HMS) indicate an extensive anthropogenic alteration, such as bank and channel resection, reinforcement, or other river engineering construction projects [25].

The macrophyte survey, including two sections (a pair of related river sections), was located inside each of the RHS sites (Fig. 4). Field surveys were conducted using the macrophyte method for river assessment (MMOR) [26]. The macrophyte survey was conducted along river reaches of 100 m in length. Only river macrophytes growing in the water were recorded. The survey includes a list of species and estimated vegetation cover. The presence of each species was recorded with their percentage cover using the following nine-point scale: <0.1%, 0.1-1%, 1-2.5%, 2.5-5%, 5-10%, 10-25%, 25-50%, 50-75%, and >75% [26]. Both sections of MMOR were contained in one RHS site and varied only according to shadow rate (Fig. 2). Other habitat conditions (physico-chemical parameters of water, hydromorphological conditions, hydrology, bottom substrate) were similar within the RHS section. Thanks to this uniformity, the evaluation of the impact of shadow on macrophyte biodiversity was possible. Both macrophyte-related sections, differing in the degree of shading within one RHS section, were tested the same day by the same person.

Water samples for the physico-chemical analysis were collected during the same field surveys when botanical and hydromorphological surveys were carried out. Surface water samples were taken mid-stream below the surface. All samples were filtered using Sartorius cellulose filters with a nominal pore size of 0.45 µm, except for those used for determining total phosphorus. Water samples were cooled and analysed in a laboratory within a 12-h period. Electrical conductivity and pH were measured by digital potentiometers (Elmetron CP-401, CC-551). Concentrations of phosphate (molybdenum blue method), total phosphorus (molybdenum blue method after mineralisation in an open system), and nitrate nitrogen (cadmium reduction method) were determined using a spectrophotometer Hach-Lange DR/2400.

Mathematical and Statistical Procedures

Based on the collected data, as many as 9 biodiversity metrics were calculated: total number of taxa, number of species of macroalgae, bryophytes, vascular hydrophytes and vascular helophytes, Shannon-Wiener diversity index, evenness, Simpson diversity index,
and total vegetation cover. Next, the contribution of four groups of plants was analysed (i.e., macroalgae, aquatic bryophytes, hydrophytes, and helophytes). Furthermore, three macrophyte indices of ecological status were computed: (1) Polish Marophyte Index for Rivers, MIR [26]; (2) French Indice Biologique Macrophytique en Rivière, IBMR [27]; and (3) British River Macrophyte Nutrient Index, RMNI [28]. These indices reflect river degradation, especially eutrophication level (concentration of phosphate and total nitrogen).

To evaluate the homogeneity of the four analysed groups according to physico-chemical and hydromorphological parameters, a variance analysis was made. The analysis was preceded by estimating normal distribution of data using the Shapiro-Wilk W test. This test showed that, in the case of three parameters (phosphates, total phosphorus, nitrates), distribution of data is significantly right-skewed and therefore nonparametric Kruskal-Wallis variance analysis was used to evaluate the homogeneity of the groups. The influence of shading on macrophyte diversity and ecological state were estimated using the Wilcoxon Z test for the related pairs of observations. The choice of nonparametric test as an alternative for t-Student test was due to the significant right-skewed distribution of data for several parameters (number of macroalgae and aquatic mosses taxa and its cover contribution) and lack of proper transformations to obtain normal distribution. In the next step, statistical analyses were utilised to find relationships between particular macrophyte species cover and the rate of shadow conditions using Spearman’s rank correlation. Only macrophytes with five or more appearances in different sites were used in the analyses.

**Results and Discussion**

**Similarity of Abiotic Conditions in the Studied Rivers**

The four analysed group of rivers were very similar in terms to physico-chemical parameters of water and hydromorphological conditions, thus Kruskal-Wallis ANOVA did not show any statistically significant differences among groups (H<5, p>0.05). The first group was characterised by higher nutrient concentrations in water and a bigger rate of modifications, but these differences were not statistically significant (Table 1). In general, all studied river sections represented high conductivity, high concentrations of phosphates, and nitrates in water as well as large hydromorphological modifications.

The four analysed groups of rivers were different only in terms of the level of shading (Table 2, Fig. 2). In Group I, the shading difference was equal to 26.2% on average (less shaded sections average 2.4% and more shaded sections average 28.7%). In Group II, the difference in shade was equal to 27.7% on average (less shaded sections average 25.1% and more shaded sections average 52.8%). In group III, the shading difference was equal to 25.0% on average (slightly less shaded sections average 50.6% and more shaded sections average 75.6%). In contrast, in Group IV the shading difference was higher and was equal to 58.3% on average (within pairs of less shaded sections they average 16.9% and more shaded sections average 75.2%).

**The Impact of Shading on Macrophyte Biodiversity**

Using the Wilcoxon test for associated pairs of river sites allows the elimination of influence on macrophytes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Group of rivers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td><strong>Physico-chemical parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>7.33-8.24 (7.68)</td>
</tr>
<tr>
<td>Conductivity</td>
<td>µS·cm⁻¹</td>
<td>378-1750 (778)</td>
</tr>
<tr>
<td>Phosphates</td>
<td>mg PO₄³⁻·dm⁻³</td>
<td>0.19-1.33 (0.42)</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>mg P·dm⁻³</td>
<td>0.10-1.09 (0.62)</td>
</tr>
<tr>
<td>Nitrates</td>
<td>mg N-NO₃⁻·dm⁻³</td>
<td>0.02-2.60 (0.66)</td>
</tr>
<tr>
<td><strong>Hydromorphological indices</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HQA ¹</td>
<td>Quantitative (0-100)</td>
<td>35-64 (45)</td>
</tr>
<tr>
<td>HMS ²</td>
<td>Quantitative (0-100)</td>
<td>0-90 (41)</td>
</tr>
</tbody>
</table>

¹HQA – Habitat Quality Assessment [25]
²HMS – Habitat Modification Score [25]
by environmental factors other than shading. In the case of 9 studied variables, a lack of statistically significant differences were found. For two variables (total cover and cover of hydrophytes), significant differences were observed Krved in the four analysed groups (Table 3). The highest number of significant variations were found in group IV, where strong differences in shading were observed in pairs of sites (approximately 60%). In this group of pairs, sites with higher shading had a significantly smaller number of taxa, number of helophytes, total cover (p<0.005), cover of macroalgae, hydrophytes, Shannon-Wiener index (p<0.05), and cover of helophytes (p<0.01).

During these studies, a limitation of macrophyte cover area and number of taxa were found, especially for pairs represented by group IV and to some extent also group III. Similarly, in surveys undertaken by Kohler [29], the limitation of macrophyte biomass with shading conditions exceeding 55% of the area corresponded with results presented in this paper. In contrast, the number of species and abundance of mosses was higher when the shaded area was larger. Such a phenomenon was also observed in studies carried out by Vieira [30].

On the basis of the obtained results, it was found that shading of the river channel is limiting the number of macrophyte taxa, especially emerged plants (helophytes).

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### Table 2. Level of shading river channel in four analysed river groups (see Figs 2 and 3).

<table>
<thead>
<tr>
<th>Group of rivers</th>
<th>Number of pairs of river sections</th>
<th>Difference in shading between adjacent sections</th>
<th>Shading of the less shaded section (A)</th>
<th>Shading of the more shaded section (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9</td>
<td>26.2</td>
<td>2.4</td>
<td>28.7</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>27.7</td>
<td>25.1</td>
<td>52.8</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>25.0</td>
<td>50.6</td>
<td>75.6</td>
</tr>
<tr>
<td>IV</td>
<td>11</td>
<td>58.3</td>
<td>16.9</td>
<td>75.2</td>
</tr>
</tbody>
</table>

**Table 3. Results of Wilcoxon signed-rank test (Z) in four analysed river groups.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group of rivers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Number of taxa</td>
<td>0.210</td>
</tr>
<tr>
<td>Number of macroalgae</td>
<td>0.447</td>
</tr>
<tr>
<td>Number of bryophytes</td>
<td>1.604</td>
</tr>
<tr>
<td>Number of hydrophytes</td>
<td>0.085</td>
</tr>
<tr>
<td>Number of helophytes</td>
<td>0.770</td>
</tr>
<tr>
<td>Total cover</td>
<td>2.192*</td>
</tr>
<tr>
<td>Cover of macroalgae</td>
<td>0.944</td>
</tr>
<tr>
<td>Cover of bryophytes</td>
<td>1.604</td>
</tr>
<tr>
<td>Cover of hydrophytes</td>
<td>2.073*</td>
</tr>
<tr>
<td>Cover of helophytes</td>
<td>0.533</td>
</tr>
<tr>
<td>Shannon-Wiener diversity index</td>
<td>0.059</td>
</tr>
<tr>
<td>Evenness index</td>
<td>0.178</td>
</tr>
<tr>
<td>Simpson diversity index</td>
<td>0.059</td>
</tr>
<tr>
<td>MIR 1</td>
<td>0.415</td>
</tr>
<tr>
<td>IBMR 2</td>
<td>0.178</td>
</tr>
<tr>
<td>RMNI 3</td>
<td>1.244</td>
</tr>
</tbody>
</table>

1 MIR – Polish Marophyte Index for Rivers [26]
2 IBMR – French Indice Biologique Macrophytique en Riviere [27]
3 RMNI – British River Macrophyte Nutrient Index [28]

Values in bold statistically significant: * - p<0.05, ** - p<0.01, *** - p<0.005.
growing on river banks. This leads to a decrease of Shannon-Wiener index coefficient. Shading also causes a limitation of macrophyte cover of all studied groups except aquatic mosses (Table 3, Fig. 5). Earlier studies showed that according to the ecology of rivers, optimal shading level of the water table is about 80% [31]. Under conditions with shading level below 50% of the area, the increase of macrophyte biomass can be rapid and reach 300 g of dry mass per square meter, above this level of biomass the cutting of aquatic plants is generally recommended.

The Impact of Shading on Macrophyte Species

Due to the typical lowland character of the surveyed rivers (laminar current, silty bottom substrate) vascular plants were the dominant taxa, with particular contributions by *Lemna minor* L. (66% of sites), *Agrostis stolonifera* L. (58%), *Mentha aquatica* L. (56%), *Phalaris arundinacea* L. (55%), *Berula erecta* (Huds.) Coville (50%), *Myosotis palustris* (L.) Emend. RCHB. (49%), and *Sparganium emersum* Rehmann (41%). All mentioned species are perceived as very common in the eutrophic Polish Lowlands waters. Similar species were identified by Hachol and Bondar-Nowakowska [15] in small and average watercourses of Lower Silesia, where the domination of *Sparganium emersum*, *Phalaris arundinacea*, and *Lemna minor* was observed. Also, Wiegleb [32] found similar taxa in lowland rivers of northwestern Germany, such as *Phalaris arundinacea* (80% of sites), *Callitriche platycarpa* Kütz. (58%), *Sparganium emersum* (56%), *Lemna minor* (54%), *Myosotis palustris* (53%), and *Agrostis stolonifera* (47%).

The most common taxa among bryophytes were *Leptodictyum riparium* (Hedw.) Warnst. (18% of sites), *Fontinalis antipyretica* Hedw. (13%), *Platyhynidium riparioides* (Hedw.) Dixon (10%), *Brachythecium rivulare* Schimp. (10%), *Cratoneuron filicinum* (Hedw.) Spruce (10%), *Conocephalum conicum* (L.) Dumort. (10%), and *Hygroamblystegium tenax* (Hedw.) Jenn. (8%). Other mosses were observed only in single river sites.

Shadow level in river channels had statistically significant relationships only with the cover of four species of macrophytes (p<0.05). Positive correlation with the rate of shadowing was found in the case of two aquatic bryophyte species: *Hygroamblystegium tenax* (r = 0.766, N = 8) and *Fontinalis antipyretica* (r = 0.716, N = 10), which showed greater cover when the shadow rate was higher. Such a relationship can be the indirect effect of a strong linkage of mosses with the presence of trees and bushes growing along the river. Roots of trees and woody debris are the most important place for development of bryophytes in lowland watercourses [30]. Negative correlation with rate of shadow was found in the case of two vascular species: *Ceratophyllum submersum* L. (r = -0.973, N = 7) and *Sagittaria sagittifolia* L. (r = -0.745, N = 16), which showed a decrease of cover when the shadow in the river channel was greater.

The importance of shading for macrophyte diversity was observed in earlier studies, where cutting of trees and shrubs growing on river banks was used in restoration of rivers [33]. Such a method was utilised in Słupia Valley Landscape Park on Kwaczka River, and a significant increase of diversity and cover of macrophytes was observed [34]. Additionally, species characteristic of habitat 3260 (natural and semi-natural...
watercourses with floating vegetation of the *Ranunculus fluitantis* alliance) appeared [35], such as *Batrachium aquatile* (L.) Dumort. and *Callitriche cophocarpa Sendtn.* from the *Ranunculus fluitantis* community.

A common procedure during river conservation is cutting trees and shrubs along a river site. This solution is used to obtain uniformity of bank profiles and to reduce the number of objects affecting hydraulic roughness under flood conditions. At the same time, shading of the river channel prevents watercourses from macrophyte overgrowth and thus reduces the frequency of necessary weed cutting [18, 33]. A similar process was observed in studied sites with strong shading of river channels where the increased presence of bryophytes and subsequent decrease of macrophytes were observed [30].

The Impact of Shading on Macrophyte Indices of Ecological Status

The impact of shading a river channel on results of ecological status evaluated using macrophytes (MIR, RMNI, and IBMR indices) was tested. Properly developed indices based on bioindicative properties of organisms used in the estimation of trophic degradation should be independent of the rate of shading. Studies have shown that the level of shading does not significantly affect the results of macrophyte indices (p>0.05), despite a reduction of macrophyte biodiversity (Table 3). During our studies, it was found that strong river shadowing (>60%) causes a decrease in the number of macrophyte taxa in river sites, from on to six on average (Fig. 5), and this could limit the accuracy of evaluation of the ecological status of the river. Authors of macrophyte methods define the minimum number of indicative species as five, thus enhancing proper evaluation of ecological status [26]. In extreme cases, high shadowing can preclude accurate evaluation. For instance, in four analysed pairs in river sites with high rates of shadowing, the number of identified taxa was below five.

**Conclusions**

1) The impact of shading on macrophyte indices of ecological status was negligible regardless of the degree of shading, but in extreme cases high shadowing can preclude accurate evaluation due to the small number of indicator species.

2) Although increased shading was limiting macrophyte biodiversity and total cover in the studied rivers, in the case of bryophytes, both cover and number of species were higher in shaded sites.

3) For all analysed indices, higher statistical significance was observed for pairs of sites with greater shading difference.

4) A slight increase in shading of the riverbed (about 25%) results in a reduction in the coverage of macrophytes, including primarily submerged plants, but has little impact on biodiversity indicators.

5) The shading limit value, which causes a clear reaction of macrophytes, is about 50%.

6) Obtained results can be utilised both in control and enhancement of macrophyte growth in rivers.

**Acknowledgements**

The authors are deeply indebted to the anonymous reviewers for their valuable suggestions and comments on this manuscript. This research was funded by the Ministry of Science and Higher Education (contract Nos. N 304 099 31/3546 and N N305 3637 33).

**Conflict of Interest**

The authors declare no conflict of interest.

**References**


10. Wang H., Liang F., Zhang L. Composition of anticyanobacterial activity of essential oils from six different...


12. JUSIK S., SZOSZKIEWICZ K. Biodiversity of water plants in diverse conditions of morphological modifications in lowland rivers of western Poland. Nauka Przyroda Technologie, 3 (3), #84, 2009 [In Polish].


30. VIEIRA C., SENAGA A., SERGIO C., FERREIRA M.T. Bryophyte taxonomic and functional groups as indicators of fine scale ecological gradients in mountain streams. Ecological Indicators, 18, 98, 2012.


34. OBOLEWSKI K., OSADOWSKI Z., MILLER M. Renaturation methods for small river basins in the example of the Kwacz River (Słupia River basin). Nauka Przyroda Technologie, 3 (3), #95, 2009 [In Polish].