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HORIZONTAL DISTRIBUTION OF CILIATES: OPEN PEATBOG *VS.* LAGG (ROZTOCZE NATIONAL PARK)

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Summary. The primary objectives of this paper were to analyse the qualitative and quantitative structure of ciliates in the horizontal lagg- open peatbog, and to assess the influence of physical and chemical parameters on protozoa communities. Sampling was done in highmoor peatbog from May to October 2012 in a transect including two sites: 1 – lagg, 2 – open peatbog (the centre of the peatbog). The species richness and abundance of protozoa significantly differed between the studied stations, with the lowest numbers in the open peatbog and the highest in the lagg. These differences between macro-habitats may be due to differences in environmental conditions. The results of PCA analysis showed that Axis 1 and Axis 2 explained 47.8% of the total variance in composition of studied zoocenosis. The distribution of samples in ordination space led to conclude that studied habitats are distributed along the falling gradient of water level and rising gradient of total organic carbon, and nitrate nitrogen. Assemblages of investigated microorganisms showed a strong compositional gradient correlated with surface water and phosphates.

Key words: peatbog, ecotone, lagg, ciliates, abiotic parameters

INTRODUCTION

Quantifying the diversity and distribution of ciliates in aquatic habitats is important to gain a better understanding of food webs in these systems. However, ecologists have paid little attention to peatbogs, compared to other aquatic ecosystems [Gilbert and Mitchell 2006]. Peatlands are generally characterized by rich biodiversity and also play key roles in preserving the stability of ecological relationships in particular regions. At the same time, they belong to the fastest disappearing and most endangered ecosystems in Europe. This is especially disquieting in combination with progressive climate warming [Robson *et al.* 2005]. In *Sphagnum*-dominated peatbogs, the animal communities, especially invertebrate are sufficiently known [Borcard and Vaucher von Ballmoos 1997]. By contrast, little or no attention is given to abundance and biomass of protozoans and their relationships to environmental parameters in these specific ecosystems. These organisms are important consumers of pico- and nano – sized producers, as well as important food sources to metazoan [Mieczan 2010, 2012, Bielańska-Grajner *et al.* 2011]. However, our knowledge of the distribution and regulating factors of ciliates in highmoor peatbogs is still fragmentary. A highmoor peatbog is not a uniform ecosystem. Several zones can be distinguished within its area, differing with a number of physical, chemical and biological factors. The dominant area of a peatbogs is the open bog, mainly occupied by *Sphagnum* and other peat-forming vegetation. A peatbog also includes a zone separating the two areas. It is the lagg area, i.e. a transitional zone between the ecosystem of the open peatbogs and the peatbogs catchment [Herbichowa and Potocka 2004]. So far, studies concerning physical and chemical water parameters in the agricultural catchment – lagg system in peatbogs were conducted in the Mazurian Lakeland [Kruk 2003]. Due to a clear differentiation of chemical and biological conditions of the water in the horizontal arrangement, it seems that a similar differentiation should be expected in case of ciliates. The primary objectives of this paper were: to analyse the qualitative and quantitative structure of ciliates in the horizontal lagg- open peatbog; to determine whether the lagg waters differ in hydrochemical and biological terms from the waters of the central part of the peatbog.

MATERIAL AND METHODS

The study was undertaken in peatbog Międzyrzeki located in southern part of Roztocze National Park (eastern Poland). The bog is mainly open with scattered small *Pinus sylvestris* L. trees. The vegetation is heavily dominated by *Carex acutiformis* Ehrhart., *Carex gracilis* Curt., *Sphagnum angustifolium* (C.C.O. Jensen ex Russow), *Sphagnum cuspidatum* Ehrh. ex Hoffm., and *Polytrichum sp.*

Ciliate communities were examined in a transect including: 1 – lagg (LG) and 2 – open peatbog (the centre of the peatland – OP). Collected data were presented in three seasons: spring (May), summer (July) and autumn (October) 2012. During each sampling occasion 3 samples were collected from each. At each of the sites, free water (water between mosses on the surface) was sampled by means of a plexiglass corer (length 1.0 m, Ø50 mm) to a depth of 0.5 m. The plexiglass corer was closed at each end with a cork and then water samples were collected using a glass pipette. The volume of water extracted from plexiglass corer ranged between 300–500 ml. The abundance of ciliates and their community composition were determined using Utermöhls method. Protozoa samples (whole sample = 500 ml) was sedimented for 24h in cylinder, stoppered with parafilm, then the upper was gently removed. In order to determine the density, three samples were preserved with Logol's solution. Observation of live samples was used for the taxonomic identification. Morphological identifications were mainly based on works by Foissner and Berger [1996]. In three investigated season, the water samples (volume of 500 ml) for chemical analyses were taken. Temperature, conductivity and pH were determined *in situ* with a multiparametric probe, total organic carbon (TOC), BOD and COD were determined using the spectrophotometer PASTEL UV and the remaining factors (TP – total phosphorus, $P\text{-}PO_4$, $N\text{-}NO_3$, chlorophyll *a*) were analysed in the laboratory [Golterman 1969].

The differences of physical and chemical water parameters among studied habitats were analysed by means of one-way ANOVA. Tukey's multiple range test (at $P < 0.05$) was used to compare means when significant differences were found. Detrended Correspondence Analysis (DCA), was used to measure and illustrate the variability gradients indicated by ciliates. Because the length of the gradient was <2 standard deviations, PCA (Principal Component Analysis) was performed in order to specify separation between lagg and open bog habitats; RDA (Redundancy Analysis) was used to explore the relationships between the abundance of taxonomic groups and environmental variables [ter Braak and Šmilauer 2002].

RESULTS

Abiotic variables

The surface water level was highly variable among sites and samples, ranging 5–40 cm (ANOVA, F = 27.2, P = 0.001). Statistically significant differences among the investigated sites were found in conductivity, nutrients, TOC and concentrations of chlorophyll *a* (ANOVA, F = 26.22-29.71, P = 0.001). Water pH fluctuated from 4.0 in lagg to 4.9 in open peatbog. Conductivity was significantly differentiated, attaining from 20 $\mu S\ cm^{-1}$ in lagg to 43 $\mu S\ cm^{-1}$ in peatbog. The highest conductivity occurred in spring and summer, but was decidedly lower in autumn. In all sites examined, the water temperature reached the highest value in summer (22.6°C–24.8°C), and decreased in autumn (8.2°C). The highest concentrations of TOC, COD and BOD occurred in the open peatbog (>51 mg C L^{-1}, 145.5 mg O_2L^{-1} and 85 mg O_2L^{-1}, respectively) and the lowest in lagg (>33 mg C L^{-1}, 93.5 mg $O_2\ L^{-1}$ and 45 mg $O_2\ L^{-1}$, respectively). The concentration of total organic carbon fluctuated between 33.7 mg C L^{-1} in spring and 63.2 mg C L^{-1} in summer. Nutrients reached the highest values in the LG, and were the highest during the spring and autumn periods. LG had a higher concentration of nutrients in summer and chlorophyll *a* in autumn (Tab. 1).

Table 1. Physical and chemical characteristics of water in investigated peatbog (values for the May-October period 2012), n = 18

Parameters/sites	Lagg			Open peatland		
	spring	summer	autumn	spring	summer	autumn
Surface water level, cm	40	15	20	21	10	5
Temp., °C	17.56	22.6	8.28	17.86	24.8	8.2
pH	4.6	4.0	5.2	4.0	3.8	4.9
Conductiv., µS cm^{-1}	29	30	20	40	31	33
N-NO_3, mg NO_3 L^{-1}	0.747	1.034	1.399	0.606	0.947	0.999
PO_4, mg PO_4 L^{-1}	0.346	0.514	0.103	0.031	0.382	0.017
TP, mg P L^{-1}	0.536	0.643	0.778	0.448	0.395	0.632
Chlorophyl *a*, mg L^{-1}	36.13	24.78	377.73	38.4	72.88	102.97
TOC, mg C L^{-1}	33.75	41.5	37.2	51.5	63.2	59.8
COD, mg O_2 L^{-1}	74.2	93.5	86.5	118.5	145.5	138.5
BOD, mg O_2 L^{-1}	45.0	56.2	50.3	69.5	85.0	80.5

Ciliate assemblages – general results

A total of 24 ciliate taxa occurred in the studied area. Species richness of protozoa showed horizontal diversity. The highest numbers of taxa occurred in the LG (23 ciliate taxa) and became much lower in the OP (7 taxa). The numbers of ciliates varied between 19 and 45 ±4 cells ml^{-1}, respectively, with the highest mean numbers in the LG and the lowest in the OP. The highest abundances of ciliate communities were noted in spring and autumn (from 15 ±3 cells ml^{-1} in the OP to 65 ±11 cells ml^{-1} in the LG), whereas in summer we observed a remarkable decrease in ciliate abundance (from 6 ±2 cells ml^{-1} in the OP to 21 ±3 cells ml^{-1} in the LG). The most abundant species occurring in OP were *Paramecium bursaria* and *Colpidium colpoda* was dominant in LG. Among ciliates, exclusive (typical) species for the OP were: *Coleps hirtus* and *Coleps spetai.* In the LG, a significant number of typical taxa were determined, not occurring at other sites. Those were: *Lacrymaria olor*, *Paradileptus elephantinus*, *Spathidium sensu lato* and *Amphileptus* *claparedii*. The results of PCA analysis showed that Axis 1 ($\lambda = 0.745$) and Axis 2 ($\lambda = 0.252$) explained 47.8% of the total variance in composition of studied microorganisms. The first two axes showed a clear separation of the zoocenosis (Fig. 1). The redundancy analysis (RDA) for spatial distribution of ciliates showed that all environmental variables together explained 71.0% of the total variance. However, Monte Carlo permutation test ($P < 0.05$) showed the significance of water level,

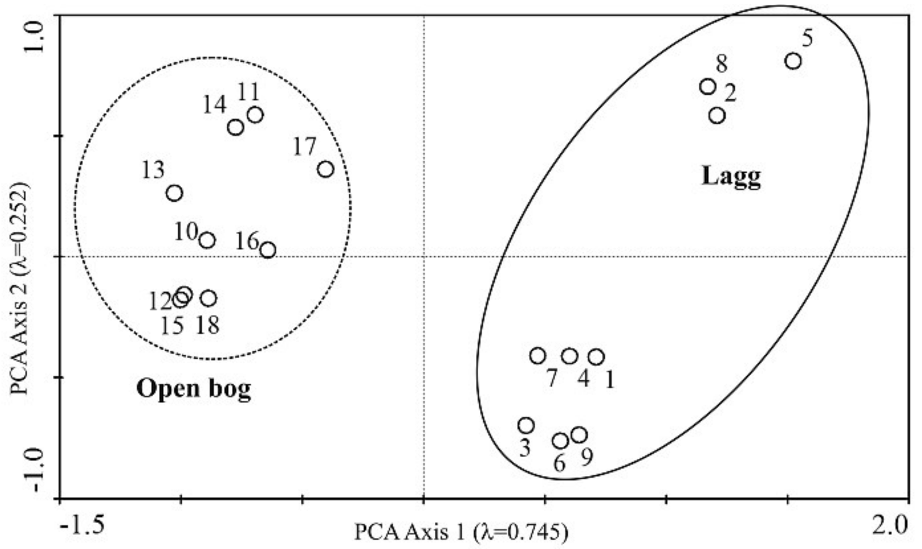

Fig. 1. Biplots of principal components analysis (PCA) for Axis 1 and 2 showing: study habitats. Samples collected in studied habitats are marked with an Arabic numeral: 1–9 Lagg; 10–18 Peatbog

chlorophyll *a* and P-PO_4 in explaining the variability of ciliates in studied habitats. At the RDA biplot (Fig. 2), Axis 2, separated collected samples into two groups (habitats): lagg and open bog. In the lagg habitat, the ciliate species – *Amphileptus cleparedei, A. pleurosigma, Aspidisca costata, Codonella crater, Colpoda steinii, Oxytrichia* sp., *Paradileptus elephantinus, Spathidium sensu lato, Spirostomum ambigum* and *Stylonychia mytilus,* correspond with rising gradient of WL and P-PO_4. The species – *Cinetochilum margaritaceum*, *Colpoda cucullus*, *Plagiopyla nasuta*, *Prorodon* sp. and *Strombidium viride* correspond with the lowering gradient of pH, chlorophyll-*a*, N-NO_3 and TP. On the ordination plot (Fig. 2), species collected in the open bog habitat showed the positive relation with rising gradient of temperature and BOD (*Drepanomonas revoluta*, *Lacrymaria olor*, *Paramecium bursaria*) or with lowering gradient of conductivity, TOC and COD (*Colpidium colpoda*, *Coleps hirtus*, *C. spetai*, *Chlamydonella* sp., *Cyrtohymena muscorum*, *Holosticha pullaster* (Fig. 2).

A

B

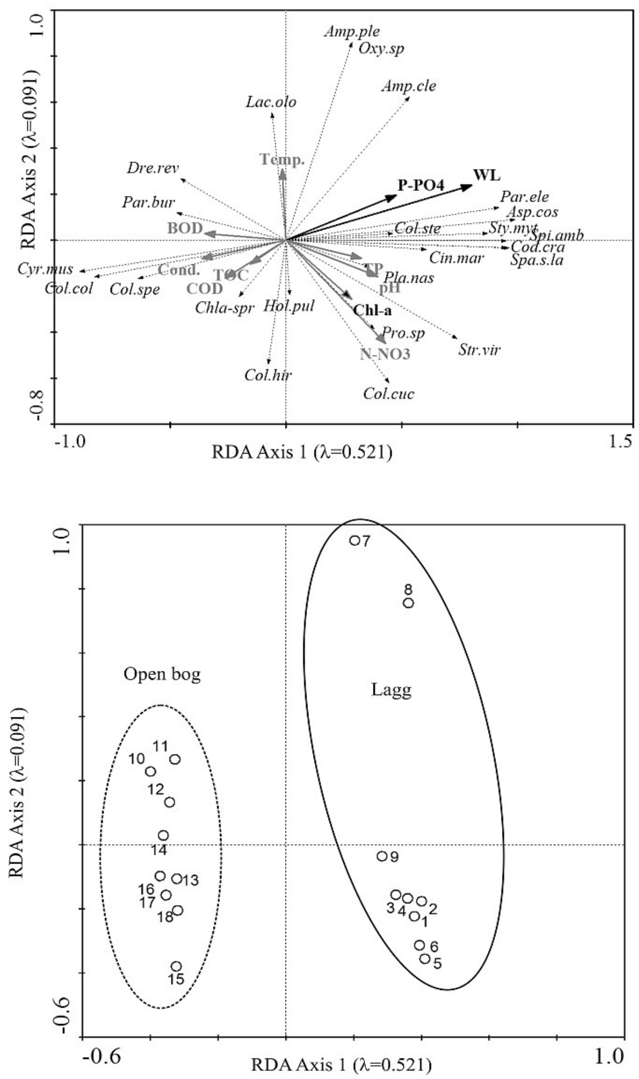

Fig. 2. Biplots of redundancy analysis (RDA) of ciliates A) species, B) samples. Arrows marked as bolded indicate significant parameters in Monte Carlo permutation test at $p < 0.05$. Samples collected in studied habitats are marked with an Arabic numerals: 1–9 Lagg; 10–18-Peatbog. Species codes: Chla-spr – *Chlamydonella-spr,* Col.ste – *Colpoda steinii,* Col.cuc – *Colpoda cucullus,* Col.col – *Colpidium colpoda,* Lac.olo – *Lacrymaria olor,* Par.ele – *Paradileptus elephantinus,* Pla.nas – *Plagiopyla nasuta,* Spa.sen – *Spathidium sensu lato,* Spi-amb – *Spirostomum ambigum,* Par.bur – *Paramecium bursaria,* Cin.mar – *Cinetochilum margaritaceum,* Asp.cos – *Aspidisca cicada,* Hol.pul – *Holosticha pullaster,* Oxy.sp – *Oxytricha* sp., Sty_myt kom – *Stylonychia mytilus*-Komplex, Cod.cra – *Codonella cratera,* Str.vir – *Strombidium viride,* Amp.cla – *Amphileptus claparedii,* Amp.ple – *Amphileptus pleurosigma,* Col.hir – *Coleps hirtus,* Col.spe – *Coleps spetai,* Pro.sp – *Prorodon* sp., Dre.rev – *Drepanomonas rezoluta,* Cry.mus – *Cyrtohymena muscorum*

DISCUSSION

So far, comparative data concerning distribution of protozoa in the horizontal transect (lagg – open peatbog) are very scarce. This study suggests significant relationships between the species richness of protozoa and the type of a habitat. In the lagg zone, a significantly higher number of species were recorded in comparison to the open peatbog. Analysis of investigated group of organisms data and environmental variables in all of the studied habitats revealed seasonal changes in species composition and environmental conditions. The number of identified taxa and abundance of ciliates is comparable with other studies examining the surface water of peatbogs [Grolière 1978, Mieczan 2009], humic lakes in Finland [Järvinen 1993]; however, there is a decidedly lower similarity, according to *Sphagnum* ponds of Simmelried in Germany [Kreutz and Foissner 2006]. These differences may be a result of the lower pH value seen in the peatbog investigated in this study. The two investigated sites had different ciliate assemblages in respect to species composition, total numbers, as well as distribution of particular dominating species. Like species richness, also numbers of ciliates were significantly higher in the lagg zone in comparison to open peatbog. The differentiation probably resulted from fertility of the microhabitat (contents of nutrients). Their concentrations indirectly condition occurrence of protozoa by affecting abundance of bacteria. Bacteria constitute the main source of alimentation for ciliates in various types of hydrogenic ecosystems [Mieczan 2009]. Maximum densities of ciliates have often been observed during mid or late spring [Mieczan 2007]. In addition to high spring densities, the abundance of ciliates reached a peak in autumn. Spring and autumn peaks of ciliates coincided with the higher concentrations of total organic carbon and/or nutrients in three studied macro-habitats. The highest contribution among ciliates was reached by species belonging to Colpodea, occurring in the highest numbers. The species was observed both in mosses in *Sphagnum* peatlands and in surface layers of soils [Grolière 1978, Foissner and Berger 1996). Strűdel-Kypke and Schönborn [1999] observed its occurrence on glass plates exposed in dystrophic lakes in Germany. In the lagg zone, a significant number of typical taxa were determined, not occurring at other sites. Those were: *Lacrymaria olor*, *Paradileptus elephantinus*, *Spathidium sensu lato*, *Aspidisca cicada*, *Oxytricha* sp., *Amphileptus cleparedei* and *Amphileptus pleurosigma*. In the case of the open peatland, only one exclusive species occurred, namely *Coleps hirtus* and *Coleps spetai*.

CONCLUSIONS

The species richness and abundance of protozoa significantly differed between the studied stations, with the lowest numbers in the open peatbog and the highest in the lagg. These differences between macro-habitats may be due to differences in environmental conditions. It seems that the lagg zone of a highmoor peatbog can fulfil the function of an ecotone zone, distinguished by a significant increase in biodiversity and species specificity of microorganisms.

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HORYZONTALNE ROZMIESZCZENIE ORZĘSKÓW: TORFOWISKO OTWARTE *VS.* OKRAJEK (ROZTOCZAŃSKI PARK NARODOWY)

Streszczenie. Celem badań było poznanie struktury jakościowej i ilościowej orzęsków w układzie horyzontalnym okrajek – otwarte torfowisko oraz określenie wpływu właściwości fizyczno--chemicznych wód na występowanie tych mikroorganizmów. Próby pobierano z torfowiska wysokiego od maja do października 2012 r. w transekcie obejmującym okrajek oraz otwarte torfowisko (strefę centralną torfowiska). Zarówno bogactwo gatunkowe, jak i liczebność pierwotniaków wykazywały istotne zróżnicowanie pomiędzy badanymi stanowiskami, osiągając najwyższe wartości w strefie okrajka. Wynikało to prawdopodobnie z wyraźnego zróżnicowania parametrów fizyczno-chemicznych. Analiza PCA wykazała, że osie ordynacyjne wyjaśniają 47,8% zmienności w strukturze taksonomicznej tych mikroorganizmów. Największy wpływ na rozmieszczenie orzęsków wykazywały gradienty zmienności poziomu wody oraz stężenia fosforanów.

Słowa kluczowe: torfowisko, ekoton, orzęski, parametry abiotyczne