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Microbial Biomass and Enzymatic Activity of the Surface Microlayer and Subsurface Water in Two Dystrophic Lakes

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Abstract

Nutrient and organic matter concentration, microbial biomass and activities were studied at the surface microlayers (SML) and subsurface waters (SSW) in two small forest lakes of different water colour. The SML in polyhumic lake is more enriched with dissolved inorganic nitrogen (0.141 mg l^{-1}) than that of oligohumic lake (0.124 mg l^{-1}), the former also contains higher levels of total nitrogen (2.66 mg l^{-1}). Higher activities of lipase (V_{max} 2290 nmol l^{-1} h^{-1} in oligo- and 6098 in polyhumic) and glucosidase (V_{max} 41 nmol l^{-1} h^{-1} in oligo- and 49 in polyhumic) were in the SMLs in both lakes. Phosphatase activity was higher in the oligohumic SML than in SSW (V_{max} 632 vs. 339 nmol l^{-1} h^{-1}) while in polyhumic lake was higher in SSW (V_{max} 2258 nmol l^{-1} h^{-1} vs. 1908 nmol l^{-1} h^{-1}). Aminopeptidase activity in the SSW in both lakes was higher than in SMLs (V_{max} 2117 in oligo- and 1213 nmol l^{-1} h^{-1} in polyhumic). It seems that solar radiation does inhibit neuston microbial community as a whole because secondary production and the share of active bacteria in total bacteria number were higher in SSW. However, in the oligohumic lake the abundance of bacteria in the SML was always higher than in the SSW (4.07 vs. 2.69×10^6 cells ml^{-1}) while in the polyhumic lake was roughly equal (4.48 vs. 4.33×10^6 cells ml^{-1}) in both layers. Results may also suggest that surface communities are not supplemented by immigration from bulk communities. The SML of humic lakes may act as important sinks for allochthonous nutrient resources and may then generate considerable energy pools for microbial food webs.

Key words: dystrophic lakes, enzymatic activity in lakes, neuston, bacteria in surface microlayer

Introduction

At the air-water interface, the surface microlayer (SML) is physico-chemically distinct compared to the subsurface water (SSW) and is characteristically enriched with nutrients and organic matter (Dietz *et al.*, 1976; Södergren, 1993; Hunter, 1997; Münster *et al.*, 1998; Franklin *et al.*, 2005; Stolle *et al.*, 2009). The presence of the surface film and surface tension properties causes the SML to be a unique ecotone inhabited by microorganisms called neuston to distinguish from the subsurface plankton (term first used by Nauman 1917 and cited in Cunliffe *et al.*, 2011). For bacterioneuston, it is considered to be a stressful habitat on the one hand, but on the other – it might favour bacterial heterotrophic activity through the accumulation of organic matter (OM) (Cunliffe *et al.*, 2011). Most organic matter inputs into water bodies are not directly utilisable by bacteria, which cannot access organic material that is larger than 600 Da (Weiss *et al.*, 1991). Therefore, bacteria induce extracellular enzymes that hydrolyse polymers and oligomers into labile monomers that can pass through cell membranes (Chróst, 1991; Weiss *et al.*, 1991; Hoppe *et al.*, 1991 cited in Williams and Jochem, 2006). Upon exposure to solar radiation, which takes place more intensively in the SML and in the photic zone, active enzymes can be released (Boavida and Wetzel, 1998). The SML is a challenging habitat in which neustonic microbial communities must rapidly respond, with possible effects on the overall bacterial metabolism (Santos *et al.*, 2009).

However, our knowledge of the biology of the SML in humic lakes is still insufficient (Södergren, 1993; Münster *et al.*, 1998; Hillbricht-Ilkowska and Kostrzewska-Szlakowska, 2004; Kostrzewska-Szlakowska, 2005). Much more work has been done in oceans (Dietz *et al.*, 1976; Hermansson and Dahlbäck, 1983; Hunter, 1997; Stolle *et al.*, 2009; Cunliffe *et al.*, 2011). It is believed that these processes that cause enrichment in the SML are very similar in marine and freshwaters (Hardy, 1997).

In this study, we quantitatively characterise the activity of bacteria living in the SML and simultaneously

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those living in the SSW, in two humic lakes of different water colours and assessed the relationship between bacterial activities and various environmental parameters of these lakes. We try to answer the following questions: (1) how do SMLs in lakes of different trophy state behave; (2) does the solar radiation inhibit bacterioneuston activities; (3) are SMLs directly supported from the underlying waters; (4) may SML act as sinks for allochthonous nutrient resources and thus may generate new energy pools for microbial food webs.

Experimental

Materials and Methods

Sampling. Two dystrophic, humic lakes (Kruczy Staw 53°39'42"N, 21°24'21"E and Smolak 53°43'29"N, 21°36'09"E) from north-eastern Poland were sampled monthly from April to October 2008 and some analyses in 2009. SML samples were collected by using Larsson plate. This method was chosen as fast and appropriate for sampling bacteria, microalgae and chemical compounds (Larsson *et al*., 1974; Hillbricht-Ilkowska and Kostrzewska-Szlakowska, 2004). Considering the great dynamics of SML, we sampled the lakes during calm weather (wind speed under 0.3 m s^{-1}) to minimize this variability. This procedure collected volumes of water corresponding to the layer thickness of 0.61 ± 0.13 mm in oligo- and 0.50 ± 0.15 mm in the polyhumic lakes. SSW samples (from 0.5 m depth) were collected using a Limnos apparatus. To compare the concentration of variables in the SML versus SSW, the enrichment factor ($Ef = SML/SSW$) was calculated (Hunter, 1997). Mean enrichment factors (Table I) were estimated as the average from monthly data. Water samples were kept in dark bottles at ambient temperature until laboratory processing within a few hours.

Table I

Mean values for total nitrogen (TN; mg l^{-1}), dissolved inorganic nitrogen (DIN; mg l^{-1}); total phosphorus (TP; µg l^{-1}), orthophosphates (PO_4-P; µg l^{-1}), total and dissolved carbon (TOC, DOC; µg l^{-1}), SUVA, chlorophyll *a* (chl *a*; µg l^{-1}), bacterial numbers (BN; 10^6 cell ml^{-1}). Mean enrichment factors (Ef) were estimated as averages from monthly calculated enrichment factors.

	Oligohumic			Polyhumic		
	SML	SSW	Ef	SML	SSW	Ef
TN	0.97	0.72	1.4	2.66	2.43	1.1
DIN	0.124	0.103	1.2	0.141	0.087	1.5
TP	60.5	86.3	0.8	106.7	110.2	1.0
PO_4-P	3.1	13.5	0.9	17.6	34.6	0.7
TOC	15.1	13.5	1.1	54.1	52.9	1.1
DOC	13.7	12.3	1.1	47.2	48.0	1.0
SUVA	8.6	9.2	1.0	31.7	32.5	1.0
chl *a*	11.1	10.4	1.2	39.3	75.0	0.5
BN	4.07	2.69	1.7	4.48	4.33	1.1

Physico-chemical analyses. During each sampling period, physical conditions (temperature, pH, which were recorded electronically OXI 195 *in situ*), inorganic and organic nutrients, chlorophyll *a* concentrations, bacterial abundance, and ectoenzyme kinetics were determined at each lake and in each water layer. Photosynthetically active radiation (PAR) was measured with a light meter [LI-COR Biosciences (LI-250A), USA] very closely over the surface of the water and a few mm below. Water colour was measured in portable photometer (Hanna Instruments HI93727) in 0-500 Platinum Cobalt Units range.

The chemical analyses were measured in unfiltered (total) and filtered (GF/F – dissolved) samples. The total and dissolved phosphorus (TP and DP), orthophosphates (PO_4-P), ammonia (NH_4-N), nitrate (NO_3-N) and nitrite (NO_2-N) were analysed according to Golterman and Clymo (1978) and total and dissolved nitrogen (TN and DN) according to Dowgiałło (1984). Dissolved inorganic nitrogen (DIN) was calculated as a sum of ammonium, nitrate and nitrite (Williams and Jochem, 2006). Direct determination of calcium (Ca), iron (Fe), magnesium (Mg) and manganese (Mn) in aqueous matrices by atomic absorption spectrometry were analysed according to Flame Atomic Absorption Spectrometry (FAAS, 2007) in Schimadzu AA 660. Total and dissolved organic carbon (TOC and DOC) were determined by high temperature catalytic combustion (Schimadzu TOC 5050A). Specific ultraviolet absorbance at 254 nm (SUVA) was defined as the UV absorbance at 254 nm divided by the DOC concentration (Chow *et al*., 2003).

Chlorophyll *a* concentration (chl *a*) as a proxy for phytoplankton biomass was analysed spectrophotometrically after extraction with 96% hot ethanol (Marker *et al*., 1980). Samples of 0.2–0.75 ml volume of water were concentrated on glass fibre filters GF/F (Whatman, 25 mm).

Bacterial analyses. The number of DAPI (4',6-diamidino-2-phenylindole) stainable bacteria (BN) was determined by direct counting of cells suspended on 0.2 µm, black polycarbonate membrane filters (Millipore) under epifluorescence microscope according to Porter and Feig (1980). For bacteria counting, a computer image analyzing system composed of a Nikon epifluorescence E450 microscope, Nikon Digital Camera DXM 1200F and NIS elements software (Nikon) was used. Bacterial biomass (BB) was calculated from the biovolume and content of C per μm^{-3} 360 fg C according to Arvola *et al*. (1996) and Tulonen (1993).

Secondary production (BP) was measured by the incorporation of ^{3}H-thymidine (^{3}H-TdR according to Chróst *et al*., 1988).

Table II
Substrates used for enzyme activity measurement.

Enzyme	Fluorogenic products of reaction	Substrate	Increasing substrate concentration in the sample (μM)
L-leucine-aminopeptidase (AMP)	AMC (7-amino-4-methylcoumarin hydrochloride (Sigma)	LMCA (L-leucine-4-methyl-7-coumarinylamide) (Fluka)	3.125; 6.25; 10; 12.5; 15; 20; 25
lipase	MUF (4-methylumbelliferone) (Sigma)	MUFB (4-methylumbelliferyl butyrate) (Sigma)	3.125; 6.25; 10; 12.5; 15; 20; 25; 50.0
β-glucosidase (Glu)	MUF (4-methylumbelliferone) (Sigma)	4-Methylumbelliferyl-*β-D* -glucoside (Sigma)	1,25; 2,5; 5,0; 7,5; 10,0; 15,0; 20,0
acid phosphatase (APA)	DiFMU (6,8-difluoro-7-hydfoxy-4-methylcoumarin) (Sigma)	DiFMUP (6,8-difluoro-4-methylumbeliferyl phosphate) (Sigma)	3.125; 6.25; 10; 12.5; 15; 20; 25; 50.0

The share of active bacteria with intact membranes (MEM+) was determined using LIVE/DEAD *Bac*Light Bacterial Viability Kits (Schumann *et al*., 2003). For 1 ml subsamples, a mixture of two *Bac*Light Kits: SYTO 9 and propidium iodide was added (1:1), then incubated for 15 min, filtered through 0.2-μm pore-size black polycarbonate membrane filters Millipore (Millipore Corporation, Billerica, MA, USA, 2013) and enumerated using an epifluorescence microscope (the same as in BN analyses). The percentage contribution of MEM+ bacteria was calculated as the ratio of MEM+ to the sum of MEM+ and MEM- bacterial cells.

Extracellular enzyme activity was measured on the base of the degradation rate of increasing concentrations of four artificial fluorogenic substrates (Table II) as described previously by Chróst (1991) and Hoppe (1993). To create the samples set for kinetics measurement, we used 3.8 ml of sample in case of buffered ones and 3.9 ml for unbuffered samples for each substrate concentration. The increasing concentration of substrates for each examinated enzyme was used (Table II). A 0.1 ml of 40 fold concentrated substrates (separately for each concentration) was used to obtain desired substrate concentration (final sample volume – 4 ml). Samples for measuring lipase and beta-glucosidase (Glu) were buffered after incubation to pH 9.0 (0.1 ml of Tris-HCL, final concentration 25 μM), to intensify the MUF fluorescence. L-leucine-aminopeptidase (AMP) and acid phosphatase (APA) were determined by the *in situ* pH. Calibration curves were created independently for each sample and water layer by preparing an increasing concentration of fluorogenic reaction products in 0.2 filtrated (Millipore polycarbonate filters), autoclaved water derived from examinated lake. Fluorescence of the reaction products (described in Table II) was determined in a Shimadzu RF 1501 spectrofluorometer at zero time and after 0.5–1.0 h of sample incubation (incubation temp.: 20°C). The tested enzyme-substrate systems followed first-order Michaelis-Menten kinetics. The plot of the reaction velocity (v) against substrate concentration [S] displayed a rectangular hyperbola relationship, described by the equation $v = Vmax * [S]/(Km + [S])$. Nonlinear regression analysis was applied to calculate the kinetic parameters of enzymatic reactions by means of PC software Origin 6.1 (OriginLab Corporation, Northampton).

Statistical analyses. To compare the mean values between both layers (surface microlayer and bulk water) or between lakes, the nonparametric Wilcoxon test for matched pairs was performed. A critical p-value of < 0.05 was always applied. Canonical Analyses (CA) was used in an effort to use water biological and chemical parameters to explain the residual variance in the four response parameters, SML and SSW in two lakes. Statistics were performed in Statistica v. 6. Due to specific method for collection of surface waters we used no replications.

Results

Physical and trophic parameters. The water temperature ranged from 3–5°C in November to 12–14°C in April to maximal over 22°C in July and August in both lakes. The pH value fluctuated around 5.0 and generally was slightly higher in SMLs. PAR reaching the surface waters during the study period varied between 120 and 2300 μmol m^{-2} s^{-1} depending on the weather and season. We calculated that the SML absorbs 60% (40–70%) of PAR radiation.

The polyhumic lake was more eutrophic (Table I). Concentrations of TP were almost twice as high in the SML in the polyhumic than in the oligohumic lake (in the SSW more than 1.3 times). Differences between lakes were statistically significant in the case of the SML ($p = 0.043$, $n = 5$, Wilcoxon test). Although, PO_4-P were also higher in the polyhumic lake-close to six times in the SML and in the SSW – 2.6 times, differences were statistically significant between both lakes in SSWs ($p = 0.028$, $n = 7$, Wilcoxon test). TN concentrations

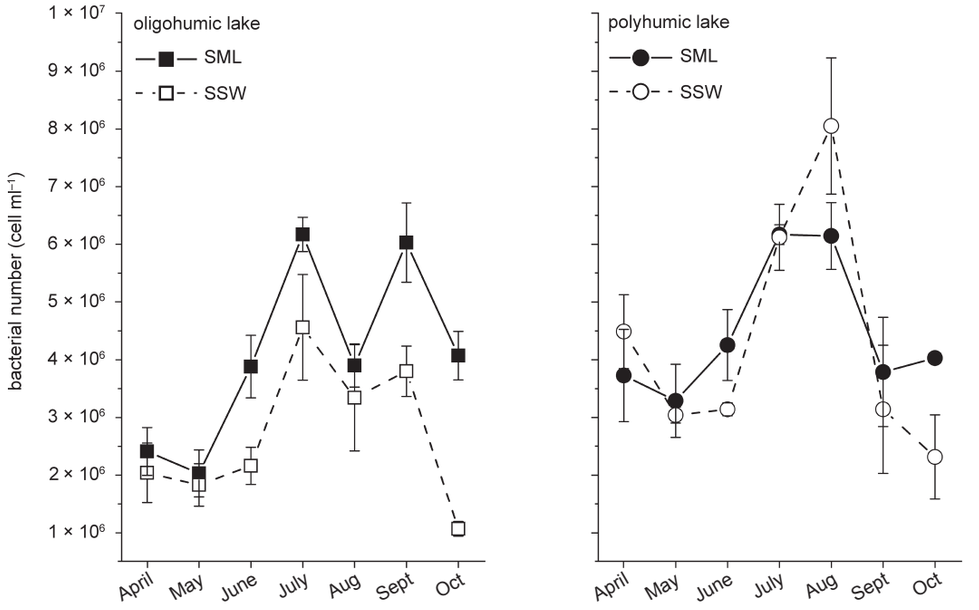

Fig. 1. Bacterial number in the surface microlayer (SML) and subsurface water (SSW) in oligohumic and polyhumic lakes.

were nearly three times higher in the SML and more than 3.0 in the SSW in polyhumic lake, statistically important differences were only in the lower layer ($p = 0.023$, $n = 7$, Wilcoxon test) (Table I). The DIN was almost at the same level in the SML in both lakes, but in the SSW it was higher in the oligohumic lake. The SML was enriched with nitrogen (Ef – ranged from 1.4 for TN in the oligohumic lake and 1.1 in the polyhumic, to 1.5 for DIN in the polyhumic and 1.2 in the oligohumic). The differences between layers were statistically significant in both lakes in the case of DIN ($p = 0.042$ and 0.043, respectively) and in TN only in the oligohumic lake ($p = 0.028$, $n = 7$). The concentrations of TP and PO_4-P were generally higher in the SSW in both lakes, but the differences were not statistically significant.

The concentrations of chl *a* were almost equal for neuston and plankton samples in the oligohumic lake. In the polyhumic lake, the concentration of chl *a* was lower in the SML (Ef = 0.5) and differences were statistically significant ($p = 0.043$, $n = 5$). The concentration of chl *a* in the polyhumic lake was higher than in the oligohumic by 3.5 times in the case of the SML and more than 7 times in the SSW (Table I).

The concentrations of TOC and DOC were almost 4.0 times higher in the polyhumic than in the oligohumic lake (Table I). To distinguish the aromaticity of DOC in the studied lakes, SUVA values were calculated. In the oligohumic lake, SUVA ranged from 8.6 in the SML and 9.2 in the SSW, while in the polyhumic lake they were 31.7 and 32.5, respectively. However, the differences between layers were not statistically significant in both lakes. These data correlated with water colour, almost transparent in the oligohumic lake (39 in the SML and 29 in SSW), and dark brown in the polyhumic (688 and 631, respectively). Through all seasons, the water colour of the SML was higher, however, only in the oligohumic lake were the differences significant ($p = 0.018$, $n = 7$).

Bacterial parameters. In the SML of both lakes and in the SSW of the polyhumic lake the BN varied between 4.0 and 4.5×10^6 cells per ml; however, in the SSW of the oligohumic lake the number was as low as 2.7×10^6 ml^{-1} (Fig. 1). Only in the oligohumic lake the differences of mean BNs between layers were statistically significant ($p = 0.018$, $n = 7$). The abundance of bacteria started from spring at a low level with the highest number during the summer and decreasing during autumn. The Ef reached 1.7 only in the oligohumic lake (1.1 in the polyhumic lake). BB changes followed the similar pattern as the BN in the polyhumic lake. During the end of the season, the SML was still enriched in BB (Fig. 2). A different situation was observed in the oligohumic lake where much higher biomasses in the SML were observed with one exception (June) throughout the season. Mean values for the studied season were 376 μg C l^{-1} in the SML and 208 μg C l^{-1} in the SSW in the oligohumic lake, while in the polyhumic lake they were 228 μg C l^{-1} and 221 μg C l^{-1}, respectively. The SML was enriched in BB in both lakes, but only in the oligohumic lake were the differences significant ($p = 0.042$, $n = 7$). The enrichment factors for biomasses were – 1.9 in the oligohumic lake and 1.2 in the polyhumic lake.

During the spring (April and May) and autumn (November), there were no differences in the contri-

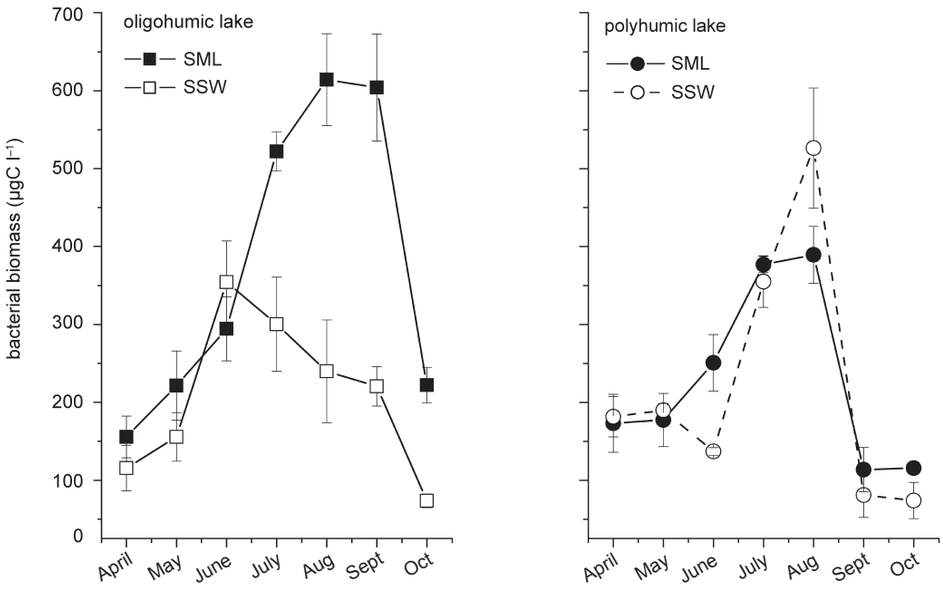

Fig. 2. Changes in bacterial biomass in the surface microlayer (SML) and subsurface water (SSW) in oligohumic and polyhumic lakes.

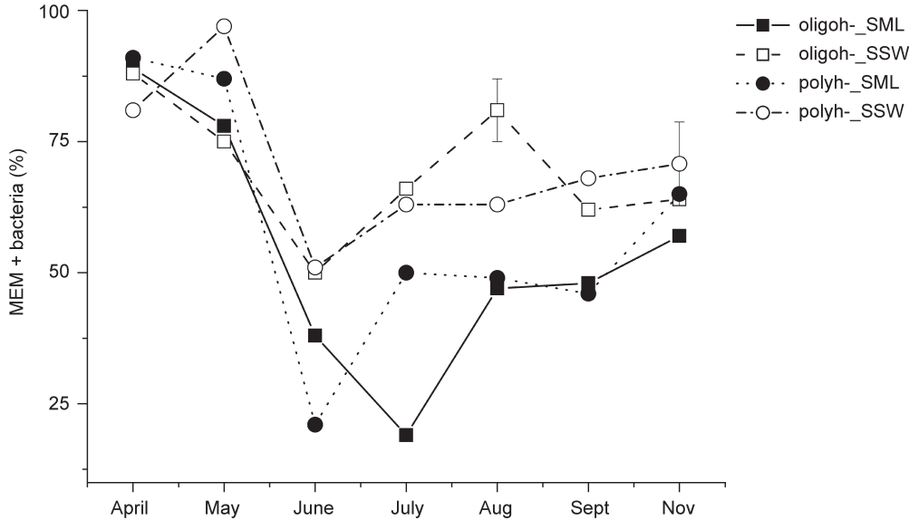

Fig. 3. Percentage contribution of active bacteria with intact membranes (MEM+) to the sum of MEM+ and MEM- bacteria in the surface microlayer (SML) and subsurface water (SSW) in oligohumic (oligoh-) and polyhumic (polyh-) lakes.

bution of active bacteria with an intact membrane to the sum of MEM+ and MEM– bacteria between the SML and SSW in both lakes (Fig. 3). The percent of active bacteria started from over 80% in the spring and fell to about 60% during the autumn. Throughout the summer, active bacteria were found in the SSW. Differences in the mean percentage of MEM+ bacteria were not important between lakes, but between SML vs. SSW they were statistically significant for the polyhumic lake ($p = 0.043$, $n = 7$).

BP was about half as much in the poly- than in the oligohumic lake (11.5 µg C l^{-1} d^{-1} in the SML of the polyhumic lake and 8.2 µg C l^{-1} d^{-1} in the oligohumic lake, while in the SSW they were 24.1 and 15.8 µg C l^{-1} d^{-1}, respectively; Fig. 4). Differences were observed only for the polyhumic lake and were statistically significant ($p = 0.046$, $n = 6$). In both lakes, ^{3}H-TdR incorporation of the bacterioplankton was up to 3 times higher compared to that of bacterioneuston throughout the study season, however, differences were not statistically significant (Efs were 0.7 in the oligo- and 0.5 in the polyhumic lake).

The relative magnitude of extracellular enzyme activities determined in this study ranked; in polyhumic

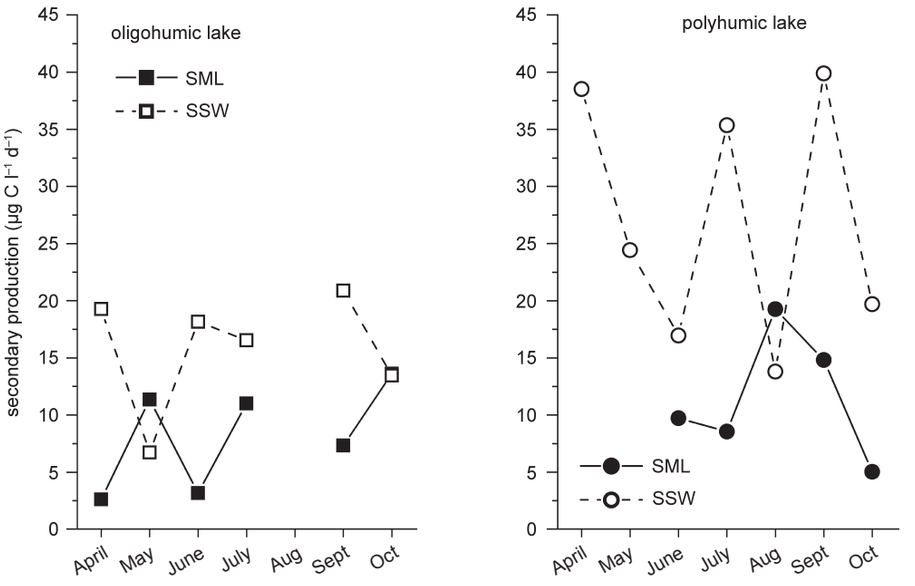

Fig. 4. Secondary production in the surface microlayer (SML) and subsurface water (SSW) in oligohumic and polyhumic lakes.

lake: lipase > APA > AMP > Glu; in oligohumic: lipase > AMP > APA > Glu (Table III). APA activity was higher in the polyhumic lake (V_{max} was 1908 nmol l^{-1} h^{-1} in the SML and 2258 nmol l^{-1} h^{-1} in the SSW, while in the oligohumic lake it was as low as 632 and 339 nmol l^{-1} h^{-1}, respectively (Fig. 5). Differences between lakes and layers were statistically significant only in the SSW ($p = 0.043$, $n = 7$). APA activity was higher in the polyhumic lake than in the oligohumic by 11 times in the SSW and ca. five times in the SML. However, the differences between APA and TP or orthophosphates were not statistically significant. The enrichment factor (Ef) was much higher in the oligohumic lake (1.9) and just below 1.0 in the polyhumic one. Of all the measured enzymes, only the potential maximal activity of AMP was higher in the oligohumic (1471 nmol l^{-1} h^{-1} in the SML and 2117 nmol l^{-1} h^{-1} in the SSW) than in the polyhumic lake (861 and 1213, respectively), but differences were statistically significant only in the SSW ($p = 0.028$, $n = 6$) (Fig. 5). In both lakes higher activity of AMP was observed in the SSW. Lipase activity was more than twice higher in the polyhumic lake (6098 nmol l^{-1} h^{-1} in the SML and 3732 nmol l^{-1} h^{-1} in the SSW) than in the oligohumic one (2290 nmol l^{-1} h^{-1} in the SML and 2238 nmol l^{-1} h^{-1} in the SSW; Fig. 5). Differences between lakes were statistically significant in both layers on the same level ($p = 0.028$, $n = 6$), and bacteria were more active in the SML of the polyhumic lake (Efs 1.6). In both lakes, glucosidase was on the same level (about 30–50 nmol l^{-1} h^{-1}) and differences between lakes were not statistically important. Neustonic bacteria were more active in both lakes (Efs 1.1–1.4).

Table III
Mean values for ectoenzyme affinity (K_m; µM), potential maximal activity (V_{max}; nM h^{-1} l^{-1}) for glucosidase (Glu), acid phosphatase (APA), aminopeptidase (AMP); and lipase (lipase).

	Oligohumic		Polyhumic	
	SML	SSW	SML	SSW
Glu				
K_m	142.0	168.3	59.3	30.7
V_{max}	41	29	49	43
APA				
K_m	0.8	2.9	22.8	36.8
V_{max}	632	339	1908	2258
AMP				
K_m	93.6	92.2	65.2	41.3
V_{max}	1471	2117	861	1213
Lipase				
K_m	44.5	90.3	59.3	30.7
V_{max}	2290	2238	6098	3732

Discussion

Bacteria are a key component in aquatic ecosystems due to their wide biodiversity, their capacity to survive in extreme environments like in the surface microlayer, and their large variety of metabolic activities (Cunliffe *et al.*, 2011). Bacteria also play a major role in OM cycling and, subsequently, in sustaining nutrient turnover (Chróst, 1991). However, conflicting results concerning surface films can be found in the literature (Dietz *et al.*, 1976; Hermansson and Dahlbäck, 1983; del

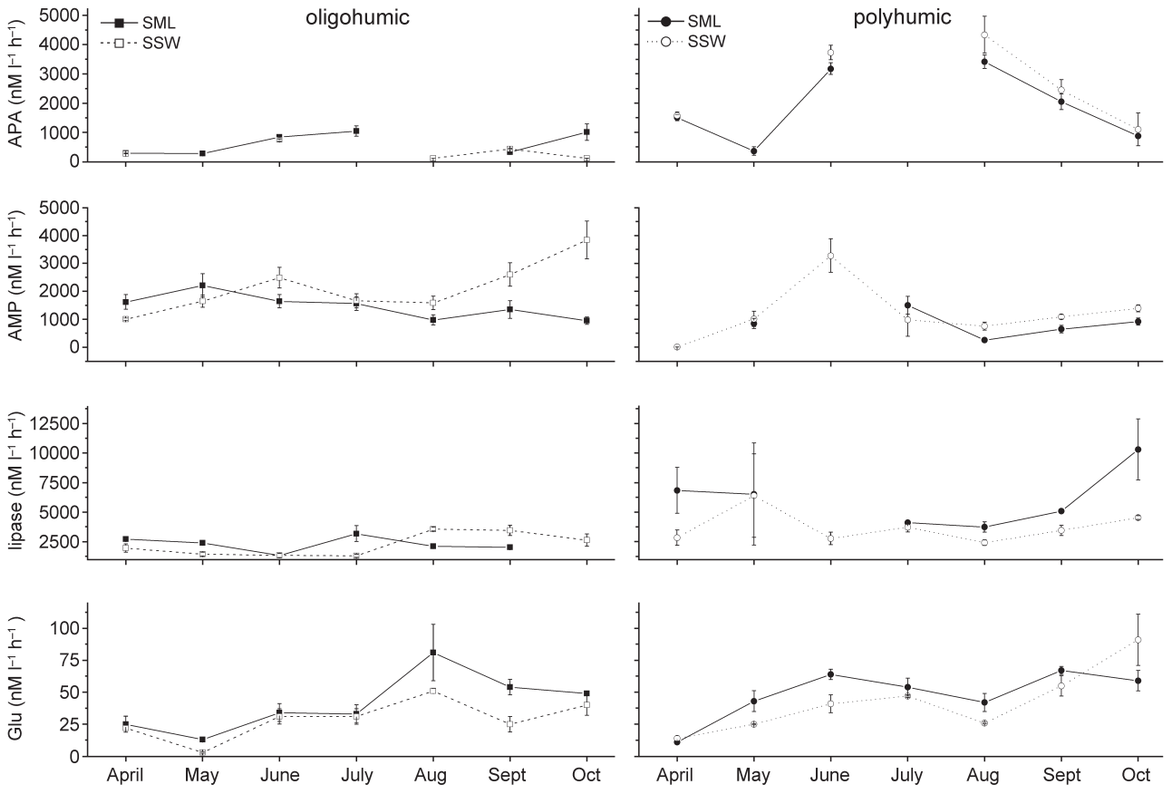

Fig. 5. Enzymatic (APA – alkaline phosphatase, AMP – aminopeptidase, Glu – glucosidase, and lipase) activity (Vmax) of the surface microlayer (SML) and subsurface water (SSW) in oligohumic and polyhumic lakes.

Giorgio and Scaraborough, 1995; Münster *et al.*, 1998; Kuznetsova and Lee, 2001; Mudryk and Skórczewski, 2004; Kostrzewska-Szlakowska, 2005; Santos *et al.*, 2009; Stolle *et al.*, 2009). We need to keep in mind that the SML undergoes highly dynamic spatial and temporal changes (Kuznetsova and Lee, 2001). The key issue may be the surface microlayer sampling methods used. Different devices sample different depths of the surface water of a microlayer, thus, influencing the collected samples (Münster *et al.*, 1998; Stolle *et al.*, 2009; Cunliffe *et al.*, 2011).

Nutrient and organic matter content (Table I) was roughly concordant with data obtained by Münster *et al.*, (1998) for Finnish, humic lakes and Södergren (1993) for Swedish lakes.

Hermansson and Dahlbäck (1983) and Joux *et al.* (2006) showed no significant differences between the fraction of active cells in the SML and in the bulk water. Kuznetsova *et al.* (2004) found that the percentage of bacteria with damaged membranes was frequently lower in the SML than in the SSW. In opposition to these data, in dystrophic lakes the percentage of bacteria with damaged membranes were higher in SMLs (Fig. 3). It seems that the function of bacteria, based on their total numbers, may have to be revised to accommodate large variations in the proportion of metabolically active cells (del Giorgio and Scaraborough 1995). However, they revealed that both based on literature data and their own from 24 studied Canadian lakes, the percent of active bacteria was between 21 and 23%. Schumann *et al.* (2003) discovered that in the eutrophic freshwater lakes bacteria with intact cells accounted for 70%, while in the mesotrophic only 42%. In our lakes, the means for the season percent of active bacteria were slightly higher in the polyhumic lake in the SML (61 versus 54%) but were similar in the SSW (about 70%). It could be concluded that (1) more active bacteria were found in the SSW and (2) variability is large (19–97%) depending on the trophic status of the waters, sampling data and investigated layer.

We cannot conclude that irradiance had detrimental effects on the neustonic algal community because chl *a* was enriched in the SML of the oligohumic lake and depleted in the polyhumic one. In addition, bacteria preferred to stay in the SML (Efs higher than 1 in both lakes; Table I). Cunliffe *et al.* (2011) showed 78% similarity of the bacterial communities of SML and SSW samples, Santos *et al.* (2009) pointed to high structural similarities between bacterial communities in both layers while Franklin *et al.* (2005) suggested that the marine bacterioneuston contains a distinct community of bacteria.

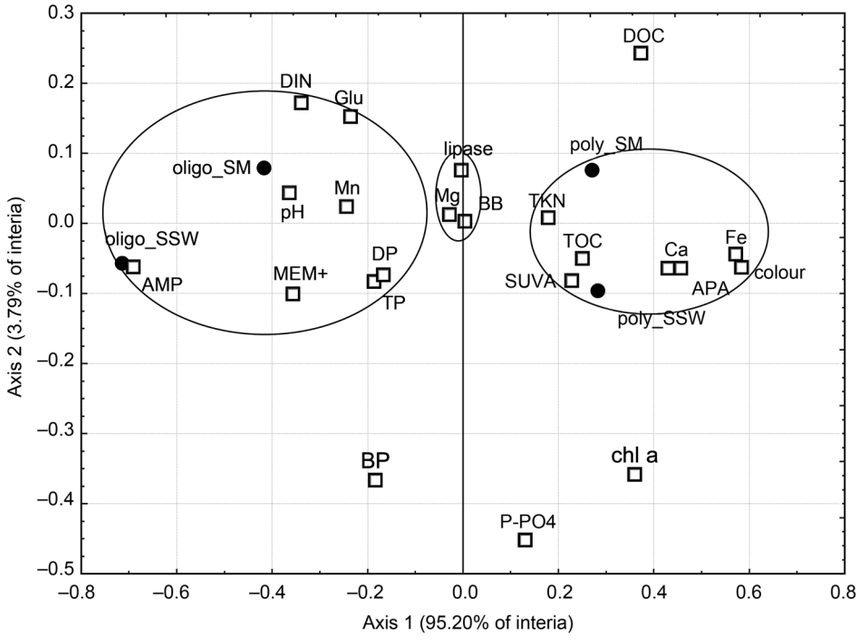

Fig. 6. CA analysis of studied variables in the surface microlayer (SML) and subsurface water (SSW) in oligohumic (oligo-) and polyhumic (poly-) lakes.

DIN – dissolved inorganic nitrogen, TKN – total Kjeldahl nitrogen, TP – total phosphorus, DP – dissolved phosphorus, PO_4-P – ortophosphorans, TOC – total organic carbon, DOC – dissolved organic carbon, BB – bacterial biomass, BP – secondary production, MEM+ – percentage contribution of active bacteria with intact membranes, Fe – iron, Ca – calcium, Mg – magnesium, Mn – manganese, AMP – aminopeptidase, Glu – glucosidase, APA – alkaline phosphatase and lipase.

Münster *et al.* (1998) stated that although the biofilm microbial communities in the SML can be assumed to receive generally higher environmental impacts like UV-irradiance, heavy metals, pollutants and others, there were significantly higher microbial activities and bacterial growth rates in the SML samples compared to those in the SSW. However, Hermansson and Dahlbäck (1983) also did not observe any effects of sunlight on the activity of bacteria in the surface samples. In the lakes studied here, this statement is only true in the case of lipase and glucosidase activity in both lakes and APA in the oligohumic one. Other activities measured such as: secondary production, AMP and APA in the polyhumic lake were higher in the SSW. However, in both lakes we observed a higher concentration of phosphorus, especially orthophosphates, in the SSW and a slightly enriched SML of DOC. In addition, Santos *et al.* (2009) stated that the activity of the studied extracellular enzymes was higher in bacterioneuston than in bacterioplankton, probably due to stimulation of extracellular enzyme synthesis by high concentrations of polymeric OM in the SML. It was also varying in the studied humic lakes, whereas some enzymatic activities were higher in the SML (lipase and glucosidase in both lakes and phosphatase in the oligohumic lake) and some in the SSW (aminopeptidase in both lakes and phosphatase in the polyhumic lake). Münster *et al.* (1992) found that the activity of extracellular enzymes produced by aquatic microheterotrophs depends on lake water pH. Phosphatases and glucosidases have their relative maxima of activity at pH = 5.4, while aminopeptidases had their maxima in the neutral pH region. Generally, we used standard fluorogenic substrates with one exception, we used an unusual substrate for APA, (DiFMUP (6,8-difluoro-4-methylumbeliferyl phosphate), which exhibits strong fluorescence at acidic pH and allowed us to directly measure the activity of acid phosphatases in the acidic water samples without alkaline buffer addition after sample incubation. In the majority of published studies a substrate for alkaline phosphatases were used so comparing the results obtained from these two methodological approaches may be inaccurate.

Stolle *et al.* (2009) have reported that ^{3}H-TdR incorporation of the bacterioneuston in the Baltic Sea was reduced by 50 to 80% compared to that in the underlying water. The same situation was observed in humic lakes and thymidine incorporation of the bacterioneuston was reduced by 50 to 70% compared to bulk water. This indicates that the DOM pool of the SML may not be readily available for bacteria (Santos *et al.* 2009). However, opposing data was reported by Münster *et al.* (1998) measuring α-Glucase, which was 4–5 times higher in bacterioneuston compared to

bacterioplankton. Joux *et al.* (2006) observed that the intensity of bacterial production in the Mediterranean Sea was highly variable.

CA analysis was used to investigate the relation between water biology and chemistry variables in two layers-SML and SSW of two humic lakes with different water colours (Fig. 6). For layers in both types of lakes, almost all of the explained variables were due to the first axis (95.20% of inertia) with a negligible influence from the second axis (3.79% of inertia). Concentrations of iron, calcium, orthophosphates, total nitrogen, total and dissolved carbon and water colour, SUVA, and some biological aspects like APA and chlorophyll *a* were positively related to both analysed layers of the polyhumic lake. Concentrations of total and dissolved phosphorus, DIN, Mn, pH and some biological elements connected with bacteria like secondary production, percent of active bacteria with intact membranes, Glu, AMP correlated with both layers in the oligohumic lake. On the contrary, the model could not explain BB, Mg or potential activity of lipase. In addition, the results suggest that inherent properties of organic matter as indicated by TOC and DOC, SUVA, Fe were related to chl *a* in the polyhumic lake and were negatively related with most attributes of bacteria like production, MEM+ and some enzymatic activity. APA, which was used as an indicator of P deficiency, correlated negatively with concentrations of phosphorus but positively with orthophosphates. Nonetheless, the low percentage of second axis (however both explaining almost 99% of cases) if we put '0' line – two layers of two lakes are placed in each quadripartite. Thus, it seems that SML and SSW showed lake-specific differences in all studied parameters and that was SML is generally supported from the underlying waters.

It could be stated that: (1) Polyhumic lake are characterised as more eutrophic with higher concentrations of organic matter and nutrients compared to oligohumic lake, then the surface microlayer of the polyhumic lake was enriched in forms of phosphorus and ammonium, while enrichments in nitrogen and bacterial abundance were higher in the oligohumic one. (2) The bacteria activity pattern in the SML versus SSW was variable. Activities expressed as lipase and glucosidase V_{max} were higher in the SMLs in both studied lakes and as phosphatase V_{max} just in the oligohumic lake. The aminopeptidase V_{max} were higher in the SSW in both lakes and phosphatase V_{max} only in the polyhumic lake. (3) Solar radiation does inhibit neuston microbial community as a whole; it has a pronounced inhibitory effect on the surface microbial activity characteristics as an bacterial production and share of active bacteria with intact membranes, however, in the oligohumic lake, we noticed stable higher total abundance of bacteria in SML. (4) SML and SSW showed lake-specific differences in all studied parameters and SMLs are not directly supported from the underlying waters (SSWs). (5) The SMLs of small humic lakes is an inhospitable microhabitat in the same way as in other water bodies (controlled by dynamic physical processes like intense solar radiation, wind, changing temperature, waves, etc.) but to a larger extent may act as an important sinks for allochthonous nutrient resources and may then generate significant energy pools for microbial food webs.

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