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EXTRACELLULAR ENZYME ACTIVITY IN THE SURFACE MICROLAYER AND SUBSURFACE WATER IN THE COASTAL LAKE DOŁGIE WIELKIE

Marta Zdanowicz*, Zbigniew Jan Mudryk, Piotr Perliński

Department of Experimental Biology,
Institute of Biology and Environmental Protection,
Pomeranian University in Słupsk, ul. Arciszewskiego 22b,
76-200 Słupsk, Poland
** e-mail: marta.zdanowicz@apsl.edu.pl*

Abstract

The paper presents studies on level hydrolytic activity of extracellular enzymes in the surface microlayer and subsurface water in the coastal lake Dołgie Wielkie. The ranking order of the potential enzyme activity rates in the studied water layers was as follows: aminopeptidase > lipase > α-glucosidase > β-glucosidase. The level of activity of all studied hydrolases was higher in the surface microlayer than subsurface water. Activity of extracellular enzymes was influenced by the season.

Key words: coastal lake, surface layers, subsurface water, enzymatic activity, bacteria

INTRODUCTION

The bacteria, mainly heterotrophic organisms, play the key role in regulating accumulation, export, re-mineralization and transformation of the largest part of organic matter in the aquatic ecosystems (Bigg et al. 2004, Momzikoff et al. 2004). These processes include microbiological biotransformation of dissolved (DOM) and particulate (POM) organic matter of auto- and allochtonus origin. In water bodies, heterotrophic bacteria are able to decompose a wide spectrum of organic material from natural and anthropogenic origins, whose molecules differ in size from monomers to polymers (Agogué et al. 2005). For heterotrophic bacteria those compounds constitute a very important source of carbon, nitrogen and energy and are used for biosynthesis or respiration processes (Joux et al. 2006).

The heterotrophic bacteria play a central role in the organic matter and energy flux through water food webs. These organisms produce new bacterial biomass by incorporating a part of dissolved organic matter (DOM) and they respire organic matter to inorganic compounds (del Giorgio and Cole 1998). A high concentration of inorganic and organic matter occurring in the surface microlayer and many aquatic organisms find optimal conditions for growth in such a biotope. The surface microlayer is the boundary layer between the atmosphere and the hydrosphere, where the transfer of material is controlled by complex physicochemical and biological processes (Wurl and Obbard 2004, Reinthaler et al. 2008).
In the spectrum of enzymes studied in the aquatic environment, special attention has been given to ectoenzymes, which were responsible for the hydrolysis of the major components of DOM (Cunha et al. 2010). Most of organic matter in aquatic ecosystems consists of compounds of a high molecular weight and polymeric structure. Macromolecular organic substrates must be enzymatically hydrolyzed to smaller subunits before they can be taken up and metabolized by the bacterial cells (Li and Chróst 2006). Enzymatic hydrolysis by extracellular enzymes is a crucial first step in bacterial utilization of the organic matter. Therefore, enzymatic activity may represent a significant fraction of the potential degradation and recycling capacity of natural systems (Misic and Fabiano 2006).
The aim of the present paper was to determine spatial and seasonal dynamics of the changes in level hydrolytic activity of extracellular enzymes in the surface microlayer and subsurface water in the coastal lake Dołgie Wielkie.

MATERIALS AND METHODS

1. Study area and sampling

The study has been carried out in the freshwater coastal lake Dołgie Wielkie which is a part of the World Biosphere Reserve (the Slovinski National Park). The lake has water area of 156.4 hectare with mean depth of 1.4 m. It's located within a typical forest catchment basin which makes a natural protection zone of this water body. Lake Dołgie Wielkie is a basin that shows an advanced natural eutrophication process. It is characterized by variable physical and chemical parameters (Table 1) which stand in close connection with its near-marine location. Lake Dołgie Wielkie was a bay of estuarine Gardno Lake, but some time ago (about 300 years ago) it separated and became an isolated non-estuarine lake.
Water samples were taken in vegetation season (spring, summer and autumn) in 2003-2006 at three sites (Fig. 1):
– site 1 – placed in the eastern part of the lake;
– site 2 – situated in the northern part of the lake;
– site 3 – located in the western part of the lake.
At each site, two layers of water were collected. Samples of surface layer (SML) (thickness of about 240 μm) were collected with Garrett net (Garrett 1965). Probs from subsurface water (SSW) were taken at the depth of about 10-15 cm. All water samples were placed in sterile glass bottles and stored in an ice-box at a temperature lower than 7°C. The time between the sampling and the analysis usually did not exceed 6-8 hours.

Table 1

Some physical and chemical parameters in the water lake Dołgie Wielkie (according to Antonowicz et al. 2010)

Parameters	Unit of measure	Mean (SD)
N-T	mg dm^{-3}	0.770 (0.090)
N-org	mg dm^{-3}	0.690 (0.090)
$N\text{-}NH_4$	µg dm^{-3}	78.750 (11.850)
P-T	µg dm^{-3}	123.300 (36.530)
P-org	µg dm^{-3}	47.080 (30.530)
$P\text{-}PO_4$	µg dm^{-3}	76.210 (11.480)
electrical conductivity	mS	0.089 (0.002)
Cl	mg dm^{-3}	15.860 (0.510)
O_2	mg dm^{-3}	9.220 (1.990)

SD – standard deviation

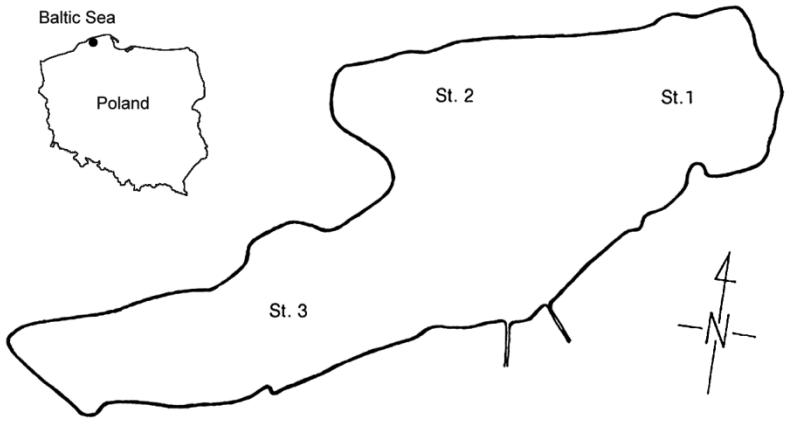

Fig. 1. Lake Dołgie Wielkie, northern Poland, with location of sampling sites
Geographic coordinates of Lake Dołgie Wielkie 54°41'29.25" – 54°42'11.10"N, 17°10'13.10" – 17°12'34.18"E

2. Estimation of enzyme activity

The level of enzymatic activity were determined according to method by Hoppe (1993). In order to estimate the activity of hydrolytic enzymes following fluorescently labelled with MUF (4-methylumbelliferyl) and MCA (4-methyl-coumarinyl-7 amide) organic substrates were used:

– L-leucine-4 methylcoumarinyl-7 amide to determine the activity of aminopeptidase (AMI) (E.C.3.4.11.1),
– 4-methylumbelliferyl butyrate to determine the activity of lipase (LIP) (E.C. 3.1.1.3),
– 4-methylumbelliferyl-α-d-glucopyranoside to determine the activity of α-glucosidase (α-GLU) (E.C. 3.2.1.20),

– 4-methylumbelliferyl-β-d-glucopyranoside to determine the activity of β-glucosidase (β-GLU) (E.C. 3.2.1.21).

From each of these substrates, the solutions initial concentration equal to 10 nM were prepared using methyl cellsolve (Sigma) as solvent. Before taking the measurements each of these solution was diluted with spectroscopically pure water (Fluka) to reach final concentrations of 0.5 μM, 2 μM, 10 μM, 20 μM, 50 μM, 200 μM, 500 μM. The water samples of 3.9 cm^3 and 0.1 cm^3 of the substrate of the appropriate concentration were transferred into quartz microcuvettes which were placed in a Hitachi spectrofluorometer F-2500 with FL Solutions software. The measurements with the spectrofluorometer were taken with excitation at 318 nm for MUF labelled substrates, and 345 nm for MCA labelled substrates, and the readings were done at 445 nm and 440 nm, respectively. The first reading was done immediately after the substrate was introduced into the analysed water sample (so called zero time). Subsequent readings were carried out after 20, 40, 60 min for lipase and aminopeptidase and after 30, 60, 90 min for α-glucosidase and β-glucosidase. We applied ENZFITTER software, version 1.05, to determinate maximum rate of enzymatic reaction (Vmax).

3. Statistical analysis

All statistical analysis (Spearman's rank correlation coefficient, standard deviation – SD, coefficient of variation – CV, coefficient of dispersion – CD) were calculated using Statistica 9.0 software. The normal distribution of the data was checked by using the Shapiro–Wilk test before statistical analysis. Relationships among parameters within the whole data set were examined using Spearman rank correlation. The significance of differences between layers, sites and seasons in level enzymatic activity rate was assessed using Kruskal–Wallis non-parametric equivalent of ANOVA, when mean values revealed a distribution other than normal.

RESULTS

The microbial hydrolytic potential activities of four enzymes determined in water lake Dołgie Wielkie are summarised in Table 2. The mean rate of the activity of aminopeptidase equalled 113.14 nM MCA·cm^{-3}·h^{-1}, while the mean rate of the activity of lipase equalled 26.25 nM MUF·cm^{-3}·h^{-1}.

Table 2

Spectrum of selected extracellular enzymatic activities in the water lake Dołgie Wielkie

Enzyme	Enzyme activity (nM MUF, MCA·cm^{-3}·h^{-1})					
	mean	min	max	SD	CV [%]	CD
Aminopeptidase	113.14	3.96	759.86	16.94	127.04	182.60
Lipase	26.25	1.25	123.37	32.43	123.57	40.08
α-glucosidase	1.26	0.00	11.92	0.30	204.84	5.28
β-glucosidase	1.25	0.00	6.49	0.16	111.53	1.56

Statistical tests (SD – standard deviation, CV – coefficient of variation, CD – coefficient of dispersion)

The lowest level of potential activity is α-glucosidase and β-glucosidase with the mean activity rate equalling 1.26 nM MUF·cm^{-3}·h^{-1} for α-glucosidase and 1.25 nM MUF·cm^{-3}·h^{-1} for β-glucosidase. The ranking of the activity rates of the assayed enzymes was usually: aminopeptidase > lipase > α-glucosidase > β-glucosidase.
Fig. 2 shows changes in the level of extracellular enzyme activity along the vertical profiles. It can be clearly seen that enzymatic activity is stratified and differences between surface microlayer and subsurface water. All tested hydrolytic enzymes showed higher activity in the surface microlayer than in subsurface water (for aminopeptidase 128.85 nM MCA·cm^{-3}·h^{-1}, for lipase 29.64 nM MUF·cm^{-3}·h^{-1}, for α-glucosidase 2.36 nM MUF·cm^{-3}·h^{-1}, for β-glucosidase 1.76 nM MUF·cm^{-3}·h^{-1}).

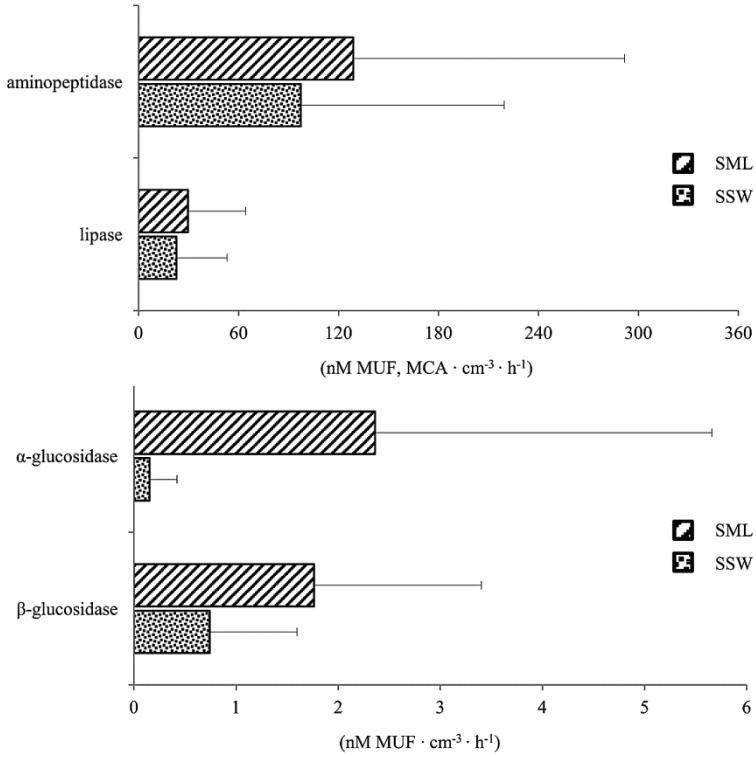

Fig. 2. Vertical distribution of enzymatic activity in surface microlayer (SML) and subsurface water (SSW) (average from the pooled data of all sites and season). Vertical bars represent standard errors of the mean, n = 36

Fig. 3 shows the seasonal variation in the activity level of the studied hydrolytic enzymes in the surface microlayer and subsurface water in the coastal lake Dołgie Wielkie. According to these data in studied seasons, the higher activity level of all tested hydrolytic enzymes was in the surface microlayer than in subsurface water. The highest activity level of aminopeptidase (419.99 nM MCA·cm^{-3}·h^{-1}) was noted in spring 2004, lipase (96.04 nM MUF·cm^{-3}·h^{-1}) and α-glucosidase (11.42 nM MUF·cm^{-3}·h^{-1}) in summer 2003 and β-glucosidase (5.69 nM MUF·cm^{-3}·h^{-1}) in autumn 2003 in the surface microlayer.

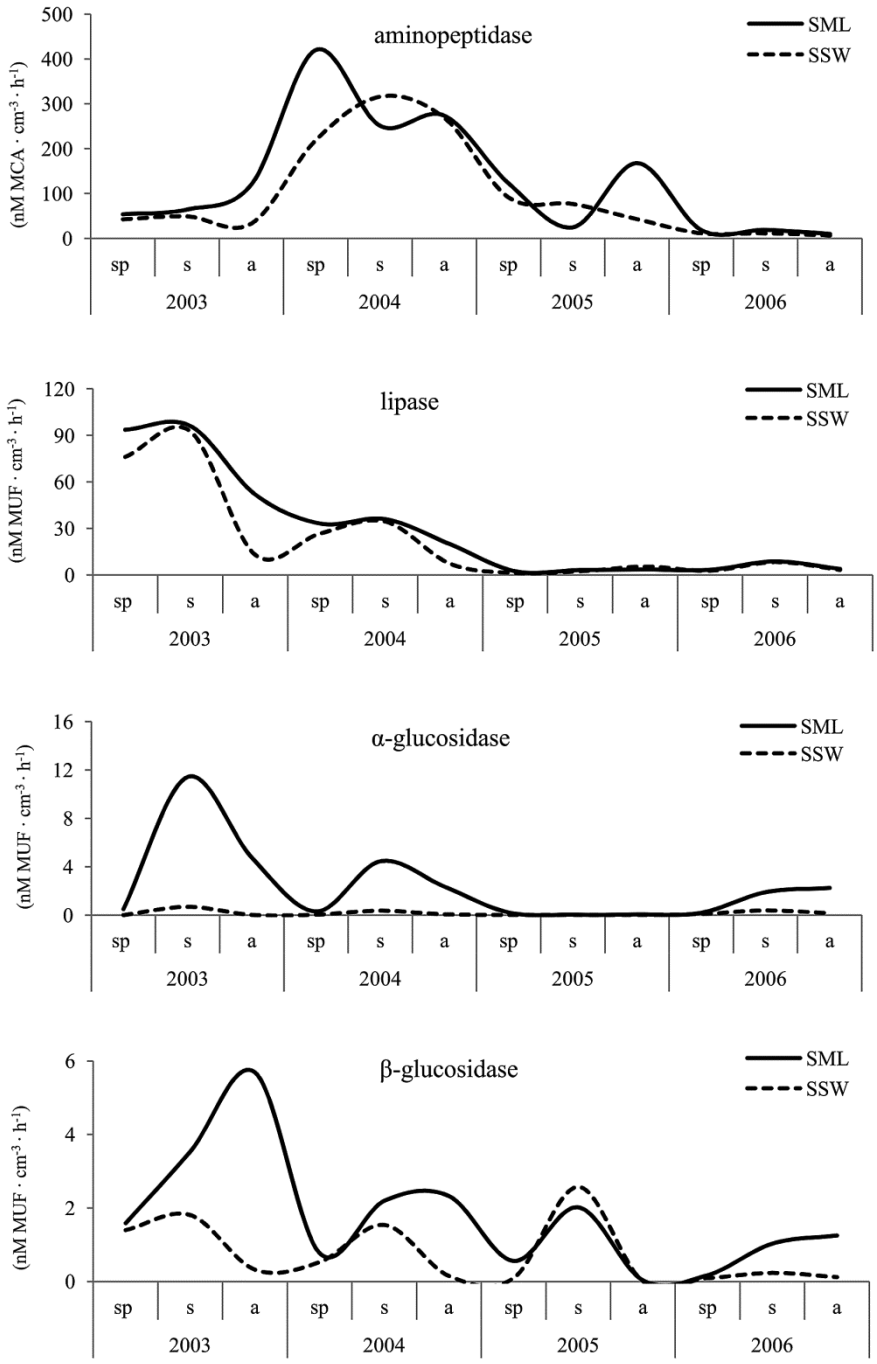

Fig. 3. Seasonal dynamics change of potential enzymatic activity in water lake Dołgie Wielkie (average from the pooled data of all sites)
(sp – spring, s – summer, a – autumn)

As a rule, the lowest activity level of the studied enzymes was noted in subsurface water – 6.46 nM MCA·cm^{-3}·h^{-1} for aminopeptidase in autumn 2006, 1.31 nM MUF·cm^{-3}·h^{-1} for lipase in spring 2005, 0.01 nM MUF·cm^{-3}·h^{-1}for β-glucosidase in autumn 2005 and absences for α-glucosidase in spring 2003.

The level of extracellular enzymatic activity along the horizontal profile of the studied lake is presented in Fig. 4. These data show that the activity of all hydrolytic enzymes along the entire horizontal profile of the basin remained at similar level. At all studied sites the highest level of enzymatic activity was observed in aminopeptidase (97.28 – 142.54 nM MCA·cm^{-3}·h^{-1}) and the lowest in α-glucosidase (1.13 – 1.50 nM MUF·cm^{-3}·h^{-1}) and β-glucosidase (1.22 – 1.30 nM MUF·cm^{-3}·h^{-1}).

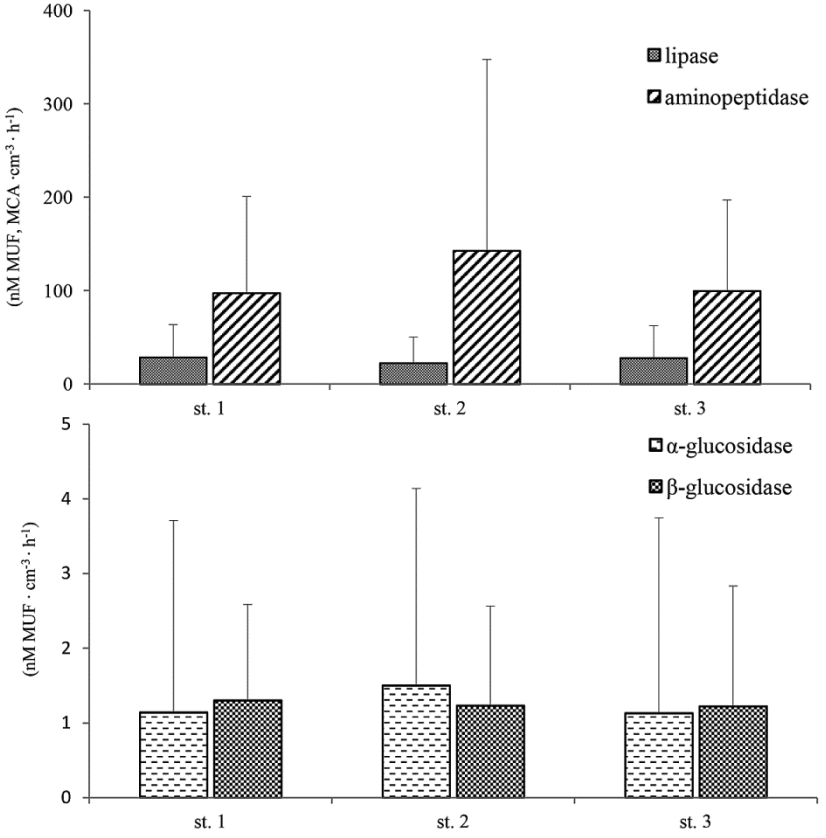

Fig. 4. Horizontal variation of potential enzymatic activity in different parts studied lake (average from the pooled data of all water layer and seasons). Vertical bars represents standard errors of the mean, n = 24

The relationships among the activity of analysed extracellular enzymes in the water lake Dołgie Wielkie are given as the correlation matrix in Table 3. Significant positive correlation between the activity of lipase and α-glucosidase (r = 0.38) and β-glucosidase (r = 0.55) was noted. Significant positive correlation (r = 0.49) in water lake Dołgie Wielkie was also found between α-glucosidase and β-glucosidase.

Table 3

Correlation coefficient enzymatic activity in the water lake Dołgie Wielkie

Nonparametric Spearman's correlation coefficients in dataset				
	AMI	LIP	α-GLU	β-GLU
AMI				
LIP	0.31**			
α-GLU	0.11	0.38***		
β-GLU	0.26*	0.55***	0.49***	

Explanations: n = 72 in all cases
significance (p) is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

By grouping the results by the sites, layers and seasons the Kruskal–Wallis nonparametric test was carried out to the level enzymatic activity (Table 4). The analyses showed significant differences for the level of α-glucosidase activity among layers, sites and layers, layers and seasons. The enzymatic activity of β-glucosidase rates were found to be significantly different between layers, seasons, sites and seasons, layers and seasons.

Table 4

Analyses of the Kruskal–Wallis test in the level of enzymatic activity in the water lake Dołgie Wielkie due to site, layer and season

Enzymes	Source of variation	H	p
Aminopeptidase	site	0.046	ns
	layer	0.169	ns
	season	0.394	ns
	site × layer	1.250	ns
	site × season	1.507	ns
	layer × season	2.344	ns
Lipase	site	0.645	ns
	layer	0.916	ns
	season	3.158	ns
	site × layer	1.699	ns
	site × season	4.071	ns
	layer × season	4.092	ns
α-glucosidase	site	0.856	ns
	layer	23.375	***
	season	4.488	ns
	site × layer	24.257	***
	site × season	6.650	ns
	layer × season	29.263	***
β-glucosidase	site	0.702	ns
	layer	9.144	**
	season	14.743	***
	site × layer	10.047	ns
	site × season	17.106	*
	layer × season	27.868	***

Explanations: significance (p) is indicated by asterisks: $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$
H – the Kruskal–Wallis test, p – significance level, ns – non-significant

DISCUSSION

On the level of intensity of decomposition and transformation processes of organic matter in aquatic ecosystems is influenced mainly bacterial enzymatic activity (Münster and Chróst 1990). The organic matter accumulated in water basins in a significant part consists of polymers of high molecular weight, which due to the limited permeability of the cell membrane cannot be absorbed directly by bacteria (Zaccone et al. 2004). Macromolecules only after enzymatic hydrolysis to monomers, such as amino acids and monosaccharides, are actively absorbed by bacteria (Unanue et al. 1999, Li and Chróst 2006) and used as a food substrate or energy source (Brown and Goulder 1996, Patel et al. 2000). Therefore, many microorganisms, mainly bacteria, are characterized by the ability to synthesize and release various hydrolytic exoenzymes that carry out the depolymerization of macromolecular compounds in the aquatic environment (Microbial enzymes… 1991, Marxsen and Witzel 1993). The role of these enzymes in decomposition of dissolved organic matter is very important (Chróst et al. 1994, Caruso and Zaccone 2000), and the level of enzymatic activity can be an indicator of the bioavailability of nutrients for bacteria (Zaccone et al. 2003). On the other hand, enzyme activity depends on a large extent on a number of physicochemical environmental factors such as substrate concentration, number of enzyme molecules, specific enzyme activity, interactions between microorganisms and bacterial counts and their production (Nausch and Nausch 2000, Hoppe et al. 2002, Chróst and Siuda 2006). Heterotrophic bacteria are able to react quickly to the inflow of organic matter through the synthesis of hydrolytic enzymes, whose activity is stimulated or inhibited by the trophic level of the environment (Microbial enzymes… 1991, Caruso and Zaccone 2000, Zaccone et al. 2004).

In the water of studied lake Dołgie Wielkie leucine aminopeptidase showed the highest potential level of activity. According to Keil and Kirchman (1992), the activity of this exoenzyme is closely related to the enzymatic activity of heterotrophic bacteria in the aquatic environment. Proteins and polypeptides are found in a significant amount in water basins and are an important source of carbon, nitrogen and energy for bacteria (Patel et al. 2000). During the multistep depolymerization of these compounds under the action of aminopeptidase they are broken down into single amino acids, which are used by bacteria during biosynthesis of cellular structures or in respiratory processes (MacCarthy et al. 1998). Martinez et al. (1996) and Mallet and Debroas (1999) have shown that this proteolytic enzyme is highly active in various water basins. In aquatic ecosystems, aminopeptidase is present along with other proteolytic enzymes, contributing to the depolymerization of numerous proteinaceous compounds originating from dead plant and animal remains (Microbial enzymes… 1991). According to Martinez et al. (1996), Mallet and Debroas (1999) and Nausch and Nausch (2000), the activity of extracellular aminopeptidases depends on the protein concentration in the aquatic environment. The level of aminopeptidase activity in water basins is mainly related to primary production and the availability of detritus of animal origin (Burney 1986). As results from research carried out by Thompson and Sinsabaugh (2000), Mudryk and Skórczewski (2004) as well as Chróst and Siuda (2006) showed the activity of aminopeptidases also depends on the degree of eutrophication of the water basin. The present study was conducted in studied

Dołgie Wielkie lake with a high nutrient content, characterized by a natural gradual but advanced eutrophication process, which can largely explain the high activity of aminopeptidase.

In the water lake Dołgie Wielkie the highest activity of aminopeptidase was noted in the surface layer. This corresponds to the results of studies on the activity of this hydrolase in the water of lake Gardno published by Mudryk and Skórczewski (2004), which showed that the highest level of aminopeptidase activity was observed in surface layers of water. Also Jones and Lock (1993) indicate a high level of proteolytic enzymes activity in these layers of water.

In the water of studied lake Dołgie Wielkie, the lower activity of aminopeptidase was characterized by the lipase. Lipase activity is closely correlated with the availability of the lipid substrate and, therefore, the level of this activity may reflect the concentration and distribution of fatty compounds in the water basins (Hoppe 1993). High activity of lipases in various water basins has been demonstrated by Martinez et al. (1996), Sinsabaugh et al. (1997), Mudryk (1998), Skórczewski (2003), and Kalwasińska (2008). In lake Dołgie Wielkie, the activity of this enzyme was much lower than in the above-mentioned studies. The reason for the low activity of the lipase in the studied lake is probably related to the fact that the lipid compounds in this water basin are broken down to a large extent by other groups of lipases, the activity of which has not been investigated in this study.

In the Dołgie Wielkie Lake, the highest level of lipase activity was found in the surface layer. This confirms the regularity that lipids most actively accumulate in surface water layers, which generates increased lipase activity (Goutx et al. 1987).

According to Sinsabaugh et al. (1997) correlation between the activity of enzymes released by microorganisms and the concentration of nutrients is related to seasonal fluctuations of hydrolase activity in water basins. In the presented study, the highest level of activity of lipase was observed in the summer, which is probably associated with a significant increase in the concentration of lipid compounds in water lake Dołgie Wielkie resulting from the intensive development of phytoplankton and zooplankton.

Among the studied in the water of lake Dołgie Wielkie hydrolytic enzymes, α-glucosidase and β-glucosidase were characterized by the lowest activity. Despite the high carbohydrate concentration in aquatic ecosystems, other authors also obtained similar results (Brown and Goulder 1996, Rulík and Spáĉil 1999). The low glucosidase activity in the water basins is probably related to the low concentration of substrates suitable for these hydrolases. According to Zaccone et al. (2002) amino acids produced as a result of depolymerization of proteins and polypeptides represent a better source of carbon and energy for bacteria than carbohydrate hydrolysis products, and protein distribution proceeds faster than polysaccharide degradation (Unanue et al. 1999, Zaccone et al. 2003). The consequence of this process is the fact that the enzymatic activity of the aminopeptidase (113.14 nM MCA·cm^{-3}·h^{-1}) was the higher than α-glucosidase (1.26 nM MCA·cm^{-3}·h^{-1}) and β-glucosidase (1.25 nM MCA·cm^{-3}·h^{-1}). A similar dependence of 100-fold lower α-glucosidase activity than aminopeptidases was demonstrated by the seawater tests conducted by Müller-Niklas et al. (1995). A low level of enzymatic activity of β-glucosidase compared to the activity of

aminopeptidase was also noted by Zaccone et al. (2003), Mudryk and Skórczewski (2004) and Kalwasińska (2008).

Organic substances in water basins are unevenly distributed in them (Carlucci and Wolgast 1992). The surface microlayer has a higher concentration of food substrates suitable for microorganisms than in subsurface water layer (Williams et al. 1986, Carlucci et al. 1991). Results of studies of the activity of α-glucosidase and β-glucosidase in lake Dołgie Wielkie showed that level these enzymes was significantly higher in the surface layer than in the subsurface water, which corresponds to the results obtained by Mudryk and Skórczewski (2000).

As with aminopeptidase and lipase, α-glucosidase and β-glucosidase activity was subject to seasonal fluctuations. These enzymes showed an increase in summer activity, which was most likely caused by the inflow of significant amounts of organic substances at this time of year and by higher temperatures (Münster et al. 1992). Research by Rochelle-Newall et al. (2004) indicate that bacteria by regulating enzyme synthesis is able to quickly adapt to the seasonal changes taking place in the pool of available food substrates.

The above considerations give the ground to formulate the following conclusion:

– The ranking order of the potential enzyme activity rates in the studied water layers was as follows: aminopeptidase > lipase > α-glucosidase > β-glucosidase.
– The level of activity of all studied hydrolases was higher in the surface microlayer than subsurface water.
– Activity of studied extracellular enzymes was influenced by the season.

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AKTYWNOŚĆ ENZYMÓW HYDROLITYCZNYCH W MIKROWARSTWIE POWIERZCHNIOWEJ I W WODZIE PODPOWIERZCHNIOWEJ PRZYBRZEŻNEGO JEZIORA DOŁGIE WIELKIE

Streszczenie

W błonie powierzchniowej i w wodzie podpowierzchniowej przybrzeżnego jeziora Dołgie Wielkie badano aktywność enzymatyczną czterech enzymów hydrolitycznych z wykorzystaniem metody spektrofluorometrycznej. Uzyskane wyniki badań wykazały, że najwyższym poziomem aktywności enzymatycznej charakteryzowały się aminopeptydaza i lipaza. Poziom aktywności wszystkich badanych enzymów hydrolitycznych był wyższy w błonie powierzchniowej niż w wodzie podpowierzchniowej. W jeziorze Dołgie Wielkie stwierdzono dynamikę zmian sezonowych poziomu aktywności badanych ektoenzymów.