

## The occurrence of lipolytic bacteria and the activity of extracellular lipases in a riverine ecosystem

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### Abstract

This study determined the numbers and distribution of heterotrophic and lipolytic bacteria and the activity of lipases in the water of the Słupia River, in the section of the river that flows through the city of Słupsk. From spring to fall the mean number of heterotrophic bacteria remained at a relatively constant level ( $158.3 \times 10^3$  cells  $\text{cm}^{-3}$  –  $169.4 \times 10^3$  cells  $\text{cm}^{-3}$ ), whereas in winter a sudden drop to only  $18.3 \times 10^3$  cells  $\text{cm}^{-3}$  was found. The numbers of heterotrophic bacteria in the whole studied section of the river were similar. The mean number of lipolytic bacteria reached a maximum in spring ( $12.9 \times 10^3$  cells  $\text{cm}^{-3}$ ), was high in summer ( $10.5 \times 10^3$  cells  $\text{cm}^{-3}$ ), and reached a minimum ( $1.3 \times 10^3$  cells  $\text{cm}^{-3}$ ) in winter. Lipolytic bacteria accounted for only 5 to 8% of the total number of heterotrophic bacteria. No marked differences in the

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numbers of lipolytic bacteria were determined within the section of the river studied. The highest levels of lipase activity were determined in spring ( $18.9 \text{ nM MUF dm}^{-3} \text{ h}^{-1}$ ) and fall ( $19.9 \text{ nM MUF dm}^{-3} \text{ h}^{-1}$ ), while the lowest ( $11.0 \text{ nM MUF dm}^{-3} \text{ h}^{-1}$ ) were noted in winter. There were no differences in the level of activity of lipolytic enzymes among the sites studied.

## INTRODUCTION

Lipids and fatty acids are integral components of all living organisms and are widely distributed in aquatic environments (Reemtsma et al. 1990). Detritus, live and dead phytoplankton, and invertebrates are the main sources of lipids in water basins (Kattner and Brokman 1990, Arts et al. 1992, Albers et al. 1996).

In aquatic ecosystems, lipid compounds constitute 3-55% of all accumulated organic matter (Chróst and Gajewski 1995, Marty et al. 1996). The concentration of dissolved and particulate lipids in water oscillates between 10 and  $500 \mu\text{g dm}^{-3}$  (Gajewski et al. 1997). Dissolved and particulate lipids are a very heterogeneous group of compounds; the most important classes include: triglycerides, diglycerides, fatty acids, sterols, phospholipids, glycolipids, sulpholipids, wax esters, and aliphatic hydrocarbons (Parrish 1988, Jaffe et al. 1995). Of those, triglycerides and fatty acids are the major components in freshwater and marine ecosystems, constituting 15-50% of all lipids, both dissolved and particle-associated fractions (Kattner and Brokman 1990).

According to Chróst and Gajewski (1995), lipid compounds are very important substrates in the metabolism of aquatic bacteria, as they constitute sources of carbon, energy reservoirs, and components of bacterial membranes (Parrish 1988, Gajewski et al. 1993, Mudryk and Skórczewski 2000). However, lipids are often a complex mixture of organic compounds that are too large to be directly incorporated into bacterial cells (Foreman et al. 1998). This explains why many bacteria are capable of synthesizing lipases which catalyze reactions of lipid depolymerization. Lipases (glycerol ester hydrolases, EC 3.1.1.3) are nonspecific esterases with a molecular mass of about 25 000; they are usually relatively small proteins (Gajewski et al. 1993, Prim et al. 2003). According to Chróst and Gajewski (1995) and Mudryk and Skórczewski (2004), lipases belong to the group of the most active enzymes produced by aquatic bacteria. They are actively exported from living bacteria, but are also released as free enzymes after the lysis of bacterial cells (Chróst 1991). These biocatalysts attack emulsified lipids and hydrolyze them, yielding glycerol and fatty acids. Only in this form can these products be actively assimilated by bacteria to be used in respiratory processes, or in biosynthesis of cellular structures (Gajewski et al. 1997).

According to Dachs et al. (1998), the specific role of bacteria in modifying and contributing to the lipid composition in water ecosystems is complex and far from understood. Therefore, the objective of the present study was to

determine the numbers and distribution of lipolytic planktonic bacteria and the level of the activity of extracellular lipases in a riverine ecosystem.

## **MATERIALS AND METHODS**

### ***Study area***

The Słupia River is a coastal river in northern Poland, flowing into the Baltic Sea. Its catchment area is strongly influenced by human activity (agricultural, industrial, hydrotechnical). The Słupia River flows through a number of lakes (Skrzynka, Gowidlińskie, Węgorzyno, Żukowskie), and through hydroelectric power stations (Krzynia, Kondradowo). It is 138.6 km long; the surface area of the river's hydrological basin is about 1623 km<sup>2</sup>. The largest part of the catchment area is occupied by arable fields (62%) and forests (32%) (Wilamski 1978). The average water discharge of the river is 15.5 m<sup>3</sup> s<sup>-1</sup> and its slope is about 1.3%, which gives it a mountainous character. The Słupia River is inhabited by a diverse ichtiofauna, mainly the migratory trout, salmon, grayling, river trout, and rainbow trout. The Słupia River flows into the town of Słupsk from the south and is about 30-40 meters wide.

### ***Sampling***

The research area covered a 10 km section of the Słupia River within the city limits of Słupsk. Water samples were taken at monthly intervals from March 2004 to February 2005 at three sites: site 1 was located at 24 RKm of the river course below the sewage treatment plant near the village Włynkówko; site 2 was located at 31 RKm of the river course in the northern part of Słupsk; site 3 was located at 34 RKm of the river course in the southern part of the town, approximately 250 m above the fishpass (Fig. 1). Water samples were collected from the middle of the river at a depth of about 20 cm directly into sterile glass bottles. Samples were transported to the laboratory in an ice container at a temperature of about 7°C. The time between sample collection and analyses usually did not exceed 2-3 h.

### ***Bacteriological analyses***

In order to determine the number of heterotrophic bacteria (HB), the collected samples were diluted with sterile buffered water (pH 7.2) (Daubner 1967) to reach final concentrations of 10<sup>-2</sup> and 10<sup>-5</sup>. Subsequently, 0.2 cm<sup>3</sup> of these diluted samples were inoculated by the spread method in five parallel replicates on iron-peptone agar (IPA) medium (Ferrer et al. 1963). After 6 days

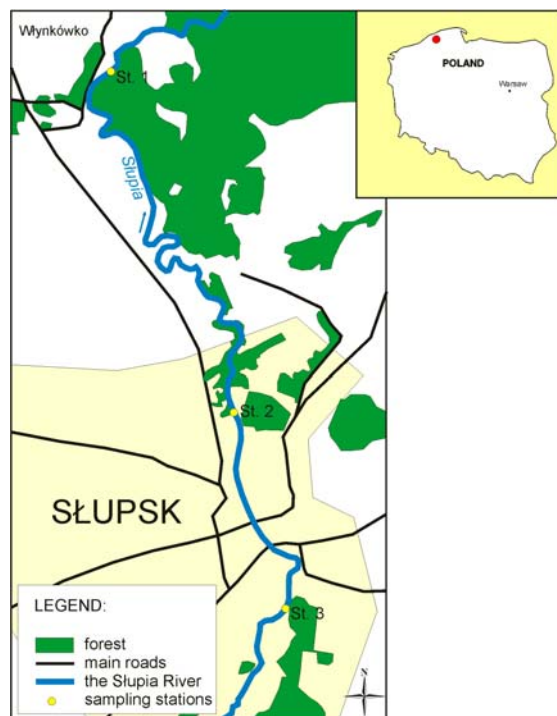


**Fig. 1.** Map of the study area and location of the sampling sites.

of incubation at 20°C, bacterial colonies were counted, and the results expressed as CFU were recalculated per 1 cm<sup>3</sup> of water.

The number of lipolytic bacteria (LB) was assayed in IPA medium enriched with 10 cm<sup>3</sup> of emulsified 50% tributyrin (Fluka) (Mudryk and Donderski 1997). After 6 days at 20°C, the presence of lipolytic bacteria was detected on the basis of clear zone formation around bacterial colonies.

### ***Estimation of lipase activity***

Quantitative measurements of potential lipase activity (LA) were carried out with the use of fluorescently labeled model substrate 4-methylumbelliferyl-butyrate (MUF-But, Sigma). MUF-But was dissolved in 10 cm<sup>3</sup> of methylcellosolve (ethyleneglycomonomethylether, EGME, C<sub>3</sub>H<sub>8</sub>O<sub>2</sub>) to a concentration of 10 mM dm<sup>-3</sup> and then stored at -20°C. Prior to the experiment, stock solutions were thawed and diluted in double distilled ultra pure water to the concentration of 1 mM dm<sup>-3</sup>.

Model fluorogenic substrate (50  $\mu\text{l}$  of a 20  $\mu\text{M}$  solution) was added directly to triplicate 2.5  $\text{cm}^3$  water samples in glass tubes. Incubation was performed in the dark for 1h with continuous shaking at *in situ* temperature. The addition of 100  $\mu\text{l}$  of a saturated solution of mercuric chloride (final concentration  $\sim 4$  mM) stopped the incubation. The substrate and  $\text{HgCl}_2$  were added to the control sample. An increase in the level of fluorescence caused by enzymatic cleavage of fluorogenic substrates was measured with a Hitachi spectrofluorometer T-2500 in 3  $\text{cm}^3$  quartz cuvettes. Excitation/emission wavelengths were centered at 318/445 nm, the optimum wavelengths for these measurements (Skórczewski et al. 1999). Fluorescence units were converted into the quantity of substrate released per unit time with the use of an internal standard curve (Mallet, Debros 1999). Before each experiment the procedure was calibrated by fluorescence reading of MUF standard solutions (20 nM – 1 mM) (Martinez et al. 1996; Hoppe, Ulrich 1999). In the results, readings from triplicate samples are given.

### Data analyses

Standard deviation SD, coefficient of variation CV, and coefficient of dispersion CD, were calculated according to Velji and Albright (1986). Simple linear regression was used to calculate the general correlation between studied parameters. Data on the number of heterotrophic bacteria, lipolytic bacteria, and lipase activity were natural-log transformed to equalize the variance. The significance of differences between sites and seasons in bacteriological parameters and enzymatic activities were assessed by a two-way ANOVA, according to Incera et al. (2003).

## RESULTS

The number of heterotrophic bacteria (HB) in the Słupia River oscillated between  $0.3 \times 10^3$  cells  $\text{cm}^{-3}$  and  $1180 \times 10^3$  cells  $\text{cm}^{-3}$  (Table 1). Their mean number from spring to fall remained at a relatively constant level ( $158.3 \times 10^3$  cells  $\text{cm}^{-3}$  –  $169.4 \times 10^3$  cells  $\text{cm}^{-3}$ ), whereas in winter a sudden drop to only  $18.3 \times 10^3$  cells  $\text{cm}^{-3}$  was observed.

The dynamics of the monthly changes in the numbers of heterotrophic bacteria in the Słupia River at the three study sites is given in Fig. 2A. These data indicate that during the annual study cycle the level of HB remained fairly constant, with the exception of a higher number of HB observed in March and June at site 1, and a rapid increase in bacterial density in September at site 2.

The results given in Table 1 indicate that the mean number of lipolytic bacteria (LB) reached a maximum in spring ( $12.9 \times 10^3$  cells  $\text{cm}^{-3}$ ), was high in

**Table 1**

Number of heterotrophic bacteria (HB) ( $10^3$  cells  $\text{cm}^{-3}$ ), lipolytic bacteria (LB) ( $10^3$  cells  $\text{cm}^{-3}$ ), and lipase activity (LA) ( $\text{nM MUF dm}^{-3} \text{ h}^{-1}$ ) in water of the Słupia River.

| Parameter | Season | Mean                    | Range        | SD    | CV %  | CD    |
|-----------|--------|-------------------------|--------------|-------|-------|-------|
| HB        | winter | 18.3                    | 0.3 - 56.7   | 17.4  | 95.3  | 16.6  |
|           | spring | 158.3                   | 42.7 - 600.0 | 185.3 | 117.0 | 216.7 |
|           | summer | 169.4                   | 50.0 - 333.0 | 110.2 | 65.1  | 71.7  |
|           | autumn | 158.7                   | 8.3 - 1180.0 | 383.3 | 241.5 | 925.7 |
| LB        | winter | 1.3 (7.1) <sup>a</sup>  | 0.04 - 5.7   | 1.8   | 137.1 | 2.5   |
|           | spring | 12.9 (8.1) <sup>a</sup> | 0.6 - 38.3   | 12.3  | 95.8  | 11.8  |
|           | summer | 10.5 (6.2) <sup>a</sup> | 1.5 - 33.3   | 9.7   | 92.5  | 9.0   |
|           | autumn | 7.5 (4.7) <sup>a</sup>  | 0.5 - 40.0   | 12.3  | 163.6 | 20.1  |
| LA        | winter | 11.0                    | 6.3 - 15.3   | 3.3   | 30.5  | 1.0   |
|           | spring | 18.9                    | 7.6 - 29.3   | 8.2   | 43.1  | 3.5   |
|           | summer | 14.2                    | 4.3 - 26.5   | 6.9   | 48.5  | 3.3   |
|           | autumn | 19.9                    | 8.5 - 42.6   | 12.1  | 60.8  | 7.3   |

<sup>a</sup> percentage of lipolytic bacteria in the number of heterotrophic bacteria

summer ( $10.5 \times 10^3$  cells  $\text{cm}^{-3}$ ), and reached a minimum in winter ( $1.3 \times 10^3$  cells  $\text{cm}^{-3}$ ). Lipolytic bacteria accounted for only 4.7 to 8.1% of the total number of heterotrophic bacteria.

The numbers of LB during the annual study cycle remained relatively constant at all study sites (Fig. 2B). The only exceptions are the increased numbers of lipolytic bacteria observed in March at all study sites, and in August and September at site 2.

Data concerning seasonal changes in the level of the activity of lipases (LA) in the water of the Słupia River are given in Table 1. The highest mean activity of lipases was observed in spring ( $18.9 \text{ nM MUF dm}^{-3} \text{ h}^{-1}$ ) and in fall ( $19.9 \text{ nM MUF dm}^{-3} \text{ h}^{-1}$ ). The lowest mean level of the activity of those enzymes was observed in winter ( $11.0 \text{ nM MUF dm}^{-3} \text{ h}^{-1}$ ). As can be seen in Figure 2C, there were no big differences in the activity of lipolytic enzymes between sites. At all study sites, the highest level of the activity of those enzymes was observed in September.

Linear regression of the studied bacteriological parameters and the activity of lipases showed that there was a significant correlation between the numbers of heterotrophic bacteria and lipolytic bacteria ( $R=0.14$ ,  $p<0.05$ ), and lipase activity and numbers of lipolytic bacteria ( $R^2=0.23$ ,  $p<0.01$ ). No significant correlation between numbers of heterotrophic bacteria and lipase activity was noted (Fig. 3).

Grouping the results by seasons and sites, a factorial ANOVA test was carried out for the number of heterotrophic and lipolytic bacteria, and for lipase activity (Table 2). No significant differences in the studied parameters among grouped data using two-way ANOVA were shown.

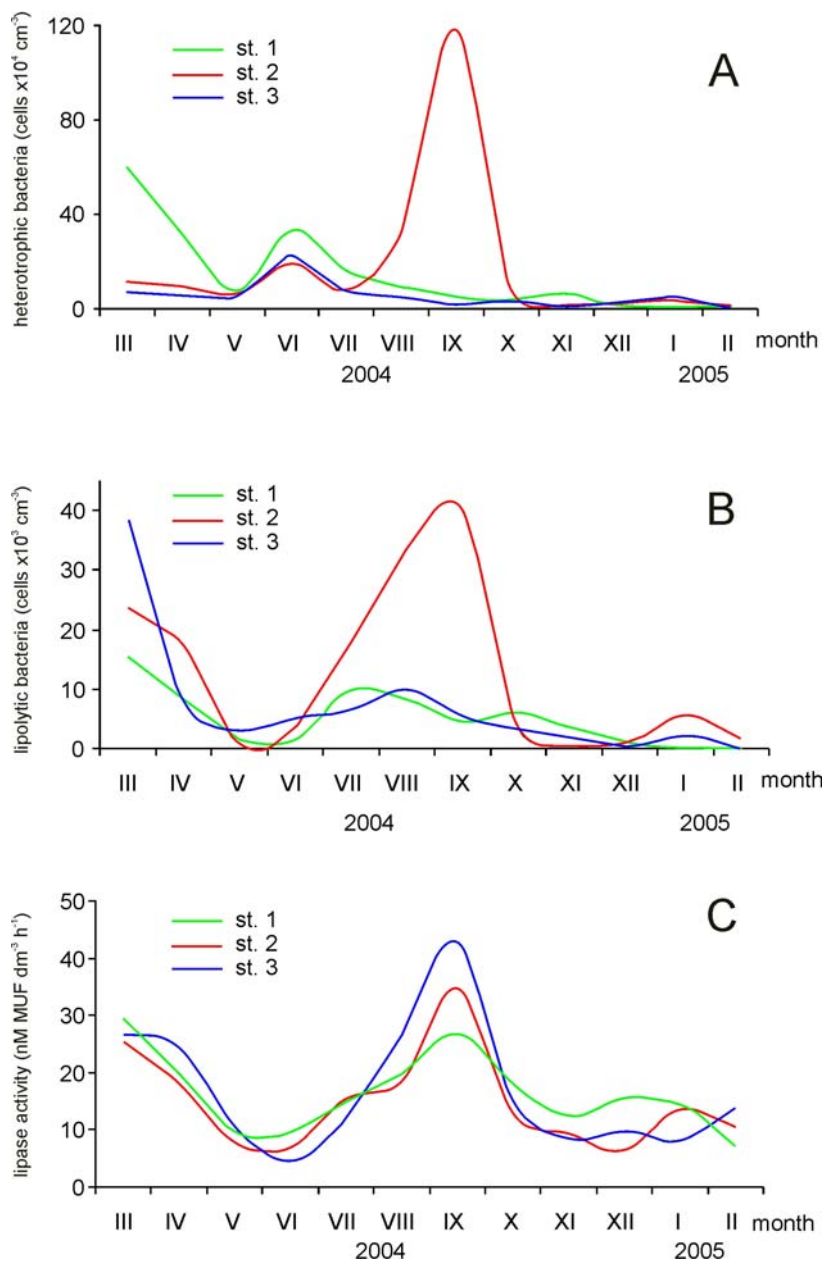


**Fig. 2.** Temporal variation of number of heterotrophic bacteria (A), lipolytic bacteria (B) and extracellular lipolytic activity (C) in the Słupia River.

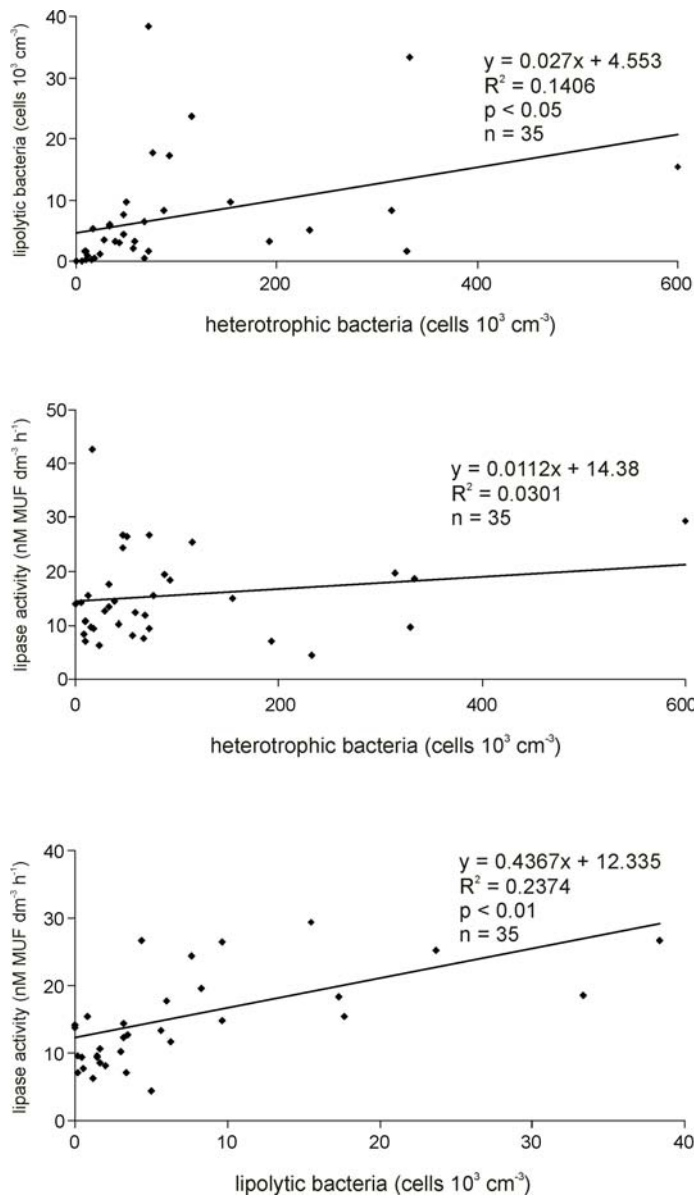


**Fig. 3.** Correlation between studied parameters in the Słupia River. (The solid line represents linear regression including all data.)

**Table 2**

Analyses of 2-way - ANOVA of variance in the number of heterotrophic bacteria (HB), lipolytic bacteria (LB), and lipase activity (LA) due to site and season.

| Parameter | Source of variation | df | MS      | F   | p     |
|-----------|---------------------|----|---------|-----|-------|
| HB        | site                | 2  | 53938.3 | 1.2 | 0.320 |
|           | season              | 2  | 48005.6 | 1.1 | 0.383 |
|           | site x season       | 5  | 57947.9 | 1.3 | 0.302 |
| LB        | site                | 2  | 179.1   | 1.6 | 0.213 |
|           | season              | 2  | 226.4   | 2.1 | 0.129 |
|           | site x season       | 5  | 40.2    | 0.4 | 0.891 |
| LA        | site                | 2  | 10.5    | 0.1 | 0.889 |
|           | season              | 2  | 157.1   | 1.8 | 0.179 |
|           | site x season       | 5  | 3.8     | 0.0 | 1.000 |

df - degrees of freedom

MS - mean squares

F - Fisher test

p- significance level

## DISCUSSION

In running water ecosystems, a large part of the processes of the degradation and transformation of organic matter is mediated exclusively by heterotrophic bacteria using specific enzymatic systems (Liu et al. 2004, Wilczek et al. 2004). Hence the enrichment of river water with dissolved and particulate organic matter, both autochthonous and allochthonous in origin, is reflected in the number of heterotrophic bacteria (Rulik, Spacil 2004). According to Tryland and Fiksdal (1998), the numbers of heterotrophic bacteria in water can be considered a useful general indicator of water quality.

The numbers of heterotrophic bacteria in the water of the Słupia River oscillated between  $0.3 \times 10^3$  cells  $\text{cm}^{-3}$  and  $1180 \times 10^3$  cells  $\text{cm}^{-3}$ . A similar number of heterotrophic bacteria ( $66.6 \times 10^3$  cells  $\text{cm}^{-3}$  –  $1427 \times 10^3$  cells  $\text{cm}^{-3}$ ) was determined by Małecka and Donderski (2006) in the Brda River. In the Słupia River during the whole vegetation season (spring, summer and fall), the number of heterotrophic bacteria remained at a constant high level, while in winter it dropped drastically. This indicates that the number of heterotrophic bacteria in the Słupia River was regulated by such environmental factors as availability of organic matter or water temperature. Substrates available to heterotrophic bacteria consist mainly of the products of photosynthesis. Substrate transfer from primary to secondary producers in aquatic communities is mediated by algal cell lysis or by exudates from live algae (Coveney, Wetzel 1989; Jones, Lock 1993). Algal exudates are organic sources easily assimilable by heterotrophic bacteria (Romani, Sabater 2000). As during the vegetation season phytoplankton proliferates in the Słupia River, algal exudates can be an

important stimulator of the growth of heterotrophic bacteria. Decrease in the number of planktonic bacteria in the Słupia River in winter is most probably related to a low concentration of organic matter and low temperature of the water.

Heterotrophic bacteria inhabiting water ecosystems do not represent a homogeneous group of organisms. On the contrary, they are a population of various physiological groups displaying different enzymatic activities (Krstulović, Solić 1988). Recent research (Chróst, Gajewski 1995; Martinez et al. 1996; Dachs et al. 1998; Podgórska, Mudryk 2003; Mudryk, Skórczewski 2004; Małecka, Donderski 2006) points to the fact that lipolytic bacteria constitute a very numerous physiological group in marine, estuarine and freshwaters basins.

Earlier studies of the water of the Słupia River (Mudryk 1981) have shown that bacteria hydrolyzing lipids were numerous and constituted 15-55% of all heterotrophic bacteria. These results, obtained nearly 25 years ago do not correspond with the data presented in this study. Presently, only 5-8% of bacteria have lipolytic properties. Similarly, Wu (1993) has determined that lipolytic bacteria in the Tansui River were not numerous and constituted only 12% of all bacteria. Possibly, the marked decrease in the number of lipolytic bacteria in the water of the Słupia River over 25 years can be related to a drastic decrease in the concentration of lipids, caused most probably by changes in the biocoenotical structure of this ecosystem or a decrease in the inflow of allochthonous lipids.

The intensity of decomposition of lipids in water basins is usually determined not only by the number of heterotrophic bacteria capable of decomposing lipids, but also by the actual activity of lipolytic enzymes (Mudryk, Skórczewski 2000). Boetius (1995), Boschker and Cappenberg (1998), and Mudryk and Skórczewski (2004) draw attention to the high level of activity of lipases in aquatic environments. Those hydrolases are capable of attacking emulsified mono-, di- and triglycerides, and of splitting them with the yield of glycerol and fatty acid residues (Gajewski et al. 1997). Tryland and Fiksdal (1998) indicated that an important portion of the measured activity of lipases in river water was brought about by aerobic and anaerobic bacteria. The mean lipase activity of the aerobic isolates from a river is  $5 \times 10^{-11} \mu\text{M MUF min}^{-1}$  and anaerobic isolates  $3 \times 10^{-13} \mu\text{M MUF min}^{-1}$ .

The activity of lipases in the water of the Słupia River was low as compared to other rivers (Jones, Lock 1993; Boon 1990; Foreman et al. 1998). This low activity of lipid-hydrolyzing enzymes might be due to the fact that in the study area only 5 - 8% of heterotrophic bacteria had lipolytic properties. Chróst and Gajewski (1995) draw attention to the fact that lipolytic enzymes are actively exported mainly by heterotrophic bacteria or are released as free enzymes after

the lysis of their cells. Additionally, according to Brown and Goulder (1999), in shallow rivers, i.e. also in the Słupia River, most extracellular enzyme activity may occur on submerged stone surfaces rather than in overlying water.

It has been shown that concentrations of lipids and activities of lipases in water bodies fluctuate strongly with season (Kattner, Brokman 1990; Gajewski et al. 1997). In the water of the Słupia River, the maxima of the activity of lipases were observed in spring and fall. Similar results were obtained by Foreman et al. (1998) in the Maumee River (northwest Ohio) and Boon (1990) in the Kiewa River (south-eastern Australia). The higher activity of lipases observed in spring in the Słupia River could have resulted from the considerable amounts of allochthonous lipids flowing into the water together with the snow melt, while an increased level of the activity of lipases in fall could have been related to heavy rainfall and increased mortality of phytoplankton and zooplankton which brings considerable amounts of lipids into the water (Arts et al. 1992). These lipids generate the activity of lipases.

## REFERENCE

- Albers C.S., Kattner G., Hagen W., 1996, *The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptation*, Mar. Chem., 55: 347-58
- Arts M.T., Evans M.S., Robarts R.D., 1992, *Seasonal patterns of total and energy reserve lipids of dominant zooplanktonic crustaceans from hyper-eutrophic lake*, Oecologia 90: 560-71
- Boetius A., 1995, *Microbial hydrolytic enzyme activities in deep-sea sediments*, Hel. Meer., 49: 177- 86
- Boon P.I., 1990, *Organic matter degradation and nutrient regeneration in Australian freshwater: II Spatial and temporal variation and relation with environmental conditions*, Arch. Hydrobiol., 117: 405-36
- Boschker H.T.S., Cappenberg T.E., 1998, *Patterns of extracellular enzyme activities in littoral sediments of Lake Gooimeer, The Netherlands*, FEMS Microbiol. Ecol., 25: 79-86
- Brown S.E., Goulder R., 1999, *Change in riverine epilithic extracellular enzyme activity in response to fish farm effluent*, Lett. App. Microbiol., 29: 385-88
- Chróst R.J., 1991, *Environmental control of the synthesis and activity of aquatic microbial ectoenzymes*, [in:] *Microbial enzymes in aquatic environments*, Ed. (Chróst, R.J.). Springer Verlag, New York, pp. 29-59
- Chróst R., Gajewski A.J., 1995, *Microbial utilization of lipids in lake water*, FEMS Microbiol. Ecol., 18: 45-50
- Coveney M.F., Wetzel R.G., 1989, *Bacterial metabolism of algal extracellular carbon*, Hydrobiologia, 173: 141-49
- Dachs J., Bayona J.M., Fowler S.W., Miquel J.C., Albaiges J., 1998, *Evidence for cyanobacterial inputs and heterotrophic alternation of lipids in sinking particles in the Alboran Sea (SW Mediterranean)*, Mar. Chem., 60:189-201
- Daubner J., 1967, *Mikrobiologia Vody*, Slov. Akad. Vied., Bratislava, pp 345.
- Ferrer E.B., Stapert E.M., Sokalski W.T., 1963, *A medium for improved recovery of bacteria from water*, Can. J. Microbiol., 9: 420 - 22

- Foreman C.M., Franchini P., Sinsabaugh R.L., 1998, *The trophic dynamics of riverine bacterioplankton: Relationships among substrate availability, ectoenzyme kinetics, and growth*, Limnol. Oceanogr., 43: 1344-52.
- Gajewski A., Chróst R., Siuda W., 1993, *Bacterial lipolytic activity in a eutrophic lake*, Arch. Hydrobiol., 128: 107-26
- Gajewski A., Kirschner A.K.T., Velimirov B., 1997, *Bacterial lipolytic activity in a hypertrophic dead arm of the river Danube in Vienna*, Hydrobiologia, 344: 1-10
- Hoppe HG., Ulrich S., 1999, *Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone*, Aqua. Microbial Ecol., 19: 139 - 48
- Incera M., Cividanes S.P., Lastra M., López J., 2003, *Temporal and spatial variability of sedimentary organic matter in sandy beaches on the northwest coast of the Iberian Peninsula*, Estuar. Coast. Shelf Sci., 58S: 55-61
- Jaffe R., Wolf G.A., Cabrera A.C., Chity H.C., 1995, *The biogeochemistry of lipids in rivers of the Orinoco Basin*, Geoch. Cosm. Acta., 59: 4507-22.
- Jones S.F., Lock M.A., 1993, *Seasonal determination of extracellular hydrolytic activities in heterotrophic and mixed heterotrophic/autotrophic biofilms from two contrasting river*, Hydrobiologia, 257: 1-16
- Kattner B., Brockman U.H., 1990, *Particulate and dissolved fatty acids in an enclosure containing a unialgal Skeletonema costatum (Greve.) Cleve culture*, J. Exp. Biol. Ecol, 141: 1-13
- Krstulović N., Solić M., 1988, *Distribution of proteolytic, amylolytic and lipolytic bacteria in the Kastela Bay*, Acta Adria, 29: 75-83
- Liu H., Dagg M., Campell L., Urban-Rich J., 2004, *Picophytoplankton and bacterioplankton in the Mississippi River plume and its adjacent waters*, Estuaries, 27: 147-56
- Mallet C., Debroas D., 1999, *Relations between organic matter and bacterial proteolytic activity in sediment surface layers of a eutrophic lake (Lake Aydat, Puy de Dôme, France)*, Arch. Hydrobiol., 145: 39-56
- Małecka M., Donderski W., 2006, *Heterotrophic bacteria inhabiting water of the river Brda on the Bydgoszcz town section*, Balt. Coast. Zone, 10: 31-46
- Martinez J., Smith D.C., Steward D.F., Azam F., 1996, *Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea*, Aqua. Microbial Ecol., 10: 223-30
- Marty Y., Quemeneur M., Aminot A., Corre P., 1996, *Laboratory study on degradation of fatty acids and sterols from urban wastes in seawater*, Wat. Res., 30: 1127-36
- Mudryk Z., 1981, *The occurrence physiological groups between bacteria isolated from water Ślupia River*, Ślup. Prace Mat-Prz., 2: 113-24 (in Polish)
- Mudryk Z., Donderski W., 1997, *The occurrence of heterotrophic bacteria decomposing some macromolecular compounds in shallow estuarine lakes*, Hydrobiologia, 342/343: 71-8
- Mudryk Z., Skórczewski P., 2000, *Occurrence and activity of lipolytic bacterioneuston and bacterioplankton in the estuarine lake Gardno*, Estuar. Coast. Shelf Sci., 51: 763-72
- Mudryk Z., Skórczewski P., 2004, *Extracellular enzyme activity at the air- water interface of an estuarine lake*, Estuar. Coast. Shelf Sci., 59: 59-67
- Parrish Ch.C., 1988, *Dissolved and particulate marine lipid classes: A review*, Mar. Chem., 23: 17-40
- Podgórska B., Mudryk Z., 2003, *Distribution and enzymatic activity of heterotrophic bacteria decomposing selected macromolecular compounds in a Baltic Sea beach*, Estuar. Coast. Shelf Sci., 56: 539-46
- Prim N., Sanchez M., Ruiz C., Pastor J.F. Diaz P., 2003, *Use of methylumbelliferyl-derivative substrates for lipase activity characterisation*, J. Mol. Cat., 22: 339-46

- Reemtsma T., Haake B., Ittekkot V., Nair R. R., Brockmann U. H., 1990, *Downward flux of particulate fatty acids in the Central Arabian Sea*, Mar. Chem., 29: 277-99
- Romani A.M., Sabator S., 2000, *Influence of algae on extracellular enzyme activity in river biofilm*, Micro. Ecol., 41: 16-24
- Rulik M. Spacil R., 2004, *Extracellular enzyme activity within hyporheic sediments of a small lowland stream*, Soil Biol. Bioch., 36: 1653-62
- Skórczewski P., Mudryk Z., Kukliński B., 1999, *Optimisation of measurement enzyme activity using fluorogenic substrates in water*, Balt. Coast. Zone, 3: 41-52
- Tyrland I., Fiksdal L., 1998, *Rapid enzymatic of heterotrophic activity of environmental bacteria*, Wat. Sci. Tech., 38: 95-01
- Velji M.J., Albright J., 1986, *Microscopic enumeration of attached marine bacteria of seawater, marine sediment, faecal matter and kelp blade samples following pyrophosphate and ultrasound treatments*, Can. J. Microbiol., 32: 121-26
- Wiliamski J., 1978, *Chemical erosion of catchment Słupia River*, IMGW Słupsk (in Polish)
- Wilczek S., Fischer H., Brunke M., Pusch M.T., 2004, *Microbial activity within a subaqueous dune in a large lowland river (River Elbe, Germany)*, Aqua. Micro. Ecol., 36: 83-97
- Wu R.Y., 1993, *Studies on the microbial ecology of the Tansui Estuary*, Bot. Bull. Acad. Sin., 34: 13-30