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ORIGINAL ARTICLE

Extracellular hydrolytic enzyme activity in the water from the ponds of a carp farm

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Abstract
The hydrolytic activity of four classes of extracellular enzymes was determined in the surface water in three fish farm ponds. These water basins were inhabited by different developmental stages of carp. The ranking order of the mean enzyme activity rates in the studied water basins was as follows: aminopeptidase > lipase > β-glucosidase > α-glucosidase. The level of activity of the tested enzymes differed among the studied ponds. Each hydrolase had its characteristic horizontal profile of activity. The level of extracellular enzyme activity characterised dynamic influenced by the seasons.

Keywords Ponds · Fish farm · Enzyme activity

Introduction

Fish farms, which nowadays account for 47% of the global food fish supply are usually highly eutrophic water basins in which large amounts of heterogeneous complex mixture of both particulate (POM) and dissolved (DOM) organic matter are accumulated (Vezzulli et al. 2002; Mancuso 2013; Caruso 2014). The large quantities of organic matter in these aquacultures occur mainly in the form of food residues and faecal plates of cultured fish (Caruso 2003; Sakami et al. 2005). Other significant sources of organic matter in the water of fish farms are exudates from benthic meiofauna, macrofauna, primary phytoplankton photosynthetic production extracted from macrophytes and also of terrestrial origin (Song et al. 2018). For biological balance and optimal functioning of aquaculture areas all accumulated autochthonic and allochtonic organic matter have to be degraded to inorganic nutrients (Salazar et al. 2016; Franzo et al. 2019). In aquatic ecosystems heterotrophic prokaryote community plays a key role in the decomposition, transformation and mineralization of organic compounds to inorganic nutrients and CO_2 (Giraldo et al. 2014; Li et al. 2019; Zaccone et al. 2019). According to several studies (Caruso et al. 2013; Baltar et al. 2016; Song et al. 2018) on heterotrophic prokaryotes, heterotrophic bacteria are main consumers of dissolved and particulate organic matter. Due to their small size, high biomass, biochemical diversity and high physiological activity and therefore they are the major players in organic matter turnover within the microbial loop. These organisms are able to react quickly to the inflow of organic matter through the synthesis of hydrolytic extracellular enzymes, which activity is stimulated or inhibited by the trophic level of the aquatic environment (Yamada et al. 2012; Giraldo et al. 2014; Song et al. 2018). Many heterotrophic bacteria by their rapid growth and high physiological diversity are known to carry genetic and metabolic potentials to synthesise and regulate activity of a wide range of extracellular enzymes, mainly hydrolases with precise substrate specificities, which can degrade and modify a large variety of organic matter in water basins (Baltar et al. 2010; Cunha et al. 2010; Kalwasińska and Swiontek – Brzezińska 2013; Caruso 2015). In aquatic ecosystems more than 90% of organic matter is accumulated in the form of chemically complex polymers and very diverse structures (Baltar et al. 2009; Zaccone et al. 2014). According to (Zaccone et al. 2014; Franzo et al. 2019) among these organic polymers the most representative are proteins, polysaccharides and lipids. All these polymers are too large (HMW >1000 Da) to be direct transported across the cytoplasmic bacterial membrane (Baltar et al. 2016; Salazar et al. 2016). Before incorporation they must be initially hydrolysed by

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extracellular enzymes into small assimilable molecules (<600 Da), mainly monomers such as amino acids, monosaccharides and fatty acids (Celussi et al. 2015; Franzo et al. 2019; Zaccone and Caruso 2019). In that form they may easily diffuse through permeases into the periplasmic space and then be used to meet bacterial energy requirements and to build up their biomass (Yamada et al. 2012; Steen and Arnosti 2013; Zaccone et al. 2014).

The results of many studies (Orsi et al. 2018; Li et al. 2019; Zaccone et al. 2019) showed that bacterial extracellular hydrolytic enzyme activity was recognized to be a fundamental step in the bacterial decomposition, turnover and utilization of biopolymers in all aquatic ecosystems. Hence, most of the previous studies on extracellular enzyme activity in aquatic environments focused on the marine environment (Baltar et al. 2009; Perliński et al., 2018), coastal zone (Astel et al. 2018; Zdanowicz et al. 2018), estuaries (Mudryk and Skórczewski 2004) and freshwater basins (Kalwasińska and Swiontek-Brzezińska 2013). However few such studies (Brown and Goulder 1996; Sakami et al. 2005) are available for aquaculture and our knowledge of the influence of fish farming on extracellular enzyme activity in the water of these specific aquatic basins is still incomplete. Therefore, the aim of this study was to determine (1) the level of extracellular enzyme activity in three ponds inhabited by different developmental stages of carp, (2) variation in enzymatic activity of four hydrolases along the horizontal profile of the studied water basins, (3) seasonal dynamics of the hydrolytic activity of extracellular enzymes in the ponds of a carp farm.

Material and methods

Study area and sampling

This study was carried out in three ponds inhabited by various developmental stages of carp, i.e., fries, fingerlings and adults, located in Wiklino (North Poland) (Fig. 1). All studied water basins are situated in the depression surrounded by arable land. Basic data on morphological, physicochemical and bacteriological characteristics of the studied ponds are presented in Table 1. A common feature of all these ponds is the cycle of filling and emptying these reservoirs with the Brodniczka River water during the period of its excess or deficit used in traditional carp farming. Fish in the studied ponds were adequately fed with commercial pelleted feed. Biochemical composition of fish food mainly based on protein rich diet. Water samples were collected from each pond at three sites (Fig. 1) in the spring, summer and autumn seasons in 2013:

site 1 - located in the zone near the river water inflow,
site 2 – situated in the central part of the pond,
site 3 – lies in the zone near the water outflow.

Fig. 1 Location of the sampling sites

Table 1 Basic data on morphological, physicochemical and bacteriological characteristics of the studied ponds

Parameters		Fry pond	Fignerling pond	Adult pond
Pond type		artificial	artificial	natural
Area		0.5 ha	3 ha	3 ha
Average depth		0.7 m	0.8 m	0.9 m
Temperature	spring	17.2	17.3	16.8
	summer	22.0	22.7	20.3
	autumn	9.3	9.7	8.7
pH	spring	7.2	7.7	7.7
	summer	7.8	7.5	7.8
	autumn	7.8	7.7	7.8
Heterotrophic psychrophilic bacteria number (10^3 CFU·cm^{-3})		51.14	28.39	21.56
Heterotrophic mesophilic bacteria number (10^3 CFU·cm^{-3})		4.75	11.53	8.39

Collected water samples were obtained from the depth of about 15 cm below the water surface directly into sterile glass bottles. Water samples were stored in an box whit ice, where the temperature did not exceed +7 °C, and immediately transported to the laboratory. Enzymatic activity measurement as a rule were conducted within 2–4 h from the sample collection time. In collected water samples we determine the number of heterotrophic bacteria (colony forming units - CFU), For this purpose collected samples were diluted with sterile buffered water to final concentrations of 10^{-2} and 10^{-3}. Subsequently, 0.2 mL of those diluted samples were inoculated on agar nutrient medium in three replications using the spread method. After 72 h incubation at 20 °C, psychrophilic bacteria and after 48 h incubation at 37 °C mesophilic bacteria were counted and the results expressed as CFU were recalculated per 1 mL of water.

Estimation of enzyme activity

The spectrofluorometric method was used to measure the level of extracellular enzyme activity (EEA) in the water of three studied ponds. It is a widespread technique used to determine this parameter in aquatic ecosystems (Cunha et al. 2010; Yamada et al. 2012; Zaccone and Caruso 2019). Qualitative measurements of EEA in collected water samples were carried out using fluorescently labelled model substrates according to Hoppe (1983) and Perliński and Mudryk (2018). In order to determine the activity spectrum of four classes of hydrolytic enzymes: α-D-glucosidase (EC 3.2.1.20), β–D-glucosidase (EC 3.2.2.21), lipase (EC 3.1.1.3) and aminopeptidase (EC 3.4.1.1), the following methyl-umbelliferyl substrates (MUF and MCA derivatives) were used: MUF-α-D-glucoside, MUF-β-D-glucoside, MUF-butyrynate and MCA- leucine (L-leucine-4 methylcoumarinyl - 7 amide). All MUF and MCA substrates were purchased from Sigma and Fluka Chemical Company. MUF and MCA substrates were dissolved in 10 mL of methylcellosolve (ethylene glycol monomethyl ether, EGME, C3H8020, Sigma) to a concentration of 10 mM L and then stored at −20 °C. Prior to the experiment, stock solutions were thawed and diluted in double distilled ultra pure water to a final concentration of 0.05 μM, 0.2 μM, 0.5 μM, 2 μM, 10 μM, 50 μM. Calibration was performed with known concentrations of MUF and MCA standards (Song et al. 2018). 3.9 mL of the water samples of and 0.1 mL of the substrate of the appropriate concentration were transferred into quartz cuvettes, which were placed in a Hitachi spectrofluorometer, model T-2500 with FL Solution software. The samples were incubated in the dark continuous shaking at in situ temperature. The fluorescence released by enzymatic cleavage of the artificial substrates was measured fluorometrically, in triplicate, at excitation/emission wavelengths of 320/440 nm for MUF, and 345/440 nm for MCA. The first reading was done immediately after the substrate was introduced into the analysed water sample (zero time). Subsequent readings were carried out after 30 and 90 min for aminopeptidase and lipase and after 60 min and 24 h for α-glucosidase and β-glucosidase. The increase in fluorescence units during the incubation time was converted into hydrolytic activity with a standard curve of the end product of the reaction of MUF and MCA substrates (Sala et al. 2005; Yamada et al. 2012). ENZFITTER software, version 1.05 was applied to determine the maximum rate of enzymatic reaction (Vmax). The results were converted using a Lineaweaver–Burke linear transformation and expressed in M MCA/MUF·$L \cdot h^{-1}$ (Caruso et al. 2003).

Statistic analysis

Statistical tests (standard deviation - SD, coefficient of variation- CV, coefficient of dispersion - CD) were calculated using Statistica 9.0 software. Relationship among enzymatic activity in studied ponds were examined using no-nparametic Sperman's correlation coefficients. The significance of different between ponds, seasons and sites in level enzymatic

activity was assessed using Kruskal-Wallis non-parametric test according to Incera et al. (2003).

Results

Extracellular enzyme activity (EEA) in the water of the studied ponds was presented in Table 2. In all studied ponds, out of four determinate enzymes, aminopeptidase showed the highest EEA (35.97–79.51 nM MCA· L · h^{-1}). Lipase was also fairly active (3.71–5.35 nM MUF nM · L · h^{-1}), yet its activity was about 15-fold lower compared to aminopeptidase. β-glucosidase showed the lowest level of potential activity, with the mean activity equal to 0.15–0.83 nM MUF nM · L · h^{-1}, which was about 140-fold lower than the activity of aminopeptidase. The pattern of EEA in the water of the studied ponds was as follows: aminopeptidase > lipase > α- glucosidase > β-glucosidase, with mean ratio of about 141:96:15:1. According to the data presented in Table 2, the level of activity of the tested enzymes differed among the studied water basins. The highest activity level of all studied hydrolases was noted in fry pond and the lowest in fingerling pond.

Figure 2 presents the variability in the level of activity of bacterial enzymes at the sampling sites in the studied ponds. These data shows that each enzyme had its characteristic horizontal profile of activity. The maximum level of aminopeptidase activity in the water samples collected from all studied ponds was noted in the zone near the river water outflow (site 3). The highest level of lipase activity in fry and fingerling ponds was noted at site 3, while in adult pond at site 1 (near the river water inflow) and site 2 (central part of the pond). The maximum level of α- glucosidase activity was noted in fry pond at site 3, in fingerling pond at site 1 and in adult pond at site 1 and 2. According to the results of the present study, the highest level of activity of β-glucosidase in fry and adult ponds was recorded in the water collected from site 1, while in fingerling pond - from site 2. The maximum level of α- glucosidase activity in the water fry pond was noted in site 2, in fingerling pond in site 2 and 3 and adult pond in site 1 and 2. According to the present data, the highest level activity of β-glucosidase in fry ponds was recorded in the water collected from sites 2, while in fingerling and adult pond was recorded from site 1.

Data presented in Fig. 3 showed that in different seasons the level EEA trends were rather irregular. The maximum activity of aminopeptidase in fry pond was noted in autumn, while in fingerling pond in summer and in adult pond in the spring season. The highest level of activity of enzymes hydrolysing lipids in all three studied ponds was recorded in summer. α-glucosidase and β-glucosidase activity in fry pond increased from spring till autumn. In fingerling ponds the maximum level of activity of these hydrolases was recorded in autumn and in adult ponds in summer.

The relationships among activity of tested extracellular enzymes in studied ponds are given as the correlation matrix in Table 3. In all studied water basins we noted significant ($\alpha \leq 0.5$, $\alpha \leq 0.01$) positive correlation between activity of α-glucosidase and β-glucosidase (r = 0.77–0.83). Also positive correlation ($\alpha \leq 0.05$) in fry pond was found between activity of β-glucosidase and aminopeptidase (r = 0.62) and in fingering pond between lipase and aminopeptidase (r = 0.76).

From the linear regression we found significant ($R^2 = 0.47$, $p \leq 0.001$) correlation between level activity of lipase and water temperature (Fig. 4). It did not show any correlation between temperature and level activity of aminopeptidase, α-glucosidase and β-glucosidase.

By grouping the results by the ponds, seasons and sites using Kruskal-Wallis test was carried out to detect significant

Table 2 Spectrum of selected extracellular enzymatic activities [nM · dm^{-3} · h^{-1}] in the water studied ponds (average from the pooled data of all season and sites)

Pond	Enzyme	Mean	Range	SD	CV(%)	CD
Fry	aminopeptidase	79.51	26.5–181.50	43.27	54.43	23.55
	lipase	5.35	2.62–10.18	2.78	51.87	1.44
	α-glucosidase	1.44	0.10–4.29	1.70	117.81	2.00
	β-glucosidase	0.82	0.00–1.56	0.79	96.05	0.76
Fingerling	aminopeptidase	35.97	0.18–60.72	17.00	47.23	8.02
	lipase	3.71	2.02–6.86	1.92	51.79	1.00
	α-glucosidase	0.09	0.02–0.20	0.07	78.15	0.05
	β-glucosidase	0.16	0.01–0.78	0.24	151.49	0.37
Adult	aminopeptidase	44.87	8.90–119.5	31.54	70.28	22.17
	lipase	4.83	2.17–9.62	2.77	57.48	1.60
	α-glucosidase	0.21	0.06–0.63	0.21	101.88	0.22
	β-glucosidase	0.15	0.01–0.39	0.12	80.28	0.10

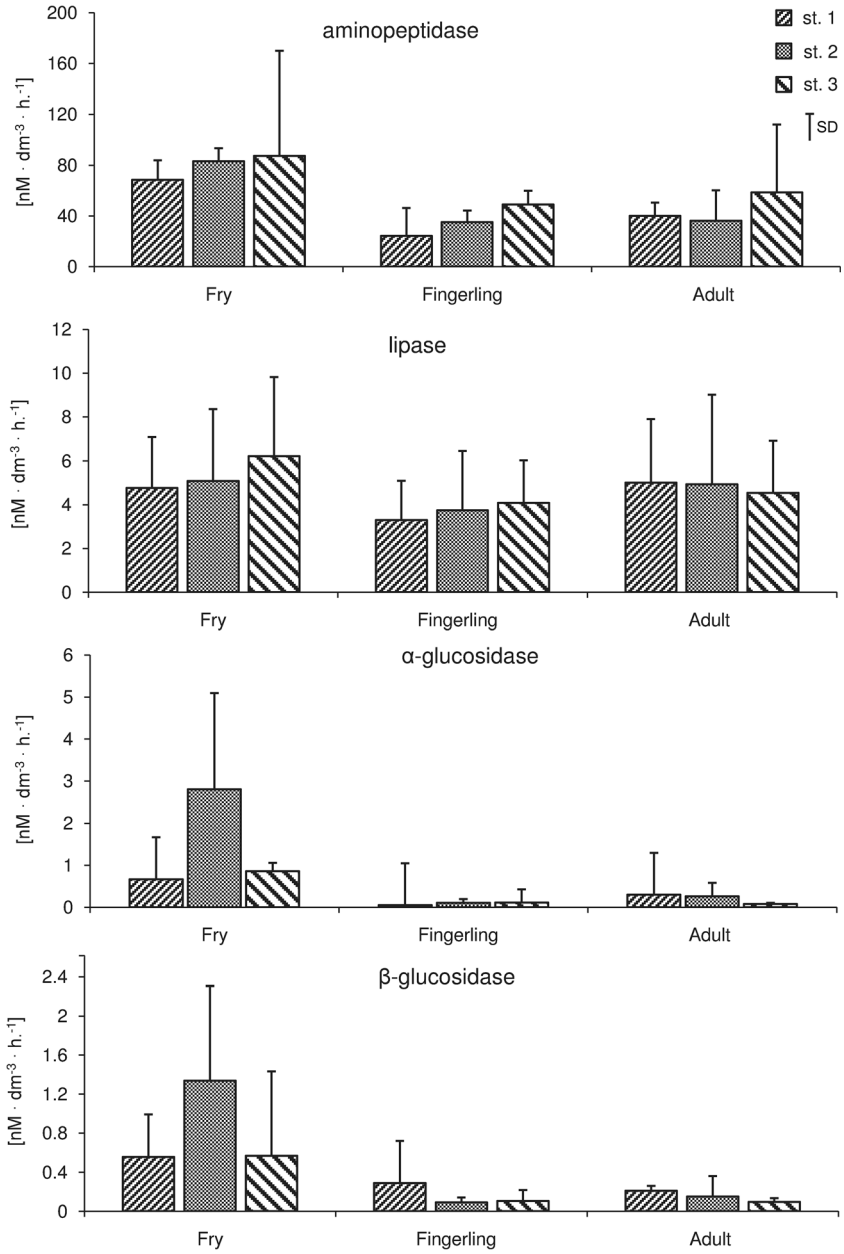

Fig. 2 Spatial variations of potential bacterial enzymatic activity in different parts of studied ponds. Each bar show mean enzymatic activity all seasons. Vertical lines represented ± SD ($n = 27$)

differences in level studied enzymatic activity (Table 4). There were significant differences in the level activity of α-glucosidase, β-glucosidase and aminopeptidase between ponds and seasons. Also significant differences in the level lipase activity found between seasons, ponds and seasons, seasons and sites.

Discussion

As a key process in microbial ecology, enzymatic activity has received considerable attention because this parameter can be a good indicator of bioavailability of nutrients for microbial community which is necessary to understand the dynamics of the carbon and nitrogen cycle in aquatic ecosystems (Tiquia 2011; Caruso 2016; Zaccone et al. 2019). Enzymatic hydrolysis of polymeric compounds by microorganisms is the first critical step in the decomposition, transformation and mineralization of organic matter in all water basins (Zaccone et al. 2012; Caruso 2014). According to Sakami et al., (2005) the measure level of extracellular hydrolytic enzyme activity would be useful to characterize the decomposition intensity of high load of organic matter accumulated in aquaculture, mainly in fish farms. The most comprehensive studies (Cunha et al. 2010; Franzo et al. 2019; Ziccone and Caruso 2019) on

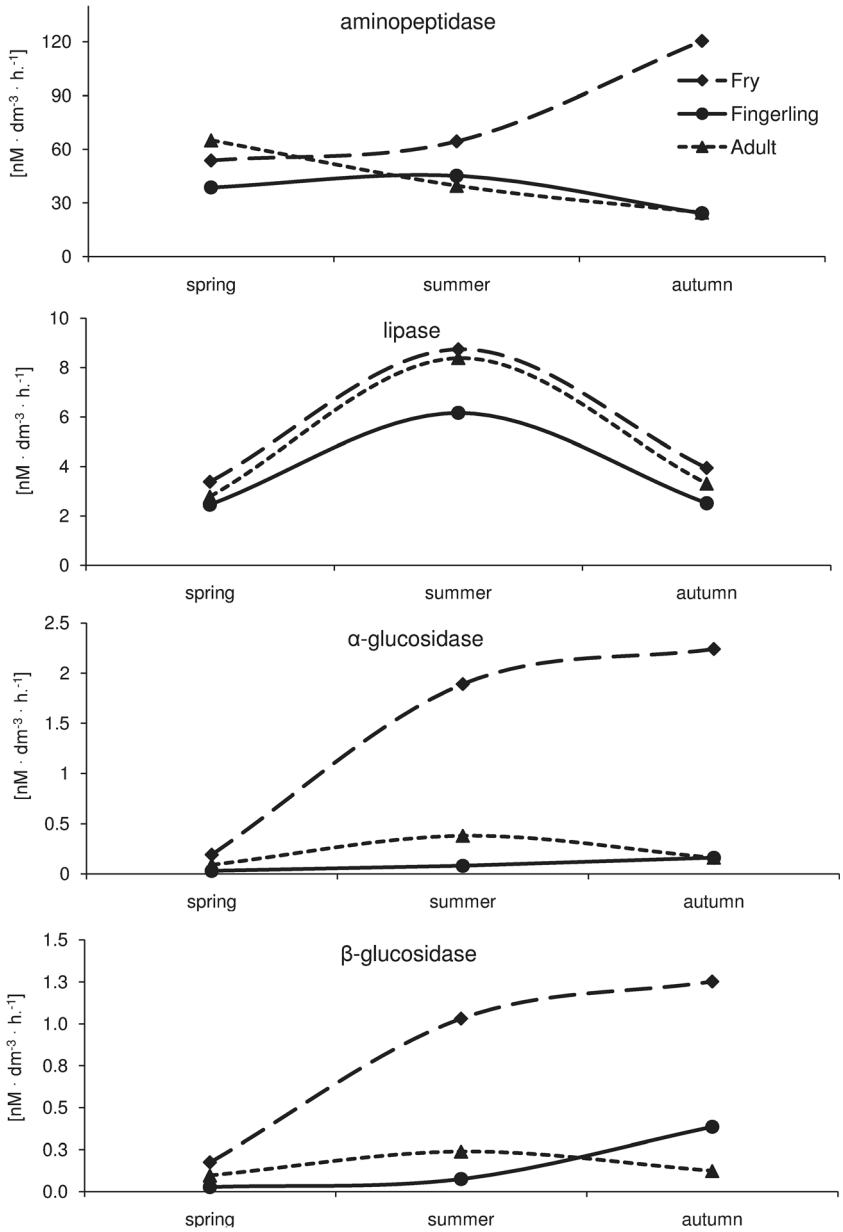

Fig. 3 Seasonal difference of level bacterial enzymatic activity. Data represented mean of all sites

enzyme activity in the aquatic environment focused on the determinated of the activity such hydrolases as leucine aminopeptidase, lipase, α and β-glucosidase. These enzymes representing model of enzymes which catalyse chemical bond cleavage in proteins, lipids and polysaccharides, i.e., three main compounds in organic matter accumulated in water basins.

Our study showed that among four extracellular enzymes assayed in the water of three ponds of the carp farm it was leucine aminopeptidase (LAP), which preferentially cleaves leucine from the N- terminus of proteins or peptides that showed the highest potential activity level. The variation in the LAP activity found in the present study (35.97–79.51 nM MCA· L · h^{-1}) was similar to the range reported in the central Tyrrhenian Sea (17.55–35.72 nM MCA· L · h^{-1}; Caruso et al. 2003) and higher than in the Baltic Sea 0.6–9.3 nM MCA· L · h^{-1} (Baltar et al. 2016) but lower than in the aquaculture in the Gulf of Castellammare (11.75–455.87 nM MCA· L · h^{-1}; Caruso et al. 2003). Sala et al. (2005), Franzo et al. (2019) and Zaccone and Caruso (2019) have shown that leucine aminopeptidase is widespread in many marine and freshwater environments. This hydrolase may be synthesized by almost all species of aquatic bacteria but also by cyanobacteria, phytoplankton, ciliates and zooplankton (Caruso 2010, 2015; Kiersztyn et al. 2012). According to Sakami et al. (2005) and Zdanowicz et al., (2018) the level of activity of LAP, which is a proteolytic enzyme, depends on the protein concentration in aquatic ecosystems. In water basins

Table 3 Correlation coefficient enzymatic activity in the water carp fish ponds (n = 27 in all cases)

Pond	Enzymes				
Fry		α-GLU	β-GLU	AMI	LIP
	α-GLU				
	β-GLU	0.77**			
	AMI	0.55	0.62*		
	LIP	0.07	0.33	−0.25	
Fingerling		α-GLU	β-GLU	AMI	LIP
	α-GLU				
	β-GLU	0.83**			
	AMI	0.00	−0.33		
	LIP	0.17	−0.18	0.76*	
Adult		α-GLU	β-GLU	AMI	LIP
	α-GLU				
	β-GLU	0.77*			
	AMI	0.57	0.40		
	LIP	0.23	0.57	0.10	

proteinaceous compounds originating mainly from dead plant and animal remains constitute 35–50% of dry mass of organic matter and are very important carbon, nitrogen and energy sources mainly for heterotrophic bacteria (Kalwasińska and Swiontek-Brzezińska 2013; Zaccone et al. 2014; Caruso 2015). Leucine aminopeptidase is exopeptidase (cuts the extreme amino acids of a polypeptide chain), which hydrolyses proteins, polypeptides, and peptides to monomers such as amino acids (Sala et al. 2005; Kalwasińska and Swiontek-Brzezińska 2013). These monomers can be assimilated by bacteria and used during biosynthesis of cellular structures or in respiratory processes (MacCarthy et al. 1998). The results of several studieand higher than in the Baltic Seas carried out by Patel et al. (2000), Mudryk and Skórczewski (2004) as well as Chróst and Siuda (2006) show that the activity of aminopeptidases depends on the degree of trophy of the water basin. This is probably the reason why a high potential activity level of LAP was also noted in heavily eutrophicated ponds, due to very intensive carp farming, in the present study. The high LAP activity detected in the studied fish farm may be also ascribable to the biochemical composition of fish feed, which is mainly based on proteins. These results correspond with the study by Caruso et al. (2003) carried out in fish farms of shore mariculture located on the Tyrrhenian Sea, Castellammare Gulf and Mediterranean Sea.

According to the results of our study lipase showed a lower activity level compared to leucine aminopeptidase. These hydrolases are usually relatively small proteins of

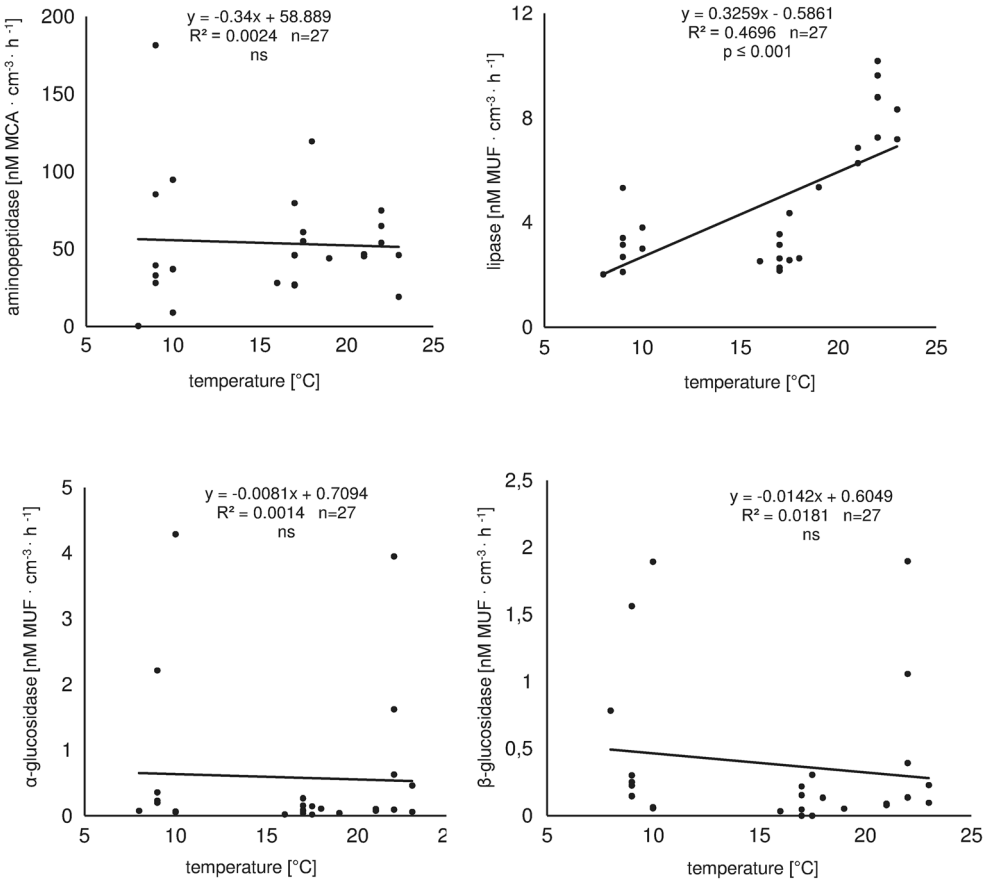

Fig. 4 Relationships between enzymes activity and temperature in the water carp fish ponds. Solid line represents linear regression including all data (y – regression equation, R^2 – coefficient of determination, n – number of samples, p – significance level)

Table 4 Analyses of the Kruskal - Wallis test in the level of enzymatic activity in the water fish carp ponds due to site, layer and season. Significance (p) is indicated by asterisks: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Enzymes	Source of variation	H	p
α-glucosidase	pond	10.310	**
	season	3.495	ns
	site	0.617	ns
	pond×season	16.349	*
	pond× site	13.513	ns
	season× site	5.937	ns
β-glucosidase	pond	6.611	*
	season	6.170	*
	site	2.320	ns
	pond×season	16.115	*
	pond× site	10.870	ns
	season× site	9.970	ns
aminopeptidase	pond	8.522	*
	season	0.522	ns
	site	0.183	ns
	pond×season	15.619	*
	pond× site	12.085	ns
	season× site	2.138	ns
lipase	pond	3.630	ns
	season	17.675	***
	site	0.667	ns
	pond×season	21.587	**
	pond× site	4.762	ns
	season× site	20.815	**

Explanations: H – the Kruskal – Wallis test; p – significance level; ns – non-significant

molecular mass equal to about 25,000, which are capable of attacking emulsified mono- di and triglycerides and splitting them, yielding glycerine and fatty acid residues (Gajewski et al. 1997; Mudryk and Skórczewski 2004). Lipids are integral components of all living organisms; in aquatic ecosystems they constitute 3–55% of all organic matter (Reemtsma et al. 1990; Perliński et al. 2017). Live as well as dead phytoplankton, zooplankton, meiofauna, macrofauna, microflora and detritus are the main sources of lipids in water bodies (Skórczewski and Mudryk 2009; Kalwaśinska and Swiontek-Brzeźińska 2013). The lipase activity is closely correlated with the availability of lipid substrate and, therefore, the level of its activity may reflect the concentration and distribution of lipids in water basins (Hoppe 1993; Li et al. 2019). According to Kalwasińska and Swiontek-Brzezińska (2013) and Perliński et al. (2017) heterotrophic lipolytic bacteria, which can actively synthesize lipases and release them as free enzymes after the lysis of their cells play a key role in the processes of the decomposition and transformation of lipid compounds in water basins. The results of the present study showed that the population of heterotrophic bacteria (Table 1) inhabiting the water of the studied ponds was numerous and probably these organisms were able synthesized lipases, which actively hydrolysed lipids accumulated in these water basins.

Among four hydrolytic enzymes assayed in this study, β-glucosidase (β-GLU), which is used as an indicator of polysaccharide hydrolysis showed the lowest level of activity. Acorrding to Salazar et al. (2016) this enzyme influences the last step of cellulose decomposition and exhibits substrate specific for the hydrolysis of glycoside bonds of the β-type of glucose, cellobiose, carboxymethylcellulose, glycoproteins and glycolipids. This hydrolase is known to be mainly associated with heterotrophic bacteria, and it is produced in waters and sediments of freshwater and marine ecosystems (Steen and Arnosti 2013; Giraldo et al. 2014). A low level of the β-glucosidase activity in the studied ponds may be due to the fact that amino acids produced as a result of depolymerization of proteins represent a better source of carbon, nitrogen and energy for bacteria than polysaccharide hydrolysis products. This thesis may be supported by the LAP/ β-GLU ratio which ranged from 96 to 299, and showed that the aminopeptidase activity was consistently higher than that of β-glucosidase**.** A low level of enzymatic activity of β-glucosidase compared to the activity of aminopeptidase was also noted by Zaccone et al. (2002), Yamada et al. (2012) and Zaccone and Caruso (2019). According to Lackland et al. (1982) another reason of a low level of enzymatic activity of β-glucosidase in water bodies is the fact that cellulose is relatively resistant to the processes of bacterial degradation, and its transformation into glucose requires a considerable amount of energy. For a microbiological depolymerization of cellulose, a synergistic activity of many hydrolytic enzymes, synthesized not only by bacteria but also by actinomycetales, fungi and protozoa, is needed (Münster and Chróst 1990; Perliński and Mudryk 2018).

The results of the present study indicated different activity level of the tested enzymes along the horizontal profile in the water of the studied ponds. Such pattern is a well-known global phenomenon in aquatic ecosystems (Kalwasińska and Swiontek-Brzezińska 2013; Perliński and Mudryk 2018; Zaccone and Caruso 2019). According to Caruso (2010) and Cunha et al. (2010) such horizontal variation in enzymatic activity may be caused by the differences in the composition, concentration, availability and degradability of organic compounds, as well as the changes in the metabolic patterns of microbial community. Therefore, zonation of enzymatic activity along the horizontal profile is expected to mirror the distribution of organic matter and composition and level of metabolic activity of microorganisms inhabiting water basins (Mudryk and Podgórska 2005; Li et al. 2019).

In the water from the ponds of the studied carp farm extracellular enzyme activity was strongly influenced by the season. Usually the highest level of activity of analysed enzymes was observed in summer and autumn. It may be associated with a significant increase in the concentration of organic matter in the water of the studied ponds in these seasons

resulting from the extraction of photosynthetic products during phytoplankton bloom and from macrophytes (Zaccone et al. 2019). According to Rochelle-Newall et al. (2004), Perliński and Mudryk (2018) and Zdanowicz et al. (2018) a correlation between enzyme synthesis by microorganisms and the concentration of organic matter is related to the seasonal variation in the hydrolase activity in water basins. Also Brown and Goulder (1996) observed in trout-farm effluents that increases in level extracellular-enzyme activity was accompanied by increase phytoplankton chlorophyll concentrations. Beside quantity and quality of organic matter that influence the seasonal variation in extracellular enzyme, it may be also affected by physicochemical environmental factors such as the concentration and availability of inorganic nutrients, pH, run-off, snow melt, wind patterns, dissolved oxygen concentration, acidification, salinity, UV radiation and trophic status of the water basin (Giraldo et al. 2014; Caruso et al. 2016; Zaccone et al. 2019). According to Yamada et al. (2012), Baltar et al. (2017) and Franzo et al. (2019) the main environmental factor which influences the seasonal variation in level of extracellular enzyme activity in water basins is temperature. An important indirect effect these abiotic parameters is its influence on the affinity of enzyme systems since, at low temperatures, the affinity of enzymes decreases, while it increases along with increasing temperature (Perliński and Mudryk 2018). Zdanowicz et al. (2018) noted a positive effect of warm temperature of water in summer on enzymatic decomposition of organic matter. This regularity is also confirmed in various aquacultures basins (Sakami 205, Caruso 2015, Banerjee and Ray 2018). In our study such pattern concerned the activity of lipases in all three studied ponds and the activity of aminopeptidase in fingerling pond, and α and β -glucosidase in adult pond.

Conclusion

In conclusion, we hope that the results of the present study may bring important information on the potential role of enzymes in the processes of organic matter transformation and that a measurement of extracellular activity can be used as an indicator of microbial decomposition of organic matter in aquacultures.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This study was conducted following the accepted principles of ethics and professional conduct.

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