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INDICATION OF TEMPERATURE INVERTED MICROBIAL ASSIMILATIVE CAPACITIES (EXTRACELLULAR ENZYMES ACTIVITIES) IN THE PELAGIC OF LAKE SEVAN (ARMENIA)

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Abstract

Pioneering records of extracellular enzymes activities (EEA) in Lake Sevan waters highlight dependence of heterotrophic functioning on physicochemical characteristics and bacterial assemblage. Values of EEA, ranged 0.11-30.39 μg C/P $L^{-1}h^{-1}$, were higher in upper layers compared to the omission in deeper parts. Particles associated (ecto-) enzymes mainly predominated over free dissolved (exo-) enzymes. In June activities of all studied enzymes followed similar pattern, particularly, decreasing at thermocline and increasing twice/more in cold deeper waters. Regardless higher bacterial density and temperature in June, with no similar records up to now, EEA revealed reverse relationship to temperature and bacteria data and were significantly lesser than in March. Our finding might be suggested as temperature inverted impact to heterotrophic activities in eutrophic conditions. We assume that observed, with temperature raise, declined EEA was due to blocked enzymatic active center from colloids and DOM components interaction, which, in overall, may suppress organic substrate utilization and result in weakening of first and rate limiting step of biological self-purification in Lake Sevan waters. Therefore, since temperature is co-regulator of assimilative/carrying capacity of aquatic ecosystems, climate warming might have unexpected negative feedbacks also through lowering assimilative capacities of water bodies, jeopardizing their quality and ecology.

Keywords: freshwater alpine lakes, Lake Sevan, climate change, eutrophication, temperature, bacteria, extracellular enzymatic activity

1 INTRODUCTION

In all aquatic ecosystems autochtonous and allochtonous inputs of organic matter are the essential link in the energy and nutrients cycles, driven by microbial decomposers (Wetzel, 1992). Organic matter of aquatic ecosystems, mostly possesses heterogeneous nature based on molecular weight, chemical composition, its availability and origin, turnover, as well as significance to the microorganisms (Münster&Chròst, 1990), and is only partly (< 5 %) assimilable (Chróst, 1991). The initial degradation of those complex molecules into for bacteria easily utilizable oligo- to monomeric compounds via depolymerization is induced by extracellular enzymes (Hoppe, 1991; Hoppe et al., 1988; Meyer-Reil, 1991; Karrasch et al., 2003a, b). This is the first step of microbial self-purification processes, which is the key mean to regulate the turnover of in- and organic compounds in aquatic environments (Hoppe, 1983). The activity of a certain microbial extracellular enzymes can provide useful insights into rates of nutrient mineralization and organic matter processing (Sinsabaugh el al., 2008). Microbial extracellular enzyme activities in aquatic environments represent the microbial physiological processes that have a direct influence on ecosystem level transformations of carbon and nutrients (Jackson et al., 2013). Moreover, the capability of microorganisms in aquatic environments to uptake a certain compound can depend indirectly from quantities and functioning of extracellular enzymes serving to liberate the compound from POM and polymeric DOM and lift the monomers into the cells (Chróst, 1991; Hoppe, 1991; Karrasch et al., 2003a, b).

For free-living microbes utilizing DOM, the ambient substrate field is radically different (Traving et al., 2015). Enzyme significant activities have been measured on particles (Grossart et al., 2007; Ziervogel&Arnosti, 2008) as due to high nutrient concentrations on particles (Vetter et al., 1998) the free-enzyme strategy is profitable for particle-associated microbes. In contrast, surface-attached enzymes represent the most cost-efficient strategy, as free enzymes should not be profitable due to the dilute nature of DOM (Chróst, 1990; Somville&Billen, 1983). The enzymatic hydrolyzation processes in aquatic ecosystems and the rate of them are influenced by a complex of environmental factors (e.g. physicochemical parameter, including temperature, colloid sorption, surface charge of particles, etc.), that affect the activity and availability of extracellular enzymes, and by that mean, may directly impact the ecological stability of entire aquatic ecosystems (Arnosti, 2003). Although, investigations in various aquatic environments have characterized enzymes to give different responses to water temperature changes, the majority of scientists agreed for enzymes to

follow the "Q_{10}" rule for temperature dependent enhanced catalytic rate. Thus, Christian&Karl (1995) have found activities of leucine-aminopeptidase and β-glucosidase to significantly vary in positive response to temperature changes in subtropical North Pacific, the equatorial Pacific and the Southern Ocean regions with very pronounced differences among latitudinal and climatic zones. This supports similar results of latitudinal gradient in the relative activities of proteases and polysaccharases in marine microbiota from Kriss et al. (1963). Study in Antarctic has showed activity of extracellular chitinases, regardless their high barotolerance at neutral pH, to reduce with low temperature (Helmke&Weyland, 1986), meanwhile Hoppe and Gocke (1993) that demonstrated for N-S Atlantic a clear dependency of heterotrophic activity on temperature, suggested probability of compensatory adaptation of enzymes to low temperature over nutrients supply for bacterial growth and functioning to a specific extent. Schweitzer&Simon (1994) have defined low temperature being one of the limiting factors to bacterial heterotrophic functioning for Lake Constance. Also Meyer-Reil&Köster (1992) marked activities of enzymes to depend positively on the temperatures. Study from Sinsabaugh&Linkins (1988), Chappel&Goulder (1994) determined temperature, as well as light (Sinsabaugh&Linkins, 1988; Sabater et al., 1998) to control enzymatic degradation rate in the streams. In the study from Kirschner et al. (1999) of the Alte Donau (Danube), temperature and carbon supply were suggested as the main limiting factors for bacterial functioning during the cold seasons and the highest values of EEA recorded for hot season (especially during phytoplankton bloom) and consequently lower EEA at the cold weather. Other data demonstrate substrate availability being the major factor for extracellular enzymatic activities (Meyer-Reil, 1987) and bacterial activities; concentration and discharge of bioavailable nutrients (Romaní&Sabater, 1999), and the humic material (Freeman et al., 1990). Chappel&Goulder (1994), as well as Sabater&Romaní (1996), as a biological indicator for heterotrophic processes in aquatic ecosystems, have outlined microbial activities being positively aligned to environmental traits.

In light of altogether, the main aims of the present work were to study the dependence of activities of certain extracellular enzymes in correlation to water physicochemistry upon the quantitative characteristics and distribution of bacterioplankton. The goal of this investigation was to examine whether extracellular enzymes display characteristics of local adaptation on latitude level on Lake Sevan example. This task is very important because of the adaptation of certain microbial enzymes to a particular temperature regime that may or not sustain microbial decomposer activity under global warming conditions in freshwater lakes. For that

purpose EEA rates as an indicator of waters assimilative/carrying capacities have been quantified in Lake Sevan waters to indicate OM load limits depending on physicochemical traits to outline standing stocks of the baseline structure. We hypothesized that microbial EEA in deep alpine lakes (such as Lake Sevan) would be more sensitive to temperature seasonal changes, and that EEA changes would show temperature dependence sensitivity with increasing water temperature. The pioneering results derived from this study suppose to improve our ability to model the effects of global warming on decomposition of DOM in alpine freshwater lakes, to serve for development of appropriate management strategies in further monitoring and for better elucidation of the remediation program of the freshwater reservoirs conservation worldwide, and for Lake Sevan, particularly.

2 MATERIAL AND METHODS

2.1 Study site

Lake Sevan is one of the largest freshwater alpine lake in the world at 1900 m above sea level situated approximately 60 km north of Armenia capital Yerevan, possesses a surface area of 1270 km^2 and a maximum depth of 80 m. Lake Sevan, the largest freshwater lake in the South Caucasus region and nearby territories (Iran, Turkey). Hrazdan River is the only outflowing river of Lake Sevan that becomes Lake Yerevan (an artificial water body in the southwestern part of the city) on the territory of c. Yerevan (the capital of Armenia). Lake Sevan - River Hrazdan - Lake Yerevan water cascade continues again as River Hrazdan joining River Arax in the Ararat valley, along with Armenian-Turkish borders, then flowing along the Armenian-Iranian and Iranian-Azerbaijan borders to meet with the Kura River and inflow into the Caspian Sea. It has soft water with mineralization = 700 mg L^{-1}. Salinity is about 0.36 - 0.37 ppt, total hardness of its water is 4.65 - 4.90 meq L^{-1}, conductivity 598 - 634 µS cm^{-1}. Lake Sevan with its huge socioeconomic importance for Armenia is very unique itself also due to its endemic fishes: e.g. Sevan trout (*Salmo ischchan gegarkuni*), Sevan koghak (*Capoeta capoeta sevangi*) and Sevan beghlou (*Barbus geokschaicus*). Due to artificial decline of its water level (down to 20.2 m), started in 1933, the morphometric, physicochemical and biological parameters of the lake were heavily impacted. Hypolimnion total volume decreased for up to 40 %, and basin water average temperature increased for 2-3˚C (Lind&Taslakyan, 2005). Decomposition of loading organic matter decreased the oxygen content in water column in general with oxygen saturation tending in the lake floor zone to low values, especially for hot

seasons during the lake stratification. The Lake started its evolution toward from oligo- to meso-eutrophic stage. Altogether collapsed entire balance of the lake through affecting the natural limnological properties of it. Moreover, the changing climatic conditions and new strategy on water management started since 2002, contributed to a quick rerise of the lake's water level up to 3.6 meters that caused problems of flooding numerous settlements and forestlands, which can threaten water quality of the Lake.

2.2 Material collection

The geographical coordinates of the sampling points are 40° 29' 35.50' N and 45° 11' 31.34" E (Figure 1). Water sampling of the Lake north-western sector (Minor Sevan) along vertical profile (0; 5; 10; 15; 20; 25; 30; 35; 40; 45; 50; 55; 60m) was done with a Ruttner sampler.

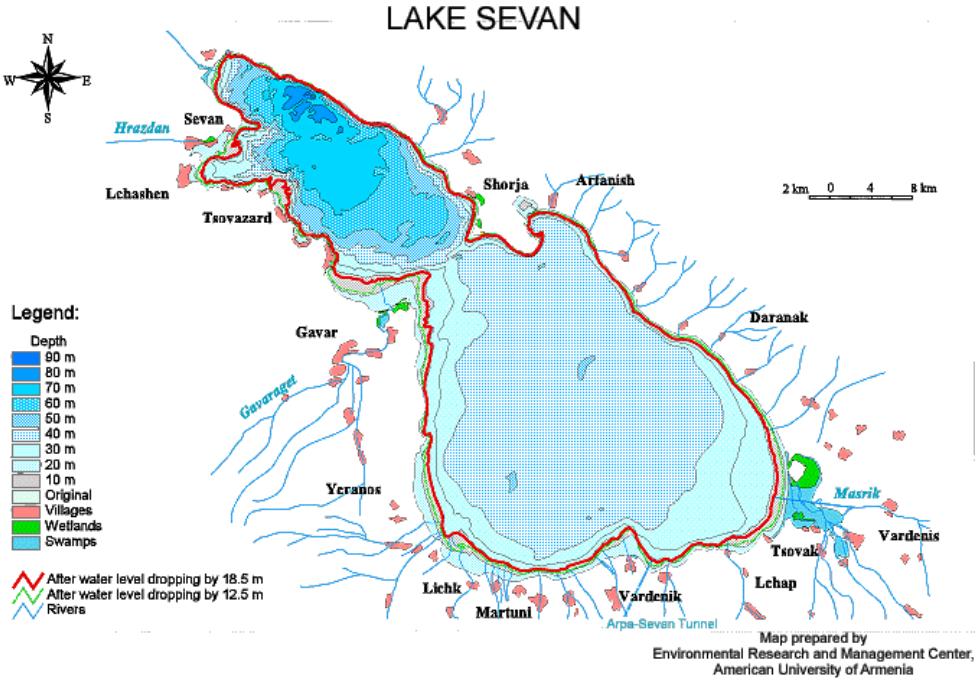

Figure 1. Map of Lake Sevan catchment basin ("X" is the illustration of the position of the sampled vertical profile)

For the microscopic analyses 40 ml of the samples were immediately fixed with 0.2 µm filtered formalin (final concentration 2 % v/v) and stored at 4ºC for further determination of the abundance and biomass of bacteria.

The non-preserved samples were collected for the quantification of

the EEA. As no negative effects on the quantification of EEA after several days of storing the samples in the dark at 4ºC were observed by the authors, we had followed the same procedure. All samples were transported (in cold and dark) to the host institute in Germany (UFZ -Helmholtz Centre for Environmental Research, Department of River Ecology) for proceeding (started on 15-th March and 15-th June for samples collected on 12th March and 13th June respectively).

2.3 Quantification of bacterial abundance and biomass

Total abundance and biomass of bacteria were determined by fluorescence microscopy (acridine orange (AO) method after Hobbie et al. (1977) on 0.2 μm Irgalan black stained polycarbonate nuclepore filters). Counting was performed with a Zeiss Axioplan epifluorescence microscope (365nm excitation filter, 395nm beam splitter, 397nm emission filter). A minimum of 20 fields per filter were counted by means of a new Porton grid (Graticules, Ltd.) to obtain confidence level of 95%. The cell volume (V) was calculated as follows:
cell volume = π/4 x W2 x (l - W/3) (with W: width in μm and L: length in μm)

Bacterial biomass in carbon was determined using the non-linear equation derived from Simon&Azam (1989):
cell carbon / fg = (cell volume/μm^3) 0.59 x 88.6 x 1.04878

2.4 Quantification of extracellular enzymes activities

The EEA were determined as a measure in accordance with the method published by Hoppe (1983) modified by Karrasch (2005). A technique based on 4-methylumbelliferyl (MUF)α- and β-D-glucopyranoside, phosphate, β-D-galactopyranoside, N-acetyl-β-D-glucosaminide) and L-leucine7-amino-4-methyl-cumarin (AMC) labeled enzyme substrates was used to quantify rates of extracellular breakdown of dissolved and particulate organic macromolecules (Karrasch et al., 2003a, b). The rates of EEA are expressed in terms of maximum velocity of hydrolysis (μg C L^{-1}h^{-1}) or (μg P L^{-1}h^{-1}in the case of MUF-phosphate). 10 μl of substrate analogue (final concentration 500 μM, MUF-phosphatase 125 μM L^{-1}) was supplied to the samples. To divide the total EEA into ectoenzymes (enzymes associated with bacteria and particles) and free in the water dissolved enzymes activities, a filtration was performed using 0.2 μm

nuclepore polycarbonate filters whereby the <0.2 μm fraction defines free dissolved enzymes. 200 μl subsamples (0.2 μm filtered and non-filtered sample) were added into each well of microplate (4 replicates) and supplemented with previously added AMC and MUF-substrates. Incubation time was 24h at ambient sample temperature in the dark. Standard of AMC and MUF solutions were used for calibration (10 μl of 0.5 μmol L^{-1}; internal standard addition method). Fluorescence quantification was done using a microplate fluorescence reader (Labsystems, Ascent software) at λ=364 nm (excitation) and λ=445 nm (emission).

3 RESULTS

In March transparency of Lake Sevan water reached up to 13 m and dropped down to 8 m in June. Temperature fluctuated from +3.5˚C - +18˚C. Dissolved oxygen was decreasing from surface (14.2 mg L^{-1}) up to the upper edge of thermocline at 20 m (12.4 mg L^{-1}) with further lowering at bottom. The detection limit for nitrite ion that quickly converts into nitrate in natural waters, was below <0.023 mg L^{-1} for over the water column. Concentrations of nitrate ion were in the range of 22 μg N L^{-1} up to 277 μg N L^{-1}, ammonium was of 34 μg N L^{-1} - 152 μg N L^{-1} (data of March). Phosphate was accounted for 58.6 μg P L^{-1} up to 82.0 μg P L^{-1}.
In summer total phosphorus content had increased up to values typical for eutrophic waters (Minasyan, 2010).

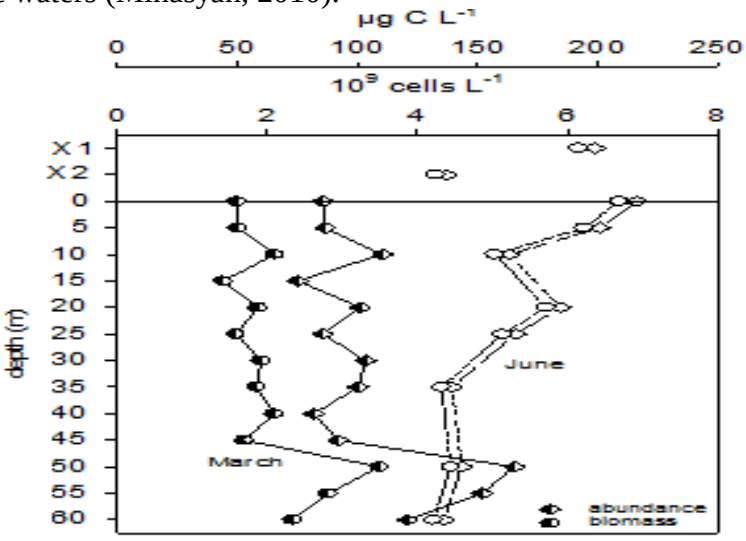

Figure 2. Vertical distribution of the total bacterial abundance (*10^9 cells L^{-1}) and biomass (μg C L^{-1}) in Lake Sevan waters

In March bacteria were counted from $2.4*10^9$ cells L^{-1} up to $5.3*10^9$ cells L^{-1} (see Fig.2), with minimal numbers for the surface waters. At 50 m depth was registered the highest value for spring followed with gradual drop down at bottom. Reverse situation of higher bacterial density starting with the highest values for surface ($6.9*10^9$ cells L^{-1}) and gradual decrease for deeper waters was counted in June. Insignificant oscillations in vertical distribution were observed with decrease started at the thermocline = $5.3*10^9$ cells L^{-1} (25 m) and continued up to the lowest for the bottom waters.

Bacterioplankton dominated by small-size single cells with mean volume in March equaled to 0.08 μm^3. Comparatively medium-size bacterial cells were observed in June (cell mean volume 0.20 μm^3) with biomass values accounted for 68.4 - 108.54 µg C L^{-1} (Figure 2).

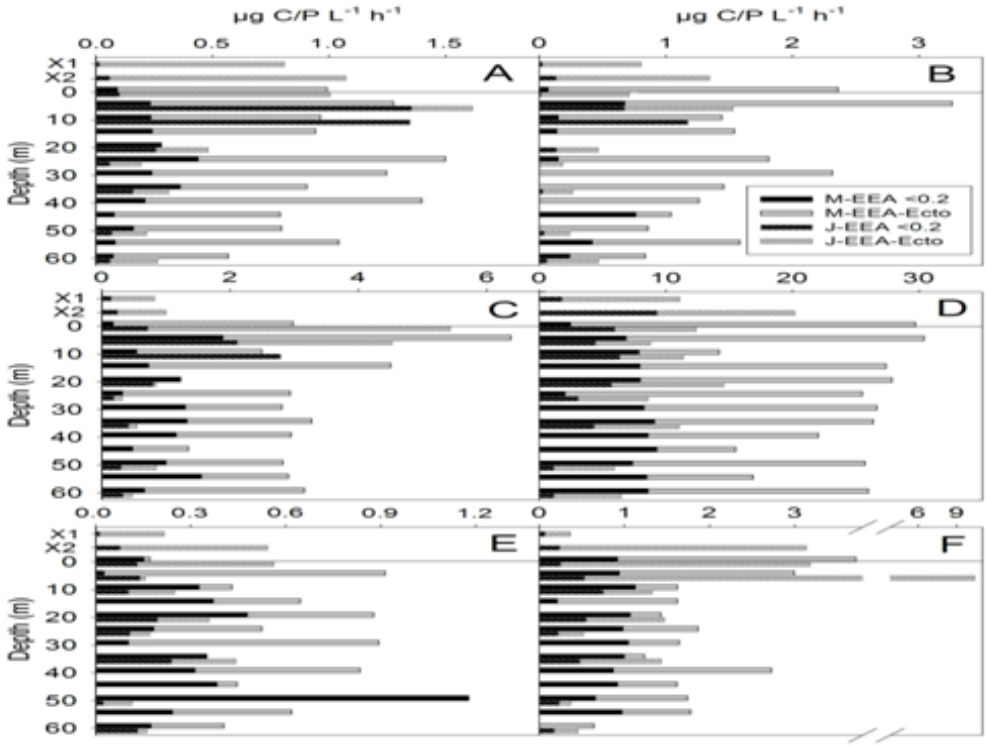

Figure 3. Vertical distribution of hydrolysis rates of EEA in Lake Sevan in 2010 March/June.

A = α-glucosidase; B = ß-glucosidase; C= phosphatase; D = leucin aminepeptidase; E = ß-galactosidase; F = N-acet;

EEA <0.2 = external enzymes; EEA-Ecto = ectoenzymes; M = March; J = June

The EEA, except for leucine-aminopeptidase, ranged from 0.11 to 10.41 μg C/P L^{-1} h^{-1}, with the lowest values for β-D-galactosidase of the maximal up to 2 % share (Figure 3). Activity of leucine-aminopeptidase was one magnitude higher (from 5.93 up to 30.39 μg C L^{-1} h^{-1}), with up to 74 % proportional share in the total EEA. In March started from 15 m depth up to the bottom increasing bacterial density was in concordance with increasing enzymatic activities (relatively high for β-D-glucosidase (2.36 - 3.26 μg C L^{-1} h^{-1}) and N-acetyl-β-D-glucoseaminidase (3.00 - 3.73 μg C L^{-1} h^{-1}) up to 6.37 μg P L^{-1} h^{-1} for phosphatase and the highest of 30.39 μg C L^{-1} h^{-1} for leucine-aminopeptidase). In the upper layers, particularly for 0-5 m linked to relatively low bacterial densities nearly for all enzymes were registered higher activities and relative decrease at 10 m (euphotic zone with water lower temperature) and furthermore.

In June both values of bacterial densities and EEA were higher for upper layers up to 10 m (euphotic zone with water higher temperature). The values of activity of phosphatase, β-D-galactosidase were higher at surface and α- and β-D-glucosidase and N-acetyl-β-D-glucoseaminidase at 5 m depth. The rates of activities for all 6 studied enzymes followed similar pattern. Started from thermocline all enzymes, except of leucine-aminopeptidase, had demonstrated decreased activities up to the range of β-D-galactosidase measured in March. Their activities decreased at thermocline (25 m) and tend to increase approximately twice or more in hypolimnion. The hydrolysis rates of α-D-glucosidase, β-D-glucosidase and phosphatase at the bottom layer were up to 2.5 times higher than at the thermocline. Activity of N-acetylglucoseaminidase, registered "above" - "at" - "below" thermocline, differs approximately three times.

4 DISCUSSION

The concentration of nitrogen and phosphorus in Lake Sevan waters was in range typical for mesotrophic lakes stepping into the first stage of eutrophy (Wetzel, 2000). Low oxygen content at bottom characterized Lake Sevan being at meso- toward first stage of eutrophy too. The relatively equal distribution of bacteria in March was with low values at the upper layers and increase in hypolimnion. Windy weather and typical spring overturn that equalized also water temperature could be the main reasons for homogeneous distribution of bacteria in Lake Sevan. In June in the epilimnion of Lake Sevan the water higher temperatures were registered. Together with more organic matter approaching from enhanced human

summer activities in the Lake basin, such as recreation, agricultural, sewage discharge, etc., higher concentrations of available nutrients of autochtonous and allochtonous origin, increase mainly N and P inputs (Hovhannisyan, 2010). Additionally, a solid amount of organic matter entering the Lake Sevan ecosystem from the flooded territories (reports of the Ministry of Nature Protection of Republic of Armenia) can be essential for development of microbial community.

According to Lind&Taslakyan (2005) algal "blooming" in Lake Sevan during the hot seasons causes TN/TP ratio increase toward P-limitation in the upper layers and P-trapping in the Lake floor, and a lower nutrient availability in the epilimnion clearly enhance the "bottom-up" control reflected in the relatively increased bacterial density in epilimnion and decreased in hypolimnion. Our past studies (Kosolapov et al., 2010; Hahn et al., 2012) revealed similar tendency of increasing quantitative characteristics (abundance and biomass) of bacterial community, especially in hot seasons together with higher algal numbers (Minasyan&Karrasch, 2015), demonstrate continuing evolution of Lake Sevan from meso- toward eutrophic stage. Altogether confirm high oxygen demand and could be one of the reasons of its depletion in Lake Sevan hypolimnion.

Extracellular enzymes activities as a first step of biological self-purification capacity of aquatic ecosystems undergo the influence of a multitude of physicochemical, as well as biological processes and interactions existing therein (e.g. Karrasch et al., 2003a, b). The majority studies indicate temperature (the "Q_{10} rule") as one of the environmental trait to regulate cell metabolism, which was attributed also for EEA (Münster et al., 1992), with seasonal character of extracellular enzymes sensitivity over temperature (Fenner et al., 2005; Koch et al., 2007; Trasar-Cepeda et al., 2007). The affinity of enzyme systems decreases at low temperatures, therefore, their interaction has an indirect effect on enzymatic activity (Zweifel, 1999; Pomeroy&Wiebe, 2001; Reyes et al., 2008) of certain aquatic ecosystem.

Registered in Lake Sevan waters reduced EEA with increased temperatures was quite contrary with no similar records in the existing literature. In June water higher temperature and higher bacterial density were coupled with the mean values of activities of total enzymes being lower, than those in March (an exceptions of N-acetylglucosaminidase) (Figure 3). Reduced activities of 6 studied enzymes in both fractions (ecto- and exoenzymes) was quantified especially right at the thermocline level (20-25 m) - "above" - "at" - "below", differing approximately three times. Meanwhile, the abundance of bacteria of those depths did not differ so dramatically ($5.91\text{-}4.47*10^6$ cells L^{-1}), with less difference in

picocyanobacterial density (0.18-0.16*10^6 cells L^{-1}) (Minasyan&Karrasch, 2015). Activity of leucine-aminopeptidase that had the highest share in EEA in Lake Sevan also comparatively declined in summer. This observation is in contrast to the statement of Hoppe (1983) of observed typical EEA to reach its maximums during the hot periods, when water temperature, bacterial density and uptake of leucine are also the highest, together with increased nutrients input from human activities. Leucine-aminopeptidase (due to their high values activity of leucine-aminopeptidase was not included in the total calculations), with the higher proportional share in cold season (in average 2.4 times higher in March) are similar to data from Münster (1991) of the higher activity of ecto- fraction of leucine-aminopeptidase to be in oligotrophic waters.

Observed in Lake Sevan dominance of aminopeptidase versus glucosidase is common for other aquatic ecosystems (Stursova et al., 2006; Karrasch et al., 2011) too. Lancelot&Billen (1984) showed tight coupling of aminopeptidase activity to primary production, similar to data of Lake Sevan. Hoppe (1986) also showed extracellular glucosaminidase activities, being with the highest values during the spring phytoplankton bloom mainly in March with higher dependency of EEA on total bacterial density. Rath et al. (1993) demonstrated activity of α-D-glucosidase (associated with phytoplankton biomass and dissolved monomeric carbohydrates achieved from forestlands) and N-acetyl-P-D-glucosaminidase (chitobiase) to decrease from eutrophic to oligotrophic conditions, which is in contrast to our observation. In Lake Sevan high activity of N-acetylglucosaminidase was accompanied with high activity of β-glucosidase (hydrolyze cellulose) and phosphatase, more likely associated with deciduous leaf litter decomposing, approaching Lake Sevan from flooded forestland areas (March).

Chróst et al. (1989) offered potential close correlation between ectoenzymatic activity of α-glucosidase and algal community, which is similar to what we seen in Lake Sevan in June. This indicates the particle associated self-purification capabilities in Lake Sevan waters to be higher in March (mesotrophic stage). If to couple these observations and finding from Cunha et al. (2010) about ectoenzymes being responsible for the hydrolysis of the major components of DOM consisting of labile oligo- to monomeric molecules originating mainly from autochthonous processes like viral lysis (Fuhrman 1999) exudations from phytoplankton (Fogg, 1977, Baines&Pace, 1991) and zooplankton excretions (Lampert, 1978; Jurmars et al. 1989), that are easily accessible for extracellular enzymes, linked with data on high algal and viral densities in Lake Sevan (Minasyan&Karrasch, 2015), we can assume tendency of increasing concentration of DOM, originating from

algal exudation of increased grazing, especially in hot seasons.

General biochemical considerations upon the role of temperature on reaction rates and physiology within the zone of biokinetic temperatures (e.g. Arrhenius, 1989, the empiric Q_{10}, derived from the vant' Hoff equation) stimulates the cell growth (including bacterial and algal cells) and their metabolic rates. With increased primary production the presence of high DOM concentrations (e.g. exudation, sloppy feeding, etc.) is very likely raises the probability of the occurrence of enzyme inhibiting compounds that might block the enzymatic active centers and/or the significantly changed tertiary structures of them (Tietjen&Wetzel, 2003). Finding of Karrasch (2005), Wetzel (1991) of the increased occurrence of charged particle surfaces (e.g. clay and POM), an intensified of colloids and further DOM components interaction with the extracellular enzymes, e.g. creating van der Waals dipole linkages, covalent and hydrogen bonds, ionic linkages, aggregation and precipitation, e.g. by organic acids and cross-linking with reactive functional groups in proteins, nucleic acids and other organic matter components, showed high potential to suppress enzymatic activities.

In June higher abundances of bacteria, viruses and picocyanobacteria (Minasyan&Karrasch, 2015) in Lake Sevan, were in concordance with the similar tendency registered for primary productivity (up to 3 times higher) and bacterial production (up to 8 times higher) quantifications (data not shown). In a number of studies in deep lakes Chróst&Siuda (2006); Kisand&Tammert (2000) have demonstrated a close coupling between enzyme activities and primary productivity. If to link these to the observed bloom by cyanobacteria genera of *Microcystis*, *Anabaena*, and *Aphanizomenon* (Minasyan et al., 2012) together with summer increased standing stocks and applying temperature related physiological axiom, we have to expect with increasing temperature raised EEA, which was the opposite in Lake Sevan. Registered maximums of EEA (3.26; 6.37; 10.41; 30.39 $\mu g\ P/C\ L^{-1}\ h^{-1}$; for β-glucosidase; phosphatase, N-acetylglucosaminidase and leucine-aminopeptidase, respectively) that demonstrates the upper limit of the self-purification capacities of Sevan waters indicates also that increased discharge of substrates would impact the ecosystem via accumulating and induce significant changes in the community structure and by that threaten the ecology of Lake Sevan. Presumably, the occurrence of organic matter equipped partially with high reactive surface act/impacting inhibiting enzymes availabilities or activities in the waters of Lake Sevan, coupled with water temperature and its water mixing intensity are the most probable explanations of corresponding fluctuations in enzymatic activity during our study, with displaying restrict

functionality of free dissolved enzymes versus their ecto- fraction. Since EEA-rates are representing the maximum self-purification limits, our findings of their considerable reduction in Lake Sevan observed at thermocline, would probably encompass far-reaching consequences for the ecology of freshwater lakes with typical thermal stratification (e.g. Jeppesen et al., 2014) to demonstrate possible negative feedbacks of global warming from reduced assimilative capacity. The world is warming as a result of anthropogenic activities raising the atmospheric CO_2 concentrations (IPCC, 2007). Discovering the inner processes that could determine water quality through assessing assimilative/carrying capacities might help in defining appropriate experimental sets for further monitoring of the ecological situation and the trophic stage of aquatic ecosystems, especially in summer period, when weakens the first and rate limiting step of the biological self-purification leading the evolution of aquatic ecosystem to raised trophic levels.

5 CONCLUSION

The occurrence especially of surface charged DOM could interact with the extracellular enzymes either directly via blocking the zone around the active center of enzymes or by allosteric inhibition thus disturbing the access of substrate to it. Indirectly the autochthonously produced organic matter with a high electrostatically potential could mediate with their charged surfaces the adsorption of extracellular enzymes to colloids and particles whereby, depending on the accessibility of the active center (enzyme orioentation), the creation of an enzyme substrate complex could be interfered. Both cases may decline EEA and suppress organic substrate utilization and by that weaken the first and rate limiting step of biological self-purification in Lake Sevan waters. Therefore, since temperature is co-regulator of assimilative/carrying capacity of aquatic ecosystems, climate warming might have unexpected negative feedbacks also through lowering assimilative capacities of water bodies, jeopardizing their quality and ecology.

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