Limnology and plankton diversity of salt lakes from Transylvanian Basin (Romania): A review

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ABSTRACT

In the present work, we review the current knowledge on genesis, limnology and biodiversity of salt lakes distributed around the inner contour of Eastern Carpathian arc (Transylvanian Basin, Central Romania). Transylvanian salt lakes formed on ancient halite (NaCl) deposits following natural processes or quarrying activities. Most of these lakes are located in eastern (Sovata area), southern (Ocna Sibiului), and western (Turda-Cojocna) parts of the Transylvanian Basin, have small surfaces (0.1-4 ha), variable depths (2-100 m), are hypersaline (>10%, w/v, total salts, mainly NaCl) and permanently stratified. As consequence of steady salinity/density gradient, heat entrapment below surface layer (*i.e.*, heliothermy) develops in several Transylvanian lakes. The physical and chemical water stratification is mirrored in the partition of plankton diversity. Lakes with less saline (2-10% salinity) water layers appear to harbor halotolerant representatives of phyto- (*e.g.*, marine native *Picochlorum* spp. and *Synechococcus* spp.), zoo- (*e.g.*, *Moina salina*), and bacterioplankton (*e.g.*, Actinobacteria, Verrucomicobia), whereas halophilic plankton communities (*e.g.*, green algae *Dunaliella* sp., brine shrimp *Artemia* sp., and members of Halobacteria class) dominate in the oxic surface of hypersaline (>10% salinity) lakes. Molecular approaches (*e.g.*, PCR-DGGE, 16S rRNA gene-based clone libraries, and DNA metabarcoding) showed that the O_2-depleted bottom brines of deep meromictic Transylvanian lakes are inhabited by known extremely halophilic anaerobes (*e.g.* sulfate-reducing Delta-Proteobacteria, fermenting Clostridia, methanogenic and polymer-degrading archaea) in addition to representatives of uncultured/unclassified prokaryotic lineages. Overall, the plankton communities thriving in saline Transylvanian lakes seem to drive full biogeochemical cycling of main elements. However, the trophic interactions (*i.e.,* food web structure and energy flow) as well as impact of human activities and predicted climate changes are yet to be assessed in these unique ecosystems with little or no match to analogous salt lakes worldwide.

Key words: Biodiversity; halite; halophilic; hypersaline; meromictic lake; picophytoplankton; zooplankton.

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INTRODUCTION

Saline environments (*i.e.*, those milieus exceeding sea water salinity of ~3.5%, w/v, total salts) occur worldwide either as terrestrial (*e.g.*, salt marshes, saline soils) or aquatic habitats (*e.g.*, inland saline lakes, deep-sea brines, solar evaporation ponds, etc.) (Hammer, 1986; O'Leary and Glenn, 1994; Oren, 2016). Based on their origin, saline aquatic systems are classified into thalassohaline (derived from sea water) or athalassohaline (*i.e.*, of land origin) (Hammer, 1986). Additionally, inland saline aquatic systems can be classified into pH-neutral (enriched in Na^+ and Cl^-; *e.g.*, Great Salt Lake in Utah, USA,

and Dead Sea in the Middle East) (Belovsky *et al.*, 2011; Oren, 2016), alkaline (with elevated Na^+ and HCO_3^-/CO_3^{2}; *e.g.*, soda lakes in the Siberian steppe or Eastern African Rift Valley) (Sorokin *et al.*, 2015; Deocampo and Renaut, 2016) and acid saline lakes (*e.g.*, the ephemeral sulfate-rich brines in SW Australia) (Benison and Bowen, 2015). According to the above classifications, all inland salt lakes in Transylvanian Basin (Central Romania) are pH-neutral, athalassohaline systems (Baricz *et al.*, 2014; 2015). On the contrast to numerous neutral salt lakes investigated to date in North America (Belovsky *et al.*, 2011), Middle East (Makhdoumi-Kakhki *et al.*, 2012; Oren, 2016), Australia (Podell *et al.*, 2013) or Antarctica (Laybourn-

Parry and Bell, 2014), the limnology and biology of salt lakes in Romania are only scarcely visible in the main stream of scientific literature. Previous works reported on morphometry and limnogeology (Bulgăreanu (1993; 1996), zoo- and phytoplankton diversity (Ionescu *et al.*, 1998), enzymatic activity associated to organic-rich sediments (Muntean *et al.*, 1996) and potential for novel microbial taxa in Romanian salt lakes (Enache *et al.*, 2007). The aim of the present work is to review the current knowledge on the origin, hydromorphological, physicochemical, and biological particularities of most representative salt lakes situated in Transylvanian Basin, with emphasis on recent advancements in the understanding of ecological complexity of these extreme aquatic habitats.

Brief history of salt lakes research in Transylvania

Salt lakes are distinctive components of the Transylvanian Basin landscape; they have been extensively observed and exploited over time. The physico-chemical peculiarities of salt waters (*e.g.*, heliothermy) and the therapeutic value of organic-rich sediments (or 'sapropels') are most relevant features of Transylvanian salt lakes that led to early capitalization and scientific interest.

The first documented observation of the salt water heating phenomenon took place at the end of 19th Century in a salt basin near Miserey (Besançon, France) where temperature value of 62°C was recorded at 1.35 m depth without any further scientific explanation (Ziegler, 1898, commented by Lang, 1898). Shortly afterwards, von Kalecsinszky (1901) was first to methodically study how brines can store heat as consequence of lens effect of freshwater surface, performing a series of observations and measurements on Ursu Lake (Sovata). He suggested that the thin layer of low-saline or freshwater at the surface of the lakes topping the deeper hypersaline horizon favoured significant increase of temperature at a depth of 1.5-3 m. Following these observations, Ursu and its analogous saline lakes in Sovata were termed as '*wärmeaccumulatoren*' ('heat reservoirs', von Kalecsinszky, 1901). According to Sonnenfeld and Hudec (1980), I.A. Maxim conceived the term 'heliothermal' for lakes entrapping a warmer layer between the upper and deeper strata with lower temperature, a phenomenon latter coined 'thermal anomaly' by Touchart (2002).

The curative effects of salt lakes of Transylvania (mainly in Turda, Ocna Sibiului, and Sovata) have been testified since 16th Century by princes, nobles or physicians that invited various acquaintances to heal infertility, skin or rheumatic ailments (Alexe, 2010). During the 19th and 20th Century, respectively, intermittent scientific observations (1820-1896) were followed by systematic surveys on limnology, geochemistry, and morphometry of most if not all Transylvanian salt lakes (Supplementary Tab. S1).

Genesis of salt lakes in the Transylvanian Basin

The basement of Transylvanian Basin belongs to the Eastern Carpathian type, being composed of crystalline schist and Mid-Cretaceous conglomerates lying at a depth of 4500 m. This area slowly subducted during Late Cretaceous as indicated by the Paleogene and Neogene sediments arranged in continuous succession, which filled up the basin (Sanders *et al.*, 2002). Several genetic phases configured the present-day Transylvanian Basin (Supplementary Fig. S1):

- the phase of Paleogene basin situated inside the Eastern Carpathians arc and bordered by crystalline massifs of Rodna, Preluca, Dealu Mare, Dealu Codrului, the Apuseni Mountains and the Meridional Carpathians, where thick packages of Eocene and Oligocene sandstones were stored (Pop, 2000); during this phase, the northern and western parts of the basin were covered by marine waters, being affected by a gradual descent and an important sedimentation;
- beginning with the Middle Miocene (Badenian), after a tectonic fragmentation, the eruptive chain Oaş-Gutâi-Ţibleş was born, the old intra-Carpathian basin being divided into two compartments: a smaller area in the north (Maramureş Basin) and a larger one in the central part (Transylvanian Plateau); around 14 Ma ago, following the Central Paratethys Sea regression, massive evaporite sediments (mostly halite and gypsum) formed in Transylvanian Basin and Carpathian Foredeep, respectively, in a relatively short geological period (0.2 Ma) (Ciupagea *et al.*, 1970; Báldi, 2006);
- upper Pliocene marks the third phase, with sea level drop and retreat of marine waters to the south-east followed by the subaerial shaping of north and centre sub-basins; sediments (Pliocene) were deposited further on the edges, in conjunction with the areas where the Pliocene sea withdrew (the actual corridor of the Someş, Mureş, and Olt rivers).

The rise of the mountain area triggered the development of diapiric folds on the edges (Praid, Turda, Ocna Dej, Cojocna, Ocna Mureş, Ocna Sibiului) (Pop, 2000).

Due to the geological evolution, the Transylvanian Plateau consists of two relatively concentric areas with distinct geological structure, morphology and spatial organization: quasi-horizontal structures in central area, arched as domes from place to place and a folded structure on the outside (milder on the western and southern and more pronounced on the eastern part), corresponding to the alignments of diapirs (Irimuş, 1998).

Salt lakes are an evolutionary and residual component of salt horizons from the base of sedimentary deposits, which under the pressure of sediments have migrated to the periphery in contact with the mountain area. As a result of migration, numerous salt domes formed and subsequently affected by subaerial agents, thus salt appearing

on surface (Irimuş, 1998; 2006). The appearance of salt led either to mining since Roman period or the occurrence of surface pseudokarst, resulting in numerous artificial or dissolution lakes (Gâştescu, 1971). All saline lakes presently found in the Transylvanian Basin formed by salt dissolution of underground halite deposits (Har *et al.*, 2010). They are located in Sovata, Ocna Sibiului, Ocna Mureş, Turda, Cojocna, Sic, Ocna Dej, and Jabeniţa areas (Alexe, 2010; Alexe and Şerban, 2014a, 2014b) (Fig. 1).

Limnology of most representative saline lakes

Nowadays, 41 salt lakes are counted among which 7 lakes (five in Sovata and two in Ocna Sibiului) appeared

by natural processes (or 'karstosaline' – a term proposed by Bulgăreanu, 1993; 1996) and the others being situated in ex-underground or surface salt mining areas (man-made or 'anthroposaline' lakes). Generally, Transylvanian salt lakes have small areas but with significant depths (Tabs. 1 and 2) (Alexe, 2010; Şerban *et al.*, 2005; Alexe and Alexe, 2008; Alexe and Şerban, 2014a).

Eleven Transylvanian saline lakes with significant depths (>-10 m) and salinity (>30%, w/v, total salts, at the bottom layer) developed ideal conditions for permanent density stratification and, therefore, are heliothermal. While the amplitude of this phenomenon remains quite low in most lakes (up to 5°C), in the Ursu Lake (Sovata)
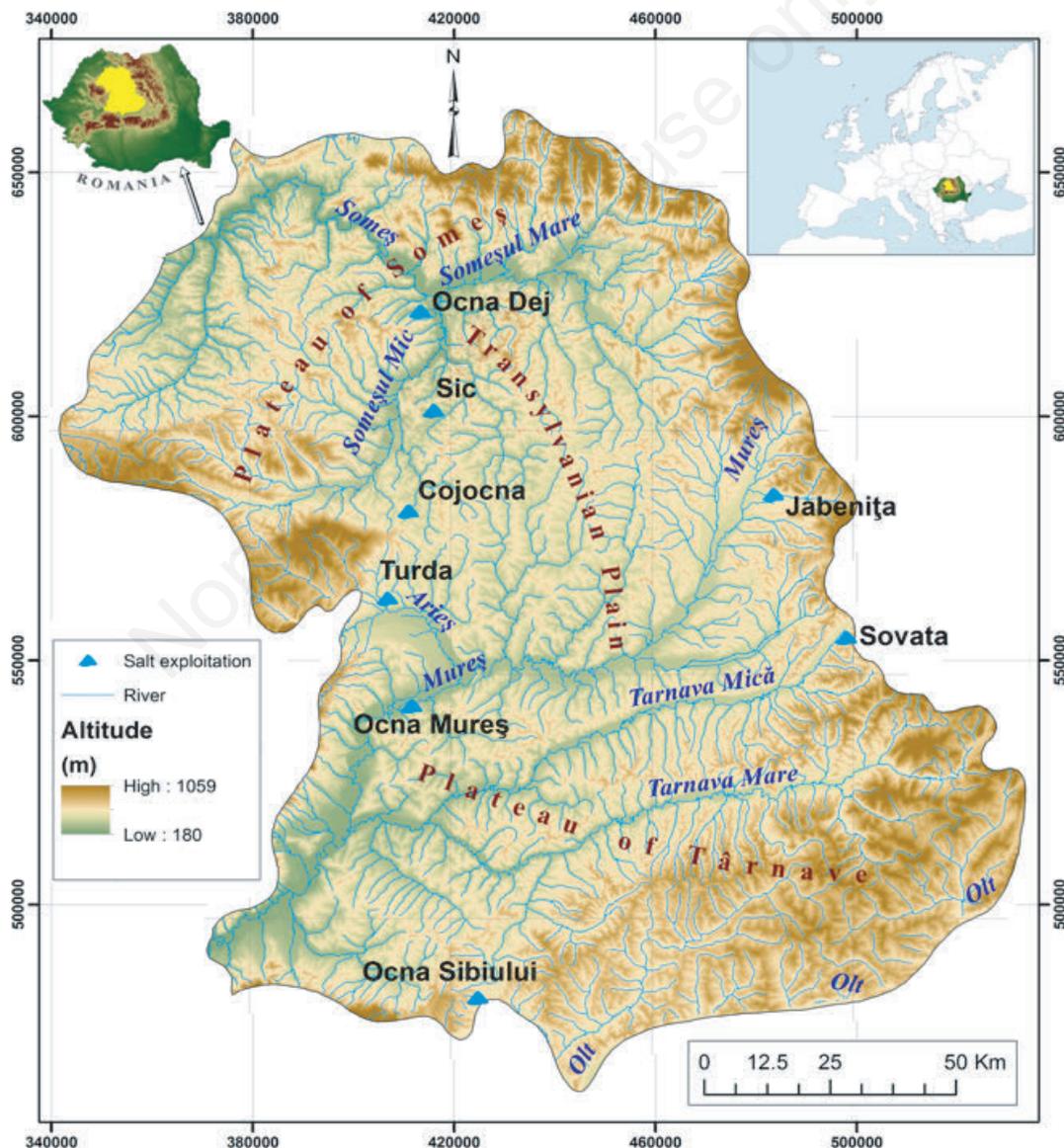

Fig. 1. Locations of salt lakes in the Transylvanian Basin at local (center), Romania (upper left) and Europe scales (upper right).

the temperature difference between the surface and heliothermal layer (1.5-3 m depth) is frequently exceeding 12°C and may reach up to 25°C during summer (Alexe and Şerban, 2006, 2014a, 2014b). Moreover, by its morphometry, Ursu Lake appears as one of the the largest saline heliothermal lakes in the world. Although rarely encountered, heliothermal bodies of water were reported on all continent and climate zones in Asia (*e.g.*, in Siberian and Mongolian steppes) (Sonnenfeld and Hudec, 1980; Egorov, 1993), Africa (Solar Lake, Sinai, Egypt) (Cohen *et al.*, 1977), North America (Hot Lake, WA, USA; Mahoney Lake, BC, Canada) (Zachara *et al.*, 2016; Northcote and Hall, 1983), Antarctica (Ekho Lake, Vestfold Hills) (Bowman *et al.*, 2000), Australia and Pacific Islands (Hudec and Sonnenfeld, 1989).

Brief descriptions of the saliferous Transylvanian areas with best investigated salt lakes are given below.

The basin of Sovata is a combe developed and formed by erosion along the diapiric anticline as a result of the epigenetic sinking processes of the Târnava Mică River and its tributaries in the diapiric folds (Alexe *et al.*, 2006). The most representative lake in Sovata basin is Ursu - or 'Bear' - (46°36'15 N; 25°05'09 E) (Fig. 2). It appeared following the accelerated dissolution of underground salt deposit that created a huge abruption, causing a landfall. The two nearby rivulets filled up this gap resulting in the present lake (with a contour resembling the shape of a bear fur). Unofficial sources (*i.e.*, not supported by cadastral information) indicated that the lake started to form between 1875 and 1879 (Lengyel, 1898).

In Turda area, the lacustrine complexes (Durgău-Valea Sărată, and Băilor complex) are situated in the vicinity of Turda city and lie on the western border of the Transylvanian Basin, in a diapiric area which appears in the open under the form of salt massifs (Alexe, 2010) (Figs. 1 and 2). The two lakes discussed in this review (Ocnei - 'Salt Mine's'*;* and Rotund - 'Round') are located in the Durgău-Valea Sărată lacustrine complex and exploited for outdoor bathing during warm seasons. Both lakes are fed by rainfall and freshwater springs and main-

Tab. 1. Main features of Transylvanian salt lakes.

Characteristic	Description
Number	- a total number of 41 salt lakes are charted in Transylvanian Basin (Central Romania).
Origin	- formed on the Miocene halite (NaCl) deposits dating from Badenian salt crisis event; - most of lakes (34) originated from collapsed salt mines (man-made or 'anthroposaline'), a few lakes (7) derived from natural dissolution of subterranean halite deposits (natural or 'karstosaline').
Surface and depth	- small surfaces (0.1 to ca. 4 ha); variable depths: from shallow (1-3 m) to deep lakes (10-100 m), the latter developed meromixis (see main text);- Ursu Lake (Sovata) is the largest (41,270 m^2) and deepest (18.20 m) karstosaline lake; the Ferdinand – 1 Mai (Ocna Mureş) and Ocniţa - Avram Iancu (Ocna Sibiului) lacustrine complexes are the largest (143,875 m^2), and deepest (127.10 m) man-made lakes, respectively.
Hydrology and circumlacustrine vegetation	- most are land-locked basins relying on meteoric water; only a few (*e.g.*, Ursu Lake) are fed with permanent water sources (*e.g.*, rivulets); - most lakes are surrounded by scarce (cut-grass) vegetation and only a few (*i.e.*, lakes in Sovata area) are surrounded by lush (forest) vegetation.
Salinity	- the majority of lakes (37) are brackish to moderately saline (1-10 % salinity) in surface, whereas 4 lakes are hypersaline (10-20% salinity) from surface.

Tab. 2. Morphometric elements of the representative lakes from Transylvania.

Lake	Elem./Year	Surface(m^2)	Lenght(m)	B$_m$(m)	B$_{max}$ (m)	h$_m$(m)	h$_{max}$ (m)	A(m)	a(m)	Perimeter(m)	C$_s$	Volume (m^3)
Ursu	1955[a]	41702	457.53	91.14	183.81	12.65	18.90	366.76	243.05	1257.2	1.74	489,966
	2002[b]	41270	455.95	90.51	204.61	11.84	18.20	366.02	238.78	1223.3	1.70	488675
Ocnei	1972[c]	1653.8	53.75	30.64	45.75	11.67	34.7	53.75	45.75	151.5	1.05	19,289.1
	2005[b]	2134.3	59.7	35.75	51.3	12.30	33.3	59.7	51.3	176.4	1.08	26249.4
Rotund	1972[c]	470	28.75	16.35	23.25	4.86	15.6	28.75	23.25	78.15	1.02	2243.3
	2005[b]	624.8	32.8	19.05	25.5	3.28	13.8	32.8	25.5	97	1.09	2050.6
Fără Fund	1973[d]	1655.0	55.0	30.10	47.8	6.72	32.95	55.0	47.75	151.5	1.05	11,114
	2000[b]	1672.2	52.2	32.03	59.02	6.04	32.0	52.2	59.02	156.3	1.08	10098.4
Brâncoveanu	2000[b]	1601.2	54.1	29.6	42.9	4.88	14.5	51.4	42.9	151.3	1.07	7811.6

B$_m$, average width; B$_{max}$, maximum width; h$_m$, mean depth; h$_{max}$, maximum depth; A, long axis; a, short axis; P, perimeter; C$_s$, sinuosity coefficient.
[a]Pişota, 1960; [b]Alexe (2010); [c]Pânzaru, 1974; [d]Pânzaru, 1977.

tained at a constant level by an artificial drainage channel towards the Sărata Valley. Lake Ocnei (46°35' N; 23°47' E) resulted from the collapse of an old salt mine around 1800 (Pânzaru, 1974). It has a circular shape, characteristic to bell-shaped salt lakes, with a small surface area and steep morphological profile (Fig. 2, Tab. 2). Ocnei Lake is permanently density-stratified (meromictic) with hypersaline monimolimnion and thus, develops heliothermy (*i.e.*, >30°C at 1.5-3 m depth) during summer. Situated nearby Ocnei Lake, Rotund Lake has a circular shape and a depth over 10 meter (Fig. 2, Tab. 2). It is heliothermal during summer and is one of the few saline lakes with steady morphological stability over time (*i.e.*, no substantial changes in depth and surface) (Tab. 2).

Ocna Sibiului is situated in the southern Transylvania (15 km NW of Sibiu city) (Fig. 2) and is place of the largest salt lake complexes in Romania that currently includes 14 lakes out of which 12 being of man-made origin. Among these lakes, Fără Fund ('Bottomless' - 45°52'34'' N; 24°04'03'' E) and Brâncoveanu (45°52'18'' N; 24°03'56'' E) are best explored to date (Andrei *et al.*, 2015, 2017a). Both lakes resemble in their origin, morphometry, and limnology. They resulted by the collapse of bell-shaped salt mines following water seepage and salt dissolution during 17th (Brâncoveanu) and 18th Cemtury (Fără Fund). Both are land-locked (*i.e.*, no permanent water input), small-surfaced, circular lakes with steep shores and significant depths (Fig. 2, Tab. 2). The distinctiveness of these lakes resides in their unusual hypersaline (>17%, w/v, total salts) surface due of the direct contact between water and salt massif. Brâncoveanu and Fără Fund lakes have been long time (since ca. 150 years ago) capitalized for recreational and therapeutic purposes for their hypersaline and heliothermal water alongside sapropelic muds. Similarly to Ursu Lake in Sovata, Fără Fund Lake is currently a nature reserve with a strict conservation status.

Numerous deep Transylvanian salt lakes are meromictic with the water column perenially layered into three physico-chemically distinct strata: a surficial one (mixolimnion, down to ca. 3 m depth), usually less saline (2-10% salinity), oxygenated and prone to pH, temperature and dial light fluctuations; a transition layer (chemocline, at 3-4 m depth) characterized by a sharp shift of salinity, dissolved oxygen concentration, pH, and, sometimes, oxidoreduction potential (ORP); and a bottom layer (monimolimnion, below 4 m depth) that is permanently hypersaline (>30% salinity), suboxic/anoxic (<0.1 mg·L^{-1} O$_2$), aphotic, slightly acidic (pH~6), and, in some cases such as Ursu Lake, highly reduced (>-200 mV) (Fig. 3a). In addition, water temperature of the monimolimnion is constantly around 20°C (Fig. 3a) (Andrei *et al.*, 2015; personal observations). Besides the three-layered model presented above, there are a few examples of Transylvanian lakes such as Fără Fund and Brâncoveanu that features a double-layered water column mainly because the water mass is extremely salty (>17% salinity) starting from the
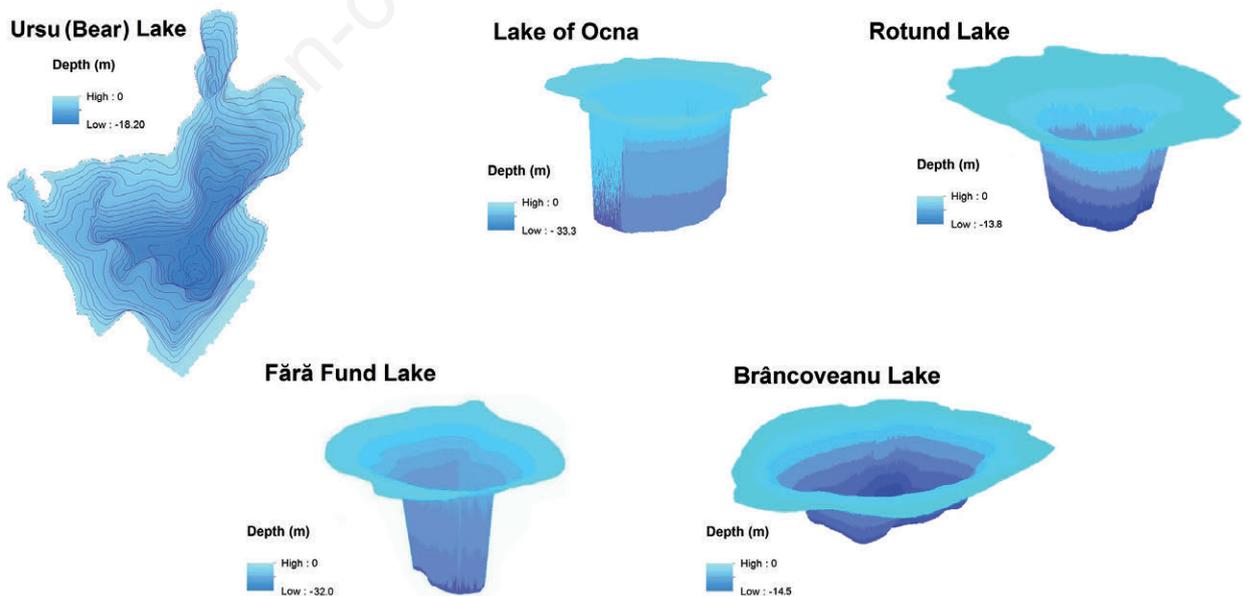

Fig. 2. Three dimensional images of the best investigated lakes to date: Ursu Lake (Sovata area), Ocnei and Rotund lakes (Turda area), Fără Fund and Brâncoveanu lakes (Ocna Sibiului area).

surface (Fig. 3a). Therefore, salinity gradient is only slightly increasing along the water column, with a minor halocline situated at ca. 2-2.5 m depth, at a lesser depth than in other meromictic lakes (*e.g.*, such as Ursu, Ocnei or Rotund) where halocline is at ca. 3-3.5 m depth (Baricz *et al.*, 2014; 2015; Andrei *et al.*, 2015; 2017a).

The meromictic nature of Transylvanian salt lakes such as Ocnei, Rotund, Brâncoveanu, Fără Fund, and Ursu lakes was revealed by *in situ*, multi-year (2009-2016) survey using a field multiparameter (pH/ORP/T/DO/salinity-meter, HI9828 model, Hanna Instruments) (Alexe, 2010; Baricz *et al.*, 2014; Andrei *et al.*, 2015, 2017a) and it was corroborated by the chemical analysis of water samples collected from different depths (Baricz *et al.*, 2014; Máthé *et al.*, 2014; Andrei *et al.*, 2015, 2017a, our unpublished data). Overall, the water chemistry indicated the dominance of Na^+ and Cl^- that

contribute to >90% of the total salts, followed by K^+, Ca^{2+}, Mg^{2+} among cations, and SO_4^{2-} and HCO_3^-/CO_3^{-2} among inorganic anions. Sulfate (SO_4^{2-}) and ammonium (NH_4^+) profiles followed that of salinity, with highest values detected in the deeper strata. The highly-reduced sulfides (mostly as H_2S) and methane (CH_4) were in significant (~5 mM and 0.06-0.3 mM, respectively) concentration in the bottom brine of Ursu Lake (Bulgăreanu *et al.*, 1978; Máthé *et al.*, 2014; Andrei *et al.*, 2015) while sporadically detected in trace amounts in monimolimnion of Fără Fund Lake (Andrei *et al.*, 2015; our unpublished data). The depth-dependent increase of highly reduced compounds is common among perennially stratified saline lakes. In meromictic brackish or saline lakes, sulfide- and ammonium-enriched bottom waters were reported in Lake A (Arctic Canada) (Gibson *et al.*, 2002), Lake Mahoney (British Columbia, Canada) (Klepac-Ceraj *et al.*, 2012),
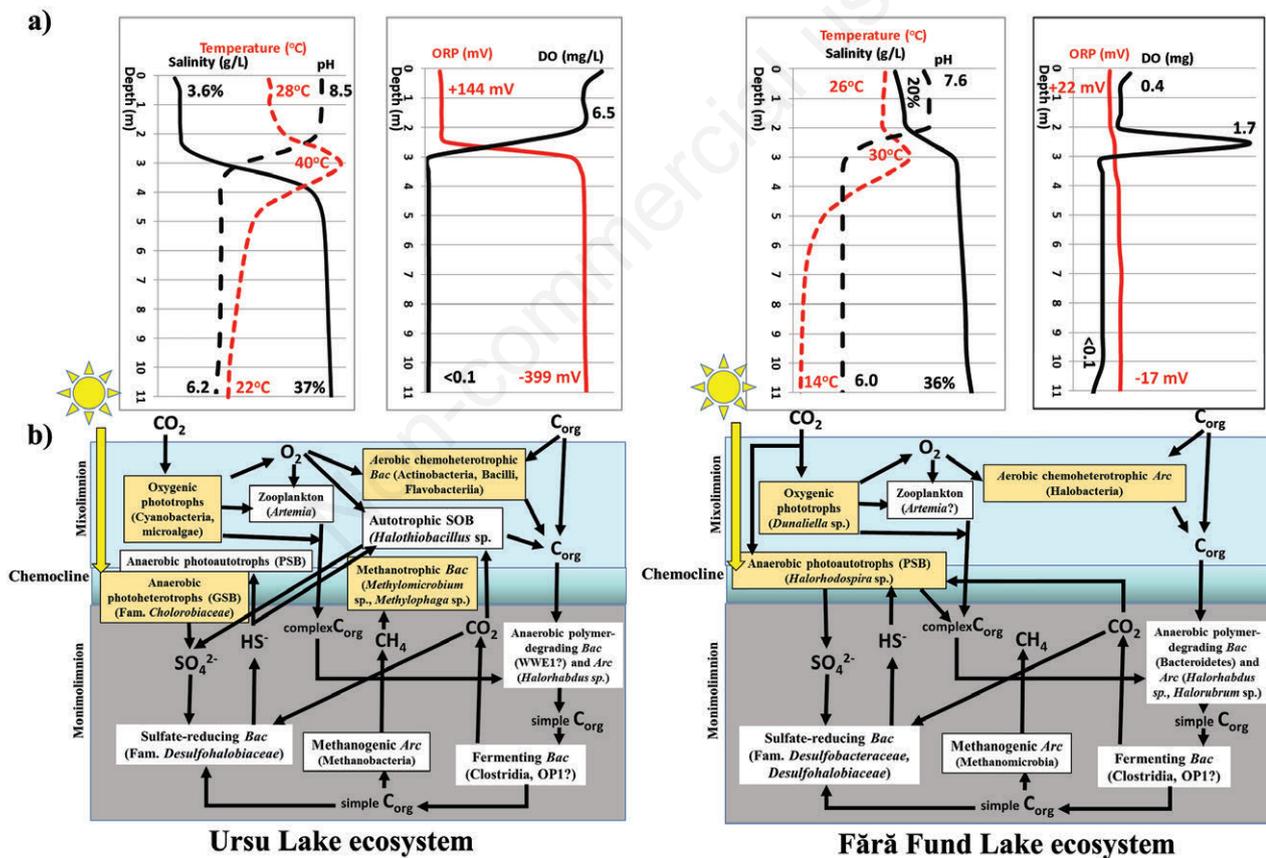

Fig. 3. a) Physico-chemical stratification of Ursu (left) and Fără Fund lakes (right) during 2015 summer season. The heliothermy is observable as temperature peak below surface; relevant values for salinity (black line), temperature (red dotted line), pH (black dotted line), DO (black line), and ORP (red line) are shown. b) The proposed biogeochemical cycling of carbon and sulfur in two aquatic meromictic systems, Ursu (left) and Fără Fund (right), based on DNA metabarcoding approach employed by Andrei *et al.* (2015). The metabolic groups that differed among two lakes are highlighted in red background. DO, dissolved oxygen concentration; ORP, oxidoreduction potential; C_{org}, organic carbon (complex C_{org} - polymeric substances of plant or animal origin; simple C_{org}, monomers used by fermenters and 1C or 2/3C fermentation products, respectively); Arc, *Archaea*; Bac, *Bacteria*; SOB, sulfur-oxidizing bacteria.

Kaiike (SW Japan) (Koizumi *et al.*, 2004b), Shira and Shunet lakes (Southern Siberia) (Baatar *et al.*, 2016); methane and ammonia are dominant reduced species in the anoxic monimolimnion of Ace Lake (Eastern Antarctica) (Laybourn-Parry and Bell, 2014) whereas ammonia (NH_3), sulfides and, sometimes, methane coexist at high concentration in anoxic monimolimnia of saline alkaline Big Soda, Mono, and Soap lakes (Kharaka *et al.*, 1984; Joye *et al.*, 1999; Humayoun *et al.*, 2003; Sorokin *et al.*, 2007; Dimitriu *et al*, 2008) (see also Tab. 3).

The main factor driving the permanent stratification of water column is the steady density gradient favoring the surface, less dense horizon to lay on the top of much denser water. The high depth and the stagnancy (that is, the lack of water intermixing) are the additional ingredients to make true meromictic aquatic systems (Boehrer and Schultze, 2008; Zadereev *et al.*, 2017). Thus, in such systems, dramatic change of physico-chemical properties (see above and Fig. 3a) occurs at relatively short distance (meters or even centimeters) resulting in the formation of largely distinct micro-habitats. Niche differentiation along

water column was observed in the meromictic saline Transylvanian lakes particularly when nutrient (bicarbonate/carbonate, sulfate, sulfides, ammonium, total phosphorous, total organic nitrogen, total organic carbon, *etc.*) and biological parameters (total photosynthetic pigments, total cell), were evaluated. In this regard, the zonated water column allowed predicting roughly three distinct ecological niches: i) the surface, less or moderately saline, oxic, and euphotic zone, highly impacted by environmental fluctuations favorable to non-halophilic and halotolerant aerobic macro- and microorganisms and to light-dependent aerobic primary producers (*e.g.*, algae, cyanobacteria); ii) the intermediate, chemocline zone that, on an approximate distance of 1 m, harbors a sudden transition from oxic to suboxic/anoxic, from euphotic to aphotic, and from saline to hypersaline conditions; this layer is apparently hosting several sub-niches where subpopulation of halotolerant and halophilic aerobic, microaerophilic and anaerobic photo- and chemotrophs might flourish; iii) the deep, dark, suboxic/anoxic, and hypersaline water layer, most probably to be inhabited by

Tab. 3. Main limnological and physico-chemical features of meromictic brackish, moderately saline, hypersaline, and alkaline saline lakes with substantial records in the literature. Ursu Lake was chosen as representative meromictic lake from the Transylvanian Basin.

Characteristics	Mahoney	Ace	Organic	Kaiike	Shunet	Solar*	Mono	Soap	Ursu
Area (km^2)	0.20	0.18	0.047	0.15	0.47	0.72	160	3.4	4.1
Max. depth (m)	18	25	7.5	11.6	6.2	5	45	27	18.6
Origin	Athalassic°	Thalassic	Thalassic	Thalassic	Athalassic	Thalassic	Athalassic#	Athalassic#	Athalassic
Heliothermy observed	Yes (2 m)	Yes (8 m)	No	Yes (4 m)	No	Yes (2.5-3 m)	No	No	Yes (2.5-3 m)
Mixolimnion									
Salinity (%)	1.7-2.9	1.3 - 2	16.6-18	0.2-0.3	1.76	3.5-5	7.7-9.4-	1.2-1.8	6-13
pH	8.9	8.2-8.3	7.7	8.2	7.05	8.0-8.2	9.8	7.7	7.6-8.7
Chemocline depth (m)	7-10	12.7	5-5.5	4.5	5	1-1.25	10-17 (oxicline), 23-26 (pycnocline)	18.5-21	3.5
Monimolimnion									
Salinity (%)	6-7	2.2 4.3	20-23	3.4	8.1	18-20	8.8-9.8	14	35
pH	7.35	7.0	7.0	7.7	5.8	6.9	9.8§	9.9	6.0
S^{2-} (mM)	30-35+ PS 0.2-0.4 mM	4-8	as DMS (5 µM)	1-2	13-15	1.2	0.4-3	125-200	2.5-5.0
NH_4^+ (mM)	5-8.8	Nm^	0.13-0.3	0.3	Nm	0.001-0.005	0.1	Nd	2
NH_3 (mM)	Nd	0.035	Nm	Nd	Nm	Nd	0.5	59-65	Nm
CH_4 (mM)	Nd	~ 0.02	Nd	Nd	Nm	0.006	0.002-0.055§	1§	0.06-0.3
ORP (mV)	Nm	Nm	+	Nm	-350	-185	Nm	Nm	-300

*During stable stratification period; °sulfate and divalent cation (Mg^{2+} and Ca^{2+})-rich ecosystem; #Na^+ is main cation, CO_3^{2-} + HCO_3^- and Cl^- (in roughly equal proportion) are major anions; §no published values for pH in deep water were found, however, the entire water mass is strongly buffered at pH 9.8-10.0; ^originally, the reduced nitrogen species reported was ammonia (NH_3); $high methane (alongside low molecular weight alkanes) is explained partly by thermogenic gas seeps in Mono Lake whereas no clear explanations exist for Soap Lake. PS, polysulfides (S_n^{2-}); DMS, dimethylsulfides; Nm, not measured; Nd, not detected. References: Mahoney Lake –Northcote and Hall, 1983; Hall and Northcote, 1986; Klepac-Ceraj et al., 2012; Organic Lake – Franzmann et al., 1987; Gibson, 1999; Yau et al., 2013; Ace Lake – Gibson, 1999; Rankin et al., 1999; Laybourn-Parry and Bell, 2014; Kaiike Lake – Matsuyama, 1977; Koizumi et al., 2004b; Ohkouchi et al., 2005; Shunet Lake – Degermendzhy et al., 2010; Baatar et al., 2016; Solar Lake – Cohen et al., 1977; Cytryn et al., 2000; Mono Lake – Jellison and Melack, 1993; Joye et al., 1999; Humayoun et al., 2003; Soap Lake – Walker, 1974; Oremland and Miller, 1993; Dimitriu et al., 2008; Ursu Lake – Máthé et al., 2014; Andrei et al., 2015; our unpublished results.

extreme halophilic anaerobic microorganisms that derive energy from chemical reactions only. This picture was indeed, largely confirmed by the assessment of microbial diversity by culture-dependent and molecular techniques (see below).

Eukaryotic and prokaryotic diversity in saline Transylvanian lakes

Early evaluations of biodiversity associated with ecosystems of Transylvanian salt lakes mostly concerned littoral, planktonic and benthic flora and fauna (Maxim, 1931, 1936, 1941; Todor, 1947; Bulgăreanu *et al.*, 1978; Pop and Hodişan, 1980; Spiridon *et al.*, 1984; Ionescu *et al.*, 1998). The circumlacustrine flora of saline lakes was represented by members of up to 60 families, with *Poaceae* and *Asteraceae* as most frequently encountered (Pop and Hodişan, 1980; Ionescu *et al.*, 1998; Frink *et al.*, 2013). A few halophytes (*i.e.*, salt-adapted plants) were identified such as *Puccinellia distans* (Jacq.), *Artemisia maritima* (L.), *Aster tripolium* (L.), *Inula britannica* (L.), *Suaeda maritima* (L.) Dumort., and *Salicornia europaea* (L.) whereas the vast majority of vegetation surrounding investigated saline lakes comprised non-halophilic species (or 'glycophytes'). The listed halophytic species are typical components of vegetation associated with wet and highly saline (*i.e.*, *Puccinellietea*) or extremely saline (*i.e.*, *Salicornietea*) soils in Central and South-Eastern Europe, respectively (Piernik, 2005; Eliáš *et al.*, 2013; Stevanović *et al.*, 2016).

In contrast to salt lakes located in Cojocna, Turda, and Ocna Sibiului that are bordered by meadow or cut grass vegetation, the Sovata lakes are surrounded by lush, forest-like vegetation of '*Quercetum robur petrae*' type comprised of deciduous trees (*Quercus robur*, *Q. petraea*, *Alnus glutinosa*, *Fagus syilvatica*, *Carpinus betulus*) and numerous grass species. Littoral or aquatic macrophytes are mostly represented by hydrohalophytes such as *Potamogeton pectinatus* (L.), *Phragmites australis* (Cav.) Trin. ex Steud., and *Typha latifolia* (L.) (Ionescu *et al.*, 1998; Frink *et al.*, 2013). These aquatic species have wide geographical range in wetlands with varying salinity (Hammer and Hesseltine, 1988; Santamaria, 2002). It is worth mentioning that the abundance of shore vegetation might have a consistent contribution to the allochtonous input of organic matter to the lake ecosystem (Fig. 3b) that ultimately would enhance the formation of organic-rich sediments (sapropels) of economic value (Bulgăreanu *et al.*, 1978; 1983; 1989; Bulgăreanu, 1993). Following a survey on six tropical lakes, Talbot (1988) concluded that the accumulation of oil prone (*i.e.*, organic-rich) lacustrine sediments is favored by a combination of high surface productivity (both as phytoplankton and higher plants input) and stable stratification regime of the water column.

Halotolerant (or 'euryhaline') representatives of green

algae (*Chlamydomonas* sp., *Picochlorum* sp., *Mantoniella* sp. *Enteromorpha* sp.) and diatoms (*Amphora* sp., *Navicula* sp., *Nitzschia* sp., *Surirella* sp.) were identified both by microscopic (Bulgăreanu *et al.*, 1978; Ionescu *et al.*, 1998; our unpublished results) and molecular investigations (*i.e.*, PCR-DGGE) (Keresztes *et al.*, 2012; Máthé *et al.*, 2014) in the phytoplankton of brackish or moderately saline (1-10% salinity) shallow lakes or mixolimnia of meromictic Transylvanian lakes. Considered as marine native, the microalgae *Picochlorum* spp. were previously detected in inland saline pans (Henley *et al.*, 2004), hypersaline lake in Tibet (Wu *et al.*, 2009), and African soda lakes (Krienitz *et al.*, 2012). By using pyrosequencing approach, Lauro *et al.* (2011) obtained *Mantoniella* sp.-related 18S rRNA gene sequences at high abundance in the mixolimnetic communities of Antarctic stratified Ace Lake. Analogous to the marine-derived Ace Lake's phytoplankton community, most of the unicellular green algae (*Chlorophyta*) and diatoms detected in Transylvanian salt lakes appeared as marine species that adapted to lacustrine conditions. However, the Transylvanian saline lakes are far more distant from any sea source than their equivalent Antarctic lakes (Keresztes *et al.*, 2012).

Halophilic (or 'stenohaline') green algae *Dunaliella* sp. were abundantly observed (up to 7 x 10^3 cells·mL^{-1}) in hypersaline photic layer of lakes in Ocna Sibiului area (Ionescu *et al.*, 1998; Keresztes *et al.*, 2012; Somogyi *et al.*, 2014; Andrei *et al.*, 2015) (Fig. 4). Genus *Dunaliella* comprised obligate halophilic species (*D. viridis* and *D. salina*) with a broad salt range for growth from 0.9% (w/v) NaCl up to saturation (Oren, 2014). *Dunaliella* sp. cells are thriving as major if not sole components of phytoplankton communities in hypersaline habitats such as high-salt lakes (*e.g.*, Great Salt Lake where it reached peak density of 3-10 x 10^3 cells·mL^{-1}, or Dead Sea where it recently vanished due to increase of divalent cation contents) and crystallizer ponds (*e.g.*, Eilat, Israel – up to 3 x 10^3 cells·mL^{-1}, and Santa Pola, Spain - up to 10 x 10^3 cells·mL^{-1}) (Oren, 2014). Noteworthy, the *Dunaliella salina* (Dunal) Teod. was introduced as novel genus and species by the Romanian botanist Emanoil C. Teodorescu following his morphological and habitat description on living specimens collected from salt coastal lakes in SE Romania (Oren, 2014). A few observations on presence of *Dunaliella* sp. were made in lower euphotic layer (or upper chemocline) of deep, stratified saline lakes such as the alkaline saline Big Soda Lake (with a maximum density of 9.7 x 10^3 cells·mL^{-1}) (Priscu *et al.*, 1982) and Antarctic ice-covered Organic Lake (up to 28 x 10^3 cells mL^{-1}) (Franzmann *et al.*, 1987).

The prokaryotic members of phytoplankton present in upper, photic and moderately saline (2-13% salinity) layers of Transylvanian salt lakes include cyanobacteria of *Synechococcus* sp. (classified into marine Red Sea clade

VIII by Keresztes *et al.*, 2012), *Chroococcus* sp., *Cryptomonas* sp., *Chrysococcus* sp. (Bulgăreanu *et al.*, 1978), *Cyanothece* sp., and *Leptolyngbya* sp. (Andrei *et al.*, 2015, our unpublished results). These cyanobacteria are ubiquitous in freshwater, brackish or marine habitats and evidences on their occurrence in (hyper)saline environments (*i.e.*, with salinity exceeding 7%) are rather sparse.

For example, *Chroococcus* spp., *Leptolyngbya* spp., and *Synechoccocus* spp. alongside diatoms such as *Nitzschia* sp. were reported in crystallizer ponds with 11-18% (w/v) total salts at Port Fouad, Egypt (Madkour and Gaballah, 2012); surficial microbial mats in shallow water (~8% salinity) of hypersaline, Solar Lake (Sinai, Egypt) harbored *Synechococcus* spp. and diatoms assigned to
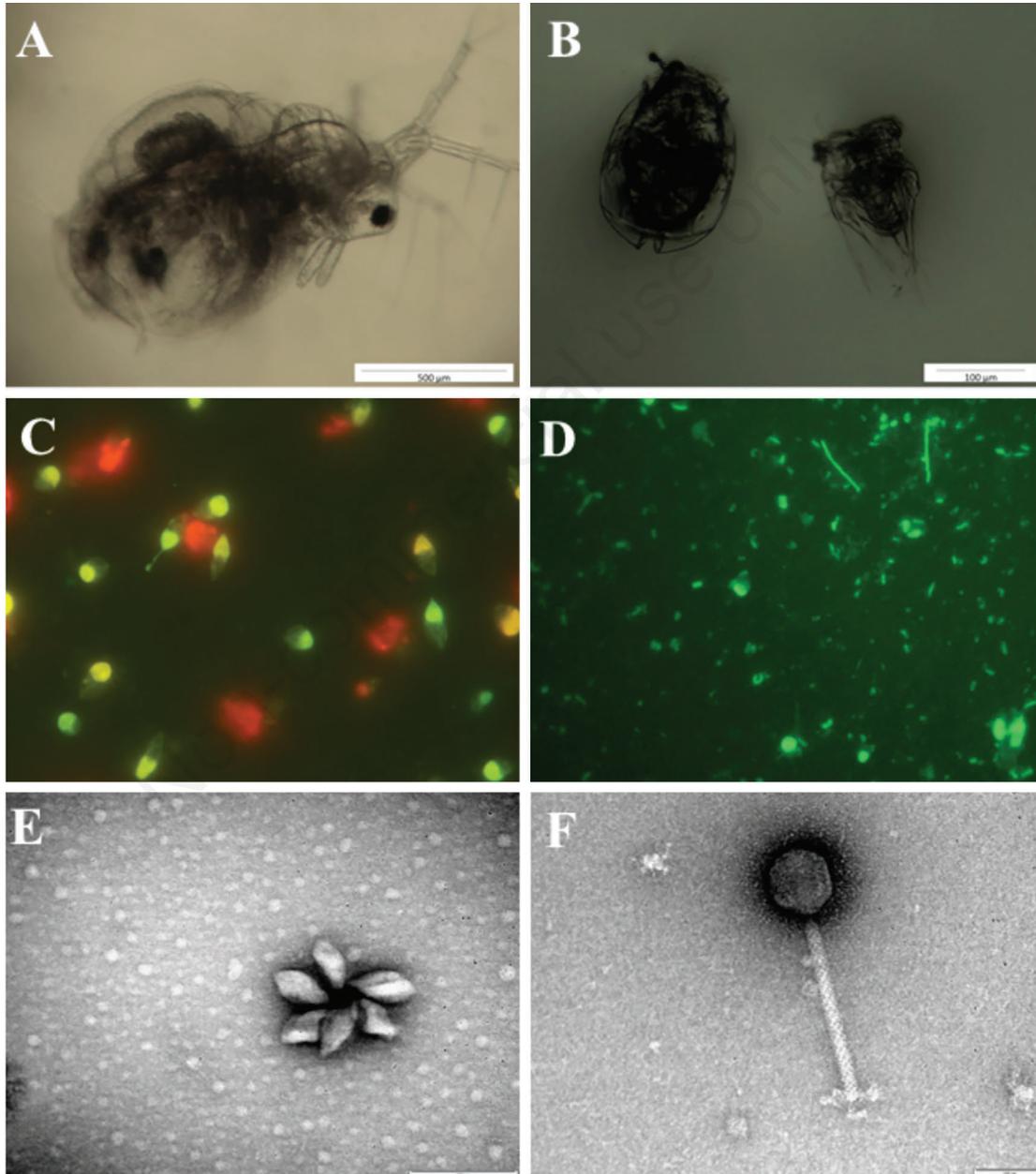

Fig. 4. Images of zoo-, phyto-, bacterio-, and virioplankton diversity in salt lakes of Transylvania: cladocerans (*Moina salina*, female) (A) and rotifers from Ursu Lake (Sovata) (B); epifluorescence microscopy of unicellular algae from Brâncoveanu Lake (Ocna Sibiului) (C); various microbial morphotypes in Tarzan Lake (Turda) observed in DAPI-stained samples (D); transmission electron microscopy of spindle-shaped (E) and isometric (F) viruses from Fără Fund lake (Ocna Sibiului). Scale bars: A) 500 µm; B), 100 µm; E) 200 nm; F) 100 nm.

Navicula, *Nitzschia*, and *Amphora* genera (Jørgensen *et al.*, 1983) whereas *Cyanothece* spp. were observed as dominant cyanobacterium in the microbial mat (~18% salinity) of hypersaline, seasonally stratified Lake Hayward, SW Australia (Burke, 1995).

In surface layers (2-13% salinity) of Transylvanian salt lakes, Somogyi *et al.* (2014) reported significant densities (*i.e.*, 0.1 - 7.6 x 10^6 cells·mL^{-1}) of picophytoplankton cells (*i.e.*, with diameter <2 μm and comprising mostly cyanobacteria but also microalgae such as *Picochlorum* sp. or *Dunaliella* sp.) suggesting a crucial role in the oxygen formation and light-dependent primary productivity in these saline aquatic ecosystems. In comparison, the brackish upper layers (~2-3% salinity) of marine-derived meromictic boreal Kaiike and Antarctic Ace lakes, the *Synechococcus* sp.-dominated phytoplankton peaks to 3 x 10^6 and 8 x 10^6 cells·mL^{-1}, respectively (Koizumi *et al.*, 2004b; Laybourn-Parry and Bell, 2014). It was suggested that the high abundance of picophytoplankton communities in Transylvanian salt lakes might be favored by the absence of grazers such as heterotrophic nanoflagellates that were, indeed, not observed in these lakes (Somogyi *et al.*, 2014).

The zooplankton dwelling the oxic, brackish or moderately saline (2-13% salinity) water layers of Transylvanian salt lakes include freshwater and marine copepods (*Canthocamptus sp.*.), halotolerant cladocerans (*Moina salina*), calanoid (*Arctodiaptomus salinus*) and harpaticoid copepods (*Cletocamptus retrogressus*), and rotifers (*Brachionus plicatilis*) (Fig. 4) (Ionescu *et al.*, 1998; personal observations). Dense populations of brine shrimp *Artemia salina* (Crustacea, Anostraca), a well-documented inhabitant of salt waters worldwide (Triantaphyllidis *et al.*, 1998), were observed in the highly saline (up to 20% salinity) surface water of lakes located in Ocna Sibiului, Ocna Şugatag, Turda, and Sovata but further detailed investigations are lacking (Botnariuc and Orghidan, 1953; Bulgăreanu *et al.*, 1996; Demeter and Stoicescu, 2008). The diversity of halotolerant and halophilic zooplankton in Transylvanian salt lakes is similar to that of coastal saline lakes in Spain (Alonso, 1990), salt pans and shallow saline lakes in Italy, Albania, and Crimea (Moscatello and Belmonte, 2009), and inland shallow saline lakes in SW Siberia (Litvinenko *et al.*, 2013). A general distribution pattern was suggested for salt-adapted metazoans in which Anostraca (*Artemia* spp.) are frequently present in marine-derived (thalassic) salt lakes or in hypersaline conditions, whereas halotolerant representatives of Copepoda (mainly calanoids) and Cladocera are likely distributed in continental (athalassic) and moderately saline bodies of water (Belmonte *et al.*, 2012). Noteworthy, the cladoceran *Moina salina* was first described from Turda and Ocna Sibiului salt lakes, the Transylvanian Basin being indicated as its 'terra typica' (Negrea, 1984).

To predict the ecosystem functioning, several attempts were carried out to elucidate the abundance, diversity and possible metabolic roles of microorganisms in Transylvanian saline lakes. For example, the archaeal communities in Rotund, Ocnei, Fără Fund, Brâncoveanu, and Ursu lakes were assessed by traditional (culture-based) and molecular (culture-independent) methods (Baricz *et al.*, 2014, 2015; Máthé *et al.*, 2014; Andrei *et al.*, 2015, 2017a) (Tab. 4). The quantitative Real-Time PCR (qPCR) analysis evidenced high archaeal cell densities (x 10^6 cells mL^{-1}) in hypersaline Ocnei (6-110), Brâncoveanu (6-60), and Fără Fund lakes (0.44-140), a finding corroborated by the increased diversity of *Archaea* in these lakes (Baricz *et al.*, 2014; Andrei *et al.*, 2015; 2017a). High archaeal cell abundances were detected in hypersaline chemocline and monimolimnion of Ocnei Lake, throughout entire water mass in Brâncoveanu, and in mixolimnion and chemocline of Fără Fund Lake in contrast to Ursu Lake where bacterial cell densities (3.6-66.5 x 10^6 cells mL^{-1}) exceeded that of *Archaea* (0.8-2.4) along the entire water column. High ratio of archaeal to bacterial cell numbers was shown in hypersaline anoxic sediments of Salton Sea (California), where Archaea accounted for up to 99% of the total prokaryotes (Swan *et al.*, 2010).

Best represented *Archaea* group in hypersaline Transylvanian lakes pertained to Halobacteria class (or 'haloarchaea') within Euryarchaeota phylum (Tab. 4). Haloarchaea include obligate halophiles relying on aerobic, facultatively or obligately anaerobic chemoheterotrophic metabolism (Andrei *et al.*, 2012; Sorokin *et al.*, 2017). At least 18 different haloarchaeal genera including the *Haloferax* sp., *Halobacterium* sp., *Halorubrum* sp. as most frequently encountered genera were retrieved both by culture and molecular approaches (Baricz *et al.*, 2014, 2015; Andrei *et al.*, 2015, 2017a). The culturable diversity of aerobic haloarchaea reported in the saline Transylvanian lakes exceeded that reported for other inland hypersaline habitats in Turkey, Iran, and Spain (Özcan *et al.*, 2007; Makhdoumi-Kakhki *et al.*, 2012; Luque *et al.*, 2012). The most hypersaline lake Brâncoveanu (>20% salinity starting from surface) was found to harbor the highest diversity of archaeal community possibly including the recently described *Halanaerorchaeum sulfurireducens* with the unusual anaerobic S^0-reducing metabolism (Sorokin *et al.*, 2016). The O$_2$-depleted hypersaline monimolimnia and bottom sediments of investigated salt lakes are preferred niches for microaerophilic *Halomicrobium* sp. or anaerobic, polymer-degrading haloarchaeon *Halorhabdus* sp. (Baricz *et al.*, 2015; Andrei *et al.*, 2015, 2017a). Based on the retrieved small-subunit ribosomal RNA (SSrRNA) gene sequences other archaeal groups were inferred including methanogens from *Methanosarcinales* (*e.g.*, *Methanohalobium* sp.), MSBL1 candidate division, and Thermoplasmata (Máthé *et al.*, 2014; Andrei *et al.*, 2015)

in Ursu and Fără Fund lakes, whereas reads related to uncultured archaeal divisions were detected in anaerobic layers of Ursu (Parvarchaea), Fără Fund (Parvarchaea and Nanohaloarchaea) (Andrei *et al.*, 2015) and Brâncoveanu (Nanohaloarchaea) (Andrei *et al.*, 2017a) lakes, respectively. Additionally, Parvarchaea was suggested as abundant component of microbial communities from hypersaline organic-rich sediments (sapropels) of Ursu and Fără Fund lakes (Andrei *et al.*, 2017b). The ultra-small-sized (<0.8 μm) Nanohaloarchaea were identified by *de novo* assembly of metagenomes from hypersaline lakes (Lake Tyrell, Australia) and halite nodules (Atacama Desert) where they appear to thrive under aerobic and hypersaline conditions alongside members of Halobacteria (Ghai *et al.*, 2011; Narasingarao *et al.*, 2012; Podell *et al.*, 2013; Crits-Cristoph *et al.*, 2016). Whereas Nanohaloarchaea seem to occur in the hypersaline environments worldwide (Zhaxybayeva *et al.*, 2013), members of Parvarchaea have not been yet evidenced in such systems. In this light, our finding of occurrence of Parvarchaea in hypersaline systems is unprecedented (Andrei *et al.*, 2015).

Comprehensive efforts on revealing bacterial diversity in Transylvanian salt lakes showed a coherent picture of niche-based distribution of bacterial communities (Tab. 4). For example, numerous aerobic halotolerant or slightly halophilic chemoheterotrophic strains belonging to Gamma-Proteobacteria (*Halomonas* sp., *Marinobacter* sp., *Salinivibrio* sp., *Idiomarina* sp., *Pseudoalteromonas* sp.), or Firmicutes (*Bacillus* sp.) were isolated from the shallow and moderately saline Mierlei and Roşu lakes (2-15% salinity, 2 m maximum depth) as well as from the mixolimnion of Ursu lake (2-10% total salinity) (Borsodi *et al.*, 2013; Crognale *et al.*, 2013; Máthé *et al.*, 2014). Insights into bacterial community composition were further retrieved by 16S rRNA gene clone library and DGGE in Mierlei, Roşu, and Ursu lakes (Borsodi *et al.*, 2013; Crognale *et al.*, 2013; Máthé *et al.*, 2014) whereas a deeper resolution of microbial diversity along the water columns and sediments of Ursu and Fără Fund was gained by DNA metabarcoding analysis (Andrei *et al.*, 2015; Andrei *et al.*, 2017b). Thus, in the oxic, moderately saline (2-13% salinity) water horizon of investigated lakes (Mierlei, Roşu, Ursu) in Sovata,

Tab. 4. Summary of bacterioplankton diversity of Transylvanian salt lakes explored to date.

Water horizon/layer (depth)	Bacteria	Archaea
Shallow lakes and mixolimnia of meromictic lakes (0-2.5 m) with low-to-moderate salinity, oxic conditions and fluctuating environmental parameters (temperature, pH, light)	**Culture-dependent methods**[1,2,3] Gamma-Proteobacteria (*Vibrio* sp., *Marinobacter* sp., *Salinivibrio* sp., *Halomonas* sp., *Idiomarina* sp.) and Firmicutes (*Marinoccocus* sp.) **Molecular methods** (PCR-DGGE[1], DNA metabarcoding[3,5]) Actinobacteria, Flavobacteriia *(Polaribacter* sp., *Psychroflexus* sp.), Mollicutes, Bacilli, Planctomycetia *(Planctomyces* sp.), Alpha-Proteobacteria (*Roseovarius* sp., Beta-Proteobacteria, Gamma-Proteobacteria (*Halorhodospira* sp., *Pseudoalteromonas* sp.), Cytophagia	Members of Halobacteria (*e.g. Halorubrum* sp., *Haloferax* sp.) were detected by culture-dependent[1,4] and molecular methods (DNA metabarcoding[5] and 16S rRNA-gene clone libraries[6,7]) mostly in hypersaline mixolimnia.
Transition zone (2.5-4 m) with steep salinity, O$_2$, light, and pH gradients	**Culture-dependent methods**[2,3] Gamma-Proteobacteria (*Salinivibrio* sp*., Halomonas* sp*., Chromohalobacter* sp.) Firmicutes (*Salimicrobium* sp*., Halobacillus* sp.); **Molecular methods** (PCR-DGGE[2], DNA metabarcoding[3,5]) Actinobacteria, Nitriliruptoria, [Rhodotermi] *([Balneola]* sp.), Chlorobia *(Prosthecochloris* sp.), Cyanobacteria *(Synecochoccus* sp.), Deferribacteres, Clostridia, Alpha-Proteobacteria, Gamma-Proteobacteria (*Salinivibrio* sp., *Vibrio* sp., *Halothiobacillus* sp.)	Members of Halobacteria were detected by traditional[4] and molecular methods (DNA metabarcoding[5] and 16S rRNA-gene clone libraries[6,7]). [Parvarchaea] candidate group was detected by DNA metabarcoding[e].
Monimolimnion (>4 m depth) with constant physico-chemical conditions (*i.e.*, hypersaline, suboxic/anoxic, aphotic)	**Culture-dependent methods**[3] Gamma-Proteobacteria (*Salicola* sp., *Idiomarina* sp.) and Firmicutes (*Marinococcus* sp.) **Molecular methods** (DNA metabarcoding[3,5]): Actinobacteria, Firmicutes (Clostridia: *Halanaerobium* sp., *Acetohalobium* sp.), Chlorobi, Alpha-Proteobacteria, Gamma-Prroteobacteria (Chromatiales, *Halorhodospira* sp.), Delta-Proteobacteria *(Desulfovermiculus* sp., *Desulfovibrio* sp.), [OD1], [OP1], [ABY1], [KB1]	**Culture-dependent methods**[4] Halobacteria (*Halarchaeum* sp*., Natrinema* sp*., Haloferax* sp*., Halorubrum* sp*., Haloarchaeobius* sp*., Halomicrobium* sp.)**Molecular methods** (DNA metabarcoding[5] and 16S rRNA-gene clone libraries[6,7]) [Parvarchaea], Methanobacteria, Methanomicrobia, Thermoplasmata, Halobacteria (*Salarchaeum* sp., *Halorhabdus* sp., *Halonotius* sp., *Halarchaeum* sp., *Haloarcula* sp., *Halobacterium* sp. and others)

[1]*Borsodi* et al*., 2013;* [2]*Máthé* et al*., 2014;* [3]*our unpublished data;* [4]*Baricz* et al*., 2015;* [5]*Andrei* et al*., 2015;* [6]*Baricz* et al. *2014;* [7]*Andrei* et al*., 2017a.*

most bacterial OTU$_{0.03}$s (Operational Taxonomic Units clustered at 97% cutoff similarity) pertained to aerobic heterotrophic representatives of Actinobacteria (*Microbacteriaceae*), Bacteroidetes (*Balneolaceae*, *Cryomorphaceae*), Alpha- (*Rhodospirillaceae*, *Rhodobacteraceae*), Beta- (*Alcaligenaceae*), and Gamma-Proteobacteria (*Halomonadaceae* and Altermonadales OM60 clade) (Borsodi *et al.*, 2013; Crognale *et al.*, 2013; Máthé *et al.*, 2014; Andrei *et al.*, 2015). The intermediate zone of Ursu Lake (that showed a steep halocline from 10 to 25% salinity paralleled by a sharp drop of dissolved oxygen concentration and photosynthetically active radiation over just 1 m vertical length) (Fig. 3a; Máthé *et al.*, 2014; our observations), is shared by oxygenic phototrophs (*Synechococcaceae*, Cyanobacteria) (upper chemocline), green sulfur bacteria (GSB) of *Chlorobiaceae* (Cholorobi) (lower chemocline), Actinobacteria, Bacteroidetes, and Proteobacteria (Máthé *et al.*, 2014; Andrei *et al.*, 2015). Noteworthy, the moderately saline upper water layer of Ursu lake is largely inhabited by *Bacteria* (~98% of OTU$_{0.03}$s relative abundance) in opposite to the hypersaline (20-25% salinity) mixolimnion and chemocline of Fără Fund lake that appeared as ideal ecological niche for *Archaea* from Halobacteria class (~95% relative abundance) seconded by Bacteroidetes (2-3%) and Gamma-Proteobacteria (1-2%). Reads belonging to the latter group were mostly assigned to extreme halophilic purple sulfur bacteria (PSB) of *Ectothiorhodospiraceae* (*Halorhodospira* sp.) that were scarcely found in Ursu water column. The bottom brines (~35%, w/v, total salts) of Ursu and Fără Fund lakes accommodated roughly similar microbial communities with *Bacteria* (65-70% relative abundances) prevailing over *Archaea* (ca. 25-30%). OTU$_{0.03}$s assigned to anaerobic sulfate-reducing Delta-Proteobacteria (*Desulfovermiculus* sp.), extremely halophilic heterotrophic Clostridia (*Halanaerobiaceae*, *Halanaerobacteroidaceae*, and *Clostridiaceae*) and Spirochaetes (*Spirochaetaceae*) dominated among DNA metabarcoding-inferred taxa. Intriguingly, sequences pertaining to uncultured or unclassified bacterial divisions (*e.g.*, OP1/Acetothermia, OD1/Parcubacteria) were found at significant (1-2%) abundances in the water columns and organic-rich sediments of Ursu and Fără Fund lakes (Andrei *et al.*, 2015; 2017b).

To our knowledge, the Transylvanian deep, hypersaline, and perennially stratified aquatic systems have no match with analogous lakes worldwide. Besides freshwater systems such as European (Faro, Cadagno, Carinthian) or African tropical lakes, the best investigated meromictic lakes to date are either brackish (*e.g.*, Antarctic lakes, Kaiike and Shira lakes), moderately saline (*e.g.*, Shunet and Organic lakes), shallow hypersaline with holomixis episodes (Solar Lake, Sinai) or alkaline saline lake (*e.g.*, Big Soda, Mono, and Soap lakes) (Zadereev *et al.*, 2017). In most of the systematically explored meromictic brack-

ish or saline lakes, the aerobic and versatile heterotrophic (*e.g.*, Actinobacteria, Bacteroidetes, Verrucomicrobia, *Alteromonadaceae* and *Halomonadaceae* within Gamma-Proteobacteria) and autotrophic (Cyanobacteria) bacterial communities prevailed in the euphotic mixolimnia (Lauro *et al.*, 2011; Comeau *et al.*, 2012; Yau *et al.*, 2013). Generally, plates of low-light phototrophic PSB (*Chromatiaceae*) (Kaiike, Mahoney, Shira, and Shunet lakes) (Koizumi *et al.*, 2004a; 2004b; Klepac-Ceraj *et al.*, 2012; Baatar *et al.*, 2016) and/or anoxygenic GSB (*Chlorobiaceae*) (*e.g.*, Ace Lake, Arctic Lake A) (Lauro *et al.*, 2011; Comeau *et al.*, 2012) were found in the lower chemocline, at or just below oxic/anoxic transition zone. In most perennially stratified waters, the anoxic or euxinic (*i.e.*, anoxic and H$_2$S-rich) bottom layer is more taxonomically diverse than surface stratum. Sulfate-reducing Delta-Proteobacteria, members of Firmicutes (Bacilli, Clostridia) (Mono, Mahoney, and Organic lakes) (Humayoun *et al.*, 2003; Klepac-Ceraj *et al.*, 2012; Yau *et al.*, 2013), and candidate divisions OD1 (Organic and Ace lakes) (Lauro *et al.*, 2011; Yau *et al.*, 2013) or OP1 (Mahoney Lake) (Klepac-Ceraj *et al.*, 2012) were detected as prevalent bacteria in anoxic bottom layers of brackish and saline meromictic lakes.

Not at least, it is worth mentioning that our attempts to shed light on the microbial diversity and its role in the trophic webs of the Transylvanian salt lakes has led to preliminary evidences for high-salt specific viral communities ('haloviruses') in the hypersaline Fără Fund lakes both by metagenomic and imaging tools (Fig. 4). Haloviruses may have significant role in controlling the abundance of halophilic microbial communities (Atanasova *et al.*, 2015).

Two models of ecosystem functioning in hypersaline meromictic lakes

Permanently stratified, pH-neutral saline aquatic systems apparently feature very similar limnological and environmental conditions. Considerable depth and stagnancy favor establishment of a density gradient and strong stratification of water column into three main layers and, implicitly, three distinct ecological niches (as described above and outlined in Tab. 1 and Fig. 3a). Thus, one would assume that analogous water layers would harbor similar diversity among meromictic lakes. However, our observation on the vertical distribution of microbial community composition in the two saline meromictic Ursu and Fără Fund lakes (Andrei *et al.*, 2015) revealed major differences among related water layers, namely mixolimnion and chemocline. As consequence, distinct ecological functioning of the apparently similar aquatic systems could be envisaged. Although DNA metabarcoding approach is a good estimate for microbial diversity at a very fine scale, functionality of microbial communities

can only be inferred by matching known taxa to taxon-specific metabolic function. Large pictures of biogeochemical cycling of main elements (C, N, P, and S) were drawn for meromictic Transylvanian salt lakes (Andrei *et al.*, 2015) allowing distinction of two models of ecosystem functioning: 'Bacteria-dominated' and 'Archaea-dominated' saline meromictic lakes with Ursu and Fără Fund lake, respectively, as model lakes (Fig. 3b).

The distinctiveness of the 'Bacteria-dominated' saline (Ursu) Lake as compared to the 'Archaea-dominated' (Fără Fund) lake is given by the significantly high abundance of bacteria-related sequences in the mixolimnion (0.5-2.5 m) and chemocline (3.5 m), whereas *Archaea* became apparent only in the lower chemocline (3.5 m) and monimolimnion (9 m). On the opposite, the epilimnion and chemocline of Fără Fund lake is clearly dominated by archaeal community whereas the monimolimnetic microbial diversity (with *Bacteria* slightly prevailing over *Archaea*) is roughly similar in both lakes. It seems that the combination of oxic, photic, and moderately saline conditions (2-15% total salinity) in upper stratum of Ursu lake support dense aerobic chemoheterotrophic and halotolerant bacteria that might actively degrade organic matter of various origins, types and complexities as also demonstrated by community-level physiological profiling using BIOLOG Ecoplates^{TM} approach (Cristea *et al.*, 2014). Primary production in this type of lake is mainly driven by oxygenic phototrophic cyanobacteria and microalgae (Máthé *et al.*, 2014; Andrei *et al.*, 2015). The analogous but hypersaline (>17% salinity) water layer of Fără Fund lake is populated almost exclusively by haloarchaea (88-96% relative abundance). Bacteria are scarcely (4-10% relative abundance) represented by $OTU_{0.03}$s assigned to Bacteroidetes (*Balneola* sp. and *Salinibacter* sp.) and Proteobacteria (*Halorhodospira* sp.). Primary production is most probably achieved by the typical halophilic *Dunaliella* cells (Somogyi *et al.*, 2013; Andrei *et al.*, 2015) (Fig. 3b).

At chemocline (3.5 m and 2 m depth, respectively), alongside decrease in light intensity and DO, paralleled by increase in salinity and ORP seconded by presence of hydrogen sulfide (Máthé *et al.*, 2014; Andrei *et al.*, 2015), key steps in the sulfur and carbon cycling are driven by anoxygenic phototrophic GSB (*Chlorobiaceae*) in Ursu lake and by the PSB (*Ectothiorhodospiraceae*) in Fără Fund lake (Fig. 3b). Why GSB and PSB are solely present in each of the two lakes might be explained by an interplay of local conditions. In Ursu lake, the GSB requirements for lower light intensity (Damsté and Schouten, 2006; Zadereev *et al.*, 2017) is fulfilled by the dense cyanobacteria 'plate' overlying GSB niche, whereas the PSB layer seemed better light-exposed in the presence of a sparse overlying population of phototrophic algae (*Dunaliella* sp.) thriving in hypersaline Fără Fund Lake

(Borsodi *et al.*, 2013; Andrei *et al.*, 2015).

Similar conditions in the bottom layer (*i.e.*, dark, O_2-deprived hypersaline water) are seconded by eutrophication that presumably feed in consistent organic carbon input in both lakes. Thus, apparently similar halophilic anaerobic communities dominated by sulfate-reducing bacteria (*Desulfobacteraceae* and *Desulfohalobiaceae*) and fermenters (*Halobacteroidaceae* and *Halanerobiaceae*) are harbored in analogous monimolimnia. Large quantities of organic matter of autochthonous (biomass formed by local primary producers added to debris of phyto- and zooplankton) and allochthonous (higher plant material, mainly leaves, from circumlacustrine vegetation) origins are sinking and slowly degraded (*i.e.*, as deduced from the higher values of total organic carbon in the monimolimnion as compared to mixolimnion; Andrei *et al.*, 2015; unpublished results). Complex polymers of plant and zooplankton origin are probably hydrolyzed by heterotrophic anaerobes such as the archaeon *Halorhabdus* to smaller C compounds (*i.e.*, monomers). Monomers are further metabolized to CO_2, formate, acetate, propionate *etc.* by fermenters (*e.g.*, *Halanaeobiales*), whereas compounds such as methylamines derived from organic compatible solutes of decayed biomass may be consumed by methanogenic archaea (*e.g.*. *Methanohalobium* sp.) or by members of Acetothermia (OP1) and Parcubacteria (OD1) (detected in Ursu Lake). The latter, candidate lineages have been also demonstrated to occur in various anaerobic milieus including hypersaline mat of Guerrero Negro, Mexic (Ley *et al.*, 2006) and water column of saline meromictic Shunet lake, Central Asia (Baatar *et al.*, 2016). As result of intertwined metabolic activities of microbial communities inhabiting the three main layers, complete turnover of C, S and, possibly, N and Fe, might be inferred in the both model meromictic lakes (Andrei *et al.*, 2015) (Fig. 3b).

Current conservation status and future challenges

Fără Fund and Ursu (including its surrounding woodland) are the only Transylvanian salt lakes that have a protected status. Both lakes have been declared natural reserves since 1968 and 1970, respectively, and are currently included in the World Database on Protected Areas (WDPA ID 183748) and classified as 'natural monuments' by International Union for Conservation of Nature (IUCN Management category III). Fără Fund Lake is the only lake strictly prohibited for any sort of capitalization. The saline lakes in Transylvania are administered by local authorities and freely accessible to public or by public-private partnerships (*i.e.*, exploited for recreational purposes by private or mixed private-public companies such as S.C. Salina Turda S.A., S.C. Balneoclimaterica S.A. in Sovata or S.C. Lacurile Naturale Ocna Sibiului S.A.). The touristic activities linked with salt lakes significantly contribute

to the development of local economy in small cities such as Turda, Cojocna, Sovata or Ocna Sibiului. Most of the Transylvanian salt lakes are closed basins fed with meteoric water only and, therefore, particularly prone to alterations due to both anthropogenic activities (*e.g.*, large influx of swimmers, modifications of the catchments) and change in Global climatic pattern (Williams, 1998). A plausible climate change scenario suggests the progression of the semi-arid Mediterranean climate towards central and east Europe with increasing of droughts periods and large variability of weather extremes (Schär *et al.*, 2004). Through warming and negative water balance, enhanced evaporative concentration is expected to severely impact saline lakes especially (Benison and Bowen, 2015; Larson *et al.*, 2016; Shadkam *et al.*, 2016; Zachara *et al.*, 2016). In this perspective, shallow saline lakes may desiccate, whereas the large or deep stratified water basins are susceptible to a more complicated evolution, possibly including eutrophication and hypoxia/euxinia (Raini, 2009; Lyons *et al.*, 2009). However, short- to long-term water management plans need to be implemented to mitigate the climate change effects on inland aquatic systems (Shadkam *et al.*, 2016). To preserve the most economically-valuable characteristics (*i.e.*, gradient stratification, heliothermy, and potential for sapropel genesis), short-term water management strategies (*e.g.*, water quality monitoring, short bathing periods, water level control) are implemented to most saline lakes used for recreational purposes in Turda, Sovata or Ocna Sibiului areas (Bulgăreanu, 1993; 1996; Alexe, 2010).

CONCLUDING REMARKS

In this review, we highlighted the abiotic and biotic features of pH-neutral salt lakes from the Transylvanian Basin, Central Romania. The thick halite deposits undelying Transylvanian Basin since Mid-Miocene Paratethys Sea regression were prerequisite for the genesis of salt lakes following mining activities or natural dissolution of salt rock. As consequence of the water chemistry (*i.e.*, salt-rich) and morphometry (*i.e.*, closed basins with significant depths), most of these lakes are meromictic and thus, harboring distinct micro-habitats along the water column. Recent studies in several saline Transylvanian lakes employing culture-dependent and molecular approaches pointed on the high ecological complexity with discrete niche-based distribution of plankton communities. Shift in the plankton community structure and composition is generally driven by the salinity and the availability of electron donors. In saline meromictic Transylvanian lakes, the bottom brines with stable abiotic conditions are apparently inhabited by similar, highly-diverse microbial assemblages relying on the downward flux of nutrients. A more lake-specific plankton diversity was described in the surface waters (mixolimnion and chemo-

cline) that are likely impacted by environmental changes. During decades of investigations, all components of trophic chains were identified suggesting that these lakes are stable ecosystems with full biogeochemical cycling of major elements. Moreover, by their combined abiotic (*i.e.*, limnology, geochemistry, and geographical positioning) and biotic (*i.e.*, highly-diverse microbial communities and potentiality for novel prokaryotic lineages), the Transylvanian salt lakes appear as unique saline aquatic systems that may inspire future field- or laboratory-based experiments. Nonetheless, thorough understanding of relationships among trophic chain components that play major roles in ecosystem functioning as well as of adverse human and changing climate impacts is needed in support of local policies for durable management and protection of Transylvanian salt lakes.

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