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# Lake La Salada de Chiprana (NE Spain), an Example of an Athalassic Salt Lake in a Cultural Landscape

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Additional information is available at the end of the chapter

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## Abstract

On a global scale, athalassic inland salt lakes are abundant, albeit restricted to semiarid and arid climates. La Salada de Chiprana is unique in Western Europe, because it is a permanent and relatively deep (up to 5.6 m) hypersaline lake (40–90 g total dissolved salt L<sup>-1</sup>) since 1700 AD. It forms part of a cultural landscape, which imposes a challenge for management. The aim of this paper is to describe the specific microbial biota and how they interacted with both animals and plant species during the last 25 years. The deeper parts regularly showed salinity stratification with an anoxic sulfide-rich hypolimnion and a bloom of green sulfur bacteria (*Prosthecochloris aestuarii* and *Chlorobium vibrioforme*) at the pycnocline. Despite highly eutrophic conditions, often the top water layer is transparent due to top-down control of phytoplankton populations by the brine shrimp, *Artemia parthenogenetica*. This allows for the development of submerged aquatic vegetation of the endemic foxtail stonewort *Lamprothamnium papulosum* var. *papulosum* f. *aragonense*, and microbial mat communities build by the cyanobacterium *Coleofasciculus (Microcoleus) chthonoplastes* coexisting with green filamentous nonsulfur bacteria (Chloroflexaceae). The microbial mats show photosynthetically induced precipitation of high-Mg calcite, which by incorporating viruses represents a mechanism for their fossilization.

**Keywords:** microbial mat, multiple stable states, regime shift, charophyta, cyanobacteria, *Coleofasciculus (Microcoleus) chthonoplastes*, Chloroflexaceae

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## 1. Introduction

Saline aquatic environments on the Earth include the ocean and the transitional environments (lagoons, estuaries, coastal wetlands, river mouths, etc.) on the coast where the salinity is derived

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from the sea, and epicontinental streams and lakes without a recent connection to the sea. The former are called thalassic (i.e., derived from the sea) and the latter athalassic. On a global scale, athalassic inland salt lakes are widely distributed (see **Figure 1**). These areas are characterized by their arid or semiarid climate, with annual rainfall values of 25–200 and 200–500 mm, respectively, which are largely insufficient to compensate for evaporation. In a landscape setting, such a structural water deficit does not allow the formation of permanent rivers that slow down the formation of river valleys. In many cases, the water simply flows to depressions in the landscape from where it escapes to the atmosphere by evaporation. Such inland areas that drain to a depression in the landscape with no outlet to rivers flowing to the sea are called endorheic basins. Permanent and temporal salt lakes are typically found in these endorheic basins where they often occupy the lower parts. In addition, athalassic salt lakes occur in Antarctica, where high salinities in the lake are a consequence of outfreezing. During freezing of water, ice crystals of almost pure water are formed while the dissolved salts are expelled from the freezing volume to the remaining water that becomes progressively more concentrated in total dissolved salts. Lake Bonney (77°43'S 162°22'E) is a permanent ice-covered athalassic saline lake on the Antarctic continent in the McMurdo Dry Valleys. On a global scale, inland saline lakes represent a volume of  $85 \times 10^3 \text{ km}^3$ , which is not much lower than the global volume of freshwater lakes of  $105 \times 10^3 \text{ km}^3$  (Shiklomanov 1990 cited by Williams [1]). However, the Caspian Sea represents almost 75% of the total volume of inland salt lakes. Other well-known salt lakes include the Dead Sea (Middle East), the Aral Sea (Central Asia), Mono Lake in California (USA), Mar Chiquita (Argentina), Sambhar Salt Lake (India), and Lake Eyre (Australia). In Western Europe, Spain is the only country where inland athalassic saline lakes occur in three different regions. The majority of these saline lakes are ephemeral showing alternations between shallow water bodies during wet periods and dry salt flats during dry periods. Lake La Salada de Chiprana is a unique salt lake in Western Europe because it is permanent and deep (up to 5.5 m depth).



**Figure 1.** Global distribution of athalassic salt lakes (shaded areas indicate the regions where such lakes occur naturally) from Williams [1]. With kind permission of Cambridge University Press, Environmental Conservation.

Limnology is the scientific study of the physical, chemical, geological, and ecological dynamics of inland lakes and other inland water bodies. The term was introduced by François-Alphonse Forel (1841–1912), who studied Lake Geneva. Most limnologists neglected the study of inland salt lakes for a long time, despite their global importance. Even today, the salt lakes do not receive the interest they merit as it is reflected by the fact that limnology is sometimes incorrectly described as the discipline studying freshwater systems. The original focus on freshwater lakes in limnology can be explained by historic reasons as this discipline really started to develop in Western Europe around the turn of the twentieth century, where inland salt lakes are scarce as mentioned above. Even around the 1920s, limnologists were mainly active in Western and Central Europe as well as in the eastern part of the USA where freshwater lakes predominate. By the end of the 1940s, Ramón Margalef (1919–2004) initiated the study of the inland Spanish salt lakes and American and Australian researchers became interested in the salt lakes in the semiarid and arid parts of their large countries. William David Williams (1936–2002) has been particularly active in promoting the study of salt lakes at a global level and highlighted the threats to inland salt lakes and advocated the need for conservation measures [2].

The salinity is highly variable among athalassic saline lakes both in terms of concentration and composition of dissolved salts. The salt often derives from the soils in the endorheic basin, which are lixiviated by rainwater that carries the dissolved salts to the terminal lake. Hence, the salt composition of the inflowing water is strongly determined by the parent material and the sedimentary deposits that form the soils in the endorheic basin. The concentration of salts in salt lakes is generally expressed in units of total dissolved salts per liter (TDS L<sup>-1</sup>). Inland lakes are considered as saline lakes when the concentration of total dissolved salts exceeds 3 g TDS L<sup>-1</sup>. However, the lakes that have a salinity exceeding 10 g TDS L<sup>-1</sup> are home to biotas that are clearly different from that of freshwater lakes. Inland lakes with salinities between 3 and 10 g L<sup>-1</sup> are often referred to as subsaline. On the other side, lakes with salinities exceeding 40 g TDS L<sup>-1</sup> are considered as hypersaline. Dissolved salts in inland lakes mainly include the anions carbonates (CO<sub>3</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup>), chloride (Cl<sup>-</sup>), sulfate (SO<sub>4</sub><sup>2-</sup>), and silicate (SiO(OH)<sub>3</sub><sup>-</sup> and other forms), and the cations sodium (Na<sup>+</sup>), magnesium (Mg<sup>2+</sup>), calcium (Ca<sup>2+</sup>), and potassium (K<sup>+</sup>). The final composition in the lake water depends on both, the composition of the inflowing water and the mineral precipitation processes. Hence, high Ca<sup>2+</sup> and low Mg<sup>2+</sup> give rise to lakes dominated by halite (NaCl), while high Ca<sup>2+</sup> combined with high Mg<sup>2+</sup> gives rise to Na<sup>+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup> and often SO<sub>4</sub><sup>2-</sup> dominated lakes ranging in pH from 6 to 8.5. Lake Chiprana is an example of the latter and is characterized by a high pH for this group, i.e., a pH around 8.5. Interestingly, low Ca<sup>2+</sup> and low Mg<sup>2+</sup> give rise to so-called soda lakes dominated by Na<sup>+</sup> and CO<sub>3</sub><sup>2-</sup> and a high pH of 10–11 [3]. A precise determination of TDS requires a chemical analysis of the major ions. Complete evaporation of saline water and gravimetric determination of precipitated salt are inadequate for TDS determinations, because water molecules are incorporated in different crystals during the precipitation. Moreover, the use of a measure of conductivity may be problematic. At first, the relation between TDS and conductivity depends on the composition of dissolved salts and the relationship is, therefore, different among lakes. Second, the relation between TDS and conductivity breaks down at higher salinities, particularly when bivalent ions are abundant in solution [4].

Inland saline lakes in a pristine landscape are ideal sentinels for variations and changes in the climate. Hence, in permanent salt lakes, draught periods are reflected by the lowering of the water table, decreasing water volume, and increasing salinity as well as a decreasing surface area. The latter is due to exposure of the shallowest parts. From the littoral zone into the lake, the shallow areas are designated as playas. These playas typically alternate between emersion after dry periods and submersion following wet periods. The exposed bottom of playas is often covered by a salt crust. However, prolonged exposure for many years may result in colonization by halophyte vegetation. During wet periods, the reverse occurs. These variations can potentially be monitored by remote sensing. For temporal salt lakes, increasing draught results in shorter submersion and longer exposure where the lake bottom appears as a salt pan. For example, in the temporal lake La Dehesilla (Spain), during the water years 1989 and 1990, the lake was filled with water during 5 months and completely dry during 7 months. The salinity of the water varied from 20 g TDS L<sup>-1</sup> to 90 g TDS L<sup>-1</sup> during the wet periods [5]. Potentially, the duration of the submerged and exposed periods can be monitored by remote sensing.

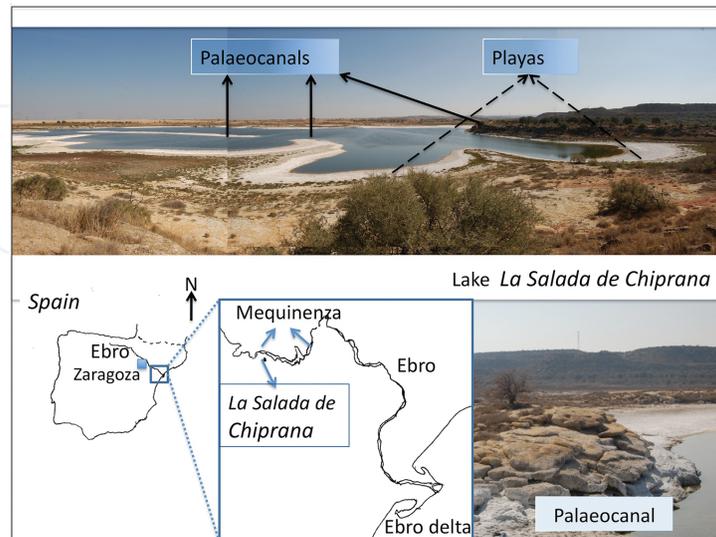
Nevertheless, humans nowadays heavily impact the inland salt lakes and jeopardize the conservation of the unique values of these ecosystems [1]. The human threats include inflow diversions, irrigation, mining, pollution, and biological disturbances, e.g., the introduction of exotic species. In many salt lakes, the variations of water volume and salinity do no longer faithfully reflect the natural climatic variation alone, but are rather modified by many of these human impacts. There is an urgent need for conservation measures that are particularly adapted to inland salt lakes. A good example for conservation measure is Mono Lake in California [1]. In this chapter, I describe some of the unique values of the athalassic salt lake La Salada de Chiprana, with a particular focus on the microbial communities. In addition, I discuss the management actions put in place for the conservation of this lake. However, Chiprana Lake is not a pristine saline lake as humans have heavily impacted the catchment since 1700. For centuries, this allowed the existence of this permanent lake in a cultural setting. Major changes in agriculture since the 1950s have represented a threat to the unique values of this lake. This poses the question on how to develop conservation measures for this interesting salt lake in a changing cultural context.

## 2. Lake La Salada de Chiprana as a specific case of an athalassic salt lake in a cultural landscape

### 2.1. Localization and history of lake La Salada de Chiprana

“La Salada de Chiprana” (41°14′30″N, 0°10′50″W, NE Spain) is the only permanent athalassic shallow salt lake in Western Europe. This salt lake (**Figure 2** and **3**) is located in an endorheic basin in the Ebro Valley 6 km SW of the village of Chiprana, and occupied a total surface of 31.5 ha in 1991 and varied in surface between 36 ha in 1993 and 22 ha in 2008. Today, the lake La Salada de Chiprana is a protected area which benefits from the following qualifications: (1) Ramsar wetland site since 1994, (2) site of community interest (SCI) according to the Habitat

Directive of the European Union since 1997, and (3) natural reserve of the Gobierno de Aragón since 2006.



**Figure 2.** Lake La Salada de Chiprana (Aragón, Spain) located in NE Spain in the valley of the Ebro River close to the Mequinenza Reservoir ( $41^{\circ}14'30''N$ ,  $0^{\circ}10'50''W$ ; see maps). Panoramic view of lake La Salada de Chiprana from the southwestern shore of the lake showing palaeocanals and playas. Close-up of paleocanal with playa in the back.

This salt lake lies upon the upper Oligocene-Miocene Caspe formation that mainly comprises sand and siltstones deposited by a river. Hence, the area where the salt lake occurs is located in a former river valley, where the river meandered. Gravel and sand were being deposited in the riverbed, while silt was deposited in the floodplain during flooding of the river. The differences in compaction and erodability of the sand and siltstones have resulted in an inversion of topography in the landscape, with the meandering sandstone formations emerging as ridges in the landscape. These sandstone formations are, therefore, called paleocanals (see **Figure 2**).

A paleolimnological study [6] has shown that during most of the Mid and Late Holocene, lake La Salada de Chiprana was a temporal lake. Human activities in the surroundings included deforestation, irrigation, and olive tree planting. In the thirteenth century, a small irrigation canal, the Civán canal, was created to carry water from the *Guadalupe* River into the surroundings of the lake. All these changes resulted in increased flow of water into the lake and it thus became a permanent hypersaline lake since 1700 AD. Irrigation practice was formally organized since 1838, when a committee of 40 landowners was formed. The irrigated surface reached 3912 ha during the 1950s and was reduced by 400 ha because of the construction of a dam and the large Reservoir of Mequinenza in the Ebro River in 1967. In 1988, a smaller reservoir was built in the *Guadalupe* River to allow for an increase of irrigation in the region. A new Civán canal was built, running parallel to the old canal, which obtains its water directly from this reservoir. Since then, an additional 200 ha has been irrigated. Olive trees were the main crops until 1970. Nowadays, most of the crops include cereals and alfalfa [6]. Hence, for the last 300 years, lake La Salada de Chiprana has been subjected to both climatic and anthropogenic

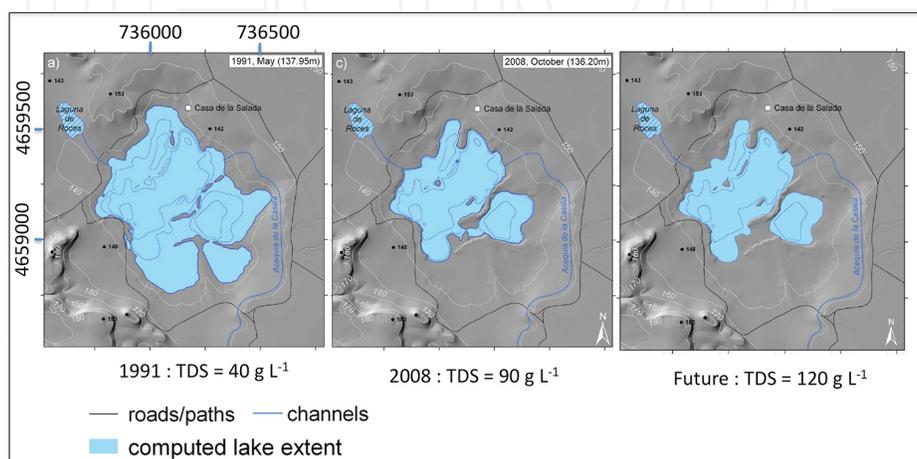
influences. The watershed *sensu stricto* of the lake is of 768 ha of which 515.7 ha benefit from protection measures, i.e., 154.8 ha as a natural reserve and 360.9 ha as a peripheral buffer zone. However, due to the irrigation canals, the watershed receives water inputs from the outside of this watershed. In the early 1990s, scientists expressed their concern about the conservation of the unique properties of the lake and called for regulation of the water flows in the area.

## 2.2. Regulating the water flows and management of the Salada de Chiprana Lake

Irrigation water for agriculture in the watershed of the Salada de Chiprana is drawn from the reservoir in the *Guadalupe* River and brought to the watershed of the lake via open irrigation canals. These comprise the Civán canal that connects to the smaller Acequia de Farol and the Acequia de la Casilla canal in the surroundings of the Salada de Chiprana. Moreover, overflow of an upstream irrigation zone also feeds into the Acequia de Farol and the Acequia de la Casilla, thus, representing an additional input. Often, more water is channeled to the watershed of the lake than requested for irrigation. Before 1994, this excess irrigation water was directly channeled into the Salada de Chiprana Lake. This practice created major environmental problems (see below) and, therefore, it was decided to build a dam, which allowed diverting this surplus flow via the Arroyo del Regallo River into the Reservoir of Mequinenza [6, 7]. In 1993, more than 100,000 m<sup>3</sup> freshwater from Civán canal entered the lake in 1993 [6]. The hydraulic works have been used since 1994. In addition, measures were taken to prevent the water from a small irrigation canal, i.e., the small Acequia de la Casilla, entered the lake directly [7].

Between 1989 and 1993, the lake level rose due to dumping of surplus irrigation water and irrigation returns in the watershed. As a result, by 1993, the lake salinity had been reduced to 30–40 g TDS L<sup>-1</sup> and the surface area of the lake achieved a maximum of 36 ha. Between January 1994 and September 1995, the lake level decreased because the diversion and salinities increased again to 80 g TDS L<sup>-1</sup>. Between 1995 and 1999, the lake level rose again, while after 2000, there was a persistent trend of decreasing lake levels. In the early 1990s, the maximum depth reported for the lake was 5.6 m [8]. Between the early 1990s and early 2009, the lake level decreased overall by 1.75 m [9]. Hence, on 4 August 2007, the Confederación Hidrográfica del Ebro recorded a maximum depth of 3.7 m. The surface areas and volumes of the salt lake have been calculated from the topography of the landscape and the level and bathymetry of the lake [9, 10]. Superimposed on these trends, since 1998, the lake also showed repeatable intra-annual fluctuations with amplitude of 30–50 cm [9]. Such behavior is natural for these salt lakes as water levels rise during winter and spring and decrease during summer, thus achieving their annual minimum levels in late summer or autumn. The decreasing lake level between 1991 and 2008, corresponded to a 42% reduction in surface (from 36 to 21 ha) a 55 % reduction in volume (from 893,000 to 405,000 m<sup>3</sup>) [9]. The decrease in the surface is illustrated in **Figure 3**. In accordance with the lake level, the salinity increased from 40 to 90 g TDS L<sup>-1</sup> (**Figure 3**). Nevertheless, the ionic composition remained remarkably constant during the entire 17 years and was dominated by Na<sup>+</sup>, Mg<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup>, and Cl<sup>-</sup>. The molar ratios of dissolved ions ( $n = 53$ ) were  $1.13 \pm 0.24$  SO<sub>4</sub><sup>2-</sup>/Cl<sup>-</sup>,  $1.38 \pm 0.34$  Na<sup>+</sup>/Cl<sup>-</sup>, and  $0.97 \pm 0.19$  Mg<sup>2+</sup>/Cl<sup>-</sup>. Magnesium exceeded calcium showing more variable molar ratios for Mg<sup>2+</sup>/Ca<sup>2+</sup>, i.e.,  $21.4 \pm 10.5$  ( $n = 53$ ). The total

amount of salts in the lake was estimated to be equal to 44,750 tons. After 1995, when the water level dropped below 137.6 m (European Datum georeference system for W Europe, ED50) above mean sea level (Alicante), a small basin in the SE corner became separated from the main lake (**Figure 3**). This separated 822 tons of dissolved salt representing a loss of 2% of salt for the main lake. Nevertheless, the salinities in the lake showed remarkable conservative behavior, i.e., the mass of total dissolved salt in the lake was rather constant and variations of its salinities were directly related to the volume of the lake [9].



**Figure 3.** Lake extent of the lake La Salada de Chiprana and updated bathymetry calculated from the bathymetric map of Guerrero et al. [10] and based on monitoring of water height at a gauge and cartography (IGN Spain), adapted from De Wit et al. [9], with kind permission of John Wiley. TDS = concentration of total dissolved salts. The right panel contains a prediction for a future extent when salinity would have increased to 120 g TDS L<sup>-1</sup> and that can be expected to occur before 2020, if the trend initiated since 2000 persists.

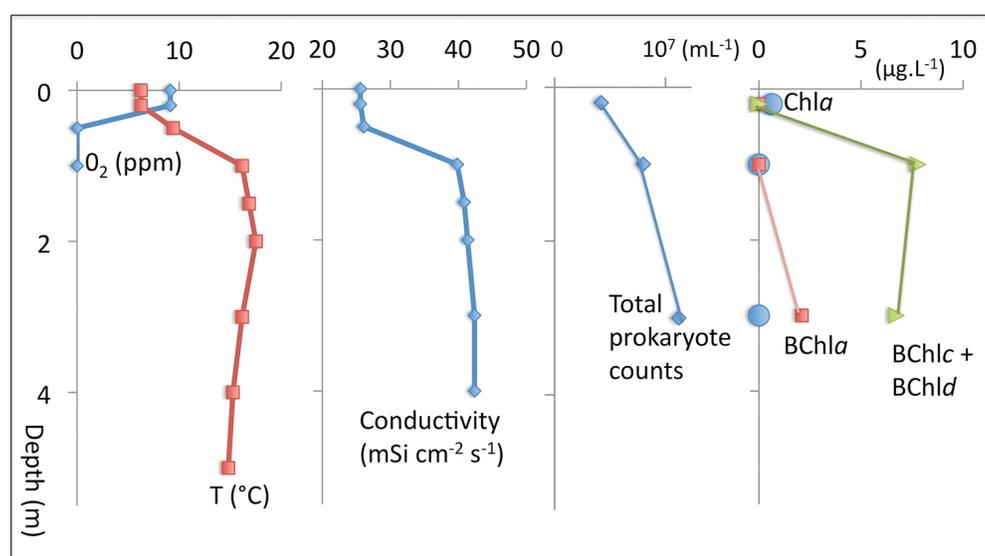
In conclusion, after a period of excessive water discharges in the lake in the early 1990s, since the introduction of the measures in 1993, the lake hydrology has been governed by high evapotranspiration (1000–1500 mm year<sup>-1</sup>), low rainfall (200–400 mm year<sup>-1</sup>), water runoff in its endorheic watershed, irrigation returns and groundwater flows. This resulted, however, in persistently decreasing lake levels and increasing salinities since 2000. To counteract this trend, the managers have decided in 2013 to supplement clean freshwater into the lake from the Cíván River.

## 2.3. Variations in microbiological communities during the last 25 years in La Salada de Chiprana

### 2.3.1. Planktonic communities and stratification of the water column

During the early 1990s, when the lake contained its highest amount of water and showed some parts where depths reached more than 5 m, stratification of the water column was often observed [8]. **Figure 4** describes the stratification that I observed on 24 January 1990. The stratification was based on a salt gradient. The upper water layer had been diluted by the freshwater inflow and showed lower salinities than the bottom layer. The top layer or

epilimnion was separated from the bottom layer (hypolimnion) by a density gradient or pycnocline. Temperature also showed a gradient from 6°C in the epilimnion to 17°C at 2 m depth. This is surprising as distilled water has a maximum density at 4°C and above 4°C its density decreases with temperature. One would, thus, expect that the water of 6°C sinks to the bottom in a lake where temperature achieves 17°C. Nevertheless, this was not the case as the cold epilimnion water had a higher density than the warmer hypolimnion, due to the very strong differences in salt concentrations. Temperature effects counterbalanced the salt gradient but were clearly not sufficient to destroy the stratification. This phenomenon is known as an inverse temperature gradient and can, sometimes, be observed in hypersaline systems. The highly saline water has a strong tendency to absorb the energy from solar radiation. In addition, the heat capacity of water decreases with increasing salinity and, thus, for the same amount of energy absorbed, saline water increases more strongly in temperature than freshwater.



**Figure 4.** Stratification of physicochemical and microbiological variables in the deepest part (5.5 m) of the lake observed on 24 January 1990. The stratification is based on a gradient of salt concentrations (pycnocline), which is reflected by conductivity. The upper part of 50 cm thickness (epilimnion) is oxic and low concentrations of Chlorophyll *a* (*Chla*) reflect the presence of microalgae. Green sulfur bacteria, which are strict anaerobes, and phototrophic bacteria develop in the anoxic zone at the pycnocline. These organisms contain bacteriochlorophylls *c* and *d* (*BChlc* and *BChld*) as light-harvesting pigments, together with minor proportions of bacteriochlorophyll *a*.

The bottom layer and the pycnocline were completely anoxic, while only the epilimnion was oxic. A low density of phytoplankton was reflected by a low chlorophyll *a* content of 1 µg L<sup>-1</sup>. At the pycnocline, still sufficient light was present to favor the blooming of phototrophic bacteria, i.e., green sulfur bacteria. These bacteria use H<sub>2</sub>S as the electron donor in anoxygenic photosynthesis. Morphological observations with light microscopy indicated that cells similar to *Chlorobium vibrioforme* were dominant, although they often coexisted with minor populations of cells resembling *Prosthecochloris aestuarii*. The latter was often located deeper in the lake. Xavier Vila and his colleagues at the University of Girona (NE Spain) succeeded in isolating these green sulfur bacteria and obtained strains in pure culture, which were related

to *Prosthecochloris aestuarii* SK413 and *Chlorobium vibrioforme* DSM260 [11]. Green sulfur bacteria contain chlorosomes with bacteriochlorophylls capable to efficiently harvest light at low intensities. Both isolated strains contained different allomers of bacteriochlorophylls *c* and *d* (BChlc and BChld) and the carotenoid chlorobactene [11]. In addition, the green sulfur bacteria also contain minor amounts of bacteriochlorophyll *a* (BChla) that occurs (i) in the photosynthetic reaction centers, (ii) in the baseplate of the chlorosomes, and (iii) as associated with the Fenna-Matthews-Olsen protein, all involved in the photosynthetic light processing. Hence, the BChla measured has probable originated from these green sulfur bacteria in the lake.

Interestingly, the sequencing of the 16S rRNA genes and the *fmoA* gene, the gene coding for the Fenna-Matthews-Olsen protein, indicated that specific lineages of green sulfur bacteria exist, which are adapted to marine and saline environments. Nonetheless, within such a lineage, the *Prosthecochloris* strain isolated from Chiprana was actually quite different from the type strain of *Prosthecochloris aestuarii*. The *Prosthecochloris* strain from Chiprana clustered with a strain isolated from another inland salt lake in the Death Valley indicating that these inland saltwater lake bacteria were separated from their marine relatives early during evolution [12].

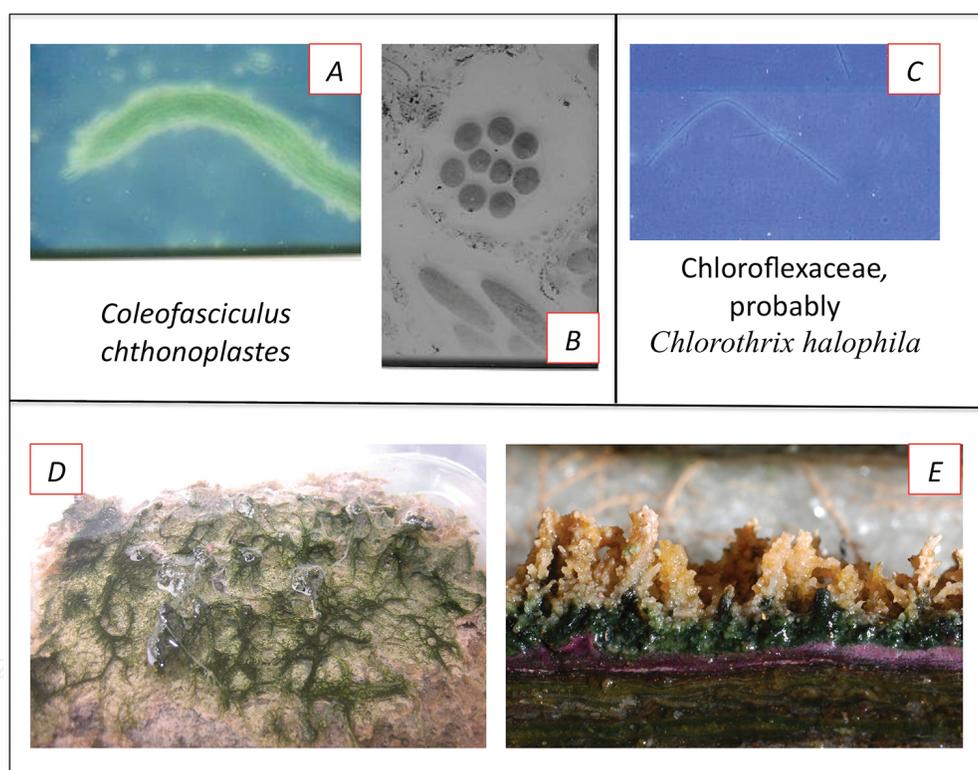
The strong variations in salinities observed in the lake since the 1990s, triggered important changes in the water column communities. In 1990, the Chla concentrations in the top layer were low due to a strong top-down effect of the water column grazer, the brine shrimp *Artemia parthenogenetica* (Bowen & Sterling, 1978). During 1992 and 1993, the strong input of freshwater resulted in decreasing salinities with a wane of the brine shrimp populations as several species in this genus do not occur below 35 g TDS L<sup>-1</sup> [13]. Hence, in the absence of the top-down control by brine shrimps, phytoplankton was able to develop high densities (with Chla values up to 100 µg L<sup>-1</sup>), which was probably further enhanced by the high nutrient input into the lake from the surplus irrigation water [14]. Fortunately, after 1994, with increasing salinities the brine shrimps returned to the lake and the top-down control was reestablished. The decreasing lake level and depth also resulted in less frequent occurrence of stratified periods. This area is subjected to strong winds that act on the lake surface to induce strong mixing in the lake and destruction of the pycnocline. In addition, the reduced freshwater input from the watershed also implies that the top layer of the lake is exposed to a lesser degree of dilution than before, which slows down the buildup of a pycnocline.

### 2.3.2. Benthic communities and their variations

Between 1989 and 1991, a large part the submerged playas were covered by benthic microbial mats [8] build by the cosmopolitan cyanobacterium *Coleofasciculus chthonoplastes* (Thuret ex Gomont) Siegesmund, Johansen et Friedl 2008. This is a filamentous cyanobacterium that forms bundles in a common sheath (see **Figure 5A**). The species was previously known as *Microcoleus chthonoplastes*, but a detailed phylogenetic study showed that among the filamentous *Microcoleus* species, the marine and halotolerant species including *M. chthonoplastes* formed a lineage that was clearly different from the freshwater *Microcoleus* branch; therefore, the species has been renamed by Siegesmund et al. [15]. Flowering plants (Magnoliophyta) were also observed as *Ruppia* sp. that formed clear meadows. The development of *Ruppia*

meadows is clearly antagonistic to the microbial mats as the plants occupy space, modify the sediment surface and reduce light intensities at the sediment surface by shading of the canopy. During this period, the extension of the *Ruppia* meadows, a phenomenon that was attributed to decreasing salinities [8], resulted locally in replacement of the *C. chthonoplastes* mats.

Diaz et al. [14] observed that the continuously decreasing salinities between 1990 and 1993 in combination with increased eutrophication resulted in heavy epiphytic growth on *Ruppia*, often inducing their decline. Simultaneously, the microbial mats continued their demise, probably because of reducing light at the sediment surface due to increasing shading by phytoplankton (see Section 4.1). The excessive low salinities in the lake, together with the progressive disappearance of the microbial mats and the poor quality of the *Ruppia* meadows, motivated Spanish researcher to ring the bell and call for targeted management measures that resulted in the hydraulic works achieved in May 1993 [7] (see Section 3).



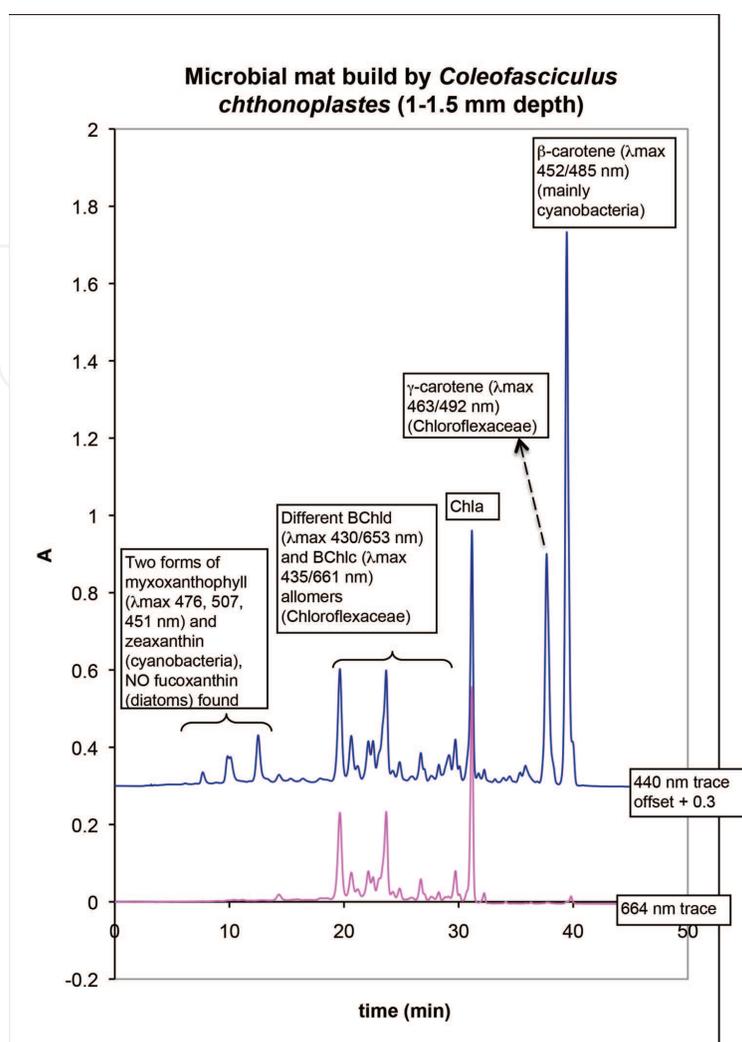
**Figure 5.** (A) Phase contrast light microscopic image of *Coleofasciculus chthonoplastes* (Thuret ex Gomont) Siegesmund, Johansen et Friedl 2008 (formerly *Microcoleus chthonoplastes*) showing multiple filaments in a common bundle. (B) Transmission electron microscopic image of a cross section of a bundle of *Coleofasciculus chthonoplastes*. (C) Phase contrast light microscopic image of a filamentous green nonsulfur bacterium from the Chloroflexaceae family, probably *Chlorothrix halophila*. (D) Macroscopic image of the surface of a biofilm growing on the top of the debris of the foxtail stonewort (*Lamprothamnium papulosum* var. *papulosum* f. *aragonense* (Prósper) Wood). The biofilm was mainly composed of green nonsulfur bacteria from the Chloroflexaceae family. (E) Macroscopic image (macrophotography) of a cross section of a mat build by *Coleofasciculus chthonoplastes* in 2010 under very high salinities (>90 g TDS L<sup>-1</sup>). The purple layer indicates the development of purple sulfur bacteria in these mats. Note a fine layer of fine-grained high-Mg calcite crystals at different depths in the mat. Due to high salinities, gypsum started to precipitate on the surface of the mats where it formed beautiful crystals.

The situation was clearly reversed after the inauguration of these hydraulic works. Valero-Garcés et al. [6] reported that since 1995, a particularly dry year in this region (200 mm rainfall), water column transparencies had recovered in the lake. During a visit in September 1996, I observed reestablishment of *C. chthonoplastes* microbial mats. Recolonization by microbial mats continued and by 1999, the extent of microbial mats was similar to that described for the lake in the late 1980s and early 1990s [8]. However, the *Ruppia* meadows had completely disappeared by 1996 as a result of the increased salinities.

More detailed studies by Jonkers et al. [16] described the laminated structure of these mats as multilayered microbial mats build by *C. chthonoplastes* living in association with filamentous green nonsulfur bacteria from the Chloroflexaceae family. Chloroflexaceae show thin filament morphologies of light green color, which do not fluoresce red light upon blue excitation and can, thus, be distinguished under the microscope from the thin cyanobacteria filaments, e.g., *Leptolyngbya*. As green sulfur bacteria (see **Figure 4**), the filamentous green nonsulfur bacteria also contain BChlc and BChld. These bacteriochlorophylls are involved in light harvesting and are special, because rather than representing a single macromolecule, they comprise mixtures of molecules known as allomers that may differ by the alcohol esterified to the macrocycle and substituents on the macrocycle. The different allomers can be separated by HPLC, using a reverse-phase protocol as that described by Buffan-Dubau et al. [17] (see **Figure 6**). The phylogenetic study by Bachar et al. [18] has confirmed the presence of 16S rRNA gene sequences affiliated to the Chloroflexaceae family some of them very closely related to the Candidatus *Chlorothrix halophila* (see **Figure 5C**). Multiple white layers of calcium carbonate were observed in the mat (see **Figure 5E**), which have been identified as high-Mg calcite [19]. In addition, diatoms sometimes occurred in the mat, particularly on the top surface layer, particularly during spring. These diatoms are favored by high inputs of inorganic nitrogen, i.e.,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  [20]. In contrast, the cyanobacteria were stimulated by the combined addition of inorganic nitrogen and phosphate [20].

The microbial mats fixed  $\text{N}_2$  [21]. In general, *C. chthonoplastes* does not possess the genes for  $\text{N}_2$  fixation, although one strain of this species was shown to contain these genes, which it probably obtained through lateral gene transfer [22]. Hence, it appears most likely that the  $\text{N}_2$  fixation is attributed to the heterotrophic bacteria in the mat [21], although  $\text{N}_2$  fixation by cyanobacteria cannot be excluded [22].

Between 2000 and 2006, there was a spectacular development of charophytes, i.e., a specific form of the foxtail stonewort *Lamprothamnium papulosum* var. *papulosum* f. *aragonense* (Prósper) Wood. This charophyte has been described originally as a distinct species by Eduardo Reyes Prósper in 1910 as a typical plant for the semiarid steppe lakes in Aragon [23, 24]. This form of *Lamprothamnium* is able to thrive at extremely high salinities (70–90 g TDS  $\text{L}^{-1}$ ). In 2000–2001, sparse populations coexisted with the *C. chthonoplastes* microbial mats. Some of the larger individuals were overgrown by slimy biofilms comprising diatoms and Chloroflexaceae, which at the sediment interface coalesced with the microbial mats. However, from 2002, this charophyte dramatically increased its surface and at many places supplanted the existing *C. chthonoplastes* microbial mats. This colonization culminated during 2005 in extensive prairies covering virtually all sediments between 0.2 and 1.8 m water depths.



**Figure 6.** HPLC traces of lipophilic pigments extracted from the 1–1.5 mm depth layer of a microbial mat build by the cyanobacterium *Coleofasciculus chthonoplastes* associated with Chloroflexaceae. The pigment analysis was performed after the protocol described by Buffan-Dubau et al. [17] using a diode array detector (350–800 nm). By comparing the chromatograms selected at 440 and 664 nm, we can directly see whether the peaks correspond to a carotenoid (no absorption at 664 nm) or to a green chlorophyll (Chla) and bacteriochlorophylls (BChlc and BChld). Note the different peaks of BChlc and BChld, which correspond to different allomers that may differ by the alcohol esterified to the macrocycle and substituents on the macrocycle.  $\lambda_{max}$  gives the absorption maxima of the separated pigments measured in-line with the diode array detector.

In September 2006, it was observed that the shallower *L. papulosum* populations had become exposed to air [9] as the water level had further decreased by 30 cm since September 2005. Full sunlight and drying out of the top part triggered massive decay of *Lamprothamnium* and detritus of this charophyte accumulated in the lake. In the shallower parts of the lake, photosynthetic biofilms developed on top of this *Lamprothamnium* detritus (see **Figure 5D**). These biofilms were dominated by Chloroflexaceae together with different species of filamentous cyanobacteria. The remaining multilayered microbial mats build by *C. chthonoplastes* and Chloroflexaceae that had been restricted in area in 2005 to the littoral zone up to a depth of 20 cm, were now completely emerged and dried out. Hence, healthy *C. chthonoplastes* mats had

almost completely disappeared from the lake. The biofilms, mainly comprising Chloroflexaceae, persisted until the spring of 2007. After an almost complete wane of the *C. chthonoplastes* builds microbial mats in 2006, there was again a new colonization by young mats of the shallower parts of the lake since the summer of 2007. However, during this period, salinities continued to increase. This resulted, in 2010, in the precipitation of gypsum ( $\text{CaSO}_4$ ) at the surface of the newly established multilayered microbial mats build by *C. chthonoplastes* (see **Figure 5E**). During the 25-year-period, the microbial mats build by *C. chthonoplastes* waxed and waned, and this chronology has been described in more detail by De Wit et al. [9].

### 2.3.3. Interactions between the pelagos and the benthos

When combining the observations about the pelagos and the benthos, it appears that regime shifts are a characteristic feature in La Salada de Chiprana and may, thus, occur commonly in hypersaline lakes. Hence, we can clearly discern different forms of an ecosystem state characterized by low water turbidity, low phytoplankton and strong development of benthic communities. This low turbidity and low phytoplankton state seems to be obtained via top-down control of phytoplankton by the brine shrimp *Artemia parthenogenetica*. Within these benthic communities, the microbial mats build by *C. chthonoplastes* associated with Chloroflexaceae occupy a conspicuous role. In some cases, these mats completely dominate the benthic community in the lake, particularly at the shallower areas. Nonetheless, macrophytes may enter in competition with these microbial mats for space at the lake bottom. At lower salinities, probably up to  $45 \text{ g TDS L}^{-1}$ , the microbial mats may suffer from competition with *Ruppia* species. At higher salinities, the microbial mats may enter in competition with the extremely halotolerant foxtail stonewort *Lamprothamnium papulosum* var. *papulosum* f. *aragonense* (Prósper) Wood. Nevertheless, the observations after 2007 indicate that this charophyte cannot colonize the lake when salinities are above  $90 \text{ g TDS L}^{-1}$ .

Hypersaline systems are often, by nature, eutrophic. The athalassic lakes represent the terminal for runoff and groundwater flows that carry both dissolved salt and nutrients. The water budget is equilibrated by high evaporation from the lake. As a result, salt lakes accumulate dissolved salts and nutrients. Therefore, in the long-term, they tend to become eutrophic and more saline. During the low turbidity and low phytoplankton state, most of the nutrients will be captured by the benthic communities, i.e., the microbial mats or the macrophytes, both Magnoliophyta as *Ruppia* sp. and charophytes as *Lamprothamnium papulosum*. However, when the top-down control is being relieved due to disappearance of brine shrimps from the lake, the fast growing phytoplankton may quickly build up high densities and the water becomes turbid. The increasing turbidity will have a negative impact on the performance of the benthic mats and macrophytes. As a result, more and more nutrients will become available for the phytoplankton and the system may flip over into a turbid state as it had been observed in 1993 and 1994 by Diaz et al. [14]. Such an interaction scheme is characterized by a positive feedback loop for phytoplankton, i.e., the development of phytoplankton decreases the competitive abilities of the benthic communities and, thus, favors its own growth. This poses the question whether multiple stable states do exist for hypersaline lakes as these have been described for freshwater lakes by Scheffer et al. [25], for which the alternate states are characterized by (1)

phytoplankton-rich high turbidity without submerged benthic vegetation and (2) highly transparent (clear) water, low phytoplankton with extensive meadows of submerged aquatic vegetation, respectively. Nevertheless, to prove the presence of multiple stable states, it is not sufficient to document regime shifts, but rather it needs to be shown that for a given window of environmental conditions, two stable states do potentially exist, each of them stabilized by positive feedback loops.

To define the windows for the different ecosystem states in this hypersaline lake, it appears most important to consider the ecology of the brine shrimp, because of its role in top-down control. Brine shrimps from different geographic origin have been reported to survive at salinities ranging from 35 to 110 g TDS L<sup>-1</sup>. Assuming that this salinity range also applies for the growth and survival of the species in the lake *Artemia parthenogenetica*, we can expect that in this salinity window, a highly transparent water layer will favor benthic communities, i.e., most often microbial mats build by *C. chthonoplastes* co-occurring with *Ruppia* and/or *Lamprothamnion*. When moving outside this window of salinities, we expect regime shifts. This has, indeed, been observed during decreasing salinities in the early 1990s and I expect it to happen in the future, if the water level continues to drop down. However, both shifts do not proof the existence of multiple stable states. Multiple stable states could exist in the salinity window ranging from 35 to 110 g TDS L<sup>-1</sup>, if certain conditions exist that keep the densities of the brine shrimp very low. However, I am not aware of an efficient top-down control mechanism that could keep the brine shrimps in check as the high salinities prevent the occurrence of fish or invertebrate predators.

#### 2.4. *Coleofasciculus chthonoplastes* microbial mats as stromatolites

The laminated structure of the *C. chthonoplastes* (see **Figure 5E**) shows that this benthic microbial mat system can be considered a living stromatolite, which is a layered benthic microbial community that accretes due to the trapping, binding and cementation of sedimentary grains. These mineral grains incorporated in the organic matrix can be derived from the substratum, from external inputs or through the precipitation of mineral particles. The biofilms show the clear presence of fine layers of carbonate grains (**Figure 5E**), which turned out to be high-Mg calcite [19]. Ludwig et al. [26] reported that the precipitation of these grains was driven by photosynthesis that results in higher pH values, locally, in the mat. Hence, the precipitation of high-Mg calcite can be described by the following reaction equation:  $\text{Mg}^{2+} + 4 \text{Ca}^{2+} + 5\text{HCO}_3^- + 5 \text{OH}^- \rightarrow \text{MgCa}_4(\text{CO}_3)_5 + 5\text{H}_2\text{O}$ . This represents the stoichiometry for a high-Mg calcite crystal with 20% (molar) proportion of Mg. In practice, the values of Mg content in the high-Mg calcite are variable [19].

Viruses are engulfed into these high-Mg calcite crystals, which represents a mechanism for the fossilization of viruses and their long-term conservation in the geological record. This was shown recently by using three different microscopy techniques (epifluorescence, electronic and atomic force microscopes). These viruses were 50–80 nm in diameter, while energy-dispersive X-ray spectrometer analysis showed that they contain nitrogen and phosphorus in a molar ratio of approximately 9, which is typical for viruses of that size. Nucleic acid staining revealed that they contain DNA or RNA. As characteristic for hypersaline environments, the

concentrations of free and attached viruses were very high (more than  $10^{10}$  viruses per g of mat). Acid treatment that dissolved the high-Mg calcite showed that there were approximately  $15 \rightarrow 10^9$  viruses per g of high-Mg calcite [19].

The observation in lake La Salada de Chiprana are also interesting in the way that it gives additional ideas why the extension of stromatolites has decreased dramatically during geological times. The current paradigm is that the evolution of metazoans has resulted in increased grazing on the microbial communities of the stromatolites and that, therefore, the present stromatolites are restricted to extreme environments where grazing pressure is low. Such extreme environments include the hypersaline environments. The observations show that the *C. chthonoplastes* build microbial mats can also be exposed to competition for space with macrophytes, which includes both the Magnoliophyta *Ruppia* sp. as well as the charophyte *Lamprothamnium papulosum* var. *papulosum*.

### 3. Conclusions and perspectives

Athalassic saline lakes are home to interesting biota, and their conservation is most important for scientific research, potential economic exploitation and aesthetics. When located in a pristine landscape, these lakes are superb sentinels for detecting climatic variations through monitoring of lake level, extent, salinities and biota. However, on a worldwide scale, many athalassic salt lakes have suffered severe degradation and it has been predicted that during this decade, the surface and volume of these lakes will decrease with increasing salinities, and that this will result in further degradation of their ecosystem values. Despite the fact that the Salada de Chiprana represents a strongly anthropogenic-influenced salt lake that forms part of a cultural landscape, its specific ecosystem biodiversity and ecosystem properties merit full protection in this global context. Therefore, management actions taken in 1993 allowed preventing direct introduction of surplus irrigation water into the lake. Although these management actions have resulted in beautiful, albeit variable, ecosystem properties from 1995–2006, I think that the current trend of increasing salinities and decreasing lake surface may jeopardize its conservation in the future. **Figure 3** includes a prediction of surface and bathymetry when salinities will reach  $120 \text{ g TDS L}^{-1}$ , which will imply the demise of the brine shrimps and the disappearance of the *C. chthonoplastes* microbial mats. This is expected to occur before 2020, if no further measures were taken.

The main question is now whether a conservation scheme for the lake La Salada de Chiprana should be based on the conservation of specific communities and ecosystem properties or should priority be given to reducing the human impacts and reestablishment of the natural climatic and hydrological factors? The latter is not really feasible in the context of the human influenced landscape. Therefore, it has been envisioned to add additional clean water from the Civán canal into the lake to compensate for the systematic loss of volume and first experiments have been performed in 2014.

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