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*Conservation of a permanent hypersaline lake: management options evaluated from decadal variability of Coleofasciculus chthonoplastes microbial mats*

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## ABSTRACT

(1) “La Salada de Chiprana” (NE Spain) is a permanent athalassic saline lake, and as such unique in Western Europe. More than 20 years ago, it was recognized that this lake should be protected; particularly, because it supported extensive microbial mat communities built by the cyanobacterium *Coleofasciculus (Microcoleus) chthonoplastes* and submerged aquatic vegetation of an endemic form of the foxtail stonewort (*Lamprothamnium papulosum* var. *papulosum* f. *aragonense*).

(2) For centuries, the lake hydrology, salinity and water levels have been driven by the combined effects of anthropogenic activities (e.g. irrigation in the watershed) and climatic factors.

(3) In the early 1990s, the water level increased and salinity dropped as a consequence of excessive fresh water inputs from direct overflow via irrigation canals. As a result, the microbial mats and aquatic vegetation perished and water column turbidity increased. Based on these observations, since 1994 management measures were introduced aimed at reducing the fresh water input by stopping the direct overflow from irrigation canals. This resulted in a rapid recovery of the hypersaline features of the lake, particularly of the microbial mat communities and the aquatic vegetation.

(4) Since 2000, there has been a persistent trend of decreasing lake level and increasing salinity. The population of the endemic foxtail stonewort collapsed in 2006 and the area occupied by *C. chthonoplastes* mats has drastically decreased. This raises the important question on how to manage athalassic saline lakes in their agro-cultural context and calls for a review of the management option taken in the early 1990s. While it could be argued that the

current situation is closer to natural hydrological functioning than 20 years ago, we propose that management should seek to conserve the original microbial and aquatic vegetation communities.

## INTRODUCTION

The majority of the inland athalassic saline lakes in Western Europe are ephemeral, shallow and restricted to endorheic basins in the semiarid regions of the Iberian Peninsula (Guerrero and De Wit, 1992; Guerrero *et al.*, 1994). “La Salada de Chiprana” (NE Spain) is unique in Western Europe because it is permanent with a reported maximum depth of 5.6 m (Guerrero *et al.*, 1991; Vidondo *et al.*, 1993). One of the most conspicuous features of this permanent hypersaline lake is the wide-spread occurrence of benthic microbial mats built by the cyanobacterium *Coleofasciculus chthonoplastes* (Thuret ex Gomont) Siegesmund, Johansen et Friedl 2008, formerly known as *Microcoleus chthonoplastes* (Siegesmund *et al.*, 2008). These mats have been intensively studied over the last two decades (Guerrero *et al.*, 1991; Guerrero and De Wit, 1992; Vidondo *et al.*, 1993; Camacho and De Wit, 2003; Jonkers *et al.*, 2003; De Wit *et al.*, 2005; Ludwig *et al.*, 2005, 2006; Bachar *et al.*, 2007; Hinck *et al.*, 2007; Polerecky *et al.*, 2007; De Beer *et al.*, 2008). Other major features are the proliferation of a special form of the foxtail stonewort (*Lamprothamnium papulosum* var. *papulosum* f. *aragonense*) (Prósper) Wood, which is endemic in Aragón, and the abundant populations of the brine shrimps *Artemia parthenogenetica* Bowen & Sterling. The latter exert a strong top-down control on phytoplankton and thus contribute to maintaining high water transparencies (Diaz *et al.*, 1998). This area is currently a protected area and identified as a Ramsar wetland

site (since 1994), a Site of Community Interest according the Habitat Directive in the European Union (SCI since 1997) and declared a natural reserve since 2006 by the Gobierno de Aragón to conserve its unique natural and cultural values, which explicitly include the microbial mats and the hypersaline conditions.

A paleolimnological study (Valero-Garcés *et al.*, 2000) has shown that during most of the Mid and Late Holocene, this lake was ephemeral and that anthropogenic activities, i.e. deforestation, olive tree planting and irrigation, resulted in its conversion into a permanent hypersaline lake since 1700 AD. Therefore, for 300 years, this environment has been subjected to both climatic and antropogenic influences which are reflected in variations of salinity, water volume and level. The combined impact of human cultural practices and climatic influences raise the difficult question as to how this unique aquatic ecosystem should be managed in the face of changes in anthropogenic and climatic drivers (e.g., Williams, 1993a, b; Florin and Montes, 1999; Kallis *et al.*, 2010). Should such a conservation scheme be based on the conservation of specific communities and ecosystem properties or should priority be given to reducing the human impacts and re-establishment of the natural climatic and hydrological factors? Agricultural practices in the area have intensified since the 1950s. In the early 1990s the lake was subjected to increasing inputs of fresh water from irrigation returns and scientists advised that there should be a reduction in the input of irrigation water into the watershed of the lake (Diaz *et al.*, 1998; Valero-Garces *et al.*, 2000) in order to protect the unique ecosystem properties of the permanent hypersaline lake. In general, worldwide many athalassic salt lakes have suffered severe degradation and it has been predicted that their extension and ecosystem values will further decrease during the following decades (Williams, 2002). 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.

In this paper we briefly review the field observations from the last 20 years reported by different microbial ecology researchers on the *C. chthonoplastes* microbial mats in “La Salada de Chiprana” and discuss them in the context of the variations of the levels, surface area, volumes and salinity of this lake. This allows to gain understanding about the variability of *C. chthonoplastes* microbial mats on decadal time scales and the drivers that explain their wax and wane. We predict that the lake levels will continue to drop if current management practices are maintained (“management as usual” scenario), which will result in decreasing water surface area and increasing salinity. Such a situation is a threat for the *C. chthonoplastes* mats, as well as for the *Artemia parthenogenetica* and for *L. papulosum* var. *papulosum* f. *aragonense*. Alternative management options and related scenarios for this salt lake ecosystem are discussed.

## STUDY AREA

“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 (Figures 1 and 2) 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 (Vidondo *et al.*, 1993). The watershed of the lake is 768 ha of which 515.7 ha benefit from protection measures, i.e. 154.8 ha as nature reserve and 360.9 ha as a periferal buffer zone. The salt lake lies upon the Upper Oligocene-Miocene Caspe formation that mainly comprises sand and silt stones. The lake's hydrology is governed by high evapotranspiration (1000-1500 mm year<sup>-1</sup>), low rainfall (200-400 mm year<sup>-1</sup>), water runoff, irrigation returns and groundwater flow. The climate is Mediterranean characterized by very hot summers and cold dry winters. The lake water chemistry is dominated by Na<sup>+</sup>, Mg<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup> and TDS have been reported as high as 80 g l<sup>-1</sup> (e.g. Jonkers *et al.*, 2003)

although values in the surface water layers often decrease after periods of heavy rainfall and the lake has shown large variations during the last two decades. A small part of the littoral zone of the lake shows *Phragmites australis* vegetation, particularly in the area close to the outflow of a canal connecting with the oligohaline Laguna de Roces, (Figure 1).

## METHODS

### Measurement and quantification of the level, surface, volume and salinities of the lake

Variations of the lake level were measured at a geometric gauge located in the NE part of the lake. The zero level of the gauge corresponds to 137.85 m height (ED 50) above mean sea level (Alicante). To calculate the corresponding area and volume of the lake, a digital elevation model is computed in combining the available topographic map of the area (Spanish Instituto Geografico Nacional, 2011) and a bathymetric map of the lake measured in 1988 (Guerrero *et al.*,1991). Documents have been georeferenced (according to the TRSS89; European Terrestrial Reference System) and digitized using a commercial GIS software (Arcgis 9.3-ESRI) . The original bathymetric map document does not include coordinates. In order to position and correctly align the data on the geographic terrestrial reference system, we identified remarkable objects that were identifiable on available aerial photographs (Spanish Instituto Geografico Nacional, 2011; GoogleEarth) and could be located on the bathymetric map. The precise geographic location of some of these objects was also revealed by GPS measurements during field work in April 2011. The original bathymetric map document does not provide any reference point and, in addition, variations of the lake level have only been measured since 1990 which makes it hard to convert water depth measurements – made in 1988 - to absolute height values above mean sea level (Alicante). However, by comparing with 1991 data, and assuming a conservative behaviour of

salinities, it was estimated that the 0-level of the lake on the map of Guerrero *et al.* (1991) corresponded to 137.8 m height. Finally, the digital elevation model of the lake and surroundings was calculated using the interpolation method and algorithm developed by Hutchinson (1989). This latter is based on the digitized elements comprising: i) isobaths of the bathymetric map (Guerrero *et al.*, 1991), ii) contours from the topographic map (Spanish Instituto Geografico Nacional, 2011), iii) stream and ridge lines, and iv) sinks that could be recognized and plotted on the topographic map and/or available aerial photographs. Such an interpolation procedure is specific for the treatment of topographies and ensures a consistent drainage pattern along the resulting interpolated surface (Hutchinson, 1989). Based on this topographic digital grid, water volume and surface area of the lake can be calculated for any given water height assuming a totally horizontal plane for the water in the lake. To test the robustness of the digital elevation model, the shoreline contour of the lake was computed and mapped for different water levels and compared with satellite/aerial images or landscape pictures of the lake for some selected dates.

Salinity measurements from different researchers have been compiled when these were based on complete analyses of cations and anions. Annual rainfall data were calculated for calendar years from a meteorological station located in Chiprana since 1995. Descriptions of microbial mat communities were obtained from literature and completed for recent years by our own observations based on light and epi-fluorescent microscopy, TEM and SEM microscopy, and HPLC analyses of pigments. The first author visited the lake in 1989, 1991, 1993, 1996, 1999, 2000, 2001, 2002, 2004, 2005, 2006, 2007, 2009, 2010 and 2011. General information on the structure and functioning of the pelagic communities were obtained from literature.

### **Oxygen profiles and saltwater freshwater switch experiments**

On 23 September 2005, we observed that *C. chthonoplastes* mats in the field adjacent to the reed zone (*Ph. australis*), in the area of the outlet of the small canal linking to Laguna de Roces, were exposed to extremely rapid changes in salinity, due to irregular ground water upwelling and surface runoff flows combined with irregular wind-driven currents. At the local scale of the mats, the salinity of the 10-20 cm overlying water column changed from that of the bulk of the lake (estimated 96 g/l TDS from conductivity measurements) to 0 g/l TDS within 15 minutes before lake salinity was re-established very suddenly and quickly, dependent on water movement by winds. We simulated the impact of such rapid short-term changes in the laboratory on 3 and 4 October 2005. Samples of these mats were taken on 25 September and brought to the laboratory in Bremen. These were immersed in Lake Chiprana water and incubated under a natural light/dark regime in a greenhouse. On the afternoon of 3 October a subsample was taken and incubated under artificial light at  $330 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $21 \text{ }^\circ\text{C}$ . Oxygen profiles were measured with custom-made oxygen micro-electrodes using standard techniques (Jonkers *et al.*, 2003). Overlying water was siphoned off and replaced by MilliQ water of 0 g/l TDS and  $21^\circ\text{C}$  to monitor the changes in  $\text{O}_2$  profiles upon a shift from saline to fresh water. Fresh water was maintained for 19 hours and during that period the mats were exposed sequentially to 4 h light, 10 h dark and 5 h light before the fresh water was replaced again by Chiprana lake water to monitor the switch from fresh to saline under light conditions ( $330 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $21 \text{ }^\circ\text{C}$ ).

## **VARIATION OF THE LAKE LEVEL WATER BODY SURFACE AND SALINITIES**

Variations of the recorded lake level, and the salinities measured by various research groups in the Salada de Chiprana between 1991 to 2009 are shown in Figure 3. Water levels were highest in the early 1990s and decreased strongly after 1994. Irrigation water for agriculture in the surroundings is drawn from a reservoir in the Guadalope river and

channelled to the watershed of the Salada de Chiprana through open irrigation channels, i.e. the Civán channel which connects to the Acequia de Farol and the Acequia de la Casilla in the surroundings of the Salada de Chiprana. An additional irrigation water input occurs through overflow of the upstream Regallo irrigation zone that also feeds into the Acequia de Farol and the Acequia de la Casilla (Figure 1). Before 1994, surplus water from the irrigation channel that was not used for irrigation was directly channelled into the Salada de Chiprana, mainly via the Acequia de la Casilla. It was concluded that this practice created major environmental problems (see below) and, therefore, it was decided to channel the surplus water directly into the Arroyo del Regallo river by constructing a hydraulic works in San Marcos. Valéro-Garcés *et al.* (2000) mention that  $104 \cdot 10^3 \text{ m}^3$  fresh water from Civán channel entered the lake in 1993. The hydraulic works (Berga *et al.*, 1994) have been used since 1994 to regulate inflow of irrigation water into the watershed of La Salada de Chiprana and to divert the excess irrigation water to the Arroyo del Regallo river, which drains directly to the Reservoir of Mequinenza in the Ebro river. In addition, measures were taken to prevent that water from a small irrigation channel, i.e. the small Acequia de la Casilla, entering the lake directly. As a result, the lake level dropped very rapidly between January 1994 and September 1995. The lake has been fed since 1994 by groundwater flows (Baquer Barriendos, 1999), surface runoff resulting from rain and irrigation returns, as well as from overflow from a oligohaline water pond, *i.e.* Laguna de Rocés, located NW of the Salada (Figures 1, 2b, c), which itself also receives water from runoff and irrigation. Annual rainfall ranges from less than 200 to slightly above 400 mm/year with the observation of the exceptionally high value of 666 mm in 2005. Between 1996 and 1999, the lake level rose again by about 1 m. Since 1998, the lake shows repeatable intrannual fluctuations with an amplitude of 30 to 50 cm. Water levels rise during winter and spring and decrease during summer achieving their minimum levels in late summer or autumn. Since 2000, the lake has shown a persistent trend of a decreasing level. A

particularly strong tendency was observed between 1999 and 2002 (about 20 cm per year) that seems related to low rainfall from 1999 to 2001 (about 200 mm yr<sup>-1</sup>). Between the early 1990s and early 2009 the lake level decreased overall by 1.75 m. This observed decrease is in relatively good agreement with reports of the maximum depth reported, i.e. 5.6 m in the early 1990s (Vidondo *et al.*, 1993) and 3.7 m recorded on 4 August 2007 by the Conferación Hidrográfica del Ebro.

Figure 4 gives the surface areas and volumes of the salt lake calculated from the topography and lake levels. It can be seen that the 1.75 m decrease of water level between 1991 and end 2008 corresponded to a 42 % reduction in surface, i.e., from 36 ha to 21 ha, and a 55 % reduction in volume (from 893,000 to 405,000 m<sup>3</sup>). In accordance with the lake level the salinity fluctuated in the top 50 cm from 30 g/L TDS to 90 g/L (Figure 3a). Nevertheless, the ionic composition remained remarkably constant and was dominated by Na<sup>+</sup>, Mg<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup>. The molar ratio of SO<sub>4</sub><sup>2-</sup>/Cl<sup>-</sup> was 1.13 ± 0.24, of Na<sup>+</sup>/Cl<sup>-</sup> 1.38 ± 0.19, and magnesium exceeded calcium with more variable molar ratios of Mg<sup>2+</sup>/Ca<sup>2+</sup> 21.4 ± 10.5 (n = 53). The total amount of salts in the lake was estimated to equal 44,750 tons. After 1993, a small basin in the SE corner became separated from the main lake when the water level dropped below 137.6 m (ED 50) above mean sea-level (Alicante) (Figure 2e). This separated 822 tons of dissolved salt from the lake. The variations of the salinities in the lake with the variations of its volume were compared to the conservative relationships (Figure 5). Lower salinities than predicted by conservative behaviour could be explained by loss of salt from the lake through deposition of evaporites on emerging playa areas as the lake progressively decreased in extent. On the other hand we have no reliable data on how much salt enters the lake through subsurface water flows. Other deviations from conservative behaviour can be due to non-homogeneous distributions of salt concentrations in the lake. For example, during the early nineties, the lake frequently showed a halocline with water masses

of lower salinities stratified above more saline water (Vidondo *et al.*, 1993). It appears that halocline formation has become less frequent as the average depth of the lake has been decreasing. Finally, horizontal heterogeneities can be due to localised inputs from fresh or less saline waters. Nonetheless, the salinities in the lake are quite well described by conservative behaviour (Figure 5).

Lake extents have been computed for the highest (May 1991) and lowest (October 2008) water levels and reported on the digitized topographic map of the area (Figure 6). The model clearly shows the narrow connection that, in May 1991 still connected the main lake with the small basin in the SE corner (Figure 6a). In 2008, this former SE basin represented a salt flat completely separated from the lake (Figure 6c), which was often dry but occasionally with a water layer from rainfall. In general, the lake extent had considerably decreased in October 2008 (Figure 6c), and particularly showed the emersion of shallow slope playa zones along the SW-NE lake border and in the southern bay. In addition, in the interior of the lake, several parts of the paleochannels that were submerged at shallow water depths in 1991 had emerged as islands within the lake. The calculated maximum water depth was 3.40 m in 2008. In the eastern part, a subbasin was under way of becoming separated from the lake as its connection with the main lake showed less than 50 cm water depth.

### **TIME LINE OF BENTHIC COMMUNITIES**

Figure 3 also depicts the timeline of the major ecosystem changes particularly those that affected the benthic communities. Between 1989 and 1991, the benthic microbial mats built by the cosmopolitan cyanobacterium *Coleofasciculus chthonoplastes* covered large parts of the shallower sloping areas (playas) up to a depth of 150 cm (Vidondo *et al.*, 1993) (Figures 2h , j). Vidondo *et al.* (1993) described the presence of *Ruppia* prairies and highlighted that the *Ruppia* and microbial mats interact negatively. Thus, the extension of the

*Ruppia* meadows, a phenomenon which was attributed to decreasing salinities (Vidondo *et al.*, 1993), resulted in partial replacement of the *C. chthonoplastes* mats.

Diaz *et al.* (1998) suggest that continuing decreasing salinities in combination with increased eutrophication resulted in heavy epiphytic growth on *Ruppia*. The nutrient input into the lake in concert with a drastic decrease of the population densities of the water column grazer, the brine shrimp *Artemia parthenogenetica*, resulted in dense phytoplankton blooms and reduced light penetration. As a result, microbial mats had virtually disappeared from the deeper parts of the littoral zone and dramatically changed their composition in the shallower waters by 1993 (Diaz *et al.*, 1998). This phenomenon gave rise to conservation measurements by the local authorities, who became aware of the negative impact of the direct overflow from the irrigation channel into the lake on the original pelagic and benthic communities (see above).

The situation was apparently reversed by the inauguration of the hydraulic work in San Marcos after 1993. Valero-Garcés *et al.* (2000) reported that since 1995, a particularly dry year in this region (200 mm rainfall), water column transparencies recovered. During a visit in September 1996 we 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, early 1990s by Vidondo *et al.* (1993).

Very well developed multilayered microbial mats built by *C. chthonoplastes* and *Chloroflexus*-like bacteria (CLB) with multiple white layers of calcium carbonate were observed from 1988 - 1990 (Vidondo *et al.*, 1993) and from 1996-2005, in the shallower parts of the lake (playas). The microbial structure of these mats has been described in detail by Jonkers *et al.* (2003). The presence of different allomers of bacteriochlorophyll *c* and *d* (BChlc and BChld) and thin filament morphologies in the mat that do not fluoresce red light upon blue excitation indicated that *Chloroflexus* like bacteria (CLB) were present in these

mats in high densities. The presence of these CLB has been further corroborated in a phylogenetic study (Bachar *et al.*, 2007), and Polerecky *et al.* (2007) have determined how these organisms contribute to oxygen cycling in these mats. In addition, diatoms occurred in the mat and were generally more abundant in the top layer and particularly during spring. Camacho and De Wit (2003) performed an experimental study on the effect of N and P additions on the competitive responses of cyanobacteria and diatoms in the mat. They found that diatoms were stimulated by N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) additions, while the joint addition of N and P resulted in stimulation of growth of cyanobacteria. Small amounts of *Beggiatoa* have also been observed in these mats (Hinck *et al.*, 2007). Furthermore Ludwig *et al.* (2005) reported that calcification in these mats was driven by photosynthesis which increases pH values locally in the mat.

In 2000, we observed sparse populations of a specific form of the foxtail stonewort i.e. *Lamprothamnium papulosum* var. *papulosum* f. *aragonense* (Prósper) Wood (Figure 2f). This charophyte has been described originally as a distinct species by Eduardo Reyes Prósper in 1910 as a typical plant for the semi-arid steppe lakes in Aragon (Prósper, 1910; Cirujano *et al.*, 2007). The sparse populations coexisted with the *C. chthonoplastes* microbial mats. Some of the larger individuals were overgrown by slimy biofilms comprising diatoms and CLB (Figure 2g), which at the sediment interface coalesced with the microbial mats. From 2002 we observed many new shoots of the *L. papulosum* var. *papulosum* f. *aragonense* (Figure 2f). It should be highlighted that this form of *Lamprothamnium* is able to thrive at extremely high salinities (TDS 70 to 90 g/l). As these new shoots grew larger they formed prairies, which replaced the existing microbial mats. This phenomenon culminated during 2005 in extensive prairies covering virtually all sediments between 0.2 and 1.8 m water depths. At some locations, these charophytes were overgrown with microbial mats (Figure 2g). In September 2006, the water level was about 20 cm lower than in September 2005. As a result, in large

shallow areas the top part of the *L. papulosum* populations were exposed to air and full sunlight apparently triggering their massive decay. Large amounts of organic debris from *Lamprothamnium* subsequently accumulated in the lake (Figure 2h) resulting in widespread development of photosynthetic biofilms on top of the *Lamprothamnium* detritus in the shallower parts of the lake, which were dominated by the *Chloroflexus*-like bacteria together with different species of filamentous cyanobacteria (Figure 2i). In some places at 30 to 40 cm water depth, the detritus layer had been removed, probably by water movement, where characteristic microbial mats dominated by the cyanobacteria *Lyngbya* spp. had developed on the exposed sediments (Figure 2h). The multilayered microbial mats built by *C. chthonoplastes* and CLB that covered the littoral zone in 2005 to a depth of 20 cm was completely emerged and dried out. Healthy *C. chthonoplastes* mats had almost completely disappeared from the lake. However, in some sheltered places along the shores of the lake we still found the characteristic mats built by *C. chthonoplastes* and CLB that can be considered as relics of the mats that had been studied previously (Vidondo *et al.*, 1993; Jonkers *et al.*, 2003; Ludwig *et al.*, 2005).

In March 2007, we still found significant amounts of degraded *Lamprothamnium* detritus covered with mats dominated by the CLB. These biofilms had virtually disappeared in September 2007. Both in March and September 2007, we observed young microbial mats built by *C. chthonoplastes* and CLB along the shore of the lake. Such relatively young *C. chthonoplastes* mats were also observed along the littoral zone in September 2009 (Figure 2j).

The shallow playa areas up to 1.5 m depth are particularly favourable for microbial mat development (Vidondo *et al.*, 1993). Figures 6b and 6d illustrate their location, computed respectively for the maximal (in May 1991) and minimal (in October 2008) water depths measured during the studied time window. In 1991, the area covered with 0-0.5 m water represented 6 ha and those covered with 0.5-1.5 m water represented 8 ha (their sum

represented 38 % of the lake surface). In 2008 with the decrease of water level, the area covered with 0-0.5 m water represented 3.70 ha (a 39 % loss of surface since 1991) and those covered with 0.5-1.5 m water represented 4.5 ha (a 43 % loss of surface since 1991). The extension of mats depends mainly on light availability at the surface of the sediment and depth distribution of mats is, therefore, strongly dependent on the turbidity of the water column. During periods of very low water turbidity, microbial mats could cover the entire lake bottom.

### **SHORT TERM IMPACT OF EXTREME SALINITY VARIATION ON OXYGEN PROFILES IN *C. CHTHONOPLASTES* MATS**

Shifts from saline to almost freshwater conditions and back, were observed in the field on 23 September 2005 for the *C. chthonoplastes* mats occurring adjacent to the reed zone (*Ph. australis*) close to the outlet of the small canal linking to Laguna de Rocés. The impacts of such extreme fluctuations were studied experimentally in the laboratory (see Methods). Oxygen profiles show that in the light, oxygen accumulates below the sediment surface due to oxygenic photosynthesis by the cyanobacteria in the mat. Upon a shift from saline lake water to freshwater, the maximum oxygen concentration below the surface decreased rapidly from 1000  $\mu\text{M}$  to 450  $\mu\text{M}$  (Figure 7). This indicates that the net photosynthesis rate in the mat decreased upon this shift. It appeared, however, that the community metabolism adapted rapidly to the new situation as within 45 minutes a new steady state was established with a constant net production rate. This implies that the *C. chthonoplastes* of the mat are continuing to photosynthesize under freshwater conditions, albeit probably at a lower rate than under the saline conditions. An alternative explanation can be that respiration by the mat community strongly increased under freshwater conditions, while photosynthesis was hardly affected.

Reversing the conditions, *i.e.* a backward shift from freshwater to saline conditions, resulted in a 12 % increase of oxygen concentration caused by increased net oxygenic photosynthesis rates within 1.5 hours. The transient profile recorded 0.5 hour after the salinity shock shows that the net photosynthesis rate initially decreased. It thus appears that the photosynthetic community can cope with the short-term alternations between hypersaline and freshwater, although the oxygen concentrations in the mat did not recover to the high values observed in the light before starting the shifting experiment. This indicates that the community shows a partial adaptation to freshwater exposure.

## **CONCLUSIONS AND RECOMMENDATION FOR MANAGEMENT OPTIONS**

### **Wax and wane of *C. chthonoplastes* mats in La Salada de Chiprana**

Regular observations in lake Chiprana during two decades have provided valuable information on factors that explain the wax and wane of *C. chthonoplastes* mats in hypersaline environments. These *C. chthonoplastes* mats thrive well at elevated salinities (TDS 60-90 g/l) in the Mg<sup>2+</sup> and SO<sub>4</sub><sup>2-</sup> rich waters in this lake, provided that sufficient light arrives at the sediment water interface. The brine shrimp, *Artemia parthenogenetica*, exerts top-down control on phytoplankton communities and thus contributes to maintaining the high water column transparencies that facilitate the growth of these *C. chthonoplastes* mats. As observed in 1993 (Diaz *et al.*, 1998), the brine shrimp disappears at low salinities (30 g TDS), which suppresses this indirect facilitation of the *C. chthonoplastes* mats thus contributing to their wane. However, our observations also show that the *C. chthonoplastes* mats can cope with sudden decreases of salinities, which occur in the littoral zone. In the short-term (hours)

these mats remain net autotrophic at zero salinity albeit showing a lower rate of net photosynthesis.

The observations also show that *C. chthonoplastes* mats compete for space with macrophytes and that prolific growth of such macrophytes contributes to the wane of the mats. Hence, the wane of the *C. chthonoplastes* mats between 1991-1993 was related to replacement by *Ruppia* meadows (Vidondo *et al.*, 1993), and their wane between 2002 and 2006 was explained by the proliferation of the foxtail stonewort (*Lamprothamnium papulosum* var. *papulosum* f. *aragonense*), thriving at extremely high salinities (TDS 70 to 90 g/l). The crash and degradation of *Lamprothamnium* biomass in 2006 created particular phenomena in the lake as it contributed to the wane of the *C. chthonoplastes* mats and favoured conditions for biofilms rich in *Chloroflexus*-like bacteria that grew on the *Lamprothamnium* detritus. Finally, characteristic intra-annual water level fluctuations (30-50 cm) imply that a belt of *C. chthonoplastes* mats often emerges during summer in the littoral zone, while new conditions for rewetting old mats and novel colonisation are created during rising lake levels in autumn and winter.

### **Future perspectives and management options**

Since 2000 there has been a general tendency of decreasing lake levels and shrinking lake surface (Figure 4). The decrease has been more pronounced during dry years (about 200 mm rainfall per year) and 30 to 50 cm fluctuations occur between winter and summer periods. Nevertheless, extrapolation of the current trend clearly predicts that the lake is losing about 22,000 m<sup>3</sup> per year and accordingly the lake surface and level will continue to drop and that salinities will continue to rise, threatening the characteristic communities, which were recognized in the early 1990s as representing a particular natural heritage that should be

protected. The emblematic *Lamprothamnium papulosum* var. *papulosum* f. *aragonense* (Prósper) Wood, which is endemic to Aragon, collapsed in the lake in 2006 and has not yet re-established its under water meadows suggesting that it cannot thrive at salinities exceeding 90 g/L TDS. Brine shrimps (*Artemia* sp.) from different geographic origins have been reported to survive at salinities ranging from 35 to 110 g/L TDS (Vanhaecke *et al.*, 1984). In solar salterns, microbial mats change composition along the salinity gradients and the *C. chthonoplastes*-CLB mats are replaced by gypsum-incrusted mats of *Halotheca/Phormidium* with dense purple sulfur bacterial layers (De Wit and Grimalt, 1992; Caumette *et al.*, 1994; De Wit and Caumette, 1994). Hence, the character of the lake is going to change if current management is maintained. To illustrate this we computed the predicted lake extent in the future when salinity could reach 120 g/L TDS (Figure 8). Hence, the lake surface would be 19.65 ha and at this salinity we can expect major ecosystem changes comprising i) the *C. chthonoplastes* communities would have disappeared and might be replaced by the other mat communities and ii) brine shrimps would have disappeared from the lake. The hydraulic works and introduction of strong control of irrigation in the watershed of the Chiprana lake since 1994 have been very successful during the 1990s early 2000s as these measures allowed the macrophyte and microbial mat communities to recover. The current situation appears to represent also a more natural situation with less direct human influences than observed in the early 1990s. However, more detailed geohydrological studies are needed to fully understand and quantify the natural hydraulics and separate clearly the anthropogenic effects from climatic variations. But, in this respect, it can also be argued that La Salada de Chiprana shows a similar negative development as it has been predicted for many other permanent athalassic salt lakes worldwide, i.e. shrinking volumes and surfaces and increasing salinities (Williams, 2002). Worldwide this trend is mainly related to increased diversion of freshwater inflows due to developments in the watersheds (Williams, 2002). For the specific case of La

Salada de Chiprana it is the current management that has resulted in decreasing lake levels and increasing salinities, which has induced drastic community changes in the lake since 2006. These changes will be exacerbated if current management continues, thus threatening the disappearance of its recognized natural heritage values and replacing it by smaller more saline lakes with different communities. This poses the question on how to manage the lake in the near future.

We believe that the managers are now facing clear philosophical and conceptual problems. Should such a conservation scheme be based on the conservation of specific communities and ecosystem properties or, alternatively, should priority be given to reducing the human impacts and re-establishment of the natural climatic and hydrological factors as the main objectives? We suggest that it is most important to conserve the lake as a permanent lake with a large surface area and with salinities ranging between 60-90 g/L TDS in order to protect i) the *C. chthonoplastes* microbial mats, ii) the endemic form of the *Lamprothamnium papulosum*, and iii) the brine shrimp. Such a management option requires the artificial addition of freshwater in the watershed of the lake, which can be done by using the existing irrigation system. However, extreme care should be taken to ensure that only non-contaminated water is used, and particularly to avoid the input of water from the Regallo irrigation zone (Figure 1). For implementing such an approach, it is most important to recognise that the lake forms anyhow part of a cultural-natural landscape as shown by Valero-Garcés *et al.* (2000), and that artificial measures may thus be considered.

Alternatively, the managers may wish to reduce the human impacts and re-establish the natural climatic and hydrological factors as major drivers for the lake hydrology and ecology. In this case they should be ready to accept dramatic community changes, shrinking lakes and increased salinization in the future with in the long-term the aim of re-establishing temporal lakes as was the case for Chiprana during the early medieval period (Valero-Garcés

*et al.*, 2000). However, re-establishment of the natural hydrological functioning appears unrealistic, because currently 32 % of the watershed of the lake is excluded from any protection measurements while only 20 % of its surface is included in the strict nature reserve. In addition, the watershed of the lake is not entirely independent of the regional landscape that has been largely modified by irrigation and creation of reservoirs. Thus it appears impossible to restore the early medieval conditions.

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## LEGENDS TO FIGURES

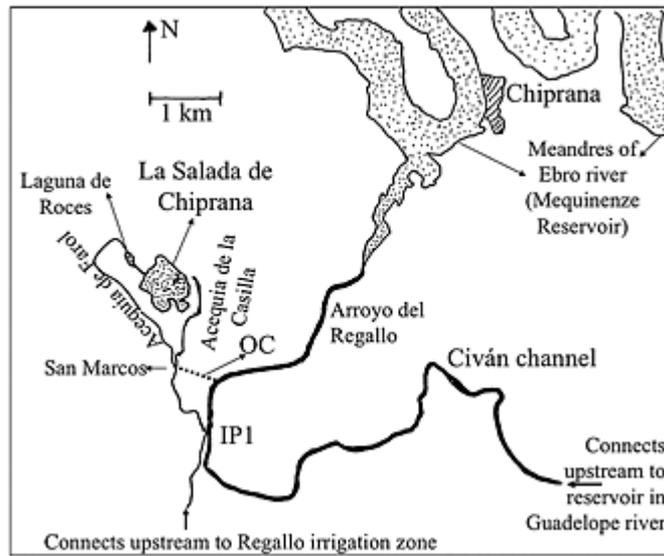


Fig. 1 Map of Chiprana (NE Spain) and its surroundings. The hypersaline lake La Salada de Chiprana is located 6 km to the SW of the village of Chiprana. Irrigation water has been channelled to the watershed of the hypersaline lake through the Acequia de Farol and Acequia de la Casilla irrigation canals. At the intersection point IP1, sluices permit to feed both canals either through the Civán channel from the reservoir in the Guadalupe river or to use the overflow from the Regallo irrigation zone. Hydraulic works were executed in San Marcos in 1994 and have been used since to regulate the inflow into the Acequia de Farol and the Acequia de la Casilla irrigation canals. In addition a blast pipe (OC) has been constructed to divert the excess irrigation water into the Arroyo del Regallo creek. The latter drains directly to the Reservoir of Mequinenza in the Ebro river. Laguna de Rocés is a small oligohaline lake in the watershed of La Salada de Chiprana, which connects to the latter through a small canal.

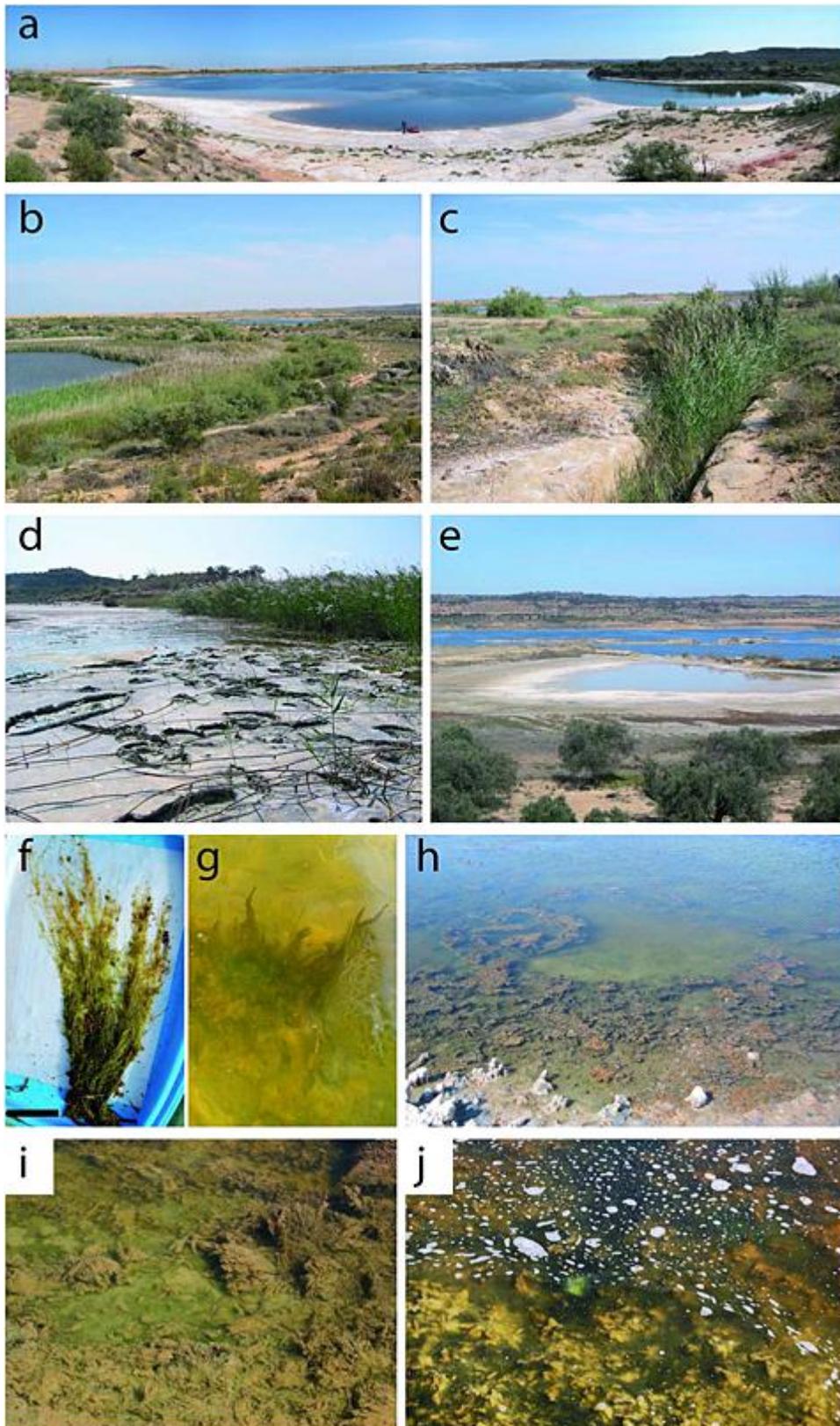
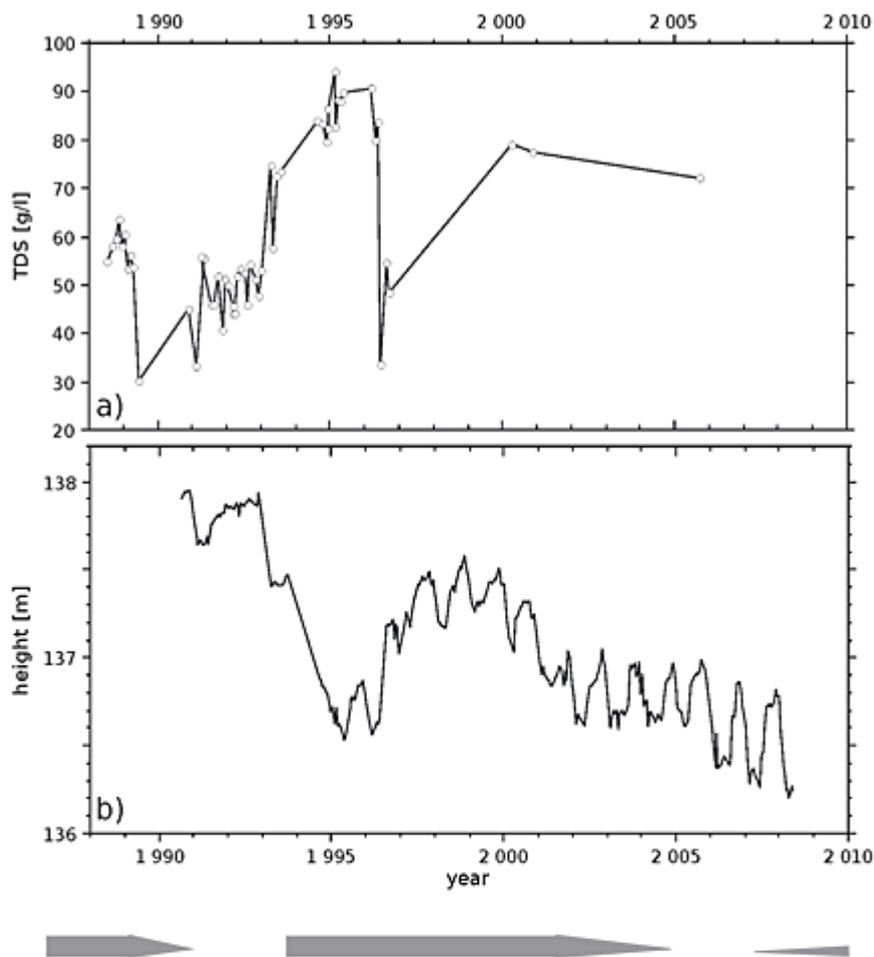


Fig. 2 Hypersaline lake La Salada de Chiprana and peripheral watersheds (Aragón, Spain). a) Panoramic view (SW-NE playas) in September 2006; b) Oligohaline water pond, Laguna de Rocés, located NW of the Salada; c) Canal which links la Laguna de

Roces and the Salada de Chiprana; d) Emerged *Coleofasciculus chthonoplastes* mats drying-out following lake-level decrease along the littoral zone with the stands of *Phragmites australis*; e) Small basin in the SE corner separated as a small lake from the main lake, after 1994, when the water level dropped below 137.6 m; f) foxtail stonewort *Lamprothamnium papulosum* var. *papulosum* f. *aragonense* (Prósper) Wood, collected in 2005 (scale bar: 5 cm); g) Living shoots of *Lamprothamnium* covered by photosynthetic biofilms, which mainly comprised diatoms and *Chloroflexus*-like bacteria (March 2006); h) Large amounts of organic debris from *Lamprothamnium* in September 2006: the detritus layer had been removed in some places, and microbial mats dominated by the cyanobacteria *Lyngbya* spp. had developed on the exposed sediments; i) Widespread development of photosynthetic biofilms on top of the *Lamprothamnium* detritus, dominated by the *Chloroflexus*-like bacteria (September 2006); j) Young re-established *C. chthonoplastes* mats observed along the littoral zone in September 2009



*Coleofasciculus chthonoplastes* mats

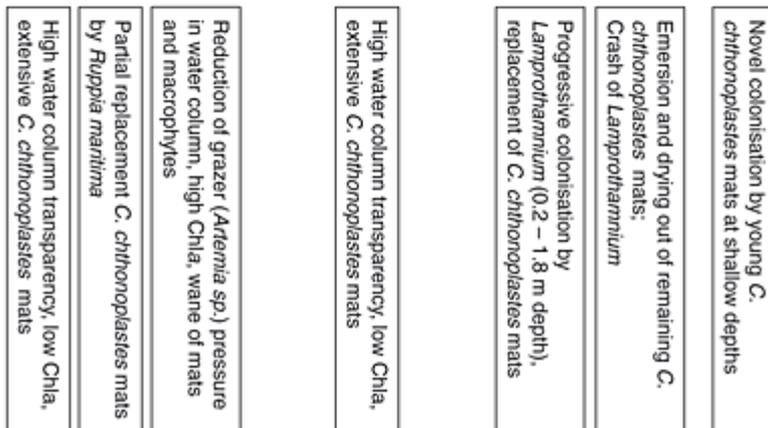


Fig. 3 Two decades chronology (1990-2009) of a) total dissolved salt concentrations (TDS), b) height of the water surface level (altitude above mean sea level, ED 50, middle panel and time line of *Coleofasciculus chthonoplastes* microbial mats and major ecological phenomena (bottom) in lake La Salada de Chiprana.

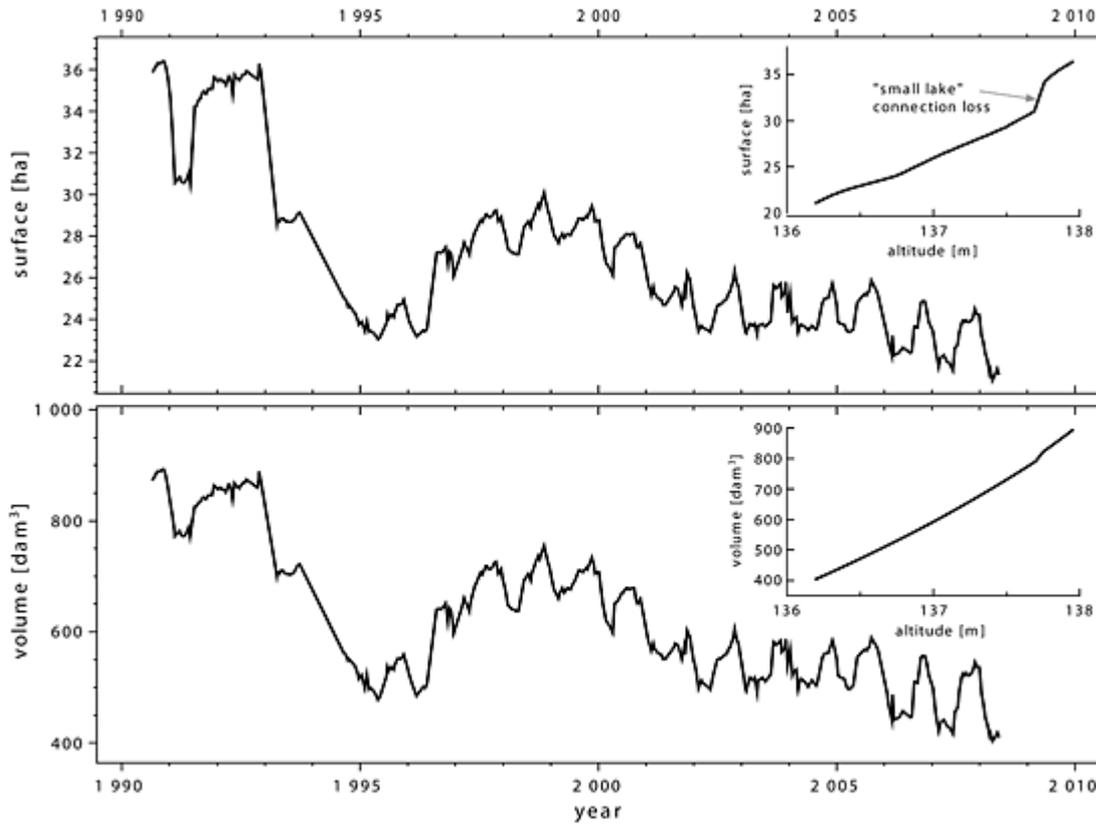


Fig. 4 Two decades chronology of the lake area and water volume of lake La Salada de Chiprana (Aragón, Spain). The curves are calculated from water height and a bathymetric map of the lake (see methods)

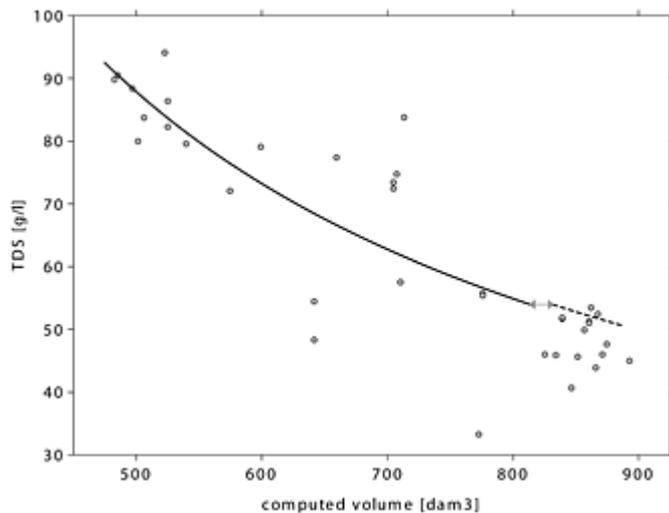


Fig. 5 Total dissolved salt concentrations (TDS) plotted against water volume in La Salada de Chiprana (Aragón, Spain) and comparison with conservative dilution-concentration lines. Broken line, conservative behaviour based on a total salt stock of

44,750 tons (including the small basin that became separated as a small lake) and Full line, conservative behaviour based on a total salt stock of 43,928 tons

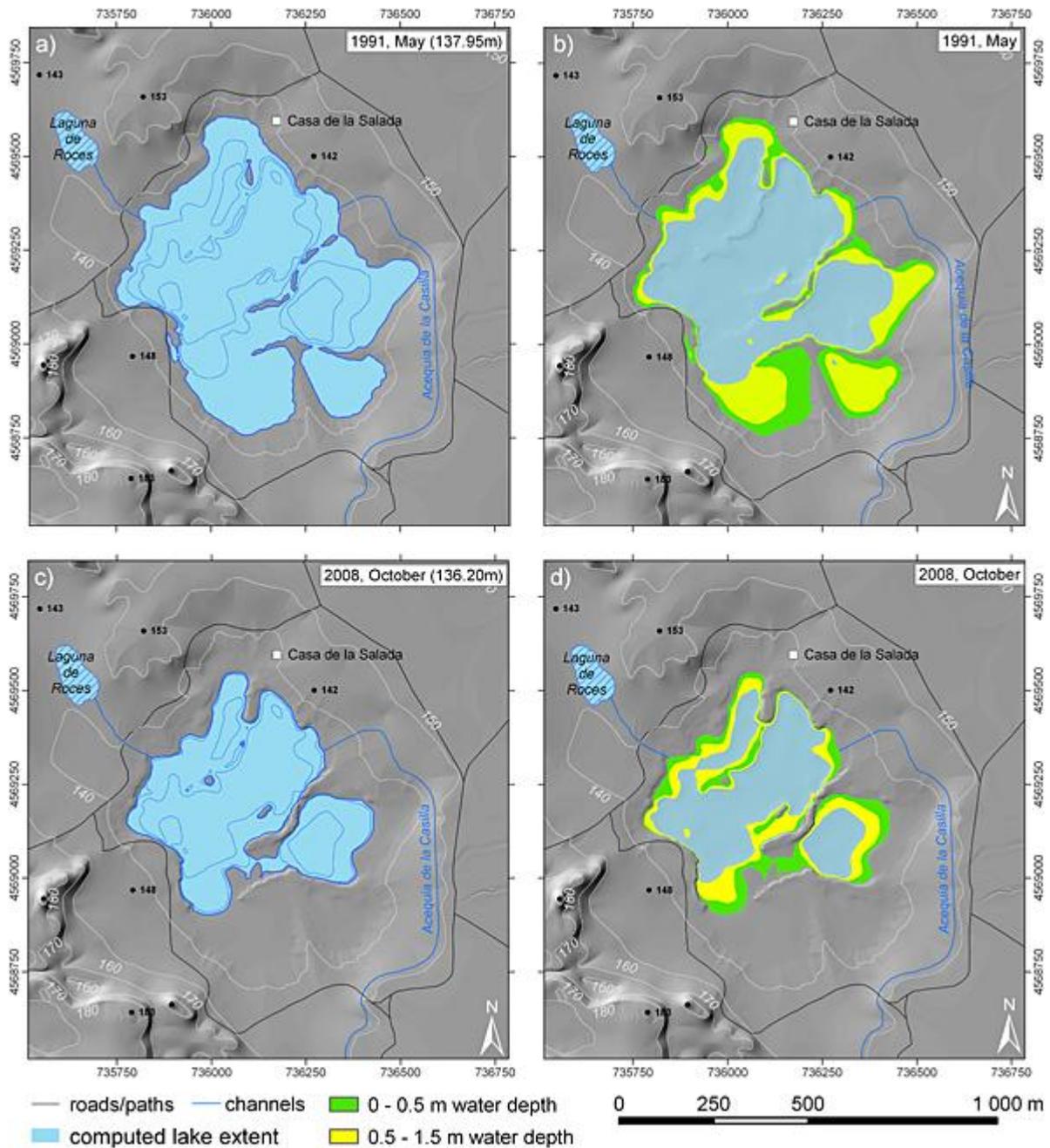


Fig. 6 Computed bathymetric maps of La Salada de Chiprana (Aragón, Spain). a) Lake extent corresponding to the water height in May 1991; b) Computed shallow water (0-1.5 m) areas indicating the extension of playas in the same period; c) Lake extent corresponding to the water height in October 2008; d) Computed shallow water (0-1.5 m) areas indicating the extension of playas in the same period. The playa areas

are particularly favourable for microbial mat developments. The extension of the cyanobacterial mats depends mainly on light availability at the surface of the sediment and depth distribution of mats is, therefore, strongly dependent on the turbidity of the water column.

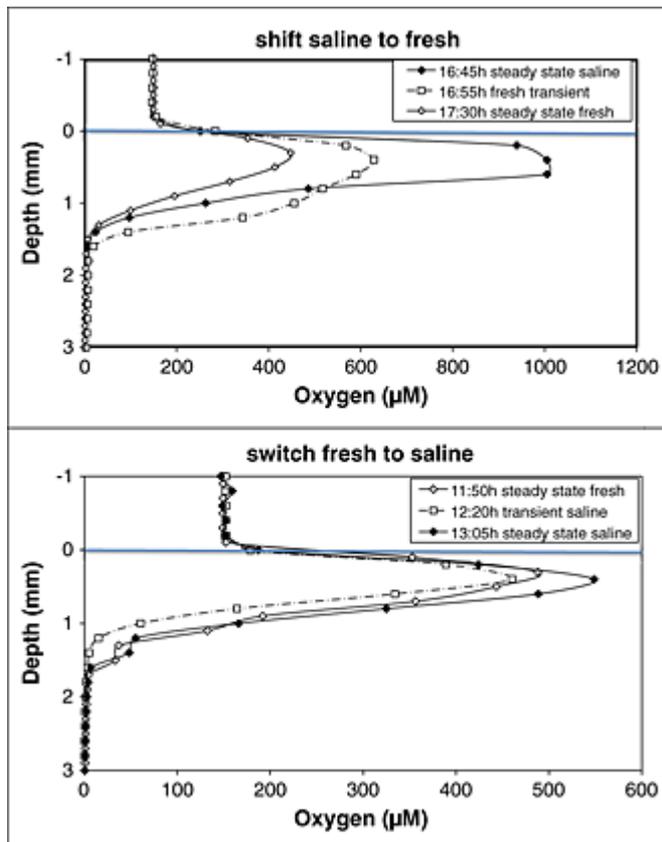


Fig. 7 Oxygen profiles measured in *Coleofasciculus chthonoplastes* mats sampled from the littoral zone in La Salada de Chiprana (Aragón, Spain) and incubated in the laboratory at  $330 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $21^\circ\text{C}$  during a shift from saline lake water to freshwater (top panel) and during a backward shift from freshwater to saline lake water (bottom panel). See Methods for details.

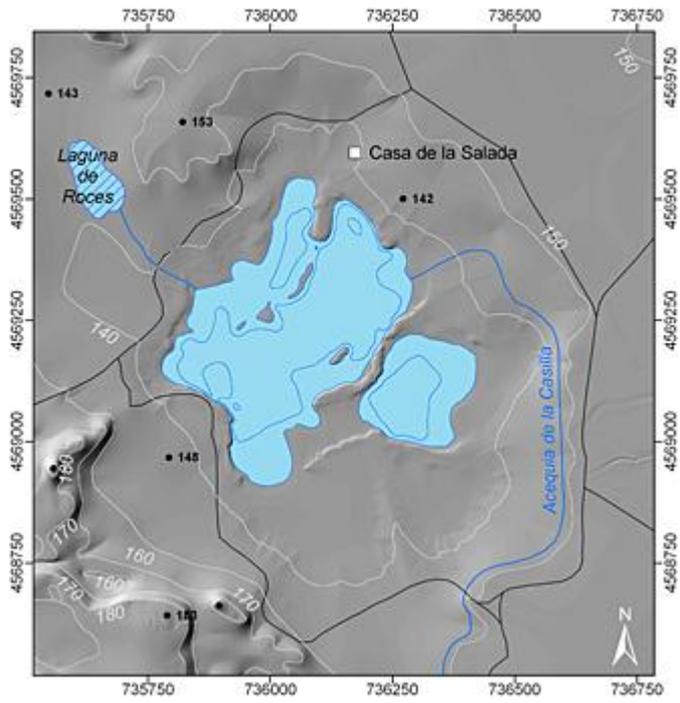


Fig. 8 Prediction of a future Salada de Chiprana lake with a salinity of 120 g/L TDS and a lake volume of 363,950 m<sup>3</sup> (assuming conservative behaviour).