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Water quality of Lake Sanabria according to phytoplankton. A comparison with historical data

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ABSTRACT

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Lake Sanabria is a Spanish lake that holds the largest volume of water in the country. It is a terminal moraine, monomictic, acidic lake that is typically oligotrophic to oligo-mesotrophic. The current situation of the lake was assessed using phytoplankton data from the growing seasons of 2013. In addition, data from the 1980s were used to evaluate any variation from former conditions. The planktonic communities were analyzed using biomass measurements (Chlorophyll-*a* and biovolume) and several composition metrics (IGA, PTSI, PTI and IGA2). Considering the applicable Spanish legislation for ecological quality assessment, the ecological status in 2013 was High. According to both the biomass and composition metrics, no change in the trophic status was apparent, although there was some evidence of a shift in species contribution to biomass from the 1980s samples to the current ones. The shift from cyanobacterial taxa to diatoms was one of the changes observed, but this does not necessarily imply a change in the trophic status.

Key words: Lake Sanabria, trophic status, phytoplankton composition, biomass, ecological status.

RESUMEN

Calidad del agua del lago Sanabria de acuerdo al fitoplancton: una comparación con datos histórico

*El lago de Sanabria es el lago que contiene el mayor volumen de agua de todos los lagos españoles. Se trata de un lago glaciar de morrena terminal. Es monomíctico, silíceo y típicamente oligotrófico u oligo-mesotrófico. Con datos del periodo mayo-octubre de 2013 se evaluó el estado actual del lago. Adicionalmente se emplearon datos de los años 80 para detectar posibles variaciones. Además de métricas de biomasa (clorofila-*a *y biovolumen total) se aplicaron diversas métricas de composición (IGA, PTSI, IGA2 y PTI) y se analizaron las comunidades fitoplanctónicas. Según la legislación Española actual, la calidad ecológica del lago en 2013 es "Muy Buena". En función de la biomasa y la composición de fitoplancton, no se observan cambios aparentes en el estado trófico, pese a que hay evidencias de un cambio significativo al comparar la composición de las comunidades fitoplanctónicas de los 80 con las actuales. La sustitución observada de taxones de cianobacterias por otros de diatomeas no representa necesariamente un cambio en el estado trófico.*

Palabras clave: *Lago Sanabria, estado trofico, composición de fitoplancton, biomasa, estado ecologico.*

INTRODUCTION

The composition of the phytoplanktonic community as an indicator of nutrient loads and, therefore, eutrophication, is widely accepted (Hörnström, 1981; Rott *et al.*, 1984; Brettum, 1989; Reynolds *et al.*, 2002), and biomass parameters are used for similar purposes (OECD, 1982; Nixdorf *et al.*, 2001). Phytoplankton biomass and composition are also used as tools for the evaluation of water quality through the study of ecological status in lakes and reservoirs in all of Europe (Directive, W. F. 2000). In this legislative context, the assessment of ecological status is based on the quantification of the deviation from natural or undisturbed conditions (Carvalho *et al.*, 2008; Poikane *et al.*, 2010). The achievement of a sufficient ecological status is a major agenda for water management authorities in European countries (Directive, W. F. 2000).

Many phytoplankton studies were conducted after the publication of the WFD. The biomass metrics included in the methods to assess eutrophication in most European countries are chlorophyll-*a* and total biovolume (Poikane *et al.*, 2015). Composition metrics used for the same assessment are more varied, and many different phytoplankton indices have been developed. These methods are based on functional groups (Padisák *et al.*, 2006), species (Mischke *et al.*, 2008; Wolfram *et al.*, 2013; Salmaso *et al.*, 2006) or superior taxonomic groupings (Catalan & Ventura, 2003; Phillips *et al.*, 2013). The probability of ecological quality misclassification, depending on the amount of samplings conducted, was also studied (Carvalho *et al.*, 2006; Mischke *et al.*, 2012; Carvalho *et al.*, 2012).

The Spanish territory has a limited number of lakes above 50 ha of surface area (a requirement established by the WFD to define a water body), and one of these is Lake Sanabria. Lake Sanabria is the Spanish lake that has the largest volume of

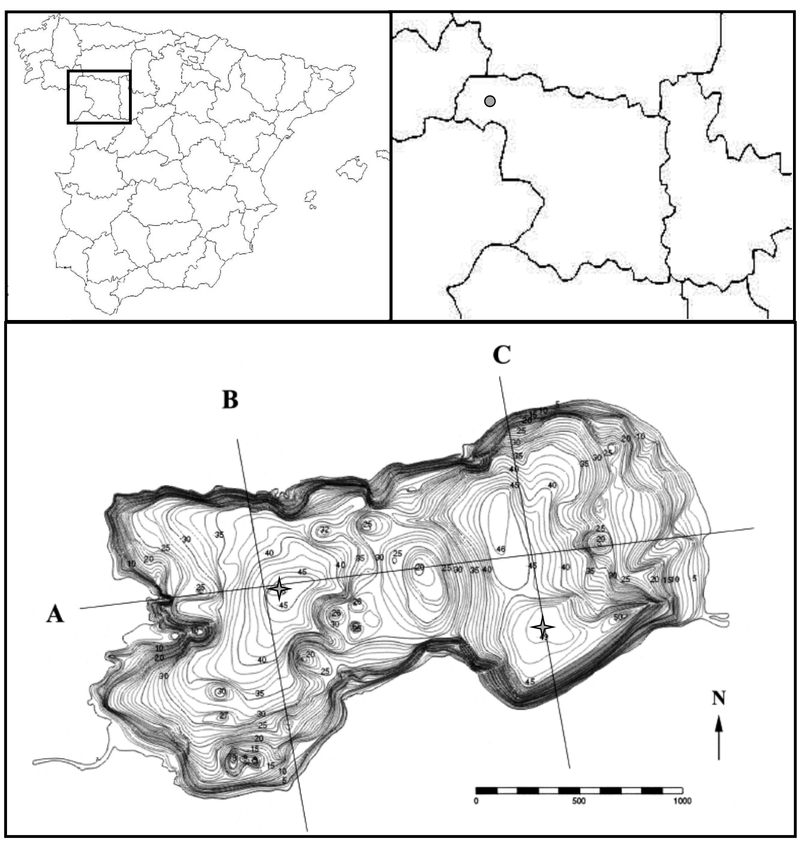

Figure 1. Location within the Iberian Peninsula and within the Zamora region, together with the bathymetry of Lake Sanabria. The bathymetry is from Vega *et al.* (2005). Samples from 2013 were taken in the deepest area of the western basin, and samples from 1987-1989 were taken from the deepest point in the eastern basin (Marked with four-point stars). *Situación del Lago de Sanabria en la península Ibérica y en la provincia de Zamora, junto con una batimetría del mismo. La batimetría es de Vega* et al. *(2005). Las muestras de 2013 se tomaron en el punto más profundo de la cubeta oeste, mientras que las de 1987-89 se tomaron en el punto más profundo de la cubeta este (marcadas con estrellas de cuatro puntas).*

water. The importance of maintaining the quality of this lake is primordial, not only because it is the largest representative of lentic natural water bodies in Spain but also because it is the centrepiece of a natural park of high ecological value in the region, which has been legally recognised (BOE, 1978).

Previous studies regarding Lake Sanabria described it as oligotrophic or oligo-mesotrophic (depending on the classification criteria), with low chlorophyll-*a* and phytoplanktonic biovolume values (Planas, 1991; Vega *et al.*, 1992; De Hoyos, 1996; De Hoyos & Comín, 1999) and no anoxic episodes during the year (Vega *et al.*, 1992; De Hoyos, 1996).

Lake Sanabria is a very important socioeconomic resource for the inhabitants of its surroundings. It offers different environmental and ecosystem services (such as downstream flood control), and it represents the main tourist attraction of the region. For these reasons, many different agents are very interested in its uses and management. In the year 2013, the Spanish national press published that the lake was irreversibly contaminated, giving rise to a debate about the quality of the water body. In this context, and in the frame of the work developed by CEH-CEDEX for the European Normalization Committee (CEN) (CEN Mandate M424 WP6), the results of five samplings conducted in the growing season of 2013 were used to evaluate the water quality of the lake.

The objectives of the study were the following: *i*) evaluate the present trophic and ecological status of the lake based on the chlorophyll-*a* concentration, total biovolume and phytoplanktonic composition during the summer of 2013 and *ii*) compare the results with some historical data available in order to analyze the changes that may have occurred.

STUDY AREA AND METHODS

Study area

Lake Sanabria is located 1000 m.a.s.l. in the northwest of Spain, in the Province of Zamora (42°07′30″N, 06°43′00″W) (Fig. 1). It is situated in a glacial depression in the Tera Valley, which belongs to the Duero River Basin.

The lake was formed by glacial erosion in the Pleistocene. It is the only lake formed by a terminal moraine in the Iberian Peninsula. It is divided longitudinally into two different basins, the western one (46 m maximum depth) and the eastern one (51 m maximum depth) (Fig. 1) (Vega *et al.*, 2005). The lake is monomictic. Due to the influence of its basin's siliceous geology, the mineral content of the water, alkalinity and pH are low (De Hoyos, 1996).

According to the Spanish typology, the lake is classified as type 6: "mid-mountain, acidic and deep lake" (BOE, 2015). The coldest month is typically January, and the warmest month is July (mean monthly temperatures of 3.7 °C and 18.6 °C, respectively). The mean annual precipitation in the catchment is 1421.7 mm (de Hoyos, 1996).

The drainage basin has a surface area of 127.3 km^2, and the surface of the lake is 3.46 km^2 (De Hoyos, 1996). The large size of the drainage basin in relation to the surface of the lake, together with an intense precipitation regime, drive some basic characteristics of the lake, like the residence time (approximately 9.16 months calculated from the mean of the 1942-1992 period) (De Hoyos, 1996), which is one of the main aspects that conditions the dynamics of the system. Several reservoirs are located in the catchment, upstream of the lake, whose management may affect different aspects of the lake's function.

Methods

The current study was based on temperature, oxygen, secchi depth, chlorophyll-*a* and phytoplankton data obtained from May to October of 2013 and on historical Secchi depth, chlorophyll-*a* and phytoplankton data from the same months during the period from 1987 to 1989. The former were obtained from the project for the development of the CEN standard for phytoplankton sampling in lakes and reservoirs (CEN Mandate M424 WP6), in which the CEH-CEDEX was responsible for the sampling of the Spanish water bodies. One of the water bodies selected for the project was Lake Sanabria. The historical data used came from samplings and analyses conducted for a Ph.D. thesis (de Hoyos, 1996).

A total of five samplings were conducted in 2013, starting in late May and ending in late October, which ensured full coverage of the stratification period. The samplings were carried out at the deepest point of the western basin (Fig. 1) always between 10:00 h and 11:00 h (GMT+2). The Secchi depth was measured using a 20 cm diameter disc. The vertical profiles of water temperature, oxygen concentration and chlorophyll-*a* concentration were measured with an YSI 6600 multi-parameter probe (Xylem, Rye Brook, New York, USA). The integrated water samples for the chlorophyll-*a* analysis and phytoplankton counts were gathered from the euphotic zone with an UWITEC depth integrator sampler. The euphotic zone was derived by multiplying the Secchi depth 2.5 times (Reynolds, 1984).The chlorophyll-*a* concentration analyses were performed in the CEH-CEDEX laboratory (Parsons & Strickland, 1963). Cell count data (Utermöhl, 1958) and biovolume estimations (Hillebrand *et al.*, 1999; MAGRAMA, 2013b) were completed for all samples, except the one in May, at the Limnologie-Büro Hoehn (Germany). The sampling and analysis methodologies from the 1987-1989 period were compiled in de Hoyos (1996). These samples were taken at the deepest

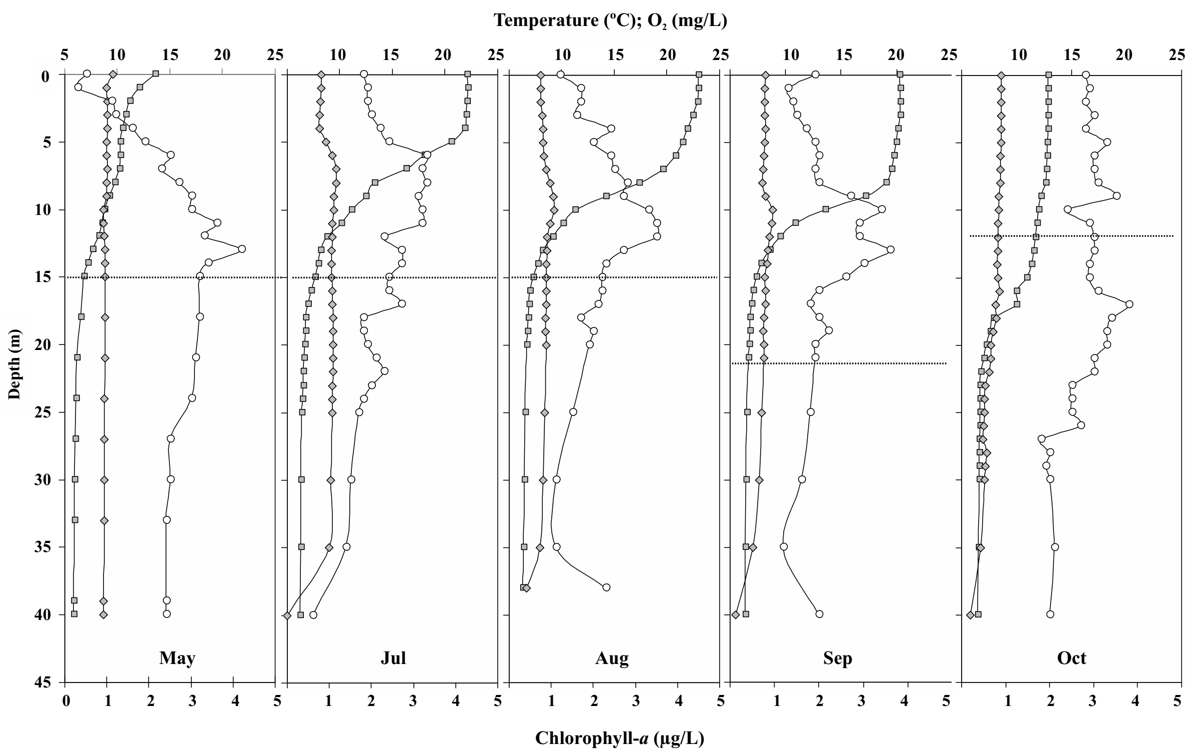

Figure 2. Temperature, dissolved oxygen and chlorophyll-*a* concentration profiles measured in Lake Sanabria in the five different samplings; from left to right 22/05/2013, 16/07/2013, 21/08/2013, 24/09/2013 and 29/10/2013. The chlorophyll-*a* concentration was measured with a fluorometric sensor, so no absolute values should be interpreted, only the variation along the column. The horizontal dotted lines represent the photic zone (calculated as 2.5 times Secchi depth). Squares represent temperature, rhombuses represent oxygen and circles chlorophyll-*a*. *Perfiles de temperatura, oxígeno disuelto, y concentración de clorofila-a medidos en el Lago de Sanabria en los cinco muestreos realizados en 2013: de izquierda a derecha 22/05/2013, 16/07/2013, 21/08/2013, 24/09/2013 y 29/10/2013. La concentración de clorofila-*a *se midió con un sensor fluorométrico, por lo que no se deben interpretar como valores absolutos, solo su variación a lo largo de la columna. Las líneas de puntos horizontales representan la profundidad de la zona fótica (calculada como 2.5 por la profundidad del disco de Secchi). Los cuadrados representan la temperatura, los rombos el oxígeno y los círculos la clorofila-*a.

point of the eastern basin. Only samples from May to October within the euphotic zone were considered (0 m, 2 m, 5 m, 10 m and 15 m deep were combined using a weighted average).

Different phytoplankton composition based metrics were applied to both the 2013 samples and the 1987-1989 samples: *i*) the "Índex des Grups Algals" (IGA) (Catalan & Ventura, 2003), based on coloniality and general taxonomy; *ii*) the "Phytoplankton Trophic Index" (PTI) (Phillips *et al.*, 2013), based on trophic scores at the genus level; *iii*) the "Índex des Grups Algals 2" (IGA-2) (Caraballo *et al.*, 2012), which is similar to the IGA, but optimizes the taxonomic resolution where necessary and considers the coloniality only where a difference in the trophic preference was found; and *iv*) the "Phyto-Seen Index" (PTSI) (Mischke *et al.*, 2008), which is the official composition index applied in Germany and is also based on trophic scores, this time at a species level.

The ecological quality of the lake was calculated from the summer means derived only from three months (July, August and September) as established in the official phytoplankton sampling protocol for lakes and reservoirs used in Spain (MAGRAMA, 2013a). The biomass parameters (chlorophyll-*a* and total biovolume) of the summers of 1987, 1988, 1989 and 2013 were used to check if the standards required by the WFD were met, and to what extent (BOE, 2015).

Statistical analyses were used to compare the means of all of the biomass and composition parameters. Normality was checked using Levene's test, and ANOVA was used for Normal distribution samples. A Kruskall-Wallis Test was used to evaluate the differences in the non-parametric variables.

RESULTS AND DISCUSSION

Vertical distribution of phytoplankton

Depth profiles of temperature, dissolved oxygen concentration, and chlorophyll-*a* concentration were used to interpret the phytoplanktonic vertical distribution. A detailed description of the results obtained from in-situ measurements of these variables in the lake, from May to October 2013, are shown in figure 2.

As seen in the profiles, the vertical stratification started in May and progressively stabilized over time. The thermocline was located at progressively deeper layers, and this same trend could be observed in the chlorophyll-*a* maximums, which were located in the metalimnetic layer. A very similar pattern was observed in the phytoplankton of the lake during 1987-1989 (de Hoyos, 1996). In the October sampling, the thermocline was almost negligible. The depth of the euphotic zone always encompassed the chlorophyll-*a* maximum during stratification (Fig. 2).

From May to October 2013, no anoxic conditions were observed in any layer of the water column. Only in those months where there was a marked thermal stratification could a diminution in oxygen concentrations be observed in the hipolimnetic zone, usually from a depth of 35 m down to the lake bed (Fig. 2). The oxygen concentration minimum was observed at a depth of 40 m in July (4.9 mg/L). Similar values were observed during the 1987-1989 period, when no anoxic conditions were observed in the water column, and 3 mg/L was the minimum registered value (October 1988 at a depth of 50 m) (Vega *et al.*, 1992; De Hoyos, 1996). Anoxia would be a clear symptom of very high oxygen consumption rates as a consequence of intense organic matter decomposition processes and, in turn, eutrophication (OECD, 1982). In the present case, the situation observed (according to the depth profiles) was that of an oligotrophic system.

The Secchi depth was also analyzed for the five samplings in the 2013 period. It ranged from 4.8 m to 9.1 m. The high Secchi depth values during the summer (Fig. 2) could be explained by the main volume of algae located in the metalimnetic zone. This situation is typical of water systems with low productivity, in which the epilimnion becomes nutrient depleted and algae are located just below the thermocline, where the stability of the water column peaks, and there is enough light and nutrients for their development (Reynolds, 1984). The Secchi depth value observed decreased to 4.8 m in Oc-

Table 1. Chlorophyll-*a* concentration and total biovolume means, standard deviations (SD), standard error of the means (SEM), maximums (max) and minimums (min) (May-October) obtained from the integrated samples of the euphotic zone of Lake Sanabria. *Medias, desviaciones típicas (SD), errores típicos de las medias (SEM), máximos (max) y mínimos (min) (mayo-octubre) para clorofila-*a *y biovolumen de fitoplancton, obtenidos de las muestras integradas de la zona fótica del Lago de Sanabria.*

		Chlorophyll-*a* (µg/L)	Biovolume (mm³/L)
1987	Mean	2.46	0.31
	SD	1.19	0.11
	SEM	0.49	0.04
	Min-Max	1.08-4.12	0.15-0.42
1988	Mean	2.31	0.39
	SD	0.87	0.10
	SEM	0.36	0.04
	Min-Max	1.32-3.64	0.31-0.57
1989	Mean	2.05	0.31
	SD	0.67	0.07
	SEM	0.28	0.03
	Min-Max	0.71-2.59	0.23-0.41
2013	Mean	2.16	0.38
	SD	0.52	0.17
	SEM	0.23	0.08
	Min-Max	1.70-3.00	0.29-0.63

tober, which could be explained by the breaking of the stratification of the water column and the subsequent redistribution of phytoplankton throughout the upper layers, together with the input of suspended particles transported from the catchment by the early autumn rains (445.9 mm were recorded at Ribadelago from the September to the October sampling). The Secchi depth values measured in 2013 were not significantly different from those measured in the 1980s (ANOVA significance of 0.195), where a minimum value of 5 m was measured

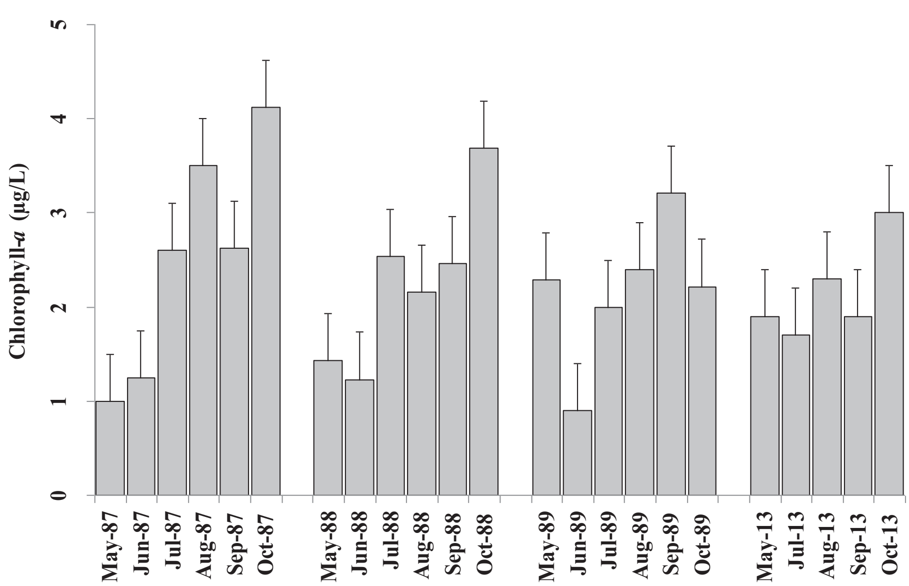

Figure 3. Chlorophyll-*a* values of the euphotic zone measured from Lake Sanabria in the May-October period from 1987, 1988, 1989 and 2013. *Valores de clorofila-*a *medidos en la zona fótica en el Lago de Sanabria en los períodos de mayo a octubre en los años 1987, 1988, 1989 y 2013.*

in November 1987 and maximum value of 9.1 m was noted in October 1989 (De Hoyos, 1996). In other studies, values of 7.2 m in July 1973 and 6.5 m in May 1974 were recorded (Margalef *et al*., 1976). Values of 6 m to 9.5 m were recorded in 1973 and values of 4.5 m to 9 m were recorded in 1974, when the May-October period was considered (Planas, 1991). This was an indicator of the high levels of transparency in the lake, like those observed in 2013.

Phytoplankton biomass results

The chlorophyll-*a* concentration of the euphotic zone ranged from 1.7 µg/L to 3 µg/L (maximum value in October) (Fig. 3), with a mean value of the period from May to October of 2.16 µg/L in the samples from 2013 (Table 1). In the 1987-1989 period, the mean concentrations of chlorophyll-*a* from the euphotic zone in the months of May to October were 2.46 µg/L, 2.31 µg/L and 2.05 µg/L (1987, 1988 and 1989, respectively) (Table 1), and the maximum values registered were 4.12 µg/L, 3.64 µg/L and 2.59 µg /L in October 1987, October 1988 and September 1989, respectively (Fig. 3). Other available data in the bibliography were 0.7 µg/L and 1.9 µg/L, which were the mean values of the top 20 m of the water column in July 1973 and May 1974, respectively (Margalef *et al*., 1976).

In 2013, the phytoplanktonic biovolumes from four dates were analyzed (July-October). The results ranged from 0.29 mm^3/L to 0.63 mm^3/L (Fig. 4), with a mean of 0.38 mm^3/L (Table 1). Data from the May-October period from the 1980s ranged from 0.15 mm^3/L to 0.57 mm^3/L (De Hoyos, 1996) (Fig. 4), and the period means were 0.31 mm^3/L, 0.39 mm^3/L, and 0.31 mm^3/L in 1987, 1988 and 1989, respectively (Table 1).

Evaluation of ecological and trophic status by chlorophyll-a and phytoplankton biovolume

According to the Spanish law RD. 817/2015 (BOE, 2015) and a report from CEDEX (CEDEX, 2010), the metrics applicable to lakes in Spain for the evaluation of ecological quality using the biological quality element phytoplankton are the chlorophyll-*a* concentration and total phytoplankton biovolume.

To evaluate the ecological status of Lake Sanabria, the mean summer values (July, August and September) of chlorophyll-*a* and phytoplankton biovolume were used (Table 2) (MAGRAMA,

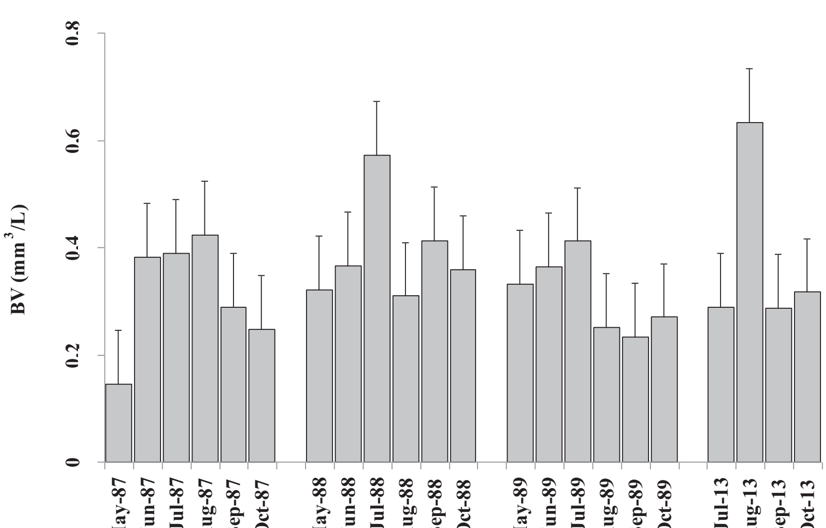

Figure 4. Total biovolume values of the euphotic zone measured from Lake Sanabria in the May-October period from 1987, 1988 and 1989, and from the July-October period in 2013. *Valores de biovolumen de fitoplancton medidos en la zona fótica en el Lago de Sanabria en los períodos de mayo a octubre en los años 1987, 1988 y1989, y en el período de julio a octubre en 2013.*

Table 2. Chlorophyll-*a* concentration and total biovolume summer means (July – September) and ecological quality class according to the thresholds and methodology established in BOE (2015) and MAGRAMA (2013a). *Medias del verano (julio a septiembre) de la concentración de clorofila-*a *y del biovolumen de fitoplancton, así como el estado de calidad ecológica según los límites y metodologías establecidos en BOE (2015) y MAGRAMA (2013a).*

	Chlorophyll-*a* (µg/L)		Biovolume (mm^3/L)	
	Mean	Class	Mean	Class
1987	2.91	**Good**	0.37	**High**
1988	2.38	**Good**	0.43	**High**
1989	2.54	**Good**	0.30	**High**
2013	1.97	**High**	0.40	**High**

2013a). The obtained values were compared with the reference thresholds established for both parameters in this type of lake (Table 3) BOE, 2015. According to the thresholds established for chlorophyll-*a*, the ecological quality status of the lake was "Good" in the 1980s and "High" in 2013. Considering the biovolume, the ecological quality status was "High" in the four years studied.

Considering the thresholds set by the OECD (OECD, 1982) for the establishment of a trophic status for water bodies, according to annual mean chlorophyll-*a* concentration values, the lake was classified as oligotrophic (< 2.5 µg/L) in all of the studied years. The means from the May-October period (Table 1) were used for this assessment.

Therefore, when comparing the 1987-1989 period to the measurements of the summer of 2013, and according to the biomass of phytoplankton metrics, the ecological status of Lake Sanabria was steadily "Good" or "High" and the trophic status was oligotrophic. We further confirmed that by comparing the 1987-1989 period mean with the 2013 mean of both biomass metrics (ANOVA), and no significant differences were found (Table 4).

Phytoplankton composition results

In 2013, diatoms were the dominant group throughout the whole study period, except in October, when dinoflagellates were dominant. The most important diatom taxa were *Aulacoseira*, which was dominant in July, and *Tabellaria flocculosa*, which was dominant in August and September. Among the dinoflagellates, the most abundant taxa were *Peridinium* sp. and *Gymnodinium uberrimum* (the dominant species in October). *Staurastrum pingue*, from the streptophytes, represented an important portion of the phytoplankton community in August and September.

The phytoplankton composition of the period 1987-1989 showed a community where diatoms were significantly less represented in terms of biovolume (Fig. 5; Table 4). *Aulacoseira* was a main contributor to diatom biovolume during this period, while *T. flocculosa* was much less represented within the group. In contrast, and notwithstanding the variations observed in these three years, the relative importance of the cyanobacterial biovolume was significantly higher than in 2013 (Table 4). Margalef *et al.* (1976) and Planas (1991) also found that, during the stratification period, colonial chroococcal cyanobacteria were dominant in the phytoplankton of Lake Sanabria, according to the cell numbers. Therefore, a change of the composition of species in the lake could be observed when

Table 3. Reference conditions and ecological quality class thresholds for the metrics chlorophyll-*a* concentration and phytoplankton biovolume (BV), for the lakes within the type 6 of the Spanish lake typology (BOE, 2015). The boundaries shown are High-Good (H-G), Good-Moderate (G-M), Moderate-Poor (M-P) and Poor-Bad (P-B). *Condiciones de referencia y fronteras entre clases de calidad ecológica para las métricas de concentración de clorofila-*a *y biovolumen de fitoplancton en los lagos del tipo 6 de la tipología oficial española (BOE, 2015). Las fronteras mostradas son Muy Bueno-Bueno (H-G), Bueno-Moderado (G-M), Moderado-Malo (M-P) y Malo-Deficiente (P-B).*

Metrics	Reference values	Type 6 threshold values			
		H-G	G-M	M-P	P-B
Chlorophyll-*a* (µg/L)	1.5	2.3	4.2	7.1	13.5
Phytoplankton BV (mm^3/L)	0.4	0.85	1.5	2.6	5.3

comparing the composition observed in 2013 with those recorded in previous samplings. The main difference consisted of a significant diminution in the proportion of cyanobacteria and an increase in the representation of diatoms (Fig. 5; Table 4), mainly the species *T. flocculosa*. Although this species was sometimes regarded as an early warning indicator of eutrophication in alpine lakes (Wessels *et al.*, 1999), studies in similar water bodies highlighted that this hypothesis should be revised. The fluctuations of the species in Lake Maggiore seemed to be controlled by climate rather than by eutrophication factors, and changes in the nutrient ratio (Si:P) after high runoff were highlighted as the main reason for the rise of *T. flocculosa* in this lake (Morabito *et al.*, 2012). Additionally, different indices based on the phosphorus optimums all located this species in the oligotrophic side of the trophic spectrum (Phillips *et al.*, 2013; Mischke *et al.*, 2008; Wolfram *et al.*, 2013), and it is currently being considered as an indicator taxon for reference conditions in Northern lakes (Järvinen *et al.*, 2012; Lepistö *et al.*, 2004; Willén *et al.*, 2007).

The change in the species composition of the phytoplanktonic community should be studied over a longer period to ascertain if it is a singular episode or of a more permanent character, or if it shows some kind of periodicity. It may have been a consequence of different factors, such as human disturbances (wildfires, changes in land use in the drainage basin, etc.) that could have induced variations in nutrient loads from the catchment and/or climatic factors, or intense rainfall that may have influenced limnological and ecological aspects of the lake. The heavy influence of climatic factors in this lake's functioning was highlighted (De Hoyos, 1996) and confirmed through sediment core studies (Luque, 2003; Jambrina-Enríquez *et al.*, 2014).

According to results obtained in paleolimnological studies of cores from the sediment of Lake Sanabria (Luque, 2003; Jambrina-Enríquez *et al.*, 2014), similar changes, like the ones addressed in this study, occurred in the geological past of the lake. Interesting data regarding diatoms could be extracted from this lake's sediment, such as the progressive increase of

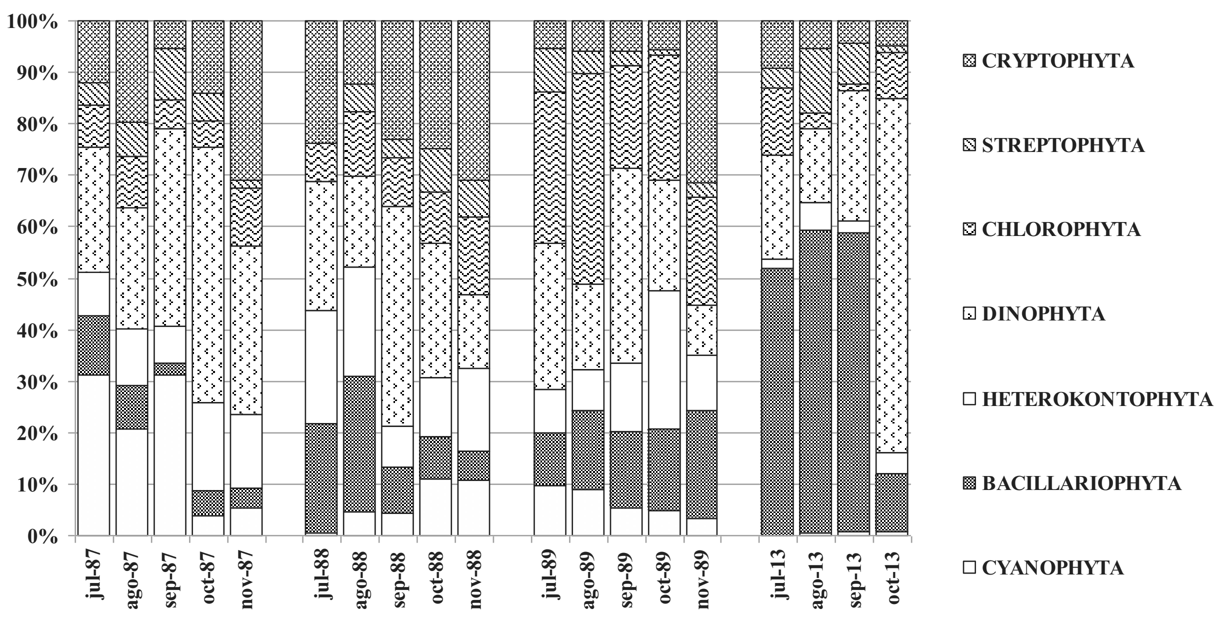

Figure 5. Phytoplanktonic composition of the euphotic zone measured from Lake Sanabria in the July-October period from 1987, 1988, 1989 and 2013. The main taxonomic groups observed are represented and calculated as percentages of biovolume. *Composición fitoplanctónica de la zona fótica del Lago de Sanabria entre julio y octubre en los años 1987, 1988, 1989 y 2013. Se representa el porcentaje del biovolumen de los grupos taxonómicos principales.*

Tabellaria during the onset of the Holocene (c.a. 11.7-10.1 cal ka BP) or its abundance in the sediments from the mid-Holocene (c.a. 6.8-4.8 cal ka BP) (Jambrina-Enríquez *et al.*, 2014). *Aulacoseira* was dominant during most of the late-Holocene; however, an episode where *T. flocculosa* represented over 60 % of the frustules in the sediment dated back to 3.8-3.2 cal ka BP (Jambrina-Enríquez *et al.*, 2014). These *Tabellaria* peaks were related to limnological changes in the lake, such as a decrease in the water level, which may have favoured benthic habitats (Jambrina-Enríquez *et al.*, 2014). Another more recent *Tabellaria* peak that was observed in the lake's sediment coincided with the period known as the *Medieval Warm Period* (800-1300 AD), characterized by relatively warm temperatures. This warm climate was followed by a cold period known as the Little Ice Age (1300-1850 AD) (deMenocal *et al.*, 2000), in which the abundance of diatoms (in general) and of *Tabellaria* (in particular) decreased significantly. This was the last of a series of cold climatic episodes that took place approximately every 1500 years throughout the Holocene in the Atlantic region, which were clearly registered in Lake Sanabria's sediment (Luque, 2003). At present, we could be witnessing the effects promoted by these climatic periodic changes. Nonetheless, this hypothesis cannot be confirmed without a continuous limnological monitoring of the lake that includes phytoplankton.

Ecological status and trophic status evaluation through phytoplankton composition metrics

Phytoplankton composition metrics are currently not considered in Spain for the evaluation of the ecological quality of lakes (BOE, 2015). Nonetheless, many composition-based indices were developed in the context of the application of the WFD and were proven useful for the evaluation of ecological quality and trophic status. In this work, several of these indices were calculated for the different samples using the data from July to October of 1987, 1988, 1989 and 2013.

Due to the conceptual attributes of the different indices used, the percentage of the biovolume of the samples considered within the taxa included in each index was very different. In the case of IGA, IGAII and PTI, the mean percentages of biovolume of the sample considered by the indices was of 99.4 %, 100 % and 88.8 %, respectively. In the case of the PTSI, this percentage dropped down to 47.4 %. This difference occurred because the PTSI is based on species and was fine tuned for German lakes, while the PTI, IGA and IGAII are based on genera or broader taxonomic groups and were intended for more

Table 4. Statistical comparison of the means of the May-October values of the 1980s samples with the 2013 samples. Variables studied were: Secchi depth, chlorophyll-*a*, biovolume, phytoplankton composition (bacillariophyte and cyanobacteria biovolume) and ecological quality indexes. [N] stands for Normality and KW stands for Kruskall-Wallis test. *Comparación estadística de mayo a octubre entre los valores mensuales de los años 80 y los de 2013. Las variables estudiadas son: Profundidad del disco de Secchi, clorofila-*a*, biovolumen, composición fitoplanctónica (biovolumen de cianobacterias y de bacialriofitas) e índices de calidad basados en el fitoplancton. [N] se refiere a Normalidad y KW se refiere al test de Kruskall-Wallis.*

	Levene	[N]	Comparison	Difference
Secchi disc	0.852	Yes	ANOVA-0.195	No
Chlorophyll-*a*	0.374	Yes	ANOVA-0.789	No
Total biovolume	0.138	Yes	ANOVA-0.473	No
Cyanophyta	0.044	No	KW-0.021	Yes
Bacillariophyta	0.010	No	KW-0.011	Yes
IGA	0.102	Yes	ANOVA-0.036	Yes
IGAII	0.749	Yes	ANOVA-0.462	No
PTSI	0.927	Yes	ANOVA-0.000	Yes
PTI	0.617	Yes	ANOVA-0.240	No

general applicability. Consequently, the results obtained from the application of the PTSI should be considered cautiously.

The results of the applied indices were compared according to pre-existing classifications, when available. In the case of the IGA, the results were compared with the existing limits (established during the first intercalibration phase) for siliceous wet reservoirs (types 1, 2 and 3 in the Spanish legislation) (BOE, 2015; De Hoyos *et al*., 2014), due to the similarities of the lake with this reservoir type. In the case of the PTSI, the results were compared with the ecological quality limits and trophic state limits established for German stratified pre-alpine lakes (Mischke *et al*., 2008). In the case of the IGA2

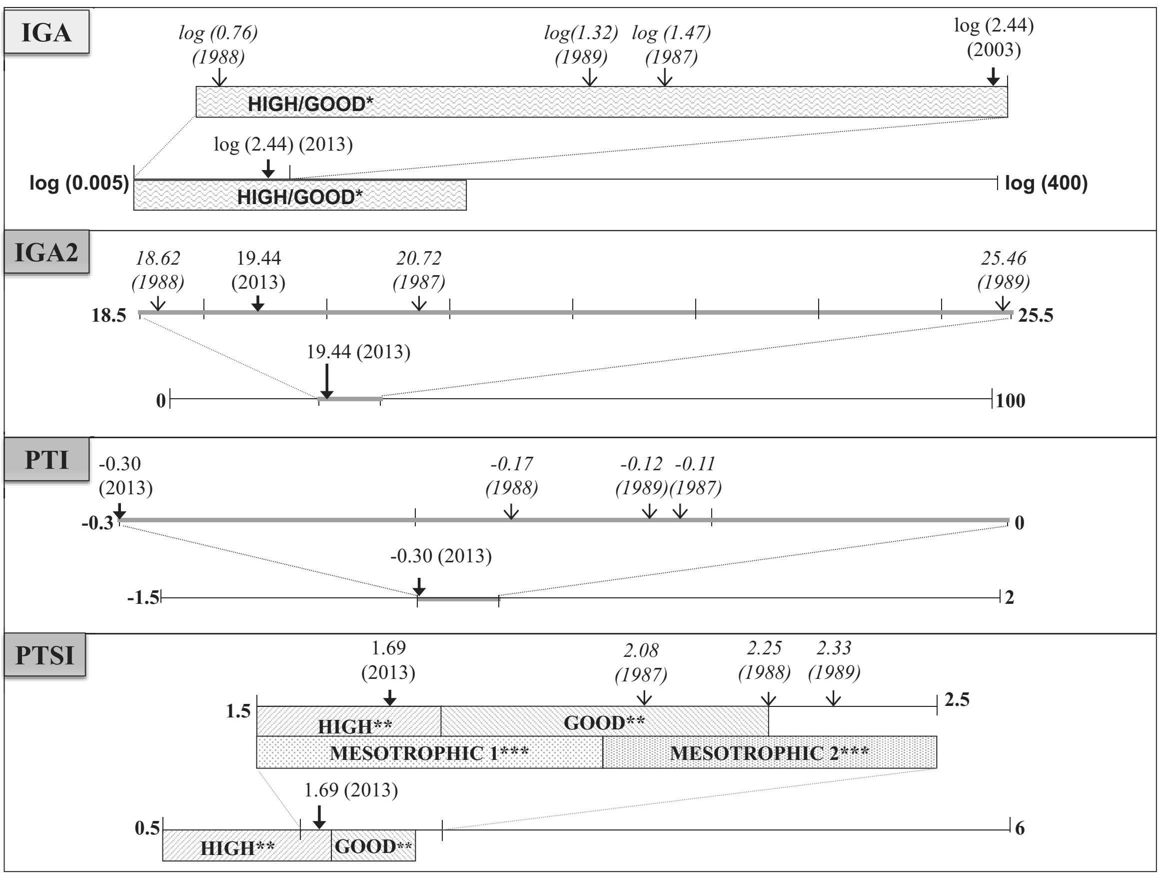

Figure 6. Calculated values for four phytoplankton composition indices applied: IGA, IGA2, PTI and PTSI. The calculations were based on the July-October mean values of 1987, 1988, 1989 and 2013. Lower lines position the 2013 values within the range of variation of the index and compare them with the reference values and thresholds established for each index, where possible. The upper line zooms in the part of the scale where both the 1980s and the 2013 values are. (➞) Index values for 2013. (→) Index values for 1987-1989 . (*) IGA High and Good ecological quality class, for Spanish reservoirs types 1, 2 and 3 (Siliceous wet reservoirs) (BOE, 2015). (**) *PTSI* High and Good ecological quality classes, for German lakes types 2 and 3 (Pre-alpine stratified lakes) (Mischke *et al*., 2008). (***) Relevant trophic status classes for *PTSI* (Mischke *et al*., 2008). *Valores calculados para cuatro índices de composición del fitoplancton aplicados: IGA, IGA2, PTI y PTSI. Los cálculos se basaron en medias de julio a octubre de los años 1987, 1988, 1989 y 2013 en todos los casos salvo en el PTSI, en el que el período utilizado va de mayo a septiembre. Las líneas inferiores se refieren a la posición de los valores determinados en la escala total de los índices, mientras que las líneas superiores enfocan la parte de la escala donde quedan los 4 valores de los distintos años. (➞) Valores de los índices para 2013. (→) Valores de los índices para 1987-1989. (*) Clases de calidad ecológica Buena o superior del IGA para los embalses españoles de tipo 1, 2 y 3 (Embalses Silíceos de zonas húmedas) (BOE, 2015). (**) Clases de calidad ecológica Muy buena y Buena del* PTSI *para lagos alemanes de los tipos 2 y 3 (lagos prealpinos estratificados) (Mischke* et al.*, 2008). (***) Clases relevantes de estado trófico para el* PTSI *(Mischke* et al.*, 2008).*

and the PTI, the results were simply compared and located within the range of the variation of the indices, since there are no thresholds established for them (Fig. 6).

It became apparent that according to the phytoplankton composition metrics IGA and PTSI, there was no deterioration in the ecological status in the lake from 1987-1989 to 2013 (Fig. 6). The IGA values for the four years were classified as "High" status, and those of the PTSI were classified as "Good" or "Moderate" in the1980s and as "High" in 2013 (Fig. 6). From a statistical point of view, the differences between the 1980s samples and the 2013 samples were observed in these two indices (Table 4). On the contrary, the values obtained for IGA2 and PTI, which were close to each other within their total index range (Fig. 6), were not significantly different when comparing the 1980s samples with those from 2013 (Table 4).

CONCLUSIONS

According to Spanish legislation, Lake Sanabria showed a "High" ecological quality status in the summer of 2013, based on the phytoplankton, and an oligotrophic state, based on the OECD chlorophyll-*a* thresholds. The phytoplanktonic composition was also representative of a nutrient poor system.

The phytoplankton biomass parameters during the 2013 study period were very similar to the ones registered 25 years ago and to those obtained from the literature from 1970s' samples, even though the observed composition of the phytoplankton community in 2013 was clearly different from the historical data. These changes, according to the applied composition indices, were not representative of a eutrophication process, and they could be a result of other anthropogenic driven changes or climatic factors.

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