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## CHARACTERISATION OF THE TROPHIC STATE AND RISK OF EUTROPHICATION IN THE RESERVOIRS OF THE EBRO BASIN ACCORDING TO OECD STANDARDS AND ROYAL DECREE 47/2022

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**Abstract.** One of the main problems affecting water quality in lentic ecosystems such as reservoirs is eutrophication. These systems are subject to intense anthropic pressure due to their use for water supply and the reception of pollutant discharges, both from diffuse and point sources, from industry, livestock farming or agriculture. This pressure generates an excessive supply of nutrients (especially phosphorous and nitrogen) which favours the excessive growth of primary producers and therefore leads to an imbalance in the ecosystem. In order to assess the trophic state of the reservoirs in the Ebro basin, as well as their risk of eutrophication, sampling campaigns were carried out in 47 reservoirs in 2023 and 2024, where the variables chlorophyll-a (as an indicator of primary production), total phosphorus (as the main limiting nutrient) and water transparency measured with the Secchi disc were analysed, with the aim of assessing the trophic state and the risk of eutrophication of the bodies of water. For this purpose, the assessment criteria proposed by the OECD in 1982 and Royal Decree 47/2022 were applied, as well as to identify differences in their assessment criteria. According to the OECD, five ultra-oligotrophic, 19 oligotrophic and 23 mesotrophic reservoirs were identified. However, when applying RD 47/2022, 41 reservoirs were classified as at risk of eutrophication and only six as non-eutrophic. This difference is explained by the fact that the Royal Decree incorporates a preventive approach when considering the pressures in the basin and is therefore stricter. However, its application could be improved by means of complementary techniques such as remote sensing, as different studies reinforce the idea that it would improve the early detection of alterations in the water body, as well as a better planning of sampling.

**Keywords:** Water quality, chlorophyll-a, Ebro basin, Secchi disk, reservoirs, trophic state, eutrophication, total phosphorus, OECD, Royal Decree 47/2022, eutrophication risk, transparency.

### 1. INTRODUCTION

Water is an essential resource for life due to its unique physicochemical properties, such as its high surface tension or its ability to contain essential dissolved salts (Westall & Brack, 2018). Furthermore, it is indispensable for health, agriculture and the development of civilisations, as they have historically thrived near water sources (Hosseiny et al., 2021). Although 70% of the planet is covered by water, only about 3% is freshwater and less than 1% is accessible to humans (Fernandez-Cirelli, 2012).

Therefore, it is important to talk about the complex concept of water quality, which can be approached from different perspectives depending on the criteria used and the purpose for which it is to be assessed. Thus, from a functional point of view, it is defined as the natural capacity of water to fulfil its different uses (human consumption, agriculture, industry or preservation of ecosystems), which depends on physical, chemical and biological parameters (Boyd, 2015). It can also be defined from an environmental point of view according to the definition provided by the Water Framework Directive, which defines it as the conditions of the water body to maintain the ecosystem in balance and to meet various ecological quality objectives (Ministerio de Medio Ambiente, 2000).

Studying water quality is important not only for drinking water supply, but also for keeping aquatic ecosystems in balance, as poor water quality alters their ecological balance and therefore affects the biodiversity of this ecosystem.

Among the variables used to evaluate water quality are temperature, transparency, conductivity, dissolved oxygen, turbidity, solids, taste, colour, odour, redox potential, pH, concentration of inorganic nutrients such as nitrogen and phosphorus, concentration of heavy metals, and organisms such as protozoa, microalgae and others (Betancourt & Labaut, 2013; Hassan, 2020).

Eutrophication is one of the most relevant processes that influence water quality, as it has a direct impact on water bodies by altering the composition of certain inorganic compounds, mainly due to a massive enrichment of nitrogen and phosphorus. This phenomenon causes an excessive growth of algae, which leads to increased turbidity and decreased water transparency, making it difficult for light to enter the deeper regions of the water, and can lead to areas of hypoxia, resulting in habitat degradation and adverse changes in the food web. In addition, some of these algae may be toxic, posing an additional risk to ecosystems and human health (Chislock et al., 2013; Devlin & Brodie, 2023; Rodríguez-Pérez et al., 2014).

This increase in excess nutrients is closely related to the trophic state. This term is used to classify aquatic ecosystems according to biotic productivity (Dodds & Cole, 2007). It is an important property of aquatic ecosystems as it reflects the anthropogenic influence on water quality, productivity and ecological functioning of water bodies (rivers, lakes and reservoirs) due to nutrient enrichment of the water body (Cunha et al., 2013; Kuczyńska-Kippen et al., 2025). Furthermore, it is linked to the maturity of the ecosystem, the direction of succession and the interactions of the water body and its catchment (Mineeva, 2023).

In assessing trophic state, two key concepts are (1) primary production and (2) heterotrophic processes, these are related to ecosystem structure and human influence on water quality, as they determine the balance of organic matter production and consumption. (Dodds & Cole, 2007).

Although both, nitrogen and phosphorus, contribute to eutrophication, the classification of trophic state usually depends on which nutrient is limiting. In most cases, the limiting factor in freshwaters is phosphorus and in saline waters it is usually nitrogen (except in the Mediterranean Sea where it is phosphorus) (Romero Gil, 2019). For lakes or reservoirs, the analysis will focus on phosphorus.

In the Spanish context, water quality is regulated by various regulations aimed at guaranteeing the good ecological state of water bodies, both surface and groundwater. Among these regulations is Royal Decree 817/2015, of 11 September, Establishing the Criteria for Monitoring and Evaluation of the State of Surface Water and Environmental Quality Standards (2015). This arises from the transposition of Directive 2000/60/EC and establishes the criteria for monitoring and assessing the state of surface waters and environmental quality standards. This Royal Decree seeks to classify water bodies and define the indicators for assessing their quality in order to meet the objectives established by European regulations. In Article 9 *General provisions on the assessment of state*, it mentions that the ecological state of surface waters will be classified as very good, good, moderate, poor or bad according to the articles detailed throughout the Royal Decree. To incorporate a better definition of eutrophication and to establish more precise methods for its estimation both in inland waters and in coastal and transitional waters, this Royal Decree was amended by Royal Decree 47/2022, of 18 January, on the Protection of Waters Against Diffuse Pollution Produced by Nitrates from Agricultural Sources (2022). In Section D of this Royal Decree, Characterisation of the trophic state

of water bodies, Part A: Inland waters, it is indicated that the characterisation of the trophic state will only be carried out in inland water bodies in the lake category and in heavily modified bodies that can be assimilated to lakes, since in rivers there should not be problems of eutrophication due to their hydromorphological characteristics. This RD indicates that the characterisation of the trophic state of water bodies is carried out considering the limits of change of state class established in RD 817/2015 (Annex II) applicable to water bodies that it establishes for each type of water body in the lake and highly modified categories assimilable to lake. In case no change limits are specified for certain indicators or if the water bodies are classified in a worse than good state, the criteria established by the OECD (1982) must be used. Water bodies are classified as non-eutrophic (those with very good or good ecological state) and eutrophic (those exceeding the OECD threshold values that are the minimum applicable for assessing the degree of eutrophication).

The main objective of this work is to evaluate the trophic state of the reservoirs of the Ebro basin during the sampling campaigns carried out in 2023 and 2024 according to the criteria established by the OECD, as well as to determine the risk of eutrophication in accordance with the thresholds established by RD 47/2022.

The specific objectives are (1) to compare the results obtained from both methods with the aim of identifying concordances, discrepancies and possible implications for reservoir management, and (2) to identify possible improvements in the assessment criteria of the Royal Decree.

## 2. MATERIAL AND METHODS

## 2.1 Study area

The Ebro basin, the largest in Spain with a total surface area of 85,534 km<sup>2</sup>, is located in the northeast of the Iberian Peninsula, covering French and Andorran territory. It is bounded by the Cantabrian Mountains and the Pyrenees in the north, by the Iberian System in the southeast and by the Coastal-Catalan chain in the east (CHE, 2025b). Biogeographically, it is located between the Eurosiberian and Mediterranean regions, characterised by a mainly Mediterranean climate, with oceanic influence in the northeast and continental influence in the centre of the peninsula (Pérez-González et al., 2023). This climatic context, together with human activities, such as irrigated agriculture, hydroelectric generation and urban supply, significantly affects the quality and availability of water resources (CHE, 2025b).



**Figure 1.** Approximate geographical distribution of the reservoirs sampled during 2023 and 2024

In this study, 47 reservoirs were analysed throughout the basin (Figure 1), which can be grouped into different types (E-T01, E-T07, E-T09, E-T10, E-T11, E-T12 and E-T13) according to characteristics such as their mixing regime (monomict or dimictic), lithology of the basin (calcareous or siliceous), climatology or basin area (<1000, >1000, between 1000 and 25,000, and more than 25,000 km<sup>2</sup>).

Then, to classify the reservoirs, it is necessary to know the climatic region in which they are located, as climate is the main variable considered. According to Köppen's climatic classification (1918), the reservoirs analysed are distributed throughout the territory in five different climatic regions (Figure 2): temperate climate without dry season with mild summer (Cfb), temperate climate without dry season with hot summer (Cfa), temperate climate with dry and hot summer (Csa), cold steppe climate (Bsk) and mountain climates (Dfb and Dfc).

## 2.2 Sampling methodology

The sampling of the reservoirs of the Ebro basin was carried out in two sampling campaigns, throughout 2023 (from January to November) and from June to October in 2024.

The main sample of each reservoir was obtained as established by RD 47/2022, i.e. between the dam and the tail of the reservoir (300-500 m from the dam), at the place of maximum depth, in order to detect possible situations of anoxia, and generally during the most unfavourable time (late spring, early summer).

In each reservoir, water transparency was measured in situ with the Secchi Disk (ZDS), with which the photic zone was defined as 2.5x the depth of vision of the disk. Along this zone the integrated sample was extracted using a vertical tube of 2.5 cm diameter. The water was collected in an 8 L integrator bottle where it was homogenised and distributed into PET bottles for subsequent storage at 4°C and filtering in the laboratory. Specific aliquots were collected for nutrient analysis, phytoplankton and other determinations. Water samples were transported in darkness and refrigerated for further processing and analysis in the laboratory.



**Figure 2.** Approximate representation of the climatic regions according to Köppen present in the Ebro basin and the presence in each of them of the reservoirs studied. Temperate climate without dry season with mild summer (Cfb), temperate climate without dry season with hot summer (Cfa), temperate climate with dry and hot summer (Csa), cold steppe climate (Bsk), mountain climates (Dfb and Dfc). In the Mediterranean oceanic climate (Csb) there are no reservoirs sampled during this campaign



## 2.3 Laboratory methodology

For chlorophyll-a extraction, water samples taken from each reservoir were filtered on Whatman GF/F glass fibre discs. Subsequently, they were extracted with a 1:1 solution of dimethyl sulfoxide and 90% acetone, using the method of Shoaf and Lium (1976), obtaining their concentration using the equations proposed by Jeffrey and Humphrey (1975).

To determine the concentration of total phosphorus, the method of Murphy and Riley (1962) was used by acid digestion and molybdenum blue, and the absorbance was measured at 882 nm (Golterman, 1970).

## 2.4 Statistical methods

For statistical analyses, the statistical software PAST (version 4.03) (Hammer et al., 2001) was used (Hammer et al., 2001). First, a descriptive statistical analysis was carried out for each variable. Then, the normality of the data was studied with the Shapiro-Wilk test and the values were found to be non-normal. To study the differences between the different types of reservoirs, the Kruskal-Wallis test for non-parametric values was performed followed by a pairwise test (Dunn's post hoc test). Then, the correlation between the variables was studied, and they were normalised by log-transforming them. Finally, with the normalised values, multivariate analysis methods such as principal component analysis (PCA) were used to identify patterns and relationships between the variables studied.

## 2.5 Trophic state assessment methods

For the evaluation of the trophic state of the reservoirs, the criteria established by RD 47/2022 and the criteria proposed by the OECD (1982) were used, with the help of Microsoft Excel software. The WFD indicates that the annual mean and maximum value of the indicators must be calculated from a minimum of 6 annual samples and at least one sample must be taken every 3 months, however, on this occasion this was calculated without complying with this premise.

### 2.5.1 OECD methodology

The OECD (1982) defines 5 categories of trophic state in lake environments (Table 1). This classification considers variables such as total phosphorus concentration, chlorophyll-a and water transparency. To calculate the trophic category, the average of the data is considered.

The trophic state obtained for each parameter is associated with a numerical value that assigns each category on a scale of 1 to 5 (Table 2). For each reservoir, these values were averaged, and the trophic state was assigned according to the scale shown in Table 3.

**Table 1.** Trophic state classification according to OECD

<b>Trophic category</b>	<b>Total phosphorus (µg/L)</b>	<b>Chlorophyll-a (µg/L)</b>	<b>Secchi disc (m)</b>
<b>Ultraoligotrophic</b>	< 4	< 1	> 6
<b>Oligotrophic</b>	4 - 10	1 - 2,5	6 - 3
<b>Mesotrophic</b>	10 - 35	2,5 - 8	3 - 1,5
<b>Eutrophic</b>	35 - 100	8 - 25	1,5 - 0,7
<b>Hypertrophic</b>	> 100	> 25	< 0,7

**Table 2.** Numerical assessment for each trophic category

Trophic state	Numerical value
Ultraoligotrophic	1
Oligotrophic	2
Mesotrophic	3
Eutrophic	4
Hypertrophic	5

**Table 3.** Trophic state change limit

Trophic state	Mean numerical number
Ultraoligotrophic	<1,8
Oligotrophic	1,8 - < 2,6
Mesotrophic	2,6 - < 3,4
Eutrophic	3,4 - < 4,2
Hypertrophic	≥ 4,2

### 2.5.2 Methodology RD 47/2022

Royal Decree 47/2022 establishes the criteria for characterising the trophic state of surface water bodies, classifying them as non-eutrophic and eutrophic (Table 4).

**Table 4.** Indicator variables of eutrophication risk according to RD 47/2022

Variables – Trophic state		Non eutrophic	Eutrophic
Total phosphorus (µg/L) annual mean		≤ 35	> 35
Chlorophyll-a (µg/L) annual mean		≤ 8	> 8
Annual maximum	chlorophyll-a (µg/L)	≤ 25	> 25
Secchi disk (m) annual mean		≥ 2	< 2

Given the number of possible situations, in some cases it is difficult to determine the state based on this table, and therefore, expert judgement on eutrophication must be used. In addition, it is necessary to consider the presence of significant pressures that may cause an increase of nutrients in the water body, mainly of anthropic origin (point and diffuse sources, linked to urban, industrial and agricultural activities, which release compounds such as organophosphates, metals, biocides or suspended matter), as indicated in Directive 2000/60/EC of the European Parliament and of the Council, of 23 October 2000 (2000). The determination of these pressures was based on the reports of the IMPRESS database of the Ebro Hydrographic Confederation (CHE, 2025a).

## 3. RESULTS

For the purpose of determining the trophic state of the different reservoirs in the Ebro basin, three variables were analysed: chlorophyll-a, transparency (ZDS) and total phosphorus, with a total of 99 observations per variable, including one to five replicates of different dates per reservoir.

Overall (Table 5), the mean chlorophyll-a was 4.15 µg/L, with values ranging from 0.4 µg/L for El Grado reservoir to 33.9 µg/L for El Val reservoir. The ZDS showed a mean of 3.33 m, with a range between 0.3 and 15.1 m, corresponding to Yesa and Llauset reservoirs respectively. Finally, total phosphorus showed a mean of 0.011 mg/L, with a minimum of 0 (corresponding to the Baserca, Yesa, Rialb, Santa Ana, Flix, Caspe, Calanda, Gallipué, Sallente, Llauset and La Loteta reservoirs) and a maximum of 0.099 mg/L in Utchesa.

**Table 5.** Statistical summary of the variables analysed. Chlorophyll-a (CHL-A) in  $\mu\text{g/L}$ , transparency measured with the Secchi disc (ZDS) in m and total phosphorus (PTOT) in  $\text{mg/L}$

	CHL-A	ZDS	PTOT
<b>N</b>	99	99	99
<b>Min</b>	0.40	0.30	0.000
<b>Max</b>	33.90	15.10	0.099
<b>Mean</b>	4.15	3.33	0.012
<b>Std Error.</b>	0.50	0.26	0.002
<b>Variance</b>	24.65	6.55	0.000
<b>Std. Dev.</b>	4.96	2.56	0.017
<b>Median</b>	2.80	2.50	0.007
<b>25 Percentile</b>	1.50	1.70	0.003
<b>75 Percentile</b>	4.50	4.10	0.013
<b>Coeff. Var.</b>	119.49	76.88	146.554

For a more detailed analysis, descriptive statistics were performed separating by type of reservoir (type 1, 7, 9, 10, 11, 12 and 13).

In relation to the mean chlorophyll-a concentration (Table 6; Figure 3A), the highest mean value corresponded to reservoir type 7, with a mean of  $7.28 \mu\text{g/L}$  and a standard deviation of  $6.43 \mu\text{g/L}$ , showing a high dispersion of the data, with a variance of 85.15 and a coefficient of variation of 126.75%. This could suggest eutrophic conditions for reservoirs of this type and heterogeneity among them. The reservoir with the highest chlorophyll concentration (El Val) is in this group. In contrast, the rest of the reservoirs showed lower mean concentrations ( $< 4 \mu\text{g/L}$ ), except for type 12, whose mean is above  $5 \mu\text{g/L}$ .

**Table 6.** Summary of the values obtained for chlorophyll-a according to the type of reservoir

	1	7	9	10	11	12	13
<b>N</b>	2	20	10	28	18	14	7
<b>Min</b>	1.1 0	1.00	0.60	0.60	0.40	1.50	1.00
<b>Max</b>	1.2 0	33.90	6.60	11.6 0	7.80	11.3 0	10.70
<b>Mean</b>	1.1 5	7.28	3.03	3.40	2.70	5.05	2.66
<b>Std Error.</b>	0.0 5	2.06	0.72	0.50	0.40	0.85	1.34
<b>Variance</b>	0.0 1	85.15	5.23	6.96	2.87	10.0 7	12.65
<b>Std. Dev.</b>	0.0 7	9.23	2.29	2.64	1.69	3.17	3.56
<b>Median</b>	1.1 5	3.65	2.40	2.50	2.35	3.85	1.40
<b>25 Percentile</b>	1.1 0	2.13	1.05	1.63	1.48	2.43	1.00
<b>75 Percentile</b>	1.2 0	8.33	5.35	4.35	3.60	7.45	1.70
<b>Coeff. Var.</b>	6.1 5	126.7 5	75.4 5	77.5 8	62.7 0	62.8 4	133.8 7

As for transparency (Table 7; Figure 3B), the highest mean values were observed in type 13 lakes (9.81 m), with the maximum at 15.1 m (Llauset reservoir) and the minimum at 1.5 m (Santa Ana reservoir), which shows that generally this type of reservoir will be more transparent, although its variance (17.17 m<sup>2</sup>) indicates that we can find less transparent lakes. This is followed by type 1 reservoirs, with an average of 6.85 m, with a maximum of 8.1 m (Lanuza reservoir) and a minimum of 5.6 m (Pajares reservoir). The reservoirs with the lowest transparency are those of type 10, with an average of around 2.1 m, with a minimum of 0.8 m (Margalef, La Loteta and La Sotonera reservoirs), indicating lakes with possibly low trophic quality.

**Table 7.** Summary of the values obtained for transparency measured with the Secchi disk (ZDS) according to the type of reservoir

	1	7	9	10	11	12	13
<b>N</b>	2	20	10	28	18	14	7
<b>Min</b>	5.60	0.80	0.30	0.80	1.50	1.40	1.50
<b>Max</b>	8.10	6.30	6.80	5.90	6.40	5.30	15.10
<b>Mean</b>	6.85	2.91	2.68	2.11	3.27	3.18	9.81
<b>Std Error.</b>	1.25	0.31	0.60	0.26	0.31	0.32	1.57
<b>Variance</b>	3.13	1.90	3.60	1.88	1.72	1.39	17.17
<b>Std. Dev.</b>	1.77	1.38	1.90	1.37	1.31	1.18	4.14
<b>Median</b>	6.85	2.45	2.55	1.65	3.10	3.20	10.00
<b>25 Percentile</b>	5.60	1.90	1.10	1.13	2.35	2.10	9.40
<b>75 Percentile</b>	8.10	3.50	3.50	2.45	3.93	4.10	11.90
<b>Coeff. Var.</b>	25.81	47.50	70.84	65.04	40.16	37.15	42.22

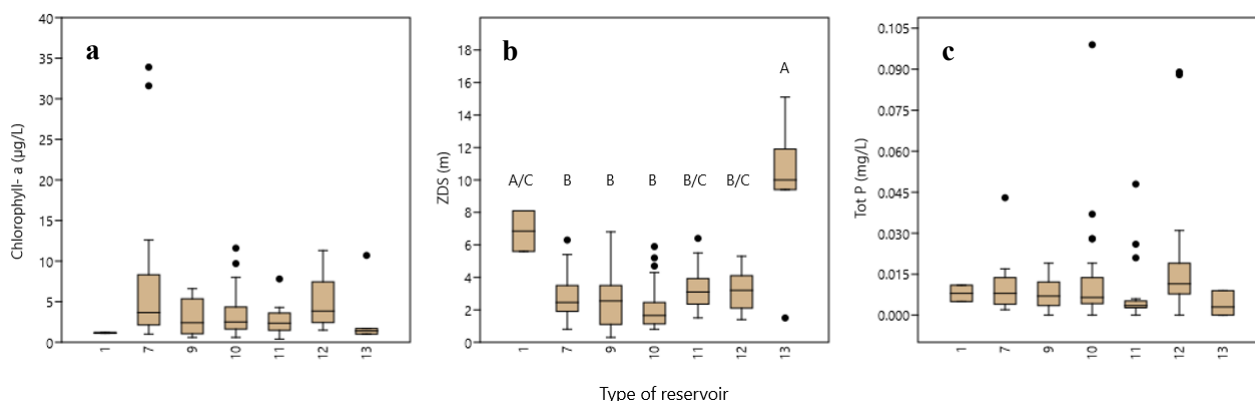
Finally, about total phosphorus (Table 8; Figure 3C), the values of all the reservoirs were generally low. The highest average corresponds to reservoir type 12 (0.0219 mg/L) with the maximum at 0.089 (Flix reservoir). This was followed by reservoir type 10, with a mean of 0.012 mg/L, with the maximum at 0.099 mg/L (Utchesa reservoir), being the reservoir with the highest phosphorus. The coefficients of variation for this variable are generally high in all types, suggesting an uneven distribution of phosphorus in reservoirs of the same type.

**Table 8.** Summary of the values obtained for total phosphorus according to the type of reservoir

	1	7	9	10	11	12	13
<b>N</b>	2	20	10	28	18	14	7
<b>Min</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	5	2	0	0	0	0	0
<b>Max</b>	0.01	0.04	0.01	0.09	0.04	0.08	0.00
	1	3	9	9	8	9	9
<b>Mean</b>	0.00	0.01	0.00	0.01	0.00	0.02	0.00
	8	0	8	3	8	2	4
<b>Std Error.</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	2	2	4	3	8	2
<b>Variance</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0	0	0	0	0	1	0



<b>Std. Dev.</b>	0.00	0.00	0.00	0.01	0.01	0.02	0.00
	4	9	6	9	2	9	4
<b>Median</b>	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	8	8	7	7	4	2	3
<b>25</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Percentile</b>	5	4	4	4	3	8	0
<b>75</b>	0.01	0.01	0.01	0.01	0.00	0.01	0.00
<b>Percentile</b>	1	4	2	4	5	9	9
<b>Coeff.</b>	53.0	88.2	76.0	148.	156.	132.	109.
<b>Var.</b>	33	04	91	346	386	935	599



**Figure 3.** Box plots for each variable studied: (a) chlorophyll-a, (b) Secchi disk depth, (c) total phosphorus. Each box delimits the interquartile range (25th-75th percentile), the horizontal line marks the median and the whiskers indicate the range of the data, excluding outliers marked with dots. For chlorophyll-a and total phosphorus no differences between groups were observed according to Dunn's test ( $p$ -value  $< 0.05$ ). For transparency, equal letters indicate groups with no significant differences and groups with different letters show significant differences

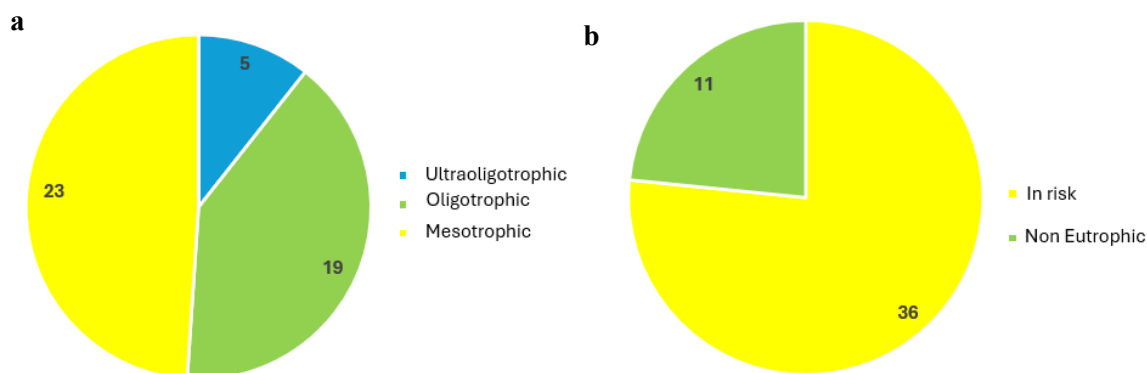
When analysing whether there are differences between groups of reservoirs (Figure 3) using the Kruskal-Wallis test for each variable, significant differences were observed in at least one group ( $p$ -value  $< 0.05$ ). However, when applying Dunn's test for pairwise comparison, significant differences between some of the reservoir types were only detected at for the transparency variable.

To study whether there is a relationship between the different trophic parameters, their correlation was studied. The Pearson correlation analysis used with the normalised values showed a significant negative linear association between the variables chlorophyll-a and ZDS ( $r = -0.21$ ;  $p$ -value = 0.0384), indicating that there is a slight but significant trend in which the higher the chlorophyll concentration, the lower the transparency of the water mass, and vice versa. In contrast, this analysis showed no evidence of a significant linear association between total phosphorus and the variables ZDS ( $p$ -value = 0.460) or chlorophyll ( $p$ -value = 0.185).

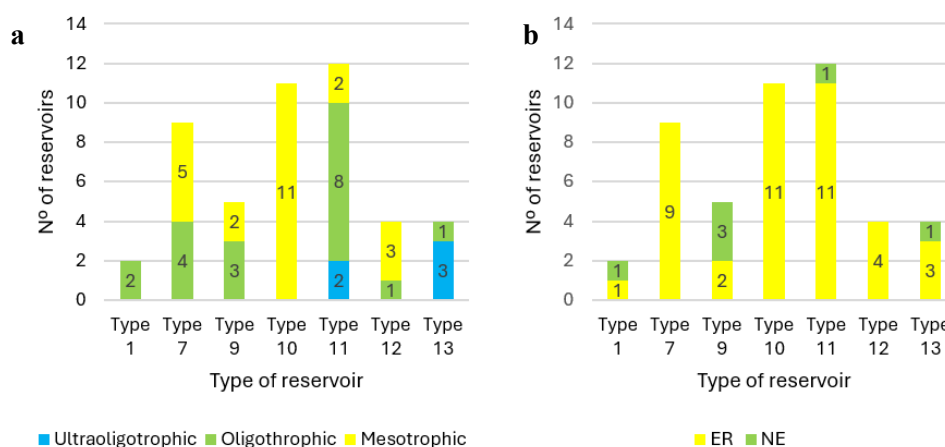
The determination of the trophic state following the criteria proposed by the OECD (1982) (Figure 4A) showed that five of the 47 reservoirs (10.6%) analysed had an ultra-oligotrophic state, corresponding to Baserca, El Grado, Calanda, Llauset and Colomers: Baserca, El Grado, Calanda, Llauset and Colomers. 40.4% (19 out of 47) correspond to reservoirs categorised as oligotrophic (Ebro, Lanuza, Yesa, Mediano, Escales, Talarn, Oliana, Barasona, Canelles, Rialb, Pajares, Camarasa, Santa Ana, La Tranquera, Caspe, Itoiz, Ortigosa, Balaguer and Sallente) and 23 out of 47 reservoirs (48.9%) were classified as mesotrophic (Ullívarri-Gamboa, Sobrón, Búbal, Terradets, La Sotonera, San Lorenzo, El Val, Mequinenza, Margalef, Flix, Moneva, Guiamets, Cueva Foradada, Santolea, Lechago, Gallipué, Ribarroja, Alcañiz, Utchesa Seca, La Loteta, Las Fitass, Maidevera and San Salvador). These data are shown in detail in Table 5.

When studied by groups (Figure 5A), of the oligotrophic reservoirs, the majority were type 11 (42%), followed by type 7 (21%), type 9 (16%), type 1 (11%) and finally type 12 and 13 (5% each). In the case of mesotrophic reservoirs, 48% corresponded to type 10 reservoirs, followed by type 7 with 22% and type 12 with 13%, and finally type 11 and 9, both with 9%. Ultra-oligotrophic reservoirs were formed only by type 13 (60%) and type 11 (40%).

Royal Decree 47/2022 classified 41 out of 47 reservoirs as being at risk of eutrophication (ER) (87.23%). Of this percentage, 76% are type 7, 10 and 11 reservoirs. The remaining six of the 47 reservoirs were considered as non-eutrophic (NE) (12.76%): Baserca, Yesa, Mediano, Oliana, Pajares and San Lorenzo reservoirs, which are distributed between types 1 (17%), 9 (50%), 11 (17%) and 13 (17%) (Figure 4B; Figure 5B).



**Figure 4.** Summary of trophic state results according to OECD (a) and Royal Decree 47/2022 (b). ER (At risk of eutrophication). NE (Non eutrophic)



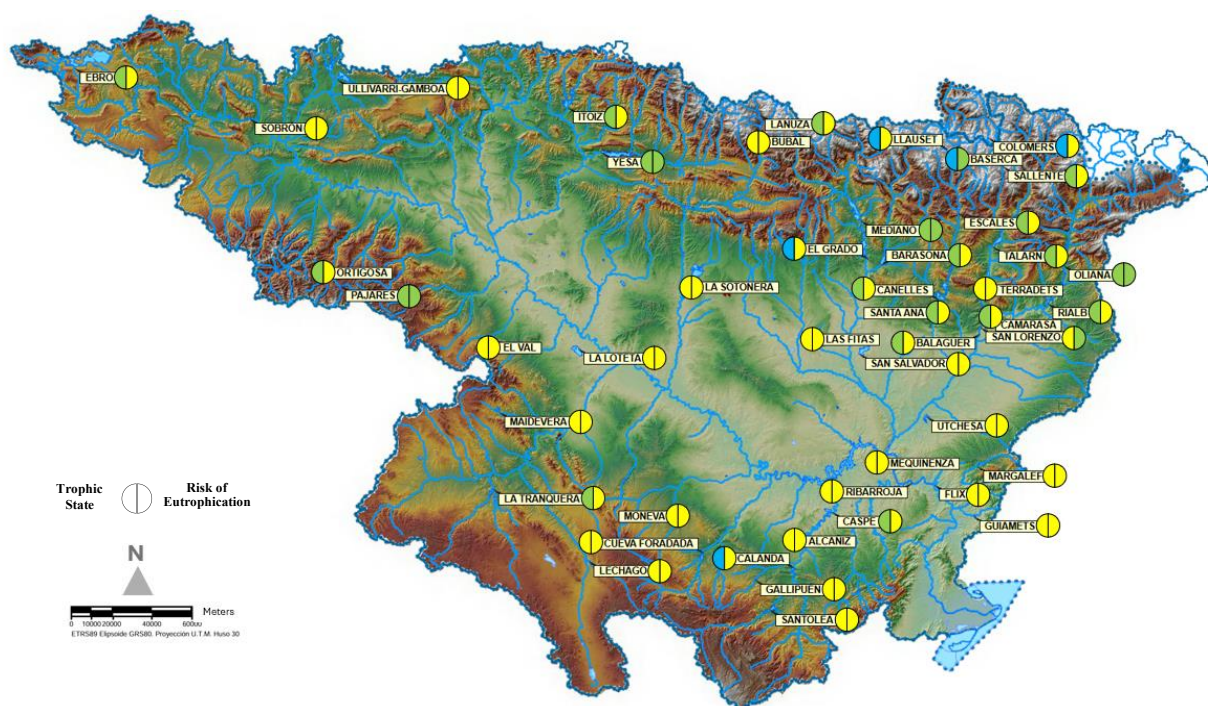
**Figure 5.** Summary of results obtained by trophic state groups according to OECD (a) and Royal Decree 47/2022 (b). ER (At risk of eutrophication). NE (Non eutrophic)

To assess the underlying patterns in the data, a principal component analysis (PCA) was performed using the normalised values of the trophic variables already discussed (Figure 7). Thus, this analysis allows us to visualise whether the reservoirs are grouped according to their characteristics (reservoir types).

The first two components (PC1 and PC2) explained 78.28% of the variance. Component 1 (42.77%) was positively correlated with chlorophyll-a (0.697) and total phosphorus (0.697), representing the productivity-eutrophy gradient. On the other hand, component 2 (35.5%) was positively related to transparency (0.493) and total phosphorus (0.671); and negatively related to chlorophyll (-0.554).

Therefore, in the upper left quadrant were found the oligotrophic or ultra- oligotrophic reservoirs, i.e. those with higher transparency and lower chlorophyll and phosphorus concentration. This is the case of type 13 reservoirs, represented in the PCA by the blue water dots, which show a clear grouping in this quadrant: Baserca, Llauset, Sallente and Colomers, except for the Sallente reservoir sampled during the month of July, which showed eutrophic conditions. In the lower right quadrant, those with primary production, phosphorus and low transparency were grouped together, as is the case of Mequinenza (dates 22/07 and 19/09), among others. In the lower left quadrant, the reservoirs with a low concentration of phosphorus, chlorophyll and transparency were found, highlighting the case of Las Fitass, Búbal, among others. Finally, in the upper right, there were transparent reservoirs, but with positive loads of total phosphorus and chlorophyll- a, such as the case of Ribarroja, sampled in March and which presented a eutrophic state according to Royal Decree 47/2022.

Of the reservoirs that were categorised as oligotrophic and ultra-oligotrophic according to OECD criteria (24), only five remained non-eutrophic, the remaining 19 were classified as at risk. As for those categorised as mesotrophic, 23 out of 23 remained at risk in the DR (Figure 6), apart from San Lorenzo reservoir (Type 11), which was considered non-eutrophic despite its mesotrophic state. This can also be seen in Figure 5, where the comparison by groups can be observed. In type 9, Yesa, Mediano and Oliana remained oligotrophic and not eutrophic; in type 13, it is Baserca that remains as not at risk.



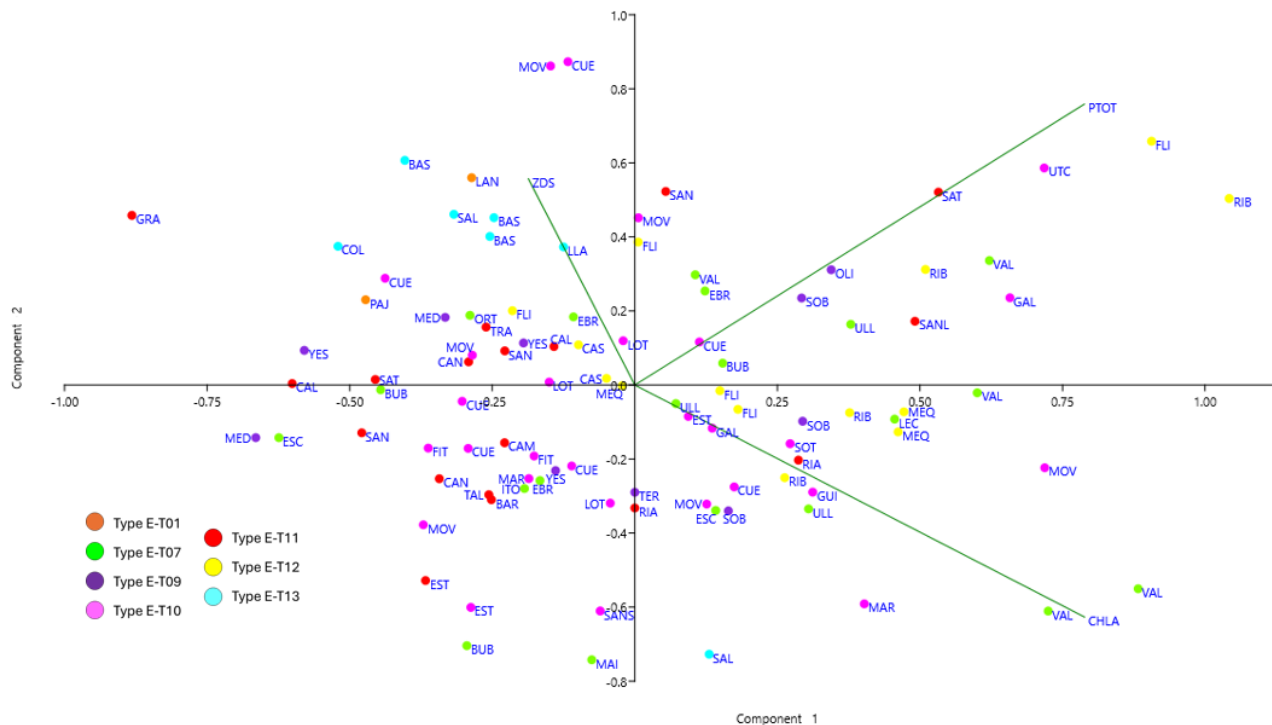
**Figure 6.** Comparison between trophic state (left semicircle) according to OECD and eutrophication risk (right semicircle) according to RD 47/2022

This distribution is in accordance with the classification of water bodies according to the OECD, as mesotrophic reservoirs are in the negative part of component 2, and oligotrophic and ultra-oligotrophic reservoirs are in the positive part of component 2.

## 4. DISCUSSION

During the sampling campaigns of the years 2023 and 2024 in the reservoirs of the Ebro basin, variables related to primary productivity were studied, such as the maximum and mean annual

concentration of chlorophyll-a, mean annual concentration of total phosphorus and transparency measured with the Secchi disk. These data allowed the trophic state of these water bodies to be categorised as proposed by the OECD (1982), resulting in five ultra- oligotrophic reservoirs, 19 oligotrophic reservoirs and 23 mesotrophic reservoirs. At the same time, the risk of eutrophication was assessed in accordance with the criteria established by Royal Decree 47/2022, of 18 January (2022), which, in addition to the parameters, also considers the pressures in the basin, resulting in 41 reservoirs at risk of eutrophication, and only six classified as non-eutrophic.



**Figure 7.** Principal Component Analysis of the reservoirs sampled during the years 2023 and 2024

It is important to note that, at present, the regulation for eutrophication risk assessment is the one proposed by RD 47/2022, which introduces a preventive approach to eutrophication risk assessment, replacing the descriptive methodology of the OECD (1982). While the OECD classifies water bodies according to their trophic state without considering external pressures, RD 47/2022 incorporates factors such as diffuse nutrient loads, land use and the susceptibility of the system, aiming to anticipate degradation processes and improve water quality management, in line with European directives. Furthermore, this regulation reduces the number of variables to two main variables (chlorophyll-a and total phosphorus), giving more weight to the latter, and using transparency in a complementary way through expert judgement, as it can be affected by factors not related to the trophic state, such as inorganic solids. However, this simplification leads to a loss of valuable information on the actual ecological state and uses of water.

Royal Decree 47/2022 introduces a precautionary approach to eutrophication risk assessment, replacing the descriptive methodology of the OECD (1982). Whereas the OECD classified water bodies according to their trophic state (oligotrophic, mesotrophic, eutrophic) without considering external pressures, RD 47/2022 incorporates factors such as diffuse nutrient loads, land uses and the susceptibility of the system, aiming to anticipate degradation processes and improve water quality management, in line with European directives.

This new model reduces the number of variables to two main variables (chlorophyll-a and total phosphorus), giving greater weight to the latter. Transparency is used in a complementary way through expert judgement, as it can be affected by factors not related to trophic state, such as inorganic

solids. However, this simplification leads to a loss of valuable information on the actual ecological state and uses of water.

A comparison of the two approaches shows that, out of 24 reservoirs classified as oligotrophic or ultra-oligotrophic according to the OECD, only five (Yesa, Mediano, Oliana, Pajares and Baserca) remain non-eutrophic under RD 47/2022. The other 19 are considered at risk due to significant pressures in the basin. A prominent case is the Calanda reservoir, with ultra-oligotrophic state, but high pressure, which classifies it as at risk despite being a catchment area for human consumption (CHE, 2025a). This may generate uncertainty in the prioritisation of actions and lead to inefficient management.

The results obtained in this study show differences with respect to previous campaigns, evidencing a trend towards mesotrophic conditions in several reservoirs. In comparison with sampling data from 2010 and 2011 (CHE, 2010, 2011), five of the 30 reservoirs analysed have shown a deterioration in their trophic state, going from oligotrophic to mesotrophic, as is the case of the Ullívarri-Gamboa or Flix reservoir. This may be due to an increase in nutrient loading associated with significant pressures in the catchment, attributed to diffuse sources derived from agricultural activities, livestock or contaminated soils (CHE, 2025a). Fertiliser runoff in catchments such as the Ebro increases the organic load in waters (Devlin C Brodie, 2023), which may accelerate the eutrophication process, as has been observed in other comparable systems (Ledesma et al., 2013). In contrast, nine reservoirs have improved their trophic state, such as the Ebro reservoir and Barasona, which have gone from a mesotrophic to an oligotrophic state. Other cases, such as El Val and Utchesa, have gone from being eutrophic to mesotrophic, and only one (Calanda) has gone from oligotrophic to ultra-oligotrophic. In the case of El Val, this is due to an improvement in the management of the reservoir (CHE, 2017), although livestock contributions persist. On the other hand, several reservoirs maintain a stable trophic state, such as the Sobrón or Mequinenza reservoir, which may be due to the stability in the characteristics of the reservoir and moderate anthropic pressure.

If we compare the situation of the reservoirs in the Ebro valley with other river basins, such as the Segura, where in 2023 80.85 % of the reservoirs were not eutrophic (Confederación Hidrográfica del Segura, 2023), the Ebro basin presents a more worrying situation, with 87.23 % of its reservoirs at risk of eutrophication and only 12.76 % remaining non-eutrophic. This trend towards eutrophication in the Ebro reservoirs is supported by studies such as those of Pérez-González et al. (2023), who analysed the presence of cyanobacteria, and Muñoz-Colmenares et al. (2021), who used zooplanktonic bioindicators, where they demonstrate a generalised trophic degradation in the basin. Furthermore, they reinforce that the response of reservoirs to eutrophication depends on their location, human pressures and water availability (European Environment Agency, 2018).

In terms of reservoir type, types 1 and 13 have oligotrophic or ultra-oligotrophic states. They are located in cold climatic regions (Cfb and high mountains), which are associated with lower temperatures, less solar radiation and reduced evaporation, limiting the accumulation of nutrients in the water (Camargo et al., 2005; Toro et al., 2006).

In contrast, type 10 reservoirs are mesotrophic, despite being in the headwaters. Their location in warmer and drier climates (Csa, Cfa, Bsk), together with high solar radiation and evapotranspiration, favours biological productivity (Dodds C Whiles, 2020). In addition, the anthropic pressure in the form of hydromorphological alteration and diffuse agricultural pollution, such as in the Alcañiz or Gallipué reservoirs (CHE, 2025a), favours this state.

Type 11 reservoirs show a predominance of oligotrophic conditions. They are located in cold regions (Bsk), with low population density, which reduces pressure on aquatic ecosystems (Dodds C Whiles, 2020; Navarro et al., 2010). Although the main pressures are hydromorphological, cases such as Santolea show impacts due to poor waste management (CHE, 2025a).

For their part, type 12 reservoirs, located in lower stretches of rivers, receive the dragging of pollutants and nutrients from the entire basin, which, added to a longer residence time of the water, increases the risk of eutrophication (Camargo et al., 2005). This is reflected in the fact that 75% of them are classified as mesotrophic. Among the pressures, hydromorphological and agricultural

diffuse pollution are found in all cases, with additional contributions from livestock (Ribarroja) or contaminated soils (Flix) (CHE, 2025a).

Finally, reservoirs type 7 and 9 confirm the climatic influence. Those located in a Cfb climate tend to be oligotrophic, such as Escales or Ebro, while those in a Csa climate, such as Terradets, show mesotrophic states. Exceptions such as Ullívarri-Gamboa and Búbal, despite their Cfb climate, show mesotrophic states, probably due to diffuse pollution from livestock activity (CHE, 2025a).

Alternative methodologies exist to assess the trophic state of water bodies, such as remote sensing. In China, recent studies (Hu et al., 2024; Liu et al., 2023; Zhou et al., 2021) have used Sentinel-2 and Landsat-5 TM images, highlighting their low cost and capacity for continuous and spatially wide monitoring. Compared to the Ebro basin, similar distributions are observed, approximately 60% of the reservoirs are mesotrophic and 30% eutrophic (Zhou et al., 2021). Furthermore, in the United States, Meyer et al. (2024) classified more than 55,000 lakes using Landsat and advanced computer models, achieving an accuracy of 74%. This methodology has also been applied by the Limnology group of the Universitat de València (Pérez-González et al., 2021, 2023; Pompêo et al., 2021).

Although Royal Decree 47/2022 requires in situ methods, these studies show that combining sampling and remote sensing improves accuracy and usefulness for hydrological planning.

## 5. CONCLUSIONS

This study has determined the trophic state for the Ebro basin reservoirs studied during 2023 and 2024, based on OECD criteria. The results show that 10.6% are ultra-oligotrophic, 40.4% are oligotrophic and the remaining 48.9% are mesotrophic. However, when applying the criteria established in RD 47/2022 to assess the risk of eutrophication, 87.23% of the reservoirs are considered to be at risk, while only 12.76% are classified as non-eutrophic. After analysis and discussion of the results, the following conclusions have been reached:

The analysis concludes that the trophic state is conditioned by multiple factors, pressures in the basin (diffuse pollution, morphological alteration), location in the course of the river and climate, with no single determining variable. There is also a trend towards mesotrophic states with respect to the 2010-2011 samples.

The difference between classifications is explained by the precautionary approach of RD 47/2022, which includes external pressures, but may not fully reflect actual water use. It is proposed to complement this regulation with remote sensing techniques to detect early changes and improve the trophic management of reservoirs, especially in a basin with high anthropic pressure such as the Ebro.

## 6. REFERENCES

- Betancourt, C., & Labaut, Y. (2013). La calidad físicoquímica del agua en embalses, principales variables a considerar. *Revista Agroecosistemas*, 1(1), 78–103.
- Boyd, Claude. E. (2015). *Water quality*. Springer International Publishing AG.
- Camargo, J. A., Alonso, Á., & Puente, M. de la. (2005). Eutrophication downstream from small reservoirs in mountain rivers of Central Spain. *Water Research*, 39(14), 3376–3384. <https://doi.org/10.1016/j.watres.2005.05.048>
- CHE. (2010). *Confederación Hidrográfica del Ebro. Informe Final de Embalses Año 2010* [Documento Memoria]. Universitat de València. [https://www.chebro.es/documents/20121/54000/Memoria\\_Informe\\_embalses\\_2010.pdf](https://www.chebro.es/documents/20121/54000/Memoria_Informe_embalses_2010.pdf). Accessed 15 Jun 20205.
- CHE. (2011). *Confederación Hidrográfica del Ebro. Informe Final de Embalses Año 2011* (p. 215) [Documento Memoria]. Universitat de València.



- [https://www.chebro.es/documents/20121/54000/Memoria\\_Informe\\_embalses\\_2011.pdf](https://www.chebro.es/documents/20121/54000/Memoria_Informe_embalses_2011.pdf). Accessed 15 Jun 20205.
- CHE. (2017). *Confederación Hidrográfica del Ebro. Estudio del estado trófico del embalse de El Val (Zaragoza) y Programa de medidas* (p. 71) [Memoria]. Ministerio para la Transición Ecológica y el Reto Demográfico. [https://www.chebro.es/documents/20121/55149/EC16013\\_SENSIBLES\\_VAL\\_IF\\_vD.pdf](https://www.chebro.es/documents/20121/55149/EC16013_SENSIBLES_VAL_IF_vD.pdf). Accessed 15 Jun 20205.
- CHE. (2025a). *Confederación Hidrográfica del Ebro. Análisis de presiones e impactos. Fichas de resultados IMPRESS*. Portal CHEbro. <https://www.chebro.es/fichas>. Accessed 18 Jun 20205.
- CHE. (2025b). *Confederación Hidrográfica del Ebro. La cuenca del Ebro*. Portal CHEbro. <https://www.chebro.es>. Accessed 13 Jun 20205.
- Chislock, M. F., Doster, E., Zitomer, R. A., & Wilson, A. E. (2013). Eutrophication: Causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge*, **4**(4), 10.
- Confederación Hidrográfica del Segura. (2023). *Servicios de asistencia técnica para el desarrollo del programa de seguimiento para determinar el estado de las aguas continentales y el control adicional de las zonas protegidas en la Demarcación Hidrográfica del Segura*. (p. 116). Ministerio para la Transición Ecológica y el Reto Demográfico. <https://www.chsegura.es/es/cuenca/redes-de-control/calidad-en-aguas-superficiales/informes/>. Accessed 15 Jun 20205.
- Cunha, D. G. F., Calijuri, M. do C., & Lamparelli, M. C. (2013). A trophic state index for tropical/subtropical reservoirs (TSItsr). *Ecological Engineering*, **60**, 126–134. DOI: 10.1016/j.ecoleng.2013.07.058
- Devlin, M., & Brodie, J. (2023). Nutrients and Eutrophication. In A. Reichelt-Brushett (Ed.), *Marine Pollution – Monitoring, Management and Mitigation* (pp. 75–100). Springer Nature Switzerland. DOI: 10.1007/978-3-031-10127-4\_4
- Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000, Pub. L. No. 2000/60/EC, OJEU-L-2000-82524 (2000).
- Dodds, W. K., & Cole, J. J. (2007). Expanding the concept of trophic state in aquatic ecosystems: It's not just the autotrophs. *Aquatic Sciences*, **69**(4), 427–439. DOI: 10.1007/s00027-007-0922-1
- Dodds, W. K., & Whiles, M. R. (2020). *Freshwater ecology: Concepts and environmental applications of limnology* (Third edition). Elsevier, Academic Press.
- European Environment Agency. (2018). *European waters. Assessment of status and pressures 2018* (EEA Report No. 7). <https://www.eea.europa.eu/en/analysis/publications/state-of-water>. Accessed 20 Jun 20205.
- Fernández-Cirelli, A. (2012). El agua: Un recurso esencial. *Química Viva*, **11**(3), 147–170.
- Golterman, H. L. (1970). Possible consequences of phosphate eutrophication of surface water. *H2O*, **3**(17).
- Hammer, Ø., Harper, D. A. T., & Ryan, D. R. (2001). Past: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, **4**, 9.
- Hassan, N. O. (2020). Water quality parameters. *Water Quality-Science, Assessments and Policy*, 89657. DOI: 10.5772/intechopen
- Hosseiny, S. H., Bozorg-Haddad, O., & Bocchiola, D. (2021). 9—Water, culture, civilization, and history. In O. Bozorg-Haddad (Ed.), *Economical, Political, and Social Issues in Water Resources* (pp. 189–216). Elsevier. DOI : 10.1016/B978-0-323-90567-1.00010-3
- Hu, M., Ma, R., Xue, K., Cao, Z., Xiong, J., Loiselle, S. A., Shen, M., & Hou, X. (2024). Eutrophication evolution of lakes in China: Four decades of observations from space. *Journal of Hazardous Materials*, **470**, 134225. DOI: 10.1016/j.jhazmat.2024.134225
- Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*1 and *c*2 in higher plants, algae and natural phytoplankton. *Biochimie Und Physiologie Der Pflanzen*, **167**(2), 191–194. DOI: 10.1016/S0015-3796(17)30778-3
- Kuczyńska-Kippen, N., Zhang, C., Mleczek, M., & Špoljar, M. (2025). Rotifers as indicators of trophic state in small water bodies with different catchments (field vs. Forest). *Hydrobiologia*, **852**(10), 2669–2685. DOI: 10.1007/s10750-024-05760-7

- Ledesma, C., Bonansea, M., Rodriguez, C. M., & Sánchez Delgado, A. R. (2013). Determinación de indicadores de eutrofización en el embalse Río Tercero, Córdoba (Argentina). *Revista Ciência Agronômica*, **44**, 419–425. DOI: 10.1590/S1806-66902013000300002
- Liu, Y., Ke, Y., Wu, H., Zhang, C., & Chen, X. (2023). A satellite-based hybrid model for trophic state evaluation in inland waters across China. *Environmental Research*, **225**, 115509. DOI: 10.1016/j.envres.2023.115509
- Meyer, M. F., Topp, S. N., King, T. V., Ladwig, R., Pilla, R. M., Dugan, H. A., Eggleston, J. R., Hampton, S. E., Leech, D. M., Oleksy, I. A., Ross, J. C., Ross, M. R. V., Woolway, R. I., Yang, X., Brousil, M. R., Fickas, K. C., Padowski, J. C., Pollard, A. I., Ren, J., & Zwart, J. A. (2024). National-scale remotely sensed lake trophic state from 1984 through 2020. *Scientific Data*, **11**(1), 77. DOI: 10.1038/s41597-024-02921-0
- Mineeva, N. M. (2023). Production Characteristics of Phytoplankton as Indicators of the Trophic State of Artificial Reservoirs. *Inland Water Biology*, **16**(2), S333–S346. DOI: 10.1134/S1995082923080096
- Ministerio de Medio Ambiente. (2000). *Libro blanco del agua en España*.
- Muñoz-Colmenares, M. E., Soria, J. M., & Vicente, E. (2021). Can zooplankton species be used as indicators of trophic status and ecological potential of reservoirs? *Aquatic Ecology*, **55**(4), 1143–1156. DOI: 10.1007/s10452-021-09897-8
- Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, **27**, 31–36. DOI: 10.1016/S0003-2670(00)88444-5
- Navarro, E., García-Berthou, E., & Armengol, J. (2010). La calidad ecológica de los embalses. *Investigación y ciencia (Spanish edition of Scientific American)*, **401**, 80–87.
- OECD. (1982). *Eutrophication of waters: Monitoring, assessment and control*. <http://lakes.chebucto.org/TPMODELS/OECD/OECD1982.pdf>. Accessed 15 Jun 2025.
- Pérez-González, R., Sòria-Perpinyà, X., Soria, J. M., Delegido, J., Urrego, P., Sendra, M. D., Ruíz-Verdú, A., Vicente, E., & Moreno, J. (2021). Phycocyanin Monitoring in Some Spanish Water Bodies with Sentinel-2 Imagery. *Water*, **13**(20), Article 20. DOI: 10.3390/w13202866
- Pérez-González, R., Sòria-Perpinyà, X., Soria, J., Sendra, M. D., & Vicente, E. (2023). Relationship between Cyanobacterial Abundance and Physicochemical Variables in the Ebro Basin Reservoirs (Spain). *Water*, **15**(14), Article 14. DOI: 10.3390/w15142538
- Pompêo, M., Moschini-Carlos, V., Bitencourt, M. D., Sòria-Perpinyà, X., Vicente, E., & Delegido, J. (2021). Water quality assessment using Sentinel-2 imagery with estimates of chlorophyll a, Secchi disk depth, and Cyanobacteria cell number: The Cantareira System reservoirs (São Paulo, Brazil). *Environmental Science and Pollution Research*, **28**(26), 34990–35011. DOI: 10.1007/s11356-021-12975-x
- Royal Decree 47/2022, of 18 January, on the Protection of Waters Against Diffuse Pollution Caused by Nitrates from Agricultural Sources, Pub. L. No. Royal Decree 47/2022, BOE-A-2022-860 5664 (2022). <https://www.boe.es/eli/es/rd/2022/01/18/47>
- Royal Decree 817/2015, of 11 September, establishing the criteria for monitoring and evaluating the status of surface waters and environmental quality standards, Pub. L. No. Royal Decree 817/2015, BOE-A-2015-9806 80582 (2015). <https://www.boe.es/eli/es/rd/2015/09/11/817>
- Rodríguez Pérez, M. J., Soria García, J. M., & Durán Lalaguna, C. (2014). El seguimiento de los embalses en la demarcación hidrográfica del Ebro. El estado de los embalses aragoneses. *Naturaleza Aragonesa*, **31**, 44–52.
- Romero Gil, I. (2019). *Carga crítica de fósforo* [Departamento de Ingeniería Hidráulica y Medio Ambiente, Universitat Politècnica de València]. <https://riunet.upv.es/server/api/core/bitstreams/97296a23-7280-46d6-817c-dc15734932c2/content>. Accessed 29 May 2025.
- Shoaf, W. T., & Lium, B. W. (1976). Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. *Limnology and Oceanography*, **21**(6), 926–928. <https://doi.org/10.4319/lo.1976.21.6.0926>

- Toro, M., Granados, I., Robles, S., & Olmo, C. (2006). High mountain lakes of the Central Range (Iberian Peninsula): Regional limnology & environmental changes. *Limnetica*, **25**, 217–252. DOI: 10.23818/limn.25.17
- Westall, F., & Brack, A. (2018). The Importance of Water for Life. *Space Science Reviews*, **214**(2), 50. DOI: 10.1007/s11214-018-0476-7
- Zhou, Y., He ,Baoyin, Fu ,Congju, Giardino ,Claudia, Bresciani ,Mariano, Liu ,Hui, Feng ,Qi, Xiao ,Fei, Zhou ,Xinmeng, & and Liang, S. (2021). Assessments of trophic state in lakes and reservoirs of Wuhan using Sentinel-2 satellite data. *European Journal of Remote Sensing*, **54**(1), 461–475. DOI: 10.1080/22797254.2021.1960201