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Establishing Total Phosphorus Boundaries to Support Good Ecological Status of Greek Lakes and Reservoirs in Accordance with the Water Framework Directive

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Abstract

Eutrophication, driven by nutrient enrichment, represents substantial anthropogenic pressure with harmful consequences for aquatic ecosystems. The Water Framework Directive provides a structured approach to addressing this challenge as it requires European Union Member States to achieve at least good ecological status for their surface waters. The establishment of realistic nutrient boundaries, above which negative effects become pronounced, is essential to guide regulatory intervention aimed at securing long-term water sustainability in Europe. Greece is one of the Member States which should determine nutrient boundaries supporting the good ecological status of lakes. Two statistical approaches, ranged major axis regression and binomial logistic regression, were applied for setting appropriate nutrient boundaries for Greek natural lakes and reservoirs, using datasets of phytoplankton and total phosphorus concentrations, retrieved from the national monitoring program (2016–2023). The predicted boundary values for total phosphorus supporting good ecological status ranged from 32 to 76 µg/L, with stricter boundaries corresponding to deep lakes. Nutrient boundaries that reflect the environmental pressures on Greek natural lakes and reservoirs are fundamental to ensure proper design of lake management strategies.

Keywords: nutrient boundaries; water framework directive; good ecological status; Greek lakes; total phosphorus

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1. Introduction

Human-induced pressures, such as agricultural practices and intensive use of fertilizers, wastewater discharge, fossil fuel combustion, and climate change, are adversely impacting aquatic environments worldwide, jeopardizing their ecological integrity and services. These pressures have profound consequences in lake ecosystems, often leading to phenomena such as eutrophication, acidification, and salinization. Human welfare can be negatively influenced as well, through numerous impacts on ecosystem services [1–4].

Eutrophication, caused by the excessive input of nutrients, such as phosphorus and nitrogen, into freshwater and especially into lake ecosystems, needs to be regulated. While moderate nutrient concentrations can potentially benefit biodiversity and fish production [5], excessive nutrient enrichment of water bodies leads to substantial environmental deterioration. Harmful algal blooms, submerged vegetation decline, and significant reductions in dissolved oxygen concentrations are only a few of the environmental downgrading aspects, directly affecting ecosystem services as well, such as the supply of drinking water and recreation activities [6,7].

To regulate or even prevent eutrophication, it is necessary to reduce nutrient supply into aquatic environments through holistic, stringent management [4,8]. While some efforts have led to improved water quality, the corresponding ecological quality recovery rate of some impacted lakes, especially for lake macrophyte communities, is hindered due to the high sedimentary phosphorus fluxes of shallow lakes [8].

In European Union (EU) countries, the establishment of the Water Framework Directive 2000/60/EC (WFD) [9] had a crucial role towards the protection of water bodies and aquatic ecosystems. According to WFD, the surface waters, including lakes, of all Member States, are obliged to conform to at least "good" ecological status. In order to achieve WFD goals towards the protection of lake ecosystems, it is fundamental to set nutrient targets. Specific boundaries for good ecological status could be used to maintain healthy biological communities. For this, biological quality elements (BQE; e.g., phytoplankton, macrophytes, phytobenthos, benthic invertebrate fauna, and fish) provide significant insights, combined with physicochemical parameters (e.g., nutrients, oxygen condition, temperature, water transparency, and salinity) and hydromorphological parameters (e.g., quantity and dynamics of water flow), which act as supporting quality elements. However, different BQEs and assessment methods are indicative of different pressures. Thus, it is important to choose nutrient-sensitive elements and methods to effectively evaluate nutrient impacts on ecological status. Assessment methods based on the BQE of phytoplankton are mostly sensitive to eutrophication [4,10–12]. The ecological status of the BQEs can be expressed using the Ecological Quality Ratio (EQR) by comparing observed ecological metrics to reference conditions. Ranging from 0 (bad status) to 1 (high status), EQR indicates how closely a water body matches minimally impacted sites, with intermediate values reflecting poor, moderate, or good status [4,13,14].

Water bodies in moderate or worse status are required to be restored by EU Member States to good or better status. Establishing nutrient thresholds plays a key role in achieving this objective [1,4,12]. Once the boundary value has been set, water bodies can be classified as either sites of concern or no concern, depending on whether their nutrient concentrations exceed or fall below the boundary value, respectively [4]. Subsequently, appropriate management strategies should be implemented to reduce environmental stressors, support ecosystem restoration, and promote the long-term health and sustainability of aquatic ecosystems [3,15].

Among the physicochemical parameters that significantly affect lake water quality, the concentration of Total Phosphorus (TP) is often selected as a key parameter due to its relation to eutrophication. Algal blooms in lakes have been linked to increased TP concentrations, and phytoplankton biomass usually responds quickly to changes in phosphorus content and availability [1,16,17]. Phosphorus exhibits a strong pressure–response relationship with phytoplankton metrics, which are often used as an expression of eutrophication. Thus, it is common among EU Member States to establish TP boundaries for lakes, based on these metrics [1,8]. While other BQEs can also be used, phytoplankton can be particularly effective for early detection as it exhibits a more immediate response to eutrophication, due to its short generation time periods and nutrient intake from the water column, while it is comparatively less sensitive to other pressures, such as chemical or hydromorphological [10,18,19].

Multiple studies have been performed for the establishment of boundary values for TP associated with good ecological status in lakes, many of which are under the WFD, providing empirical data and guidance. Dolman et al. [20] compiled water quality and phytoplankton data from German lakes and estimated that good ecological status was achieved at TP concentrations of 20–35 μg/L for stratified lakes and 35–75 μg/L for polymictic lakes. According to the implication of Free et al. [21] for setting TP boundaries in Irish lakes focusing on phytoplankton data, the TP concentration at the threshold of "good-moderate" status was between 22 and 28 μg/L. For Danish deep and shallow lakes, boundaries for good ecological status have been suggested at 12.5–25 μg/L and 25–50 μg/L of TP, respectively [22]. The only comprehensive approach proposed so far regarding Greek lakes comes from Kagalou et al. [23]. In this work, the phytoplankton results collected through the national monitoring program, during the period of 2015–2020, were used. For deep and shallow natural lakes, the proposed TP boundary values supporting good ecological status were 32 μg/L and 41 μg/L, respectively.

The aim of this study was to determine scientifically derived nutrient boundaries delineating good from moderate ecological status, focusing on TP concentrations in relation to phytoplankton, a sensitive BQE demonstrated by EQR. For this, we applied statistical models, provided by the Shiny toolkit [24], which is available online (https://shiny.freshwater-ecology.com/Tkit_NEW/ (accessed on 12 May 2025), ensuring reproducibility of the analyses. This work addresses five types of Greek lakes, i.e., deep, shallow, and very shallow natural lakes and deep and shallow reservoirs, and constitutes the first attempt to establish nutrient boundaries for the latter three types.

2. Materials and Methods

2.1. Datasets

In this study, data from 19 natural lakes and 26 reservoirs of the Greek national monitoring network were utilized. Natural lakes were classified into three types: (a) warm monomictic, deep lakes (type GR-DNL, 7 lakes), (b) polymictic, shallow lakes (type GR-SNL, 8 lakes), and (c) very shallow lakes (type GR-VSNL, 4 lakes) [23,25,26]. Reservoirs were categorized into two types: (a) deep reservoirs (type GR-DR, 20 lakes) [27,28] and (b) shallow reservoirs (type GR-SR, 6 lakes). The spatial distribution of lakes is depicted in Figure 1, while geometric parameters are provided in Supplementary Table S1. The Shiny toolkit [24] was employed to perform data analysis aimed at determining nutrient boundaries for each lake type. The datasets contained TP concentration (μg/L) and phytoplankton EQR values. Water and phytoplankton samples were collected with a Nansen-type sampler (Free Flow Water Sampler-436340, HYDRO-BIOS, Altenholz, Germany) from the euphotic zone of the water column (2.5 × Secchi disk depth) in the pelagic zone [28], at the deepest point of the lake, to ensure representativeness of the lake basin and consistency across samplings. Water samplings for TP and phytoplankton were conducted 2–4 times during the growing season of each year (May to October), while TP was additionally measured on a quarterly basis [26,28], during the period of 2016–2023. TP concentrations were determined with the persulfate digestion method [29], using an autoclave (AES-28, Raypa Metrolab, Barcelona, Spain) and a spectrophotometer (U-5100 UV/VIS, Hitachi High-Technologies Corporation, Tokyo, Japan). To prevent uneven distribution of data across and within months, seasonal measurements were averaged to obtain mean annual TP values, which were subsequently utilized in statistical analyses [23]. The phytoplankton EQR values were normalized and calculated as mean values using national assessment methods [17,30], in compliance with the WFD and its associated guidelines, to ensure comparability across lake types and years, reduce site–specific bias, and provide a robust representation of ecological status. Specifically, EQR values were calculated using the NMASRP assessment method for the

GR-DR lake type, the HelPhy assessment method for GR-DNL and GR-SNL lake types, and the GR-PTI assessment method for GR-SR and GR-VSNL lake types [17,26,31]. Microscopic analyses were performed using an inverted trinocular fluorescence microscope DMIL (Leica Microsystems GmbH, Wetzlar, Germany) and the concentrations of chlorophyll-a were measured spectrophotometrically according to standard methods [29], with both analyses required for the determination of EQR values. All chemical reagents used for the analyses were obtained from Sigma-Aldrich (St. Louis, MO, USA). The datasets comprised a total of 24, 51, 25, 52, and 27 lake-years for lake types GR-DNL, GR-SNL, GR-VSNL, GR-DR, and GR-SR, respectively. These corresponded to 87, 197, 73, 136, and 58 samples of phytoplankton and 161, 359, 143, 270, and 133 samples of TP, respectively, for the same lake types.

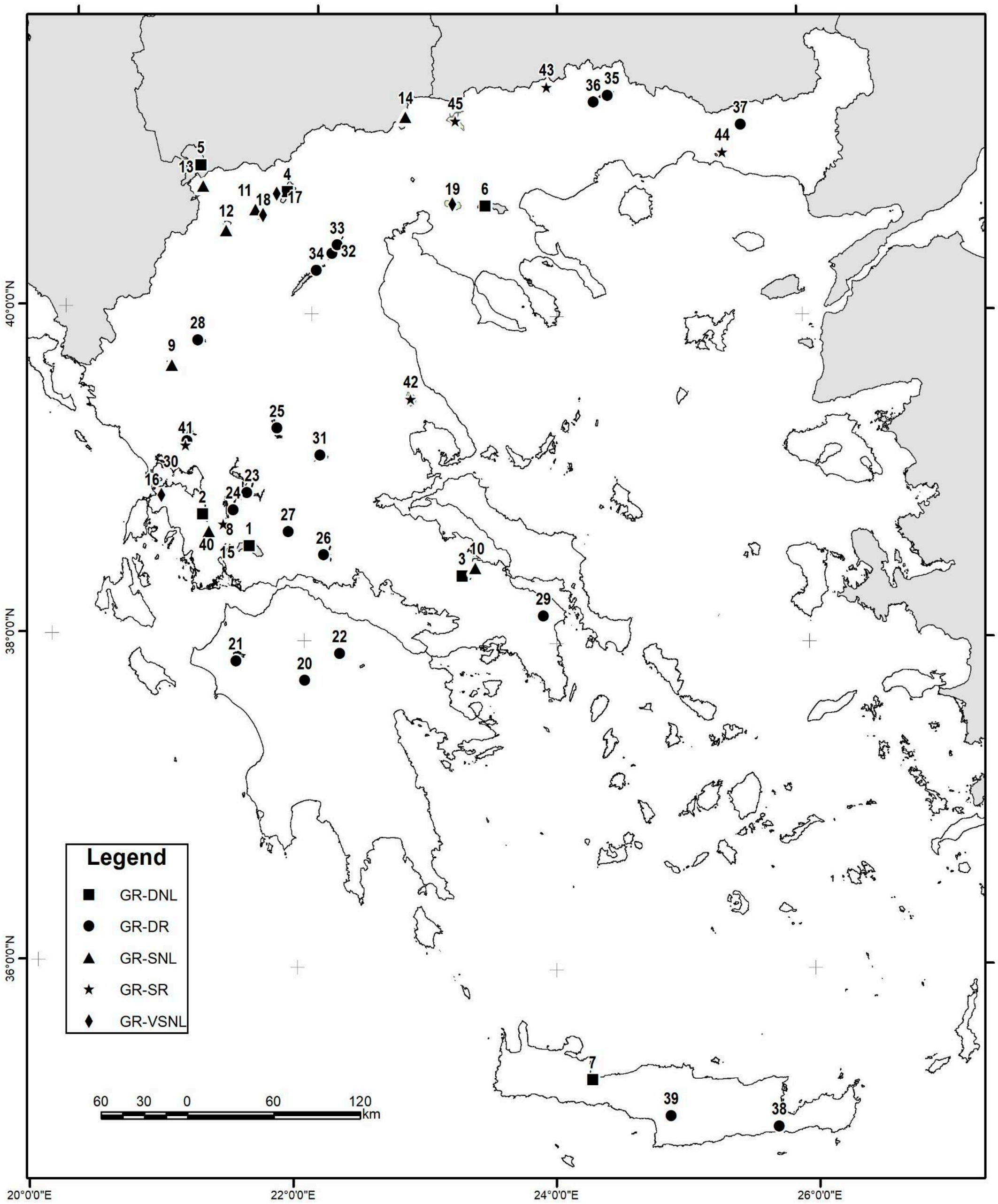

Figure 1. Lake types of the Greek monitoring network: Greek deep natural lakes (GR-DNL; 1–7); Greek shallow natural lakes (GR-SNL; 8–15); Greek very shallow natural lakes (GR-VSNL; 16–19); Greek deep reservoirs (GR-DR; 20–39); Greek shallow reservoirs (GR-SR; 40–45).

2.2. Data Check

The datasets used in this study captured the spatial and temporal variability of water bodies of similar types with a comparable number of years for each water body. Regarding data visualization, scatterplots (EQR vs. TP) were generated using the toolkit, with the option to exclude outliers if needed. For further inspection of the data, box plots were used, where data could be distributed across the following ecological quality classes: High (H), Good (G), Moderate (M), and Poor (P), with class boundaries of 0.8, 0.6, 0.4, and 0.2, respectively. Preliminary visualizations revealed the distribution of data along the gradient of interest and the potential need for axes transformation to achieve linearity, identified signs of heteroscedasticity, and finally, facilitated the selection of the method for the determination of the most appropriate boundary value for TP concentration at the threshold of good–moderate (GM) status, based on the available data.

2.3. Statistical Approaches

Two methods were used for estimating TP boundaries: linear regression analysis and binomial logistic regression. Where a single stressor has a decisive influence on the response of biology, as it applies in the case of TP concentration and phytoplankton [23,32], these two methods are considered the most reliable approaches [33]. Linear regression assumes a linear response between biology and nutrient concentration, i.e., EQR and TP, where EQR is a continuous variable. A variance test and a Breusch–Pagan test were performed, along with the inspection of the standard residuals plot, in order to check the assumptions of a linear model, i.e., homoscedasticity and normality of residuals. Both EQR and TP values are subject to measurement error, as they are simplified expressions of the complex interplay between biological communities and environmental chemistry, as explained by Kelly et al. [32]. For the minimisation of the variation in both variables, reduced major axis regression (RMA) was performed, as recommended by Phillips et al. [24]. RMA assumes equal uncertainty in the measurements of EQR and nutrient concentration. In linear modeling, the correlation coefficient R^2 was employed to assess the strength of the relationship between the two variables, with a threshold of $\geq$0.36 required for acceptance.

The most suitable alternative to linear regression is considered the Binary Logistic Model (BLM) [32,33]. In BLM, EQR is handled as a categorical variable, and thus, data are divided into two categories, on either side of the boundary of interest, i.e., biology «good or better» (G+) represented by 1 and «moderate or worse» (M−) represented by 0. The boundary values can be derived using different threshold probabilities (Prob) of biology being in «good or better» status, other than the default value of 0.5, but in any case, threshold probabilities should remain below 0.9. Additional metrics used in BLM are the Area Under the Curve (AUC) and the pseudo-r^2, both used to assess model performance. An AUC greater than 0.70 is required for demonstrating sufficient discriminatory capacity in binary classification, while a pseudo-r^2 of at least 0.15 indicates an acceptable model fit [4,24].

2.4. Confusion Matrix

To evaluate the effectiveness of models used in determining nutrient boundary values, a confusion matrix was used, as proposed by Phillips et al. [4,24]. A confusion matrix is a two-by-two table that compares biology and nutrient classifications and enables the measurement of the uncertainty associated with a proposed boundary value. Biological status is predicted from nutrient concentrations, with expectations shaped by whether their values fall below or exceed the proposed boundary. The predicted biological status is subsequently compared to the observed one. If these match, there is a true prediction; otherwise, there is a false prediction. Predictions for good and not good status are referred to as positive and negative, respectively. Therefore, there are four types of predictions: true positive—where both biota and nutrients are good; true negative—where both biota and nutrients are not good; false positive—where nutrients falsely predict the biota to be at a good status; and false negative—where nutrients falsely predict the biota to be at a not good status.

A number of measures of classification accuracy accompany the confusion matrix, as presented in detail by Phillips et al. [4,24]. The classification measures mentioned in this study are explained below. Correct Classification Rate (CCR) and Misclassification Rate (Misclass) are the proportions of correct and incorrect classifications, respectively. The latter can be divided into false positives and negatives. False Positive (conditional) rate or commission (Comm) is the proportion of false positives in all true negatives. False negative (conditional) rate or omission (Omis) is the proportion of false negatives in all true positives. Both are measures of "badness of fit". Prevalence (Prev) refers to the proportion of good biota in the dataset, highlighting potential imbalances that may affect model outcomes. Kappa (kp) serves as a measure of overall classification accuracy, and should exceed 0.32 to suggest model adequacy, while a minimum of 0.21 indicates fair agreement between the binary classifications. All measures should be evaluated collectively to ensure a comprehensive assessment.

2.5. Selection of the Most Appropriate Approach

The most suitable approach in order to determine TP boundaries was selected in accordance with the recommendations outlined by Phillips et al. [4,24]. The methods BLM and/or RMA were applied to the datasets. For each predicted boundary value, a confusion matrix was constructed in order to evaluate classification performance. Results were assessed using the specified measures, and finally, the most appropriate boundary values were selected, considering the minimisation of commission errors without significantly increasing omission errors (i.e., Omis $\leq 2 \times$ Comm), and the maximization of the overall classification accuracy (i.e., maximum kp).

3. Results

The analyzed datasets contained results of TP concentrations and phytoplankton EQRs from five lake types of the Greek national monitoring network, i.e., GR-DNL, GR-SNL, GR-VSNL, GR-DR, and GR-SR (Figure 1). In order to investigate the relationship between the two variables, scatterplots and boxplots were employed as part of exploratory data analysis (Figure 2). The TP values were log10-transformed to improve data distribution. Each lake type contained a sufficient number of records (i.e., >10), as shown in Table 1, to support meaningful comparisons.

Table 1. Summary of the predicted boundary values of TP concentrations (μg/L) at the threshold of good-moderate (GM) status, with lower (GML) and upper (GMU) values per lake type, obtained by linear (RMA) and categorical (BLM) methods, along with model performance and classification accuracy measures. N: Number of records.

Lake Type	Method	N	GM	GML	GMU	AUC	R^2 (Pseudo-r^2)	Prob	Prev	CCR	Misclass	Omis	Comm	kp
GR-DNL	BLM	24	39	30	50	0.97	(0.84)	0.43	0.58	0.92	0.08	0.07	0.10	0.83
GR-SNL	BLM	51	42	28	73	0.77	(0.27)	0.28	0.26	0.75	0.25	0.23	0.26	0.43
GR-VSNL	BLM	25	76	51	266	0.82	(0.36)	0.26	0.28	0.68	0.32	0.29	0.28	0.39
GR-DR	BLM	52	32	18	49	0.86	(0.51)	0.89	0.89	0.85	0.15	0.15	0.17	0.48
GR-SR	RMA	27	50	31	65	NA	0.61	NA	0.41	0.85	0.15	0.18	0.13	0.69

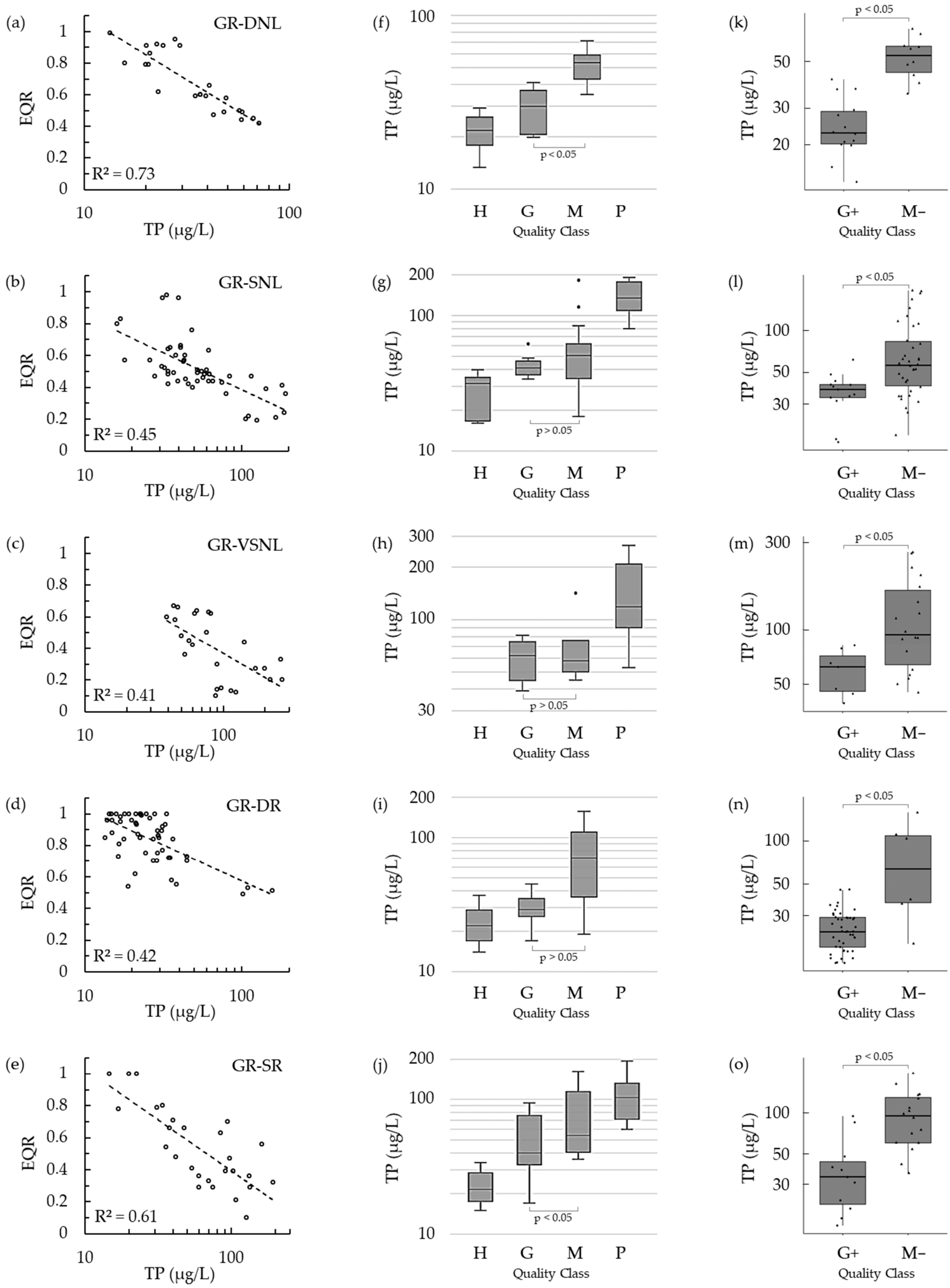

Figure 2. Scatterplots showing the relationship between phytoplankton EQR and TP concentration (µg/L) (**a**–**e**), and boxplots showing the range of TP (µg/L) across ecological quality classes High (H), Good (G), Moderate (M), Poor (P) (**f**–**j**) and binary quality classes Good or better (G+) and Moderate or worse (M−) (**k**–**o**) in lake types: GR-DNL, GR-SNL, GR-VSNL, GR-DR and GR-SR. The *p*-values represent the significance of the Wilcoxon test results.

The scatterplots from the datasets across all lake types (Figure 2a–e) revealed a relationship between phytoplankton EQR and TP concentration. A sufficiently strong linear relationship was identified in lake types GR-DNL and GR-SR ($R^2 > 0.6$), while a moderate correlation was observed in lake types GR-SNL, GR-VSNL, and GR-DR ($R^2 = 0.4$). Data were distributed into three or four ecological quality classes (High, Good, Moderate/Poor) in the datasets of four lake types (Figure 2f,g,i,j), but this was not the case for GR-VSNL, where the high class was absent (Figure 2h). Around the boundary of interest, which was the GM boundary in this study, a balanced distribution of data was evident only in the dataset corresponding to the GR-SR lake type, as the boxplots of Figure 2 revealed. Moreover, the assumptions underlying linear regression were satisfied only for the same lake type. For the lake types GR-SNL, GR-VSNL, and GR-DR, there was a great overlap between the good and moderate classes with no significant difference between them ($p > 0.05$, Figure 2g–i). However, when classes were merged into G+ and M− classes, a significant difference between the two appeared ($p < 0.05$, Figure 2l–n), justifying the use of BLM in these cases. For GR-DNL, there was a significant difference between the good and moderate classes ($p < 0.05$, Figure 2f), but the assumptions of linear regression were not met. Nevertheless, the G+ and M− classes differed at a significant level ($p < 0.05$, Figure 2k), supporting the use of BLM in this case, as well. Overall, preliminary data exploration suggested that the linear regression method (RMA) may not be suitable for deriving TP boundaries for the four lake types GR-DNL, GR-SNL, GR-VSNL, and GR-DR, using the available data. For the GR-SR lake type, good and moderate classes differed significantly ($p < 0.05$), as shown on the relevant boxplot (Figure 2j), as well as good or better and moderate or worse classes ($p < 0.05$, Figure 2o). Consequently, both RMA and BLM appeared to be suitable methods for establishing TP boundaries for this type.

Table 1 summarizes the proposed GM boundary values for TP concentration (μg/L) for all lake types, determined following the guidance of Phillips et al. [4,24]. Regression models offer the most reliable estimation of the average response of water bodies within a given dataset. However, individual water bodies may deviate from this estimation due to the uncertainty of both data and the model used [32]. Therefore, the GM boundary value is supplemented by a range defined by lower (GML) and upper (GMU) values, as presented in Table 1. The measures of model performance (i.e., R^2 for RMA, AUC, and pseudo-r^2 for BLM), along with the key classification accuracy measures of the confusion matrix, which was developed for each proposed boundary value, are also included in Table 1. The comparative relationship between commission and omission values is presented in Figure 3. A boundary value may be considered sufficiently precautionary, i.e., neither excessively conservative nor inadequately protective, when the ratio between the two errors lies within the designated green zone, where the probability of commission error remains acceptably low while omission has not markedly escalated [4,24].

To begin with, the GR-DNL dataset exhibited quite a balanced distribution (Prev = 0.58). The GM boundary for TP concentration was 39 μg/L (range: 30–50 μg/L), as shown in Table 1. BLM was fitted to this dataset with exceptional model performance (AUC = 0.97, pseudo-r^2 = 0.84). The threshold probability of being in good or better status was set to 43%. The overall success of classification was high, as indicated by CCR, Misclass, and kp values (0.92, 0.08, and 0.83, respectively), reflecting that the majority of classifications had been carried out correctly. Moreover, there was a balance between the misclassification rates (Omis = 0.07 and Comm = 0.10), leading to a precautionary boundary value, as illustrated in Figure 3. As a result, TP appeared to be a good indicator of phytoplankton status for the lake type GR-DNL.

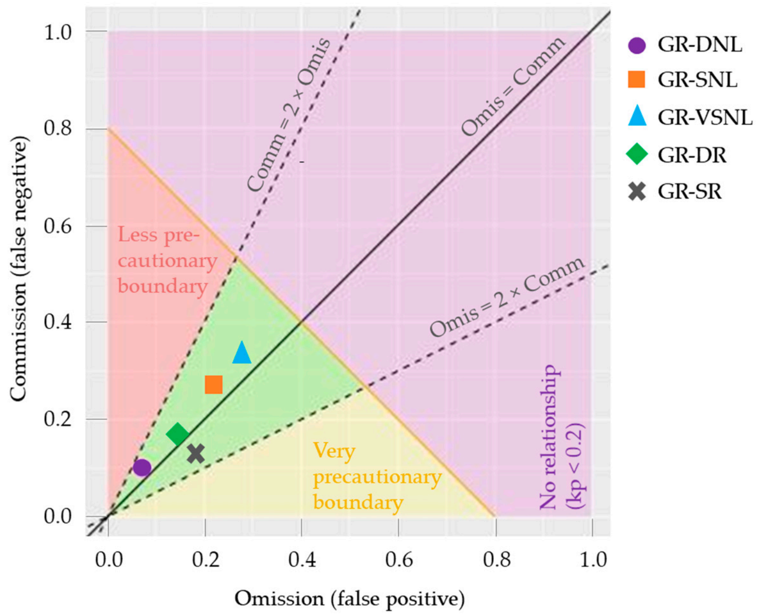

Figure 3. Comparison between Commission and Omission for lake types: GR-DNL, GR-SNL, GR-VSNL, GR-DR, and GR-SR.

Proceeding with the dataset of the lake type GR-SNL, the GM boundary for TP concentration was 42 µg/L (range: 28–73 µg/L), as presented in Table 1. BLM was fitted to this dataset as well, with acceptable model performance (AUC = 0.77, pseudo-r^2 = 0.27). This dataset was quite unbalanced (Prev = 0.26), with moderate or worse records dominating. Due to the very low prevalence value, omission and commission tend to become more sensitive and potentially less reliable, as data distribution influences the probability threshold and the classification accuracy [4]. Therefore, it was examined whether a more appropriate GM boundary could be derived using the model with the best overall classification, i.e., maximum kappa. The model of maximizing kappa generated the same boundary value as the model of balancing omission and commission, with identical classification measures. Consequently, the most appropriate boundary value based on the available data appeared to be the previously mentioned, accompanied by a threshold probability of 28%. The overall classification success was moderate as the CCR, Misclass, and kp values indicated (0.75, 0.25, and 0.43, respectively). The misclassification rate was equally divided into false positives (Comm = 0.26) and false negatives (Omis = 0.23), resulting in a precautionary boundary (Figure 3), despite the relatively low accuracy.

For the GR-VSNL lake type, BLM was applied for the determination of the boundary value for TP. The one suggested by the toolkit was generated by the balance between omission and commission (0.29 and 0.28, respectively), which was 76 µg/L (range: 51–266 µg/L), as shown in Table 1. The performance of the model was very good (AUC = 0.82, pseudo-r^2 = 0.36), while the overall classification accuracy was moderate (CCR = 0.68, Misclass = 0.32) with fair agreement between classifications (kp = 0.39). The threshold probability of being in good or better status was set to 26%. The dataset exhibited a notable imbalance (Prev = 0.28) with the moderate or worse class being the dominant, implying the possibility of overestimation of the predicted boundary value. Therefore, the model of maximum kappa was considered additionally, in order to estimate if its predicted GM boundary value was more appropriate in this case. The value obtained by this model used a lower threshold probability (18%) and was higher (TP = 88 µg/L, range: 63–266 µg/L), with better overall classification accuracy (kp = 0.53, CCR = 0.72, Misclass = 0.28). However, false positives (Comm = 0.33) greatly exceeded false negatives (Omis = 0.00), demonstrating that such a value may not be precautionary enough, probably failing to protect impacted sites. Thus, in this instance, the initially proposed GM boundary value for TP (76 μg/L) appeared to be the most suitable, as it was more protective (Figure 3), although at the expense of overall accuracy. The broad range of the predicted boundary value reflects considerable classification uncertainty. Enhancing the dataset may lead to a significant reduction in misclassification rate, ultimately contributing to improved model accuracy and reliability.

For the lake type GR-DR, the dataset was also unbalanced, with a predominance of the records classified as good or better (Prev = 0.89), implying a possible underestimation of the boundary value. In addition to that, there was a large number of truncated EQR values equal to 1 (Figure 2e). BLM was fitted to this dataset with very good model performance (AUC = 0.86, pseudo-r^2 = 0.51). The boundary value for TP suggested by the toolkit corresponded to the one derived from the balanced omission and commission (0.15 and 0.17, respectively), which was 32 μg/L (range: 18–49 μg/L), as presented in Table 1. The threshold probability was set to 89%. The overall classification accuracy was adequate (CCR = 0.85, Misclass = 0.15, kp = 0.48). As mentioned previously, the occurrence of extreme prevalence, as is also the case here, requires caution regarding the proposed boundary value. For that, the boundary value generated by the model with the best overall classification was evaluated, as well. Selecting the maximum kappa model (kp = 0.67) led to a lower probability threshold (Prob = 85%) and a higher boundary value for TP, which was equal to 35 μg/L (range: 23–64 μg/L). The misclassification rate decreased (Miscl = 0.08), along with the false negatives (Omis = 0.07), while no change occurred at the false positives (Comm = 0.17). The initially proposed boundary seems sufficiently precautionary (Figure 3) in contrast to the alternative, making it potentially more appropriate, albeit with some compromise in accuracy. Nonetheless, caution is warranted due to signs of bias arising from the highly imbalanced distribution of binary classifications (Prev = 0.89) and the substantial number of truncated EQR values present in the dataset. An expanded dataset in future revisions may enhance the reliability of the estimation.

The GR-SR dataset was balanced (Prev = 0.41), encompassing the full spectrum of disturbance in a relatively uniform way. It showed no signs of heteroscedasticity, class overlap, or truncated EQR values, suggesting it was likely the only case where RMA could be reliably applied to determine TP boundary using the available data. The GM boundary obtained for TP, assuming a balance between omission and commission (0.18 and 0.13, respectively), was 50 μg/L (range: 31–65 μg/L), considered as precautionary enough (Figure 3). This resulted in a misclassification rate of 0.15. The model performance was adequate (R^2 = 0.61), and the kappa value showed substantial agreement (kp = 0.69). For comparative purposes, BLM was applied as well to this dataset, with very good model performance (AUC = 0.89, pseudo-r^2 = 0.56). The proposed boundary value for TP using a threshold probability of 43% was equal to 54 μg/L (range: 33–88 μg/L). The values of omission and commission were similar (0.18 and 0.19, respectively), and the kappa value indicated a substantial model performance (kp = 0.62). However, the confusion matrix revealed a higher misclassification rate (Misclass = 0.19), implying increased uncertainty, further reflected by the wider range of this proposed boundary. BLM yielded a boundary value comparable to that of RMA, but due to higher overall classification accuracy, lower misclassification rate, and commission value, the RMA-derived boundary value was selected as more appropriate for this case, as shown in Table 1.

4. Discussion

In this study, TP boundary values were established for natural lakes and reservoirs of the Greek national monitoring network, grouped into five types. The BLM was used for deriving boundaries of TP in four lake types (GR-DNL, GR-SNL, GR-VSNL, and GR-DR), and RMA was used in the fifth type (GR-SR). The water bodies of each type are likely to achieve good ecological status, below the predicted GM boundary value. The GM boundaries for TP, based on available data, were as follows: 39 and 32 μg/L for GR-DNL and GR-DR types, 42 and 50 μg/L for GR-SNL and GR-SR types, and 76 μg/L for GR-VSNL. The lowest values were derived using data from deep water bodies (GR-DNL and GR-DR), intermediate values using data from shallow water bodies (GR-SNL and GR-SR), and the highest value using data from very shallow water bodies (GR-VSNL). In general, TP boundaries were lower in deep lakes than in shallow lakes, confirming the influence of depth, as reported by Cardoso et al. [16] and Zhou et al. [34]. Lake depth relates as well to the ecoregion and land use of lake ecosystems. Shallow lakes lie, in general, in lowland regions and are more exposed to agriculture and urban development, leading to extensive nutrient input. On the contrary, deep lakes are often found in upland regions, where human disturbance is low or absent. Lake productivity is largely determined by human activity, which is more intense in lowland fertile regions, where many shallow lakes are encountered, than in mountains and highlands [34].

Lake ecosystems are susceptible to eutrophication, since the majority act as sinks accumulating external nutrient loads. Deep lakes generally exhibit greater dilution capacity and seasonal thermal stratification, which restricts nutrient availability to phytoplankton in surface waters by trapping nutrients below the photic zone. In contrast, shallow and very shallow lakes, due to their smaller volume and lower capacity for nutrient input dilution, seem to be more sensitive to anthropogenic disturbances. Additionally, strong interactions between water and sediment are typically observed in shallow lakes, where sediment resuspension occurs more frequently. As a result, internal nutrient loading can be considered a significant nutrient input, thereby contributing to higher productivity [35–37]. Numerous studies have identified internal phosphorus release from sediments as a key driver of eutrophication, often exerting a stronger and more persistent effect than external inputs from the catchment. Internal loading can sustain elevated nutrient concentrations even under conditions of reduced external supply, thereby prolonging eutrophic states and hindering management efforts [38,39]. This highlights the need to account for sediment–water interactions when assessing nutrient dynamics and setting ecological boundaries.

According to Kagalou et al. [23], the proposed boundary for TP concentrations supporting good ecological status in Greek deep and shallow natural lakes was 32 and 41 μg/L, respectively. These values are in line, although lower, with the predicted values for types GR-DNL and GR-SNL, presented in our study. Phillips et al. [4] used phytoplankton and TP concentration data from very shallow lakes located in Europe. The suggested GM boundary value range for TP was 60–83 μg/L. Our approach for the type GR-VSNL falls within this range. Poikane et al. [1] in their study about setting nutrient thresholds in lakes from several European countries, used, among others, phytoplankton data from reservoirs found in the Mediterranean region, characterized as deep, large calcareous (type LM8), to which our lake type of GR-DR could be compared [27,28]. Their predicted TP boundary value for achieving good ecological status was 43 μg/L, lying over the boundary proposed for GR-DR in our study. This suggests that our assessment adopts a more precautionary approach compared to the broader nutrient limits presented in their analysis. Although European standards provide a general reference point, regional ecosystems often necessitate more stringent standards to safeguard resilience under region-specific environmental conditions [4]. Unfortunately, a comparative boundary value for the type GR-SR could not be located. Overall, the proposed boundaries of this study appear to align well with those established in other European countries, as outlined previously, supporting their relevance and applicability.

This study is subject to certain limitations that merit consideration in the interpretation of its outcomes. Long-term datasets were used, derived from samplings that capture the spatial and temporal variability of water bodies within each lake type. However, while these datasets offered a solid foundation for analysis, data distribution was not balanced in most cases, with a possible impact on the predicted boundary value (i.e., over- or underestimation). Additionally, the predicted nutrient boundary values were derived based on the current conditions and may require future revision, considering the dynamic nature of aquatic ecosystems [40]. Extreme climatic events (e.g., droughts, floods) are expected to become more frequent in the future, intensifying their impact on nutrient load in aquatic ecosystems [41]. Moreover, our datasets continue to expand over time, incorporating more lake years, but also, more water bodies per lake type due to the extension of the national monitoring network. This expansion is expected to improve the robustness of the statistical analyses, reduce the influence of isolated extreme events or atypical water bodies, and enhance the reliability and representativeness of the nutrient boundary values derived. Furthermore, other biological quality elements, such as macrophytes, along with additional environmental parameters (e.g., chlorophyll-a and water transparency expressed as Secchi disk depth), could probably provide useful data leading to further analyses and results.

The co-limitation of phosphorus and nitrogen should also be considered in the future. Phytoplankton is known to be particularly sensitive to phosphorus, but the significant influence of nitrogen has to be acknowledged, as well [42,43]. Moreover, the co-limitation of these two nutrients has been observed in many Greek lakes [44]. In this study, however, it could not be taken into consideration due to data unavailability. It remains, nevertheless, a priority for future investigation, as under projected climate scenarios, nitrogen cycling and eutrophication processes in Mediterranean lakes are expected to be significantly affected [45].

The determination of boundary values is influenced by the selected BQEs, the performance of the applied method, and expert evaluation concerning the ecological validity and acceptability of the proposed limits [23]. Careful consideration of local variation and lake typology is required. Boundaries should reflect both the regional background and the ecological responses of water bodies. In countries of Southern Europe, such as Greece, higher TP levels may be considered acceptable due to warmer climates, longer growing seasons, and different lake typologies [19]. Such factors can influence nutrient cycling and biological productivity [40], rendering direct comparisons with boundaries from cooler climates potentially inaccurate. Consequently, adjusting boundary values to reflect regional conditions is essential for achieving accurate classification and ensuring that water management strategies are ecologically appropriate and locally relevant. Additionally, as climate change increasingly affects hydrological regimes, thermal conditions, and biological responses, future boundary definitions may need to become more stringent to safeguard ecological integrity. This aligns with the conceptual approaches proposed by Free et al. [40], which emphasize the need to reassess reference conditions and classification boundaries in response to climate–driven shifts in aquatic ecosystems. Greece is regarded as one of the European regions most vulnerable to climate change, with rising temperatures and declining rainfall exacerbating existing water scarcity. Recent studies have documented warming and altered precipitation trends in Greek lakes [46], and projected alterations in hydroperiods of northern wetlands [47], underscoring the need for adaptive boundary setting.

5. Conclusions

In conclusion, the establishment of nutrient concentration boundary values within the framework of achieving WFD goals can be performed successfully using the pressure–

response relationship between nutrients and biological communities. In lakes, phytoplankton is considered a reliable biological quality element for the assessment of eutrophication due to its fast response to changes in nutrient concentrations in the water column, and it clearly participates in a strong relationship with phosphorus. This structured the basis of our study, which drew upon a complete dataset and, with the application of the Shiny toolkit, led to realistic GM boundary values for TP concentration. Despite its robustness, the proposed methodology is constrained by imbalances in data distribution, sensitivity to extremes and atypical water bodies, and reliance on present ecological conditions. Consequently, the derived nutrient boundaries could be further refined as monitoring datasets expand and climatic variability intensifies. Furthermore, the toolkit could be effectively applied to set boundaries for both water transparency and nitrogen concentrations, considering the condition of nutrient co-limitation observed in many Greek lakes. Overall, this toolkit provides a transparent and reproducible framework for defining nutrient boundaries in lakes. Such boundaries can support water authorities in prioritizing interventions, evaluating compliance with ecological objectives, and supporting adaptive management. In this way, it strengthens evidence—based policy and enhances the effectiveness of long—term monitoring programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w17233349/s1, Table S1: Summary of geometric parameters of the studied natural lakes and reservoirs (R.), based on data collected during the sampling period [17,25,48–51].

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