



THE EFFECT OF HYDRAULIC RESIDENCE TIME ON CHLOROPHYLL A RESPONSE TO TOTAL PHOSPHORUS IN DANISH LAKES

Scientific Report from DCE - Danish Centre for Environment and Energy

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Abstract:	The aim of this study was to assess how hydraulic residence time influences the eutrophication response (response of chlorophyll <i>a</i> (Chl _a) to total phosphorus (TP)) across different Danish lake types. Four residence time categories were defined: very short (≤ 4.5 days), short (4.5 – 38 days), medium (39 – 222 days) and long (> 222 days). The analyses showed that these categories significantly affected the Chl _a response to TP, with lakes having shorter residence times generally exhibiting a stronger response in Chl _a with increasing TP concentrations, compared to lakes with longer residence times. In addition, the Chl _a -TP relationship varied among lake types within the different residence time categories. Based on the results, target TP concentrations were estimated for different Chl _a class boundary. The findings highlight the importance of considering residence time when setting nutrient targets and assessing ecological status under the Water Framework Directive.
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Preface

This report examines how lake hydraulic residence time influences eutrophication processes across different lake types in Denmark, focusing on how chlorophyll *a* (Chl_a) concentrations respond to increasing total phosphorus (TP) levels. The report was prepared by Aarhus University at the request of the The Danish Agency for Green Transition and Aquatic Environment (Styrelsen for Grøn Arealomlægning og Vandmiljø, SGAV). SGAV supplied the lake residence time dataset and also had the opportunity to comment on a draft report.

Sammenfatning

Den hydrauliske opholdstid i søer påvirker potentielt deres respons på eutrofiering, specielt i forhold til fytoplanktondynamik og næringsstofretention. Selvom den hydrologisk opholdstid er en af vandrammedirektivets hydro-morfologiske støtteparametre, er det imidlertid stadigvæk ukendt, i hvilken grad opholdstid påvirker effekten af næringsstoffer, og hvordan dette eventuelt påvirker opfyldelsen af de danske miljømål.

Formålet med denne rapport var at undersøge, hvordan søers respons på eutrofiering påvirkes af den hydrauliske opholdstid, når der er taget højde for opholdstidskategorier og søtyper. Til dette formål blev opholdstidskategorier defineret for søerne, der indgik i projektet, hvorefter forholdet mellem totalfosfor (TP) og klorofyl *a* (Chla, indikator for fytoplanktonbiomasse) blev analyseret for både opholdstidskategori og søtype. Dernæst blev modelestimer brugt til at bestemme målkoncentrationer af TP for de gældende grænseværdier for klorofyltilstandsklasser (BEK nr. 792, 2023). Til analyserne anvendtes NOVANA-data omfattende data fra i alt 397 søer. Ved anvendelse af data fra flere prøvetagningsår resulterede dette i 3082 sø-år i alt.

Analysen identificerede fire relevante opholdstidskategorier: meget kort ($\leq 4,5$ dage), kort (4,5-38 dage), medium (39-222 dage) og lang (> 222 dage). Opholdstidskategorierne havde en statistisk signifikant effekt på forholdet mellem Chla og TP, som desuden var påvirket af søtype. Resultaterne viste et komplekst mønster. Generelt havde søer med kortere opholdstid højere Chla koncentrationer ved høje TP-koncentrationer end søerne med længere opholdstid. Denne følsomhed varierede dog mellem søtyperne.

Søtype 11 og 13 havde forskellig respons i forholdet mellem Chla og TP sammenlignet med de mere almindelige søtyper 9 og 10, og disse forskelle varierede mellem opholdstidskategorier. I søtype 9 og 10, var responsen stærkere (hurtigere stigning i Chla ved øgede TP-koncentrationer) med stigende TP når opholdstiden var kort. Til sammenligning, havde søtype 11 svagere (en langsommere stigning i Chla ved stigende TP-koncentrationer) Chla-TP-forhold ved meget kort opholdstid og stærkere responser ved kort, medium og lang opholdstid. Søtype 13 havde et mere komplekst mønster, med lavere følsomhed for Chla til TP ved kort opholdstid, men med meget høj følsomhed ved meget kort opholdstid.

Baseret på resultaterne blev modelberegninger anvendt til at bestemme målkoncentrationerne for TP baseret på de gældende klorofylgrænseværdier for danske søer (BEK nr. 792, 2023). Samlet set var de estimerede TP-mål for det meste lavere end de eksisterende grænseværdier i BEK nr. 792 (2023), hvor opholdstiden ikke er taget i betragtning. På trods af den store variation i datasættet, som i visse tilfælde medførte brede konfidensintervaller for de estimerede TP målkoncentrationer, anses de estimerede målkoncentrationer for TP at være pålidelige og kan anvendes som grundlag for videreudvikling og implementering.

Resultaterne af dette studie understøtter relevansen af at inddrage opholdstid ved fastsættelse af næringsstofmål og vurdering af økologisk tilstand i henhold til vandrammedirektivet. I fremtiden anbefales det, at opholdstidens betydning for andre økologiske indikatorer undersøges. Dette bør ske med

udgangspunkt i større datatilgængelighed, specielt i mindre almindelige søtyper. Sammenlignelige studier med andre europæiske lande vil også kunne hjælpe med at fastsætte, om disse målkoncentrationer er passende for bredere implementering.

Summary

The hydraulic residence time of lakes may affect their response to eutrophication, particularly regarding phytoplankton dynamics and nutrient retention. Although hydraulic residence time (RT) is one of the hydromorphological characteristics required under the EU Water Framework Directive, its role in shaping the response of Danish lakes to nutrient pollution – thus their ability to meet ecological quality requirements – remains unknown.

The main purpose of this investigation was to examine how hydraulic RT influences eutrophication responses in Danish lakes, considering relevant RT categories and lake typology. To achieve this, we first defined the relevant RT categories for the study lakes, then analysed the relationship between total phosphorus (TP) and chlorophyll *a* (Chla), used as an indicator of phytoplankton biomass, across both RT categories and lake types. Finally, model results were used to estimate TP target concentrations corresponding to Chla-defined ecological quality classes (BEK nr. 792, 2023). The analyses were based on NOVANA data, comprising 397 lakes and a total of 3082 lake-years from multiple sampling years.

The analysis identified four relevant RT categories: very short (≤ 4.5 days), short (4.5–38 days), medium (39–222 days) and long (more than 222 days). These categories significantly influenced the phytoplankton Chla response to TP, which was also affected by lake typology. The results revealed a complex pattern. In general, lakes with shorter RT tend to show a notably higher increase in Chla with increasing TP compared to longer RT lakes. However, the sensitivity of Chla to TP also varied among the lake types examined.

Lake types 11 and 13 showed different Chla to TP responses compared with the more common lake types 9 and 10, and these differences varied across RT categories. In lake types 9 and 10, Chla responded more strongly to increasing TP at shorter RT (faster increase in Chla with increasing TP concentrations). In contrast, lake type 11 showed the opposite trend, with a weaker Chla to TP response (slower increase in Chla with increasing TP concentrations) at very short RT and stronger responses at short, medium and long RT. Lake type 13 exhibited a more complex pattern, with lower sensitivity of Chla to TP in the short RT category but a steeper response at very short RT.

Based on these results, model outputs were used to back-calculate TP target concentrations for each Chla boundary of ecological quality for Danish lakes (BEK nr. 792, 2023) by lake type and RT category. Overall, these estimated TP targets, which account for residence time, were mostly lower than the standards set in BEK nr. 792 (2023) where residence time is not considered. Despite the large variation in the data, which in some cases resulted in broad confidence intervals for the estimated TP targets, these TP estimates are considered reliable and can be used as a basis for further evaluation and implementation.

The results of this study emphasise the relevance of considering RT when setting nutrient targets and evaluating ecological status under the Water Framework Directive.

Future research should examine how residence time influences other ecological indicators. This should be based on a higher data availability, especially in less common lake types. Comparative studies with other EU countries would also help establish whether these target values are suitable for broader implementation.

1 Introduction

1.1 Background

The rate at which water flows through a lake, and the average time it takes to flush the whole lake volume of water with new water, known as hydraulic residence time ($RT = \text{total water loading} / \text{lake volume}$), has an important influence on lake functioning (Søndergaard et al., 2018). RT affects nutrient retention, leaching and biological activity and therefore determines how lakes respond to eutrophication, particularly with respect to phytoplankton dynamics.

Classical mass-balance models indicate that the relative phosphorus retention increases with longer RT , thus providing a better opportunity for phytoplankton biomass to accumulate. In contrast, short RT may reduce average algal biomass by limiting colonization time and increasing wash-out of phytoplankton (Søballe and Kimmel, 1987). Therefore, lakes with short RT are often expected to tolerate higher nutrient concentrations, as rapid flushing should reduce nutrient retention and limit phytoplankton growth. Nevertheless, other studies also highlighted the classical mass-balance can be more complicated, resulting in an opposite trend, with increased phytoplankton growth at shorter RT , especially if the in-lake conditions are favourable for phytoplankton growth and if there is high nutrient availability (e.g. Søballe and Kimmel 1987; Kalff 2002).

Modelling studies by Jones and Elliott (2007) and Elliott et al. (2009) also showed that the effect of RT on $Chl a$ depends on the dominant nutrient source. When phosphorus inputs come mainly from point sources, phosphorus amount is largely independent of the water volume entering and leaving a lake. In this case, increasing flushing (short RT) leads to higher losses of nutrient and phytoplankton, resulting in lower $Chl a$ concentrations. In contrast, when diffuse sources dominate, phosphorus inputs can increase with water volume, partly compensating for losses due to flushing and sedimentation. If this is the case, shorter RT can lead to higher $Chl a$ because phosphorus inputs can be higher than losses (Elliott et al. 2009).

Characterising lake hydromorphology, including RT , is essential for understanding ecosystem processes and is required under EU Water Framework Directive (WFD). However, relatively few methods are available for assessing RT , highlighting the challenges of including this parameter in assessments (Argillier et al., 2023).

The current project was initiated because many of the lakes included in the 2021-2027 River Basin Management Plan³ (VP3) have short (<0.1 years) or even very short (<0.01 years) RT . According to the condition assessments in VP3, most of these lakes do not meet the targets for achieving good ecological state.

In lakes with short RT , rapid flushing can limit the ability of phytoplankton to develop due to accelerated loss. Consequently, in such systems, higher nutrient loads may have only a limited effect on phytoplankton growth and, therefore, on chlorophyll a ($Chl a$) concentrations. If lakes with short RT can tolerate higher nutrient concentrations, it becomes necessary to account for RT when assessing the ecological status of different lake types. This is particularly relevant for calculating the necessary phosphorus reductions in lakes for the forthcoming River Basin Management Plans 2027-2033 (VP4).

1.2 Purpose

The purpose of this study is to examine whether and to which extent RT influences the sensitivity of Danish lakes to eutrophication, particularly whether lakes with very short or short RT respond differently from lakes with longer RT. Specifically, the study focused on how Chl_a in lake water, a general indicator of phytoplankton biomass associated to eutrophication, responds to total phosphorus (TP) concentrations in water in relation to RT, while accounting for potential differences across lake types. This study also aimed to estimate TP target concentrations for each Chl_a boundary of ecological quality for Danish lakes (BEK nr. 792, 2023), for each lake type and for each RT category.

2 Methods

2.1 Data retrieval

RT data were made available by SGAV for 448 lakes of the 985 lakes included in VP3 across Denmark, comprising 10 different lake types (1, 5, 6, 9, 10, 11, 12, 13, 14, 15), as well as 24 lakes without lake type classification. Lake type followed the typology classification for Danish lakes (Søndergaard et al., 2018). Yearly RT values were used due to relatively similar monthly variations (Appendix B, Figure B1).

Water chemistry data were collected as part of the Danish national monitoring program for lakes, NOVANA. The monitoring data, stored in the VanDa database (<https://vanda.miljoportal.dk/>), were retrieved from the database Overfladevanddatabasen (ODA - ODA.dk). For lakes with RT data, TP and Chla concentrations were calculated as summer averages (May-September) for each sampling year, to be used in further analysis. In total, 418 lakes from 10 lake types were included in the RT dataset.

For this report, and due to analytical requirements, only lake types with more than seven records (types 9, 10, 11 and 13) in the RT dataset and lakes present in both the RT and the water chemistry datasets were included in the analysis, resulting in a final dataset of 397 lakes (3082 lake-years). Additionally, lake water chemistry data from several years (1989-2023, where available) were linked to the corresponding RT for each lake. This means that each lake had one RT value but several years of water chemistry observations, resulting in a one-to-many relationship between RT and chemistry data.

The lake types used in this report were:

- Lake type 9: high alkalinity, shallow, low colour, low salinity
- Lake type 10: high alkalinity, deep, low colour, low salinity
- Lake type 11: high alkalinity, shallow, low colour, high salinity
- Lake type 13: high alkalinity, shallow, high colour, low salinity

2.2 Data analyses

The data analysis consisted of four main steps:

First, the data structure was examined in terms of frequency, distribution and variation in RT for all 418 lakes and across lake types. This step was carried out to identify the main data structure, variability and potential analytical constraints, such as limited amount of data for certain lake types or specific RT categories by lake type.

Second, to determine the optimal number of RT categories, 397 lakes were grouped based on similarities in RT using *k*-means clustering method (e.g. Carvalho et al., 2016). This method iteratively assigns each data point to the

nearest cluster centroid until the centroids stabilise or a maximum number of iterations is reached. The optimal number of clusters was determined using a combination of the Silhouette Score (Rousseeuw, 1987) and the Davies-Bouldin Index (Davies and Bouldin, 1979).

Third, to analyse how RT and lake typology influences Chla responses to TP, linear mixed-effects models (LMMs) were performed using the lme4 package (Bates et al., 2015), in general for all lake types and accounting for the effects of lake typology (in total 3082 lake-years). These models make it possible to account for repeated observations from the same lakes. Model significance was evaluated using full-reduced model comparisons, and models were compared based on likelihood ratio test (LRT) between nested models using their log-likelihoods. Pairwise comparisons of Estimated Marginal Trends were conducted for significant results to determine specific groups differing significantly from each other, using the emmeans package (Lenth et al., 2025). This method estimates and compares adjusted slopes (trends) for each factor level while accounting the other variables in the model. Chla and TP variables were log₁₀ transformed, and model residuals were checked for normality (normal probability plots) and homogeneity of variance (residuals versus fitted values).

Finally, using the LMM model results, the estimated targets of TP values were back-calculated for each Chla classification value of ecological class, according to the levels defined for Danish lakes under the EU Water Framework Directive (WFD) (BEK nr. 792, 2023). These values were calculated as the predicted means and confidence intervals for each lake type and RT category. Uncertainty (95% confidence intervals) was estimated via parametric bootstrapping of the fixed effects using the lme4::bootMer() function. Random effects were not included in the predictions. Percent uncertainty was then calculated as the width of the confidence interval divided by twice the estimated target TP value (i.e. the fixed-effect estimate).

Furthermore, all the aforementioned analyses were also conducted using summer mean RT data to account for potential seasonal differences in RT. However, this did not show any difference compared to the analysis based on yearly RT. The results of the analysis based on summer mean RT are given in Appendix B.

All statistical analyses were performed using R Statistical Software (v4.5.1; R Core Team, 2025).

3 Results

3.1 Distribution of residence time

Hydraulic residence time (RT) showed considerable variation across the Danish lake types (Figure 3.1). Moreover, the distribution of calculated RT was uneven, some lake types having far more lakes with calculated RT than others (Table 3.1).

Many of the examined lakes ranked in the lowest range of the RT distribution (Figure 3.1a). Of the 418 lakes considered, almost 50% had an RT shorter than 0.15 year (< 55 days), while approximately 9% had an RT longer than 2 years (Figure 3.1a). Among the lakes in the low RT range (< 0.15 year), 26% had an RT below < 0.015 year (< 5.5 days) (Figure 3.1b).

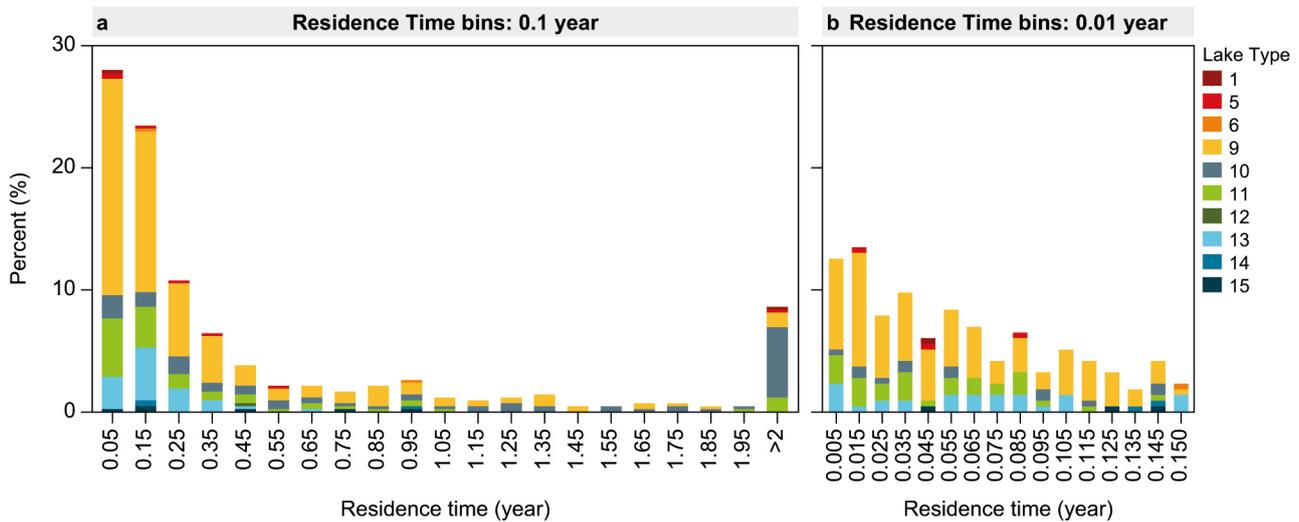
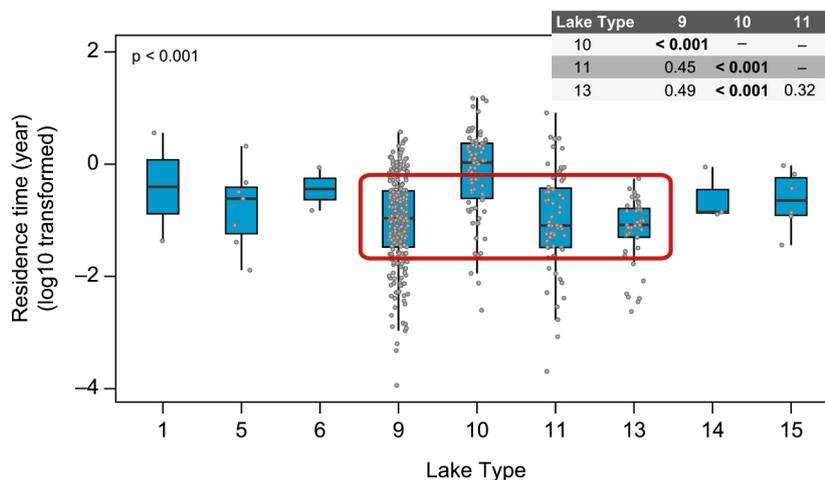


Figure 3.1. Distribution of RT of Danish lakes. **a:** All 418 lakes with data divided into 0.1-year intervals (last column shows the proportion of lakes with RT > 2 years). **b:** Distribution of RT among 215 lakes, where the RT is less than 0.15 year (divided into 0.01-year intervals, corresponding to 3.7 days).

3.2 Residence time across lake types

A one-way ANOVA followed by pairwise *t*-tests showed significant differences in RT across lake types ($p < 0.001$). The main significant differences occurred between lake type 10 (deep lakes) and the shallow lake types 9, 11 and 13 (Figure 3.2), deeper lakes having significantly longer RT. Lake types with less than eight data points were not included in the analysis.

Figure 3.2. RT across lake types. Only lake types 9, 10, 11 and 13 were considered for one-way ANOVA and pairwise t-test (indicated by red rectangle). Pairwise t-test results are shown in the table; bold font indicating significant differences. Lake type 12 is not shown, because it only had one data point.



3.3 Residence time categorisation

K-means clustering analysis, in combination with the Elbow method, Silhouette Score and the Davies–Bouldin Index (DBI), was employed to determine the optimal number of RT categories. This approach enabled us to determine the optimal number explaining the most differences in RT in the dataset with the lowest number of groups.

The Elbow method aims to determine the point where the rate of decrease in the within-cluster sum of squares shows a sharp change, indicating that adding more clusters beyond this point provides little additional explanatory power. The Silhouette Score assesses separation and cohesion of clusters, with values close to 1 reflecting dense and well-separated clusters. The Davies–Bouldin Index measures the average similarity between clusters, where lower values indicate better separation.

Based on these metrics, the four-cluster solution did not achieve the highest Silhouette Score or the lowest DBI value. However, it provided the best overall balance between the two metrics, explaining the most variance with the fewest groups (Figure 3.3).

Based on the k-means clustering, the optimal number of RT categories was four, defined as follows:

- Very short : $RT \leq 0.012$ years (≤ 4.5 days)
- Short : $0.012 < RT \leq 0.102$ years (4.5 – 38 days)
- Medium : $0.102 < RT \leq 0.608$ years (39 – 222 days)
- Long : $0.608 < RT (> 222$ days)

The histogram of the four-clusters shows that the clusters are relatively well separated, with some overlap between adjacent groups, indicating a gradual transition rather than sharp boundaries (Figure 3.3, Table 3.1). In general, these clusters provided simplified categories for subsequent analyses. It should be noted that in particular lake type 10 only includes a few lakes ($n=3-7$) in the very short and short RT categories. For lake type 13, there are only six lakes and for lake type 11 only nine lakes with very short RT. For these lakes, general conclusions are more uncertain.

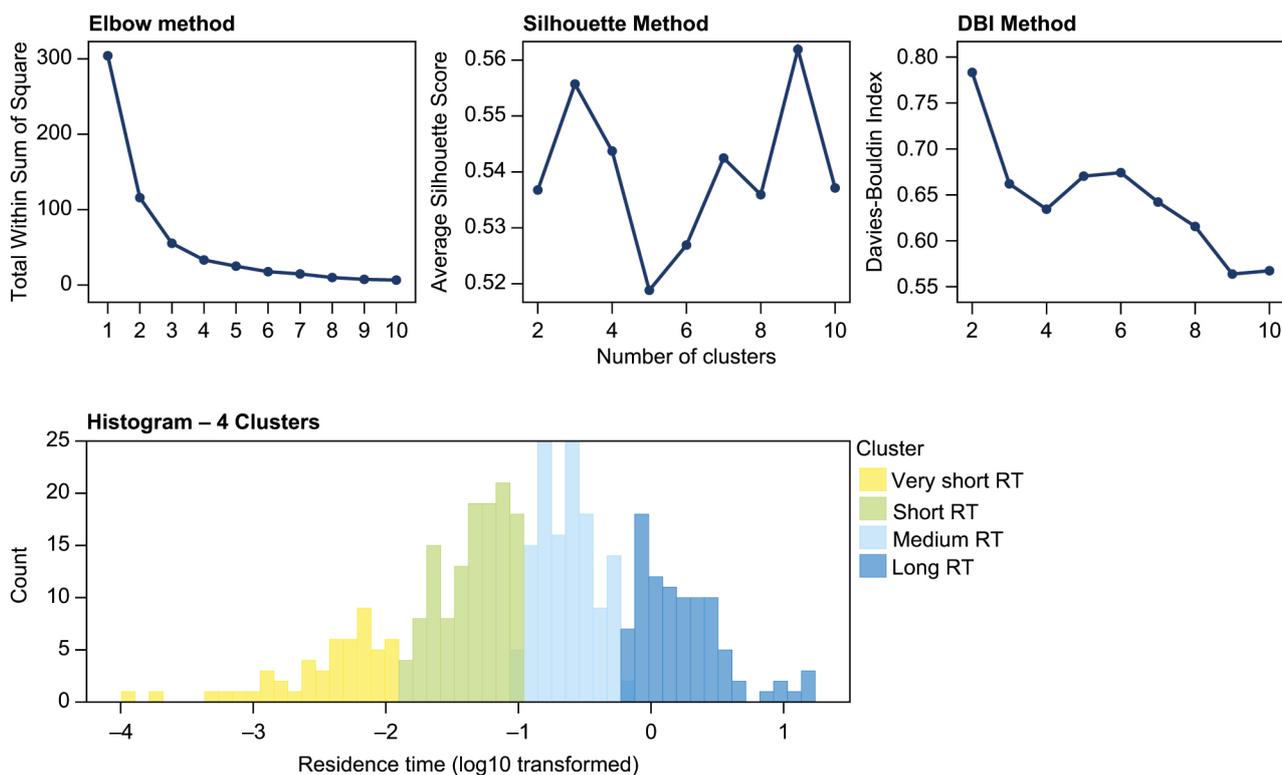


Figure 3.3. Plots for the Elbow, Silhouette and DBI methods, along with a histogram showing the distribution of log10-transformed RT across four clusters identified by k-means clustering.

Table 3.1. Summary table showing the number of lakes, lake-years, mean (min-max) of residence time (RT) categories, total phosphorus (TP) and chlorophyll *a* (Chla) concentrations (from summer mean water chemistry data) for the lake types considered in the analysis.

Lake type	RT category	n (Lake)	n (Lake year)	RT (year)	TP (mg/L)	Chla (µg/L)
9	Very short	33	171	0.006 (0.0001 - 0.012)	0.14 (0.03 - 1.77)	55.0 (3.9 - 259.5)
9	Short	75	595	0.048 (0.014 - 0.100)	0.22 (0.02 - 1.32)	98.6 (2.7 - 470.5)
9	Medium	77	571	0.257 (0.106 - 0.607)	0.25 (0.02 - 4.19)	69.3 (1.6 - 423.5)
9	Long	37	420	1.304 (0.694 - 3.759)	0.17 (0.01 - 1.34)	52.5 (2.5 - 272.3)
10	Very short	3	24	0.007 (0.002 - 0.011)	0.19 (0.03 - 1.32)	60.3 (4.7 - 186.2)
10	Short	7	61	0.052 (0.024 - 0.089)	0.08 (0.03 - 0.18)	37.4 (6.8 - 81.3)
10	Medium	19	224	0.304 (0.108 - 0.571)	0.12 (0.02 - 0.49)	55.1 (5.4 - 262.6)
10	Long	44	525	2.746 (0.638 - 15.35)	0.08 (0.01 - 0.72)	34.5 (2.1 - 511.8)
11	Very short	9	34	0.006 (0.0002 - 0.011)	0.29 (0.03 - 0.95)	105.7 (5.0 - 450.4)
11	Short	23	122	0.055 (0.014 - 0.094)	0.20 (0.03 - 0.74)	57.9 (4.7 - 255.2)
11	Medium	16	105	0.335 (0.115 - 0.576)	0.26 (0.03 - 1.66)	90.4 (3.9 - 342.6)
11	Long	11	62	2.263 (0.746 - 8.200)	0.17 (0.03 - 0.73)	76.9 (2.1 - 227.7)
13	Very short	6	28	0.004 (0.002 - 0.008)	0.08 (0.02 - 0.3)	18.1 (3.2 - 144.5)
13	Short	20	72	0.067 (0.017 - 0.102)	0.35 (0.03 - 2.23)	84.8 (6.5 - 769.6)
13	Medium	17	68	0.219 (0.146 - 0.552)	0.38 (0.02 - 5.26)	88.6 (3.5 - 574.1)

3.4 Chlorophyll α response to total phosphorus by residence time: all lake types

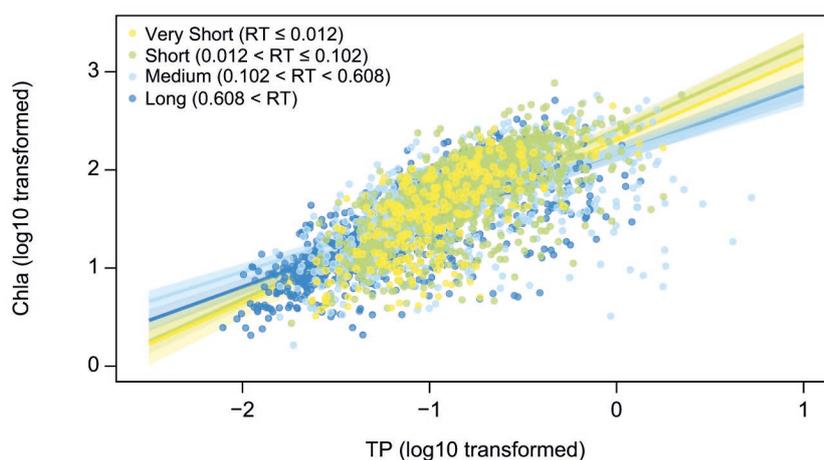
Linear mixed-effects models (LMMs) were applied to assess whether the responses of Chla ($\log_{10}\text{Chla}$) to TP ($\log_{10}\text{TP}$) differed among RT categories. The analysis was conducted using the full dataset (3082 lake-years), excluding lake type as a fixed effect. Lake identity (LakeID; VandomraadeNr) was included as a random effect to account for the repeated measurements (non-independence) within the same lake and to capture between-lake variability in baseline Chla (Formula 3.1).

$$\text{Formula 3.1 } \log\text{Chla} \sim \log\text{TP} * \text{Residence_Time_category} + (1 | \text{LakeID})$$

As a first step, two models were compared: one without the RT effect (lmer0) and one including the interaction between TP and RT category (lmer1) (Appendix A Table A1). The model considering the interaction between TP and RT category provided a better fit, indicating that the relationship between TP and Chla differs across RT categories. These results suggest that RT plays a role in shaping Chla responses to TP and therefore should be considered when establishing TP target concentrations.

The interaction model results showed a stronger increase in Chla (faster increase in Chla) with increasing TP in lakes with very short (< 4.5 days) and short (4.5 – 38 days) RT compared to lakes with medium (39–222 days) or long (more than 222 days) RT. This indicates that the strength of the relation declines at medium and long RT. Lakes with long RT exhibited a slightly stronger response than lakes in the medium category, but the difference was not statistically significant (Figure 3.4, Appendix A Table A1). Additionally, Figure 3.4 shows that very short and short RT lakes have lower Chla at low TP, but higher Chla at high TP due to the faster increase in Chla with increasing TP compared to medium and long RT lakes.

Figure 3.4. Linear mixed-effects model estimates and confidence intervals for the response of Chla concentrations to TP in Danish lakes (types 9, 10, 11 and 13). Different colours correspond to the different residence time categories defined by k-means clustering.



3.5 Effects of lake typology on the chlorophyll α response to total phosphorus

Additional linear mixed-effects models (LMMs) were fitted separately for each RT category to examine whether the relationship between TP and Chla varied among lake types (Formula 3.2).

$$\text{Formula 3.2 } \log\text{Chla} \sim \log\text{TP} * \text{LakeType} + (1 | \text{LakeID})$$

For each RT category, two models were compared: one without lake type (lmer0) and one including the interaction between TP and lake typology (lmer1) (Appendix A Tables A2–A5). The results showed that the strength of the Chla to TP relationship differed between lake types in the short and long RT categories ($p < 0.001$), whereas no statistically significant differences were found for the very short and medium RT categories. The magnitude of the differences varied among categories.

Within the very short RT category (≤ 4.5 days), the Chla to TP relationships were generally similar across lake types 9, 10 and 13, whereas lake type 11 showed a weaker Chla to TP slope, pointing out to a slower increase in Chla with TP (although this difference was not statistically significant) (Figure 3.5a, Appendix A Table A2).

For the short RT category (4.5–38 days), the effect of lake type was stronger. Lake type 13 exhibited a significantly weaker ($p < 0.001$) Chla to TP relationship than the other types, which had comparable slopes. Pairwise slope comparisons supported this result, with significant differences between lake type 13 and lake types 9 ($p < 0.01$) and 11 ($p < 0.05$) (Figure 3.5b, Appendix A Table A3).

In the medium RT category (39–222 days), the Chla response to TP was consistent across lake types, with no significant differences in slope (Figure 3.5c, Appendix A Table A4).

For the long RT category (> 222 days), no lakes of type 13 were present. However, lakes with higher salinity (type 11) displayed a stronger Chla response to increasing TP than the other lake types (Figure 3.5d, Appendix A Table A5). This effect was significant compared with type 9 lakes ($p < 0.05$) and not statistically significant compared with type 10 lakes.

Taken together, these findings suggest that phytoplankton biomass (Chla) sensitivity to TP concentrations is influenced by both lake typology and water residence time. Lake types 9 and 10 generally exhibited similar Chla responses to TP across all RT categories, whereas high-salinity lakes (type 11) and high-colour lakes (type 13) showed either weaker or stronger Chla-TP responses depending on RT category.

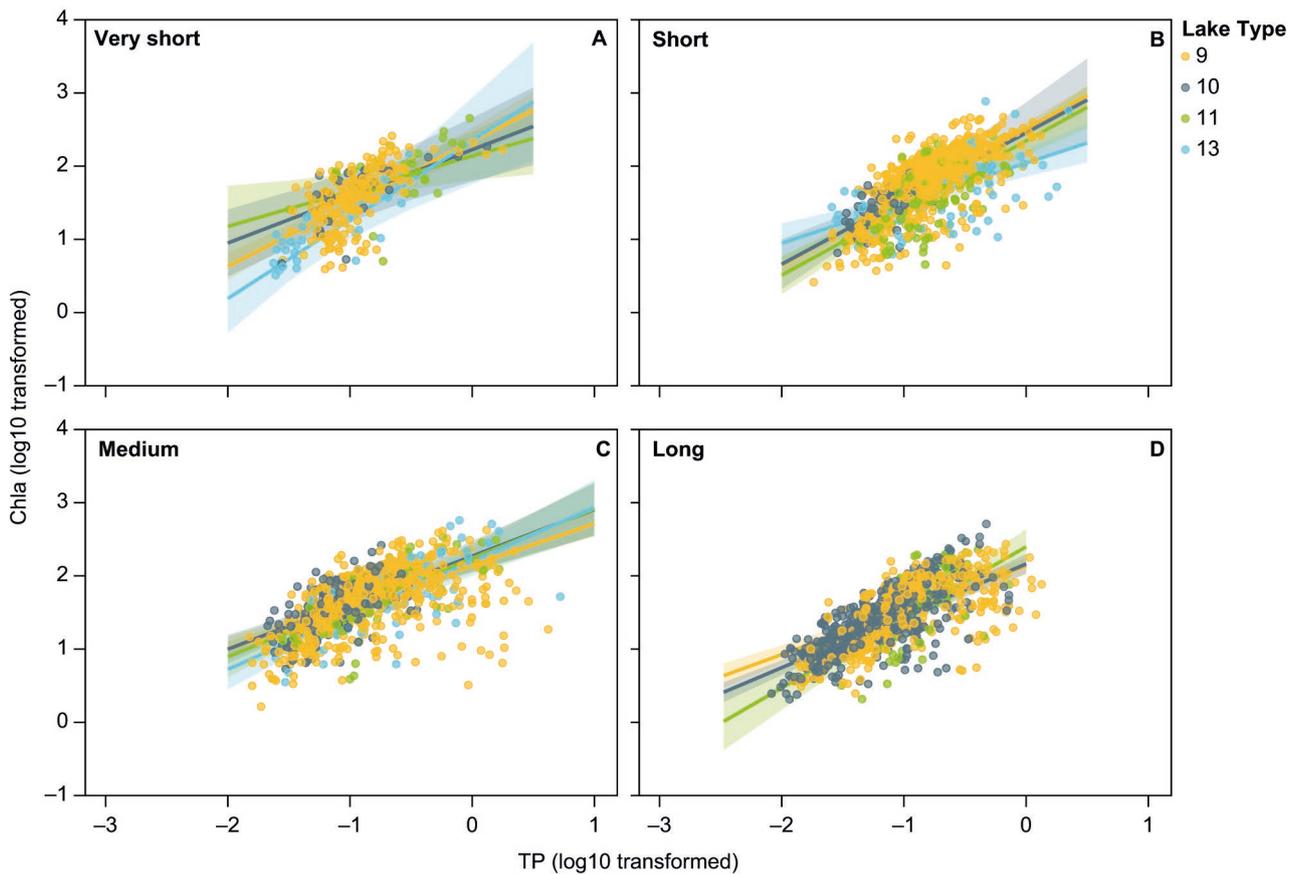


Figure 3.5. Linear mixed-effects model results for Chla concentration responses to TP across RT categories, A: very short, B: short, C: medium and D: long. Colours represent the different lake types.

The slopes of the Chla to TP relationships were further compared across RT categories within each lake type (Figure 3.6). Lake type 13 showed different responses at increasing RT than the three other lake types examined, particularly at very short or short RT.

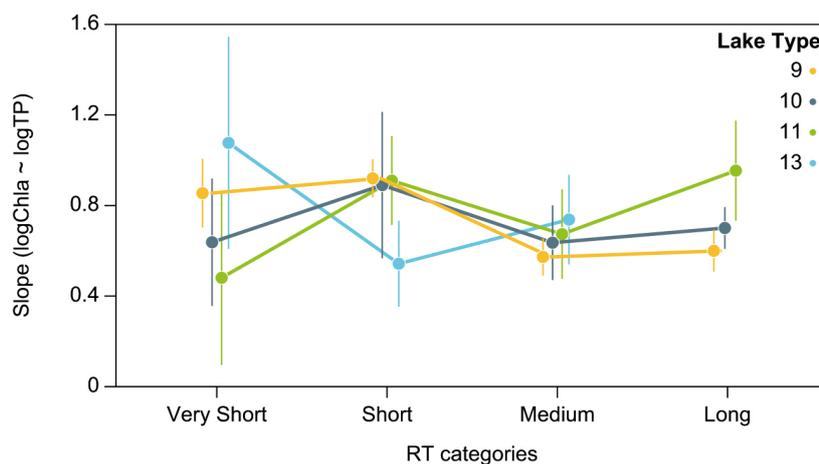
For lake types 9 and 10, short and the very short RT categories, steeper Chla to TP slopes emerged, indicating stronger Chla responses to increasing TP with shorter RT (Figure 3.6).

Lake type 11 displayed a partly opposite pattern to lake types 9 and 10, with a weaker Chla to TP relationship in the very short RT category but steeper slopes in the short, medium and long RT categories (Figure 3.6). Moreover, among the latter three RT categories, short and long RT lakes had similar Chla-TP slopes, while the slope for medium ones were slightly flatter.

Lake type 13 exhibited the flattest slope in the short RT category, suggesting weaker sensitivity of Chla to TP. However, at very short RT, the Chla response was steeper compared to the other RT categories (Figure 3.6).

Overall, these results suggest that the Chla response to TP was stronger at shorter RT for lake types 9 and 10, whereas stronger TP sensitivity in high-salinity lakes (type 11) was observed especially at short and long RT (Figure 3.6).

Figure 3.6. Comparison of Chla to TP response slopes estimated with linear mixed-effects models for each RT category and lake type. Colours represent lake types.



3.6 Target values of total phosphorus concentrations relative to lake typology and residence time categories

Using the log₁₀-log₁₀ regression model (Formula 3.2), target TP concentrations were estimated for each Chla class defined under the Danish implementation of the EU Water Framework Directive (BEK nr. 792, 2023). The predefined Chla boundaries (BEK nr. 792, 2023) used for TP estimation were:

- Lake type 10: High/Good = 7, Good/Moderate = 12, Moderate/Poor = 27, Poor/Bad = 56 µg/L
- Lake types 9, 11, 13: 11.7, 25, 56 and 90 µg/L, respectively.

Uncertainty was calculated using a parametric bootstrap. For each Chla class, a sample of TP estimates was generated by repeatedly resampling the fixed effects of the mixed model. The 2.5th and 97.5th percentiles of this sample were used as lower and upper confidence limits, respectively. Percent uncertainty was then calculated as the width of this interval divided by twice the estimated-TP target value (i.e. fixed-effect estimate). This indicated that estimated uncertainties ranged widely, from approximately 14% to more than 100%, with the largest uncertainties generally observed in the very short RT categories for lake types 10, 11 and 13 (Figure 3.7). Since uncertainty was extremely high (>100%) for these lake types in the very short RT category, the estimated target values are not presented here.

It should also be noted that higher uncertainties caused overlaps in confidence intervals, but not between the Good/Moderate and Moderate/Poor boundaries, except for short RT of type-10 lake. A subsequent adjacency-based check was done to assess whether two neighbouring TP estimates could be distinguished. Adjacent TP estimates were considered distinct when their 95% confidence intervals (CI) did not have beyond 10% overlap. Estimates that did not meet this criterion are also shown in Table 3.2 with grey shading.

In summary, given the aforementioned information, table 3.2 presents estimated-TP target concentrations for the predefined Chla boundaries (BEK nr. 792, 2023). For each boundary, the table also shows the 95% CI for each estimated-TP target concentration. Some CI overlap across adjacent class boundaries (grey shading). Overall, the estimated-TP targets with lower uncertainties and that are not overlapping with adjacent classes are considered reliable (the values not highlighted in Table 3.2).

The table also shows how estimated-TP target values vary across RT categories for each lake type. At lower Chla class boundaries (e.g. the Good/Moderate boundary), shorter RT lakes correspond to higher estimated-TP targets than longer RT lakes for some lake typologies (Table 3.2). This occurs because shorter RT lakes have lower baseline Chla (lower intercepts), meaning that more TP is required to reach a given lower Chla boundary (Figures 3.4 and 3.5; Appendix A, Tables A1-A5).

At higher Chla boundaries (e.g. Moderate/Poor), the steeper Chla-TP slopes in shorter RT lakes become more influential, so estimated-TP targets are similar to or lower than the ones for longer RT lakes.

Therefore, higher estimated-TP targets for shorter RT lakes at lower Chla class boundaries reflect differences in the Chla-TP relationships. It is also important to note that this pattern varies based on the lake typologies (Appendix A Tables A2-A5).

Table 3.2. Estimated-TP target concentrations (mg/L) calculated based on the Chla class boundaries defined for ecological quality assessment (BEK nr. 792, 2023), shown for each RT category and lake type. Data are presented as “TP estimated (confidence interval)”. Highlights indicate the adjacent TP estimates with more than 10% overlap in their 95% confidence intervals – hence class boundaries are less clearly defined.

Lake Type	RT category	High/Good		Good/Moderate		Moderate/Poor		Poor/Bad	
		TP estimated (CI lower- CI upper)							
9	Very short	0.033 (0.022-0.045)	0.080 (0.059-0.109)	0.205 (0.155-0.292)	0.356 (0.262-0.554)				
9	Short	0.027 (0.021-0.033)	0.062 (0.052-0.072)	0.149 (0.128-0.173)	0.249 (0.215-0.289)				
9	Medium	0.013 (0.009-0.020)	0.051 (0.038-0.064)	0.207 (0.163-0.265)	0.474 (0.361-0.663)				
9	Long	0.017 (0.010-0.024)	0.059 (0.041-0.079)	0.225 (0.168-0.311)	0.496 (0.353-0.737)				
10	Short	0.015 (0.005-0.029)	0.028 (0.013-0.048)	0.070 (0.042-0.120)	0.158 (0.097-0.352)				
10	Medium	0.006 (0.002-0.011)	0.013 (0.006-0.023)	0.048 (0.028-0.070)	0.151 (0.100-0.238)				
10	Long	0.013 (0.009-0.017)	0.028 (0.022-0.036)	0.090 (0.072-0.118)	0.256 (0.191-0.357)				
11	Short	0.039 (0.024-0.055)	0.090 (0.064-0.118)	0.217 (0.167-0.286)	0.366 (0.272-0.521)				
11	Medium	0.018 (0.007-0.036)	0.056 (0.030-0.091)	0.186 (0.120-0.295)	0.376 (0.241-0.661)				
11	Long	0.040 (0.023-0.062)	0.090 (0.059-0.130)	0.209 (0.149-0.313)	0.343 (0.239-0.539)				
13	Short	0.015 (0.004-0.034)	0.063 (0.028-0.105)	0.277 (0.167-0.513)	0.664 (0.384-1.529)				
13	Medium	0.029 (0.013-0.049)	0.082 (0.050-0.123)	0.245 (0.170-0.380)	0.467 (0.305-0.828)				

Lake type 9 (shallow, freshwater, high alkalinity, low colour) consistently showed lower uncertainty (ca. <50%, mean ca. 32%) and clearer distinction between ecological classes. Estimated High/Good boundaries were generally lower than the values in Table 13 of BEK nr. 792 (2023) (0.06 mg/L). Good/Moderate boundaries were also lower, although the difference was smaller – especially for lakes with very short RT, where the estimated-TP target was 0.080 mg/L compared with 0.082 mg/L in BEK nr. 792 (2023) (Table 4.1). The estimated-TP targets also indicated that back-calculated TP thresholds for short and very short RT lakes were higher at the High/Good and Good/Moderate class boundaries than for the longer RT lakes (Figure 3.8).

Lake type 10 (deep, freshwater, high alkalinity, low colour) exhibited relatively high uncertainty for short (50–100%) and medium (40–80%) RT categories, while the uncertainty for long RT was <32%. Compared to the TP concentrations given in Table 13 of BEK nr. 792 (2023), the estimated High/Good boundaries were lower, while the Good/Moderate boundaries were comparable for short and long RT, and both boundaries were lower for medium RT

category (Table 4.1). It should be noted that lake type 10 was likely underrepresented in the short RT categories (7 lakes out of 73, Table 3.1).

Lake type 11 (shallow, high salinity, high alkalinity, low colour) displayed narrower ranges for short and long RT (with 27–50% uncertainty) but wider ranges for medium RT (47–80% uncertainty). Estimated-TP targets of High/Good boundaries for short and long RT were slightly higher than the ones specified in BEK nr. 792 (2023), whereas for the medium RT, the suggested target value was lower. Estimated Good/Moderate boundaries were lower across all RT categories (Table 4.1).

For lake types 10 and 11, the comparison of the short and long RT categories showed similar estimated-TP target concentrations (short vs long) at the High/Good and Good/Moderate boundaries. This suggests that, at these boundaries, the Chla-TP relationships in lakes with short and long RT produce similar TP thresholds, whereas medium-RT lakes reach the same boundaries at lower TP.

Lake type 13 (shallow, freshwater, high alkalinity, high colour) also showed high uncertainty (ca. 42–96%), especially for short RT, while medium RT exhibiting lower uncertainty at the Good/Moderate and Moderate/Poor boundaries. Estimated-TP target values for High/Good and Good/Moderate boundaries were markedly lower than in BEK nr. 792 (2023) (Table 4.1). Additionally, at High/Good and Good/Moderate boundaries, estimated-TP target concentrations in the short RT category were lower compared to the medium category.

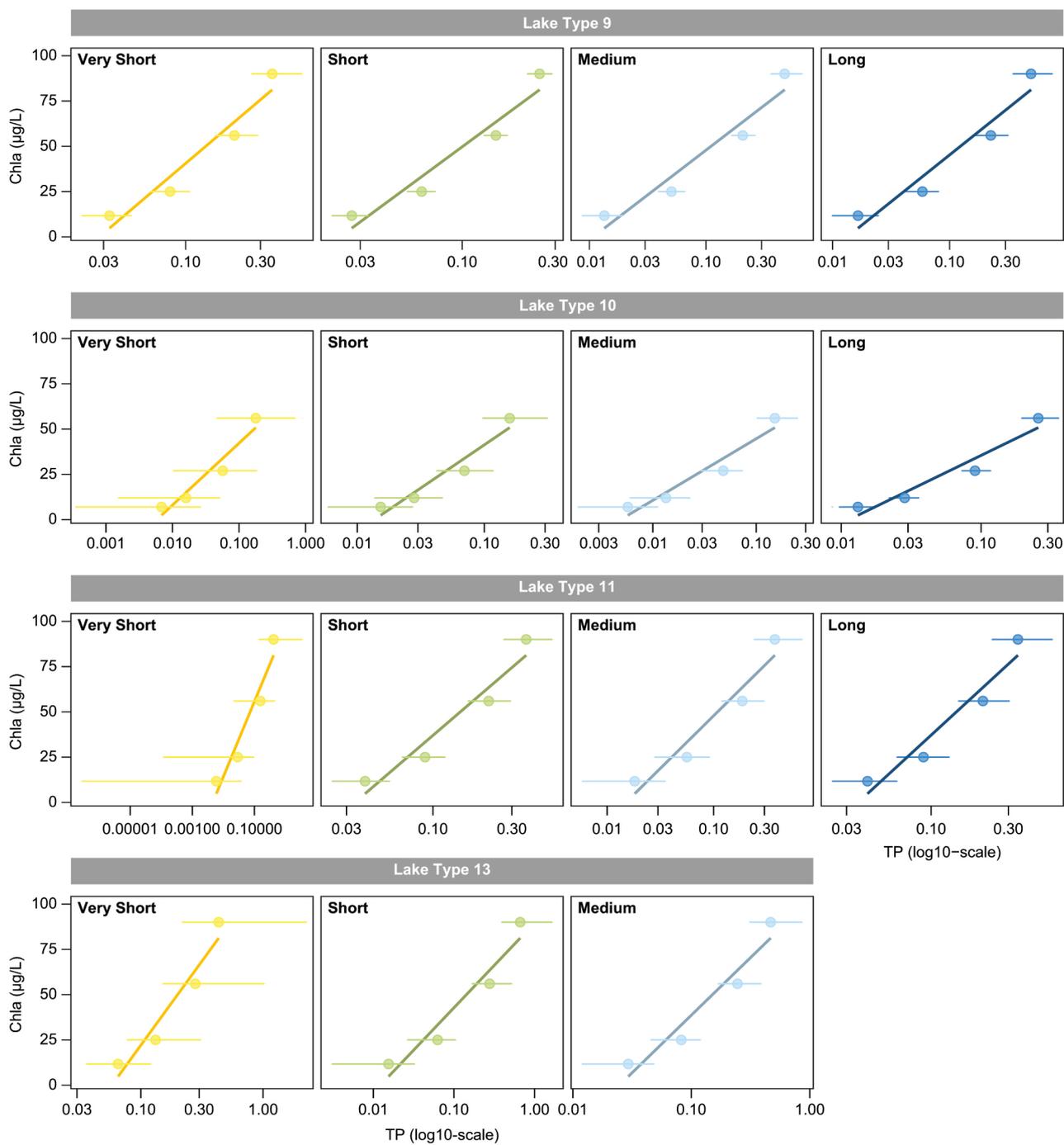


Figure 3.7. Estimated-TP target concentrations (log₁₀-scale) relative to Ch_la class boundaries, shown for each defined RT category and lake type. Error bars indicate minimum–maximum TP limits at log₁₀-scale.

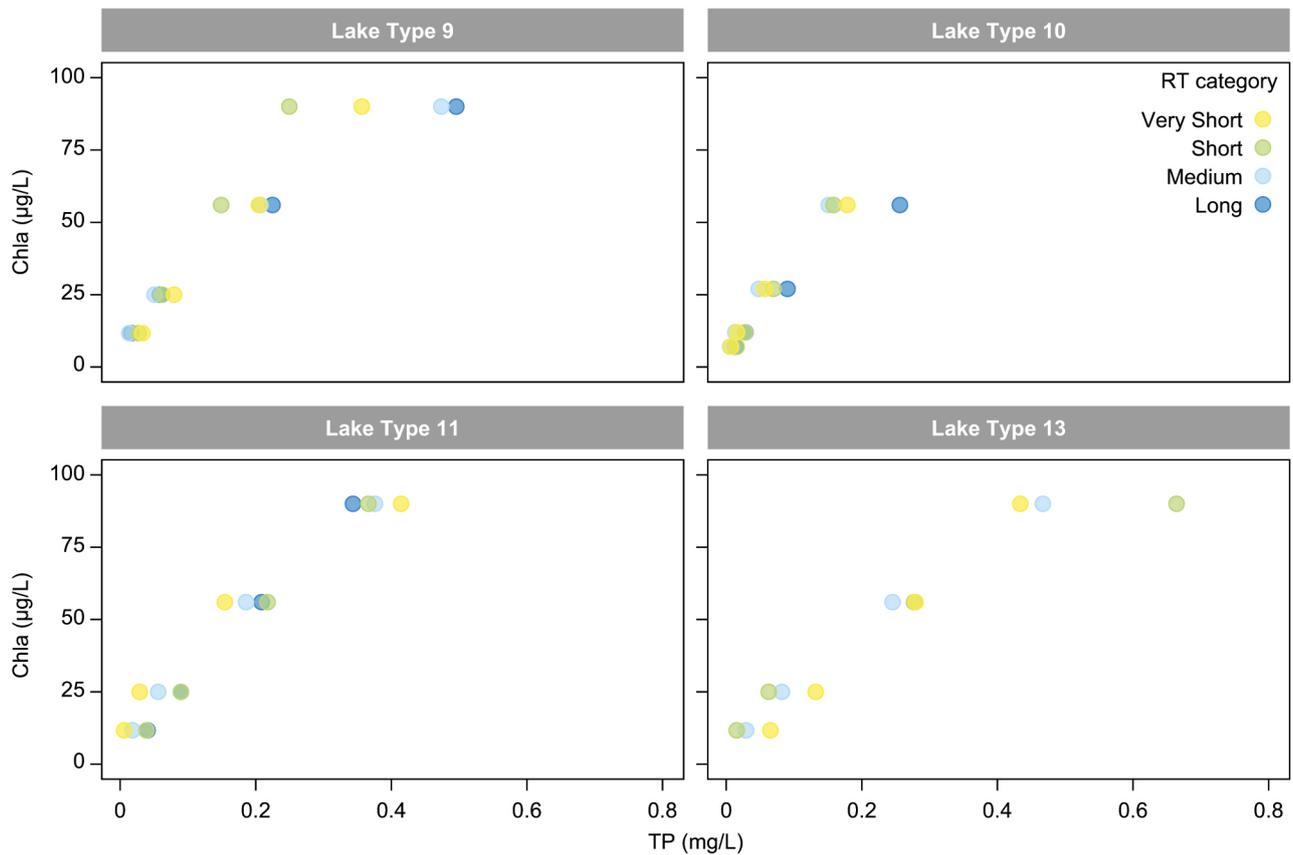


Figure 3.8. Estimated-TP target concentrations relative to Chl a class boundaries, shown for each defined RT category and lake type. Plot also includes the estimated-TP targets with very high (>100%) uncertainties.

The high uncertainties suggest that caution is needed when applying strict TP targets. Moreover, the estimated boundaries for successive ecological classes showed some overlap, but this was minimal to absent when uncertainties were lower (<50%).

Large uncertainties, such as cases with broad confidence intervals, are largely attributable to data-related factors. When lakes are classified by both residence time and type, the number of lakes in each category decreases markedly compared with broader classifications. Small sample sizes due to limited data availability increase the uncertainty of the models and, consequently, of the calculated target values. Even though the methods applied here were intended to reduce this effect by excluding cases where data availability was too low, the small sample size effect can still influence the uncertainty of the estimates. Furthermore, confounding environmental factors that were not considered in this study could also contribute to this high variability, apart from differences in lake colour, salinity or morphology. For example, RT may show marked temporal variation, with year-to-year fluctuations (Johansson et al., 2022, Figure 2.14), and variability in physical and chemical characteristics among lake types may further affect observed relationships.

4 Conclusions

This study defined relevant hydraulic residence time (RT) categories for Danish lakes and assessed how differences in RT influence eutrophication responses across lake types.

The analysis showed that many of the assessed Danish lakes have short RT (46% with RT < 38 days), and that deeper lakes (lake type 10) generally have longer RT (86% with RT \geq 39 days and 60% with RT > 222 days).

Four RT categories were identified for the examined lakes:

- Very short : RT \leq 0.012 years (\leq 4.5 days)
- Short : 0.012 < RT \leq 0.102 years (4.5 – 38 days)
- Medium : 0.102 < RT \leq 0.608 years (39 – 222 days)
- Long : 0.608 < RT (> 222 days)

The results from the analyses indicate that RT category significantly influences the response of Chla to increases in TP.

Even though classical mass-balance models indicate that the relative phosphorus retention increases with longer RT, the current study indicated a more complex pattern. Overall, lakes with shorter RT showed a stronger Chla response to TP. Very short and short RT lakes, which were dominated by shallow, low colour and low salinity lake type 9 (Table 3.1), generally had lower Chla at low TP concentrations, but higher Chla at high TP concentrations, resulting in a steeper increase in Chla with increasing TP compared to lakes with medium and long RT.

Søballe and Kimmel (1987) also highlighted a possibility of an opposite trend to classical mass-balance model, where short RT may promote algal growth. For example, they pointed out that high flushing rates (short RT) can increase turbulence, keeping phytoplankton suspended (reduced sinking losses), consequently increasing phytoplankton nutrient uptake and exposure to light. They also pointed out to the tendency of higher nutrient levels in systems with high flushing rates (Søballe and Kimmel 1987).

Moreover, Elliott et al. (2009) showed that when the dominant nutrient source is diffuse, shorter RT (ca. < 40 days RT) combined with higher nutrient concentrations in the inflow can cause phosphorus inputs to exceed sedimentation losses, resulting in increased Chla concentrations despite short RT.

Compatibly, in the current study the observed steeper Chla-TP relationship at shorter RT is consistent with patterns reported in other studies, where higher nutrient availability, may support phytoplankton growth despite shorter RT. However, because the Chla-TP relationship can be influenced by several external factors, the main cause of this steeper increase cannot be determined from the available data, and any explanation of potential mechanisms would be speculative.

Lake-type specific patterns in Chla-TP relations were also observed, where both shallow (type 9) and deep (type 10) low colour, low salinity lakes showed

steeper Chla-TP slopes at shorter RT, while types 11 (high salinity) and 13 (high colour) showed different sensitivities depending on RT category. These differences indicate that the interaction between lake typology and RT plays an important role in shaping eutrophication responses.

Table 4.1. Proposed estimated target TP concentrations (mg/L) compared to the TP concentrations in BEK nr. 792 (2023) for each RT category and lake type. Data are presented as “TP estimated (confidence interval)”.

Lake Type	RT category	High/Good		Good/Moderate	
		TP estimated (CI lower- CI upper)	TP (BEK nr. 792)	TP estimated (CI lower- CI upper)	TP (BEK nr. 792)
9	Very short	0.033	(0.022-0.045)	0.080	(0.059-0.109)
9	Short	0.027	(0.021-0.033)	0.062	(0.052-0.072)
9	Medium	0.013	(0.009-0.020)	0.051	(0.038-0.064)
9	Long	0.017	(0.010-0.024)	0.059	(0.041-0.079)
10	Short				
10	Medium		0.029	0.013	(0.006-0.023)
10	Long	0.013	(0.009-0.017)	0.028	(0.022-0.036)
11	Short	0.039	(0.024-0.055)	0.090	(0.064-0.118)
11	Medium		0.033	0.056	(0.030-0.091)
11	Long	0.040	(0.023-0.062)	0.090	(0.059-0.130)
13	Short		0.114	0.063	(0.028-0.105)
13	Medium	0.029	(0.013-0.049)	0.082	(0.050-0.123)

The proposed estimated-TP targets (Table 4.1), which account for RT, were mostly lower than the standards set in BEK nr. 792 (2023) where RT is not considered. Even though the methods applied here were intended to reduce the effect of limited data availability, large variation in the data (e.g. due to small sample size) still resulted in broad confidence intervals and high uncertainties for the estimated TP targets. Targets for which uncertainties were lower and had no overlap with adjacent classes are considered reliable (the values not highlighted in Table 3.2) and can be used as a basis for further evaluation and implementation.

Overall, this study highlights RT as an important parameter influencing Chla responses to TP and provides estimated-TP target ranges for management purposes. The results emphasise the importance of considering RT when setting nutrient targets and evaluating ecological status under the Water Framework Directive.

Future research should explore the effects of residence time on other ecological indicators. This should be based on a higher data availability, especially in less common lake types. Comparative studies with other EU countries would also help establish whether these target values are suitable for broader implementation.

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Appendix A

Table A1. Linear mixed-effects model results for the full data set, followed by pairwise comparison. Formula: $\log Chla \sim \log TP + Residence_Time_category + (1 | LakeID)$

Likelihood ratio test (common vs. group-specific slopes)							
term	npar	AIC	BIC	Chisq	df	p.value	signif
lmer0	4	361.5	385.6	NA	NA	NA	
lmer1	10	332.3	392.7	41.1	6	<0.001	***
Mixed model coefficients							
effect	term	estimate	std.error	t-value		p.value	signif
fixed	(Intercept)	2.304	0.074	31.203		<0.001	***
fixed	logTP	0.831	0.066	12.538		<0.001	***
fixed	RT_upd - short	0.102	0.083	1.233			
fixed	RT_upd - med	-0.132	0.082	-1.611			
fixed	RT_upd - long	-0.133	0.086	-1.535			
fixed	logTP:RT_upd - short	0.031	0.075	0.408			
fixed	logTP:RT_upd - med	-0.226	0.073	-3.090		<0.01	**
fixed	logTP:RT_upd - long	-0.149	0.074	-2.009		<0.05	*
Pairwise slope comparisons							
term	contrast	estimate	std.error	t.ratio	df	adj. p.value	signif
RT category	very_short – short	-0.03	0.08	-0.41	2793		
RT category	very_short - med	0.23	0.07	3.09	2739	<0.05	*
RT category	very_short - long	0.15	0.07	2.01	2763		
RT category	short - med	0.26	0.05	5.47	2848	<0.001	***
RT category	short - long	0.18	0.05	3.71	2891	<0.01	**
RT category	med - long	-0.08	0.05	-1.70	2775		

Table A2. Linear mixed-effects model results for the very short RT category, followed by pairwise comparison. Formula: $\log Chla \sim \log TP * LakeType + (1 | LakeID)$

Very short RT: Likelihood ratio test (common vs. group-specific slopes)							
term	npar	AIC	BIC	Chisq	df	p.value	signif
lmer0_vs	4	11.5	25.7	NA	NA	NA	
lmer_vs	10	12.8	48.3	10.7	6	<0.1	.
Very short RT: Mixed model coefficients							
effect	term	estimate	std.error	t-value		p.value	signif
fixed	(Intercept)	2.337	0.094	24.839		<0.001	***
fixed	logTP	0.854	0.077	11.092		<0.001	***
fixed	LakeType 10	-0.112	0.239	-0.468			
fixed	LakeType 11	-0.199	0.191	-1.041			
fixed	LakeType 13	0.008	0.313	0.024			
fixed	logTP:LakeType 10	-0.217	0.162	-1.336			
fixed	logTP:LakeType 11	-0.374	0.209	-1.788		<0.1	.
fixed	logTP:LakeType 13	0.222	0.248	0.895			

Table A3. Linear mixed-effects model results for the short RT category, followed by pairwise comparison. Formula: $\log\text{Chla} \sim \log\text{TP} * \text{LakeType} + (1 | \text{LakeID})$

Short RT: Likelihood ratio test (common vs. group-specific slopes)							
term	npar	AIC	BIC	Chisq	df	p.value	signif
lmer0_s	4	142.299	161.280	NA	NA	NA	
lmer_s	10	131.273	178.726	23.025	6	<0.001	***
Short RT: Mixed model coefficients							
effect	term	estimate	std.error	t-value		p.value	signif
fixed	(Intercept)	2.509	0.046	54.792		<0.001	***
fixed	logTP	0.920	0.043	21.496		<0.001	***
fixed	LakeType 10	-0.048	0.214	-0.224			
fixed	LakeType 11	-0.158	0.108	-1.459			
fixed	LakeType 13	-0.458	0.104	-4.424		<0.001	***
fixed	logTP:LakeType 10	-0.030	0.170	-0.176			
fixed	logTP:LakeType 11	-0.010	0.109	-0.092			
fixed	logTP:LakeType 13	-0.377	0.105	-3.578		<0.001	***
Short RT: Pairwise slope comparisons							
term	contrast	estimate	std.error	t.ratio	df	adj. p.value	signif
LakeType	LakeType 9 - LakeType 10	0.030	0.170	0.176	784		
LakeType	LakeType 9 - LakeType 11	0.010	0.109	0.092	841		
LakeType	LakeType 9 - LakeType 13	0.377	0.106	3.564	548	<0.01	**
LakeType	LakeType 10 - LakeType 11	-0.020	0.193	-0.103	806		
LakeType	LakeType 10 - LakeType 13	0.347	0.191	1.817	842		
LakeType	LakeType 11 - LakeType 13	0.367	0.139	2.640	714	<0.05	*

Table A4. Linear mixed-effects model results for the medium RT category, followed by pairwise comparison. Formula: $\log\text{Chla} \sim \log\text{TP} * \text{LakeType} + (1 | \text{LakeID})$

Medium RT: Likelihood ratio test (common vs. group-specific slopes)							
term	npar	AIC	BIC	Chisq	df	p.value	signif
lmer0_m	4	241.310	260.811	NA	NA	NA	
lmer_m	10	247.488	296.241	5.822	6		
Medium RT: Mixed model coefficients							
effect	term	estimate	std.error	t-value		p.value	signif
fixed	(Intercept)	2.140	0.047	45.580		<0.001	***
fixed	logTP	0.573	0.042	13.762		<0.001	***
fixed	LakeType 10	0.130	0.115	1.130			
fixed	LakeType 11	0.101	0.112	0.901			
fixed	LakeType 13	0.058	0.112	0.518			
fixed	logTP:LakeType 10	0.063	0.094	0.673			
fixed	logTP:LakeType 11	0.101	0.109	0.925			
fixed	logTP:LakeType 13	0.165	0.109	1.519			

Table A5. Linear mixed-effects model results for the long RT category, followed by a pairwise comparison. Formula: $\logChla \sim \logTP * LakeType + (1 | LakeID)$

Long RT: Likelihood ratio test (common vs. group-specific slopes)							
term	npar	AIC	BIC	Chisq	df	p.value	signif
lmer0_l	4	-80.640	-60.981	NA	NA	NA	
lmer_l	8	-83.669	-44.351	11.028	4	<0.05	*
Long RT: Mixed model coefficients							
effect	term	estimate	std.error	t-value		p.value	signif
fixed	(Intercept)	2.137	0.058	36.974		<0.001	***
fixed	logTP	0.600	0.047	12.896		<0.001	***
fixed	LakeType 10	0.025	0.090	0.282			
fixed	LakeType 11	0.260	0.135	1.933		<0.1	.
fixed	logTP:LakeType 10	0.101	0.066	1.526			
fixed	logTP:LakeType 11	0.354	0.122	2.913		<0.01	**
Long RT: Pairwise slope comparisons							
term	contrast	estimate	std.error	t.ratio	df	adj. p.value	signif
LakeType	LakeType 9 - LakeType 10	-0.101	0.066	-1.520	886		
LakeType	LakeType 9 - LakeType 11	-0.354	0.122	-2.908	1000	<0.05	*
LakeType	LakeType 10 - LakeType 11	-0.253	0.122	-2.078	1000	<0.1	.

Appendix B

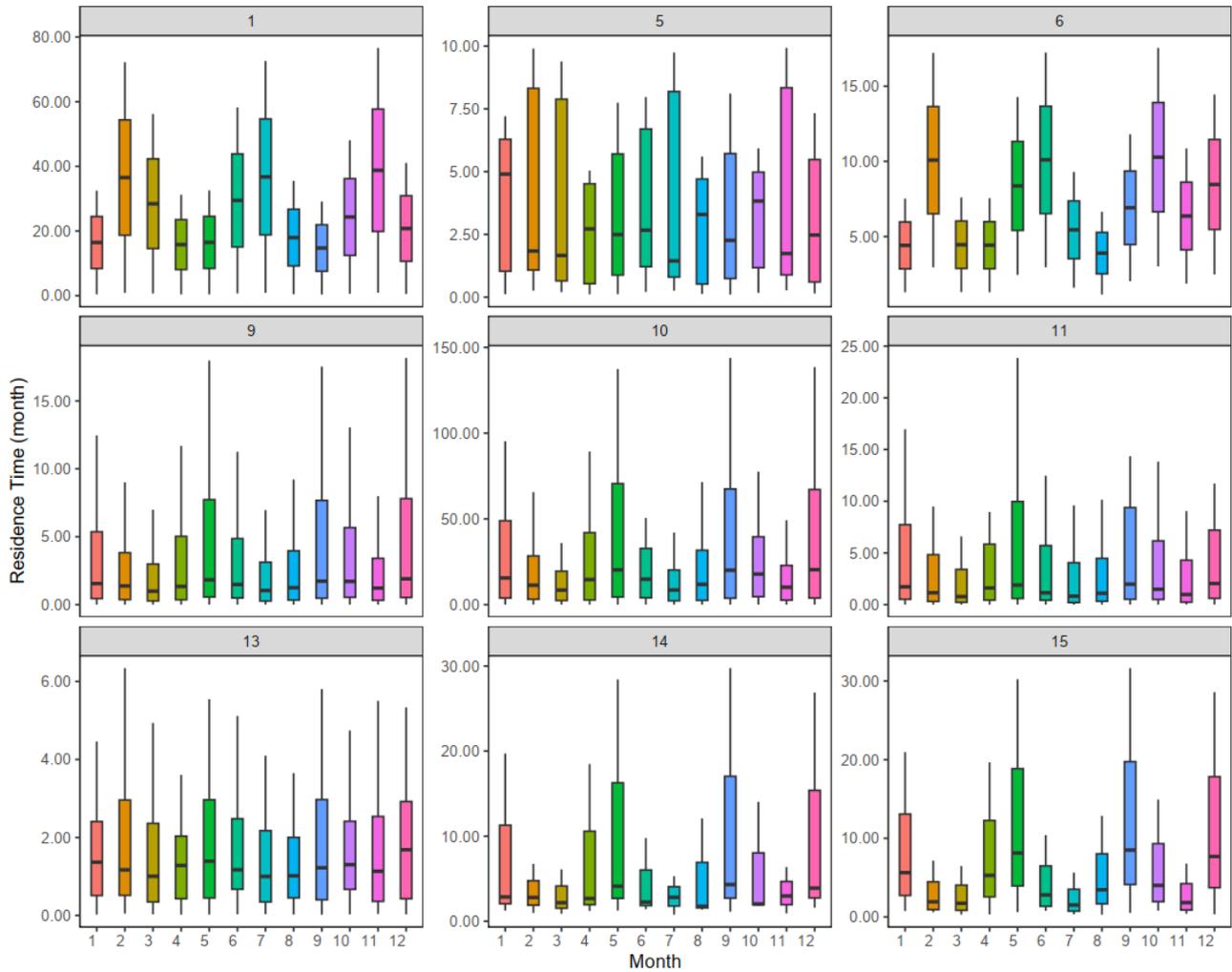


Figure B1. Monthly RT plots for different lake types. Note the different scales on the y-axis.

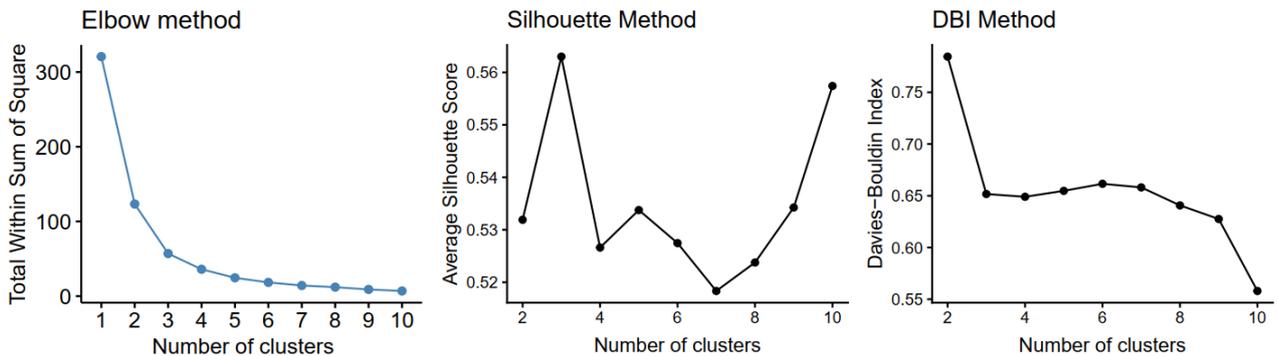


Figure B2. Summer mean RT plots for the Elbow, Silhouette and DBI methods used to identify the optimal number of clusters of k-means clustering. Data include lake types 9, 10, 11 and 13.

Figure B3. Results from the linear mixed-effects model showing the Chl-a to TP response relative to summer mean RT categories. Colours represent different residence time categories defined by k-means clustering. Data include lake types 9, 10, 11 and 13.

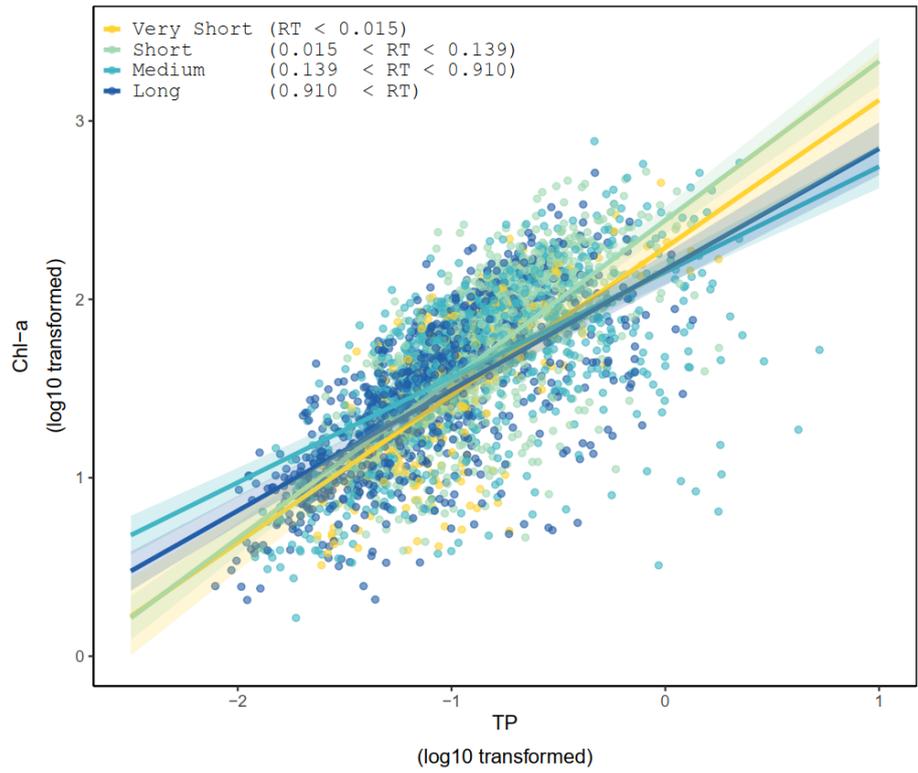


Figure B4. Linear mixed-effects model results for Chl-a concentration responses to TP for each summer mean RT category and lake type. a: Very short, b: Short, c: Medium and d: Long. Colours represent lake types.

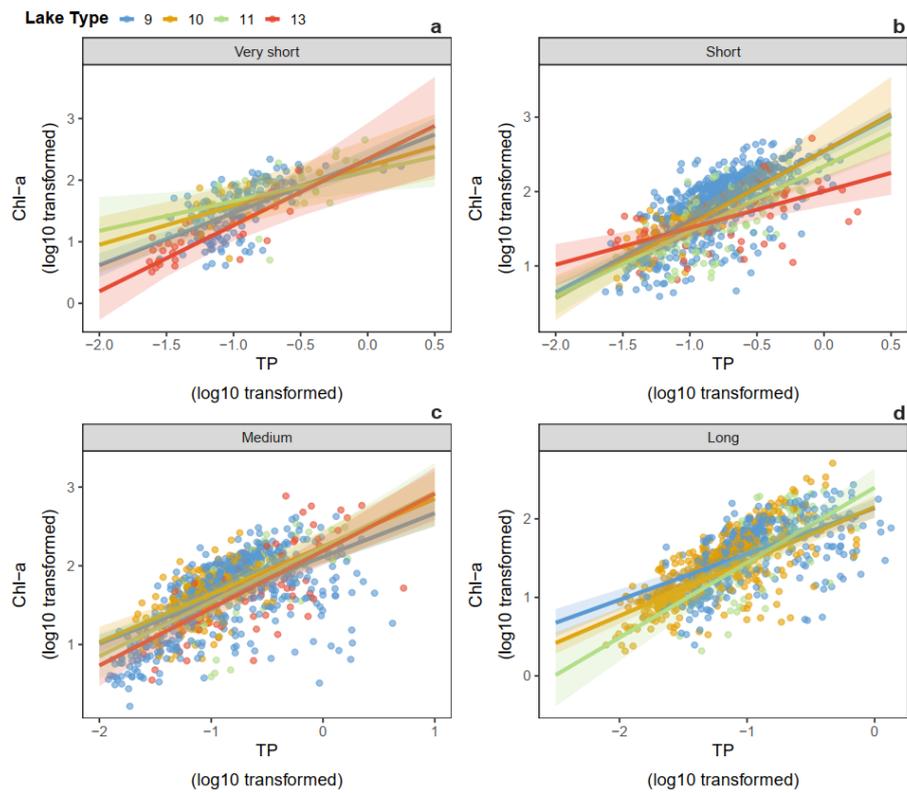
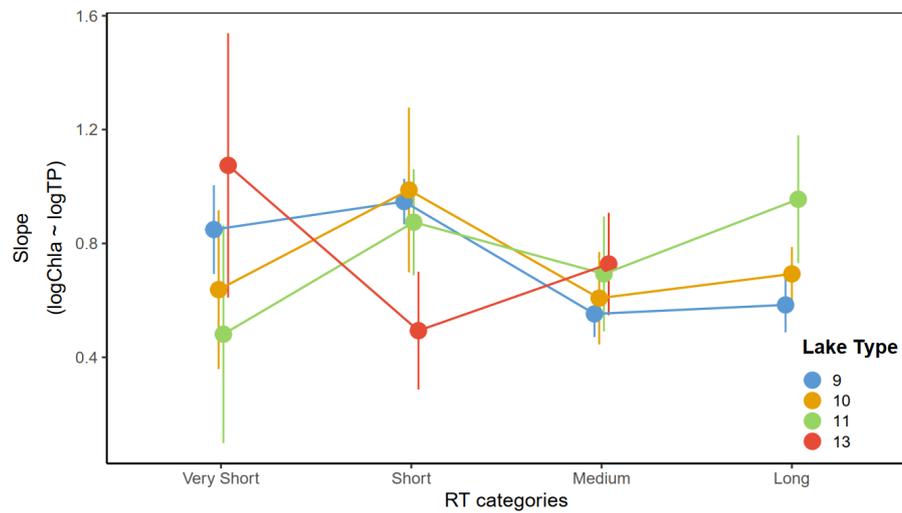


Figure B5. Comparison of Chla to TP response slopes estimated with linear mixed-effects models for each summer mean RT category and lake type. Colours represent lake types.



THE EFFECT OF HYDRAULIC RESIDENCE TIME ON CHLOROPHYLL A RESPONSE TO TOTAL PHOSPHORUS IN DANISH LAKES

The aim of this study was to assess how hydraulic residence time influences the eutrophication response (response of chlorophyll *a* (Chla) to total phosphorus (TP)) across different Danish lake types. Four residence time categories were defined: very short (≤ 4.5 days), short (4.5 – 38 days), medium (39 – 222 days) and long (> 222 days). The analyses showed that these categories significantly affected the Chla response to TP, with lakes having shorter residence times generally exhibiting a stronger response in Chla with increasing TP concentrations, compared to lakes with longer residence times. In addition, the Chla-TP relationship varied among lake types within the different residence time categories. Based on the results, target TP concentrations were estimated for different Chla class boundary. The findings highlight the importance of considering residence time when setting nutrient targets and assessing ecological status under the Water Framework Directive.