BIOLOGICAL QUALITY INDEXES IN NON-INTERCALIBRATED DANISH LAKE TYPES: PHYTOBENTHOS, PHYTOPLANKTON AND MACROINVERTEBRATES

Scientific Report from DCE – Danish Centre for Environment and Energy No. 694 2026

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Abstract:	This report evaluates whether existing ecological indices can be applied to non-intercalibrated Danish lake types, focusing on phytobenthos, phytoplankton and benthic macroinvertebrates. Using national NOVANA monitoring data and multivariate statistical analyses, the study assesses how well the indices respond to phosphorus and other key environmental gradients across lake types. Results show that the IPS and DLMI are generally applicable to freshwater non-intercalibrated lake types with a minor type-specific correction for IPS in lake type 11, while the phytoplankton index DSPI is suitable for type 10 lakes even at low alkalinity, supporting their use in future river basin management planning.

Keywords:	Ecological indices; Non-intercalibrated lake types; Biological quality elements; Water Framework Directive; IPS; DSPI; DLMI;
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Preface

This scientific report was prepared at the request of Styrelsen for Grøn Arealomlægning og Vandmiljø (SGAV) with the aim of evaluating the potential application of bioindicators for non-intercalibrated Danish lake types. Specifically, it focuses on ecological indexes for benthic diatoms (IPS) and benthic macroinvertebrates (DLMI) in lake types other than 9 and 10 according to the typology for Danish lakes (Søndergaard et al., 2018). For phytoplankton, the report assesses the applicability of the ecological index DSPI to lakes of type 10 with low alkalinity levels (0.2–1.0 mEq/L). These low-alkalinity lakes currently fall below the threshold for inclusion in the corresponding intercalibrated group of the Central-Baltic GIG, which starts at higher alkalinity levels (>1.0 mEq/L); consequently, the phytoplankton index cannot currently be applied to them. This report analyses the most extensive and up-to-date datasets available for each community up to 2023 and compares index responses across lake types for phytobenthos and benthic macroinvertebrates as well as across alkalinity conditions for phytoplankton.

The results of this project have been presented to the SGAV, who have had the opportunity to comment on a draft version of this report.

Forord

Denne videnskabelige rapport er udarbejdet efter anmodning fra Styrelsen for Grøn Arealomlægning og Vandmiljø (SGAV) og har til formål at vurdere anvendelsen af bioindikatorer for ikke-interkalibrerede danske søtyper. Rapporten fokuserer på det økologiske indeks for bentiske kiselalger (IPS) og bentiske makroinvertebrater (DLMI) i søtyper, der ikke omfatter type 9 og 10, i henhold til typologien for danske søer (Søndergaard et al., 2018). For planteplankton vurderer rapporten anvendeligheden af det Danske Sø Planteplankton Indeks (DSPI) i søer af type 10 med lav alkalinitet (0,2–1,0 mEq/L). Disse lavalkaliske søer ligger i øjeblikket under grænsen for indlemmelse i den tilsvarende inter-kalibrerede gruppe i Central-Baltic GIG, som begynder ved et højere alkalini-tetsniveau (>1,0 mEq/L), og som følge heraf kan planteplanktonindekset på nuværende tidspunkt ikke anvendes på disse søer.

Rapporten analyserer de mest omfattende og opdaterede datasæt, der er tilgængelige for hver organismegruppe frem til 2023, og sammenligner indeksenes respons på tværs af søtyper for henholdsvis fytobentos og bentiske makro-invertebrater samt indeksresponsen i forhold til alkalinitetsforhold for plante-plankton.

Resultaterne af dette projekt er blevet præsenteret for SGAV, som har haft mu-lighed for at kommentere et udkast af rapporten.

Summary

This report evaluates the applicability of existing ecological indexes for non-intercalibrated Danish lake types (lake types other than 9 and 10) in compliance with the EU Water Framework Directive, focusing on three biological quality elements: phytobenthos, phytoplankton and benthic macroinvertebrates. For phytoplankton, it assesses the applicability of the ecological index DSPI (Dansk Sø Planteplankton Indeks) in lakes of type 10 with low alkalinity levels (0.2–1.0 mEq/L). These assessments aim to support Denmark's obligations to determine ecological status for lakes ahead of the 2027–2033 River Basin Management Plans (VP4), particularly for lake types lacking intercalibration for these indexes.

The analyses were based on national monitoring data from the NOVANA programme. These included multivariate analyses of community composition, environmental variables and nutrient concentrations, as well as comparative analysis of the responses of the indexes to phosphorus concentrations across lake types (or alkalinity conditions for phytoplankton). The methods applied included non-metric multidimensional scaling (NMDS) and PERMANOVA to evaluate differences in species composition among lake types, linear mixed-effects models to test index responses to phosphorus and canonical correspondence analysis (CCA) to identify key environmental drivers of community structure. Index applicability was assessed based on the ability of existing indexes to capture ecological responses to phosphorus consistently across lake types.

For phytobenthos, the benthic diatom index IPS (Indice de Polluosensibilité Spécifique) showed consistent negative responses to total phosphorus across most non-intercalibrated lake types (1, 2, 5, 11 and 13). All these responses were consistent with intercalibrated lake types 9 and 10. Lake type 11 exhibited lower IPS values, which was due to high conductivity rather than eutrophication. Consequently, it is recommended to use a type-specific correction (+0.7 IPS units) when applying IPS to this lake type. For phytoplankton, the DSPI responded similarly to total phosphorus in low-alkalinity type 10 lakes compared to intercalibrated type 10 lakes, supporting its use without modification. For benthic macroinvertebrates, the Danish littoral macroinvertebrate index (DLMI) performed reliably in freshwater lake types (5, 9, 10, 13), but not in brackish type 11 lakes, where high salinity constrained index applicability.

The results indicate that IPS and DLMI can be applied to most non-intercalibrated freshwater lake types, with minor adjustments for specific types (IPS +0.7 in type 11). Additionally, DSPI can be used for type 10 lakes, even at low alkalinity levels (0.2-1.0mEq/L). This report highlights the need for additional data collection in rare lake types to enable broader validation and application of these indexes, ensuring consistent and accurate assessment of ecological status across Danish lakes.

Sammenfatning

Denne rapport vurderer anvendeligheden af eksisterende økologiske indekser for ikke-interkalibrerede danske søtyper (andre søtyper end 9 og 10) i overens-stemmelse med EU's vandrammedirektiv, med fokus på tre biologiske kvalitetselementer: fytobentos (IPS), planteplankton (DSPI) og bentiske mak-roinvertebrater (DLMI). For planteplankton vurderes anvendeligheden af DSPI i søer af type 10 med lavt alkalinitetsniveau (0,2–1,0 mEq/L).

Analyserne er baseret på nationale overvågningsdata fra NOVANA-programmet. Analyserne bestod af multivariate analyser af sammensætnin-gen af arter, miljøkemidata samt sammenlignende analyser af indeksenes re-sponser på koncentrationen af fosfor på tværs af søtyper (eller niveau for planteplanktons vedkommende). Metoderne omfattede ikke-metrisk multidimensionel skalering (NMDS) og PERMANOVA til at evaluere forskelle i artssammensætning mellem søtyper, lineære mixed-effects modeller til at teste indeksenes respons på fosfor og kanonisk korrespondanceanalyse (CCA) til at identificere de drivende faktorer bag artsdiversitet. Indeksenes anvendelighed blev vurderet ud fra deres evne til at registrere økologisk respons på fosfor på tværs af søtyper.

For fytobentos udviste det bentiske kiselalgeindeks (IPS) konsekvent en negativ respons på total fosfor på tværs af de fleste ikke-interkalibrerede søtyper (1, 2, 5, 13). Denne respons var i overensstemmelse med de interkalibrerede søty-per 9 og 10. Kun type 11 viste lavere IPS-værdier pga. denne søtypes højere ledningsevne, og derfor anbefales en typespecifik korrektion (+0,7 IPS-enheder) for type 11 søer. For planteplankton responderede DSPI på en ensartet måde for total fosfor i lavalkaliske type 10 søer (Typ10_LA) sammenlignet med interkalibrerede type 10 søer (L_CB1), hvilket støtter uændret anvendelse af DSPI for disse. For bentiske makroinvertebrater fungerede det danske litto-rale makroinvertebratindeks (DLMI) pålideligt i ferske søtyper (5, 9, 10, 13), men ikke i brakvandssøer af type 11, hvor høj ledningsevne begrænsede in-deksets anvendelighed.

Resultaterne støtter, at IPS og DLMI kan anvendes i de fleste ikke-interkalibrerede ferske søtyper, med mindre justeringer for specifikke typer (IPS +0,7 i type 11). DSPI kan ligeledes anvendes i søer af type 10, selv ved lave alkalinitetsniveauer (0,2–1,0 mEq/L). Rapporten fremhæver behovet for yder-ligere dataindsamling i sjældne søtyper for at muliggøre en bredere validering og anvendelse af disse indekser og dermed sikre en ensartet og præcis vurdering af økologisk tilstand på tværs af danske søer.

1 Introduction

Under the EU Water Framework Directive, Denmark is required to ensure that Danish lakes achieve good ecological status. This is an environmental objective defined in Executive Order No. 796 of 13 June 2023 and covers rivers, lakes, transitional waters, coastal waters and groundwater. According to the EU Water Framework Directive, ecological status must be determined based on the following quality elements: biological, physical-chemical and hydromorphological elements, as well as the presence of a range of environmentally hazardous chemical substances.

The biological quality elements that must be assessed under the Water Framework Directive are phytoplankton, other aquatic flora, comprising phytobenthos and macrophytes, fish and benthic macroinvertebrates.

Ahead of the preparation of the River Basin Management Plans for the period 2027–2033 (VP4), an assessment of the ecological status of lakes must be performed. Indexes for biological quality elements (bioindicators) in Denmark have been intercalibrated for the most common lake types, corresponding to categories 9 and 10 in the Danish lake typology (Søndergaard et al., 2018). Since indexes for biological quality elements cannot currently be applied in non-intercalibrated lake types, it is necessary to investigate whether it is possible to use or adapt the existing indexes tested for intercalibrated lake types (9 and 10) to other lake types.

1.1 State of Danish ecological indexes for lakes

Each EU Member State is, under the directive, required to develop indexes that enable the use of its quality elements across all lake types. Two lake types, type 9 and type 10, have been intercalibrated in Denmark. In these lake types, all quality elements can therefore be applied, except for a small number of type 10 lakes where, during the intercalibration within the Central-Baltic GIG, a slightly different alkalinity range was used. As a result, the phytoplankton index cannot be applied to lakes with an alkalinity between 0.2 and 1.0 mEq/L (Søndergaard et al., 2020).

For the nine non-intercalibrated lake types, suitable ecological indexes are still lacking, either because no type-specific index has yet been developed, or because the existing indexes used for lake types 9 and 10 could not be tested for their applicability to these other lake types, often due to absence of sufficient data. Since 2003, efforts have been made to develop indexes for all lake types, and this has gradually permitted applying more biological quality elements when assessing the ecological status of lakes. Table 1.1 shows whether an existing and applicable index is available for each lake type.

Table 1.1. Overview of proposed index application. Brackets around "Yes" indicates that a proposal exists, but its relationship with nutrient content is weak or not significant. Table adapted from table 7.1 of Søndergaard et al. (2020).

Lake type	Alk., colour, sal., depth	Macrophyte community composition used	Index used	Phytoplankton community composition used	Index used	Fish community composition used	Index used
1	Low, low, low, shallow	Yes	Yes	Yes	Yes	(Yes)	(Yes)
2	Low, low, low, deep	Too few data		Too few data		Too few data	Too few data
5	Low, high, low, shallow	No	Yes	Yes	Yes	Too few data	
6	Low, high, low, deep	Too few data		Too few data		Too few data	Too few data
9	High, low, low, shallow	Intercalibrated		Intercalibrated		Intercalibrated	
10	High, low, low, deep	Intercalibrated		Intercalibrated		Intercalibrated	
11	High, low, high, shallow	No	(Yes)	Yes	Yes	(Yes)	(Yes)
12	High, low, high, deep	Too few data		Too few data		Too few data	Too few data
13	High, high, low, shallow	Yes	(Yes)	Yes	Yes	(Yes)	(Yes)
14	High, high, low, deep	Too few data		Too few data		Too few data	Too few data
15	High, high, high, shallow	No	(Yes)	Too few data		Too few data	Too few data

To fulfil Denmark's obligations under the EU Water Framework Directive, there are still several lake types for which the existing indexes based on multiple biological quality elements must be tested for their applicability in non-intercalibrated lake types. This is particularly the case for the quality elements benthic macroinvertebrates and the sub-element phytobenthos, for which indexes have so far only been developed and tested for the two intercalibrated lake types 9 and 10. Intercalibrated lake type 10 is limited to lakes with alkalinities higher than 1.0 mEq/L, whereas this categorisation includes lakes with alkalinities > 0.2 mEq/L.

The potential for extending index applicability to non-intercalibrated lake types was most recently evaluated in DCE Scientific Report No. 365 (Søndergaard et al., 2020 (Table 1.1). Since then, additional data have become available, and further work is now needed to adapt and validate the use of the existing indexes for lake types that have not yet been intercalibrated.

This report investigates the applicability of the existing ecological indexes developed for intercalibrated lake types 9 and 10 to non-intercalibrated Danish lake types. Specifically, the established indexes for benthic macroinvertebrates and phytobenthos are assessed regarding their suitability for other lake types, while the phytoplankton index is evaluated for its applicability in type 10 lakes with alkalinity values between 0.2 and 1.0 mEq/L.

2 Methods

2.1 Data retrieval

Data on biological quality indicators and water chemistry were collected as part of the national monitoring programme (NOVANA) for lakes included in VP3 across Denmark, representing 10 different lake types (1, 5, 6, 9, 10, 11, 12, 13, 14, 15). The monitoring data, stored in the VanDa database (https://vanda.miljoeportal.dk/), were retrieved from the database Overfladevandsdatabasen (ODA, ODA.dk). Physical and chemical data, including nutrient concentrations, chlorophyll α and other relevant environmental variables, were calculated as yearly summer averages (May-September) for further analysis. For this report, and due to analytical requirements, only lake types represented by more than seven lakes with both biological index data and water chemistry data were included in the analysis.

2.2 Data analysis

The analysis of each sub-element presented in this report follows the structure presented in Figure 2.1.

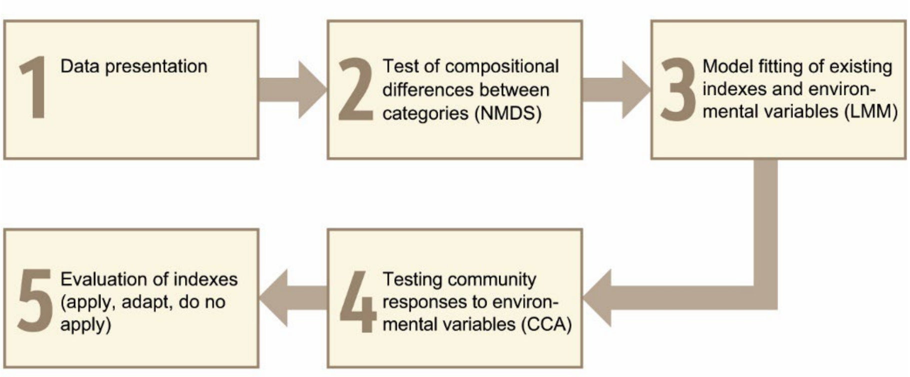


Figure 2.1. Illustration of analysis flow of sub-elements in this report. The primary meth-od applied was non-metric multidimensional scaling (NMDS) in step 2, linear mixed-effect models (LMM) in step 3, and canonical correspondence analysis (CCA) in step 4. Step 5 is further explained in figure 2.2.

2.2.1 Step 1: Data presentation

In the first step of the analysis, the data for each lake type were visualised using bar charts to show the number of lakes within each type and boxplots to il-lustrate the distribution of index values across lake types. This step provides an overview of data distribution and the variability of the indexes across lake types and helps to identify potential differences in index responses among lake types. During this step, lakes with too few data for analysis were identified (< 7 lakes) and excluded from further analysis, while the boxplots revealed preliminary patterns in the data.

2.2.2 Step 2: Compositional differences

All indexes tested in this report rely on community composition as, at least, a sub-component. Because community composition cannot be expected to be similar across lake types, we first examined whether it differed significantly among them. If significant compositional differences were present, these could help explain potential differences in index responses between lake types.

Differences in community composition were assessed for each sub-component of the report using non-metric multidimensional scaling (NMDS). The primary goal of NMDS is to reduce the number of dimensions describing a multivariate compositional dataset and order the data into a two- or three-dimensional datasets with minimal distortion (stress). In this report, NMDS is used to visualise differences in community composition among lake types based on multivariate datasets containing numerous taxa abundance variables.

To support NMDS, PERMANOVA was applied to evaluate whether lake types differ significantly in their biological community composition. This method tests for differences in multivariate centroids among groups based on ecological distance measures. In general, even little variation among categories may cause significant differences in a PERMANOVA test, and it is therefore important to consider how much variation is explained by the category (R2). For this report, we considered $R2 > 0.15$ and p-value < 0.001 as indicating an adequate fit with significant differences.

2.2.3 Step 3: Model fitting

After index calculation, linear mixed-effects models (LMM) were fitted to assess differences in index values and total phosphorous between lake types. These models account for repeated observations from the same lakes rather than assuming independence between all observations. Model significance was evaluated using full–reduced model comparisons, and nested models were compared based on likelihood ratio tests (LRT) and log-likelihood comparisons. Model residuals were checked for normality (normal probability plots) and homogeneity of variance (residuals versus fitted values), and log10 transformations were applied when needed.

After fitting the linear mixed models, estimated marginal means (EMMs) and slopes were compared among typologies to identify potential significant differences (Lenth et al., 2025). This method computes adjusted means (or predictions) for each factor level by averaging over other variables in the model. Pairwise contrasts were performed using Tukey-adjusted comparisons for both typology and total phosphorus levels, and emtrends was used to assess whether the response slopes of each typology differed significantly among typologies.

2.2.4 Step 4: Community response to environmental variables

After identifying potential differences in how index values responded to total phosphorus, we determined which environmental variables drove the observed variation among lake types.

To explore these relationships, canonical correspondence analysis (CCA) was performed. This constrained multivariate analysis estimates the variance in the community composition matrix explained (i.e., constrained) by key environmental variables, avoiding spurious or arbitrary correlations that may occur in unconstrained ordination methods.

CCA allows assessment of how environmental gradients structure biological communities and identifies the main variables contributing to differences between lake types. In a CCA plot, arrows represent variables and their direction shows how they relate to the main shared pattern between the two data groups. Longer arrows indicate stronger influence, while arrows pointing in the same direction are positively related and those pointing in opposite directions are negatively related. Dots that appear close together are more closely associated than those farther apart. For the purpose of this report, CCA analysis allows to determine how lake types differ from each other.

This analysis made it possible for us to evaluate which environmental variables were most strongly associated with differences among lake types and how these factors influenced the relationship between the ecological index and eutrophication, expressed as total phosphorus.

2.2.5 Step 5: Index evaluation

In the final step, the index was evaluated for each non-intercalibrated lake type. For each index and lake type, it was determined whether the existing index could be applied without modification, whether it required modification before application or whether it could not be applied due to lack of data a different response to total phosphorus compared to intercalibrated lakes. This decision process is illustrated in the flowchart in Figure 2.2.

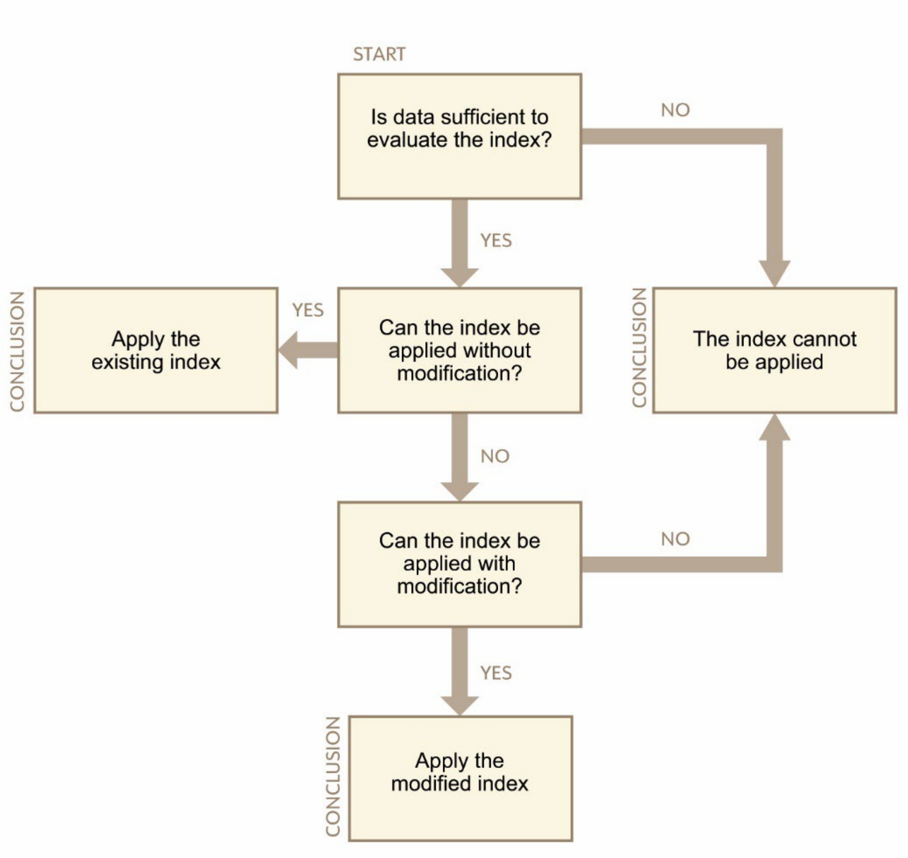


Figure 2.2. Flowchart illustrating the new decision process for determining whether an index can be applied to non-intercalibrated lake types.

One of the objectives of this project was to update Table 1.1 from Søndergaard et al. (2020) by simplifying the classification scheme. Instead of distinguishing between categories such as "insufficient data" and "weak relationships," the updated version presents a binary evaluation indicating whether an applicable index exists or not for each lake type. The decision framework illustrated in Figure 2.2 was applied to determine whether existing indexes could be used (with or without modification) or whether no suitable index was available. The outcome was a revised Table 1.1 providing a clear yes/no classification of index applicability across lake types (see chapter 6).

3 Phytobenthos

3.1 Introduction

Phytobenthos, the community of benthic photoautotrophs, is one of the biological elements selected for the assessment of ecological status of Danish lakes. Among the four quality elements (phytoplankton, other aquatic flora, benthic invertebrates and fish) phytobenthos is integrated with aquatic macrophytes into the quality element referred to as "other aquatic flora".

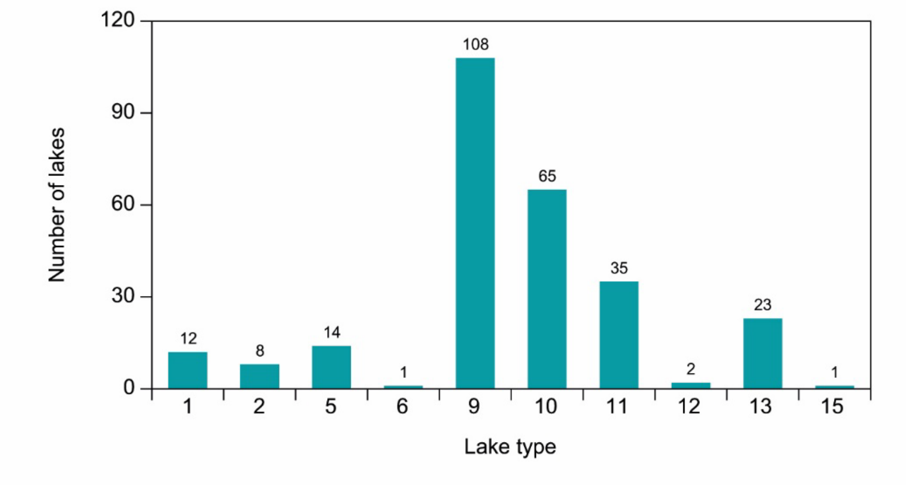
Diatoms are an abundant and diverse taxonomic group within the community of benthic autotrophs in freshwaters. Because benthic diatoms constitute a major and ecologically responsive component of the phytobenthos community, they are commonly used as a practical proxy for phytobenthos, enabling assessment of ecological status to benefit from their sensitivity and well-established indicator value. Their high taxonomic diversity and well-defined sensitivity to environmental gradients have made diatoms a widely applied tool for assessing the ecological status of freshwaters, in line with the requirements of the Water Framework Directive (Schaumburg et al., 2004; Kelly et al., 2008; Stevenson et al., 2010; Poikane et al., 2016).

The use of phytobenthos as an indicator of ecological status is recent in Danish lakes. This became possible through the testing and adoption of the IPS (Indices de Polluosensibilité Spécifique, CEMAGREF 1982) as the most effective index for capturing ecological responses of benthic diatoms to total phosphorus in Danish lakes (Johansson et al., 2019). This diatom-based index focuses on the ecological responses to total phosphorus (TP) gradients as the main nutrient indicating organic contamination and a key limiting nutrient for primary producers, including lake phytobenthos. However, other environmental factors may also affect the composition of benthic diatoms, and, consequently, interpretations of ecological status based on this index. The IPS has only been tested and intercalibrated for lake types 9 and 10, as these are the most common lakes in Denmark and have the most monitoring data (Johansson et al., 2019, Søndergaard et al., 2018). This currently limits the use of the IPS, and therefore of phytobenthos, in Danish lakes for types other than 9 and 10. In this chapter, we analyse the potential use of IPS for non-intercalibrated lake types (lakes other than 9 and 10) following the methods described in section 2.2.

3.2 IPS in non-intercalibrated lake types

A total of 269 lakes were analysed for the period 2013–2023, based on the availability of both phytobenthos and physicochemical data, including nutrients. Most of these lakes (64%) belonged to lake types 9 (40%) and 10 (24%) (Figure 3.1).

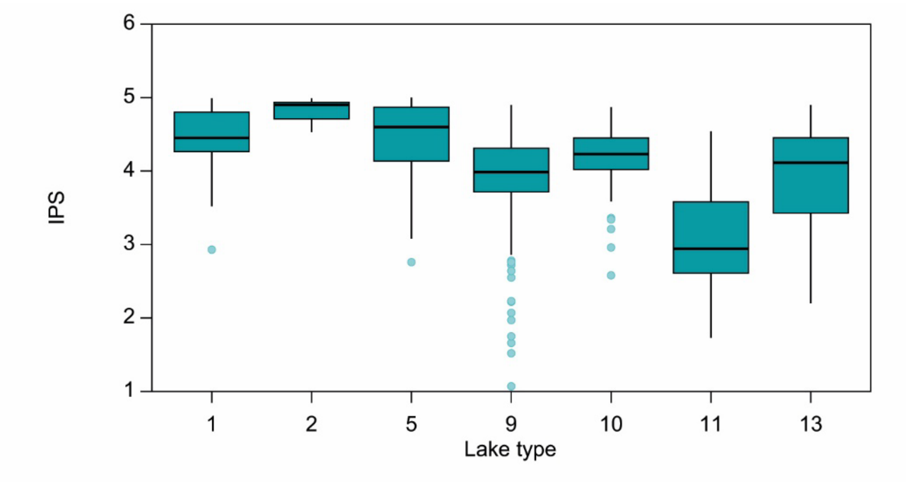
Figure 3.1. Lake number by lake type category according to the Danish lake typology (Søndergaard et al., 2018) for the period 2013-2023.



Some lake types had very few lakes, particularly lake types 6 and 15 with only one lake each, and type 12 with two lakes (Figure 3.1). These lake types were excluded from further analysis because the small sample size does not allow generalisation of responses at lake type level. All other lake types were included in the analysis of IPS responses to TP, considering their repeated measurements over time.

The range of IPS variation was compared across lake types to identify potential relative to intercalibrated lake types 9 and 10 (Figure 3.2). The IPS was calculated based on the species composition of benthic diatoms using the software OMNIDIA 6.1 (Lecointe et al., 1993). Because IPS values can vary between years within the same lake, the analysis considered each lake–year observation, thereby capturing temporal variation within lake type categories.

Figure 3.2. IPS by lake type category in Danish lakes for the period 2013-2023. (Horizontal solid line indicates median of the distribution, the box represents the lower to upper quartile values of the data, the whiskers extend to the last data point beyond 1.5 * the Interquartile Range, circles represent outliers beyond this range).



Kruskal-Wallis analysis of variance indicated differences in the IPS variation across lake types ($\chi2$= 172.2, $p < 0.001$). Post hoc pairwise comparisons using the Dunn test (with Benjamini-Hochberg adjustment) indicated no significant differences in IPS between lake types 9 and 10, or between 9, 10 and type 13. Lake types 1, 2, and 5, showed no differences in IPS but differed significantly from lake types 9 and 10, as well as from lake types 11 and 13. Also, that IPS in lake type 11 was also significantly different from other lake types (Table A1).

These results suggested that IPS responses cannot be directly generalized across all lake types and highlight the need to investigate the factors underlying the distinct IPS variation compared to intercalibrated lake types 9 and 10.

They also indicate a significantly lower range of IPS in lake type 11 compared to all other lake types (Figure 3.2).

To understand how potential compositional differences among lake types could influence IPS, we performed an NMDS analysis of the benthic diatom species, for all analysed lake types (Figure 3.3). This analysis allowed for visual identification of clusters, or groups of lake types based on their benthic diatom composition. For this analysis, data on diatom abundance were log-transformed, and rare species present in less than 5% of samples were excluded.

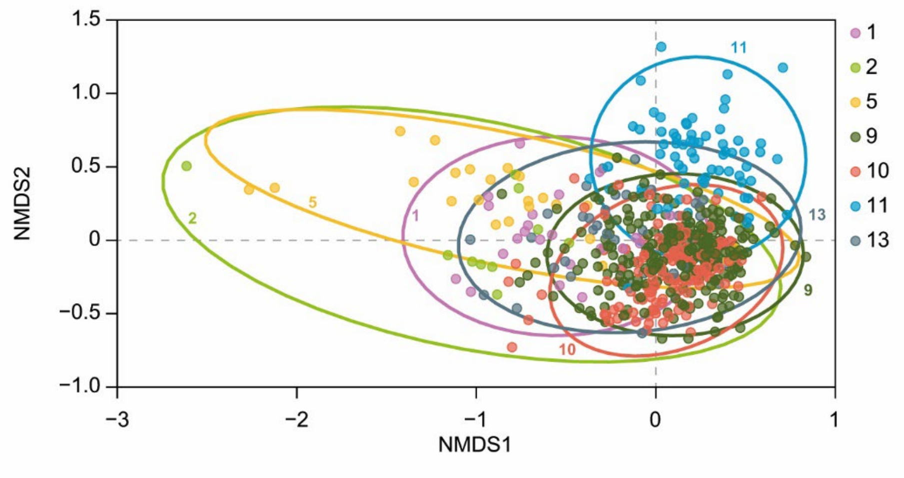


Figure 3.3. Non-metric multidimensional scaling analysis (NMDS) of the diatom composition across Danish lake types, indicated with colour. Stress = 0.24.

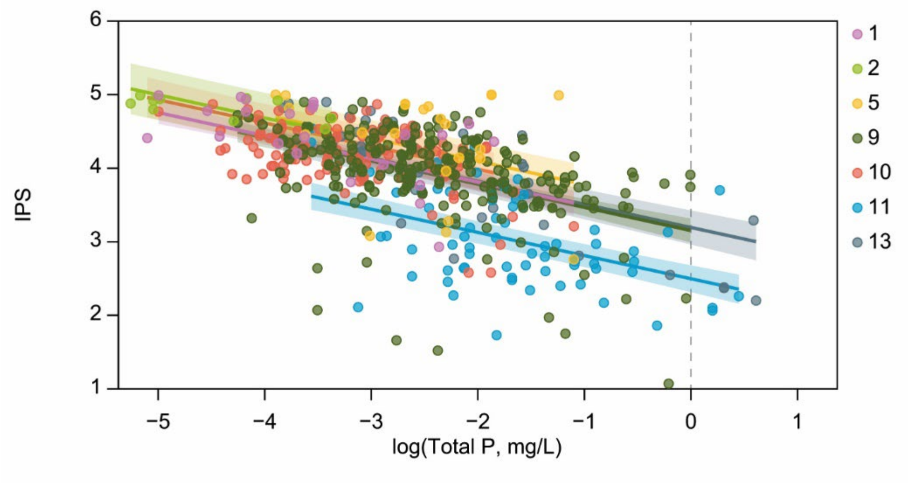
The stress value of the NMDS analysis indicated that groups were not markedly separated, and there was substantial similarity in composition. In general, lake types 2 and 5 seem to share a similar composition, distinct from other lake types. Likewise, lake type 11 differed from the cluster formed by lake types 9, 10 and 13. These compositional differences between lake types were analysed using PERMANOVA (Permutational Multivariate Analysis of Variance) and confirmed by ANOSIM (Analysis of Similarities), both based on 999 permutations and Bray-Curtis distances. PERMANOVA indicated significant differences in benthic diatom composition among lake types (R^2= 0.17, F= 11.58, p= 0.001), and these differences were confirmed by ANOSIM (R= 0.42, p= 0.001). These results suggest that compositional differences in benthic diatoms may contribute to variations in IPS among lake types.

Considering these differences in IPS by lake type and related compositional differences, we analysed how IPS responded to TP while accounting for potential lake type differences using linear mixed-effects models. These models included the effects of TP and lake type as fixed effects and lake identity as random effect to account for repeated measurements in lakes over time.

$$IPS \sim log(TP) * Lake\ type + (1 \mid Lake\ ID) \qquad \text{(Equation 3.1)}$$

IPS declined significantly with increasing TP in all lake types (estimate = -0.32 ± 0.03, T= -10.6, p < 0.001), indicating a similar response to TP across lake types (Figure 3.4). Lake type 11 exhibited consistently lower IPS values compared to the reference lake types 9 and 10 (estimate = -0.86 ± 0.16, T= -5.3, p < 0.001), while other lake types did not differ significantly compared to lake types 9 and 10 (Figure 3.4).

Figure 3.4. Linear mixed-effects model (LMM) estimates and 95% confidence intervals of IPS responses to TP (log-transformed) for all examined lake types, indicated with colour.



These models were then analysed using emtrends analysis, which computes adjusted predictions (means) of IPS in response to TP for each factor level (lake type). This allowed to test whether lake types differ significantly in their IPS responses to TP (different slopes) after accounting for random effects. The comparison of the IPS–TP slopes showed no differences among lake types, indicating that the magnitude of the IPS response to TP was consistent among all lake types, and not even lake type 11 exhibited different IPS-TP slopes (Table A2).

Estimated Marginal Means was used to compare the adjusted means in the IPS-TP response among lake types. Estimated marginal means of IPS showed no significant differences and little variation across most lake types (mean IPS ≈ 4.0–4.3) (Table A3). In contrast, lake type 11 had a substantially lower adjusted IPS (3.31 ± 0.08, 95% CI: 3.15–3.46), being ~ 0.7–1 lower compared to other lake types (Table A3). Tukey-adjusted pairwise comparisons confirmed that lake type 11 differed significantly ($p < 0.001$) from all the other types, while no significant differences occurred among the remaining lake types. These results indicate that IPS responds similarly to log(TP) across all the analysed lake, with the exception of lake type 11 where IPS is 0.7 to 1 unit lower.

Direct comparisons of IPS values from lake type 11 to those of other lake types are therefore not appropriate. To ensure a comparable assessment of ecological status based on IPS, a type-specific correction for lake type 11 can be applied so that its IPS–log(TP) relationship aligns with the responses observed in the other lake types. A simple additive correction (+0.7 IPS units) could technically harmonise these values. However, such a correction assumes only a systematic bias in the IPS for lake type 11 and does not account for underlying ecological differences. Therefore, ecological validation is required, particularly to identify the factors explaining these differences in the IPS–log(TP) relationship for lake type 11 compared to other lake types. This step is essential to rule out the possibility that the lower IPS values in lake type 11 are genuinely caused by organic contamination or eutrophication. If other environmental factors, unrelated to organic pollution or nutrient enrichment, are found to ex-plain this different response, then a type-specific correction can be applied, al-lowing the use of an adjusted IPS for lake type 11.

To test this, we performed multivariate analyses to identify the main environmental variables influencing benthic diatom composition among different lake types, with particular focus on lake type 11. To estimate species responses along environmental gradients, we first performed a detrended correspondence analysis (DCA). This revealed a long gradient (5.6 standard deviations), indicating unimodal species responses. Consequently, canonical correspondence analysis (CCA) was selected as the appropriate constrained multi-variate method to explore the factors shaping benthic diatom composition. The environmental variables included in the model were log-transformed and stepwise selected based on their significance in the CCA model. Collinearity among environmental variables was tested by Pearsons's correlation and variance inflation factor (VIF) inspection, where variables with a high VIF (>10), overexplaining variance, were sequentially removed from the model. The significance of the model and the selected environmental variables was tested by Monte-Carlo permutational test with 999 iterations. The CCA model adequately explained benthic diatom composition in Danish lakes of different types ($F= 4.7$, $p< 0.001$) (Figure 3.5).

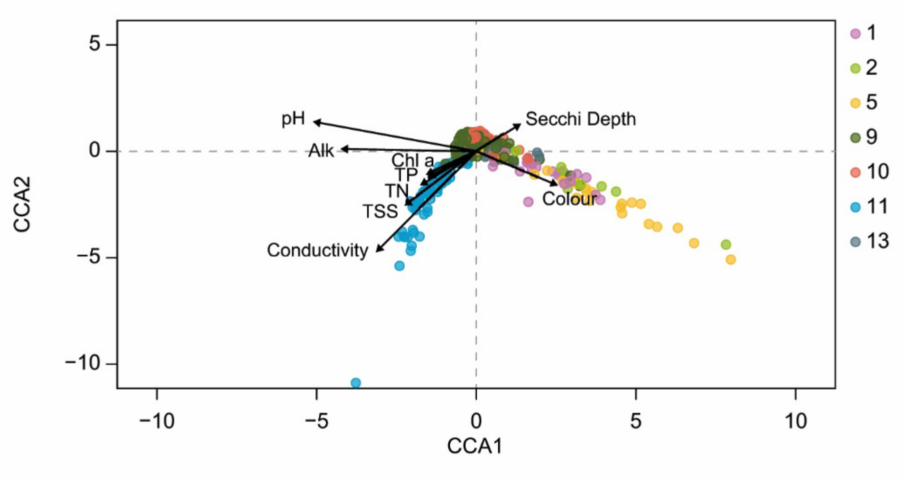


Figure 3.5. Canonical Correspondence Analysis of the benthic diatom composition in different Danish lake types. Arrows represent contribution from environmental variables to explaining species composition. Included environmental varia-bles: Secchi depth, suspended solids (TSS), total nitrogen (TN), total phosphorous (TP), pH, conductivity, color and total alkalinity (Alk).

CCA indicated that among the several variables influencing benthic diatom composition for different lake types, conductivity is the main environmental variable explaining the benthic diatom composition in lake type 11 (Figure 3.5). Other variables such as pH, alkalinity, colour and those related to eutrophication, such as nutrients, chlorophyll a (estimator of phytoplankton biomass), suspended solids and Secchi depth were relevant in explaining the benthic diatom composition for all lake types but had less influence than conductivity for lake type 11 (Figure 3.5).

Lake type 11 is characterised by high salinity, and accordingly, conductivity compared to intercalibrated lake type 9 (Søndergaard *et al.*, 2018). This elevated conductivity of lake type 11 (Figure A1) influenced the diatom composition (Fig. 3.5) and was therefore tested as the main factor influencing its different IPS-log(TP) response.

To address this, the environmental factors explaining the compositional differences in benthic diatoms was first tested, by comparing lake type 11 to intercalibrated lake types 9 and 10. The PERMANOVA indicated that, among all tested environmental variables, conductivity was the most relevant in explaining the differences in the benthic diatom composition in lake type 11.

Next, to evaluate the role of conductivity in explaining the lower IPS range in response to TP in lake type 11, two LMMs were compared:

the initial LMM including lake type as an explanatory factor of IPS:

$$IPS \sim log(TP) * \ Lake\ type\ +\ (1\,|\,Lake\ ID)$$

and a model including both lake type and conductivity as explanatory variables of IPS

$$IPS \sim log(TP) * \ Lake\ type\ + \log(Conductivity) + \ (1 \mid Lake\ ID)$$

This comparison showed that including conductivity as an environmental variable influencing IPS substantially improved the model fit (AIC: 697 to 683, BIC: 740 to 730, logLik: 639 to 630). The previously significant effect of lake type 11 was overridden by conductivity, losing its significance once conductivity was added to the model. Likelihood ratio tests (LRT) indicated that the model including conductivity explained the IPS-log(TP) relationships better than the model including lake type (L ratio= 16.2, p < 0.001).

Together, this evidence supports that conductivity is the only environmental variable explaining the lower range of IPS variation in lake type 11 compared to other lake types, independent of TP. Compared with intercalibrated lake type 9, type 11 lakes are characterised by their higher salinity, which is reflected in a significantly higher conductivity. Water conductivity reflects the concentration of dissolved ions (salts) in the water and is therefore an indicator of the salinity of the lake. Salinity is widely recognised as a relevant factor influencing diatom biomass and composition in inland waters (Stenger-Kovács et al., 2023). The results indicate that conductivity not only structures diatom communities differently in lake type 11 but also constrains the IPS metric, thereby explaining the apparent type-specific differences in the IPS-log(TP) responses.

3.3 Conclusions

The analysis of 269 Danish lakes during the period 2013–2023 indicated that IPS responds consistently to TP across the majority of non-intercalibrated lake types. Linear mixed-effects models showed a significant decline in IPS with increasing TP in all lake types, with no differences in the magnitude of the response among lakes, indicating a similar sensitivity of IPS to TP. Direct comparisons of adjusted IPS further indicated that lake types 1, 2, 5 and 13 have comparable IPS-log(TP) responses to intercalibrated lake types 9 and 10, supporting the use of IPS in these lake types without any restriction or type-specific adjustment.

Lake type 11 represents an exception, with consistently lower IPS values relative to other lake types. This is due to higher conductivity influencing benthic diatom composition rather than nutrient levels. The results indicate that IPS can still be applied to lake type 11, provided a conservative correction of IPS +0.7 units to align its estimated values with those of other lake types. This adjustment ensures comparable ecological assessment while avoiding overestimation of IPS and reflects the systematic influence of conductivity rather than eutrophication. These results support the broader applicability of IPS across non-intercalibrated lake types, with a simple, validated adjustment for lake type 11.

4 Phytoplankton

4.1 Introduction

Phytoplankton in Danish lakes account for a large share of total primary production and play a major role in the overall environmental status. In most cases, phosphorus is considered the most limiting nutrient, and despite large natural variations, there is a clear positive relationship between phosphorus concentration and phytoplankton biomass.

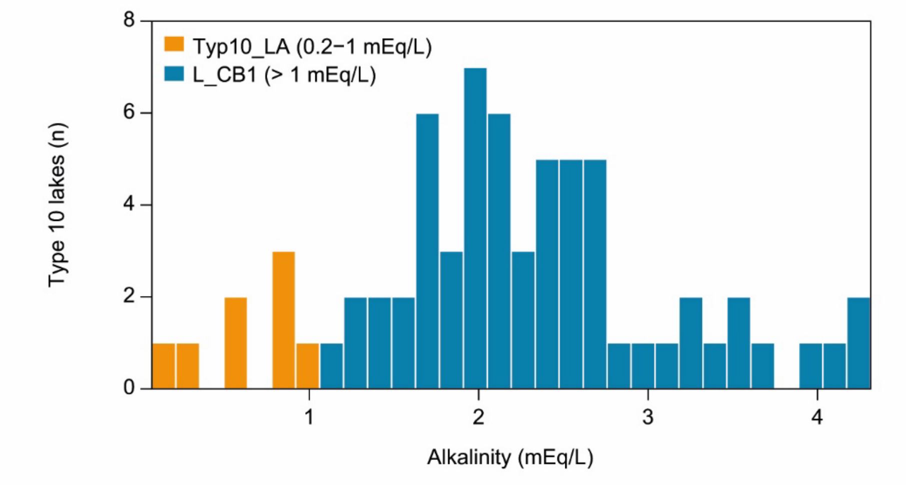
The two EU-intercalibrated lake types currently included in the Danish freshwater management plans are L_CB1 (Danish type 10) and L_CB2 (Danish type 9). However, Danish type 10 lakes are not directly equivalent to the inter-calibrated L_CB1 type, as they are defined by a lower alkalinity threshold of 0.2 mEq/L compared to 1 for L_CB1. This means that in previous analysis of the Danish phytoplankton index (Dansk Sø Planteplankton Indeks, DSPI), only type 10 lakes corresponding to L_CB1 (alkalinity >1 mEq/L) have been included. As a result, several lakes have been excluded and categorised as unknown regarding phytoplankton ecological status assessment. The DSPI is specifically designed to respond negatively to eutrophication (Søndergaard et al., 2013). Its components consist of chlorophyll a in water (µg/L) as an estimator of total phytoplankton biomass, relative biovolume of cyanobacteria (%), chrysophytes (%) and a selection of indicator species (Søndergaard et al., 2013). It is reasonable to assume that each of these index parameters could be influenced by low alkalinity, skewing DSPI values.

The purpose of this chapter is to test whether the phytoplankton index currently used for L_CB1 can also be applied to type 10 lakes with low alkalinity (0.2-1 mEq/L), hereafter referred to as Typ10_LA. To address this, we applied the method described in section 2.2.

4.2 DSPI in low-alkalinity lakes of type 10

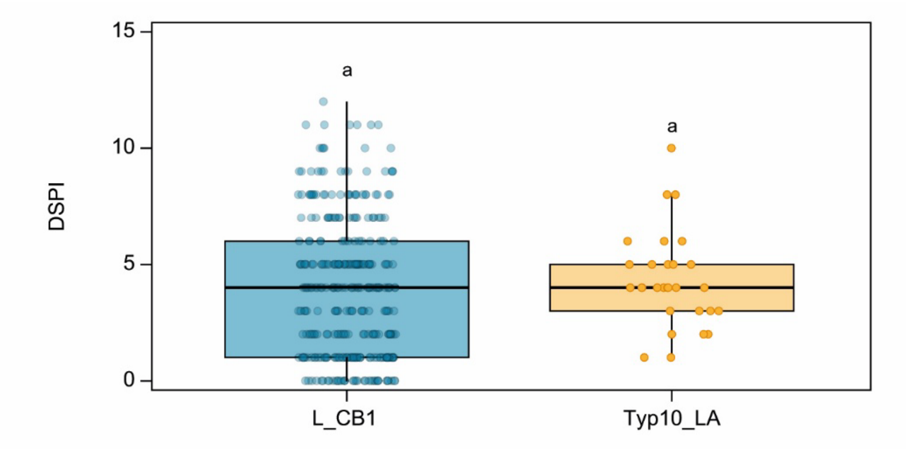
For lakes to be included in this part of the analysis, they had to meet specific criteria. Data needed to be available for both water chemistry and phytoplankton community composition. Water chemistry data were required to assign alkalinity groups and test the effect of environmental stressors on the index, while phytoplankton community composition data were necessary for calculation of the DSPI as well as testing differences in community composition between alkalinity groups.

Figure 4.1. Distribution of Danish type 10 lakes by alkalinity. Colour-coded according to intercalibrated EU lake type L_CB1 (blue) or lower alkalinity Typ10_LA (0.2-1.0 mEq/L, red).



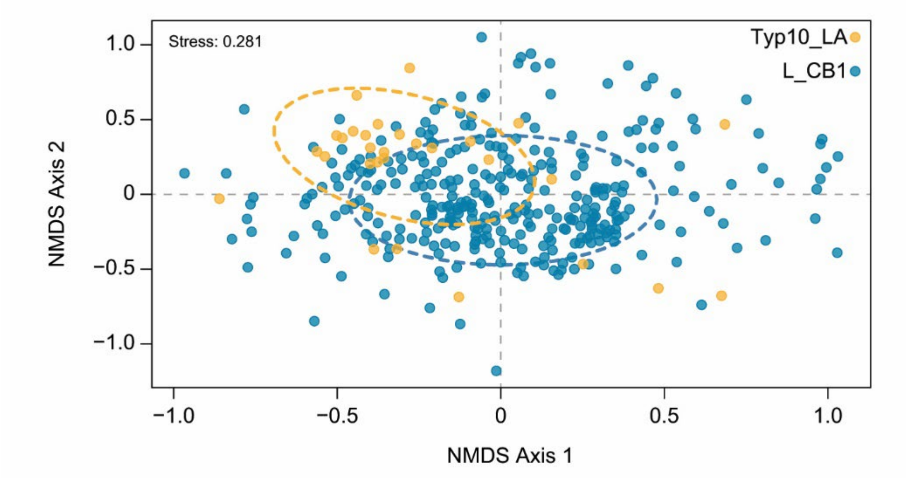
In the Danish national dataset, 67 lakes met these criteria and were included in the analysis. Of these, 60 belonged to the EU-intercalibrated L_CB1 lake type, while the remaining seven, characterised by lower alkalinity, were classified as Typ10_LA lakes (Figure 4.1).

Figure 4.2. Distribution of DSPI based on alkalinity group. Colour-coded according to intercalibrated EU lake type L_CB1 (blue) or lower alkalinity Typ10_LA (0.2-1.0 mEq/L, red). Kruskal Wallis test revealed no significant differences between alkalinity groups (N = 355, $\chi2$ = 0.78, p = 0.377). Boxplot ranges defined as de-scribed in Figure 3.2, letters indicate lack of significant differences.



All lakes had multiple observations included in the dataset spanning from the earliest measurements in 1989 to the most recent in 2021, resulting in a combined dataset of 354 observations across these 67 lakes. Of these, 325 were from type L_CB1 lakes, while 29 were from Typ10_LA lakes. After calculating DSPI, there were no significant differences between alkalinity groups (Figure 4.2).

Figure 4.3. Non-metric multidimensional scaling (NMDS) of phytoplankton community composition in Danish type 10 lakes showing differences between intercalibrated EU lakes (L_CB1, blue) and type 10 lakes in the low-alkalinity range (0.2-1.0 mEq/L, Typ10_LA, red). Stress = 0.28.



Species composition was tested using NMDS (Figure 4.3), and statistical differences between the identified groups were tested using PERMANOVA (Table 4.1). The test results exhibited statistically significant differences between the two alkalinity groups (L_CB1 and Typ10_LA); however, alkalinity ex-plains only a small proportion of the total variation in community composition (R^2 = 0.01, p = 0.001).

Although statistically significant differences were detected between alkalinity groups (Table 4.1), the R^2 value was below the threshold at which differences were considered influential (<0.15). Therefore, these differences are not sufficiently substantial to indicate significant variation in phytoplankton composition, explaining the differences in DSPI responses between alkalinity groups. To further examine potential effects of alkalinity, the phytoplankton index (DSPI) was calculated, and its relationship with TP was assessed (Figure 4.3).

Table 4.1. PERMANOVA results showing differences in community composition between EU-intercalibrated lakes (L_CB1) and lower-alkalinity lakes (Typ10_LA) within Danish type 10 lakes.

Factor	DF	Sum of Sqs	R^2	F	*p*
Alkalinity	1	2.06	0.01	5.12	0.001
Residual	354	142.13	0.99		
Total	355	144.19	1		

The index was calculated using the method presented in box 2.1.1 of Søndergaard et al. (2013) and then tested against TP concentration applying a linear mixed-effects model (Equation 4.1).

Equation 4.1: Linear mixed-effects model was fitted to assess the effects of TP and alkalinity group (L_CB1 and Typ10_LA) on the phytoplankton index (DSPI). Lake ID was added as a random effect to account for non-independence between samplings within lakes over time.

$$DSPI \sim log(TP) * \ Alkalinity\ group \ + \ (1 \mid Lake\ ID)$$

A significant negative relationship between DSPI and TP had already been established by Søndergaard et al. (2013). Therefore, the goal of this analysis was to elucidate whether the phytoplankton index for Typ10_LA lakes responded significantly differently to phosphorous than L_CB1 lakes.

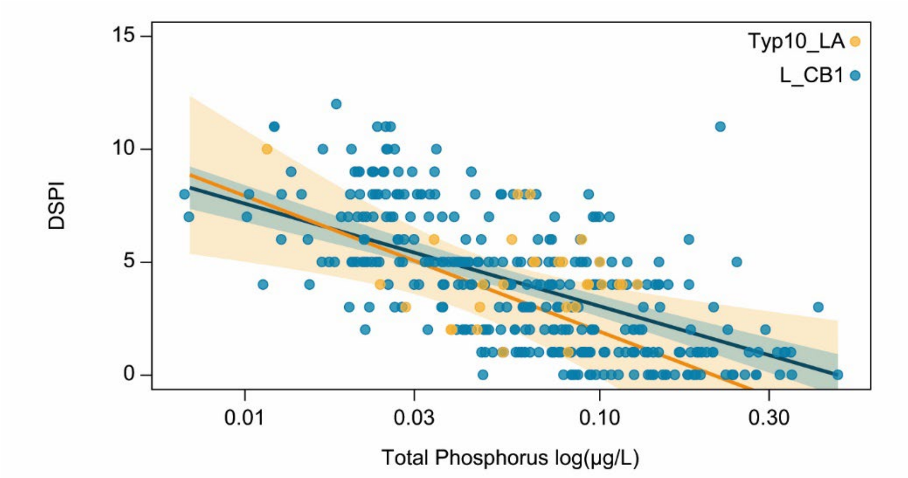


Figure 4.4. Linear mixed-effects model (LMM) estimates and confidence intervals of the Danish phytoplankton index (DSPI) in response to total phosphorous (log-transformed) in type 10 lakes. Colour indicates differences between intercalibrated EU lakes (L_CB1, blue) and lakes with lower alkalinity (Typ10_LA, red).

The phytoplankton index calculated for the two alkalinity groups responded similarly to TP, with complete overlap of standard deviations (Figure 4.4). Standard deviation for Typ10_LA was higher than for L_CB1, likely because of the relatively low number of samples compared to L_CB1. When differences in slope were compared using a Satterthwaite Type III ANOVA, no significant difference was found between alkalinity groups, but a strong relationship with TP occurred (Table 4.2).

Table 4.2. ANOVA type III, Kenward-Roger significance test of the linear mixed-effects model for the impact of total phosphorus (log-transformed) and alkalinity group on DSPI.

Factor	Sum of squares	Mean square	DenDF	F	*p*
Log(TP)	72.86	72.86	244.08	23.7	<0.001
Alkalinity group	2.2	2.2	243.28	0.46	0.398
Log(TP):Alkalinity group	1.41	1.41	243.28	0.46	0.490

It was thus established that there is no difference in the slope or confidence intervals between alkalinity groups with regard to response of index values to TP. To investigate potential environmental variables explaining the species composition of phytoplankton in lakes, a CCA was performed using community composition and the primary water environmental variables (total nitrogen (TN), suspended solids (SS), total phosphorous (TP), pH, Secchi disk depth and alkalinity (TA_env)). This CCA was performed to identify potential compositional differences between alkalinity groups that might be explained by factors other than alkalinity. For example, eutrophication-related variables such as nutrients and reduced water transparency could indicate whether relevant ecological-status differences exist between alkalinity groups.

The CCA showed that lake phytoplankton community composition was explained by two main gradients associated with canonical axis 1 and 2 (Figure 4.5). The first gradient, represented by axis 1 (horizontal), reflected eutrophication, characterised by high TN, TP, SS, higher pH and lower Secchi depth. The second (vertical) gradient, represented by axis 2, was primarily associated with alkalinity.

The CCA clearly separated the influence of alkalinity from the eutrophication axis, which indicates that any effect of alkalinity on community composition operates independently of eutrophication. This suggests that differences in community composition between alkalinity groups are not driven by other environmental variables but are attributable solely to alkalinity.

4.3 Conclusions

The results here indicate that there are no significant differences in the response of DSPI to TP between lower alkalinity (0.2 – 1.0 mEq/L) lake type 10 and higher alkalinity lakes (>1.0 mEq/L). This is supported by the relationship between DSPI and TP, which did not differ significantly between alkalinity groups. DSPI is specifically designed to respond to eutrophication, and the results here indicate that eutrophication effects operate independently of alkalinity in lake type 10. These findings strongly support that DSPI, currently ap-plied only to lakes with alkalinity levels corresponding to the intercalibrated type L_CB1, may also be used in lakes with lower alkalinity, specifically those classified in this report as Typ10_LA.

5 Macroinvertebrates

5.1 Introduction

In Denmark, an index has been developed based on the community composition of benthic (bottom-dwelling) macroinvertebrates in the littoral zone of lakes (Wiberg-Larsen & Rasmussen, 2020). DLMI, the Danish littoral macroinvertebrate index, has been intercalibrated with other similar indexes from comparable EU countries for lake types 9 and 10 but not for the other Danish lake types. The purpose of this chapter is to evaluate whether DLMI, developed for lake types 9 and 10, can be used for the non-intercalibrated Danish lake types in its current form or with modification.

The DLMI is calculated as the average of four sub-indexes, which are adapted versions of indexes originally developed for other countries and adapted for use in Denmark (Wiberg-Larsen, 2025). As with most other indexes in the Danish implementation of the Water Framework Directive (Directive 2000/60/EU), the DLMI index is designed to be sensitive to eutrophication (Wiberg-Larsen & Rasmussen, 2020).

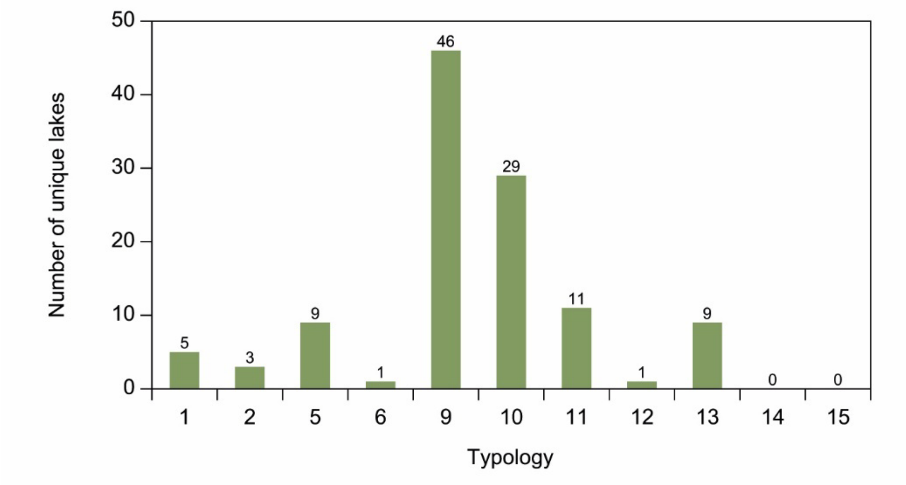
Macroinvertebrate species composition and abundance can vary between lake types, which may result in different DLMI responses as a predictor of eutrophication, particularly in relation to total phosphorus (TP).

5.2 DLMI in non-intercalibrated lake types

For lakes to be included in this part of the analysis, they had to meet specific criteria. Data needed to be available for both water chemistry and macroinvertebrate community composition. Water chemistry data were required to test the influence of environmental variables on the index, while macroinvertebrate community compositional data were necessary for calculating the DLMI and assessing differences in community composition between lake types.

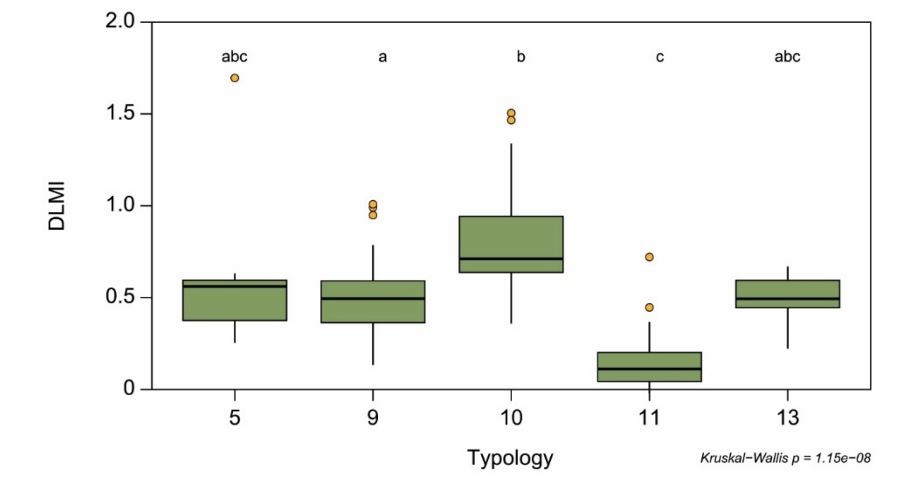
In the Danish national dataset, the most prevalent lake types were 9 and 10, which are also the types where the DLMI is currently implemented (Figure 5.1). Other lake types occur much less frequently in the dataset, and it was decided that only lake type 5, 11 and 13 contained enough unique lakes (>7) to be included in the analysis. Some lakes have multiple observations spanning from the earliest measurements in 2012 to the most recent in 2023. The result is a combined dataset of 113 observations from 104 unique lakes of types 5, 9, 10, 11 and 13.

Figure 5.1. Distribution of Danish lakes with macroinvertebrate community and chemistry data by lake type. Numbers above bars indicate the number of lakes represented in each category.



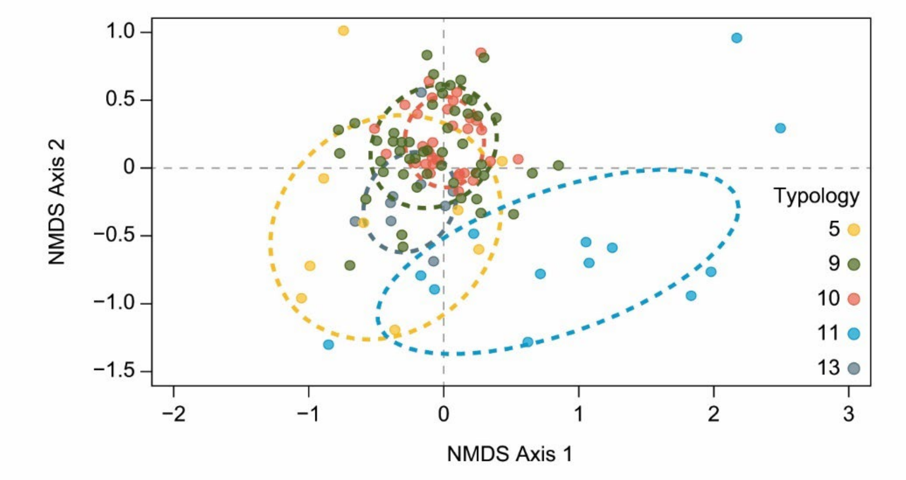
The DLMI index was calculated following the methods described by Wiberg-Larsen & Rasmussen (2020). Boxplots of index values for each lake type are shown in Figure 5.2. The results indicate that lake type 11 has significantly lower DLMI values than types 9 and 10, while type 10 has significantly higher values than both 9 and 11. In contrast, lake types 5 and 13 did not differ significantly from any of the other types (Table A4). These differences in the range of variation of the DLMI, compared with the intercalibrated lake types (9 and 10), may indicate an impact of environmental factors or compositional differences that could influence the DLMI response to TP across lake types.

Figure 5.2. Distribution of DLMI values across all lake types included in the analysis. Letters above boxplots indicate significant differences between groups based on a Kruskal-Wallis test (results in table A4). Boxplot ranges defined as described in Figure 3.2.



To explore the causes of the differences in DLMI variation across lake types, community composition was investigated using an NMDS (Figure 5.3), and the potential compositional differences were tested with a PERMANOVA test (Table 5.1).

Figure 5.3. Non-metric multidimensional scaling (NMDS) of macroinvertebrate community composition in Danish lakes illustrating differences between lake types. Stress = 0.21.



The NMDS indicated the presence of distinct compositional groups (Figure 5.3). There was substantial compositional overlap between lake types 9, 10 and 13. Lake type 5 partially overlapped with this cluster and, to a lower extent, with lake type 11. Lake type 11 showed a distinct composition, with low species overlap compared to the intercalibrated lakes 9 and 10 (Figure 5.3).

PERMANOVA results confirmed compositional differences between lake types (Table 5.1). Given the broad range of environmental adaptations among macroinvertebrates, it is unlikely that species community structure would be identical among all Danish lake types. Adaptations to light availability (colour), acidity (alkalinity), salinity (conductivity) and depth are all well established in the literature and underpin the categorisation of lake types. Some lakes with different communities may still respond similarly to certain stressors.

Table 5.1. PERMANOVA results showing differences in community composition between lake types.

Factor	DF	Sum of Sqs	R^2	F	*p*
Lake type	4	4.96	0.15	4.67	<0.001
Residual	108	28.64	0.85		
Total	112	33.6	1		

The general differences in composition between lake types were corroborated by pairwise PERMANOVA tests, exhibiting differences between all lake types (Table 5.2). The magnitude (R^2) of differences was particularly significant for lake type 11 compared to other lake types.

Table 5.2. Pairwise PERMANOVA results showing differences in community composition between lake types. The table is sorted according to highest R2.

Lake type		Sum of Sqs	R^2	F	*p*
10	11	2.49	0.18	9.7	<0.001
11	13	1.14	0.15	3.42	<0.001
5	11	1.21	0.14	3.36	<0.001
5	10	1.49	0.14	6.49	<0.001
9	11	2.19	0.11	7.57	<0.001
5	13	0.49	0.1	1.71	0.02
10	13	0.84	0.09	3.9	<0.001
5	9	1.18	0.07	4.33	<0.001
9	10	0.67	0.03	2.78	<0.001
9	13	0.5	0.03	1.9	0.01

The differences in macroinvertebrate species composition may influence the DLMI response to TP across lake types. To examine this, the relationship between DLMI and phosphorus was evaluated for different lake types. The analysis assumes that the DLMI is sensitive to eutrophication in lake types 9 and 10 (Wiberg-Larsen & Rasmussen, 2020). Accordingly, phosphorus was selected as the primary environmental variable related to eutrophication in Danish lakes, and in accordance with the effects evaluated for other indexes, DLMI was therefore tested against TP concentration (logTP) using a linear mixed-effects models (Equation 5.1).

Equation 5.1: Linear mixed-effects models for DLMI in response to TP and lake type. Lake ID was included as a random effect to account for non-independence between samplings within lakes over time.

$$DLMI \sim log(TP) * Lake\ type\ +\ (1\ |\ Lake\ ID)$$

In the original DLMI report, a weak relationship between DLMI and total phosphorus (TP) was found, and the index was therefore evaluated against a eutrophication factor (Wiberg-Larsen & Rasmussen, 2020). In the present report, a stronger relationship between DLMI and TP was observed than previously reported for DLMI versus the eutrophication factor (R^2 = 0.36 vs. R^2 =0.42 in this study). For comparability, the eutrophication factor used in the original report was also calculated; however, its relationship with DLMI was weaker than the relationship between DLMI and TP in this study. These differences are likely attributable to methodological differences between the studies. In the original report, individual samples were treated as independent observations, whereas in the present study DLMI was calculated at the lake level and methods accounting for non-independence among sites and systematic variation between years were applied. This approach is considered to provide a more robust statistical evaluation. Given the improved statistical performance and consistency with the evaluation of other biological quality indices, TP is a sufficient and appropriate pressure variable for assessing the applicability of DLMI across lake types. Biologically, littoral invertebrate communities are expected to respond to nutrient enrichment through changes in habitat structure and organic matter, which supports the observed DLMI–TP relationship.

Figure 5.4 shows regression line estimates and confidence intervals of the linear mixed-effects models for each lake type in response to phosphorous concentrations. DLMI declined significantly with increasing TP in all lake types except type 11. The relationship with phosphorous was confirmed by an

ANOVA test (Table 5.3), and the differences between the individual lake types were analysed with Tukey's posthoc test (Table 5.4). Lake type 11 exhibited consistently lower DLMI values compared to the reference types 9 and 10, while other lake types did not differ significantly (Figure 5.4, Table 5.4).

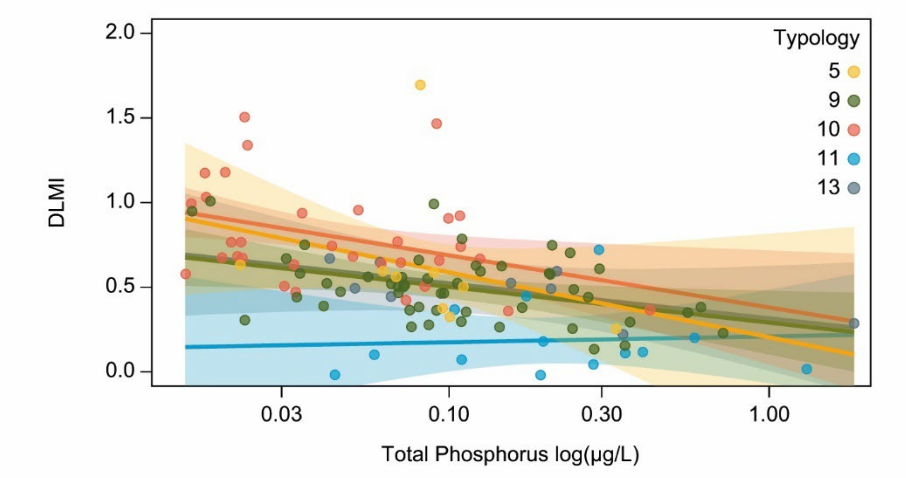


Figure 5.4. Linear mixed-effects model (LMM) estimates and 95% confidence intervals of the Danish macroinvertebrate index (DLMI) responses to total phosphorous (log transformed) in Danish lakes relative to lake type.

Table 5.3. Results from the ANOVA using Satterthwaite's method showing the effects of log-transformed total phosphorus (logTP), lake typology, and their interaction on the response variable.

Factor	Sum Sq	Mean Sq	NumDF	DenDF	F	*p*
logTP	0.34	0.34	1	97.4	7.3	0.01
Typology	0.03	0.01	4	89.27	0.18	0.95
logTP:Typology	0.14	0.03	4	93.28	0.74	0.56

Type 11 lakes are similar to the more common and intercalibrated lake type 9, except for their defining characteristic: high salinity. The threshold for classifying a lake as brackish is 0.5‰, meaning that brackish lakes exhibit a much broader range of salinity values (0.5 - 33.8‰) compared to freshwater lakes (0 - 0.5‰) (Søndergaard et al., 2018).

Table 5.4. Pairwise comparisons of LMM regressions between lake typologies. Degrees of freedom were estimated using the Kenward–Roger method, and p-values were adjusted using Tukey's correction. Significance: *** p < 0.001, ** p < 0.01, * p < 0.05.

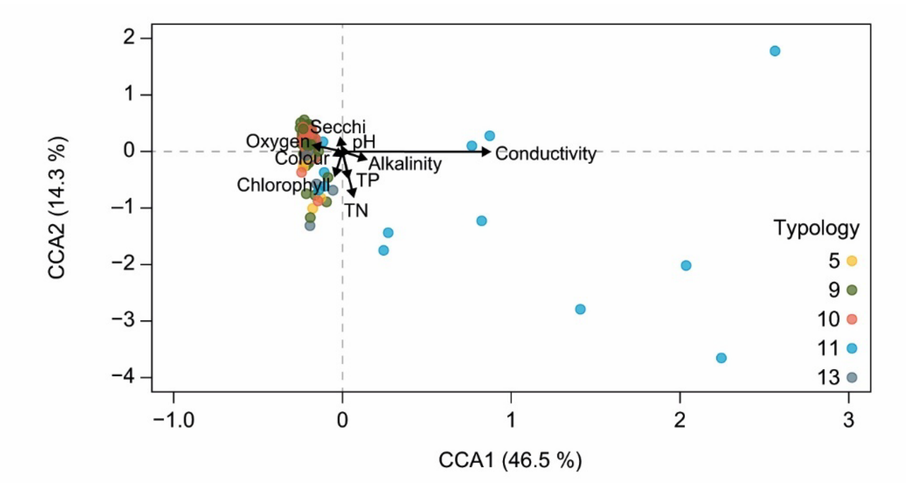
Lake type		TP	Estimate	SE	df	t.ratio	*p*	Significance
11	10	0.16	0.45	0.11	97.71	4.19	<0.001	***
11	9	0.16	0.28	0.08	93.17	3.45	0.01	**
11	13	0.16	-0.3	0.11	98.28	-2.8	0.05	*
11	5	0.16	0.33	0.13	99.62	2.55	0.09	
10	9	0.16	-0.17	0.09	100.84	-1.91	0.32	
10	13	0.16	0.15	0.11	101.52	1.3	0.69	
10	5	0.16	-0.12	0.13	101.59	-0.86	0.91	
5	9	0.16	0.05	0.12	101.5	0.45	0.99	
5	13	0.16	0.03	0.13	101.76	0.23	1	
9	13	0.16	-0.02	0.09	101.32	-0.24	1	

The structuring role played by salinity in lake communities is well established in the literature (Cunillera-Montcusí et al., 2022; Søndergaard et al., 2018) and has been associated to relevant effects on phytobenthos composition and to differential responses of the IPS ecological index in Danish lakes (Chapter 3). Salinity imposes strict osmosis requirements on water-dwelling creatures, and brackish lake types, as defined by the Danish monitoring programme, are expected to host a broader range of species with different salinity tolerances compared to freshwater lakes. Some lakes categorised as brackish may only have slightly higher salinity than freshwater lakes, while others may approach marine conditions. Furthermore, some species may be absent or occur in lower abundances in brackish lakes than in freshwater lakes, which could compromise index accuracy if they are used as indicator species. An indicator species cannot be used where its natural occurrence is unlikely.

All four sub-indexes used in the DLMI (ASPT, Hill, EPTCBO and %COP) were designed for application in freshwater lakes. Some of them may even be sensitive to brackish or saline conditions. For example, the ASPT index assigns scores based on a list of indicator species (see Wiberg-Larsen & Rasmussen, 2020), which is heavily weighted towards freshwater macroinvertebrates. The EPTCBO index represents the number of taxa belonging to Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata, while %COP reflects the relative abundances of Coleoptera, Odonata and Plecoptera. Both EPTCBO and %COP are based on taxa that show a strong association with freshwater rather than brackish environments.

A CCA was performed to investigate the causes of the macroinvertebrate compositional differences between lake types that might explain the observed poor response of the DLMI to TP for type 11 brackish lakes (Figure 5.5).

Figure 5.5. Canonical correlation analysis (CCA) of littoral macroinvertebrate community composition in Danish lake according to type. Arrows indicate environmental variables and their relative impact on community composition.



Canonical correlation analysis (Figure 5.5) showed that conductivity was by far the most important environmental variable driving macroinvertebrate compositional differences in lake type 11 compared to other lake types. These results indicate that salinity, estimated as conductivity, is the main environmental factor shaping macroinvertebrate community structure in Danish lakes and particularly explains the distinct composition observed in lake type 11. This divergence in community composition, caused by the higher salinity in lake type 11, can explain the different DLMI responses to TP in this lake type compared to others.

5.2.1 Evaluation of DLMI in intercalibrated lake types 9 and 10

Based on the results from both this, and the previous report by Wiberg-Larsen and Rasmussen (2020), the use of the existing DLMI index on the already intercalibrated lake types 9 and 10 was evaluated.

The DLMI is a multimetric index based on four sub-indexes. The sub-indexes are ASPT, Hill1, EPTCBO, and %COP, and the DLMI is an average of these four. (Wiberg-Larsen and Rasmussen, 2020). ASPT is an index developed in the U.K. to assess ecological quality of streams, Hill1 is defined as exp(Shannon-Wiener Index), EPTCBO is the number of taxa of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata, and %COP is the relative abundance of Coleoptera, Odonata and Plecoptera.

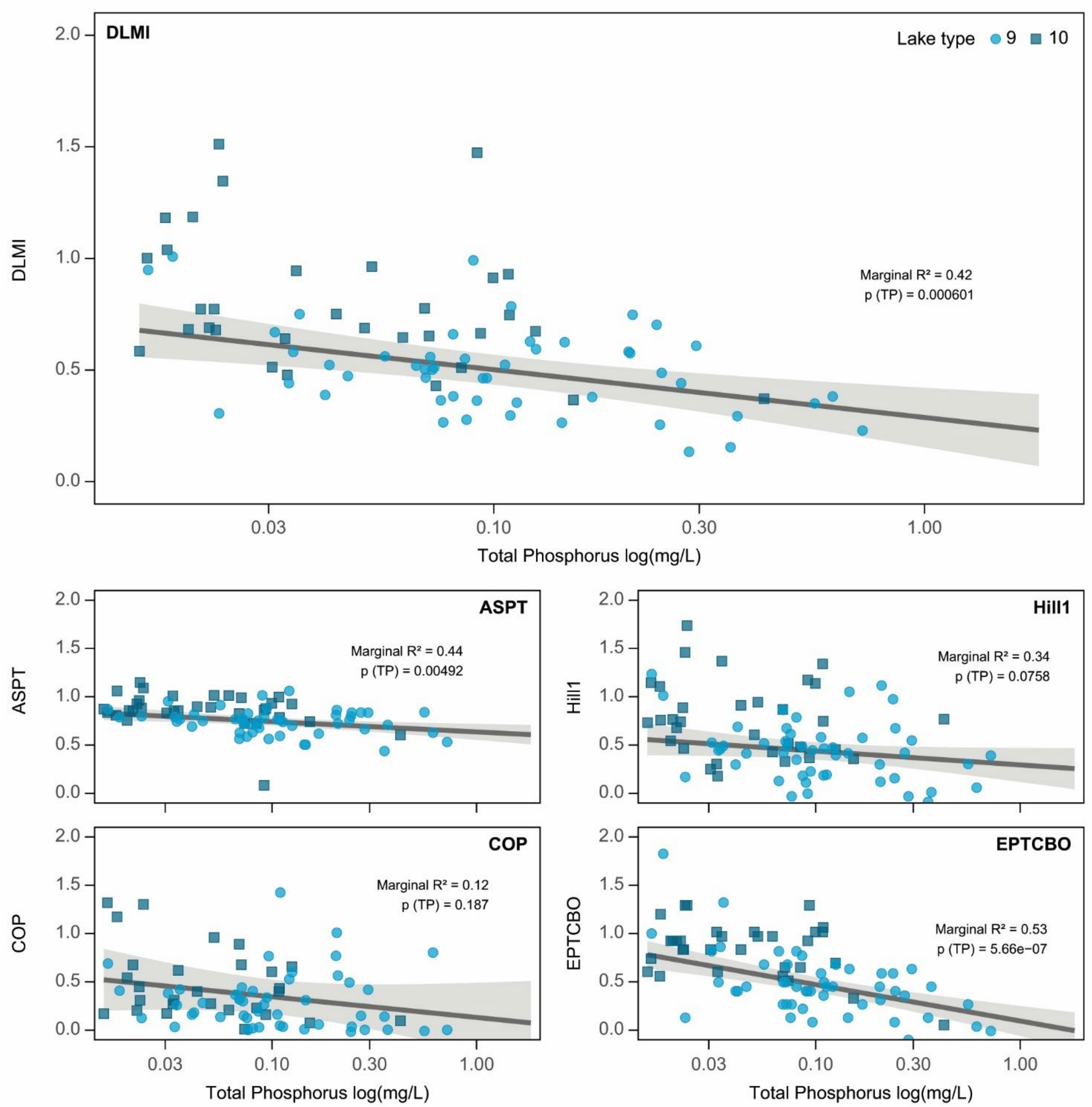


Figure 5.6. Linear mixed-effects models for sub-index scores and DLMI in response to TP. Lake ID was included as a random effect to account for non-independence be-tween samplings within lakes over time.

All sub-indexes and their relation to total phosphorous are plotted for lake type 9 and 10 in figure 5.6. The ASPT and EPTCBO sub-indexes had significant relationships with total phosphorous, but COP and Hill1 had not.

Of these sub-indexes, all except Hill1, are designed for use within streams but can also be used for lakes as they are in Denmark. This however is also the biggest concern, as several of the indexes, especially the COP index, estimates ecological state-based on organisms that are more diverse and/or prevalent in streams than in lakes. While these organisms can still serve as indicators of water quality in lakes, their relative rarity may reduce their effectiveness as consistent indicators. This was in evidence from the weak relationships between total phosphorous and the COP sub-index (figure 5.6). The Hill1 index is a common ecological diversity index. In this analysis there was only weak relationship between Hill1 and total phosphorous. This probably is because the differences in diversity of lakes across the eutrophication range is too low.

Overall, the integrated DLMI had significant negative relation to total phosphorous in lake types 9 and 10. DLMI regression (figure 5.6) fulfils the criteria established in the ECOSTAT guidelines for establishing threshold values between a biological quality element (DLMI) and a physical chemical supporting element (Total phosphorous) by having an R2 value over 0.36 (European Commission: JRC, 2025). Although further refinement of the DLMI may be possible, its current form appears robust and suitable for assessing ecological state in lake types 9 and 10.

5.3 Conclusions

The results show that all freshwater lake types exhibit a similar DLMI–TP relationship, with no significant differences in slope or confidence intervals. However, the index showed a weak corelation with eutrophication (TP) in brackish lake type 11. This suggests that DLMI can be applied to lake types 5 and 13, in addition to the already intercalibrated lake types 9 and 10, but not to type 11. Furthermore, since DLMI was never intended for use in brackish lakes and relies on indicator organisms primarily associated with freshwater conditions, its applicability may be restricted not only in type 11 but also in other brackish lake types (12 and 15, not tested in this report due to insufficient data). For the same reasons, it is not possible to adapt the DLMI to lake type 11, and it is recommended that a separate index be developed or adapted for use in brackish lake types.

6 Final conclusions

This report tested the three indexes for phytobenthos (IPS), phytoplankton (DSPI) and macroinvertebrates (DLMI) in non-intercalibrated lake types (excluding lake types 9 and 10) (Table 6.1). For phytobenthos, it was possible to evaluate the IPS and provide recommendations for lake types 1, 2, 5, 11 and 13. IPS can be applied without modification to all these lake types – except for the brackish type 11, for which modification (IPS +0.7) is required. Similarly, the DLMI could be evaluated for lake types 5, 11 and 13. For lake types 5 and 13, the index can be used without modification, whereas for lake type 11 it cannot be used due to marked differences in macroinvertebrate composition, associated with a significantly different DLMI response to TP.

For phytoplankton, the DSPI was tested for low-alkalinity type 10 lakes (0.2 to 1.0mEq/L) and was found to be applicable without modification.

Table 6.1. Recommendations for use of indexes for the Danish non-intercalibrated lake-types where sufficient data existed to evaluate the indexes.

Ecological indicator	Index	Lake type	Recommen-dation	Index modification
Phytobenthos	IPS	1, 2, 5, and 13	Use	None
		11	Use	IPS + 0.7
Macroinvertebrates	DLMI	5 and 13	Use	None
		11	Do not use	
Phytoplankton	DSPI	10 (0.2-1.0mEq/L)	Use	None

As in previous investigations (Søndergaard et al., 2020), the main limitation for testing index applicability across lake types was the lack of data for the less common lake types. Due to low data availability for rare lake types, it was not possible to evaluate either IPS or DLMI for lake types 6, 12, 14 and 15, and for types 1 and 2 in the case of DLMI. This lack of data to test the indexes for these lake types reflects the relative rarity of these lake types in the NOVANA programme and the Danish landscape.

This report recommends a targeted effort to collect sufficient data for evaluating ecological indexes in these rare lake types. Until such evaluations are possible, we advise against applying the indexes (Table 6.2).

Table 6.2. Recommendations for use of indexes for the Danish non-intercalibrated lake types where data were insufficient to evaluate the indexes.

Index	Lake type	Recommendation
IPS	6, 12, 14, and 15	Do not use
DLMI	1, 2, 6, 12, 14, and 15	Do not use

The results of this report suggest extending the applicability of the tested ecological indices to several non-intercalibrated lake types for phytobenthos and macroinvertebrates, as well as to phytoplankton in the low-alkalinity lake type 10 (Table 6.3). Future research should focus on testing the potential applicability of macrophyte, fish and phytoplankton indexes in non-intercalibrated lake types, which was not analysed in the current report (Table 6.3).

In this report, the ecological indexes were analysed regarding their responses to phosphorus as a relevant nutrient related to eutrophication in lakes. Most of these ecological indexes in lakes have been developed or intercalibrated based on their responses to phosphorus, being therefore the most adequate nutrient to test their responses among lake types. However, this does not neglect the relevant ecological effects of other nutrients such as nitrogen on lakes. Future approaches on biological indicators should integrate other relevant ecological factors related to eutrophication such as the role of nitrogen in the ecological quality of Danish lakes.

Table 6.3. Revised version of Table 1.1 based on Søndergaard et al. (2020, Table 7.1). Indicated is whether an index exists than can be used to assess ecological conditions for each biological quality element. "Yes" means that an index exists and can be used either in its original form or with modifications, whereas "-" indicates that no suitable index is available (e.g., DLMI tested but not suitable for lake type 11) or that there is insufficient data to evaluate existing indexes. * Lake type 14, macrophyte and fish indexes have not been tested in the current report.

Lake type	Alkalinity, colour, salinity, depth	Phytobenthos Index (IPS)	Macrophyte Index* (DSVI)	Phytoplankton Index (DSPI)	Macroinvertebrate Index (DLMI)	Fish Index* (DFFS)
1	Low, low, low, shallow	Yes	Yes	Yes	-	-
2	Low, low, low, deep	Yes	-	-	-	-
5	Low, high, low, shallow	Yes	Yes	Yes	Yes	-
6	Low, high, low, deep	-	-	-	-	-
9	High, low, low, shallow	Yes	Yes	Yes	Yes	Yes
10	High, low, low, deep	Yes	Yes	Yes	Yes	Yes
11	High, low, high, shallow	Yes (+0.7)	-	Yes	-	-
12	High, low, high, deep	-	-	-	-	-
13	High, high, low, shallow	Yes	Yes	Yes	Yes	-
14*	High, high, low, deep	-	-	-	-	-
15	High, high, high, shallow	-	-	-	-	-

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8 Appendix A

Table A.1. Pairwise comparisons of IPS among lake types using Dunn's test with Benjamini-Hochberg adjustment. Significance: *** p < 0.001, ** p < 0.01, * p < 0.05.

Lake type		*p*	Significance
1	2	0.0680	
1	5	0.9120	
1	9	0.0001	**
1	10	0.0723	
1	11	6.61E-15	***
1	13	0.0055	**
2	5	0.0592	
2	9	1.46E-06	***
5	9	0.0002	**
10	2	0.0007	**
10	5	0.0938	
10	9	7.15E-05	**
10	11	6.72E-23	***
10	13	0.0725	
11	2	1.16E-14	***
11	5	1.32E-14	***
11	9	7.86E-14	***
11	13	1.03E-08	***
13	2	5.55E-05	***
13	5	0.0075	**
13	9	0.4970	

Table A.2. Emmeans paired comparison of slopes from the LMM of IPS in response to log(TP).

Lake type		Slope Difference	t-ratio	*p*
1	2	-0.08967	-0.338	0.9999
1	5	0.13212	0.612	0.9964
1	9	0.07844	0.529	0.9984
1	10	0.05328	0.338	0.9999
1	11	0.13531	0.835	0.981
1	13	0.317	1.939	0.4567
2	5	0.22179	0.799	0.9849
2	9	0.16811	0.734	0.9904
2	10	0.14296	0.608	0.9965
2	11	0.22498	0.945	0.9648
2	13	0.40667	1.7	0.6166
5	9	-0.05368	-0.318	0.9999
5	10	-0.07884	-0.445	0.9994
5	11	0.00318	0.018	1
5	13	0.18488	1.014	0.9505
9	10	-0.02516	-0.306	0.9999
9	11	0.05687	0.628	0.9958
9	13	0.23856	2.561	0.1428
10	11	0.08202	0.782	0.9865
10	13	0.26372	2.461	0.1784
11	13	0.18169	1.598	0.6835

Table A.3. Emmeans paired comparison of adjusted means from the LMM of IPS in response to log(TP). Significance: *** p < 0.001, ** p < 0.01, * p < 0.05.

Lake type		**Estimate**	**t-ratio**	***p***	**Significance**
1	2	-0.0677	-0.311	0.9999	
1	5	-0.1109	-0.606	0.9966	
1	9	0.2026	1.424	0.7884	
1	10	0.1741	1.224	0.8843	
1	11	0.8582	5.332	<0.0001	***
1	13	0.1611	0.969	0.9602	
2	5	-0.0432	-0.196	1	
2	9	0.2703	1.435	0.7825	
2	10	0.2418	1.305	0.8494	
2	11	0.9259	4.518	0.0002	**
2	13	0.2288	1.104	0.9266	
5	9	0.3134	2.392	0.2063	
5	10	0.285	2.077	0.3698	
5	11	0.969	6.59	<0.0001	***
5	13	0.272	1.739	0.5906	
9	10	-0.0285	-0.384	0.9997	
9	11	0.6556	7.37	<0.0001	***
9	13	-0.0415	-0.397	0.9997	
10	11	0.6841	6.621	<0.0001	***
10	13	-0.013	-0.115	1	
11	13	-0.697	-5.669	<0.0001	***

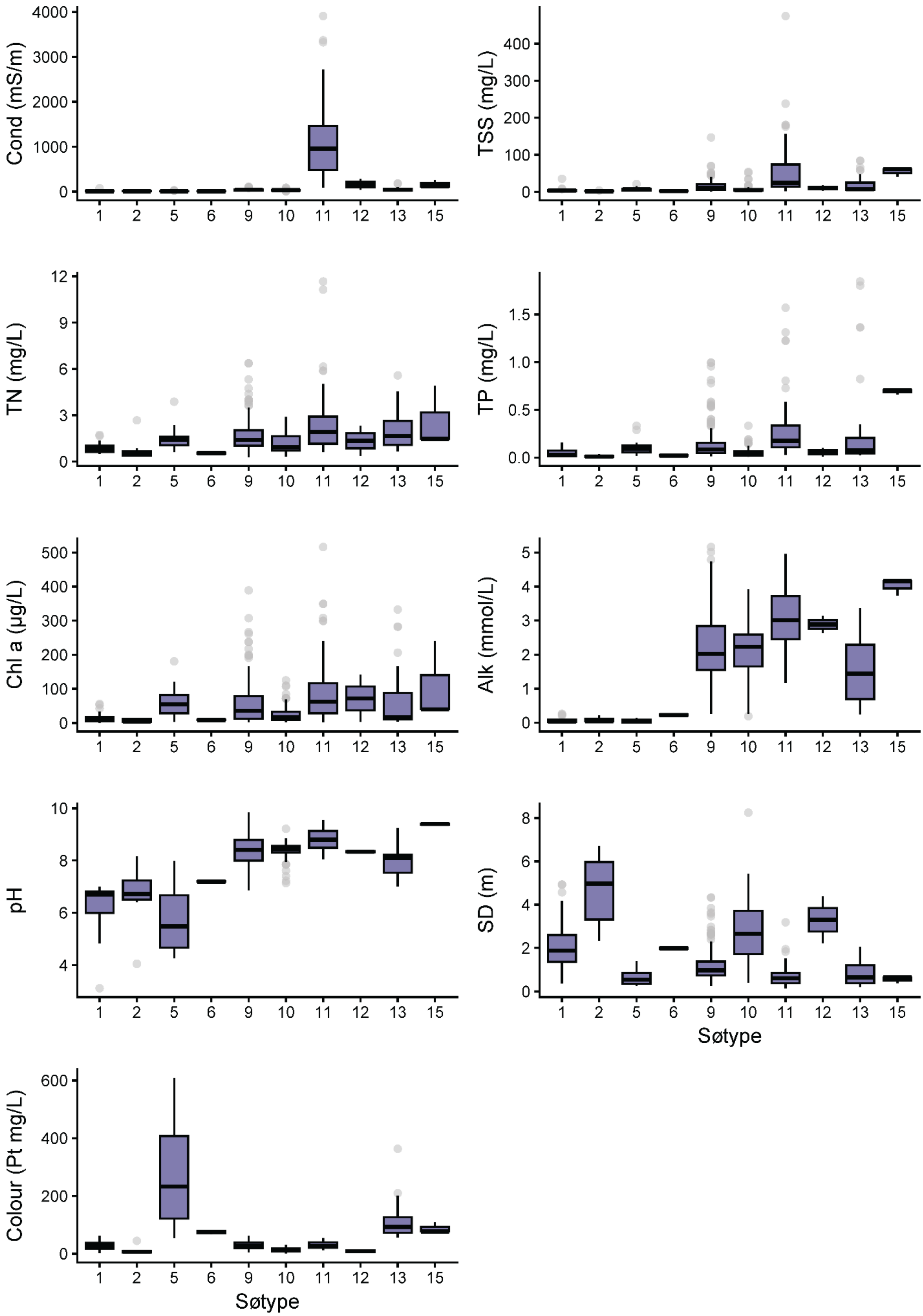

Figure A.1. Main environmental variables in Danish lake types for the period 2013-2023.

Table A.4. Results of Kruskall-Wallis test for differences in DLMI between lake types.

Lake type 1	Lake type 2	n1	n2	statistic	p	Significance
5	9	9	50	-0.465	6.42E-01	ns
5	10	9	32	2.194	2.80E-02	ns
5	11	9	13	-2.8	5.00E-03	ns
5	13	9	9	-0.36	7.19E-01	ns
9	10	50	32	4.401	>0.001	***
9	11	50	13	-3.359	1.00E-03	**
9	13	50	9	-0.003	9.97E-01	ns
10	11	32	13	-6.209	>0.001	****
10	13	32	9	-2.644	8.00E-03	ns
11	13	13	9	2.409	1.60E-02	ns

BIOLOGICAL QUALITY INDEXES IN NON-INTERCALIBRATED DANISH LAKE TYPES: PHYTOBENTHOS, PHYTOPLANKTON AND MACRO-INVERTEBRATES

This report evaluates whether existing ecological indices can be applied to nonintercalibrated Danish lake types, focusing on phytobenthos, phytoplankton and benthic macroinvertebrates. Using national NOVANA monitoring data and multivariate statistical analyses, the study assesses how well the indices respond to phosphorus and other key environmental gradients across lake types. Results show that the IPS and DLMI are generally applicable to freshwater non-intercalibrated lake types with a minor type-specific correction for IPS in lake type 11, while the phytoplankton index DSPI is suitable for type 10 lakes even at low alkalinity, supporting their use in future river basin management planning.

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