REVISED DANISH MACROINVERTEBRATE INDEX FOR LAKES

An assessment of ecological quality

Scientific Report from DCE – Danish Centre for Environment and Energy No. 373 2020

AARHUS UNIVERSITY
DCE – DANISH CENTRE FOR ENVIRONMENT AND ENERGY

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Data sheet

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Abstract: The revision was needed due to flaws in the software programme used to calculate the metrics in a previous report from 2017. The revised index ("Danish Littoral Macroinvertebrate Index", DLMI) is tested against environmental stressors like eutrophication and anthropogenic pressures in the littoral and the adjacent riparian zone, and it is documented that the index significantly correlates with these pressures. The index intercalibrates excellent with the common intercalibration metric used by other countries included in the so-called Central-Baltic Intercalibration Group to which Denmark belongs. The provision of DLMI is part of the Danish implementation of the Water Framework Directive as macroinvertebrates are required in the assessment of the ecological quality of Danish lakes in line with other biological quality elements (phytoplankton, macrophytes/phytobenthos and fish).

Keywords: Macroinvertebrates, Danish multimetric index, ecological quality, lakes, littoral zone, human pressures

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Supplementary notes: The Danish macroinvertebrate index is developed on basis of a similar Lithuanian index. The front page shows an example of "near-natural" Lithuanian lake.

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Preface

This report is a revision of a previous report published in 2017 (Wiberg-Larsen & Rasmussen 2017), being the template for introducing macroinvertebrates as an indicator of environmental quality in Danish lakes. Therefore, much of the content in the present report are similar to that in Wiberg-Larsen & Rasmussen (2017). The reason for making the present revised version is the discovery of a software flaw (discovered in autumn 2019) affecting the calculation of the four metrics that combines to the Danish multimetric Index, DLMI). Consequently the calculation programme unfortunately, only used about half of the macroinvertebrate taxa as in-data when calculating the metrics. Therefore it was urgent to recalculate all index values used previously. The primary difference between the present and the former report is, thus, of data-technical character, rather than changes in the composition of the presented multimetric national index and overall principles. Overall, present results are in agreement with the previous ones, but new results are significantly "better" (significant), concerning both intercalibration and response of the index to human pressures. Consequently, the revised report presents new so-called anchor points used to calculate EQR-values for each of the metrics. Further, the revised report suggests new boundaries between the five status classes.

The Danish Environmental Protection Agency (DEPA) financed the original study, whereas the present revision did not receive any such financial support.

During preparation of the revised report, the authors have informally discussed technical issues like the overall process, definition of anchor points, and choice of boundaries between status classes with representatives for Danish EPA. However, the content (and recommendations) of the present report is exclusively the choice and responsibility of the authors.

Summary

This report is a revision of a previous report (Wiberg-Larsen & Rasmussen 2017) that was based on a software programme that has subsequently been found to erroneously subtract parts of the data base layer used to calculate the "metrics" included in DLMI. These shortcomings have now been rectified, new so-called anchor points used to calculate EQR values for each of the metrics provided, as are the suggestion of new boundaries between the five status classes. However, much of the content in the present report are similar to that in Wiberg-Larsen & Rasmussen (2017).

According to EU's Waterframe Directive, Denmark must provide a national index based on benthic macroinvertebrates, as well as for other so-called biological quality elements (phytoplankton, phytobenthos & macrophytes and fish), to assess ecological quality in its lakes.

This report presents a revised macroinvertebrate index based on an already existing Lithuanian index (LLMI) that has been intercalibrated together with national indices from other countries within the Central-Baltic Intercalibration Group (CB-GIG) to which also Denmark belongs.

The Danish Littoral Macroinvertebrate Index (DLMI) is multimetric being composed of four different components being calculated as:

$DLMI = (ASPT + H_1 + EPTCBO + \%COP)/4,$

where *ASPT* is an index developed in the U.K. to assess ecological quality of streams, H_1 (Hill's 1) 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.

DLMI is calculated based on a composite kick-sample for 2 minutes on firm substrates (sand, gravel, stones) in the littoral zone using a standard net in the littoral zone.

The index was tested on a total 280 samples from 55 Danish lakes of which two lakes complied with generally accepted criteria for reference conditions. The data set included both shallow and deep lakes, however all being alkaline and basically with "clear" (not humic) water. Thus, the index is used for both these national lake types (9 & 10) representing about half of all Danish lakes.

The index correlated well ($r^2 = 0.45$) with a combination of both "eutrophication", assessed using a Principal Components Analysis on a suite of physical, chemical and biological parameters and expressed by primary and best explaining axis (PCA1) scores, and the "anthropogenic pressure" in the littoral and riparian zone (assessed from a large suite of different elements and activities) where macroinvertebrate sample were taken. Further, the index correlated significantly with each of the two pressures, although strongest with eutrophication.

Moreover, DLMI correlated strongly ($r^2 = 0.85$) with the macroinvertebrate common metric (index) to which all national indices within the CB-GIG were benchmarked in order to intercalibrate the national boundaries for High / Good and Good / Moderate ecological quality.

After a preliminary designation of DLMI boundary values (expressed as a Ecological Quality Ratio on a scale from 0 to 1) for High / Good (0.80), Good / Moderate (0.60), Moderate / Poor (0.40) and Poor / Bad (0.20), respectively, these boundaries were intercalibrated using exactly the same procedure as already carried out for the majority of countries within the CB-GIG (see Böhmer et al. 2014). The intercalibration showed that boundaries H / G and G / M were significantly biased, the latter exceeding 0.25, meaning that the boundary would be less strict than required. We therefore recommend the following adjusted boundaries, based on a mean bias < ±0.25 as requested by the EU (and in case close to 0) for both boundaries:

Boundary	H/G	G/M	M/P	P/B
$DLMI_{EQR}$	0.77	0.55	0.36	0.18

Taking into account that DLMI primarily reflects the pressure of eutrophication, it is estimated that obtaining at least good ecological status in shallow or deep alkaline and clear-water Danish lakes require that the total-phosphorus yearly mean should not exceed 0.045 mgL^{-1} (yearly mean). According to internal guidelines from Danish EPA, good ecological status for chlorophyll-a, fytoplankton and macrophytes may be achieved if summer mean total-phosphors does not exceed 0.053 and 0.031 mgL^{-1} for shallow and deep lakes, respectively. A conversion of the yearly mean of 0.045 mgL^{-1} for DLMI to summer mean, result in 0.051 and 0.048 mgL^{-1} for shallow and deep lakes, respectively. For deep lakes, this is above the Danish EPA boundary to obtain good ecological status.

The report recommends that DLMI is suitable as an official, national assessment method in alkaline lakes (national lake types 9 and 10). However, for several reasons (most of all the weak relationship between DLMI and total-phosphorus), it is also recommended that is more advisable to focus efforts to achieve at least good ecological status based on the boundaries established for chlorophyll-a, phytoplankton and macrophytes, rather than on a boundary estimated for macroinvertebrates.

Sammenfatning

Denne rapport er en revideret version af en tidligere rapport (Wiberg-Larsen & Rasmussen 2017), som uheldigvis baserede sig på software, der efterfølgende har vist sig (i visse tilfælde) fejlagtigt at frasortere dele af datagrundlaget til beregningen af de ”metrics”, som indgår i DLMI. Disse mangler er nu udbedret, nye såkaldte ankerpunkter anvendt til beregning af EQR-værdier for hvert metric præsenteret, ligesom der er foreslået ny grænseværdier for de enkelte statusklasser. Indholdet af rapporten svarer dog ret nøje til det i Wiberg-Larsen & Rasmussen (2017).

Ifølge EU's Vandrammedirektiv er Danmark forpligtet til at tilvejebringe et nationalt indeks baseret på bundlevende makroinvertebrater (smådyr) til vurdering af økologisk tilstand i søer (ligesom for andre biologiske kvalitetselementer som planteplankton, fytobenthos & vandplanter, og fisk).

Denne rapport præsenterer et sådant nyt makroinvertebrat indeks. Dette er baseret på et allerede eksisterende Litauisk indeks (LLMI), som er interkalibreret i forhold til sammenlignelige indices fra andre lande inden for den såkaldte Central-baltiske interkalibreringsgruppe (CB-GIG), som også Danmark tilhører.

Det danske indeks, Dansk Littoralzone Makroinvertebrat Indeks (DLMI) er multimetrisk, dvs. sammensat af fire forskellige delelementer, og beregnes som:

$DLMI = (ASPT + H_1 + EPTCBO + \%COP)/4$,

hvor *ASPT* er et indeks udviklet i U.K. til vurdering af økologisk tilstand i vandløb, H_1 (Hill's 1) er defineret som exp(Shannon-Wiener Indekset) (et matematisk udtryk for diversitet), *EPTCBO* er antallet af taksonomiske grupper af døgnfluer (Ephemeroptera), slørvinger (Plecoptera), vårfluer (Trichoptera), biller (Coleoptera), muslinger (Bivalvia) og guldsmede (Odonata), og *%COP* er den relative hyppighed af biller, guldsmede og slørvinger.

DLMI beregnes for en given sø-lokalitet på grundlag af en såkaldt “sammensat” sparkeprøve, indsamlet over 2 minutter og ved brug af en standard ketsjer, på fast bund (sand, grus, sten) i bredzonen (også kaldet littoralzonen).

Indekset blev testet på i alt 280 prøver fra 55 danske søer. To af disse søer kunne ud fra generelt accepterede kriterier karakteriseres som ”reference” søer, dvs. nærmest upåvirkede af menneskets aktiviteter. Der indgik både lavvandede og dybe søer, som var alkaliske og basalt set med klart, ikke-humusholdigt (dvs. ikke-brunt) vand. De to nationale søtyper (9 og 10) omfatter omkring halvdelen af alle danske søer.

Indekset var signifikant og ret godt korreleret (r^2 = 0,45) med en kombination af (a) en “eutrofieringsparameter” og (b) en parameter (PI), som udtrykker “menneskeskabte påvirkninger” i søens lavvandede bredzone (hvor DLMI-prøven blev indsamlet) og de nærmeste omgivelser på land. Eutrofieringsparameteren blev konstrueret på baggrund af en såkaldt “Principal Components Analysis” ud fra en række fysiske, kemiske (bl.a. fosfor og kvælstof) og biologiske faktorer (som karakteriserer den pågældende sø). Der blev ved analysen anvendt den bedst forklarende faktor, PCA1. DLMI var signifikant korreleret med hver af de to påvirkninger, men langt stærkest med eutrofiering (PCA1).

Derudover var DLMI ret stærkt og signifikant korreleret (r^2 = 0,85) med det "fælles" makroinvertebrat indeks (ICCM), som samtlige lande inden for CB-GIG er blevet interkalibreret ("benchmarked") i forhold til, således at de enkelte landes nationale grænser mellem "Høj/God" og "God/Moderat" for økologisk tilstand er sammenlignelige.

Efter en foreløbig national fastlæggelse af grænserne mellem de fem klasser (Høj, God, Moderat, Ringe, Dårlig) af økologisk tilstand (udtrykt på en EQR-skala fra 0 til 1, hvor 0 er dårligst og 1 bedst, og ligeligt inddelt således: 0,80, 0,60, 0,40, 0,20) blev disse grænser interkalibreret efter præcis samme procedure som allerede anvendt ved den allerede gennemførte interkalibrering/benchmarking for flertallet af lande inden for CB-GIG (se Böhmer et al. 2014). På grund af en høj bias for grænsen M/G i forhold til de andre lande, som betyder at de nationale grænser ville fremstå "mildere", var det nødvendigt/hensigtsmæssigt at ændre de foreløbige grænseværdier. Med udgangspunkt i en bias inden for ±0,25 (i praksis tæt på 0) foreslås følgende justerede grænseværdier, hvorved grænserne H/G og G/M bliver mere sammenlignelige med andre landes grænser:

Grænse	H/G	G/M	M/R	R/D
$DLMI_{EQR*}$	0,77	0,55	0,36	0,18

*EQR – Ecological Quality Ratio

Eftersom det er påvist, at DLMI primært afspejler eutrofiering, kan det ud fra grænserne ovenfor beregnes, at opnåelse af mindst god økologisk tilstand (det overordnede mål i Vandramme Direktivet) kræver, at en søs indhold af total-fosfor (målt som årsgennemsnit) ikke bør overstige 0,045 mgL^{-1} (uanset om der er tale om lavvandede eller dybe, alkaliske, ikke-brunvandede søer). På baggrund af en intern instruks fra Styrelsen for Vand- og Naturforvaltning (2016) kan god økologisk status imidlertid opnås for klorofyl-a, fytoplankton og makrofytter, hvis sommermiddel total-fosfor ikke overstiger 0,053 and 0,031 mgL^{-1} for hhv. lavvandede og dybe søer. En omregning fra 0,045 mgL^{-1} som årsmiddel til sommermiddel ift. DLMI medfører grænseværdier på 0,051 og 0,048 mgL^{-1} for hhv. lavvandede og dybe søer. For dybe søer er dette væsentlig højere end den nationale grænseværdi for klorofyl-a, fytoplankton og makrofytter.

Rapportens konklusion er, at DLMI kan anvendes som et nationalt indeks til vurdering af økologisk tilstand i de søtyper (9 & 10), som det er udviklet for. Desuden anbefales det af flere grunde, først og fremmest den meget svage sammenhæng mellem total-fosfor og DLMI, at målopfyldelse søges opnået på baggrund de generelle nationale grænseværdier for klorofyl-a, fytoplankton og makrofytter (for hhv. søtype 9 og 10), snarere end ud fra denne rapports tilsvarende grænseværdi/-er beregnet for DLMI.

1 Background

According to the European Union (EU) Water Framework Directive (WFD), EU member states must administer surface water surface water resources and ecosystems as irreplaceable values to be protected and managed as such (EU-Parliament and the Council 2000). In this context, ecosystems of all lakes and streams targeted by the WFD should meet the legislative requirements and obtain at least "good" ecological and chemical status by 2027. The ecological status is based on four biological quality elements (BQE's, including phytoplankton, phytobenthos + macrophytes, benthic macroinvertebrates and fish) and is categorised according to the deviation of biological communities from the community which would be expected at none or insignificant anthropogenic influence. The WFD operates with five ecological quality classes (high, good, moderate, poor and bad). One ecological quality class should be determined for each biological quality element, and the overall ecological quality class ascribed to a water body will be the lowest ecological quality class among all biological quality elements (one-out-all-out principle).

Each EU member state must develop one or more metrics or indicators for each biological quality element, and these metrics or indicators must target the dominant anthropogenic stressors (e.g. eutrophication or hydromorphological degradation). Subsequently, these ecological indicator tools must be intercalibrated with those of other EU member states to harmonise the thresholds between the ecological quality classes.

Intercalibrated indices for Danish lake ecosystems exist for all quality elements (except phytobenthos). In the following, we summarise the history leading to the national macroinvertebrate index.

Wiberg-Larsen (2014a) provided two indices for benthic invertebrates in Danish lakes. The first index (DISI) mainly targeting local physical stressors acting in the littoral and riparian zones, and the second index (LIMCO) targeting eutrophication. However, LIMCO failed to meet the criteria for successful intercalibration (Willby et al. 2014) with the common European index (ICCM), and DISI varied significantly among within-lake sampling sites, causing serious complications for lake management. Therefore, Wiberg-Larsen (2014b) advised against the use of benthic invertebrates in the classification of ecological status of lakes in Denmark. Subsequently, Denmark directed itself to the Working Group ECOSTAT under the Common Implementation Strategy to obtain its WG ECOSTAT for excluding benthic invertebrates from the Danish national assessment of ecological status in lakes (Wiberg-Larsen 2014b).This application was, however, rejected and WG ECOSTAT imposed Denmark to provide an alternative index.

In 2016, The Danish Environmental Protection Agency therefore assigned DCE/Bioscience, Aarhus University, to explore alternative indices based on benthic invertebrates in Danish lakes in order to meet the legislative requirements of the EU WFD. This resulted in an alternative index based on littoral benthic invertebrates, primarily reflecting the dominant stressor in Danish lakes, eutrophication, however also including other major stressors such as human activities in and near the littoral zone (Wiberg-Larsen & Rasmussen 2017).

The reason for the present revision of the previous work is the discovery of a flaw in the software used to calculate the metrics in the national index provided and tested in 2016/2017. The flaws were discovered in autumn 2019 and was due to inexpediencies in the ASTERICS program used to calculate the metrics included in the index (see 3.6 for further explanation).

1.1 Benthic invertebrates

In the present study, benthic invertebrates refer to macroinvertebrates, for instance worms, leeches, mussels, snails, larger crustaceans, such as isopods and amphipods, and not at least different aquatic insects. Hence, microinvertebrates, for instance Microturbellaria, Copepoda, Cladocera and Ostracoda, are not included in this study.

Danish lakes and ponds are inhabited by more than 1,000 benthic invertebrate species. A significant proportion of these are restricted to small lakes and ponds. Most benthic invertebrates are affiliated with habitats in the littoral zone that are often characterised by high macrophyte abundance and diversity and high substrate diversity, much higher than in the profundal zone. Depending on wind direction and shelter, the littoral zones are physically exposed resulting in currents and may resemble habitats found in running water. Due to exposure and restricted depth, oxygen concentrations in the water of littoral zones rarely decrease below critical thresholds. In contrast, in profundal zone oxygen concentrations may exceed such thresholds, especially during summer in stratified lakes. Consequently, benthic invertebrates occupying the profundal zone are typically characterised by traits that reduce their dependency on high oxygen concentrations (e.g. haemoglobin). Therefore, the total abundance and species richness of benthic invertebrates in littoral zones exceed by far those of the profundal zones.

1.2 Lake littoral benthic invertebrates as environmental indicators

Lake littoral benthic invertebrates are generally assumed to be sensitive to a broad range of environmental stressors including acidification (Solimini et al. 2006; McFarland et al. 2010), eutrophication (e.g. Brodersen et al. 1998) and hydromorphological degradation (Smith et al. 1987; Christensen et al. 1996; Solimini et al. 2006; Timm and Möls, 2012), and profundal benthic invertebrates are believed to better reflect gradients in eutrophication than littoral benthic invertebrates (Saether, 1979; Lang, 1985; Solimini et al. 2006; Timm and Möls, 2012). However, a former study of national monitoring data from a relatively large number of lakes indicates that profundal/soft-bottom dwelling benthic invertebrates (including those in the true profundal zone) do not reflect the gradient in eutrophication of Danish lakes well (Wiberg-Larsen et al. 2009).

A later study of littoral macroinvertebrate communities has shown a potential of using macroinvertebrate for assessing the ecological state of the littoral zones of Danish lakes (Wiberg-Larsen 2014a). Thus, it was possible to develop a multimetric index, DISI, showing that macroinvertebrates primarily responded to anthropogenic pressures in the littoral zone and adjacent riparian zones (including hydromorphometric alterations), but also to general eutrophication. Further, an alternative index, LIMCO (Miler et al. 2012), was tested for responses to eutrophication. Even though the indices based on littoral benthic invertebrates showed a potential to reflect human pressures, their use for assessing ecological quality in Danish lakes was not recommended (Wiberg-Larsen 2014b) for the following reasons: Index scores of DISI performed poorly due to a too wide variation in scores when littoral / riparian anthropogenic pressures were low and LIMCO showed poor correlation with the so-called intercalibration common multimetric index (ICCM), which is used in the intercalibration exercise conducted by a number of countries in the Central-Baltic Intercalibration Group (CB-GIG) to which Denmark belongs.

Despite the Danish arguments for not using macroinvertebrates as a BQE (Wiberg-Larsen 2014b), ECOSTAT ruled / decided that Denmark must – should better arguments not be provided – implement a national method based on macroinvertebrates (S. Poikane in litt., 2015). At the moment macroinvertebrate methods are used in six CB-GIG countries, including Lithuania and Germany. Therefore, ECOSTAT suggested that Denmark might adopt the methods applied in either Germany or Lithuania (Sidagyte et al., 2013) or, alternatively, the ICCM (S. Poikane in litt., 2015).

After discussions with the Danish Environmental Protection Agency (DEPA) of how to comply with the decision and views of ECOSTAT, it was decided to test if the Lithuanian index (Sidagyte et al. 2013) would be suitable for Danish lakes, possibly with some modifications. This resulted in the introduction of a new Danish index, DLMI (Wiberg-Larsen & Rasmussen 2017).

2 Lake categories and sampling sites

2.1 Categorisation of lake types

In the implementation of the WFD, the majority of European countries primarily include lakes with a surface area > 50 ha. However, Denmark includes all lakes with an area > 5 ha, and even some lakes with a surface area between 1 and 5 ha. This bias in size potentially influences the comparison between Danish and EU lakes as species richness and biodiversity generally increase with lake surface area.

Eleven different types of lakes have been identified in Denmark (Danish EPA 2004). About half of all Danish lakes belong to lake type 9 (alkaline, clear water, shallow and freshwater) or type 10 (alkaline, clear water, deep, freshwater) - consult table 2.1 for further details. The remaining lakes are primarily characterised by low alkalinity and / or a high content of humic substances, or being brackish.

Table 2.1. Physical and chemical characteristics of the two dominant lake types in Denmark (data from Søndergaard et al. 2003a). *Lake typology also depends on stratification.

Lake type	Alkalinity (meq L^{-1})	Colour (mgPt L^{-1})	Salinity (‰)	$Depth_{mean}$ (m)*
9	≥ 0.2	< 60	< 0.5	< 3
10	≥ 0.2	< 60	< 0.5	≥ 3

In this context, and as the data available in terms of littoral benthic invertebrate samples is sufficient only for lake types 9 and 10, we focus exclusively on these in the development of a Danish littoral benthic invertebrate index. This is further justified as intercalibration within the Central Baltic Geographic Intercalibration Group has been performed only on these lake types (Böhmer et al. 2014). Only one example of another lake type was included, namely Lake Madum (relatively close to a reference condition), characterised by low alkalinity (0.04 meq L^{-1}), non-humic conditions and near neutral pH (6.5).

2.2 Reference lakes

Due to the WFD requirements of evaluating all anthropogenic impacts on surface water bodies as the deviation of biological communities from the community expected to occur at none or insignificant anthropogenic influence (i.e. reference conditions) (EU Environmental Quality Standards Directive 2008), lakes subjected to minimal anthropogenic impact might be an essential element of a Danish index based on littoral benthic invertebrates. Several definitions of the term “reference condition” exist (Stoddard et al. 2006), presenting a pragmatic and systematised procedure in cases where no truly un-impacted ecosystems exist. As the Danish landscape is heavily utilised for agriculture (60%) and urban settlements (15%), truly un-impacted or minimally impacted lake ecosystems do not exist. Thus, Wiberg-Larsen (2014a) identified “best achievable condition” as the best alternative definition available, and we refer to Wiberg-Larsen (2014a) for a comprehensive evaluation of all alternative definitions. The specific physical and chemical characteristics defining the best achievable condition for Danish lakes have been identified by Søndergaard (2003) and are summarised in table 2.2. These threshold values were supplemented with criteria for acceptance of a maximum proportion of domestication in the lake catchment of 10% (including agriculture, urban settlements, roads, paved surfaces etc.). Moreover, a maximum threshold of anthropogenic modification of the riparian and littoral zones (defined as a Pressure Index value of 3 – see 3.3 for details of definition of the index) was included following the recommendations of Wiberg-Larsen (2014a) and Böhmer et al. (2014). Only two lakes qualified as having / fulfilling reference conditions based on all selective criteria (Lake Almind and Lake Slåen).

Table 2.2. Threshold values defining the best achievable condition for Danish lakes belonging to lake types 9 or 10 (Danish EPA 2004). Data originate from Søndergaard et al. (2003a).

Lake type	Total-P ($\mu g\ L^{-1}$)	Total-N ($mg\ L^{-1}$)	Chlorophyll a ($\mu g\ L^{-1}$)	Secchi depth (m)
9	14.6	0.4	3.7	3.8
10	7.6	0.38	3.9	5.4

2.3 Lakes and sampling sites

A total of 55 lakes were included in the analysis on which historical data are available through "grey" literature (investigations performed by the former Counties of Aarhus, Viborg, and Ringkøbing in 1984-1994 (n = 18)) and data collected specifically for the development of a Danish lake index for littoral benthic invertebrates in 2012 (n = 15) and 2013 (n = 24). The samples collected in 2013 were not included in the study by Wiberg-Larsen (2014a). Two lakes were sampled in both 2012 and 2013. To avoid pseudo-replication, we only used 2013 data for these lakes (since more benthic samples were available from all lakes this year). Hence, the total number of lakes included in our analysis exceeded that of Wiberg-Larsen (2014a) by 22. The lakes included in the analysis represent strong gradients in eutrophication and anthropogenic impacts in the riparian zone (Table 2.3).

Table 2.3. Minimum and maximum values of central physical and chemical parameters for the 55 lakes included in the analysis.

	Alkalinity ($meq\ L^{-1}$)	pH	Total-P ($\mu g\ L^{-1}$)	Total-N ($mg\ L^{-1}$)	Chlorophyll-a ($\mu g\ L^{-1}$)	Secchi-depth (m)	PI[1]
Min	0.26	7.1	12	0.26	4.0	0.45	0
Max	3.57	9.2	327	4.50	121.0	5.31	33

[1] PI=Pressure Index. See Methods section (3) for details.

The number of sampling sites per lake ranged from 1 to 8 for lakes sampled from 1984 to 1994 and from 4 to 6 for lakes sampled in 2012 and 2013. The total amount of sampling sites summed to 280 compared with 166 in the study by Wiberg-Larsen (2014a). A detailed overview of lake sizes, mean depths and average values for central physical and chemical parameters is presented in Appendix 1. For references to "grey" literature used to collect lake data from 1984-1994 see Wiberg-Larsen (2014a).

3 Methods

3.1 Benthic invertebrate sampling

One composite sample was collected at all sampling sites, mainly targeting hard substrate types (i.e. sand, gravel, and stones). “Stratified” sampling sites were selected, i.e. the sites were distributed “evenly” along the banks where suitable substrates were available. This method is described in detail in Wiberg-Larsen (2013) and Rasmussen & Wiberg-Larsen (2020). In brief, benthic invertebrates are collected for two minutes using a standard kick-sampling net (frame 25 x 25 cm, mesh size 500 µm) and by stirring up bed substrate using a kick-sampling technique. Sampling was, with a few exceptions during 1984-1994, restricted to September and October. Important parameters such as sample volume, mesh size of the standard net, and sampling effort was assessed comparable between the historical data collected in 1984-1994 and the new samples collected in 2012 and 2013 (Wiberg-Larsen 2014a).

3.2 Processing of benthic invertebrate samples and harmonisation of community data

Sample processing and the taxonomic level of identification for benthic invertebrates with respect to samples collected in 2012 and 2013 followed the procedure described by Wiberg-Larsen (2013). Moreover, the level of identification for samples collected in 1984-1994 was similar or sometimes even more detailed compared with the recommended procedure (Wiberg-Larsen 2013). No details on sample processing were available in the reports on the samples collected in 1984-1994. However, based on expert judgement (Peter Wiberg-Larsen), the taxonomic composition of the benthic invertebrate community and species abundances do not imply major incongruence that could hamper a comparison among samples collected in 1984-1994 with those collected in 2012 and 2013 (see also Wiberg-Larsen 2014a).

Since the taxonomic level was more detailed for several samples collected in 1984-1994 (e.g. many Chironomidae identified to species level), the taxonomic lists were harmonised according to the lowest level of detail to allow comparison of samples collected in 1984-1994 with those collected in 2012-2013. Moreover, several microinvertebrates were included in the taxon lists of some samples collected in 1984-1994. Such taxa were excluded and other adjustments made prior to data analysis to realign taxonomic congruence among all samples.

In summary, the total data set was harmonised according to the identification level presented in Rasmussen & Wiberg-Larsen (2020) and appendix 5.

3.3 Habitat characteristics

Physical habitat characteristics were recorded in the field for all sampling sites in 2012 and 2013 following the protocol described by Wiberg-Larsen (2013). In brief, physical habitat types were quantified within a predefined area (50 m of lake shore extending X m into the lake, where X is defined by the wadeable depth). Within this area, the proportional coverage of 8 different substrate types (e.g. sand, gravel, stones and coarse woody debris) was recorded. Moreover, areal proportions containing overhanging vegetation, roots and coverage of 11 different morphological types of macrophytes were recorded along with the total volume of submerged macrophytes. For details, see Wiberg-Larsen (2013). Based on these data, two habitat indices were calculated:

i) the Substrate Index (SI) and ii) the Vegetation Index (VI). In terms of the historical data (1984-1994), information of physical habitat characteristics (substrate composition and aquatic plant community composition) was extracted from the reports containing the benthic invertebrate data.

The SI is based on five different substrate categories (stones, gravel, sand, silt and large woody debris). Initially, each substrate category is assigned an Adjustment Factor (AF), reflecting the potential positive influence on benthic invertebrate biodiversity: stones (AF=5), gravel (AF=4), sand (AF=2), silt (AF=1), large woody debris (LWD) (AF=5). Subsequently, the AF of each substrate category is multiplied by its proportional coverage at the sampling site, and the IS index is calculated as:

$$SI = (proportional\ coverage_{(stone)} \times AF_{(boulder)}) + (proportional\ coverage_{(gravel)} \times AF_{(gravel)}) + (proportional\ coverage_{(sand)} \times AF_{(sand)}) + (proportional\ coverage_{(silt)} \times AF_{(silt)}) + (proportional\ coverage_{(LWD)} \times AF_{(LWD)}).$$

Hence, high SI values generally represent high substrate diversity with dominance of hard surfaces provided by boulder, gravel and LWD. Conversely, low SI values generally represent low substrate diversity with dominance of soft substrates as silt and sand.

The VI index is based on ten different morphological features of aquatic macrophytes. Similar to bed substrate categories, each morphological feature is assigned an AF: Submerged macrophytes with thread-forming lobes (AF=5), bryophytes and submerged macrophytes without conspicuous lobe formation and with parallel nerves (AF=3), broad-leaved macrophytes without conspicuous lobe structure and without parallel nerves (AF=2) and all other categories (AF=1). Subsequently, the proportional coverage of each morphological feature is multiplied by the AF specified for that morphological feature. Similar to the SI index, the VI index is calculated as:

$$VI = (proportional\ coverage_{(A)} \times AF_{(A)}) + (proportional\ coverage_{(B)} \times AF_{(B)}) + (proportional\ coverage_{(C)} \times AF_{(C)}) + (proportional\ coverage_{(D)} \times AF_{(D)}) + (proportional\ coverage_{(E)} \times AF_{(E)}) + (proportional\ coverage_{(F)} \times AF_{(F)}) + (proportional\ coverage_{(G)} \times AF_{(G)}) + (proportional\ coverage_{(H)} \times AF_{(H)}) + (proportional\ coverage_{(J)} \times AF_{(J)}),$$

where each capital letter (A-J) represents one of the ten morphological features. Hence, high VI values represent high diversity of morphological features of aquatic plants with dominance of submerged macrophytes with thread-forming lobe structures and bryophytes, and submerged macrophytes containing leaves without conspicuous lobes but with parallel nerves. Conversely, low VI values represent plant communities with few morphological features dominated by those assigned an AF of 1. Please consult Wiberg-Larsen (2014a) for further details concerning the categorisation and calculation of VI.

3.4 Anthropogenic pressure

Anthropogenic influence on sampling sites was recorded during the field sampling in 2012 and 2013, while the historic sites (sampled during 1984-1994) were “revisited” using Google Map or similar air photographs to quantify anthropogenic influence. In brief, anthropogenic pressures were recorded within the habitat sampling area (described in section 3.3 and Wiberg-Larsen 2013) and in a 50 m zone extending from the edge of the habitat sampling area. A comprehensive list of possible anthropogenic pressures can be found in Wiberg-Larsen (2013) and Rasmussen & Wiberg-Larsen (2020). The summed impact of anthropogenic pressures was quantified using the Pressure Index (PI) (Miler et al. 2012) calculated as:

PI = number of category 1 pressures (outside, but maximum 50 m, the habitat sampling area) + (2 × number of category 2 pressures (outside, but maximum 50 m, the habitat sampling area)) + (2 × number of category 1 pressures within the habitat sampling area) + (4 × number of category 2 pressures within the habitat sampling area),

where category 1 pressures represent low-intensity anthropogenic impacts such as forest paths, non-paved roads, public parks, conifer plantations, fruit gardens and pasture. Category 2 pressures represent high-intensity anthropogenic impacts such as industry, paved surfaces, agriculture, urban areas, lake harbours and marinas, fixation of lake shores (e.g. riprap structures), drainage canals and removal of aquatic vegetation and lake sediment.

3.5 Physical and chemical characteristics

Physical and chemical properties of the lakes were described by a series of spatial and environmental parameters including, but not limited to, catchment area, lake surface area, mean depth, alkalinity, pH, ortho-phosphate, nitrate, ammonium, total-P, total-N and phytoplankton biomass (chlorophyll-a and Secchi-depth used as proxies). These data were collected from central databases (e.g. containing data via the National Monitoring Program for Nature and Aquatic Environment, NOVANA) and regional monitoring reports (see Appendix 2 in Wiberg-Larsen 2014a). If available, physical and chemical data from the year of benthic invertebrate sampling was used (normally possible). If this requirement could not be fulfilled (historical data), the temporarily closest physical and chemical data was selected to minimise incongruence.

Yearly mean values of alkalinity, pH, ortho-phosphate, nitrate, ammonium, total-P, total-N, chlorophyll-a, and Secchi-depth were calculated and used in all analyses. Number of yearly measurements in each lake was generally ≥ 7.

3.6 Benthic invertebrate metrics and indices

Compared with the first report on the development of a lake littoral benthic invertebrate index (Wiberg-Larsen 2014a), this report contains an extended dataset. Therefore, all basic benthic invertebrate metrics and indices were recalculated, as an extended dataset may change correlations to environmental variables. The calculated metrics were primarily those included in LMMI (Sidagyte et al. 2013) and in the so-called Intercalibration Common Metric index (ICCM) used in the intercalibration process of national indices of the other countries of the Central-Baltic Intercalibration Group).

The development of a new Danish benthic invertebrate index for lakes follows the requirements of the WFD and contains elements of taxonomic composition and diversity, species-specific abundances and relationships between sensitive and tolerant taxonomic groups of relevance for the dominant stressor. Further, presence / absence of major taxonomic groups is considered by including EPTCBO taxa (although it is assessed that this parameter might not be as meaningful for lakes as for streams). Table 3.1 gives an overview of all calculated metrics and indices with references when needed.

In the previous report, i.e. Wiberg-Larsen & Rasmussen (2017), the software ASTERICS 4.04 was used to calculate multiple metrics and indices. However, due to the recent discovery of flaws in the process of feeding data into the program (see Preface), resulting in calculation of only a subset the taxa included in the total data set, and the lack of documentation/transparency of the calculations used, we decided to calculate all metrics in EXCEL in the present report. This provides full transparency and documentation of the handling of the macroinvertebrate data.

ASPT (and BMWP) is based on score values for a number of specific families. These scores have been revised several times since the introduction of the index. We used the score values presented in appendix 3.

In appendix 4, we have defined which taxa belonged to the category Lithophile taxa (i.e. taxa associated with stony substrates). We based this assessment on our own experience and knowledge, combined with information in literature.

Table 3.1. Overview of indices/metrics used in development of a national macroinvertebrate littoral index for Danish lakes.

Indices/metrics	Description	Characterisation according to WFD requirements
% ETO (abundance)	Abundance of Ephemeroptera+Trichoptera+ Odonata) (% of all taxa)	Taxonomic composition, abundance, diversity
ETO taxa	Number of taxa of Ephemeroptera+Trichoptera+Odonata	Taxonomic composition, diversity
%COP (abundance)	Abundance of Coleoptera + Odonata + Plecoptera) (% of all taxa)	Taxonomic composition, abundance, diversity
CEP taxa	Number of taxa of Coleoptera+Ephemeroptera+Plecoptera	Taxonomic composition, diversity
EPTCBO taxa	Number of taxa of Ephemeroptera+Plecoptera+Trichoptera+ Coleoptera+Bivalvia+Odonata	Taxonomic composition, diversity
% Lithophile taxa	Abundance of taxa inhabiting stony substrates (% of all taxa)	Taxonomic composition, abundance
ASPT	Average Score Per Taxon (= BMWP*/antal taxa) (Armitage et al. 1983). Based on occurrence of families, each assigned a specific indicator value	Ratio sensitive/non-sensitive taxa
Shannon-Wiener Index	SW, cf. Shannon (1948)	Diversity
Hill's 1. number (H_1)	Exp (Shannon-Wiener index)	Diversity

*British Monotoring Working Party

The Lithuanian Lake Macroinvertebrate Index (LLMI) is a combination of four metrics: (i) Average Score Per Taxon (ASPT), giving information on species sensitivities towards low oxygen concentrations (consequence of heavy eutrophication) (Armitage et al. 1983), (ii) the first Hill's number (H_1), giving the effective number of so-called operational taxa (Hill 1973), (iii) CEP taxa (number of operational taxa belonging to the orders of Coleoptera, Ephemeroptera, and Plecoptera), giving a measure of taxonomic community composition, and (iv) %COP (proportional abundance of individuals belonging to the orders of Coleoptera, Odonata and Plecoptera), giving a measure of group abundances.

The LLMI was finally derived as the average value of these four core metrics (converted to EQR) as:

$$LLMI = (ASPT + H_1 + CEP + \%COP)/4.$$

Further, an alternative version of LLMI, substituting CEP taxa with EPTCBO taxa (see 4.2 & table 4.1), was calculated as:

$$DLMI = (ASPT + H_1 + EPTCBO + \%COP)/4.$$

This version is referred to as The Danish Lake Macroinvertebrate Index (DLMI). The reason for taking this modification of LLMI into account is explained in chapter 4.2.

3.7 Data treatment and statistical analyses

The overall aim of the present report was not to differentiate between lake types 9 and 10 since a new Danish index for littoral benthic invertebrates per definition should cover both lake types. Therefore, all lake data were analysed without differentiating between lake typologies.

3.7.1 Environmental parameters

During the former attempt to elaborate a Danish macroinvertebrate index for lakes (Wiberg-Larsen 2014a), it was found that none of the variables traditionally reflecting eutrophication (such as total-P, total-N, chlorophyll-a, and Secchi depth) exhibited optimal correlations with macroinvertebrate metrics. Therefore, and in accordance with this work, a Principal Component Analysis (PCA) was performed on all measured chemical variables, depth and surface area (listed in section 3.5) to compare and quantify interdependencies between environmental parameters along the first two orthogonal axes in the ordination space. All data were log (x+1) transformed prior to analysis to obtain normality. The rationale was to obtain an eutrophication metric that would better reflect eutrophication. The PCA analysis was performed in PC-ORD for Windows. Correlations between PCA1 scores and environmental parameters (Pearson correlation) were calculated in Sigma Plot 11.0 for Windows.

3.7.2 Macroinvertebrate indices

Before calculating the multimetric indices, all calculated invertebrate metrics were converted to so-called EQR values (ecological quality ratios) on a scale from 0 to 1 according to the following formula:

$$\text{EQR} = \frac{\text{observed value - lower anchor}}{\text{reference value - lower anchor}}$$

For the conversion, it is essential to estimate two anchor points, the upper (being theoretically equal to a reference value) and lower anchor. In the CB-GIG intercalibration exercise (Böhmer et al. 2014), and due to the lack of reference sites / samples, the upper and lower anchor values were derived as 90% and 10% percentiles of all samples from the common dataset (including lake sites from Belgium, Estonia, Germany, Lithuania, the Netherlands and the UK) – see values in table 3.2. However, when these principles were applied to the Danish dataset to calculate multimetric indices, several metrics showed significantly negative values and, consequently, several negative index scores. Further, we obtained several values far exceeding 1.0. Therefore, we adjusted the upper and lower anchor points from the CB-GIG intercalibration exercise taking the actual Danish dataset into account. First approach was to estimate national upper anchor values as the 75th percentiles of the distribution of samples from reference lakes (Lakes Almind and Slåen) following recommendations by Hering et al. (2006). However, it appeared that Lake Almind, due to its extreme high number of taxa, scored exceptionally higher than Lake Slåen, resulting in a “twisting” of the upper end of the EQR scale. We therefore decided to use the 95% percentile based on all samples / lakes, this resulting in more appropriate scores, although we thereby accept that EQR values for Lake Almind exceed 1.0 (max. 1.55). Further, we estimated lower anchor values as 10% percentiles based on all samples / lakes, with the exception that minimum value was used for ASPT. Accordingly, we used 95% and 10% percentile of all samples / lakes as upper and lower anchor, respectively, for the metric %Lithophile taxa that is used in ICCM. This index is defined as:

*ICCM = (2*EPTCBOtaxa + ASPT +%ETO taxa + %Lithophile taxa)/5.*

Table 3.2. Anchor points used to scale relevant metrics to EQR values in the present study and in the CB-GIG intercalibration exercise* according to Böhmer et al. (2014), respectively. # No CB-GIG data available. **Note that these values are erroneously switched in Böhmer et al. (2014).

Anchor points	ASPT	Hill (H_1)	CEP taxa	%COP taxa	EPTCBO taxa	%ETO taxa	%Lithophile taxa
Upper (present study)	5.8	19.4	9	11.0	21.0	59.1	24.3
Upper (CB-GIG)*	5.5	#	#	#	20.1	48.1	25.1**
Lower (present study)	3.5	3.7	1	0.0	4	1.4	0.1
Lower (CG-GIG)*	3.6	#	#	#	2.8	9.8	8.7**

Generally, the national anchor points generally correspond well with those derived from CB-GIG. Main difference is that national lower anchors for %ETO taxa and %Lithophile taxa are somewhat lower than those of CB-GIG.

Calculations of LLMI, DLMI and ICCM were all carried out in EXCEL, and index scores were tested against environmental parameters (PI, VI, SI and PCA1) by use of linear and multiple linear regressions in SigmaPlot 11.0.

3.7.3 Intercalibration of the national index, DLMI, with those of other CB-GIG countries

When preparing the previous report (Wiberg-Larsen & Rasmussen 2017), intercalibration of preliminary Danish boundaries for H/G and G/M was carried out by Jürgen Böhmer, BIOFORUM GmbH, (Germany). Jürgen Böhmer further provided so-called “Intercalibration EXCEL Template Sheets” (Nemitz et al. 2011) containing the intercalibration data from the other CB-GIG countries, thus making it possible to intercalibrate the Danish data. Thus, we used this EXCEL sheets to intercalibrate the revised data presented above.

3.7.4 Description of macroinvertebrate communities

The macroinvertebrate composition of the samples was analysed using multidimensional scaling (MDS). Prior to the analyses, taxa were aggregated to obtain consistency across the whole dataset (see chapter 3.2), and fourth root transformation was performed to down-weight very abundant taxa. Subsequently, Bray-Curtis similarities were calculated for every pairwise combination of the 280 samples. These were then scaled in a MDS biplot showing the best possible expression of the species composition reflected by the two primary axes. The reliability of this expression is expressed by a so-called stressvalue. If values < 0.20, then presentation is reliable, if < 0.10 ideal (Clarke & Warwick 2001). Next, we tested for the difference between groups of samples designated to one of the five ecological quality classes (EQC) – see 3.7.3. This classification is only a labelling of the samples and does not influence the analysis described above. The actual designation (labelling) was derived from the setting of national boundaries for DLMI and corresponding ICCM values (according the results presented in chapter 4.4). The tests were carried out using ANOSIM (acceptance of significance: $P \leq 0.1\%$). Finally, using SIMPER, we assessed the taxa that contributed most to each of the five EQC’s and separated these. All analyses were carried out using the software PRIMER 6.

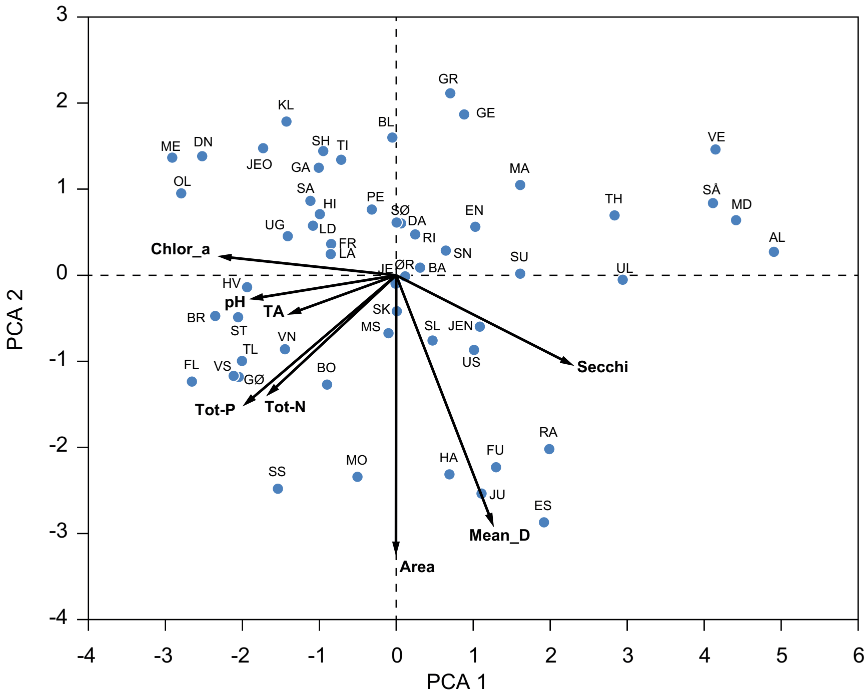
4 Results

4.1 Environmental parameters

We found strong gradients for surface area, depth, water chemistry and proxies for phytoplankton biomass. In total, 44.3% of the variation in the dataset was explained by PCA axis 1, whereas an additional 19.9% of the data variation was explained by PCA axis 2 (figure. 4.1). Overall, PCA axis 1 represented a gradient in eutrophication, primarily reflected by total-P (Pearson r = -0.76, p <0.0001), total-N (Pearson r = -0.64, p <0.0001), chlorophyll-a (Pearson r = -0.88, p <0.0001), pH (Pearson r = -0.72, p <0.0001) and Secchi depth (Pearson r = 0.87, p <0.0001). Accordingly, PCA axis 2 represented a gradient in lake size (surface area: Pearson r = 0.82, p <0.0001) and mean depth (Pearson r =0.74, p <0.0001). Data on physical / chemical parameters and PCA1 scores are presented in Appendix 1.

In the further analyses, PCA axis 1 scores were used as a “proxy” for eutrophication as these values integrate multiple elements of eutrophication rather than just one (e.g. total-P), allowing a much more comprehensive and reliable interpretation of eutrophication. This procedure has previously been suggested by Hering et al. (2006).

Figure 4.1. PCA of morphometric, chemical and biological parameters characterising the in-lake environment of 55 Danish lakes: mean depth (Mean_D), lake surface area (Area), chlorophyll-a (Chlor_a), Secchi depth (Secchi), pH, total alkalinity (TA), total phosphorus (Tot-P) and total nitrogen (Tot-N). Abbreviations of lake names are explained in Appendix 1.



The littoral habitat was described by anthropogenic pressures (PI = pressure index) as well as by substrates (SI = substrate index) and vegetation (VI = vegetation index). Among these, PI and VI had long gradients, whereas SI was relatively uniform due to the selection of sampling sites with “hard” substrates (Wiberg-Larsen 2013). Only PI and VI were significantly correlated (positively), though only slightly ($r^2 = 0.05$, $p<0.05$). None of the three indices was significantly correlated with eutrophication (PCA1).

4.2 Biological metrics

Among the calculated indices and metrics all, except %lithophile taxa, were strongly and significantly correlated with PCA1 ($P<0.001$), whereas they were all (except %ETO taxa) less - although still significantly - correlated with the pressure index, PI ($P<0.05$), see table 4.1.

ASPT, EPTCBO taxa and CEP taxa were the metrics best correlated with PCA1 (Pearson $r > 0.5$), whereas H1 and %COP taxa showed less correlation ($r = 0.42-0.44$). Both %ETO taxa and % Lithophile taxa were only slightly correlated with PCA1 ($r < 0.25$). Further, PCA1 was not at all correlated with the pressure index (PI).

All metrics except %ETO taxa were only slightly correlated with the pressure index ($r < 0.27$); %ETO was not at all correlated with PI.

The higher correlation with PCA1 of EPTCBO taxa compared with CEP taxa may justify substituting this metric in LLMI, thereby providing a new Danish version of LLMI (DLMI). Further, EPTCBO is already included in ICCM, presupposing that DLMI would intercalibrate well with this. Thus, based on this result we focussed primarily on DLMI in providing a new national method, however testing also LLMI.

Table 4.1. Correlations (r, Pearson coefficient) between macroinvertebrate indices/metrics and environmental variables for 280 specific sites in 55 Danish lakes. Indices/metrics were calculated as EQR-values after normalisation according to table 3.2, whereas pressure index and PCA1 scores were normalised using maximum and minimum values as upper and lower anchors.

Parameter	Pressure Index (PI) (r)	P-value	PCA1 (r)	P-value
ASPT	-0.200	<0.001	0.508	<0.001
H1	-0.225	<0.001	0.441	<0.001
CEP taxa	-0.160	0.007	0.533	<0.001
%COP taxa	-0.133	0.027	0.427	<0.001
EPTCBO taxa	-0.222	<0.001	0.586	<0.001
%ETO taxa	0.069	0.253	0.245	<0.001
%Lithophile taxa	-0.265	<0.001	0.185	0.002
PI			0.013	0.83

4.3 Multimetric indices tested in relation to pressures

The Lithuanian index as well as the new proposed Danish index was tested against each of the major pressures, eutrophication (represented by the proxy parameter PCA1) and the pressure index. The tests were carried out in linear as well as in multiple linear regressions (see table 4.2). We also calculated linear and multiple regressions for ICCM.

The results for DLMI are shown graphically in figure 4.2.

We further tested if inclusion of the habitat parameters SI and VI would strengthen the explanatory power of the multiple linear regressions. However, this only resulted in a minor improvement ($r^2 = 0.478$, $P<0.001$) compared with multiple linear regression without these to parameters ($r^2 = 0.450$, $P<0.001$), only VI contributing besides PCA1 and PI.

Table 4.2. Results of linear regression models for LLMI, DLMI and ICCM including PCA1 and PI as explanatory variables.

Index	Regression formula	r^2	P-value
$LLMI_{(PCA1,PI)}$	0.440 + (0.087 * PCA1) - (0.007 * PI)	0.415	<0.001
$LLMI_{(PCA1)}$	0.321 + (0.070 * PCA1)	0.348	<0.001
$LLMI_{(PI)}$	0.451 – (0.007 * PI)	0.046	<0.001
$DLMI_{(PCA1,PI)}$	0.455 + (0.094 * PCA1) - (0.008 * PI)	0.450	<0.001
$DLMI_{(PCA1)}$	0.389 + (0.094 * PCA1)	0.358	<0.001
$DLMI_{(PI)}$	0.375 – (0.006 * PI)	0.056	<0.001
$ICCM_{(PCA1,PI)}$	0.462 + (0.084 * PCA1) - (0.008 * PI)	0.407	<0.001
$ICCM_{(PCA1)}$	0.402 + (0.083 * PCA1)	0.350	<0.001
$ICCM_{(PI)}$	0.472 – (0.007 * PI)	0.052	<0.001

Overall, LLMI, DLMI and ICCM all fulfil the requirements of the WFD that r^2 must exceed 0.25 in regression with relevant pressures. This was certainly the case for PCA1 separately and in combination with PI. All indices are therefore appropriate to reflect multi-stressors, with main weight, however, on eutrophication. Correlation for all indices with PI was weak although significant.

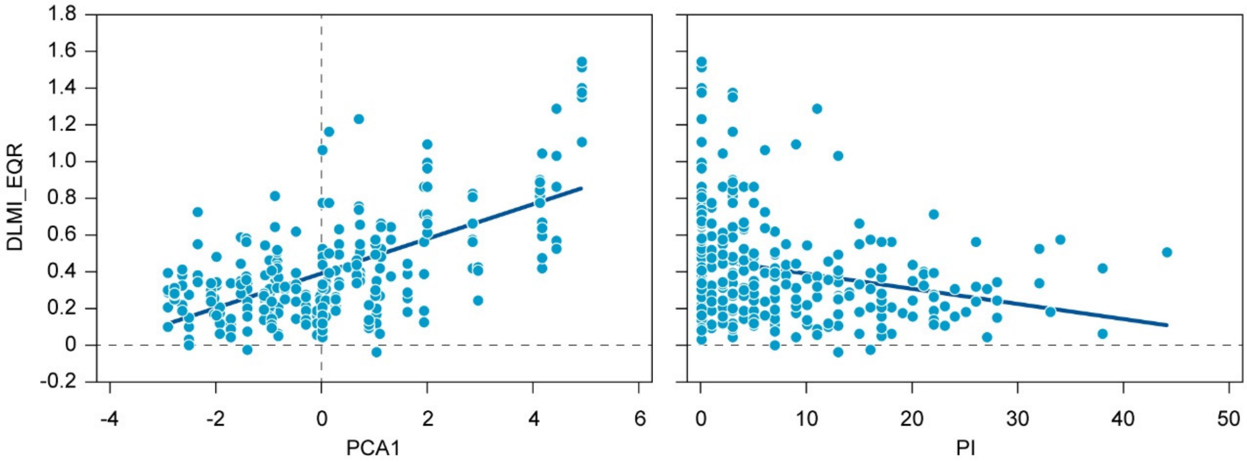


Figure 4.2. Linear regression between DLMI and PCA1, a proxy for “eutrophication” (LEFT), and Pressure Index, PI, representing anthropogenic impact in the littoral zone (RIGHT). For statistics of the linear regressions, see table 4.2.

We used PCA1 as a proxy for eutrophication. More directly, eutrophication is traditionally reflected by concentrations of the nutrients, N and P. Therefore, we also tested for linear regression between DLMI and these nutrients. Overall, correlations were weak with $r^2 << 0.25$ (results not shown), thus far from fulfilling the WFD requirement.

4.4 Difference in DLMI values according to lake type

We also tested for statistical differences in DLMI scores between the two lake types: shallow and deep lakes (see table 4.3). Overall, DLMI scored significantly highest in deep lakes.

Table 4.3. Comparison of DLMI scores from shallow and deep lakes. Differences were tested and turned out to be statistically significant by Mann-Whitney U-test (P<0.001).

Lake type	Minimum	Mean	Median	Max	N
Shallow ($D_{mean} \leq 3$ m)	-0.04	0.23	0.20	1.06	124
Deep ($D_{mean} > 3$ m)	0.05	0.54	0.48	1.55	156

4.5 Intercalibration of LLMI and DLMI with ICCM

Both LLMI and DLMI correlated strongly with ICCM (r^2 >>0.25, P< 0.001, N=280):

LLMI = -0.018 + (0.913 * ICCM) (r^2=0.80) and

DLMI = -0.007 + (0.9894 * ICMM) (r^2=0.85).

The result of the correlation for DLMI is shown in figure 4.3 (the corresponding correlation for LLMI is almost similar and therefore not shown).

Generally, values were well distributed along the regression line, except for a few outliers that only represented 5% of all samples. Values for the two reference lakes were all within the 95% prediction interval and located in upper part of the respective scales.

The result of the regression support the usefulness / appropriateness of DLMI as a new national method (or index).

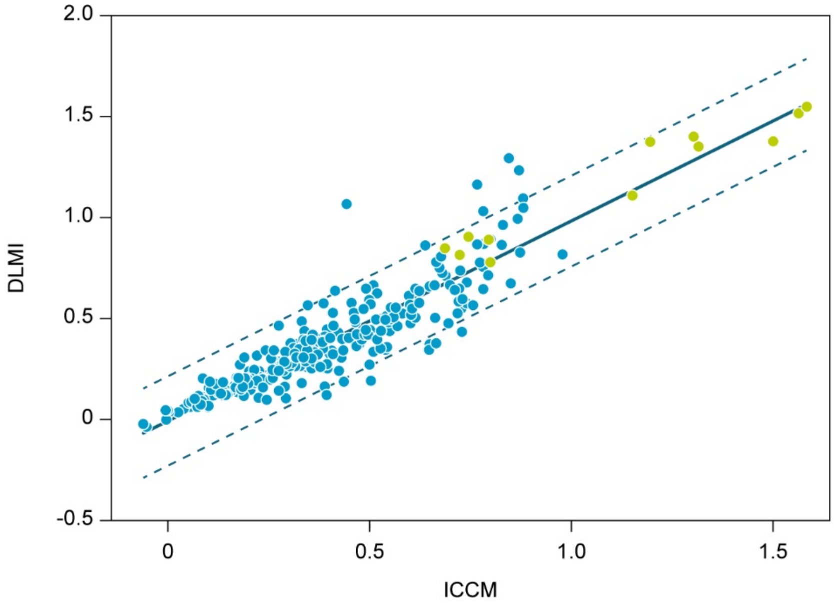


Figure 4.3. Linear regression between DLMI and ICCM based on 280 macroinvertebrate samples from 55 lakes. Shown regression line (solid line), 95% prediction interval (stippled lines). Green dots represent samples from reference lakes. For statistics of the linear regression, see text above.

4.6 "Preliminarily"selected EQC boundaries for the Danish macroinvertebrate index

Danish boundaries for the five ecological quality classes (EQC) were preliminarily derived on the basis on the national intercalibration results (see above) using an equal subdivision of the ICCM axis and subsequent estimation of preliminary boundaries for corresponding DLMI values using the equation presented in chapter 4.5. The boundaries are shown in table 4.4.

Table 4.4. "Preliminary" national boundaries of ecological quality classes (EQC) for DLMI – and corresponding classification according to the CG-GIG common intercalibration metric.

Boundaries	**ICCM**	**DLMI**
High/Good	0.80	0.80
Good/Moderate	0.60	0.60
Moderate/Poor	0.40	0.40
Poor/Bad	0.20	0.20

4.7 Intercalibration of "preliminary" EQR boundaries to those of other CB-GIG countries

The national ecological assessment methods and, thus, the present macroinvertebrate index for lakes (DLMI) must be compared (intercalibrated) with those of other countries.

In practice data from all comparable countries, in this case countries belonging to the CB-GIG, has to be included in a comprehensive analysis described in detail in chapters 6 & 7 in Böhmer et al. (2014). Overall, every national index is linearly correlated with the ICCM (see above). First of all common metric elements were standardised, normalised and combined into the multimetric common index, ICCM, expressed as EQR. Then national EQRs of status class boundaries were translated into ICCM using regression lines of the ICCM in dependence of the national EQR. Thereafter, ICCM class boundary values were averaged to get a common view, and finally the deviation of the national indices was expressed in terms of their status class width. The tolerable bias (status class width) is ± 0.25 (Böhmer et al. 2014).

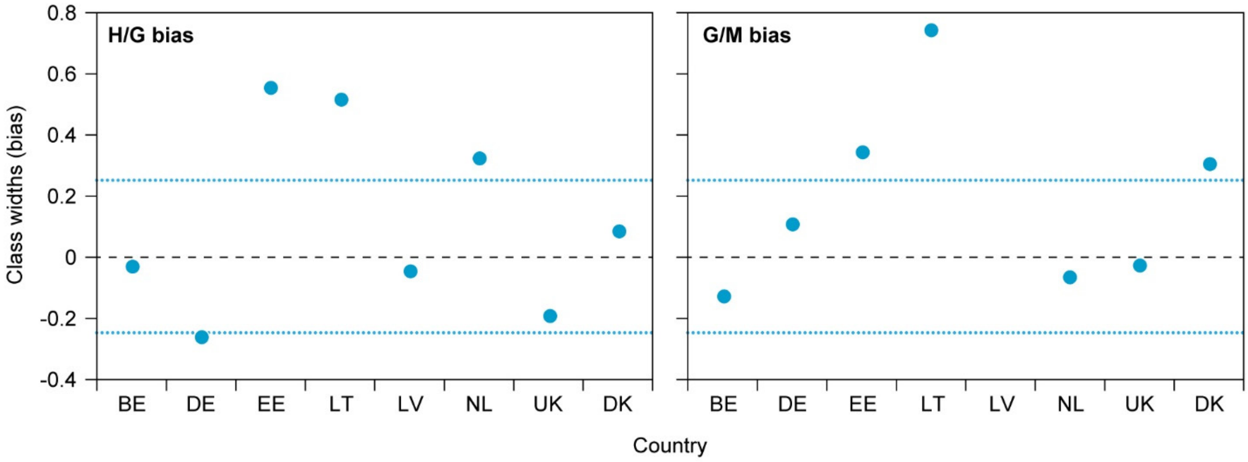


Figure 4.4. Class widths (bias) for the boundary of high/good (H/G) and good/moderate (G/M), respectively, for national macroinvertebrate indices of each of the CB-GIG countries. Dotted blue lines represents a bias of ± 0.25. Notice that boundaries for Estonia and Lithuania have been adjusted subsequently to reduce their bias.

The preliminary boundaries of DLMI (according to table 4.4) were intercalibrated as shown in figure 4.4. The result was a bias of 0.085 and 0.309 for H / G and G / M, respectively, the latter being above 0.25 and, thus, more strict than required. This means that the preliminary boundaries may be adjusted.

4.8 Description of macroinvertebrate communities related to ecological quality classes

A total of 263 macroinvertebrate taxa were recognized in the 280 samples. Of these 92 taxa were rare and recorded in less than five samples each. Thus, macroinvertebrate communities were characterised by relatively few widely occurring and generally abundant taxa. Only 12 taxa occurred in 50% or more of the samples and 26 taxa were recorded in 25-49% of the samples.

MDS analysis (based on adjusted status class boundaries – see 5.1) revealed a relatively uniform taxonomic composition as reflected in a relatively high stress value (0.26), showing only a poor presentation of the scaled similarities in a two-dimensional plot (figure 4.5). Thus, the “gradients” in taxon composition was relatively weak, suggesting that many (common) taxa occurred over the whole spectrum of samples (and lakes).

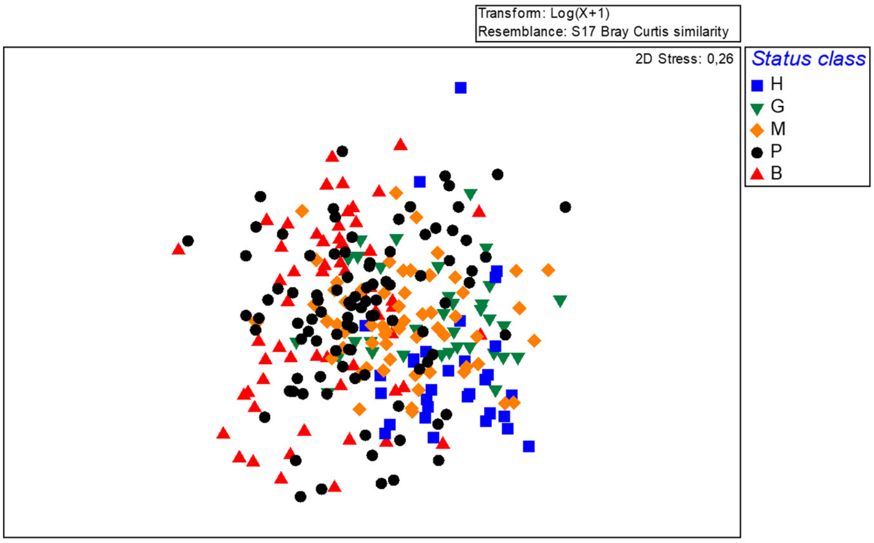


Figure 4.5. Multidimensional scaling of Bray-Curtis similarities based on 280 macroinvertebrate samples from 55 Danish lakes. The symbols show the “a priori” classification of status class ((H – high, G – good, M – moderate, P – poor, and B – bad) according to the new Danish index, DLMI. Boundaries were set to H/G = 0.77, M/G = 0.55, M/P: 0.36, and P/B = 0.18, respectively, according to chapter 5.1.

Based on the ecological quality classification (or labelling) of DLMI (according to chapter 5.1), and thus without influence on the multidimensional scaling, a further analysis showed that samples with *high quality* were significantly separated from those with *good*, *moderate*, *poor* and *bad quality* (ANOSIM, R=0.16-0.44, P=0.001). Samples with *good quality* were significantly separated from those with *poor* and *bad quality* (R=0.11-0.29, P=0.001), but only weakly (and not significantly) from those with *moderate* quality (R=0.08, P=0.02).

In the lower half of the scale, samples with *moderate quality* were significantly separated from those with *poor* and *bad quality* (R=0.21-0.30, P=0.001). However, samples with *poor quality* were not significantly separated from those with *bad quality* (R=0.04, P=0.04).

This picture of a relatively uniform taxonomic composition is supported when further characterising the macroinvertebrate communities (using SIMPER). Thus, the majority of widely occurring and abundant taxa were found in samples covering the whole spectrum of quality classes and pressures. Examples are Tubificidae (Oligochata), *Pisidium spp.* (Bivalvia), *Asellus aquaticus* (Crustacea), *Caenis horaria* (Ephemeroptera), Tanytarsini and Chironomini (Diptera).

However, samples with *high* and *good* status had higher abundancies of *Potamopyrgus antipodarum* (Gastropoda), *Gammarus pulex* (Crustacea, Amphipoda), *Caenis luctuosa* (Ephemeroptera), *Nemoura avicularis* (Plecoptera) and *Oulimnius tuberculatus* (Coleoptera) than those with *moderate*, *poor* and *bad* status. A few taxa were even potential “indicators” for high and good quality: *Ephemera vulgata*, *Leptophlebia* spp. (Ephemeroptera), *Notidobia ciliaris* and *Triaenodes bicolor* (Trichoptera), only very rarely or never found in samples with *moderate* or *poor* quality.

5 Discussion

Based on the recommendation and suggestions from ECOSTAT, received in 2014, we proposed a new national macroinvertebrate index for implementation in Danish lakes to assess ecological quality (or status) (see Wiberg-Larsen & Rasmussen 2017). The present report is a revision based on the original data set, but corrected for flaws in calculation of the metrics of the index.

The suggested index, DLMI (Danish Littoral Macroinvertebrate Index), represents a slight modification of the Lithuanian index (LLMI), which already is intercalibrated within the CB-GIG. The two indices include three common metrics (ASPT, H1, %COP taxa), whereas the fourth, and last, metric is EPTCBO taxa in DLMI instead of CEP taxa in LLMI. In both indices, the four metrics are weighted equally.

The adjustment of LLMI represented in its Danish version, DLMI, is made because (i) it reflects important pressures, eutrophication and morphometric alterations (or anthropogenic impacts) of the littoral zone slightly better for Danish lakes than LLMI, (ii) it supposedly performs more consistent as it includes more taxonomic groups (due to the substitution of metric CEP taxa with EPTCBO taxa), and (iii) EPTCBO taxa are specifically included in the multimetric index (ICCM) to which the CB-GIG countries intercalibrated their national methods.

5.1 Intercalibration of DLMI and national boundaries

Despite the obvious scarcity of available reference lakes necessary to provide the upper anchor values of the EQR scale (only two lakes fulfil the criteria), DLMI was successfully intercalibrated relative to the CB-GIG standard procedure. Thus, DLMI correlated exceptionally well (see chapter 4.5) with ICCM (r^2=0.85), in fact better than the indices of the other CB-GIG countries in the comprehensive CG-GIG intercalibration (see Böhmer et al. 2014). Among these, LLMI (in Lithuania) only had r^2=0.13, presumably due to a very small gradient in pressures (see below).

DLMI reflected anthropogenic pressures well compared with the indices of the other countries (Böhmer et al. 2014). Being able to reflect both eutrophication (expressed as PCA1 scores) and sampling site specific anthropogenic pressures (expressed as a pressure index, PI, equivalent to so-called morphometric alterations) of the littoral zone with r^2=0.45, DLMI performed well and "easily" fulfilled the requested r^2-value of at least 0.25. DLMI also performed much better than LLMI in Lithuania (r^2<0.28 for BOD_7 – a "proxy" of phytoplankton biomass, and only r^2=0.15 for the combined pressure of total-P and morphometric alterations). In comparison, the German index was less correlated with the combined pressure of total-P and morphometric alterations (r^2=0.30) than DLMI, whereas the indices of Estonia and the Netherlands correlated comparably well with land use and shore alterations, respectively (r^2=0.41 / 0.45). Only the index of the UK (CPET), targeted exclusively at eutrophication, performed better than DLMI (r^2=0.78).

Due to the convincing overall correlation with ICCM and pressures, we propose DLMI as a new national common index despite the score differences observed between shallow and deep lakes. Thus, there is no reason to believe that basic differences between the two lakes types should influence the overall relationship between DLMI and pressures as the invertebrate communities were only assessed in near-shore water with less than 1 m water depth. This is opposite to macrophytes, for which two different multimetric indices have been developed for shallow and deep lakes, respectively, since the metrics applied biologically depend on depth (Søndergaard et al. 2009). Moreover, the differences in DLMI scores observed between shallow and deep lakes are likely due to the fact that the shallow lakes were more nutrient-rich and more impacted by anthropogenic pressures in the littoral and riparian zone than the deep lakes. Statistically, the development of two different indices would be hampered by lack of fewer data in the correlations, these therefore being weaker. A final argument for using only one index is that the other CB-GIG countries also use a common index for both shallow and deep lakes (and the same boundaries between status classes).

Table 5.1. Overview of boundaries for national macroinvertebrate indices already intercalibrated within the CB-GIG – supplemented with the preliminary and adjusted boundaries for DLMI.

Member state	Intercalibrated index	H/G	G/M
Belgium (FL)	Multimetric index	0.90	0.70
Germany	AESHNA	0.80	0.60
Estonia	Multimetric index	0.86	0.70
Lithuania	LLMI (multimetric index)	0.74	0.50
The Netherlands	WFD expert-based index	0.80	0.60
United Kingdom	CPET	0.77	0.64
Denmark	DLMI (multimetric index):		
	Preliminary boundaries	0.80	0.60
	Adjusted boundaries (bias <0.25)	0.77	0.55

In conclusion, DLMI may be successfully implemented as a national method.

However, according to the intercalibration of DLMI together with data from the other CG-GIG countries, the preliminary national boundary for G / M are more strict than required, the bias (as class width) being >0.25 (see chapter 4.7). Countries are allowed to have stricter boundaries, but we recommend that the boundaries be adjusted to a bias < 0.25 for both classes. This implies that boundaries may be reduced to 0.77 for H / G and 0.55 for G / M (see Figure 5.1 and Table 5.1). Thus, with these boundaries, bias is close to 0. With the these recommended boundaries, all samples from the reference lakes achieve high quality. The boundaries for M / P and P / B are adjusted accordingly to 0.36 and 0.18, respectively.

5.2 Applying ecological status classes to Danish lakes in the present dataset

According to the proposed (adjusted) boundaries of the ecological status classes presented in table 5.1 and figure 5.1 (i.e. to a bias <<0.25), the macroinvertebrate samples were classified into five classes.

Further, mean DLMI EQR values for each of the 55 lakes were calculated and classified into status classes (see figure 5.2).

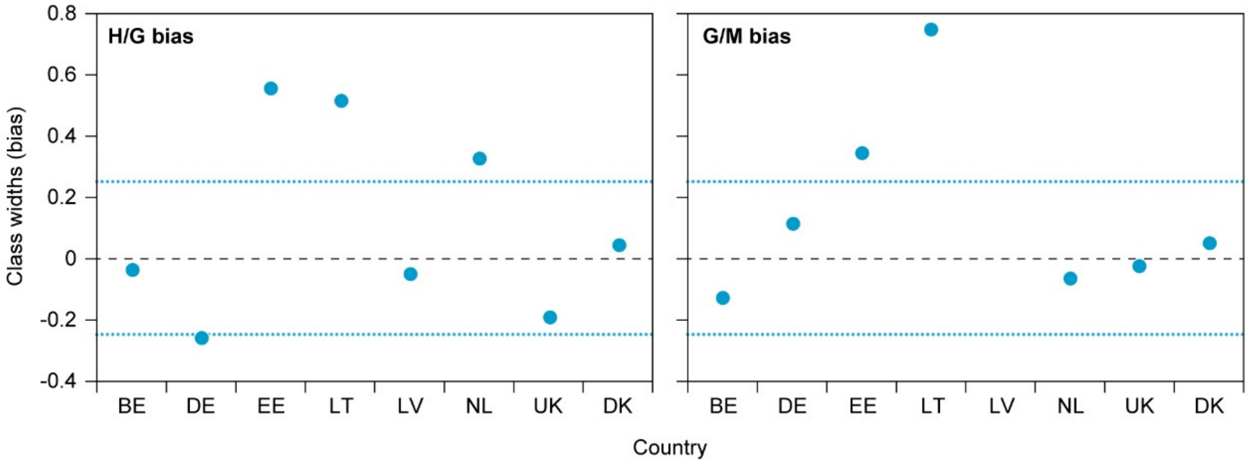
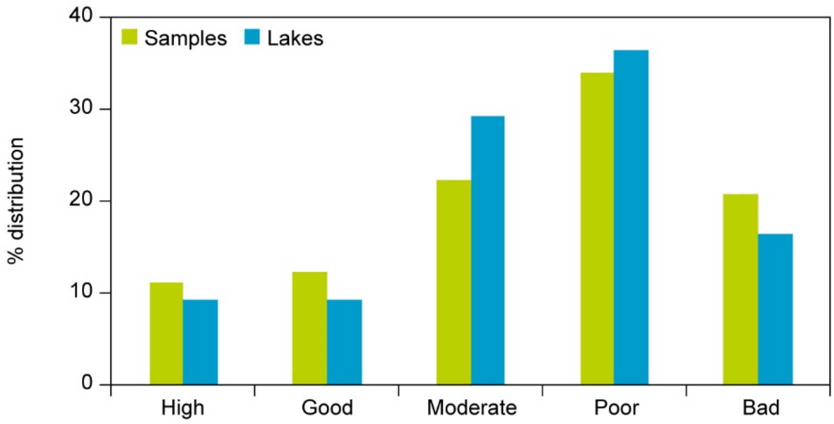


Figure 5.1. Class widths (bias) for the boundary of high/good (H/G) and good/moderate (G/M), respectively, for national macroinvertebrate indices of each of the CB-GIG countries, and after adjustment of preliminary Danish boundaries H/G (from 0.80 to 0.77) and G/M (from 0.60 to 0.55). Dotted blue lines represents a bias of ± 0.25. Notice that the presented boundaries for Estonia and Lithuania have been adjusted subsequently to reduce their biases (see text).

Figure 5.2. Classification of 280 samples and 55 lakes into the proposed (adjusted) status classes (see table 5.1).



The classification was relatively "skew". Thus, poor ecological status dominated among samples (34%) and lakes (36%). Only 23% of the samples and 18 % of the lakes obtained at least good ecological status. Both reference lakes were classified with high ecological quality.

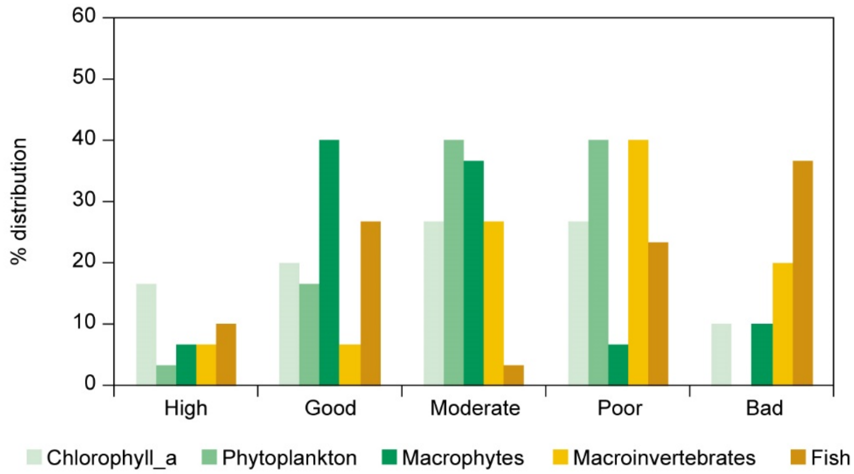
As expected, the DLMI values varied among the samples within the specific lake (Appendix 2). Standard deviation ranged 1-123% of the mean with median value 31%. Although such variation seems relatively high, it may be regarded as acceptable and unavoidable.

5.3 DLMI in relation to other national BQEs

In a national perspective, it is relevant to investigate how DLMI classifies lakes compared with the classifications of other biological quality elements (BQE). From the national monitoring programme (NOVANA) it was possible to obtain data for the complete suite of for all other available BQEs totally representing 30 (although not representative) lakes. The relative distribution of the five quality classes for the four quality elements (representing five metrics) is presented in figure 5.3, visually showing an overall bias of DLMI scores in the middle and "lower" part of the quality scale (moderate to bad status).

The bias is partly confirmed by pair-wise statistical tests (Wilcoxon Signed Ranks Test). Thus, DLMI scored poorer (median difference = one quality class) than the corresponding national indices / metrics for chlorophyll-a ($P<0.05$) and macrophytes ($P<0.001$). However, DLMI did not score statistically different from phytoplankton and fish metrics ($P>0.05$).

Figure 5.3. Classification of 30 lakes into ecological status classes according to four ecological quality elements represented by five metrics. Data derived from the national monitoring programme (NOVANA) and calculated/provided by DEPA.



The rationale behind using several biological quality elements instead of just one is that the elements may respond differently of environmental pressures. In case of Danish lakes, indices / metrics selected for all biological quality elements primarily respond to eutrophication generally expressed by the lake water concentration of total-P. However, the quality elements are part of an integrated biological structure, where e.g. phytoplankton, macrophytes and fish together with zooplankton influence each other. The interactions between the biological elements (and key species within each of these) are not straight forward. This is certainly also the case for the benthic macroinvertebrates. Therefore, one cannot expect a similar classification in respect to ecological status. Further, and also important is the response time of a quality element (and the chosen index / metric) to e.g. reduction in load of total-P. Thus, fytoplankton and macrophytes may respons fast to relatively fast, whereas fish may respond much slower. Macroinvertebrates may also respond relatively slow because taxa typical for lakes with high and good status may be totally absent in lakes with poorer status. Thus, any improvement in status strongly depends on dispersal of “lacking” taxa from other lakes. Such taxa are typically insects that may disperse by flying (facilitated by favorable winds) over-land, but dispersal distances may often be too long for succesful colonisation within a short time scale.

5.4 Implementation of DLMI

Primarily, DLMI shows relatively high correlation with eutrophication and with anthropogenic alterations in the littoral / riparian zones. Eutrophication is, however, much better reflected by the constructed eutrophication variable, PCA1, than by total-P (and total-N). Unfortunately, PCA1 is not straightforward for use in water management action plans describing how much eutrophication must be reduced to achieve at least good ecological status as defined by DLMI. However, if DLMI boundaries are transformed to specific PCA1 boundaries described by the equations in table 4.2, the latter can be further transformed into total-P boundaries (expressed as yearly means) using an exponential function between PCA1 and total-P, see figure 5.4.

The relationship is relatively well described by the exponential function (r^2 = 0.61). “Feeding” the boundary for DLMI between good/moderate status (0.55) into the linear regression between DLMI and PCA1 (figure 4.2) provides a PCA1 boundary of 1.72. This may, in turn, be transformed to a total-P value of 0.045 mg L^{-1} (yearly mean). According to the national water managements plan for the period 2015-2021 (Styrelsen for Vand- og Naturforvaltning 2016, page 54), it is assumed that at least good ecological status for the quality elements chlorophyll-a, phytoplankton and macrophytes may be fulfilled at summer-mean total-P of 0.053 mg L^{-1} in shallow lakes (type 9) and 0.031 mg L^{-1} in deep lakes (type 10). The estimated boundary value of 0.045 L^{-1} in the present study may according to formulas, also presented in Styrelsen for Vand- og Naturforvaltning (2016), be transformed into a summer-mean of 0.051 and 0.048 mg L^{-1} for deep and shallow lakes, respectively. For shallow lakes, this is only slightly below the boundary value (i.e. 0.053 mg L^{-1}) for the above-mentioned quality elements, whereas for deep lakes it is far above the boundary (i.e. 0.031 mg L^{-1}).

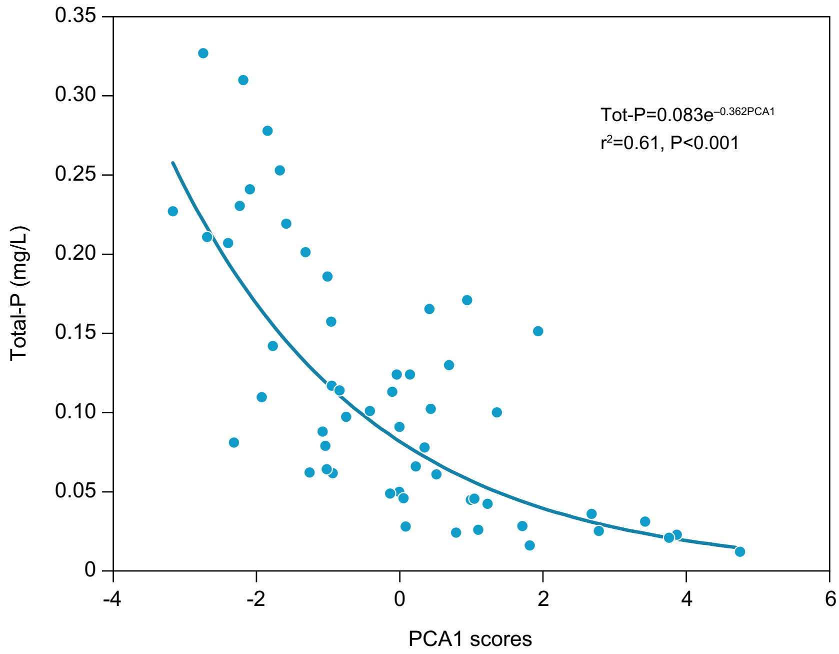


Figure 5.4. Relationship between the constructed eutrophication metric, PCA1, and total-P. Data for 55 Danish lakes included in the present study.

There are, however, good reasons for being careful when using modelled total-P (e.g. from reduced loading) to predict future DLMI values. Firstly, total-P is already included in the PCA1 scores, and the parameters are therefore not independent. Secondly, the relationship between total-P and DLMI is very weak (see 4.3). Thirdly, we only established a common relationship between DLMI and PCA1/total-P for shallow and deep lakes. Finally but yet important, macroinvertebrates may respond much slower to improved environmental condition than do phytoplankton and macrophyte vegetation (see 5.3). Consequently, we recommend that is more advisable to focus efforts to achieve at least good ecological status based on the boundaries established for chlorophyll-a, phytoplankton and macrophytes, rather than on a boundary for macroinvertebrates presented in this report.

5.5 Revision of technical guidance to macroinvertebrate sampling and sample processing

The present data set represented 55 lakes and 280 samples, the number of samples per lake varying between 1 and 8. We tested the influence of sample number and variation in % standard deviation of the mean in DLMI EQR values, and found no statistical significant difference between lakes with (3-)4 and >4 samples, respectively (Mann-Whitney Rank Sum Test, P=0.14). It is therefore acceptable to recommend four samples per lake in future monitoring. This is in accordance with the present technical guidance (Rasmussen & Wiberg-Larsen 2017).

The technical guidance document for NOVANA monitoring of macroinvertebrates in lakes must be revised to comply with the taxonomic levels used to develop and test the present version of DLMI. Furthermore, the guidance might include description of the calculation procedures and family scores used in calculation of BMWP / ASPT or alternatively in a data-technical guidance document.

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7 Acknowledgements

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Further, we acknowledge the effort and skills of the numerous consultants and others that provided the species lists from Danish lakes, not at least SBH consult that were responsible for sorting, identification and counting all the 2012 and 2013 samples.

Finally, we thank the staff at the Danish Environmental Protection Agency for sampling and assessment of habitat quality in the littoral zone of the lakes studied in 2012-2013, and for giving us constructive criticism during preparation of the report. We especially wish to acknowledge biologist, PhD, Charlotte Jørgensen for fruitful discussions.

8 Appendices

Appendix 1: Table overview of lake characteristics (surface area, mean depth and central physical and chemical parameters) for each of the 55 lakes used in the analysis.

Appendix 2: DLMI scores and ecological quality classification of samples from 55 Danish lakes.

Appendix 3: Family score values used to calculate the index BMWP / ASPT in the present study – and suggested in future calculations of DLMI.

Appendix 4: List of so-called lithophile taxa, used in calculation of %Lithophile taxa – a metric included in ICCM.

Appendix 5. Identification level used in the present study – and in future assessments of DLMI

Appendix1. Surface area, mean depth and central physical/chemical parameters for each of the 55 lakes used in the analysis. Physico-chemical variables are yearly means.

Lake name	Lake code	Area (km²)	Depth (mean) (m)	TA (meq/L)	pH	Tot-P (mg/L)	Tot-N (mg/L)	Chloro-phyll (µg/L)	Secchi depth (m)	PCA1	Year of data
Almind Sø	AL	0.53	10.4	0.58	7.54	0.012	0.29	4	5.31	4.75	1994/95
Bastrup Sø	BA	0.32	3.4	2.89	8.50	0.102	0.86	16.2	2.15	0.43	2013
Borresø	BO	1.95	4.9	1.47	8.55	0.157	2.35	27	1.05	-0.96	1985
Bromme Lillesø	BL	0.14	1.8	3.16	8.28	0.028	1.07	25.1	0.99	0.08	2013
Bryrup Langsø	BR	0.38	4.6	1.47	9.14	0.230	2.25	107	0.75	-2.23	1988
Damhus Sø	DA	0.46	1.6	1.40	8.90	0.066	1.16	13.5	1.78	0.22	2013
Dons Nørresø	DN	0.36	1.0	1.96	9.14	0.081	1.23	192.9	0.61	-2.32	2013
Engelsholm Sø	EN	0.44	2.6	1.73	8.11	0.045	0.81	21.7	2.28	0.99	2013
Esrum Sø	ES	17.3	12.3	2.60	8.43	0.151	0.52	6.7	4.96	1.93	2013
Flyndersø	FL	4.18	3.6	2.70	8.42	0.327	1.75	120	0.45	-2.75	1994
Frederiksborg Slotssø	FR	0.21	3.1	2.03	8.08	0.117	1.76	52	1.04	-0.95	2012
Fussing Sø	FU	3.33	14.6	1.69	8.70	0.100	0.94	15	4.08	1.36	1989/90
Gentofte Sø	GE	0.26	0.9	1.56	8.66	0.026	0.74	6	1.40	1.09	2012
Grarup Sø	GA	0.08	2.1	2.84	8.38	0.062	1.61	67.1	1.11	-0.94	2013
Grærup Langsø	GR	0.33	0.7	0.69	7.78	0.061	0.58	16	0.92	0.51	2012
Gødstrup Sø	GØ	4.60	1.8	2.27	7.90	0.207	4.09	94	0.90	-2.40	1990
Hald Sø	HA	3.33	14.6	1.06	8.00	0.165	1.49	41	2.72	0.41	1985
Hinge Sø	HI	0.93	1.2	1.70	8.39	0.064	1.44	49.4	0.88	-1.02	2013
Hvidkilde Sø	HV	0.61	2.0	3.51	8.78	0.278	1.16	100.1	1.39	-1.85	2013
Jels Midtsø	JE	0.25	4.1	2.69	8.29	0.050	2.75	21	1.82	-0.01	2012
Jels Nedersø	JEN	0.53	5.7	2.30	8.13	0.046	1.94	10.8	2.61	1.04	2013
Jels Oversø	JEO	0.08	1.2	3.93	8.05	0.142	1.57	75.6	0.85	-1.77	2013
Juel Sø	JU	5.70	7.8	1.85	7.52	0.130	3.63	7	2.80	0.69	1985
Klokkerholm Sø	KL	0.08	0.9	1.73	8.91	0.062	1.81	59.1	1.12	-1.26	2013
Lading Sø	LD	0.44	1.0	2.00	8.72	0.186	1.42	17.6	1.27	-1.01	2013
Langesø	LA	0.18	3.1	3.57	8.30	0.114	1.57	66	1.75	-0.84	2012
Madum Sø	MD	2.01	3.2	0.03	6.50	0.031	0.63	11.3	2.12	3.43	2013
Magle Sø v. Brorfelde	MA	0.15	3.6	2.97	8.39	0.016	0.82	10.8	2.49	1.82	2013
Maribo Søndersø	MS	8.62	1.7	2.40	8.39	0.049	1.35	22	1.44	-0.13	2012
Mellemdyb (V.Stadil Fjord)	ME	0.86	0.4	1.00	8.13	0.227	1.94	145.6	0.35	-3.17	2013
Mossø	MO	13.1	10.3	1.79	8.97	0.101	1.20	51	1.42	-0.42	1986
Ollerup Sø	OL	0.23	1.2	3.47	8.71	0.211	1.46	148.3	0.65	-2.69	2013
Peblinge Sø	PE	0.10	2.2	1.76	9.16	0.124	1.09	15	1.96	-0.04	2012
Ravn Sø	RA	1.80	15.0	2.29	7.60	0.028	3.58	9	3.66	1.71	1988
Ring Sø	RI	0.24	2.9	1.47	8.04	0.124	0.92	29	1.69	0.14	2012
Salten Langsø	SL	3.00	4.5	1.29	8.14	0.078	0.88	32	1.51	0.35	1993
Skarre Sø	SK	1.93	2.6	3.01	8.27	0.091	0.90	29	1.83	0.00	2012
Slåen Sø	SÅ	0.18	7.3	1.39	7.11	0.023	0.26	9	4.88	3.87	2012
Stallerup Sø	SA	0.24	2.1	2.20	8.55	0.079	1.19	84.0	1.07	-1.04	2013
Stigsholm Sø	SH	0.21	0.8	1.17	8.18	0.088	2.02	34	0.94	-1.08	2012
Stilling-Solbjerg Sø	SS	3.66	8.1	2.13	8.52	0.253	4.50	46	1.30	-1.67	1984
Stubbergård Sø	ST	1.54	2.3	1.91	8.60	0.241	1.64	86	0.76	-2.09	1990
Sunds Sø	SU	1.27	1.6	0.49	7.70	0.043	2.70	6	2.40	1.22	2012
Søbo Sø	SØ	0.21	3.6	3.11	8.23	0.046	1.02	61	1.73	0.05	2012
Sønder Sø	SN	1.25	3.3	2.79	8.43	0.024	0.72	22.5	1.34	0.79	2013
Thorsø	TH	0.69	4.2	0.98	7.85	0.025	0.31	9.8	2.55	2.78	2013
Tillerup Sø	TI	0.05	2.8	2.71	7.99	0.097	1.26	58.6	1.01	-0.75	2013
Tjele Langsø	TL	4.72	2.9	2.06	8.96	0.110	2.08	72	0.67	-1.93	1984
Ugledige Sø	UG	0.16	2.6	4.89	8.37	0.201	1.21	40.3	0.99	-1.32	2013
Ulse Sø	US	0.50	8.8	1.96	8.03	0.171	0.83	10.2	2.03	0.94	2013
Ulstrup Langsø	UL	0.44	4.8	1.87	7.33	0.036	0.91	5	4.34	2.68	1993
Vedsted Sø	VE	0.08	5.0	0.26	7.21	0.021	0.52	7	3.98	3.76	2012
Viborg Nørresø	VN	1.23	3.6	1.51	8.38	0.219	2.11	75	1.08	-1.58	1986
Viborg Søndersø	VS	1.46	4.2	1.59	8.68	0.310	2.19	121	0.96	-2.19	1986
Ørn Sø	ØR	0.40	4.0	0.88	7.97	0.113	1.39	43	1.48	-0.11	1988

Appendix 2. DLMI scores of samples (mean, minimum, maximum, S.D., and number of samples (N)) and ecological quality classification (EQS) according to the suggested boundaries (see table 4.4 & 5.1). Reference lakes indicated in "bold". #: SD could not be estimated due to too few samples

Lake	**Lake code**	**DLMI_mean**	**DLMI_min**	**DLMI_max**	**DLMI_SD**	**N**	**EQS**
Almind Sø	AL	1.38	1.11	1.55	0.14	7	H
Bastrup Sø	BA	0.45	0.32	0.63	0.13	6	P
Borresø	BO	0.59	0.36	0.82	0.17	3	M
Bromme Lillesø	BL	0.23	0.18	0.27	0.05	5	P
Bryrup Langsø	BR	0.47	0.34	0.72	0.16	5	P
Damhus Sø	DA	0.25	0.15	0.34	0.08	6	P
Dons Nørresø	DN	0.10	0.00	0.28	0.10	6	B
Engelsholm Sø	EN	0.15	-0.04	0.35	0.13	6	B
Esrum Sø	ES	0.47	0.13	0.86	0.29	6	P
Flyndersø	FL	0.31	0.19	0.41	0.09	6	P
Frederiksborg Slotssø	FR	0.17	0.05	0.37	0.13	5	B
Fussing Sø	FU	0.54	0.38	0.65	0.12	4	M
Gentofte Sø	GE	0.13	0.10	0.22	0.05	6	B
Grarup Sø	GA	0.25	0.19	0.39	0.08	6	P
Grærup Langsø	GR	0.48	0.35	0.66	0.12	6	P
Gødstrup Sø	GØ	0.17	0.17	0.17	#!	1	B
Hald Sø	HA	0.87	0.74	1.23	0.24	4	G
Hinge Sø	HI	0.26	0.15	0.46	0.12	6	P
Hvidkilde Sø	HV	0.15	0.07	0.20	0.05	6	B
Jels Midtsø	JE	0.38	0.09	0.78	0.27	6	P
Jels Nedersø	JEN	0.38	0.06	0.53	0.18	6	P
Jels Oversø	JEO	0.13	0.05	0.34	0.11	6	B
Juel Sø	JU	0.59	0.48	0.66	0.08	5	M
Klokkerholm Sø	KL	0.30	0.24	0.34	0.05	3	P
Lading Sø	LD	0.39	0.24	0.55	0.12	6	P
Langesø	LA	0.44	0.31	0.52	0.07	4	P
Madum Sø	MD	0.86	0.53	1.29	0.29	6	G
Magle Sø v. Brorfelde	MA	0.28	0.18	0.40	0.10	6	P
Maribo Søndersø	MS	0.15	0.06	0.19	0.05	6	B
Mellemdyb (V.Stadil Fjord)	ME	0.27	0.10	0.39	0.10	7	P
Mossø	MO	0.34	0.15	0.62	0.15	6	P
Ollerup Sø	OL	0.28	0.25	0.31	0.03	4	P
Peblinge Sø	PE	0.20	0.12	0.27	0.06	6	B
Ravn Sø	RA	0.82	0.61	1.09	0.18	8	G
Ring Sø	RI	0.26	0.19	0.41	0.08	6	P
Salten Langsø	SL	0.43	0.43	0.43	#	6	P
Skarre Sø	SK	0.35	0.04	1.06	0.43	6	P
Slåen Sø	SÅ	0.85	0.78	0.90	0.05	5	G
Stallerup Sø	SA	0.21	0.14	0.28	0.05	1	B
Stigsholm Sø	SH	0.19	0.09	0.40	0.12	6	B
Stilling-Solbjerg Sø	SS	0.40	0.28	0.59	0.12	5	P
Stubbergård Sø	ST	0.26	0.26	0.26	#	1	P
Sunds Sø	SU	0.44	0.44	0.44	0.00	2	P
Søbo Sø	SØ	0.22	0.12	0.33	0.08	6	B
Sønder Sø	SN	0.41	0.34	0.46	0.05	6	P
Thorsø	TH	0.64	0.42	0.83	0.16	6	M
Tillerup Sø	TI	0.29	0.25	0.32	0.04	3	P
Tjele Langsø	TL	0.33	0.16	0.49	0.13	4	P
Ugledige Sø	UG	0.10	-0.02	0.20	0.10	4	B
Ulse Sø	US	0.45	0.27	0.58	0.13	4	P
Ulstrup Langsø	UL	0.38	0.24	0.43	0.09	6	P
Vedsted Sø	VE	0.64	0.42	1.05	0.22	6	M
Viborg Nørresø	VN	0.47	0.36	0.58	0.12	4	M
Viborg Søndersø	VS	0.29	0.24	0.34	0.04	4	P
Ørn Sø	ØR	0.71	0.40	1.16	0.34	4	M

Appendix 3. Family score values used to calculate the index BMWP/ASPT in the present study – and suggested in future calculations of DLMI. The scores are obtained from: Centre for Intelligent Environmental Systems (Ray Martin) (2004) “Revision of the BMWP Scoring System” (http://www.cies.staffs.ac.uk/bmwptabl.htm (accessed October 2019). Wherever needed the values were supplemented by scores were taken from Freshwater Biological Association (2012): https://www.fba.org.uk/sites/default/files/BMWPLIFEtaxa_Modified.pdf.

Family	**Score**	**Family**	**Score**
Planariidae	4.2	Corduliidae	8.0
Dendrocoelidae	3.1	Libellulidae	5.0
Neritidae	7.5	Mesoveliidae	4.7
Viviparidae	6.3	Hydrometridae	5.3
Valvatidae	2.8	Gerridae	4.7
Hydrobiidae	3.9	Nepidae	4.3
Lymnaeidae	3.0	Naucoridae	4.3
Physidae	1.8	Notonectidae	3.8
Planorbidae	2.9	Pleidae	3.9
Ancylidae	5.6	Corixidae	3.7
Unionidae	5.2	Haliplidae	4.0
Sphaeriidae	3.6	Dytiscidae	4.8
Oligochaeta	3.5	Gyrinidae	7.8
Piscicolidae	5.0	Hydrophilidae	5.1
Glossiphoniidae	3.1	Scirtidae	6.5
Hirudididae	0.0	Dryopidae	6.5
Erpobdellidae	2.8	Elmidae	6.4
Asellidae	2.1	Sialidae	4.5
Corophiidae	6.1	Polycentropodidae	8.6
Gammaridae	4.5	Psychomyiidae	6.9
Astacidae	9.0	Hydropsychidae	6.6
Siphlonuridae	11.0	Hydroptilidae	6.7
Baetidae	5.3	Phryganeidae	7.0
Heptageniidae	9.8	Limnephilidae	6.9
Leptophlebiidae	8.9	Molannidae	8.9
Ephemeridae	9.3	Beraeidae	9.0
Caenidae	7.1	Leptoceridae	7.8
Nemouridae	9.1	Goeridae	9.9
Platycnemidae	5.1	Lepidostomatidae	10.4
Coenagriidae	3.5	Sericostomatidae	9.2
Lestidae	5.4	Tipulidae	5.5
Gomphidae	8.0	Chironomidae	3.7
Aeshnidae	6.1		

Appendix 4. List of so-called lithophile taxa (i.e. taxa associated with stony substrates), used in calculation of %Lithophile taxa – a metric included in ICCM.

DK-Taxon Code	**Group**	**Taxon**
6000601	Tricladida	*Bdellocephala punctata*
6000701	Tricladida	*Dendrocoelum lacteum*
22010101	Hirudinea	*Glossiphonia complanata*
22010102	Hirudinea	*Glossiphonia concolor*
22040101	Hirudinea	*Erpobdella octoculata*
22040199	Hirudinea	*Erpobdella* sp.
44042005	Ephemeroptera	*Kageronia fuscogrisea*
45020201	Plecoptera	*Nemoura avicularis*
51030301	Coleoptera	*Oulimnius troglodytes*
51030302	Coleoptera	*Oulimnius tuberculatus*
51030399	Coleoptera	*Oulimnius* sp.
51030401	Coleoptera	*Riolus cupreus*
53010101	Trichoptera	*Agraylea multipunctata*
53010502	Trichoptera	*Orthotrichia costalis*
53010699	Trichoptera	*Hydroptila* sp.
53050301	Trichoptera	*Polycentropus flavomaculatus*
53050504	Trichoptera	*Cyrnus trimaculatus*
53060101	Trichoptera	*Ecnomus tenellus*
53070203	Trichoptera	*Tinodes waeneri*
54020202	Trichoptera	*Ceraclea annulicornis*
54020205	Trichoptera	*Ceraclea nigronervosa*
54070101	Trichoptera	*Goera pilosa*
54070202	Trichoptera	*Silo nigricornis*
64066010	Gastropoda	*Radix auricularia*
64066020	Gastropoda	*Radix balthica*
65010101	Gastropoda	*Theodoxus fluviatilis*
65050101	Gastropoda	*Bithynia leachii leachii*
65050102	Gastropoda	*Bithynia tentaculata*
65090101	Gastropoda	*Ancylus fluviatilis*

Appendix 5. Identification level used in the present study – and in future assessments of DLMI

Taxon group	Level of identification
Flatworms (Tricladida)	Species
Nematodes (Nematoda)	(+)[1]
Hair worms (Nematomorpha)	(+)[1]
Oligochaete worms (Oligochaeta)	Family
Leaches (Hirudinea)	Species
Watermites (Hydrachnidia)	(+)
Spiders (Araneae)	Species[2]
Macrocrustacenas (Malacostraca)	Species
Mayflies (Ephemeroptera)	Species
Stoneflies (Plecoptera)	Species
Dragonflies/damselflies (Odonata)	Species
Aquatic bugs (Heteroptera)	Species
Beetles (Coleoptera) – adults	Species
Beetles: Elmidae – larvae	Species
Beetles: Gyrinidae, Haliplidae, Noteridae, Dytiscidae, Hydraenidae, Hydrophilidae, Donaciinae, Curculionidae – larvae	Family
Alderflies (Megaloptera)	Species[3]
Caddisflies (Trichoptera)	Species
Butterflies (Lepidoptera)	Species[4]
Non-biting midges (Diptera, Chironomidae)	Subfamily (Chironominae subdivided in tribes Chironomini/Tanytarsini
Other Diptera	Family[5]
Snails (Gastropoda)	Species
Mussels (Bivalvia)	Species/genus[6]

[1] (+) No further identification
[2] Just one truly aquatic species (*Argyroneta aquatica*)
[3] Just one species in lakes (*Sialis lutaria*)
[4] Family Crambidae: 5 species
[5] Use of identification level tribus allowed
[6] *Pisidium* just to genus

REVISED DANISH MACROINVERTEBRATE INDEX FOR LAKES

An assessment of ecological quality

This report presents a revised Danish multimetric index based on macroinvertebrates inhabiting the littoral zone of lakes. The revision was needed due to data errors in a previous report from 2017. The index ("Danish Littoral Macroinvertebrate Index", DLMI) is tested against environmental stressors like eutrophication and anthropogenic pressures in the littoral and the adjacent riparian zone, and it is documented that the index significantly correlates with these pressures. The index intercalibrates excellent with the common intercalibration metric used by other countries included in the so-called Central-Baltic Intercalibration Group to which Denmark belongs. The provision of DLMI is part of the Danish implementation of the Water Framework Directive as macroinvertebrates are required in the assessment of the ecological quality of Danish lakes in line with other biological quality elements (phytoplankton, macrophytes/phytobenthos and fish).

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