Revideret makroinvertebratindeks (DLMI) til danske søer

Bidrag til godkendelsesprocedure for det nationale indeks ved ECOSTAT

Fagligt notat fra DCE – Nationalt Center for Miljø og Energi

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	Gengivelse tilladt med tydelig kildeangivelse
Foto forside:	Flodguldsmeden *Gomphus vulgattisimus* (Ondrêj Machač) forekommer typisk på sandet/siltet bund i bredzonen af rene søer.
Sideantal:	18
Supplerende bemærkninger:	KS af selve GAP-dokumentet (appendix) gennemføres i ECO-STAT.

Indhold

1 Forord

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 de andre biologiske kvalitetselementer: planteplankton, fytobenthos & vandplanter, og fisk).

I maj 2019 blev det første danske makroinvertebratindeks (DLMI) til brug i danske søer godkendt ved ECOSTAT som en dansk metode. Med denne godkendelse kunne DLMI herefter tages i anvendelse i den pågående vandplanlægning.

Dokumentationen for godkendelsen ved ECOSTAT var hhv. en videnskabelig rapport (Wiberg-Larsen & Rasmussen, 2017) samt et interkalibreringsdokument, udfyldt i den såkaldte Gap2 platform (Wiberg-Larsen, 2019).

Udviklingen mv. af DLMI var bestilt af Miljøstyrelsen.

I forbindelse med den statslige vandplanlægning blev der i efteråret 2019 af Miljøstyrelsen beregnet indeksværdier af DLMI for en række søer, som ikke tidligere var undersøgt for dette kvalitetselement. Ligeledes blev der rutinemæssigt også beregnet indeksværdier for søer, som indgik ved udarbejdelsen af det ny indeks. Det viste sig imidlertid, at de ny-beregnede værdier for sidstnævnte søer afveg væsentlig fra de værdier, som indgik i rapportens datagrundlag.

Afvigelsen viste sig at skyldes, at programmet ASTRICS, der benyttes til beregning af de metrics/indices, som samlet indgår i DLMI, fejlagtigt kun indlæste en begrænset del af artsdatafilerne. Reelt blev kun den øvre del af artslisten i datafilen indlæst og metrics/indices beregnet herudfra. Det betyder naturligvis, at den oprindelige udvikling af DLMI ikke er retvisende. Derfor var det nødvendigt, at omregne de fire metrics/indices som indgår i det multimetriske indeks, og sammenhængen mellem DLMI-værdier og miljøfaktorer mv. skulle ligeledes genberegnes.

Dette notat er derfor en revision af det oprindelige makroinvertebratindeks, DLMI.

2 Kort beskrivelse af DLMI mv.

Det danske indeks, Dansk Littoralzone Makroinvertebrat Indeks (DLMI)er baseret på et allerede eksisterende litauisk indeks (LLMI), som er interkalibreret i forhold til sammenlignelige indices fra andre lande inden for den såkaldte centralbaltiske interkalibreringsgruppe (CB-GIG), som også Danmark tilhører.

DLMI er beskrevet detaljeret i Wiberg-Larsen & Rasmussen (2017). Der er i 2020 foretaget en genberegning af indices/metrics for DMLI (jf. forordet) og rapporten fra 2017 er således revideret (Wiberg-Larsen & Rasmussen, 2020). I konsekvens heraf er der tilvejebragt nye såkaldte ankerpunkter anvendt til beregning af EQR-værdier for hvert metric/indeks, der indgår i DLMI, ligesom der er foreslået ny grænseværdier for de enkelte statusklasser. Indholdet af den reviderede rapport svarer i opbygning og overordnet indhold ret nøje til den tidligere rapport. I det følgende er der givet en kort beskrivelse af det reviderede DLMI.

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 UK 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 sølokalitet 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 er testet på i alt 280 prøver fra 55 danske søer. To af disse søer kunne ud fra generelt accepterede kriterier karakteriseres som "referencesøer", 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. Disse kriterier definerer de to nationale søtyper 9 og 10 (jfr. vandrammedirektivet) og omfatter omkring halvdelen af alle danske søer.

Indekset er 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 er 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 er ved analysen anvendt den bedst forklarende faktor, PCA1. DLMI er signifikant korreleret med hver af de to påvirkninger, men langt stærkest med eutrofiering (PCA1).

Derudover er DLMI stærkt og signifikant korreleret ($r^2 = 0{,}85$) med det "fælles" makroinvertebratindeks (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, nedenfor forkortet til henholdsvis H, G, M, R, D) 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) er 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 G/M i forhold til de andre lande, som betyder, at de nationale grænser ville fremstå ”mildere”, har det været det nødvendigt/hensigtsmæssigt at ændre de foreløbige grænseværdier. Med udgangspunkt i en bias inden for ±0,25 er foreslået 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 vandrammedirektivet) kræver, at en søs indhold af totalfosfor (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 Miljøstyrelsen (daværende Styrelsen for Vand- og Naturforvaltning) (2016) kan god økologisk status imidlertid opnås for klorofyl-a, fytoplankton og makrofyter, hvis sommermiddel totalfosfor ikke overstiger 0,053 og 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 væsentligt højere end den nationale grænseværdi for klorofyl-a, fytoplankton og makrofyter.

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

3 Godkendelse af DLMI ved ECOSTAT

Før et nationalt indeks, i dette tilfælde DLMI, kan tages i brug som en såkaldt national standard, kræves at indekset interkalibreres i forhold til de indices, som øvrige sammenlignelige EU-lande anvender. I Danmarks tilfælde er der tale om landene i den såkaldte CB-GIG (Central-Baltic Geographical Intercalibration Group): Belgien (Flandern), Estland, Lithauen, Holland, Tyskland og UK

Formålet med interkalibreringen er at sikre, at de enkelte landes indices så at sige opererer på samme kvalitetsskala (dvs. for grænserne mellem de fem kvalitetsklasser), altså at de i praksis anvendes på en sammenligelig måde. Derved sikres, at et land fx ikke foretager vurderinger af vandområdernes (her søers) kvalitet, som er lempeligere end de øvrige landes (inden for givne beregnede og fastsatte rammer).

Selve interkalibreringsproceduren for CB-GIG følger Böhmer et al. (2014). Se også afsnit 2.

Interkalibreringen blev udført i en samlet proces for samtlige lande i CB-GIG. Da det danske indeks endnu ikke var færdigudviklet på dette tidspunkt, indgik den første udgave af DLMI i en efter-interkalibrering af kvalitetsgrænserne og blev endelig godkendt i maj 2019.

I praksis er proceduren, at det pågældende land indsender et såkaldt Gap2 dokument (platform) til godkendelse ved ECOSTAT. I forbindelse med godkendelsen foretages ved der via ECOSTAT et review af den foreslåede metode. Reviewet foretages af en kompetent fagperson. I forbindelse med den tidligere godkendelse af DLMI blev reviewet foretaget af Jürgen Böhmer, der forestod interkalibreringen af seks CB-GIG landes indices.

Selvom det oprindelige DLMI således allerede er godkendt af ECOSTAT til brug i danske søer, er det pga. de nævnte genberegninger af metrics/indices nødvendigt med en fornyet godkendelse ved ECOSTAT.

Det må forventes, at opnåelse af en sådan godkendelse vil være en mere eller mindre formssag. Indekset er således opbygningsmæssigt uændret, ligesom sammenhængen med miljøvariable er langt bedre end før, og sammenhængen med ICCM noget nær perfekt (se afsnit 2).

Ligesom for den tidligere udgave af DLMI skal der dog fastsættes nationale grænseværdier for adskillelsen af de fem statusklasser. Som det fremgår af afsnit 2, er der fra rådgiverside (DCE, Aarhus Universitet) foreslået grænser, som indebærer af Danmark i forhold til de øvrige CB-GIG lande placerer i sig midten af det såkaldte bias-bånd.

Til hjælp ved godkendelsen af den reviderede udgave af DLMI har DCE udarbejdet det nødvendige indhold til brug i Gap2-dokumentet (se appendiks, afsnit 6).

4 Nationale grænseværdier mellem statusklasser

Som nævnt har DCE – på et fagligt grundlag - foreslået grænser mellem de fem statusklasser, som opfylder kravene i forhold til interkalibrering med de øvrige CB-GIG lande.

Det er imidlertid de nationale miljømyndigheder (Miljøstyrelsen & Miljø- og Fødevareministeriet, Departementet), der beslutter præcis hvilke grænser, der ønskes anvendt. Grænserne skal dog som udgangspunkt opfylde kravene mht. bias (jf. afsnit 2 og 3).

Det er muligt for de pågældende myndigheder selv at foretage en interkalibrering af de ønskede grænser ved brug af et til formålet udviklet beregningsprogram i EXCEL (Nemitz et al. 2011).

5 Referencer

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Wiberg-Larsen, P & Rasmussen, JJ (2020) Revised Danish macroinvertebrate index for lakes - a method to assess ecological quality. Aarhus University, DCE – Danish Centre for Environment and Energy, 48 pp. Scientific Report from DCE – Danish Centre for Environment and Energy No. xxx. http://dce2.au.dk/pub/SR373.pdf

6 Appendiks: Bidrag til tekst til Gap2 dokument for DLMI

Reporting on Intercalibration of a revised, "Danish Lake Macroinvertebrate Index", according to finalized Intercalibration results (Gap 2)

INTRODUCTION

MS: Denmark
BQE: Benthic invertebrates
Category: Lakes.

This document is prepared to replace the former document (Wiberg-Larsen 2019) that was the template for the approval by ECOSTAT (7-8 May 2019) of the first Danish Lake Macroinvertebrate Index. The reason for submitting a new, revised Gap2 is detection of a flaw in the original work. Thus, due an inexpediency of the program ASTERICS, only a part of present taxa of the total macroinvertebrate data sheet was used in calculation of the metrics of the index. Thus, it has been necessary to revise the original report (Wiberg-Larsen & Rasmussen 2017), using the full macroinvertebrate data set. Overall, however, the present Gap2 and the revised report (Wiberg-Larsen & Rasmussen 2020) are based on the same approach as the previous work (and the multimetric index the same). The important differences is that metrics are now correctly calculated, and accordingly new anchor points are provided. Further, relations between DLMI and stressors/ICMM are re-calculated, and new intercalibrated status class boundaries for DLMI are provided.

DESCRIPTION OF NATIONAL ASSESSMENT METHODS

Denmark has adopted a minor modification of the Lithuanian multimetric index (LLMI), named Danish Lake Macroinvertebrate Index (DLMI), and defined as:

DLMI = (ASPT + Hill1 + EPTCBO taxa + %COP taxa)/4.

NOTE: A more detailed description of the national method, including origin of data, data treatment etc. is provided in Wiberg-Larsen & Rasmussen (2020).

METHODS AND REQUIRED BQE PARAMETERS

Table 1. Overview of the metrics/indices included in the national method (See appendix)

MS	Taxonomic composition	Abundance	Diversity	Ratio sensitive/non-sensitive taxa
DK	EPTCBO, %COP	%COP	EPTCBO, %COP, Hill1	ASPT

Normalised EQR of all metrics (i.e. standards for measurements) - including scores - are summed and divided equally by 4. The selected metrics all comply with requirements regarding parameters for benthic invertebrates in lakes.

SAMPLING AND DATA PROCESSING

Description of sampling and data processing:
Sampling time and frequency: September – October.
Sampling method: Kick sampling (composite sample) during a fixed period (2 minutes) on solid substrates (sand, gravel, stones). Thus, one sample is collected on a specific site. Number of sites should be at least four in each lake. Standard equipment is a handnet with a 25 x 25 cm opening and net mesh size 0.5 mm.
Data processing: Samples are preserved using ethanol to a final concentration of 80%. Macroinvertebrates are hand-sorted and counted in laboratory. Data may be handled in EXCEL and metrics also calculated in EXCEL (according to technical guidance documents under the monitoring program, NOVANA). BMWP family scores used to calculate ASPT are presented in Wiberg-Larsen & Rasmussen (2020).
Identification level: Groups like Ephemeroptera, Plecoptera, Odonata, Coleoptera, Trichoptera, and Bivalvia are identified to species (except for most larvae of Coleoptera), whereas most other groups (especially Oligochaeta and Diptera) are identified to family/subfamily or less (e.g. Hydrachnidia). The identification level ensures calculation of all relevant metrics. In the basic data set used in testing of the new index, the identification level of several groups were more detailed than this, e.g. Chironomidae (subfamily, tribus). However, these were only treated on family level in calculation of metrics. The identification level is described in Wiberg-Larsen & Rasmussen (2020).

NATIONAL REFERENCE CONDITIONS

Danish landscapes are heavily impacted by human activities, about 60% of the total area being used for agriculture, and in addition, urban areas (including roads) make up about 10%. Thus, true anthropogenically un-impacted or minimally impacted lake ecosystems do not exist. There are several definitions of the term "reference condition" (Stoddard et al. 2006) representing a pragmatic and systematised procedure for cases where no truly un-impacted or minimally impacted ecosystems exist. Wiberg-Larsen (2014) identified "best achievable condition" as the best alternative definition available. The specific physical and chemical characteristics defining the best achievable condition for Danish lakes have been identified by Søndergaard (2003) and are summarized in Table 2.2. These threshold values were (according to Böhmer et al. 2014) supplemented with a criteria of <10% agriculture and <10% artificial land (urban settlements, roads, paved surfaces etc.), and only minor anthropogenic modifications of the riparian and littoral zones defined as a Pressure Index value according to Miler et al. (2012), see 2.5. Thus, PI values < 3 less were accepted as reference conditions.

Table 2.2. Threshold values defining the best achievable condition for Danish lakes belonging to lake types 9 or type 10 (see Chapter 3). Data originates from Søndergaard et al. (2003).

Lake type	Total - P (µg L-1)	Total-N (mg L-1)	Chlorophyll a (µg L-1)	Secchi- depth (m)
9	14.6	0.4	3.7	3.8
10	7.6	0.38	3.9	5.4

Using these criteria, only two lakes qualified as having reference conditions based on all selective criteria: Lake Almind and Lake Slåen.

NATIONAL BOUNDARY SETTING

Denmark used linear regression between DLMI and ICCM as a template to provide national boundary classes. ICCM is defined as: (2*EPTCBOtaxa + ASPT +%ETO taxa + %Lithophile taxa)/5.

Regression showed that DLMI and ICCM were highly correlated and samples well distributed – except for a few outliers - along the line that was anchored almost in 0.0 and 1.1 (slope 0.99, intercept 0.007) – see Figure

2.1. Thus, boundary classes were obtained simply by dividing the DLMI axis in five equal intervals representing each of the five ecological status classes.

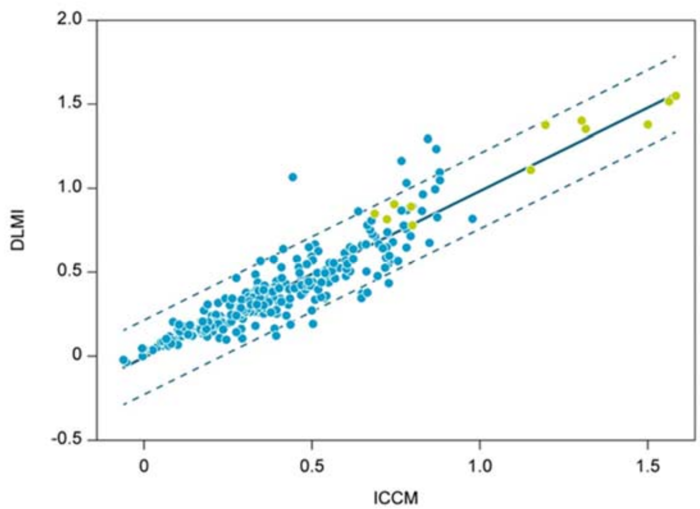

*Figure 2.1. Linear regression (solid line) between DLMI and ICCMi reflecting 280 samples from 55 Danish lakes: DLMI = -0.007 + (0.9894 * ICMM) (r^2=0.85). Shown 95% prediction interval (stippled lines) and reference lakes (green dots).*

PRESSURES ADDRESSED

Two pressures were identified to be important in Danish lakes: Eutrophication and anthropogenic impacts in the littoral zone. Eutrophication has received an overall interest during many decades, primarily being recognized as an effect of phosphorus (total-P) and to minor extent nitrogen. In comparison, littoral pressures and hydromorphological alteration have received less attention until recently.

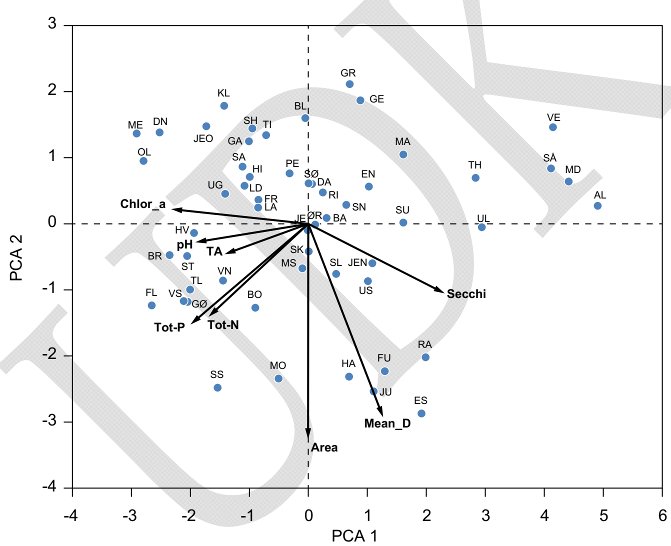

Figure 2.2. Principal Components Analysis for 55 Danish lakes. PCA1 explained 44.3% of the variation and represented parameters all indicative for eutrophication and thus significantly correlated with this axis (Pearson r): Total-P (r = -0.76, P<0.0001), total-N (r = -0.64, P <0.0001), chlorophyll-a (r = -0.88, P <0.0001), pH (r = -0.72, P <0.0001), and Secchi depth (r = 0.87 P<0.0001). PCA axis 2 represented a gradient in lake size (surface area: r = 0.82, P <0.0001) and mean depth: r =0.74, P <0.0001).

During a previous study (Wiberg-Larsen 2014), it was found that total-P was a significant, although far from perfect descriptor of eutrophication in correlation with macroinvertebrate metrics. Thus, instead eutrophication was described by a proxy parameter constructed by axis 1 scores (PCA1) in a Principal Component Analysis (PCA) including physical, chemical and biological parameters, see figure 2.2. The data were derived as "average" data for a specific lake from regional and national monitoring programs (average of at least 7 samplings during a specific year – temporarily comparable with macroinvertebrate samples). Chemical and several physical parameters were calculated and used as yearly means.

Anthropogenic impact in the littoral zone (where samples) was assessed using a Pressure index (PI) as described by Miler et al. (2012). The index was estimated for all 280 sites (from 55 lakes) included in the intercalibration.

DLMI responded significantly to both pressures, see figure 2.3. The relation was by far strongest for eutrophication (PCA1) (r^2=0.39, P<0.001) – whereas the relation to PI was minor (r^2=0.06, P<0.001) In a multiple linear regression, both pressures contributed significantly to the explanation of DLMI:

DLMI = 0.455 + (0.094 * PCA1) - (0.008 * PI)
(r^2=0.45, P<0.001)

The correlation (r) to both pressures in combination (and to PCA1 specifically) was well above 0.25 and, thus, acceptable.

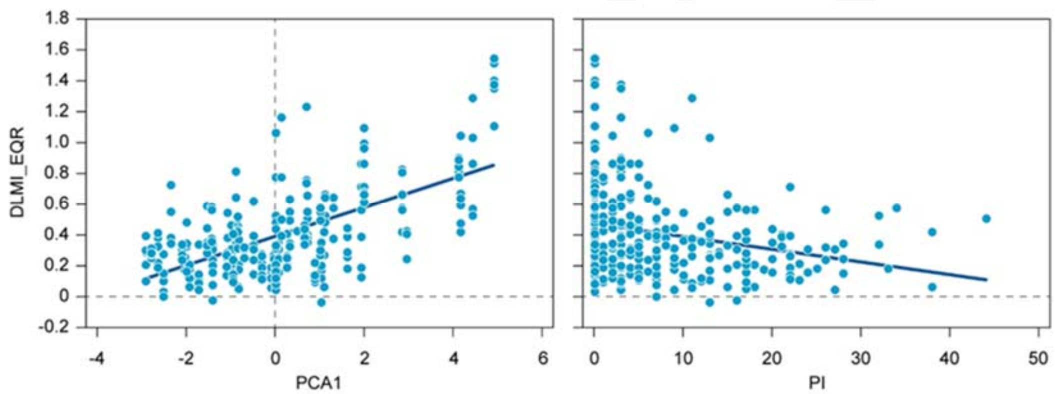

Figure 2.3. Linear regression between DLMI and PCA1, a proxy for "eutrophication" (LEFT), and Pressure Index, PI, representing anthropogenic impact in the littoral zone (RIGHT). For formulas see text above.

WFD COMPLIANCE CHECKING

Compliance criteria	Compliance checking
Ecological status is classified by one of five classes (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's normative definitions (Boundary setting procedure)	YES
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A combination rule to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES. Taxonomic composition, abundance, disturbance sensitive taxa, diversity are all included in a multimetric index by the choice of four specific indices/metrics: ASPT, Hill1, EPTCBO taxa and %COP taxa. Further, absence of major taxonomic groups is also considered by including EPTCBO taxa (although it is assessed that this parameter might not be as meaningful in lakes as in streams)
Assessment is adapted to intercalibration common types that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	YES. Only lakes belonging to Danish types 9 and 10 are included in the intercalibration, both being equivalent to those included in the CB-GIG intercalibration process (L-CB1 and L-CB2).

The water body is assessed against type-specific near-natural reference conditions	YES. It was possible to designate two out of the 55 lakes included in the intercalibration as reference lakes (according to criteria that were comparable to those used by CB-GIG). Ideally, the number of reference lakes should have been at least five, but more than two were not available.
Assessment results are expressed as EQRs	YES. Using standard procedure for upper and lower anchors: $$EQR = \frac{\text{observed value - lower anchor}}{\text{reference value - lower anchor}}$$ Upper anchor values – for all four metrics - were estimated as the 95 percentiles of their distribution from all samples/lakes. Note that values for reference lakes alone were not used to define upper anchors. This was due to the exceptionally high taxon richness of Lake Almind. Lower anchors were estimated using same CB- GIG procedures estimating these as 10 percentiles based on all samples/lakes, except that absolute minimum was used for ASPT.
Sampling procedure allows for representative information about water body quality/ecological status in space and time	YES. Overall, the 55 lakes in the data set are geographical representative, and macroinvertebrate data temporarily comparable. Number of samples of macroinvertebrates (and description of littoral pressures) were obtained from on the average 6 sites per lake (range 1-8). Water chemistry was described by at least 7 samplings during a year used to calculate a yearly mean that was used in the analyses. In each lake, data for macroinvertebrates, PI and chemistry (s.l.) were from the same year.
All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure	YES
Selected taxonomic level achieves adequate confidence and precision in classification	YES

IC FEASIBILITY CHECKING

TYPOLOGY

The national method addresses the same common lake types as were included by the other countries in the CB-GIG (L-CB1 = national type 9 and L-CB2 = national type 10), see chapter 3. The number of lakes of these types was almost equal: 26 L-CB1 and 29 L-CB2.

PRESSURES ADDRESSED

Overall, the national method addresses the same pressures as considered by the other countries in the CB-GIG. These pressures are primarily eutrophication, considered in all six countries included in the CB-GIG calibration, and hydromorphological alterations, considered in five of the six countries. It is assessed that the national interpretation of the pressures are equivalent to that used by the other countries, although Denmark have used an eutrophication proxy instead of e.g. total-P. Regarding hydromorphological alterations, Denmark has used a well-documented and reproducible method according to Miler et al. (2012).

ASSESSMENT CONCEPT

The national method follows the same assessment concept as the large majority of the countries in CB- GIG. Thus, the national method is based on macroinvertebrate communities in the eulittoral zone, depending on composite samples. Further, the method involves use of a multimetric index (DLMI) that is a slightly modified version of LLMI already being intercalibrated. Like Denmark, most countries have provided multimetric indices to reflect relevant pressures.

CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Overall the national method is considered consistent and IC feasible.

DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

Background

Six Member States (Belgium-Flanders, Estonia, Germany, Lithuania, the Netherlands and UK) were intercalibrated in CB-GIG based on their submitted benthic invertebrate assessment methods. After evaluation of WFD compliance and the IC feasibility, all methods were included in the IC exercise all addressing eutrophication and hydromorphological alterations (except UK - only eutrophication) and follow the same assessment concept focusing on the eulittoral zone (except UK – "whole lake samples"). Intercalibration "Option 2" was used, i.e. an indirect comparison of assessment methods using a common metric. This IC common metric (ICCM) was developed specifically for the IC exercise comprising four metrics, and it was benchmark-standardized using a "continuous benchmarking" approach. ICCM was formulated as: (2*EPTCBO + ASPT + %ETO taxa + %Lithophile taxa)/5.
The present intercalibration is carried out as an indirect comparison against the common metrics, ICCM, described above ("option 2"). Further, Denmark has used benchmark-standardization also as that used in the completed IC exercise.

Description of IC dataset

The National data set were obtained primarily from the National Monitoring Program for Nature and Aquatic Environment (NOVANA). The majority of the data (macroinvertebrate samples and assessment of littoral pressures (PI)) was sampled/measured specifically for the present development, test and intercalibration of the Danish method. These data were collected during 2012-2013 for NOVANA lakes that were planned to be surveyed for water chemistry. These data included 36 lakes. Further, data were compiled from older studies (monitoring) of 19 lakes during the period 1984-1994, these data being provided by the regional environmental authorities (Danish County Councils being present at that time). Despite this temporal difference of the data set, it is assumed that data from all 55 lakes are directly comparable in a comprehensive analysis. Thus, it has been possible to provide temporarily matching macroinvertebrate and pressure data for each of the 55 lakes, and to ensure that the sampling and sample processing for macroinvertebrates, assessment of anthropogenic pressures (PI), and environmental data (lake area, depth, water chemistry, chlorophyll-a and Secchi depth) were compatible (same methods for sampling and analyzing water samples).

Description of Intercalibration procedure

Benchmark standardization

Due to the scarcity of reference lakes, "continuous benchmarking" was used (Böhmer et al. 2014), using linear models with biological metrics as dependent variable and combined pressure variables as covariates. Adjustments of the dose response regressions were made by selecting standardization values. All macroinvertebrate metrics used in the DLMI and ICCM (as well as other metrics considered during the development and testing of a new Danish index) were subjected to benchmark standardization. The standard formula EQR = (Observed value – lower anchor)/(Upper anchor-lower anchor) was used. Upper anchor were calculated as 95 percentile for values from all samples/lakes. Lower anchors were calculated

according to the CB-GIG benchmarking, being estimated as the10 percentile of all samples/lakes (except absolute minimum for ASPT).
For those metrics included in ICCM (EPTCBO taxa, ASPT, %ETO taxa, %Lithophile taxa) the national values were in practice identical with those used in CB-GIG benchmarking (although here estimated as the 90 percentile). Main difference was that national lower anchors of %ETO taxa and %Lithophile taxa were somewhat lower than those of CB-GIG.
Calculation of Intercalibration Common metrics (ICCM)
After benchmarking of the relevant metrics, ICCM was calculated as: (2*EPTCBO + ASPT + %ETO taxa + %Lithophile taxa)/5.

Translation of national boundaries to ICMM.

The scores of DLMI and ICCM was correlated using linear regression according to CB-GIG procedures – see figure 2.1.

The regression line, DLMI = -0.007 + (0.9894 * ICMM) was highly significant ($r2 = 0.85$, $P<0.001$; $N = 280$) and values well distributed along the line, except for a few outliers (approx. 5% of all values/sampling sites).

Denmark has not a priori defined national boundaries for DLMI. Due to the fact that the regression line was anchored almost in 0.0 and 1.0 and with slope 0.99, national boundary classes may be obtained by simply dividing the DLMI axis according to an equal division of the ICMM axis in five equal intervals (<0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8, and >0.8) representing each of the five ecological status classes bad, poor, moderate, good and high. Thereafter, preliminary national boundaries was estimated a priori from the regression result.

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

Calculating boundary bias.

The national preliminary boundaries was intercalibrated using a template (Nemitz et al 2011) with data used in the CB-GIG intercalibration (see Böhmer et al. 2014), and supplied by Jürgen Böhmer (Bioforum, Germany) in 2016 (in relation to the previous submission of Gap2 for DLMI). Please consult chapters 6 & 7 in Böhmer et al. (2014) for explanation of the procedure. Overall, every national index is linearly correlated with the ICCM (see above). The tolerable bias (status class width) is ± 0.25 (Böhmer et al. 2014).
Harmonizing of boundaries.
The outcome of the intercalibration was that the preliminary boundaries resulted in a tolerable H/G bias (0.085), whereas the G/M (bias 0.309) were well above 0.25. This means that the boundary G/M were more strict than required. Although Denmark is, thus, not under the obligation to revise the preliminary boundaries, it is advisable to lower these allowing a bias within the band of ±0.25.

Final boundaries

The choice of the National boundaries between environmental classes are the responsibility of the ministry of environment and food, and this section of the document are therefore not completed.

National boundaries are accordingly adjusted to:

Boundaries	DLMI
High/Good	0.XX
Good/Moderate	0.XX
Moderate/Poor	0.XX
Poor/Bad	0.XX

Insert text with argumentation for the chosen boundaries……

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

Macroinvertebrate communities were defined using multidimensional scaling of Bray-Curtis similarities (in PRIMER 6). Samples were initially designated to any of the five ecological quality classes (see 5.4), and the differences between classes tested using ANOSIM, supplied by SIMPER procedure to evaluate the contribution to the differences by specific taxa.
Overall, it was possible to separate communities according to ecological status, although difficult for some status classes. Thus, communities with high status were significantly separable from those with good, moderate, poor and bad status, whereas communities with good status were significantly separated from those with poor and bad status, but only weakly from those with moderate status. In the lower end of the scale, samples with moderate status were significantly separated from those with poor and bad status.
The picture of a relatively uniform taxonomic composition is supported when further characterising the macroinvertebrate communities. Thus, the majority of widely occurring and abundant taxa were found in samples covering the whole spectrum of quality classes. Examples are Tubificidae (Oligochata), *Pisidium spp.* (Bivalvia), *Asellus aquaticus* (Crustacea), *Caenis horaria* (Ephemeroptera), *Tanytarsini* and *Chironomini* (Diptera).
Despite this high degree of conformity, it was possible to provide the following descriptions.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

Occurrence of a very high number of taxa, being higher than those with moderate, poor and bad status. Examples were *Gammarus pulex* (Crustacea, Amphipoda), *Caenis luctuosa* (Ephemeroptera), *Nemoura avicularis* (Plecoptera) and *Oulimnius tuberculatus* (Coleoptera).
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.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

Communities were characterized and separated from those with poor and bad status, based on the same characteristics as those with high status. It was not possible to point out taxa that clearly separated communities with high and good status.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Generally absence of the "indicator" taxa mentioned above. Abundances *of Gammarus pulex, Caenis luctuosa, Nemoura avicularis* and *Oulimnius spp.* low. Diversity higher than for communities with poor and bad status.

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Appendix.

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

Indices/metrics	**Description**	**Characterisation according to WFD requirements**
%COP (abundance)	Abundance of Coleoptera + Odonata + Plecoptera) (% of all taxa)	Taxonomic composition, abundance, 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) 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

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