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1 **A new diatom-based multimetric index to assess lake ecological status**

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7 **Abstract**

8 Eutrophication impairs lake ecosystems at a global scale. In this context, as benthic
9 microalgae are well-established warnings for a large range of stressors, particularly nutrient
10 enrichment, the Water Framework Directive required the development of diatom-based
11 methods to monitor lake eutrophication.

12 Here, we present the diatom-based index we developed for French lakes, named IBDL. Data
13 were collected in 93 lakes from 2015 to 2020. A challenge arose from the discontinuous
14 pressure gradient of our dataset, especially the low number of nutrient-impacted lakes. To
15 analyze the data we opted for the so-called “Threshold Indicator Taxa ANalysis” method,
16 which makes it possible to determine a list of “alert taxa”. We obtained a multimetric index
17 based on different pressure gradients (Kjeldahl nitrogen, suspended matter, biological oxygen
18 demand and total phosphorous).

19 The IBDL proved to be particularly relevant as it has a twofold interest: an excellent
20 relationship with total phosphorus and possible application to any lake metatype. Its
21 complementarity with macrophyte-based indices moreover justifies the use of at least two
22 primary producer components for lake ecological status classification.

23 **Keywords:** ecological assessment, lakes, phytobenthos, Water Framework Directive

24 **1-Introduction**

25 Eutrophication is one of the most frequent consequences of human pressure on lake
26 ecosystems at a global scale (Stenger-Kovacs et al., 2007). Primary producers are directly
27 impacted since they are the base of the aquatic food web (Brauer et al., 2012). As the ability
28 of species to compete differs according to nutrient availability, nutrient enrichment results in
29 significant changes in community structure and function (Birk, 2012). For this reason,
30 scientists and policymakers developed indices based on primary producer attributes to
31 monitor eutrophication (Stevenson, 2014). In the early 2000s, the Water Framework Directive
32 (WFD, 2000/06/EC) required all EU member states to implement bioassessment methods
33 based, among other aspects, on the biological quality of “macrophytes and phytobenthos” to
34 assess lake ecological status. This led to the development of numerous methods at the
35 European level.

36 Poikane et al. (2016) reviewed this panel of methods and observed that countries generally
37 developed separate assessment tools for macrophytes and phytobenthos, and that most of
38 them considered diatoms, which are unicellular microalgae, to be a good proxy for
39 phytobenthos. Diatoms are indeed early and well-established warnings for a large range of
40 stressors, particularly nutrient enrichment (Stevenson, 2014). As a first step, indices originally
41 dedicated to rivers were applied to lakes by the majority of member states (Kelly et al.,
42 2014b), considering that many processes influencing diatom assemblages were comparable
43 between lakeshores and shallow rivers (Cantonati and Lowe, 2014).

44 In some rare cases, diatom-based indices were developed specifically for lakes, based on
45 species composition and abundance as for rivers (Bennion et al., 2014; Poikane et al., 2016).
46 Diatoms from mud and silts were generally not considered, as they would respond to pore-
47 water chemistry rather than water quality. The recommended sampling substrate varied

48 according to authors, from macrophytes to cobbles or even artificial substrates when no
49 natural substrates are found in all water bodies (King et al., 2006).

50 To harmonize the different national approaches, a European intercalibration exercise was
51 performed, involving eleven member states (Kelly et al., 2014b). France participated in this
52 exercise with the Biological Diatom Index (BDI, Coste et al., 2009), routinely used to assess
53 river ecological status. Although previous results tended to suggest there was a good
54 correlation between BDI and the environmental pressure gradients, at least in shallow lakes
55 (Cellamare et al., 2012), this intercalibration exercise revealed a poor correlation between
56 BDI values and total phosphorous across France (Kelly et al., 2014b). This was explained by
57 the absence of many lake taxa from the list of key species used to calculate the BDI, resulting
58 in an overall poor relevance of the final status assessment.

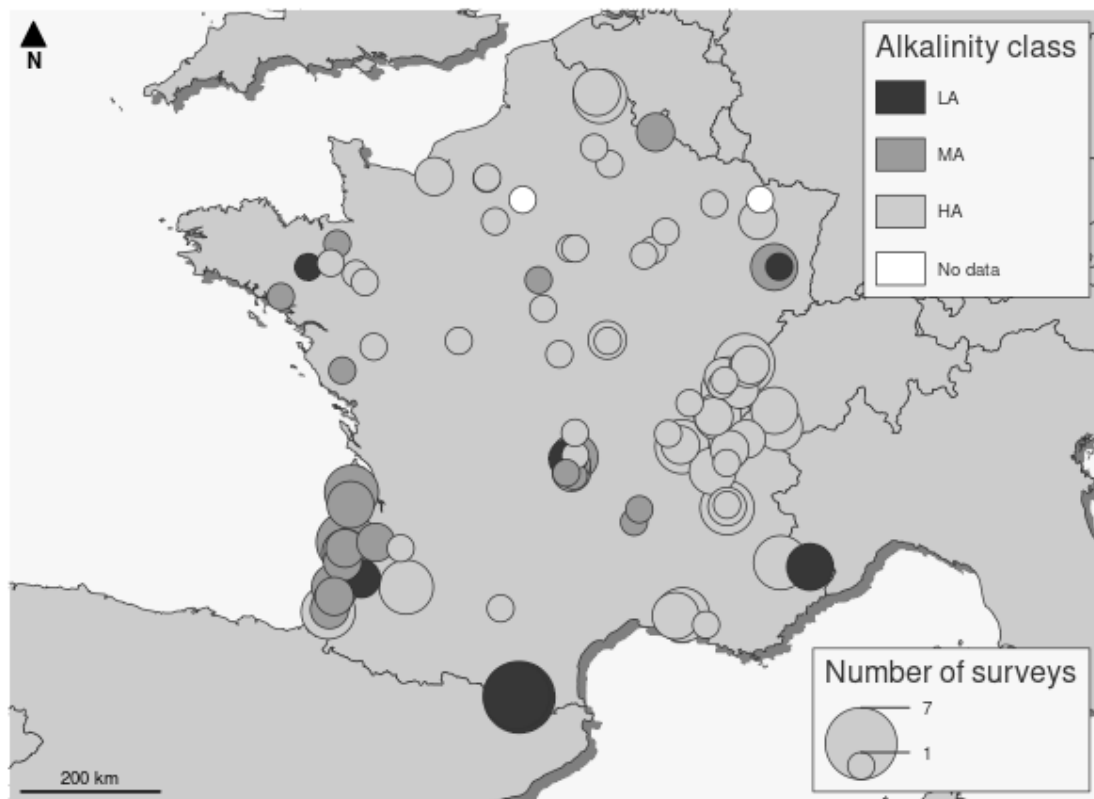
59 The aim of the present study was, therefore, to develop a new diatom-based index for lakes in
60 metropolitan France: the IBDL (*Indice Biologique Diatomées en Lac*: Diatom Biological
61 Index for Lakes). To collect the necessary data, we proposed a method (Morin et al., 2010)
62 consistent with a potential subsequent combination of this index with the existing French
63 macrophyte index IBML (*Indice Biologique Macrophytique en Lac*: Macrophyte Biological
64 Index for Lakes, Boutry et al., 2015). We detail here how diatom data were sampled and
65 analyzed and how we developed the IBDL. Finally, we discuss the relevance of this new
66 index, comparing the results obtained with index scores based on macrophytes, and assessing
67 its ability to reveal environmental gradients.

68 **2-Materials and Methods**

69 **2-1 Data collection**

70 Samples were collected from 93 French lakes (Figure 1) during the summer period, each year
71 from 2015 to 2020, according to Morin et al. (2010). The lakes were classified into three

72 metatypes based on alkalinity, according to the European intercalibration exercise previously
73 performed (Kelly et al., 2014b): low alkalinity (LA, alkalinity $\leq 0.2 \text{ meq.l}^{-1}$), medium
74 alkalinity (MA, $0.2 \text{ meq.l}^{-1} < \text{alkalinity} < 1 \text{ meq.l}^{-1}$), and high alkalinity (HA, alkalinity ≥ 1
75 meq.l^{-1}). Diatoms were collected from both mineral substrates and lakeshore macrophyte
76 surfaces in observation units (OUs), whose number and location varied according to the lake
77 surface area and the riparian zone types. Such units are defined in the French macrophyte
78 sampling protocol for lakes NF T90-328 (AFNOR, 2022).



79

80 Figure 1: Study sites, number of surveys per site, and lake alkalinity classes (LA: low
81 alkalinity; MA: medium alkalinity; HA: high alkalinity) (Kelly et al., 2014a)

82

83 2-1-1 Biological data

84 Samples from hard mineral substrates were taken from at least five boulders or cobbles
85 selected at random for each OU. The total surface area sampled was equivalent to 100 cm², as
86 defined in the NF T90-354 standard (AFNOR, 2016). Selected substrates had to be submerged
87 within the euphotic zone, at a maximum depth of 0.5 m.

88 Samples performed on macrophytes were taken from helophytes (mainly *Phragmites australis*
89 (Cav.) Trin. ex Steud.). Green stem segments submerged for at least 4 to 6 weeks were
90 collected from a minimum of 5 macrophytes chosen at random. These stem segments had to
91 be located at a maximum depth of 0.2 m.

92 Diatoms were sampled from both substrates according to the NF T90-354 protocol, in line
93 with the European standards (EN 13946, European Commission). Cells were identified at
94 100x magnification by examining permanent slides of cleaned diatom frustules (400 valves
95 per slide) using, among others, Krammer and Lange-Bertalot (1986–1991) and Lange-
96 Bertalot (1995–2015, 2000–2013). Taxonomic homogenization was performed with Omnidia
97 6 software (Lecointe et al., 1993).

98 All OUs from a single lake were sampled within a maximum of 21 days. Diatom counts had
99 to include at least 350 cells per slide, with more than 50% of the diatom cells determined at
100 the species level, to comply with the NF T90-354 requirements.

101 2-1-2 Physico-chemical data

102 Parameter values were determined in summer at the deepest point of each lake, according to
103 European standards. Data were obtained from national surveillance monitoring programs.
104 Water quality analysis was not systematically performed each year: in a few cases, the most
105 recent physicochemical data available were collected three years before the diatom samples.
106 The following parameters were recorded: biological oxygen demand (BOD₅, mg.l⁻¹), oxygen
107 (O₂, mg.l⁻¹), oxygen saturation (% O₂), conductivity (Cond, μs.cm²), Kjeldahl nitrogen (NKJ,

108 mg.l⁻¹), ammonium (NH₄, mg.l⁻¹), nitrates (NO₃, mg.l⁻¹), nitrites (NO₂, mg.l⁻¹), orthophosphates
109 (PO₄, mg.l⁻¹), total phosphorous (Pt, mg.l⁻¹) and suspended particles (SP, mg.l⁻¹).

110 **2-2 Data analysis and index settlement**

111 All analyses were performed with R version 4.1.2 (2021-11-01) (R Core Team, 2021)
112 (Platform: x86_64-pc-linux-gnu (64-bit), Running under: Ubuntu 22.04.1 LTS).

113 Considering that the final dataset revealed a discontinuous trophic gradient, we opted for the
114 so-called “Threshold Indicator Taxa ANalysis” method (TITAN2 package, Baker et al.,
115 2020), which, based on bootstrapping and permutations, makes it possible to determine a list
116 of “alert taxa”. The presence and/or increasing abundance of alert taxa reveal the existence of
117 anthropogenic pressures. TITAN replaces the community-level response along a composite
118 gradient with taxon-specific responses towards single environmental variables (Dufrière and
119 Legendre 1997). Negative and positive responses are distinguished, and cumulative
120 decreasing or increasing responses in the community are tracked. This method is particularly
121 suitable for setting up multimetric indices.

122 A three-step procedure was necessary to build our Biological Diatom Index for Lakes (IBDL):
123 identification of alert taxa, choice of relevant metrics, and aggregation of these metrics to
124 obtain the final index score.

125 **2-2-1 Identification of alert taxa**

126 For the next part of the analysis, we set an occurrence threshold ≥ 3 for taxa to be included in
127 the index calculation (the so-called “index taxa”).

128 TITAN combines change-point analysis (nCPA; King and Richardson 2003) and indicator
129 species analysis (IndVal, Dufrière and Legendre 1997). Basically, the change-point analysis
130 compares within-group vs. between-group dissimilarity to detect shifts in community

131 structure along the environmental variable considered (for further details concerning this
132 method see Baker and King, 2010). Indicator species analysis then identifies the strength of
133 association between any particular taxon and this sample grouping. At the end of the process,
134 two IndVal scores are calculated for a single taxon in a two-group classification. The
135 algorithm finally classifies taxa into three different categories: Z^+ taxa, showing a significant
136 increase in abundance along the increasing environmental gradient; Z^- taxa, showing a
137 significant decrease along this gradient; and indifferent taxa, with no significant trend.
138 Alert taxa were defined as Z^+ or Z^- taxa whose shift thresholds were greater or lesser than the
139 community shift threshold.

140 2-2-2 Building metrics and selecting the relevant ones

141 For each environmental variable, a metric was calculated at the OU scale according to (1):

$$142 \text{ Metric}_M = 1 - \left(\frac{\text{Alert}_{\text{taxa}}}{\text{Index}_{\text{taxa}}} \right) \quad (1)$$

143 Where:

144 $\text{Alert}_{\text{taxa}}$ is the number of alert taxa and $\text{Index}_{\text{taxa}}$ is the number of index taxa in the sample.

145 The metric value is bounded between 0 and 1. The lowest value (0) corresponds to a species
146 list entirely composed of alert taxa (determined for the environmental variable considered).

147 To build our index, we then selected the most relevant metrics, i.e., those with the best
148 relationship with the environmental parameter considered. We used Pearson's correlation
149 coefficients to measure this statistical association and only kept metrics showing a Pearson's
150 coefficient over 0.6. Metrics should significantly increase with impairment, significantly
151 decrease with impairment, or show no particular pattern. We obtained the response patterns of
152 the different metrics by transforming raw values into normalized deviations (Standardized
153 Effect Size: SES, Gotelli and McCabe, 2002; Mondy et al., 2012) (2). SES values made it

154 possible to obtain a single response pattern for a metric whatever the lake metatype and
155 substrate type considered.

$$156 \quad SES_M = \left(\frac{Metric_M - M_{group}}{sd_{group}} \right) \quad (2)$$

157 Where:

158 $Metric_M$ is the observed value of the metric, M_{group} and sd_{group} are the mean and standard
159 deviation, respectively, of the metric value for a given group of samples (i.e., substrate type x
160 lake alkalinity metatype) (values of M_{group} and sd_{group} are given in Table 1 S1)

161 The next step consisted of the normalization of SES values (SES_{nor_M}) to make comparable
162 metric variation ranges (3):

$$163 \quad SES_{nor_M} = \frac{(SES_M - Min)}{(Max - Min)} \quad (3)$$

164 Where:

165 SES_M is the observed value of SES for a given metric, Min its minimum value and Max its
166 maximum value in the whole dataset (values of Min and Max are given in Table 2 S1).

167 We further transformed metric values from normalized SES into the Ecological Quality Ratio
168 (EQR) (4), i.e. the ratio between the observed value of a metric (SES_{nor_M}) and its expected
169 value under reference conditions (Kelly et al., 2014a), for any lake metatype and any substrate
170 ($SES_{nor_{Mref}}$, values given in Table 3 S1).

$$171 \quad EQR = \left(\frac{SES_{nor_M}}{SES_{nor_{Mref}}} \right) \quad (4)$$

172

173 Finally, for each metric, we performed a Wilcoxon test to detect the potential influence of
174 substrate type on the EQR values obtained at the OU scale.

175 2-2-4 Aggregating metric values to obtain the final IBDL score

176 The final index score was obtained at the OU scale by averaging the selected metric values,
177 expressed in EQR.

178 For a score calculated for both mineral and macrophyte substrates, the lowest value was
179 considered the final score.

180 Each OU belongs to one of the four riparian zone types, as required in the NF T90-328
181 standard (AFNOR, 2022). These types were defined from the vegetation composition and/or
182 anthropogenic alterations of the lakeshore. The percentage of each riparian zone type was
183 estimated *in situ*, on the whole lake perimeter, during the sampling surveys. The final index
184 score for the whole lake was derived from a weighted average of the $Score_{OU}$ (5), taking into
185 account the percentage of the lake perimeter each OU represented in terms of riparian zone
186 type ($P_{C_{type}}$).

$$187 \quad IBDL = \sum_{type=1}^4 (\overline{Score_{OU}} * P_{C_{type}}) \quad (5)$$

188 Finally, the resulting IBDL scores varied between 0 (worst water quality) and 1. Relationships
189 between IBDL scores and the different environmental variables considered were tested *a*
190 *posteriori* with simple linear regressions (R "mass" package, Venables & Ripley, 2002).

191

192 2-3 Comparing IBDL and IBML scores

193 We compared IBDL and IBML scores, based respectively on diatom and macrophyte
194 communities, to evaluate their complementarity or redundancy. IBML scores were computed
195 with the online application <https://see.eaufrance.fr/api/indicateurs/IBML/1.0.1> and the "httr"
196 package (Wickham, 2022).

197 We built a multiple linear regression model ("mass" package) to test which index correlated
198 best with Pt values: IBML, IBDL or a combination of both (mean value).

199

200 2-4 Preparing intercalibration

201 Considering a future intercalibration exercise, we analyzed the relationships between IBDL
202 scores and P_t for each lake metatype. A good correlation of the candidate metric with P_t
203 constitutes a key criterion for considering the index ready for integration into the
204 intercalibration process (Kelly et al., 2014b).

205 We also plotted IBDL against CM scores (intercalibration Common Metric, i.e., the Trophic
206 Index developed by Rott et al., 1998), to check their compliance. The CM was calculated with
207 Omnidia 6 software.

208

209 **3-Results**

210 Our data revealed discontinuous pressure gradients (Table 1), with a clear lack of impacted
211 conditions and an over-representation of lakes characterized by low eutrophication levels.

Variable	% of missing values	mean	sd	median	p25	p75	maximum
ammonium (NH ₄ , mg.l-1)	0.292	0.090	0.350	0.015	0.010	0.060	3.30
biological oxygen demand (DBO ₅ , mg.l-1)	0.584	2.157	2.615	1.300	0.900	1.800	12.00
conductivity	0.309	230.108	124.368	243.500	158.000	297.000	815.00
Kjeldahl nitrogen (NKJ, mg.l-1)	0.292	0.661	0.959	0.250	0.250	0.700	6.90
nitrates (NO ₃ , mg.l-1)	0.292	1.113	1.222	0.600	0.250	1.400	6.07
nitrites (NO ₂ , mg.l-1)	0.292	0.011	0.025	0.005	0.005	0.010	0.30
orthophosphates (PO ₄ , mg.l-1)	0.292	0.015	0.026	0.005	0.005	0.010	0.22
oxygen (O ₂ , mg.l-1)	0.333	9.143	3.943	8.700	8.100	9.685	73.00
oxygen saturation (% O ₂)	0.333	110.203	20.910	108.000	101.000	117.650	187.00
suspended particles (SP, mg.l-1)	0.292	7.979	18.145	2.800	1.600	5.000	153.00
total phosphorous (Pt, mg.l-1)	0.292	0.027	0.067	0.005	0.005	0.015	0.51

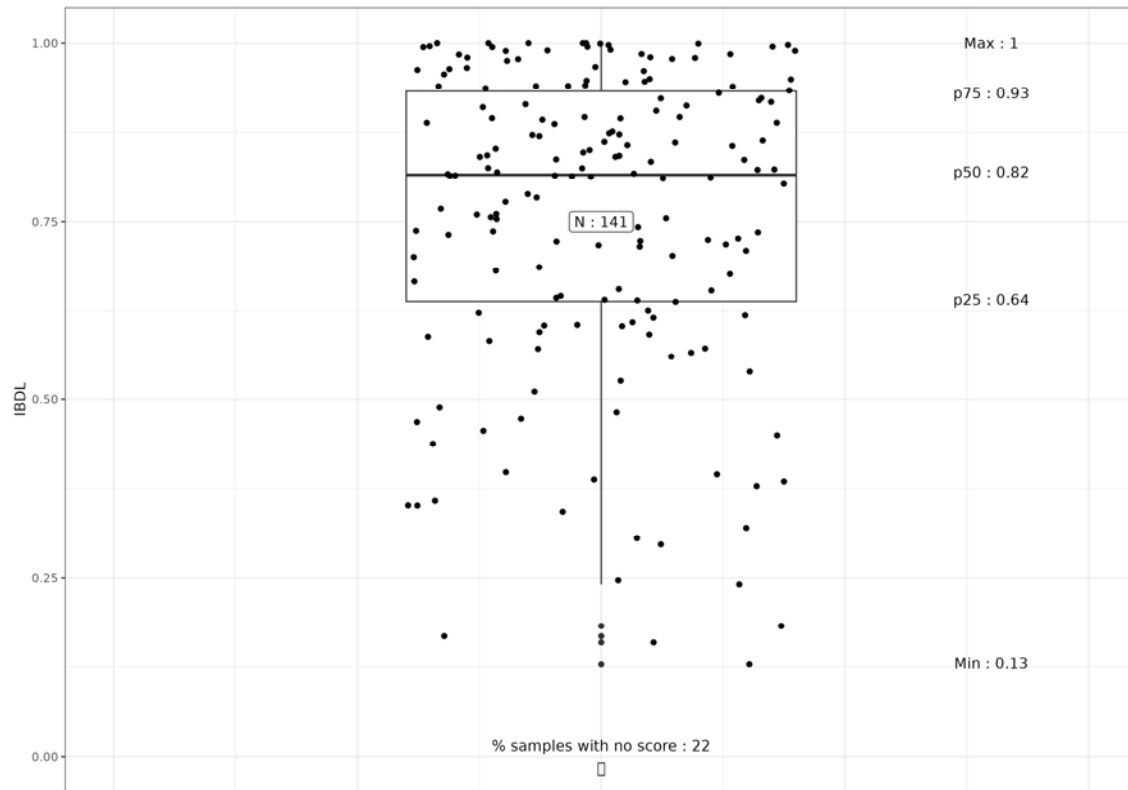
212 Table 1. Physico-chemical data available for analysis. sd: standard deviation, p25: 25th
213 percentile; p75: 75th percentile
214

215 Biotic and abiotic data were obtained for 958 samples. Considering the data validation
216 criteria, 99% of the samples were included in the analysis. Data from both substrate types
217 were available for 552 OUs. Seven hundred eighty taxa were recorded, 8% of which were
218 identified to the genus level. One hundred and twenty-one alert taxa were determined out of
219 590 index taxa (S2).

220 We obtained the following Pearson test values for the different metrics at the OU scale: R = -
221 0.715 for the metric based on the parameter NKJ, R = -0.754 for BOD₅, R = -0.688 for Pt, R = -
222 -0.666 for SP, R = -0.553 for PO₄, R = -0.329 for Conductivity, R = -0.174 for O₂, R = -0.265
223 for NO₂, and R = -0.204 for %O₂. Considering the selection rule proposed ($|R| > 0.6$), only the
224 metrics based on NKJ, BOD₅, Pt and SP were considered to build the IBDL.

225 Metric values (in EQR) calculated from the lists of taxa sampled on mineral substrates and
226 macrophytes for a single OU did not differ significantly (p-value = 0.65).

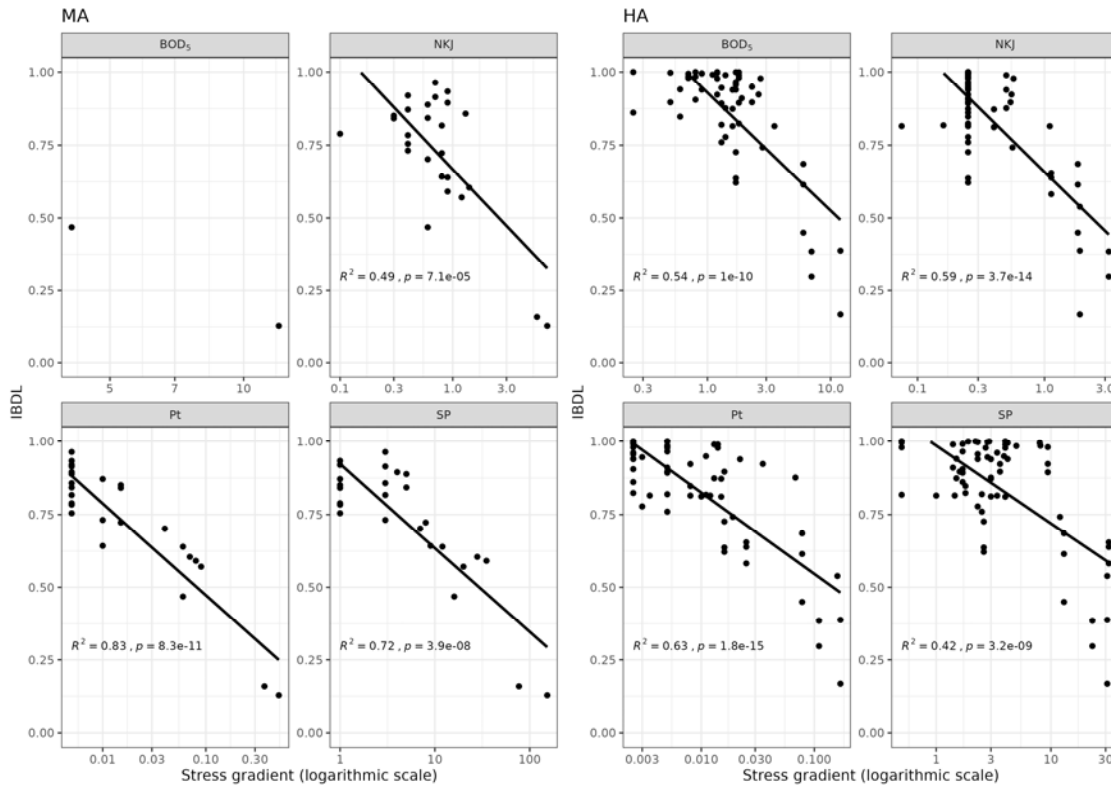
227 IBDL scores at the lake level were calculated from the selected metrics following the
228 aggregation rules proposed. The scores obtained were distributed as given in Figure 2. IBDL
229 could not be calculated for 20% of the samples due to incomplete floristic data.



230

231 Figure 2: Distribution of the IBDL scores obtained (p25: 25th percentile; p50: median value;
232 p75: 75th percentile).

233 The relationships between IBDL scores and the different environmental variables considered
234 were very good (Figure 3) in both high-alkalinity and medium-alkalinity lakes. IBDL scores
235 showed high correlations with these variables, particularly Pt, in both high alkalinity ($R^2 =$
236 0.63 , $p = 1.8e^{-15}$) and medium alkalinity lakes ($R^2 = 0.83$, $p = 8.3e^{-11}$). Note that data from low
237 alkalinity lakes were too scarce to perform such correlations.

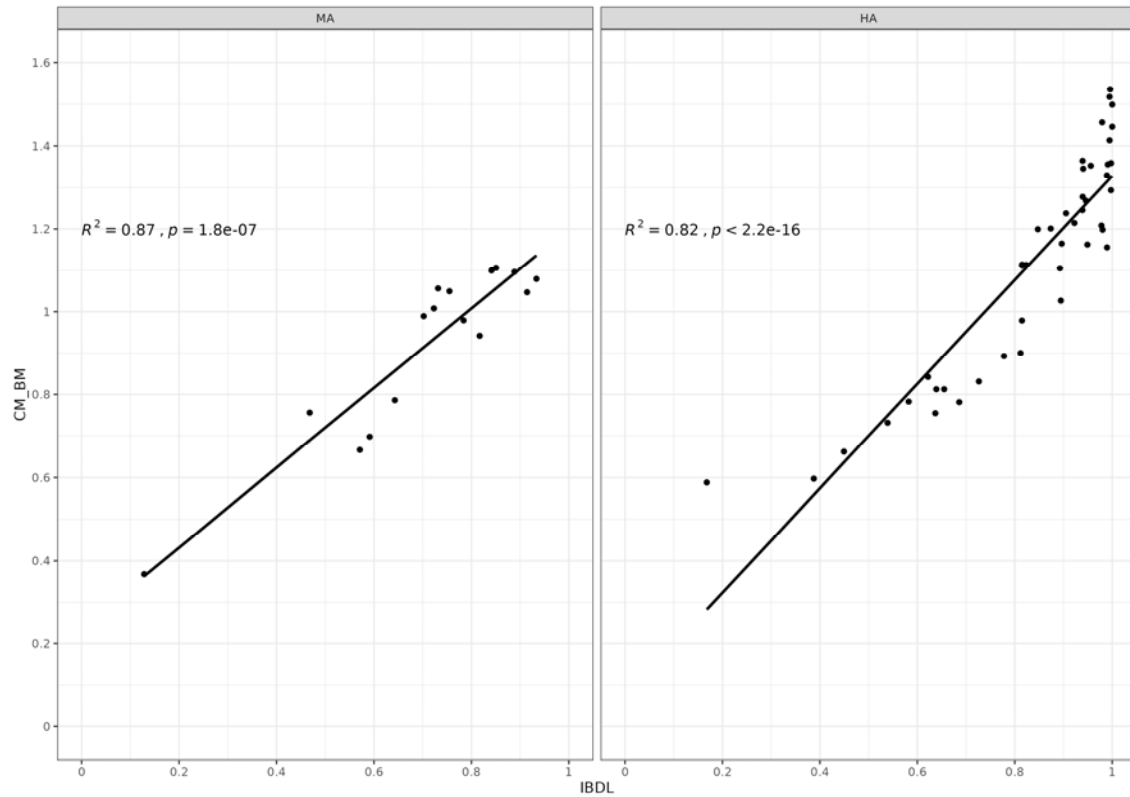


238

239 Figure 3. Relationships between IBDL and the environmental variables considered (MA:
240 medium-alkalinity lakes; HA: high-alkalinity lakes; BOD₅: biological oxygen demand; NKJ:
241 Kjeldahl nitrogen; Pt: total phosphorous; SP: suspended particles.

242

243 IBDL scores were also strongly associated with CM scores ($R^2 = 0.52$ and $p = 2.2e^{-16}$ for
244 high-alkalinity lakes; $R^2 = 0.87$ and $p = 1.8 e^{-7}$ for medium-alkalinity lakes) (Figure 4).



245

246 Figure 4: Relationships between IBDL and the common metric (CM) in medium alkalinity
247 (MA) and high alkalinity (HA) lakes.

248 IBDL scores showed a better correlation with Pt (AIC = -171.44) than did IBML (AIC = -
249 129.25) or a combination of both indices (AIC = -169.44). Nevertheless, IBDL tended to be
250 generally less stringent than IBML (in 18 out of 22 samples), especially for scores higher than
251 0.8 (clearly dominant here). Figure 5 presents the difference between IBDL and IBML scores
252 according to IBDL scores.

266 Marvan formula (Zelinka and Marvan, 1961). This method is far from optimal for datasets
267 showing discontinuous or very specific environmental conditions (Carayon et al., 2020). In
268 such cases, the identification of alert taxa seems more appropriate than considering diatom
269 communities as a whole. This has made the TITAN algorithm increasingly popular for
270 detecting specific taxa providing reliable signals of a specific stress (Khamis et al., 2014;
271 Costas et al., 2018; Gieswein et al., 2019; Carayon et al., 2020; Gonzalez-Paz et al., 2020).
272 Using this method, we built a multimetric index based on different pressure gradients (NKJ,
273 SP, BOD₅ and P_i). Although the strong influence of nutrients and organic matter on diatom
274 community composition is well established (Jüttner, 2010; Stevenson et al., 2013), diatom-
275 based metrics rarely take into account suspended particles for water quality assessment (but
276 see Larras et al., 2017). Diatoms are indeed directly impaired by turbidity, reducing light
277 availability for photosynthesis. Multimetric indices thus offer simple tools to summarize the
278 effect of multi-pressure gradients on communities (Riato et al., 2018), and can be considered
279 more effective for assessing biological conditions than a single metric (Stevenson et al.,
280 2013). However, despite their increasing use, multimetric indices suffer from the subjectivity
281 that can arise from metric selection (Reavie et al., 2008). Here, we attempted to avoid this
282 pitfall by proposing a method of selecting metrics based on the robustness of their response to
283 environmental gradients.

284 IBDL appears less stringent than IBML when assessing lakes' ecological status.
285 Literature comparing results from different indices in lakes, though scarce, tends to agree with
286 this overestimation of water quality by diatom-based methods (Kolada et al., 2016).
287 Phytobenthos has long been paid less attention than macrophytes for the assessment of lake
288 ecological status. It is true that recent diatom-based metrics barely detected newly impacted
289 lakes that would not have been detected by macrophyte metrics. Bennion et al. (2014)
290 showed, for example, that their index (LTDI) performed well for lakes with good ecological

291 status, but diatoms and other methods agreed less for lakes of lower status. This was
292 particularly the case in the presence of morphological alterations, for which diatoms are poor
293 indicators. A possible general explanation for the lower stringency of diatom-based indices in
294 lakes is the high abundance of species complexes like *Achnantheidium minutissimum* or
295 *Gomphonema parvulum*. Such complexes merge taxa that are morphologically close but with
296 different ecological preferences. Due to the existence of different taxa within the *A.*
297 *minutissimum* complex, many authors consider it an indicator of good water quality (Almeida
298 et al., 2014), whereas others consider it as tolerant towards toxic contaminants
299 (micropollutants) and hydrologic disturbances (Cantonati et al., 2014; Lainé et al., 2014).
300 Considering the generally high abundance of *A. minutissimum* in samples, this tends to blur
301 the overall pressure-response relationship between index scores and environmental variables
302 (Potapova and Hamilton, 2007). TITAN provides a means to avoid this pitfall, as such
303 complexes are not selected as alert taxa, given that their abundance dynamics do not show
304 clear response patterns to environmental gradients. Indeed, *A. minutissimum*, although highly
305 abundant in our dataset (22% of total species abundances), was not considered an alert taxon.
306 The fact remains that IBDL tends to be less stringent than IBML, despite better relationships
307 with Pt. In consequence, we have to explain why we think that the use of diatom-based
308 indices to assess lake ecological status is justified.

309 First, the discrepancy between macrophyte and diatom responses relies mainly on the
310 differences between their integration periods, given that indices provide information on
311 ecological conditions over the time an assemblage develops. Lavoie (2009) showed the
312 integration period of diatom-based indices to be about 2–5 weeks for nutrients, whereas
313 macrophytes react on yearly time scales (Kelly et al., 2016). As diatoms catch nutrients
314 directly from the water column (Wetzel, 2001), they also may be more directly sensitive to
315 rapid changes in trophic status than macrophytes (Vermaat et al., 2022). The rapid response of

316 phytobenthos should justify its routine use (Schneider et al., 2019), in particular for lakes in
317 non-equilibrium states (Kelly et al., 2016).

318 Second, diatom-based indices are essential where hydrologic pressures in littoral areas
319 prevent the development of macrophytes, and in lake typologies where macrophyte
320 communities are naturally species-poor or even absent (Schneider et al., 2019). Thus, while
321 macrophyte-based indices cannot be calculated in all lakes, this is not true for diatom-based
322 indices. Moreover, our results show that, with IBDL, water quality managers can directly
323 compare ecological status assessments from different lakes even if the substrate sampled is
324 different. Many studies highlighted that allelopathic relationships between macrophytes and
325 epiphytic diatoms may be responsible for specific associations between macrophytes and
326 diatom species and, thus, may contribute to the organization of particular assembly patterns
327 (Hinojosa-Garro et al., 2010). In any case, in terms of ecological preferences, and
328 consequently in terms of IBDL scores, our results did not show any significant differences
329 between communities sampled on mineral substrates or macrophytes at the OU level,
330 corroborating previous results obtained by Kitner and Poulíčková (2003) and Bennion et al.
331 (2014). Other studies even support the use of epiphytic diatoms as biological indicators for
332 lakes irrespective of the dominant macrophyte species sampled (Cejudo-Figueiras et al.,
333 2010). The key point is to avoid senescent material or recently grown shoots that would
334 potentially induce a colonization stage effect (King et al., 2006).

335 The next challenge was to check the consistency of the resulting classification of lakes
336 based on IBDL to the harmonized definition of good ecological status established in the
337 completed intercalibration exercise (Kelly et al., 2014b). The first step consisted in testing the
338 correlation between IBDL scores and total phosphorus in our dataset. Only HA and MA
339 typologies were considered here but, in any case, the last intercalibration exercise could not
340 be performed for LA lakes. We obtained very good correlations that are clearly an

341 improvement compared to the non-significant relationship previously obtained between BDI
342 (diatom index used for the assessment of rivers) and Pt, and even better than the pressure-
343 impact relationships observed at a pan-European scale (R^2 between national methods and Pt
344 ranged from 0.32 to 0.66 max., Kelly et al., 2014b). The second step consisted in testing the
345 correlation between IBDL scores and the intercalibration common metric (CM) scores, in
346 EQR. Here, the correlations demonstrated a very good agreement between IBDL and CM
347 scores in both medium ($R^2 = 0.87$) and high alkalinity ($R^2 = 0.82$) lakes. We are, therefore,
348 confident in our ability to match IBDL ecological status thresholds with those validated at the
349 European level.

350 **Conclusion**

351 The new diatom index proposed here meets the requirements of the WFD and makes it
352 possible to assess lakes' ecological status in metropolitan France. The IBDL has proved to be
353 particularly relevant as it has a twofold interest: an excellent relationship with total
354 phosphorus and an application in any lake metatype. Its complementarity with IBML justifies
355 the use of at least two primary producer components for ecological status classification (Kelly
356 et al., 2016).

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361 **Author's contributions**

362 All authors participated in designing the study and developing aims and research questions.
363 S.B. designed methodology, extracted data and made the analyses, supported by T.L.

364 concerning pretreatments before intercalibration. J.T.R. led the writing of the manuscript
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366 to the final version of the manuscript, and gave final approval for publication.

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