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SHORT NOTE

Using benthic diatoms for estimating lake ecological quality: Comparing different taxonomic resolution

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ABSTRACT

The Water Framework Directive asks to all Member States of the European Union to classify the ecological quality of significant waterbodies on the basis of the biological communities they host. One of the biological communities that must be used for the ecological quality assessment is the periphytic community, mainly composed by diatoms. In Italy, diatom-based lake quality assessment is performed using a specific index, named EPI-L, based on the method of weighted averages. For each species, a trophic score and an indicator weight were calculated. In order to reduce the complexity of the lake quality assessment, we calibrated a variant of EPI-L, using diatoms genera instead of species, and we compared the performance of these two variants in terms of correlation with the nutrient level and of different classification of each lake.

Key words: Diatoms; WFD; lakes.

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INTRODUCTION

The European Union's Water Framework Directive (WFD; European Union 2000) requires the management of surface water body in order to achieve (or maintain) at least good chemical and ecological status. Good ecological status is defined as a slight deviation from the ecological status normally associated with the same surface water body type under undisturbed conditions. According to annex 5 of the WFD, the ecological status should be evaluated using the so-called Biological Quality Elements (BQEs), which include fish, invertebrates, macrophytes and phytobenthos, and phytoplankton. For each BQE, the value of a quality index should be compared to a reference value indicating minimal human impact to obtain an ecological quality ratio. Macrophytes and phytobenthos are components of freshwater flora and are included by Annex 5 of the WFD in a single BQE, but they are evaluated separately, as they can respond differently to human impact, in relation to site-specific conditions (Schneider *et al.*, 2012; Kelly *et al.,* 2016).

Within phytobenthos, diatoms are considered the most representative component and are commonly used for the evaluation of the ecological status of both lotic and lentic waterbodies.

Since the pioneering work by Sladecek (1984), diatoms have been largely used for the evaluation of river quality. Among the methods used in Europe, Kelly *et al.* (2014) distinguish three types: indices based on the weighted average equation of Zelinka and Marvan (1961) and optimised against a stressor gradient, indices based on the relative proportion of taxa associated with unimpacted and impacted conditions, and multimetric indices based on a combination of these approaches. In the oldest indices, species were grouped on the basis of their tolerance or sensitivity to pollution, and river quality was estimated on the basis of the proportion of tolerant and sensitive taxa. More recent indices, such as IPS (CEMAGREF, 1982), Trophic Diatom Index (TDI, Kelly and Whitton, 1995) and the Diatom Biological Index (BDI, Coste *et al*., 2009), are based on a weighted average equation (Zelinka and Marvan, 1961) and make use of ecological information for each species, obtained from a calibration data set. During the calibration, each species is assigned a value indicating the sensitivity or tolerance of that species to a specific form of pollution, and an optional value indicating the indicator value of that species.

The development of specific indices for the evaluation of lake ecological quality (Rott, 1999; Schaumburg *et al.,* 2004; Flemish Environment Agency, 2009; Bennion *et al.,* 2014) is more recent.

Most indices using diatoms for evaluating the ecological quality of lakes are based on the trophic preferences of single species. This approach may cause two difficulties: on one hand, the large number of diatom species requires intensive training of the personnel performing the analysis and species misidentification can occur; on the other hand, a given index cannot be used when the sample is rich in species that are not included in the list that was defined when setting up the index.

Most indices were developed in the 1990s, when diatom species were grouped in large, heterogeneous genera. In the last decades, a large revision of diatoms taxonomy has occurred (Round *et al.,* 1990), splitting genera with a large number of species into smaller units, morphologically more homogenous. Although it can be expected that species belonging to smaller and probably more genetically homogenous genera would also have closer ecological preferences, it is possible that the new genera still include species with large ecological differences.

In this paper, we test the possibility to develop an index using diatom genera instead of species, for reducing the effort and the cost needed for the routine ecological classification of lakes and reservoirs and for increasing the number of waterbodies for which the index can be applied, including those that are rich in diatoms belonging to genera included in the list, even if their species are not the same used for the index calibration.

For this purpose, we develop a quality index based on diatom genera and we compare it with an existing index based on diatom species, namely the index EPI-L used in Italy (Marchetto *et al.,* 2013), and we discuss their relative ability to classify lentic waterbodies in quality classes.

METHODS

During the period 2010-2017, a total of 108 diatom samples were collected from 64 lakes in Italy (Tab. 1), following a common protocol (Buzzi *et al*., 2014), mainly based on CEN-EN standard 13946 (European Committee for Standardization, 2014). Most of the samples were collected on permanently submerged stones using a toothbrush, while 23 samples were collected on macrophyte stems. Twenty lakes were sampled more than once (Tab. 1). Samples were digested in H_2O_2 and HCl, mounted in Naphrax and a minimum of 400 diatom valves were identified under optical microscope at 1000x.

Results from a previous European project (EMERGE: "European Mountain lake Ecosystems: Regionalisation, diaGnostic & socio-economic Evaluation") allowed the addition of further 16 epilithic samples, collected in 2001 in high mountain lakes with moderate to high alkalinity (higher than 0.2 meq L^{-1}) with a very similar protocol (Marchetto *et al.,* 2009). To avoid the overrepresentation of more intensively sampled lakes, in this study we used the mean species abundance for each lake where several samples were available. The complete data set is available at: http://www.ise.cnr.it/products/datasets.

The diatom species counts were entered into OMNIDIA version 5.3 (Lecointe *et al*., 1993), a diatom database and indices calculation tool. The following indices, commonly used for lake or river quality assessment in Europe, were calculated: Biological Diatom Index (BDI; Coste, 1999), Pollution Sensitivity Index (IPS: CEMAGREF, 1982), Saprobic Index (SI; Rott *et al.*, 1997), Trophic Index (TI; Rott *et al.,* 1999), and the Trophic Diatom Index (TDI; Kelly and Whitton, 1995). The EPI-L index (Marchetto *et al*., 2013) was calculated using the tool available at: http://www.ise.cnr.it/it/wfd

Species occurring with a higher abundance than 1% in less than 3 lakes or never reaching a minimum abundance of 3% in any sample were discarded.

The EPI-L index was used for testing the effect of different taxonomical resolution. For this purpose, the index was recalibrated using the present data set, and excluding species occurring with a higher abundance than 1% in less than 3 lakes or never reaching a minimum abundance of 3% in any sample.

The calibration follows the procedure used by Marchetto *et al.* (2013) and summarised here. For each *i*-th remaining species, a trophic weight (*p'*) was obtained by the average of the logarithm of the epilimnetic total phosphorus concentration (TP, in µg L^{-1}), weighted by the abundance of that species in each *j*-th lake (*a*).

$$p'_i = \frac{\sum_j a_j \log_{10} TP_j}{\sum_j a_j} \qquad \text{(eq. 1)}$$

The indicator value (*v*) was obtained as the inverse of the average of the squared differences between the trophic weight of the species and the epilimnetic total phosphorus concentration in each *j*-th lake, weighted by the abundance of that species in the j-*th* lake itself. Indicator values higher than 30 were replaced with 30.

$$v_i = \frac{\sum_j a_j}{\sum_j a_j \left(TP_j - p'_i\right)^2} \qquad \text{(eq. 2)}$$

The re-calibrated EPI-L index (EPI-L'$_{species}$) was then calculated on the basis of the relative abundance *i*-th species using the following formula:

$$EPI\text{–}L'_{species} = 4 - 2\,\frac{\sum_i a_i p'_i v_i}{\sum_i a_i v_i} \qquad \text{(eq. 3)}$$

The index value was not calculated for those lakes for which the sum of the relative abundance of the discarded species was higher than 30%

The trophic value of each species was then corrected using a linear regression of the newly calculated index values for all lakes against the original EPI-L values.

$$EPI\text{–}L = m\, EPI\text{–}L'_{species} + n \qquad \text{(eq. 4)}$$

The slope and intercept values of the regression equation were used to rescale the trophic weights:

$$p = \frac{4-4m+2mp'-n}{2} \qquad \text{(eq. 5)}$$

Rescaling species optima (Marchetto, 1994) was used to obtain EPI-L' values close to the original EPI-L in order

Tab. 1. List of the considered lakes.

Lake	Longitude (°E)	Latitude (°N)	Altitude (m asl)	Alkalinity	Mean TP	No. of samples
Albano	41.745	12.676	293	H	20	1
Alserio	45.785	9.213	280	H	8	1
Annone (W basin)	45.817	9.333	224	H	29	1
Antholzer See	46.886	12.166	1640	M	4	1
Antrona	46.054	8.091	1083	M	5	1
Aplanersee	46.449	10.874	2367	M	3	1
Avigliana	45.065	7.387	352	H	12	3
Baratz	40.683	8.224	32	H	60	1
Bidighinzu	40.557	8.662	330	H	100	1
Bilancino	43.978	11.281	252	H	14	1
Boden Inferiore	46.442	8.453	2334	M	4	1
Boden Superiore	46.439	8.453	2343	M	4	1
Bolsena	42.583	11.933	305	H	22	4
Bracciano	42.117	12.233	164	H	16	1
Caldonazzo	46.033	11.243	450	H	7	1
Campo	46.129	8.131	2293	M	4	1
Candia	45.324	7.912	227	H	16	3
Capezzone	45.941	8.210	2100	M	4	1
Cavazzo	46.333	13.077	195	H	3	2
Cavedine	46.000	10.951	241	H	17	1
Chiusi	43.055	11.962	251	H	32	1
Cuga	40.613	8.464	642	M	24	1
Endine	45.778	9.938	334	H	15	1
Fusine Inferiore	46.482	13.669	924	H	3	1
Fusine Superiore	46.477	13.668	929	H	4	1
Garda	45.667	10.700	133	H	9	1
Garlate	45.821	9.406	198	H	12	1
Grande di Monticchio	40.930	15.610	656	H	87	1
Grünsee	46.609	12.009	2043	H	2	1
Haidersee	46.757	10.532	1449	M	13	1
Karersee	46.426	10.703	1519	H	3	1
Klammsee	46.982	12.128	2258	H	4	1
Kratzbergersee	46.705	11.286	2119	M	4	1
Ledro	45.878	10.751	655	H	9	1
Levico	46.014	11.278	440	H	5	1
Liscia	40.994	9.244	178	M	29	1
Lungo	42.475	12.849	371	H	48	2
Maggiore	45.950	8.667	194	M	7	2
Martignano	42.115	12.303	207	H	15	3
Massaciuccoli	43.833	10.333	2	H	21	1
Matogno	46.251	8.401	2067	M	4	1
Mergozzo	45.956	8.463	194	M	4	2
Mezzano	42.631	11.765	455	H	17	1
Mezzola	46.199	9.441	199	M	22	2
Milchsee	46.726	11.072	2540	M	3	1
Molveno	46.123	10.959	823	M	4	1
Montepulciano	43.090	11.920	249	H	90	1
Monterosi	42.205	12.294	237	H	55	1
Morasco	46.423	8.395	1815	H	3	1
Nemi	41.714	12.703	318	H	27	1
Orta	45.817	8.400	290	M	5	1
Paione Inferiore	46.169	8.191	2002	M	3	1
Paione medio	46.172	8.192	2147	M	6	1
Palù	46.199	9.868	1925	M	5	2

To be continued on next page

Table 1. Continued from previous page.

Lake	Longitude (°E)	Latitude (°N)	Altitude (m asl)	Alkalinity	Mean TP	No. of samples
Panelatte	46.203	8.458	2063	M	7	1
Paterno	42.382	13.014	617	H	40	1
Pattada	40.575	9.167	561	M	50	1
Piano	46.037	9.162	276	H	14	1
Piccolo di Avigliana	45.054	7.392	356	H	70	1
Piccolo di Monticchio	40.932	15.619	658	H	23	1
Piediluco	42.529	12.751	368	H	33	2
Pojala	46.329	8.335	2305	M	5	1
Posada	40.639	9.608	43	M	45	1
Predil	46.417	13.567	965	H	3	1
Pusiano	45.802	9.273	259	H	11	2
Ragogna	46.175	13.003	188	H	13	2
Ripasottile	42.475	12.815	371	H	60	2
Salto	42.279	13.024	535	H	49	2
Scanno	41.923	13.864	922	H	21	2
Segrino	45.829	9.267	374	H	11	1
Sirio	45.485	7.885	271	H	18	3
Sos Canales	40.555	9.313	711	M	28	1
Südlichter Kofferrastersee	46.576	10.940	2405	M	6	1
Tenno	45.939	12.452	570	H	3	1
Timmelsschwarzsee	46.928	11.163	2514	M	3	1
Toblino	46.063	10.967	245	H	24	1
Trasimeno	43.150	12.100	259	H	60	2
Turano	42.232	12.941	540	H	62	3
Vico	42.317	12.167	507	H	21	1
Viverone	45.401	8.051	230	H	30	3

H, alkalinity >1 $mmol_C\ L^{-1}$; M, alkalinity between 0.2 and 1 $mmol_C\ L^{-1}$; TP, total phosphorus ($\mu g\ L^{-1}$).

to avoid differences in lake classification between EPI-L and EPI-L' and to avoid the need of defining new boundaries between the quality classes for the revised index.

The same procedure was then used considering diatom genera instead of species to obtain a second index, named EPI-L'$_{genera}$.

RESULTS

All calculated diatom indices resulted significantly correlated to the trophic gradient, expressed as the mean epilimnetic total phosphorus concentration (Fig. 1), but the high correlation between EPI-L and TP is an artefact, due to the use in this paper of the same samples used for the calibration of the index.

Lakes with low phosphorous concentration can be distinguished from lakes with high concentration on the basis of the EPI-L index. On the contrary, in spite of the good correlation, for all other indices there is a large overlap between the values calculated for lakes with high and low trophic status. For this reason, EPI-L was selected for the quality assessment of Italian lakes, and is also used in this study.

Trophic weights and indicator values were obtained for 90 species and are reported in Tab. S1 in the supplementary material, after rescaling (m=1.0761, n=-0.0472). The new index (EPI-L'$_{species}$) resulted strongly correlated (r=0.874, P<0.001) with the trophic gradient, expressed as the decimal logarithm of the mean concentration of total phosphorus (Fig. 2a). Repeating the calibration for 34 genera, the index (EPI-L'$_{genera}$) also resulted strongly correlated (r=0.683, P<0.001) with the trophic gradient, expressed as the decimal logarithm of the mean concentration of total phosphorus, but some outliers were evident (Fig. 2b).

Indeed, for most genera, the trophic scores of the species lye in a relatively small range (Fig. 3), so that the use of an index based on lower taxonomic resolution should be possible. However, this was not the case for the genera *Achnanthidium*, *Discostella, Encyonema, Eunotia, Fragilaria* and *Pantocsekiella* (Fig. 3). We suspected that the presence in these genera of species with outlying trophic score could lead to a weaker performance of the index when the taxonomic resolution would be reduced. For this reason, beside an index based on diatom species ($EPI\text{-}L_{species}$) and one based on diatom genera ($EPI\text{-}L_{genera}$), we developed a hybrid index based on more homogeneous group ($EPI\text{-}L_{taxa}$).

For calibrating this latter index, we separated the relative abundance of *Achanthidium lineare* W.Smith, *Discostella stelligera* (Cleve & Grunow) Houk & Klee, *Encyonema caespitosum* Kützing and *E. ventricosum* (C.Agardh) Grunow, *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst and *Pantocsekiella ocellata* (Pantocsek) K.T.Kiss & E.Ács from the rest of their respective genera. In the case of *Fragilaria*, we considered three groups: the first formed by *F. crotonensis* Kitton, the second by *F. tenera* (W.Smith) Lange-Bertalot and *F. tenera var. nanana* (Lange-Bertalot) Lange-Bertalot & S.Ulrich, and the third including all other *Fragilaria* species.

Trophic weights and indicator values were then obtained for 40 taxa (genera and species groups) and are reported in table S2, after rescaling (m=1.2108, n=-0.1981). The correlation of this index, named $EPI\text{-}L'_{taxa}$,

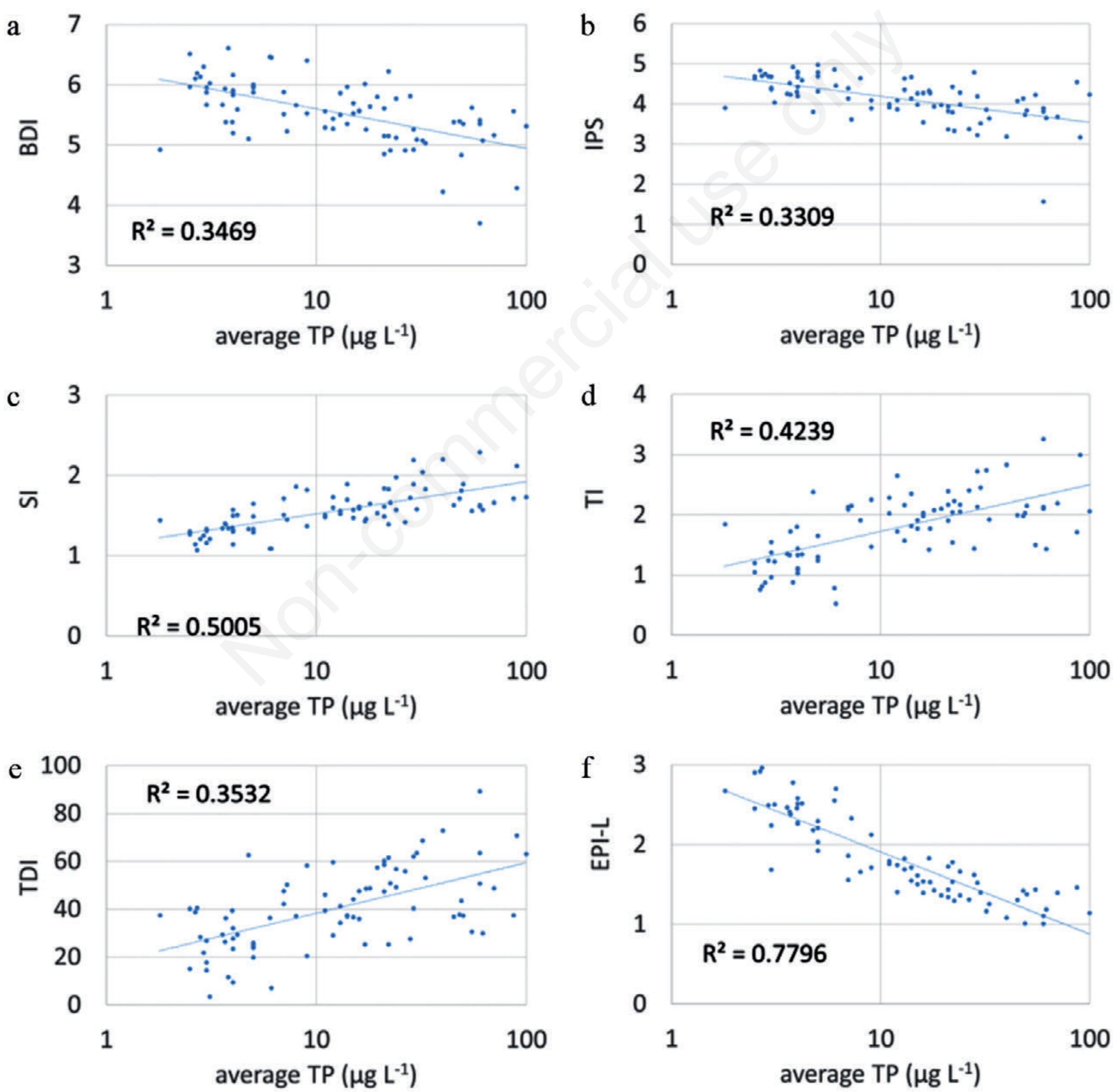

Fig. 1. Relationship between some selected diatom-based quality index and the average annual total phosphorus concentration for the lakes used in this study.

with the trophic gradient was only slightly stronger (r=0.689, P<0.001) than the one based on genera (Fig. 2c). Comparing EPI-L'$_{taxa}$ with the original EPI-L, their values compare well, with a median absolute difference of 0.02 (Fig. 2d).

DISCUSSION

In our database, the use of metrics developed for rivers for the assessment of ecological status in lakes resulted in slight correlation with the trophic gradient, probably because some diatom species do have distinct preferences for lakes over rivers (Kelly *et al.*, 2014). Among the metrics specifically developed for lakes, L-TDI (Bennion *et al.,* 2014) could not be correctly applied to our data set, as in no lakes the abundances of the species included in the L-TDI species list accounted for more than 70% of the total diatom counts. Therefore, the correlation between L-TDI and the logarithm of the total phosphorus concentration was low (r=0.44). On the contrary, Rott's (1999) TI index was well correlated with the trophic gradient (r=0.64), but it could only be used to evaluate the ecological quality of 52 out of 80 lakes in our database.

For this reason, Marchetto *et al.* (2013) developed a specific index for Italian lakes and reservoirs (EPI-L), in order to have a species list reflecting the composition of diatom assemblages in Italy and a good relationship with the trophic gradient in these lakes, which can be applied to 73 lakes in our data set. However, we expect that in a large-scale monitoring of all Italian lakes and reservoirs, some species not included in the EPI-L list will be found, making impossible to assess the ecological quality of some lakes or reservoirs. To allow the classification of those water bodies, we propose to use a revised index that does not require diatom determination at the species level. The indices based on species and on taxa was able to classify 74 out of 80 lakes, while the index based on genera was able to classify 79 out of 80 lakes.

Our results substantially confirm the finding of Bennet *et al*. (2014), who discussed the performance of diatom indices using different taxonomic resolution. They found large performance differences between species- and

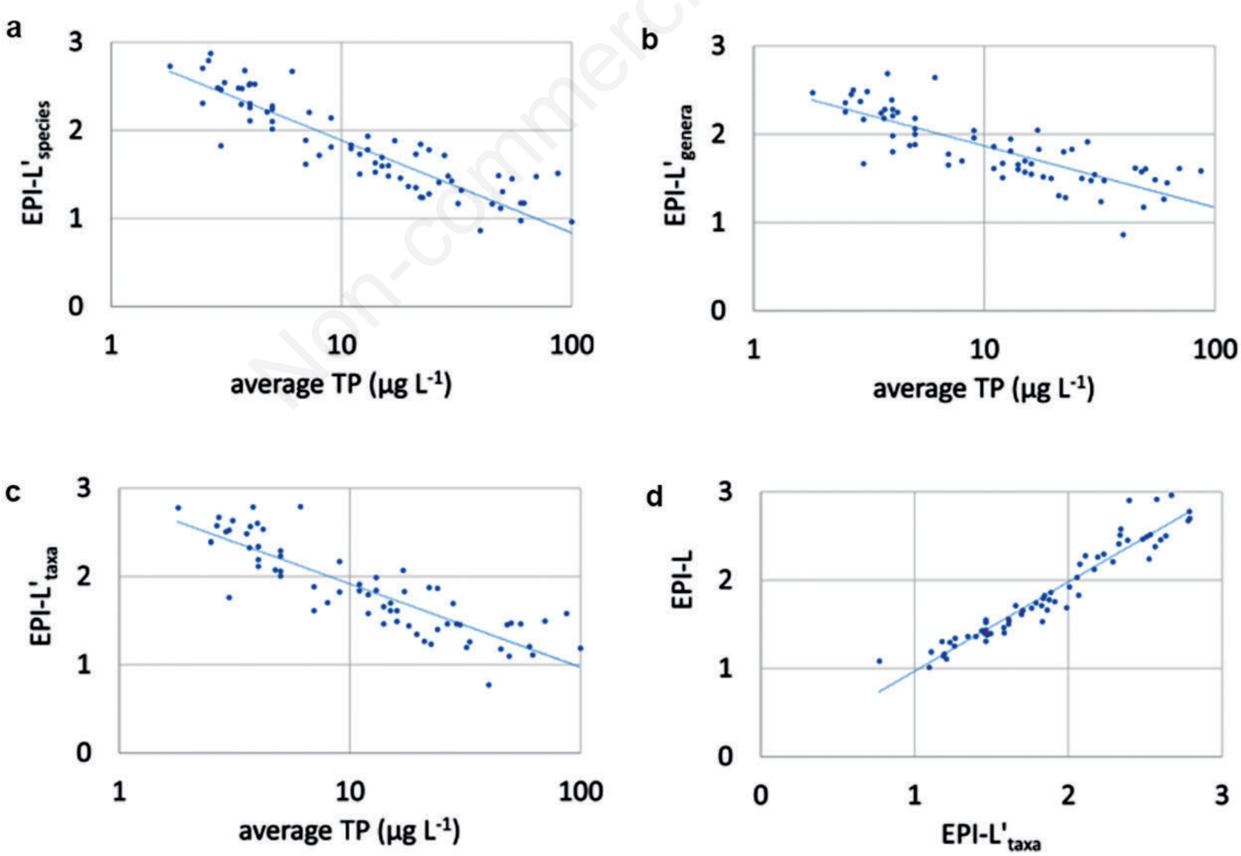

Fig. 2. Scatter plot of mean EPI-L'$_{species}$ (a), EPI-L'$_{genera}$ (b) and EPI-L'$_{taxa}$ (c) vs the average annual total phosphorus concentration, and comparison of EPI-L'$_{taxa}$ with the original EPI-L for the lakes used in this study (d).

genus-based indices calibrated along an acidity gradient, However, the differences were small when comparing indices calibrated along a trophic gradient.

Following the WFD, the boundaries between quality classes should be defined through intercalibration exercises, to assure that in the same ecoregion class boundaries are shared by all Member states. In the case of lake diatoms, the intercalibration exercise (Kelly *et al.*, 2014) was performed comparing the methods used in different countries with a common metric, namely the Trophic index (Rott, 1999), which requires diatom counts at the species level. For this reason, if the EPI-L_{genera} or EPI-L_{taxa} will be used for WFD-compliant ecological assessment, a species-level diatom determination is still needed for the data set used for the intercalibration exercise.

Based on the intercalibration exercise (Marchetto, 2014), the boundaries between the "high" and "good" quality classes were set to an EPI-L value of 1.702 for deep lakes and 1.845 for shallow lakes, while the boundaries between "good" and "moderate" ecological quality was set to 1.135 for deep lakes and 1.230 for shallow lakes.

In this study, most lakes fell in the same quality class using either EPI-L'$_{species}$, EPI-L'$_{genera}$ or EPI-L' $_{taxa}$. Differences in lake classification between EPI-L'$_{species}$, and EPI-L' $_{taxa}$ and between EPI-L'$_{genera}$ and EPI-L' $_{taxa}$ were only found for two lakes, for which the index value lied close to the boundary value. However, the number of classification mismatches was higher (6) in the case of EPI-L'$_{genera}$. These results are similar to those obtained by Bigler et al. (2010) in a similar study: they also found that reducing taxonomic resolution within the *Achnanthidium minutissimum* species complex from a series of subgroups to a single group led to changes in lake quality classification using the IPS index for a small number of lakes, only in cases when the index value was close to the class boundary.

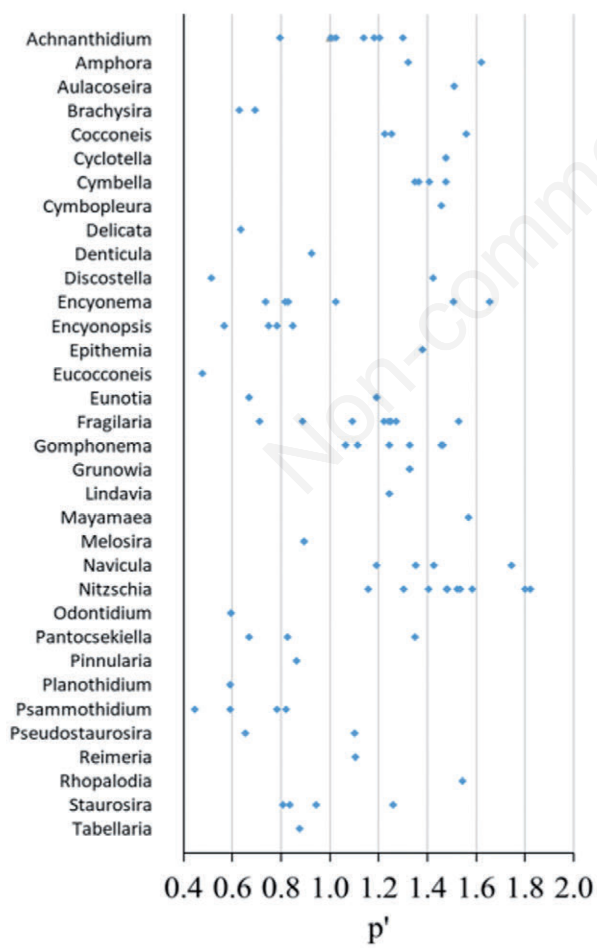

Fig. 3. Distribution of the trophic scores (*p*') for species belonging to the same genus.

CONCLUSIONS

Diatom-based indices are widely used in Europe for assessing lake ecological quality. Most of them are based on a list of indicator values assigned to diatom species. They cannot be used when a significant proportion of diatom found in a given lake are not included in their species list.

To reduce this problem, we tested the possibility to use an index based on diatom genera. However, we found that the reduction in taxonomic resolution led to an increase in classification mismatches. The results of this exercise indicate that the index based on genera can be improved in classification ability splitting some genera, when the species belonging to them have markedly different trophic preferences. The resulting index seems to compare with an index requiring diatom determination to the species level.

Finally, if adequate rescaling of the trophic scores is applied, we propose that the genera trophic scores and indicator values can be used together with the species ones, when individual specimen cannot be assigned to a given species, or when their species is not included in the species list of the original index.

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