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POND CONSERVATION

The M-NIP: a macrophyte-based Nutrient Index for Ponds

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Abstract In Swiss ponds, eutrophication represents one of the major threats to biodiversity. A biological method to assess the trophic state would, therefore, be particularly useful for monitoring purposes. Macrophytes have already been successfully used to evaluate the trophic state of rivers and lakes. Considering their colonizing abilities and their roles in pond ecosystem structure and function, macrophytes should be included in any assessment methods as required by the European Water Framework Directive. Vegetation survey and water quality data for 114 permanent ponds throughout Switzerland were analysed to define indicator values for 113 species including 47 with well-defined ecological response to total water phosphorus (TP). Using indicator values and species cover, a Macrophyte Nutrient Index for Ponds (M-NIP) was calculated for each site and assessed with both the original pond data set and a limited validation data set. The resulting index performed better when considering only species with narrow responses to TP gradient and was more applicable, but less accurate when including all species. Despite these limitations, the M-NIP is a valuable and easy tool to assess and monitor the nutrient status of Swiss ponds and was shown to be robust and relatively sensitive to slight changes in phosphorus loading with a validation subset.

Keywords Aquatic vegetation · Bioassessment · Eutrophication · Water quality · EU Water Framework Directive

Introduction

The ecological assessment of surface water quality is one of the main environmental concerns in many countries. In the European Union (EU), the Member States have set a common standard with ambitious objectives—the Water Framework Directive (WFD)—which aims to achieve at least a "good" ecological and physico-chemical status for all surface water and ground water bodies by 2015 (Bundi et al., 2000; Communities, 2000; Irmer, 2000). Although the WFD aims to protect all inland surface waters, ponds are not specifically mentioned in the Directive and for most Member States a lower size of 50 ha has been applied for standing waters to be included in monitoring programs (Davies et al., 2008). However, ponds are now increasingly recognized as very significant components of ecological quality, notably in term of their contribution to local and regional biodiversity (Murphy, 2002, Oertli et al., 2002, 2005;

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Williams et al., 2003) and, as such, require adapted tools and assessment methods supported by robust scientific knowledge (EPCN, 2007).

Among the threats to surface water, eutrophication, notably through diffuse pollution linked to the intensification of the agriculture (Havens et al., 2001), is still an important and even growing problem for freshwaters and coastal oceans (Carpenter et al., 1999; Smith et al., 1999; Bronmark & Hansson, 2002; Vadeboncoeur et al., 2003; Craft et al. 2007). With habitat destruction, eutrophication represents one of the major threats to the sustainability of biodiversity of most freshwater ecosystems; therefore, an assessment method of the nutrient status based on bioindicators and specifically designed for ponds could be a valuable tool.

The macrophyte community is one of the target groups required by the WFD in the assessment methods for lakes. In shallow systems like ponds, this group should also be included in any assessment method as it has an important potential of colonization and plays an important role in the structure and function of the freshwater ecosystem (Adams & Sand-Jensen, 1991). Moreover, macrophytes have already been widely used and are effective in the assessment and monitoring of various kinds of freshwaters ecosystems (see for e.g., Seddon, 1972; Kohler, 1975; Lehmann & Lachavanne, 1999; Melzer, 1999; Schneider & Melzer, 2003; Meilinger et al., 2005; Stelzer et al., 2005; Clayton & Edwards, 2006; Haury et al., 2006). Additionally, several trophic indexes based on macrophytes and the trophic profile of species already exists for lakes and rivers (e.g., Landolt, 1977; Melzer, 1988; Bornette et al., 1994; Robach et al., 1996; Holmes et al., 1998; Willby et al., 2000) and can serve as a basis for comparison with a pond index. Other advantages of macrophytes as bioindicator groups are the large number of taxa occurring in ponds as well as the relatively low-time investment in data acquisition, which would be particularly valuable for large scale programs (Palmer et al., 1992).

In order to build a Macrophytes-based Nutrient Index for Ponds (M-NIP), it is necessary to characterize the trophic state of the sites used to define the ecological profile of species. Eutrophication is primarily described as a regular increase of the primary productivity following larger inputs of inorganic nutrients (Naumann, 1927, 1932). Dodds (2006) gives a more general definition of the eutrophication process as an increase in nutritive factors leading to higher rates of whole system metabolism considering both the heterotrophic and the autotrophic metabolism. Independent of these definitions, the increase in nutrient concentrations enhances algal productivity and reduces light penetration in the water column, and hence the depth of colonization by submerged macrophytes, which can completely disappear with over enrichment (Phillips et al., 1978; Balls et al., 1989). The continuity of the eutrophication process complicates the establishment of well-defined limits between distinct trophic states, as well as the assignment of biological indicators values to a particular trophic state (Sondergaard et al., 2005). As a continuous measure of the whole system, metabolism is time and resource consuming and is hardly possible to perform on a large scale. For this reason, the water concentration of the main nutrients has often been used as a surrogate to define the trophic state of freshwater ecosystems (Vollenweider & Kerekes, 1982). This surrogate approach was shown to be conclusive, at least in low altitude ponds, as the water nutrient concentration significantly predicted the net periphytic primary productivity measured in nine ponds included in the present study (Sager, 2009).

By using a data set including water physico-chemistry and standardized macrophytes data for 114 ponds located throughout Switzerland [including 80 from the previous PLOCH study of Oertli et al. (2005)], the present study aims to:

- Characterize a nutrient profile of each macrophyte species using water chemistry data for ponds in which the species occurs. This profile represents the range of nutrient concentration expressed in trophic categories, where the species was recorded even if nutrient concentration is only one of the factors likely to contribute to occurrence and abundance of particular plant species. Therefore, this nutrient profile is only designed to be used for an assessment at the scale of the whole site and not to define the micro-conditions at the level of a single plant stem or macrophyte bed.
- Develop and calculate different versions of the nutrient index for a site (M-NIP) based on the nutrient profile, tolerance, and abundance of the species.

- Assess the ability of the different versions of M-NIP to correctly classify ponds in the corresponding category of nutrient status expressed in trophic state. The time investment and prerequisite knowledge of the sampler were also considered in the assessment of the different versions of the index.

In addition, an evaluation of the applicability and reproducibility of the index on newly sampled sites is presented on a subset of ponds that were not used to characterize the nutrient profile of the species.

Methods

Study area and field survey

The study area was located in Switzerland, a country of 41,244 km^2 located in central Europe, a large proportion of which incorporates the Alpine mountain chain. Despite its small size, Switzerland harbors an important variety of environmental conditions and a strong altitudinal gradient. We built a database containing the vegetation survey and environmental parameters of a set of 114 permanent ponds and small lakes located in four altitudinal belts of vegetation (see Fig. 1 and Table 1). All ponds were sampled in the summer between 1996 and 2005 (one sampling date per pond) with the standardized method developed by Oertli et al. (2005). The ponds in this data set varied in size from 6 to 96,200 m^2 (mean: 7,959 m^2, median: 2,328 m^2) and covered an altitudinal range from 210 to 2,757 m.a.s.l. (mean: 957 m, median: 642 m).

Macrophytes sampling

Vegetation sampling was carried out in square quadrats of 0.25 m^2 disposed equidistantly along transects perpendicular to the longest axis of the waterbody, and located at regular intervals according to its surface area. The total number of quadrats sampled per pond (n) was related to the water surface area (m^2) by a relationship determined by Oertli et al. (2005; $n = 1.96 - 2.8 * \log10(\text{area}) + 2.6 * (\log10(\text{area}))$ and ranged from 5 to 460 (mean = 65, median = 38). Such a strategy allows at least 70% of the real species richness to be recorded. A species list as well as water depth was drawn up for each quadrat, and this standardized list of species was completed by the observation of species located outside the quadrats. Only aquatic species were taken into account, especially the 254 species of vascular plants (Spermatophyta and Pteridophyta) listed in the highest humidity class ($F = 5$) of the Landolt (1977) index of ecological

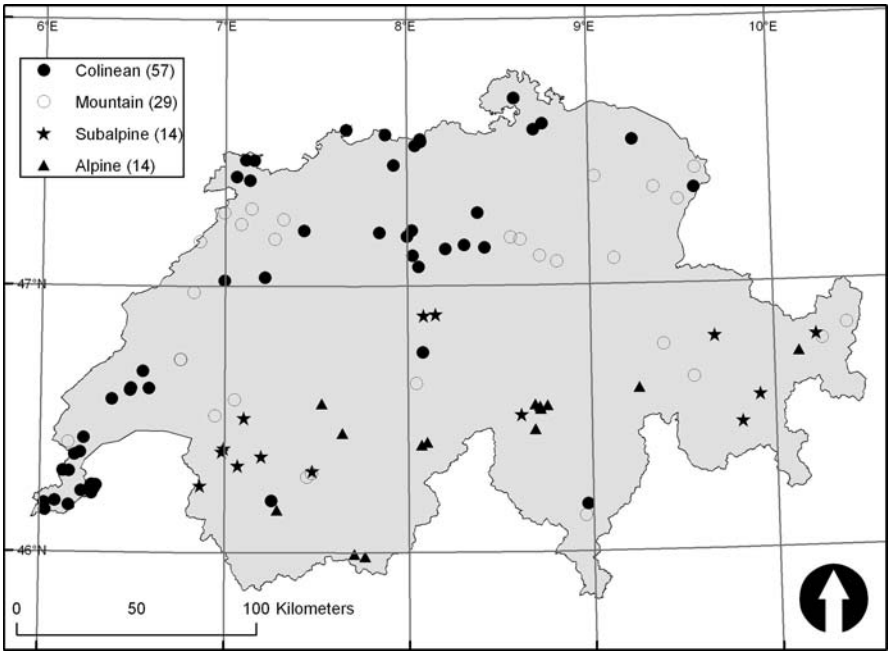


Fig. 1 Study area and locations of the 114 ponds throughout Switzerland. *Symbols* represent the four altitudinal vegetation belts with the number of sites in *brackets*

Table 1 Main morphometric and physico-chemical characteristics of the 114 ponds used to define the nutrient profiles of species and to calibrate the trophic index by site

	N	Mean	Mean std. error	Median	Minimum	Maximum
Mean depth (cm)	114	154	15	106	16	904
Sinuosity	114	1.52	0.04	1.37	0.99	3.27
Area (m^2)	114	7,959	1,320	2,328	6	96,200
Altitude (m)	114	957	59	642	210	2,757
TP (µg/l)	114	73.224	10.117	32.750	0	611
N_{min} (mg l^{-1})	114	1.060	0.135	0.551	0.036	8.790
Total hardness (méq $CaCO_3$)	112	180.2	13.1	182.5	0.8	884
Transparency S (cm)	113	41	2	47	4	60
Conductivity W (µS cm^{-1})	108	360.3	22.1	360.0	6.2	1,367

value. This standardized list was completed with 22 additional vascular species listed as $F = 4$ in the Landolt index: *Agrostis stolonifera* L., *Carex canescens* L., *Carex flava* L., *Carex lepidocarpa* Tausch, *Carex nigra* (L.) Reichard, *Eleocharis acicularis* (L.) Roem. & Schult., *Eleocharis quinqueflora* (Hartmann) O. Schwarz, *Equisetum palustre* L., *Galium palustre* L., *Juncus articulatus* L., *Juncus conglomeratus* L., *Juncus effusus* L., *Juncus filiformis* L., *Juncus inflexus* L., *Lysimachia nummularia* L., *Lythrum salicaria* L., *Lysimachia vulgaris* L., *Mentha longifolia* (L.) Huds., *Myosotis scorpioides* L., *Ranunculus repens* L., *Rorippa palustris* (L.) Besser, and *Scirpus sylvaticus* L. A plant quantity index Q was recorded for each species, and is the cube of the class of cover of the species, as explained in Table 2. This Q value is considered to be a good descriptor of the extent of a species actually present at a site (Schneider & Melzer, 2003). The size difference between sites does not seem to influence the distribution of species. The study of Oertli et al. (2002) conducted on 80 ponds included in our data set showed that among the 19 species of plants observed in >16% of the ponds, only two show a significant preference for large sites while the others are indifferent to the size.

Table 2 Correspondence between the percentage of the quadrats occupied by a species and the percentage cover classes also express as plant quantity index (Q) by cubing the class value

% Quadrats occupied	Covering classes	PQI (Q)
0–1	1	1
1–5	2	8
5–25	3	27
25–50	4	64
>50	5	125

Water physico-chemistry

Water physico-chemistry was measured in winter, when biological activity is at its minimum intensity and the concentration of nutrients in their inorganic form tends to be at its highest level (Linton & Goulder, 2000). Water sampling was carried out at the deeper central point of each pond by drilling a hole in the ice cover. Water samples were taken with a sampling bottle at 20 cm below the surface, and then immediately stocked in acid pre-rinse PE plastic bottles before being stored in the dark in a refrigerated box. Unfiltered samples were kept for total phosphorus (TP) analyses. TP was determined after potassium persulphate digestion at 121°C and under pressure for half an hour; soluble reactive phosphorus (SRP) was further measured by the ascorbate acid/molybdenum blue method (APHA et al., 1998). For this study, we only used the concentration of TP even if other physico-chemical parameters have been measured.

Classification of the ponds in trophic categories

For measuring the trophic state, water nutrient concentration was used as a surrogate for primary production, as this approach has been demonstrated to be effective by measuring the primary productivity in situ on a subset of nine of the ponds considered here (Sager, 2009). However, numerous studies have shown that the water column is not the single nutrient source for aquatic vegetation and rooted species can use the content of sediments and interstitial water for nutrient supply (Barko et al., 1991; Moore et al., 1994; Wigand et al., 1997; Vermeer et al., 2003; Engelhardt, 2006, review in Lacoul & Freedman, 2006), but the nutrient concentration of sediments or interstitial water can be highly variable within a waterbody (Wigand et al., 1997). As, we want to obtain a general value by site, the water column concentration of nutrients is, practically, more suitable than multiple measurements of the sediments. In effect, water column concentrations are generally more homogeneous at the pond scale through facilitated diffusion. For these reasons, even if the water concentration is not a measure of all available nutrients for plant growth, we consider it as representative of the actual conditions prevailing in the water body, and reliable for setting the nutrient profiles of macrophytes species usable at the site scale.

From the water concentrations of TP measured in winter, each pond was classified into a trophic state (oligotrophic, mesotrophic, eutrophic, and hypertrophic). As the most of the ponds were only surveyed once for winter physico-chemistry of the water, we choose to smooth the little variations between sites by using classes of trophic states defined by TP concentrations rather than the raw values of TP. Two different chemical scales for defining the trophic status were evaluated separately: the OECD criteria for defining the trophic state of lakes (Vollenweider & Kerekes, 1982) and a scale of ecological quality specifically defined for shallow lakes (Sondergaard et al., 2005). The latter sets limits between the first three trophic categories at a higher TP concentration than the OECD scale, and could be potentially more adapted to ponds. In addition, the shallow lake scale distinguishes a fifth category for bad status, when the TP concentration goes beyond 200 µg/l. The ranges of nutrient concentrations for these two scales are given in Table 3 along with the number of sites in each category.

Attribution of bioindication to species

In order to identify an indicator value (IV) for each species, we followed a procedure similar to that used by Schneider & Melzer (2003) in rivers. We set up a histogram for every species that showed its distribution by nutrient categories. Similarly to Schneider & Melzer (2003) and Friedrich (1990), a 20 points distribution was used to allow direct comparisons between species. Species present in less than three ponds were systematically excluded and those which occurred only in 3–6 ponds were carefully examined. An IV was calculated for the species with enough occurrences using weighted averaging (Eq. 1).

$$\mathrm{IV}_a = \frac{\sum_{i=1}^{n} O_{ai} \cdot T_i}{\sum_{i=1}^{n} O_{ai}} \qquad (1)$$

where IV_a is the indicator value of species a, O_{ai} is the number of occurrences of species a in the trophic category i, and T_i is the value of the trophic category i (from 1 = oligotrophic to 4 = hypertrophic).

One indicator value (IV) by species was calculated for each considered chemical scale of trophy, namely, IV-P and IV-Ps for the OECD scale and the shallow lakes. These IV by species are neither to design for a single use nor to define micro-conditions at the scale of a single stem. Instead, they were used on survey results fulfilling all the requirements enumerated thereafter to compute a reliable M-NIP at the pond scale.

Table 3 Ranges of water concentration for TP by trophic state for the scales used in this study

Trophic scale Classification	Oligotrophic High	Mesotrophic Good	Eutrophic Moderate	Hypertrophic Poor	Bad	*n*
OECD TP (µg/l)	0–10	10–35	35–100	>100		
n	24	35	32	23		114
Shallow lakes TP (µg/l)	0–25	25–50	50–100	100–200	>200	
n	49	22	17	15	11	114

n indicates the number of sites in the data set for each trophic category. The scales used are those proposed by OECD (Vollenweider & Kerekes, 1982) and Sondergaard et al. (2005) for shallow lakes

Table 4 Correspondence between the range of ecological amplitude and the weighting factors attributed to indicator species

a_a	W
0–0.2	16
0.2–0.4	8
0.4–0.6	4
0.6–0.8	2
>0.8	1

In order to express the ecological tolerance or amplitude of a species to a given factor, we calculated the root-mean-square-deviation weighted by the number of occurrences in each nutrient category (Eq. 2). This amplitude of tolerance permitted to weight the contributions of the species to the index by giving higher influence to the species with a narrow spectrum. The correspondence between ranges of a_a and weighting factors (W) are given in Table 4.

$$a_a = \sqrt{\frac{\sum_{i=1}^{n} (T_i - IV_a)^2 \cdot O_{ai}}{\sum_{i=1}^{n} O_{ai}}} \tag{2}$$

where $a_a$is the amplitude of species a, T_i is the value of the nutrient category i (from 1 = oligotrophic to 4 = hypertrophic), IV_a is the indicator value of species a, and O_{ai} is the number of occurrences of species a in the trophic category i.

Determination of the M-NIP and assessment of its accuracy

The M-NIP equation (Eq. 3) corresponded to one of the macrophyte trophic index (TIM) of Schneider & Melzer (2003), which was also the term used in the saprobic index of Zelinka & Marvan (1961).

$$\text{M-NIP} = \frac{\sum_{i=1}^{n} \mathrm{IV}_a \cdot W_a \cdot Q_a}{\sum_{i=1}^{n} W_a \cdot Q_a} \tag{3}$$

where M-NIP is Macrophytes Nutrient Index for Ponds, IV_a is the indicator value of species a, W_a is the weighting factor of species a, and Q_a is the plant quantity of species a in the pond.

Depending on the amplitude of tolerance of a species expressed the weighting factors (W), two distinct types of M-NIP were calculated: one considering all species (M-NIP) and the other considering only species with a weight above one ($\text{M-NIP} - W > 1$).

This calculation gave an M-NIP value for a given pond that could theoretically range from one to four. The subdivision in classes of trophic state was made further.

In order to assess the accuracy of the M-NIP for a given site, the weighted standard deviation of the indicator values of species present in the pond was calculated. If this rate of scatter (SC, Eq. 4) exceeded a fixed threshold, the computed M-NIP was not valid and could be used for the determination of the nutrient status. The thresholds were fixed as about half of the extent of the M-NIP values for the category with the narrower range. For this reason, the threshold for SC differed between the different indexes tested.

$$\mathrm{sc} = \sqrt{\frac{\sum_{a=1}^{n} (\mathrm{IV}_a - \mathrm{M} - \mathrm{NIP})^2 \cdot W_a \cdot Q_a}{(n-1)\sum_{a=1}^{n} W_a \cdot Q_a}} \tag{4}$$

Prerequisite for a consistent calculation of the M-NIP

In order to ensure that the M-NIP can be use as a reliable indicator of trophic state, the requirements are necessary as follows:

- Sampling of vegetation must have been made with the standardized method described above and during the main vegetation period (June–September), including the standardization of sampling intensity and the extrapolation of plant quantity (Q) using only species observed within quadrats.
- At least two indicator species must occur in the pond.
- The sum of the plant quantities Q must be at least 35 for the M-NIP's (one common and one infrequent species) and nine for the $\text{M-NIP} - W > 1$'s (one rare and one infrequent species), since this index includes only species with narrower tolerance.
- The rate of SC must be inferior to the fixed threshold of confidence.

Several versions of the M-NIP, based on different subgroups of species, were assessed, these were:

(1) M-XNIP (with X for the nutrient used to classify the sites by trophic states) consider all the observed species with a valid IV.

(2) M-XNIP-WC consider only the species classified as aquatic or helophyte in the red list of fern and flowering plants of Switzerland (Moser

et al., 2002). This corresponds to the pool used for the M-XNIP without Characea (WC) and few others species not classified as aquatic or helophytes.

(3) M-XNIP-AQ consider only aquatic species. This version corresponds to the group used for the M-XNIP-WC but without the helophytes species.

(4) M-XNIP-SUB consider all the submerged species including the Characea.

This choice of testing multiple indexes was dictated by the need to find the most reliable group of species by considering the effectiveness of the index to correctly reclass ponds in the corresponding nutrient status. We also considered the ecological meaning of the subgroup of macrophytes and the easiness of applicability by end users in term of time investment and skills for species identification.

Defining the ranges of M-NIP values by trophic category

The valid M-NIP values for the ponds used to define the nutrient profile of the species and fulfilling all the pre-requisite conditions were box-plotted by trophic categories to define the maximum rate of SC for considering the index result as reliable. Thresholds of SC, corresponding approximately to half of the range of M-NIP values between trophic classes, were defined separately for each tested trophic scale and macrophytes groups.

After removing the sites with a too high SC to be reliable, M-NIP values by site were box-plotted versus trophic categories. Significance of the differences between classes was assessed by a non-parametric Mann–Whitney (MW) test between the groups of M-NIP values from adjacent trophic categories. When the M-NIP values were mostly overlapping between two contiguous categories, the index was considered unable to distinguish between these two nutrient status and the MW test was performed between groups of values from non-adjacent categories (e.g., oligotrophic and eutrophic).

Validation and test on the M-NIP index

In order to test the usability of the M-NIP, the indexes were calculated with data from surveys not included in the data set used to calculate the IV by species. As the calibration data set was just large enough to define ecological values for species, only five sites were set aside for this task. Among these, we included two sites previously incorporated in the building process but with new data obtained from other surveys performed between 1 and 10 years after the initial study. This validation step permitted us to assess if the calculated index corresponded to the nutrient status determined by water physico-chemistry. It also allowed us to estimate the stability of the index for two sites that were sampled in two subsequent years and to assess the monitoring ability of the index value over a longer time period for one pond. For this purpose, the M-PNIP values of resample sites were compared along with the species lists and physico-chemical data.

Results

Bioindication of the species

A total of 168 macrophytes species and 1,702 observations were recorded in 114 ponds used to define the indicator value (IV) by species. Among this set, 113 species representing 96.4% of the observations, had sufficient occurrences to compute an indicator value. These include 45 species that were at the lower limit of inclusion with only 3–6 occurrences in the present data set. The ranges of the IV by trophic scale were 1.2–3.67 for the IV-P and 1–4.33 for the IV-Ps.

Depending on their amplitude on the histogram of occurrences by trophic state, the IV's of the species were weighted to further calculate an index by site. A large part of the species fulfilling the conditions to compute a reliable IV can be classified as eurytrophe as they are able to grow on a wide range of nutrient concentrations. Depending on the chemical parameter and scale considered to define the trophic state, 66 (IV-P)–92 (IV-Ps) species showed a wide tolerance and an inherent minimum weighting factor in the index ($W = 1$). Consequently, IV of at least 21 species and at most 47 could be further used to compute the M-NIP $- W > 1$. The full list of species present in at least three sites is given in Table 5 with their corresponding IV and W for the two chemical scales used to define the nutrient status.

Table 5 Indicator values (IV) of the species and amplitude of tolerance expressed by the weighting factors (*W*) for the two chemical scales used to define the trophic state (see Table 3)

Species names	*n*	IV-P	*W*–P	IV-Ps	*W*-Ps	GF	EG
***Acorus calamus* L.**	3	**3.67**	**4**	4.33	1	e	Aq
Agrostis stolonifera L.	9	1.89	1	1.89	1	s	Mar
Alisma lanceolatum With.	7	2.86	1	2.43	1	e	Aq
Alisma plantago-aquatica L.	34	2.82	1	2.38	1	e	Aq
***Alnus glutinosa* (L.) Gaertn.**	8	**2.88**	**2**	2.63	1	–	For
Alopecurus aequalis Sobol.	3	2.33	1	1.67	4	e	Mar
***Berula erecta* (Huds.) Coville**	6	**2**	**4**	1.17	8	e	Aq
***Callitriche cophocarpa* Sendtn.**	3	**3.67**	**4**	4	1	fl	Aq
***Callitriche palustris* L.**	4	**1.5**	**4**	1	16	fl	Aq
Callitriche stagnalis Scop.	3	3	1	2.67	1	fl	Aq
Caltha palustris L.	32	2.22	1	1.91	1	e	Mar
Cardamine amara L.	6	2	1	2.2	1	e	Mar
Carex acutiformis Ehrh.	34	2.65	1	2.24	1	e	Mar
Carex canescens L.	9	2.78	1	2.44	1	e	Mar
Carex diandra Schrank	3	3	1	2.33	1	e	Mar
Carex elata All.	45	2.78	1	2.36	1	e	Mar
Carex flava aggr.	3	2	1	1.67	1	e	Mar
***Carex flava* L.**	18	**2.06**	**2**	1.39	1	e	Mar
Carex lepidocarpa Tausch	6	2	1	0.83	4	e	Mar
***Carex limosa* L.**	3	**1.33**	**4**	1	16	e	Mar
Carex nigra (L.) Reichard	29	1.9	1	1.63	1	e	Mar
Carex paniculata L.	12	2.17	1	2	1	e	Mar
***Carex pseudocyperus* L.**	6	**2.83**	**8**	2.17	1	s	Mar
***Carex riparia* Curtis**	5	**3**	**16**	2.2	2	e	Mar
Carex rostrata Stokes	34	2.26	1	1.79	1	e	Aq
Carex vesicaria L.	25	2.88	1	2.68	1	e	Mar
Ceratophyllum demersum L.	4	2.5	1	2.5	1	s	Aq
***Chara contraria* A. Braun**	3	**2.33**	**4**	1.33	4	s	Aq
***Chara globularis* Thuillier**	18	**2.33**	**2**	1.83	1	s	Aq
***Chara major* Vaillant**	3	**2**	**16**	0.67	8	s	Aq
***Chara vulgaris* L.**	19	**2.05**	**2**	1.42	1	s	Aq
***Eleocharis austriaca* Hayek**	8	**2.75**	**4**	2.13	4	e	Mar
***Eleocharis palustris* (L.) Roem. & Schult.**	17	**2.71**	**2**	2.12	1	e	Mar
***Eleocharis palustris* aggr.**	4	**3.5**	**4**	3.5	1	e	Mar
Eleocharis quinqueflora (Hartmann) O. Schwarz	3	1.67	1	1.33	4	e	Mar
***Eleocharis uniglumis* (Link) Schult.**	7	**1.71**	**4**	0.86	16	e	Mar
Elodea canadensis Michx.	13	3.15	1	3.15	1	s	Aq
Epilobium palustre L.	10	2.3	1	2.2	1	e	Mar
Equisetum fluviatile L.	23	2.35	1	1.96	1	e	Aq
***Equisetum palustre* L.**	26	**2.23**	**2**	1.58	1	e	Mar
Eriophorum angustifolium Honck.	13	1.69	1	1.27	1	e	Mar
***Eriophorum scheuchzeri* Hoppe**	5	**1.2**	**8**	1	16	e	Mt
Galium palustre L.	34	2.74	1	2.38	1	e	Mar
Glyceria fluitans (L.) R. Br.	15	3.07	1	3.2	1	e	Aq

Table 5 continued

Species names	*n*	IV-P	*W*–P	IV-Ps	*W*-Ps	GF	EG
Glyceria maxima **(Hartm.) Holmb.**	5	**2.4**	**4**	2	1	e	Aq
Glyceria notata Chevall.	11	3.09	1	3.09	1	e	Aq
Groenlandia densa **(L.) Fourr.**	3	**1.33**	**4**	1	16	s	Aq
Hippuris vulgaris **L.**	5	**2.6**	**2**	2.4	2	e	Aq
Hydrocharis morsus-ranae **L.**	6	**2.67**	**2**	2.17	1	fl	Aq
Hydrocotyle vulgaris L.	3	2.33	1	1.33	1	e	Mar
Iris pseudacorus L.	43	2.84	1	2.56	1	e	Mar
Juncus articulatus L.	38	2.61	1	2.13	1	e	Mar
Juncus bulbosus **L.**	4	**2.25**	**4**	1.75	1	e	Mar
Juncus conglomeratus **L.**	21	**2.9**	**2**	2.57	1	e	Mar
Juncus effusus L.	40	2.9	1	2.58	1	e	Mar
Juncus filiformis L.	14	2.36	1	1.69	1	e	Mt
Juncus inflexus L.	26	2.46	1	1.96	1	e	Mar
Lemna minor L.	33	2.79	1	2.61	1	ff	Aq
Lemna trisulca L.	9	2.67	1	2.33	1	s	Aq
Lycopus europaeus **L. s.str.**	39	**3**	**2**	2.64	1	e	Mar
Lysimachia nummularia **L.**	18	**3.11**	**2**	3	1	e	For
Lysimachia vulgaris L.	41	2.85	1	2.56	1	e	Mar
Lythrum salicaria **L.**	43	**2.84**	**2**	2.49	1	e	Mar
Mentha aquatica L.	53	2.72	1	2.25	1	e	Mar
Mentha longifolia (L.) Huds.	10	2.5	1	2.2	1	e	Mar
Menyanthes trifoliata L.	14	2.64	1	2.43	1	e	Aq
Myosotis scorpioides **L.**	16	**2.69**	**2**	2.31	1	e	Mar
Myriophyllum spicatum L.	11	2.64	1	2.27	1	s	Aq
Myriophyllum verticillatum L.	4	2.75	1	2.5	1	s	Aq
Nasturtium officinale R. Br.	3	2.33	1	2.67	1	e	Aq
Nuphar lutea (L.) **Sm.**	9	**3.22**	**2**	3.11	1	fl	Aq
Nymphaea alba L.	22	2.91	1	2.64	1	fl	Aq
Nymphoides peltata (S. G. Gmel.) Kuntze	3	3.33	1	3.67	1	fl	Aq
Pedicularis palustris L.	5	2.4	1	1.8	1	e	Mar
Phalaris arundinacea **L.**	25	**2.72**	**2**	2.32	1	e	Mar
Phragmites australis (Cav.) Steud.	55	2.58	1	2.13	1	e	Aq
Poa palustris L.	9	2.44	1	2.44	1	e	Mar
Polygonum amphibium L.	14	3.07	1	2.93	1	ff	Mar
Potamogeton alpinus Balb.	12	2.33	1	2.25	1	fl	Aq
Potamogeton crispus L.	5	2.6	1	2.4	1	s	Aq
Potamogeton filiformis **Pers.**	3	**1.33**	**4**	1	16	s	Aq
Potamogeton gr pusillus	31	2.68	1	2.19	1	s	Aq
Potamogeton lucens **L.**	9	**2.56**	**4**	2	1	s	Aq
Potamogeton natans L.	30	2.2	1	1.83	1	fl	Aq
Potamogeton pectinatus **L.**	12	**2.17**	**2**	1.42	2	s	Aq
Potamogeton perfoliatus **L.**	3	**2**	**16**	0.67	8	s	Aq
Potamogeton pusillus L.	5	2.6	1	3	1	s	Aq
Potentilla palustris (L.) Scop.	9	2.56	1	1.89	1	e	Mar
Ranunculus flammula L.	9	2.67	1	2.11	1	e	Mar

Table 5 continued

Species names	*n*	IV-P	*W*–P	IV-Ps	*W*-Ps	GF	EG
Ranunculus lingua **L.**	5	**2.6**	**2**	2.6	1	e	Aq
Ranunculus repens **L.**	6	**2.83**	**2**	2.33	1	e	Rd
Ranunculus trichophyllus **Chaix s.str.**	16	**2.19**	**2**	1.38	2	s	Aq
Ranunculus trichophyllus **subsp.** ***eradicatus*** **(Laest.) C. D. K. Cook**	3	**1.33**	**4**	1	16	s	Aq
Rorippa palustris (L.) Besser	5	2.2	1	2	1	e	Mar
Salix cinerea L.	22	2.91	1	2.59	1	-	Mar
Saxifraga stellaris **L.**	4	**1.25**	**4**	1	16	e	Mt
Schoenoplectus lacustris (L.) Palla	22	2.55	1	2.32	1	e	Aq
Schoenoplectus tabernaemontani (C. C. Gmel.) Palla	11	2.36	1	2.36	1	e	Aq
Scirpus sylvaticus L.	15	2.87	1	2.6	1	e	Mar
Scutellaria galericulata **L.**	12	**3.17**	**2**	3	1	e	Mar
Sparganium angustifolium Michx.	6	1.67	1	1.33	1	fl	Aq
Sparganium erectum L. s.str.	14	2.86	1	2.93	1	e	Aq
Sparganium erectum **subsp.** ***microcarpum*** **(Neuman) Domin**	5	**3**	**2**	2.6	1	e	Aq
Spirodela polyrhiza **(L.) Schleid.**	5	**3.4**	**2**	4	1	ff	Aq
Thelypteris palustris **Schott**	3	**3.33**	**4**	3.33	4	e	Mar
Typha angustifolia **L.**	19	**2.74**	**2**	2.21	1	e	Aq
Typha latifolia L.	52	2.87	1	2.62	1	e	Aq
Utricularia australis R. Br.	16	2.88	1	2.81	1	s	Aq
Utricularia minor **L.**	3	**2.67**	**4**	2	1	s	Aq
Utricularia ochroleuca **R. W. Hartm.**	3	**2.67**	**4**	2	1	s	Aq
Veronica anagallis-aquatica L.	8	2.25	1	2	1	e	Aq
Veronica beccabunga L.	22	2.64	1	2.41	1	e	Aq
Veronica scutellata L.	6	3	1	3	1	e	Mar

In bold, species with an IV-P with $W > 1$. *n* number of occurrences within the data set. *GF* growth forms following Landolt (1977) completed for stoneworts and emerged species with *s* submerged, *fl* floating plants, *ff* free-floating and *e* emerged. *EG* ecological groups according to Moser et al. (2002) and completed for stonewort with *Aq* aquatic, *Mar* marsh, *Mt* mountain, *Rd* ruderal and *For* forest

M-NIP by site and SC thresholds

The M-NIP variants were computed for all the 114 sites used to define the nutrient profile of the species. None of the versions could be calculated for all the sites but overall the M-NIP version that incorporated the species with a low weight ($W = 1$) obviously fulfilled more often the conditions for a reliable index. On the other hand, the indexes considering subgroups of macrophytes could less often be calculated and particularly when only taking species with a weight above one into account.

According to the distribution of the M-NIP value by chemically defined trophic categories, the banding of index values by trophic states were defined separately for each index variant. For the index based on IV-Ps, the index values were overlapping between the trophic categories and mostly distributed over a small range of values leading to low SC thresholds. Therefore, this scale was considered as non-conclusive for a correct classification of the sites in trophic categories and not evaluated further.

The M-NIP based on the TP scale of OECD (M-PNIP) performed better than the one for shallow lakes and a clear pattern of correct classification appeared. The ranges of index values attributed to each nutrient status and the subsequent SC threshold are given in Table 6. After removing the sites with SC values

Table 6 Banding of the M-NIP values into trophic categories

M-NIP type	Oligotrophic 1	Mesotrophic 2	Eutrophic 3	Hypertrophic 4	SC thresholds	Ranges/differences
M-PNIP	1–2	2–2.5	2.5–2.9	2.9–4	0.2	M-NIP values
	1	0.5	0.4	1.1		Differences
M-PNIP − $W > 1$	1–1.8	1.8–2.5	2.5–3.1	3.1- 4	0.3	M-NIP values
	0.8	0.7	0.6	0.9		Differences
M-PNIP-WC	1–1.9	1.9-2.6	2.6-2.95	2.95-4	0.2	M-NIP values
	0.9	0.7	0.35	1.05		Differences
M-PNIP-WC − $W > 1$	1–1.8	1.8–2.55	2.55–3.05	3.05–4	0.25	M-NIP values
	0.8	0.75	0.5	0.95		Differences
M-PNIP-AQ − $W > 1$	1–1.8	1.8–2.3	2.3–2.95	2.95–4	0.25	M-NIP values
	0.8	0.5	0.65	1.05		Differences
M-PNIP-SUB	1–1.8	1.8–2.45	2.45–2.9	2.9–4	0.25	M-NIP values
	0.8	0.65	0.45	1.1		Differences

superior to the thresholds, the box plots of the M-NIP values by site versus nutrient status expressed by trophic categories (Fig. 2) showed distinctly the pattern of classification in trophic categories, especially for the index versions considering only species with a weight above one. The number of sites by trophic categories fulfilling the conditions for a reliable index value is given in Table 7.

In order to illustrate the different type of IV-P obtained, examples for few species are given below.

Potamogeton lucens L.

This species was recorded from nine ponds. Four were classified as mesotrophic and five as eutrophic (Fig. 3a) according to the OECD criteria and with TP concentrations ranging between 12 and 76 µg P/l. Based on these observations, an IV-P of 2.56 with a weighting factor (*W*) of 4 was calculated. In lakes, Lachavanne et al. (1988) observed similar optima in meso-eutrophic sites for *P. lucens*, likewise Melzer (1999) classified this species in the indicator group 3.5 on a scale of 5 points. In rivers, Schneider & Melzer (2003) obtained an IV but also an amplitude very close to our observation (IV 2.65/*W* 4). In the IBMR of Haury et al. (2006), the species score (Csi) for this species is slightly worse and tally at the site scale with a score of poor to bad status. Similarly, the general index of ecological values of Landolt (1977) classified *P. lucens* as four on a five point scale of affinity or tolerance to nutritive substance (“Nährstoffzahl”, N). Our observations corroborate the prior classification of *P. lucens* as nutrient tolerant species linked to meso to eutrophic conditions. In effect, both the IV of Schneider & Melzer (2003) and our calculated IV-P are in the lower part of the range of values for eutrophic sites and no occurrences were observed in oligotrophic or hypertrophic ponds.

Ranunculus trichophyllus Chaix s.str.

Ranunculus trichophyllus Chaix s.str. occured at 16 ponds with TP concentrations ranging from 3 to 63 µg P/l. Two were classified as oligotrophic, nine as mesotrophic, and five as eutrophic (Fig. 3b) according to the OECD criteria. Based on these observations, an IV-P of 2.19 with a weighting factor (*W*) of 2 was calculated. Similarly to this IV-P, Haury et al. (2006) calculated a Csi of 11 out of 20 points, in the range for a good status of IBMR at the site level. Other authors classify *R. trichophyllus* with a higher affinity for nutrients, with an IV of, respectively, 2.7 and a wide tolerance for Schneider & Melzer (2003), four on five for Landolt (1977), and 4.5 corresponding to the eighth category on a scale of nine in the macrophyte index (MI) of Melzer (1999). Our data clearly support a shift downward of one trophic category with the calculated IV-P corresponding to an optimum in mesotrophic ponds. However, almost one-third of the sites, where *R. trichophyllus* was observed, were classified as eutrophic. This, along with the large amplitude of tolerance, indicates that the species can also growth in eutrophic conditions as shown by the above indexes, but seems to have its optimum in mesotrophic ponds.

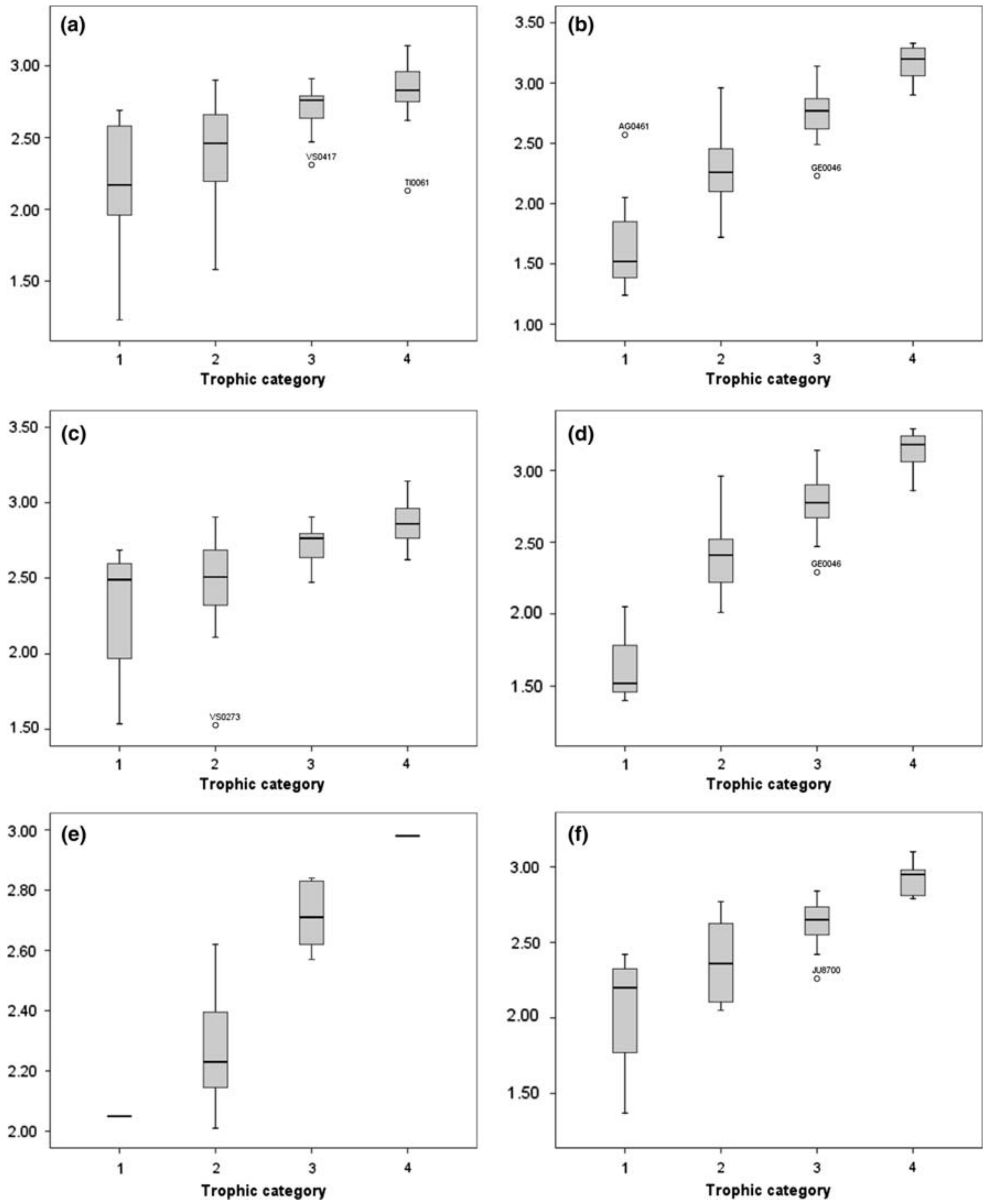


Fig. 2 Box plot of the M-PNIP index values (with an SC inferior to the thresholds defined in Table 6) by trophic categories based on TP (TP OECD trophic scale) with **a** M-PNIP, **b** M-PNIP − $W > 1$, **c** M-PNIP-WC, **d** M-PNIP-WC − $W > 1$, and **e** M-PNIP-SUB and 1: oligotrophic, 2: mesotrophic, 3: eutrophic, and 4: hypertrophic

Table 7 Number of sites fulfilling the conditions for a valid M-NIP computation in each trophic category defined by the banding of the index values (Table 6)

M-NIP		Oligotrophic	Mesotrophic	Eutrophic	Hypertrophic	*n*
M-PNIP	M-PNIP	8	25	63	8	104
	P-OECD	*21*	*31*	*31*	*21*	
M-PNIP − $W > 1$	M-PNIP	6	17	29	5	57
	P-OECD	*7*	*19*	*25*	*6*	
M-PNIP-WC	M-PNIP	4	34	53	5	96
	P-OECD	*18*	*28*	*30*	*20*	
M-PNIP-WC − $W > 1$	M-PNIP	3	15	23	5	46
	P-OECD	*4*	*13*	*24*	*5*	
M-PNIP-AQ − $W > 1$	M-PNIP	–	5	8	1	14
	P-OECD	*1*	*7*	*5*	*1*	
M-PNIP-SUB	M-PNIP	1	14	17	3	35
	P-OECD	*4*	*15*	*11*	*5*	

In italic, the number of ponds by trophic state defined by the chemical TP scale

Juncus bulbosus L.

Juncus bulbosus L. was observed in four ponds only, three were mesotrophic and one eutrophic, the resulting IV-P is 2.25 with a weighting of 4 (Fig. 3c). This value, even if calculated with few observations, clearly link this species to mesotrophic ponds, which is also in accordance with the mean *N* value (three out of five) given by Landolt (1977). The Csi of 16 out of 20 points obtained from river data by Haury et al. (2006) was a step above and fully in the range for the status "very good" of IBMR at the site level.

Berula erecta (Huds.) Coville

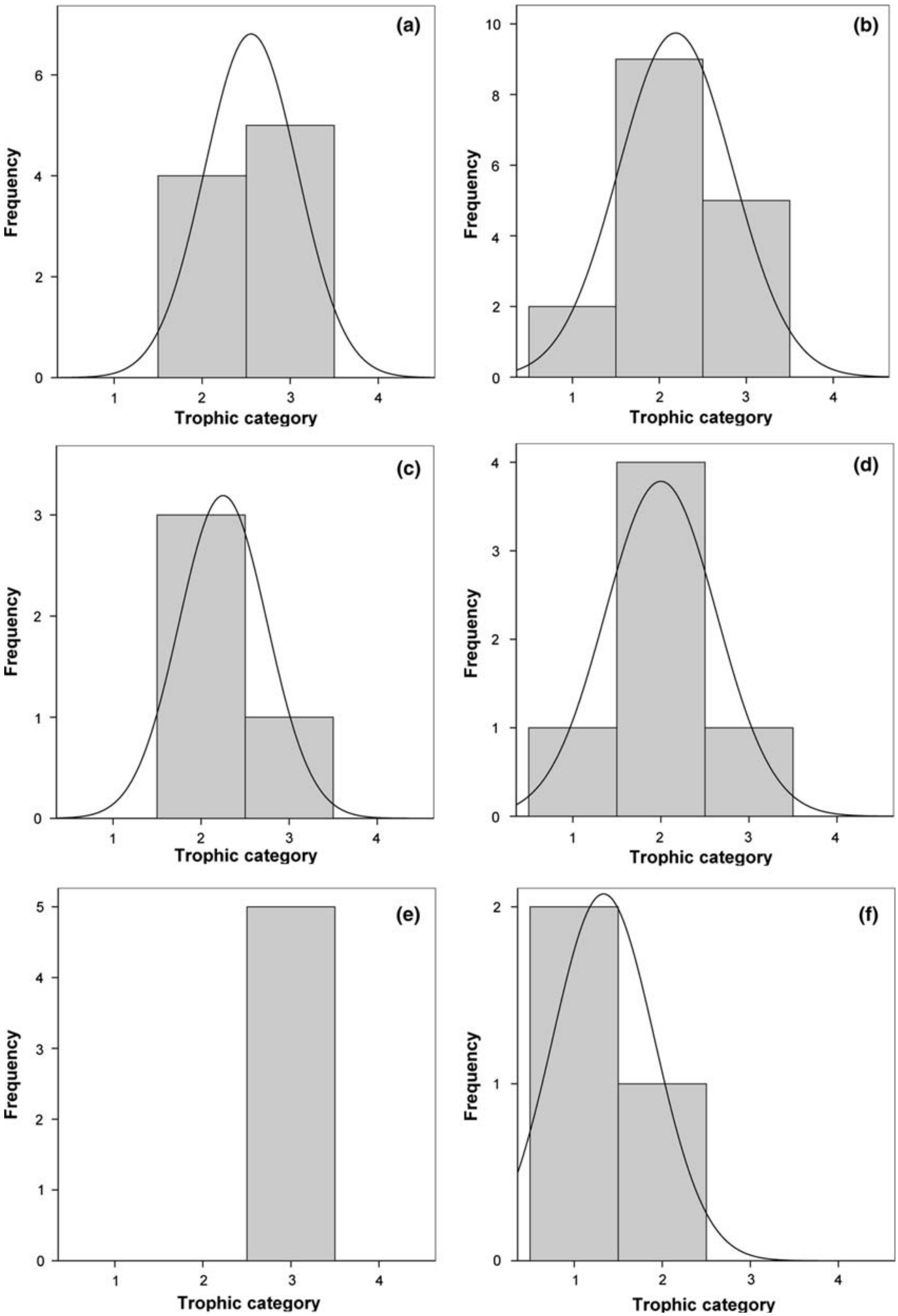
This species has been found in six ponds, four were mesotrophic, one oligotrophic, and the last stand in the lower part of TP concentration, indicating a eutrophic state (Fig. 3d). TP concentrations of the six sites ranged between 3 and 42 µg P/l. The IV-TP for *Berula erecta* is exactly two with a moderate amplitude ($W = 4$), making this species a good indicators of mesotrophic ponds. Both Landolt (1977) and Haury et al. (2006) assign also ecological values corresponding to meso-eutrophic and good conditions with an *N* value of 3 and a Csi species score of 14, respectively. However, the IV of 2.65 obtained in rivers by Schneider & Melzer (2003) classifies this species in the lower part of values indicating eutrophic conditions at the site level.

Carex riparia Curtis

The five occurrences of this sedge in the set of ponds were all in eutrophic sites with TP concentrations ranging between 39 and 76 µg/l (Fig. 3e). This narrow spectrum assigns a maximum weight *W* of 16 to that species and a strong link with eutrophic ponds ($IV = 3$) even if only five observations were available. Landolt (1977) gives also a median value of affinity to nutrients for *C. riparia* ($N = 3$) which support our calculated IV-P value.

Groenlandia densa (L.) Fourr

With only three observations, this species was poorly represented in the data set. However, TP concentrations of the colonized ponds were narrow, between 10 and 20 µg/l, two sites were at the upper limit of TP for oligotrophy, while the latter was clearly mesotrophic (Fig. 3f). The resulting IV-P of 1.33 and a weighting factor (*W*) of four make *G. densa* a good indicator of oligotrophic to oligo-mesotrophic ponds. The index proposed in the literature for this species supports this result, both Landolt (1977) and Schneider & Melzer (2003) obtain indicator values corresponding to the oligo-mesotrophic category with an *N* value of 2 and an IV of 1.83, respectively. The Csi score of Haury et al. (2006) is slightly worse, and with a value of 11 it corresponds to a moderate status for the IBMR at the site level.


(a)
Frequency
0
2
4
6
1
2
3
4
Trophic category
(b)
Frequency
0
2
4
6
8
10
1
2
3
4
Trophic category
(c)
Frequency
0
1
2
3
1
2
3
4
Trophic category
(d)
Frequency
0
1
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3
4
1
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4
Trophic category
(e)
Frequency
0
1
2
3
4
5
1
2
3
4
Trophic category
(f)
Frequency
0
1
2
1
2
3
4
Trophic category

◀ **Fig. 3** Histogram of occurrences by trophic category for **a** *Potamogeton lucens* L. (IV = 2.56/*W* = 4), **b** *Ranunculus trichophyllus* Chaix s.str. (IV = 2.19/*W* = 2), **c** *Juncus bulbosus* L. (IV = 2.25/*W* = 4), **d** *Berula erecta* (Huds.) Coville (IV = 2/*W* = 4), **e** *Carex riparia* Curtis (IV = 3/*W* = 16), and **f** *Groenlandia densa* (L.) Fourr. (IV = 1.33/*W* = 4)

Performance assessment and selection of the M-NIP index

The M-NIP derived from the trophic scale defined with TP (M-PNIP) give the most reliable index as it classifies with significant differences ponds of different chemically defined trophic categories (Table 8). However, when taking into account species with a wide amplitude ($W = 1$), the M-NIP values overlapped between categories of trophy leading to a high rate of misclassification. Table 9 summarizes the proportion of matching classification between the trophic categories defined with the M-PNIP's and the trophic state chemically defined with TP concentrations.

When the IV of all the macrophytes species present were taken into account, the sets of M-PNIP by chemically defined trophic categories were significantly different, except between oligotrophic and mesotrophic categories, where the M-PNIP values were largely overlapping. However, even if the M-PNIP values were significantly different between trophic categories, the rate of correct reclassification was overall low (53.8%), particularly for the ponds chemically defined as oligotrophic or hypertrophic which were upgraded up to eutrophic category (71.4%) or downgraded by one category (65.9%), respectively, leading to an over-representation of the eutrophic state.

Table 8 Significance of the Mann–Whitney tests performed between sets of M-NIP values grouped by chemically defined trophic categories

M-PNIP variant	o–m	m–e	e–h	o–e
M-PNIP	0.091	**0.000**	**0.011**	**0.000**
M-PNIP − $W > 1$	**0.004**	**0.000**	**0.001**	
M-PNIP-WC	0.132	**0.000**	**0.003**	**0.000**
M-PNIP-WC − $W > 1$	**0.005**	**0.000**	**0.004**	
M-PNIP-AQ − $W > 1$	ne	**0.009**	ne	
M-PNIP-SUB	0.293	**0.027**	**0.007**	**0.003**

The values in bold indicate significant differences between set at the 0.05 threshold. *o* oligotrophic, *m* mesotrophic, *e* eutrophic, *h* hypertrophic. *ne* not evaluated as there were not enough cases to perform the statistical test

The performance of the index was much better when considering only the species with a weighting factor above one (M-PNIP − $W > 1$). In that case, the sets of M-NIP values by trophic categories were always significantly different and the rates of correct reclassification overall reached 80.7% and 66.7–92% for single trophic categories. However, with this reduced pool of indicator species, only 57 out of 114 ponds fulfilled the conditions to calculate a reliable index.

When the Characea species were not included in the computation (M-PNIP-WC), the index values remained significantly different between trophic states (MW test, Table 8). Again, when counting the species with $W = 1$, the M-PNIP-WC values were not significantly different between sites chemically defined as oligotrophic and mesotrophic. In addition, the range of M-PNIP values became narrower for the mesotrophic and eutrophic categories (Table 6; Fig. 3), while the ratios of correct reclassification were low for the oligotrophic and hypertrophic ponds (Table 9). The M-PNIP-WC − $W > 1$ performed better and each set of index values by trophic categories was significantly different from the adjacent sets. With this index, the ratio of correct reclassification reached 82.6% overall and never fell under 75% for a single trophic category, while the lag between the TP scale and the M-PNIP index never exceeded one trophic category. Nonetheless, the conditions to calculate a reliable index were fulfilled for a smaller subset of ponds with only 46 sites out of 114 with valid values.

Two additional M-PNIP indexes based on subgroups of macrophytes gave values significantly different between trophic categories: the M-PNIP-AQ − $W > 1$ [species with a weighting factor superior to one and classified as aquatic in the red list of fern and flowering plants of Switzerland of Moser et al. (2002)], and the M-PNIP-SUB based on submerged species only. Even so, with these smaller numbers of species, more sites did not meet the conditions to calculate a reliable index, mainly due to an insufficient number of species with valid IV. For instance, with the M-PNIP-AQ − $W > 1$, 100 sites had an unreliable index and the valid values allocated mainly the 14 remaining ponds to mesotrophic (7) and eutrophic (5) categories (Table 7). This very low

Table 9 Number of sites and rates of matching classification between the trophic categories defined with the M-PNIP's and the trophic state chemically defined with TP concentrations

Index variants		Total	G1	G2	O	G1	G2	M	G1	G2	E	G1	G2	H	G1	G2	Total
M-PNIP	*n*	56	39	9	6	7	8	15	16	0	28	3	0	7	13	1	104
	%	53.8	37.5	8.7	28.6	33.3	38.1	48.4	51.6	0.0	90.3	9.7	0.0	33.3	61.9	4.8	
M-PNIP-W > 1	*n*	46	10	1	5	1	1	14	5	0	23	2	0	4	2	0	57
	%	80.7	17.5	1.8	71.4	14.3	14.3	73.7	26.3	0.0	92.0	8.0	0.0	66.7	33.3	0.0	
M-PNIP-WC	*n*	52	41	3	3	12	3	18	10	0	26	4	0	5	15	0	96
	%	54.2	42.7	3.1	16.7	66.7	16.7	64.3	35.7	0.0	86.7	13.3	0.0	25.0	75.0	0.0	
M-PNIP-WC $- W > 1$	*n*	38	8	0	3	1	0	11	2	0	20	4	0	4	1	0	46
	%	82.6	17.4	0.0	75.0	25.0	0.0	84.6	15.4	0.0	83.3	16.7	0.0	80.0	20.0	0.0	
M-PNIP-AQ $- W > 1$	*n*	10	4	0	0	1	0	4	3	0	5	0	0	1	0	0	14
	%	71.4	28.6	0.0	0.0	100.0	0.0	57.1	42.9	0.0	100.0	0.0	0.0	100.0	0.0	0.0	
M-PNIP-SUB	*n*	22	13	0	1	3	0	9	6	0	9	2	0	3	2	0	35
	%	62.9	37.1	0.0	25.0	75.0	0.0	60.0	40.0	0.0	81.8	18.2	0.0	60.0	40.0	0.0	

O oligotrophic, *M* mesotrophic, *E* eutrophic, *H* hypertrophic. G1 and G2 count the number of case with a gap of respectively one or two trophic category between the M-PNIP and the chemical scale

Table 10 Calculated M-NIP values of the validation data set and corresponding trophic categories

Site code	*GE0010_95*	GE0010_05	GE0010_06	*GE0048_05*	GE0048_06	ZH0002	GE0044	GE4408
TP-CATEG	*M*	E	M	*M*	E	E	E	H
M-PNIP	*2.73*	2.81	2.74	*2.71*	2.79	2.76	2.75	2.98
	E	E	E	*E*	E	E	E	H
M-PNIP $- W > 1$	*2.26*	3.00	2.72	*2.71*	2.81	2.85		3.33*
	M	E	E	*E*	E	E		H*
M-PNIP-WC	*2.79*	2.81	2.74	*2.71*	2.78	2.76	2.75	2.98
	E	E	E	*E*	E	E	E	E
M-PNIP-WC $- W > 1$	*2.55*	3.00	2.72	*2.71*	2.80	2.85		3.33
	M	E	E	*E*	E	E		H
M-PNIP-AQ $- W > 1$	*2.62*	3.09*		*2.61**	2.59	2.74*		
	E	H*		*E**	E	E*		
M-PNIP-SUB	*2.77*	2.73	2.70	*2.77*	2.88	2.72		2.52*
	E	E	E	*E*	E	E		E*

In italic the two surveys that were incorporated in the calibration process on the M-NIP. Asterisk (*) indicates unreliable index with an SC superior to the thresholds defined in the Table 6. *O* oligotrophic, *M* mesotrophic, *E* eutrophic, *H* hypertrophic

applicability makes this index inappropriate for assessment purpose even if the rate of matching classification between the chemical TP scale and the index was quiet good (71.4%). With the M-PNIP-SUB variant, the full range of trophic categories was represented but both the rate of matching classifications (62.9%) and the number of valid values were too low (35 ponds out of 114) to retain this index further.

Validation and application of the M-NIP index

Among the five sites used for the validation process, three were not in the set of ponds used to set the indicator values and the remaining two were new surveys of ponds already incorporated in the building process of the index. When possible, the M-NIP values were computed for the six indexes based on TP concentration (M-PNIP) selected for significance during the previous steps (Table 6). The calculated M-PNIP by site and the trophic categories either determined by the indicator values or with the TP concentration are given in Table 10.

For all three newly surveyed pond, the M-PNIP and M-PNIP $- W > 1$ correctly reclassified the sites in the trophic categories determined by TP. However, for one site, the SC of the M-PNIP $- W > 1$ was above the threshold of 0.3 and, therefore, the index cannot be considered as reliable. As there were no Characea species at these three sites, the versions of the index without stoneworts (M-PNIP-WC) gave the same index value. For that reason, only the index ranges defined for the general index were applied to define the trophic status. The index taking only the aquatic species into account (M-NIP-AQ $- W > 1$) could be calculated for one site but with an SC above the threshold even if it fitted in the range of the corresponding trophic category. Finally, the version of the index taking into account submerged species only (M-NIP-SUB) correctly reclassified one pond, while the index and SC values were out of the range for another and could not be calculated on the last site.

The trophic category of the pair of sites surveyed two subsequent years varied by one class between the two sampling occasions according to TP concentrations. The M-NIP values followed the same trend but remained, however, in the range of the same trophic category. This seems to indicate that the M-NIP value was able to detect slight variations in the chemical status of waterbody but with lower amplitude. This reduced response of the index to phosphorus load could possibly be explained by the resilience properties of the macrophytes community. In effect, the small decrease of the measured TP concentration between the consecutive survey of 2005 and 2006 lead to re-assign the pond GE0010 to the mesotrophic category that it had in 1995, but the M-PNIP index continues to indicate eutrophic conditions in 2006. Moreover, with a longer time period between assessments, the trophic classification based on M-PNIP follows the variation in TP concentration. In effect, between the 1995 and 2005 surveys, the physico-chemical data showed a shift from mesotrophic to eutrophic condition that was also indicated by the two more accurate versions of the MI, the M-PNIP $- W > 1$ and the M-PNIP-WC $- W > 1$.

Discussion

Significance and limitation of the indicator values (IV) by species

The nutrient profile of a large part of the species could be derived from the pond data set. Among the species dismissed, due to an insufficient number of observations, some are known for their narrow trophic profile in rivers or lakes and their inclusion could have potentially improved the performance and applicability of the index. This is notably the case of *Potamogeton plantagineus* Roem. & Schult that occurred in only one oligotrophic pond within the data set but is known for a high affinity to oligotrophic conditions from other studies and coded with an IV of 1.05 corresponding to the oligotrophic category in the river index of Schneider & Melzer (2003). Similarly, *Ranunculus circinatus* Sibth. occurred in two ponds across the whole data set, both classified as mesotrophic according to the TP concentration, and IV of Schneider & Melzer (2003) as well as *N* value of Landolt (1977) indicate a similar nutrient profile that could validate this IV. On the other hand, *Zannichellia palustris* L. was also observed in two mesotrophic ponds, but the trophic profile found by other authors are one or two degrees higher, with an IV of 2.93 in rivers (Schneider & Melzer 2003) corresponding to eutrophic conditions and a maximum *N* value for Landolt (1977) indicating nutrient rich conditions, respectively. In addition, some of the species with enough observations to contribute to a reliable index also indicated nutrient conditions differing from the profile established from lakes and rivers data, in fact, what was expected from an index based on pond data. For all these reasons, we have decided to strictly exclude any of the species with less than three observations within the present data set, even when the nutrient conditions in colonized ponds were concordant with the trophic category assigned to species from rivers or lakes or even from an expert judgment. For the index calibration step, these exclusions have only a slight influence on the results as discarded species never occupy more than two sites. By contrast, for an assessment of newly sampled ponds, the greater the number of coded species, and especially of stenotrophic species, the more chance to compute a valid M-NIP value. In order to improve the M-NIP, it is, therefore, highly recommended to include data from more sites in the calibration set. In addition, this would also allow refining of the trophic profiles for species already coded.

An important aspect of the nutrient profile of species is the amplitude of tolerance (*a*) transcribed in weighting factors for the M-NIP and SC calculations. This amplitude was wide for many species, indicating either eurytrophe species with a low bioindication potential or a too small number of observations to bring out a distinct optima. Other factors, among which the type of chemical data used to define the profile, contribute to the relatively wide amplitude observed for most species. In effect, the mean water concentration of nutrient is expressed at the site scale and does not take into account the variability of the water chemistry within the pond. For large sites with an important sinuosity and an irregular morphometry, this spatial variability of the physico-chemicals conditions can be quite important. Moreover, the content of the sediment was not measured for this study and this important source of nutrient for rooted species can show important variations even for a similar concentration of nutrients in the water. The lack of sediment data made it difficult to disentangle the effects of the variability of water chemistry from the influence of the sediment content, which both probably contribute to widen the amplitude of nutrient profiles based on mean values of water chemistry. The amplitude of tolerance expresses, however, the range of mean water nutrient concentration where the species was observed. The fact that free-floating species also had wide amplitudes, even wider that some emerged species, indicates that the observed tolerance is linked to parameters measured in the water and not only to variations or parameters not taken into account by the chemical data. This increase in the amplitude of tolerance to nutrient conditions leads to less accurate profiles of bioindication by individual species; nonetheless, the accuracy of the IV remains sufficient for an assessment at the pond level.

M-NIP as an assessment tool

The wide tolerance of most of the species also has implications on the M-NIP accuracy and particularly when the species with the larger amplitude are taken into account ($W = 1$). Despite the limitations of indicator values and considering the goal to obtain a tool for assessing the trophic status of the whole pond, the indexes adopted are able to classify most of the ponds by trophic categories albeit with an error rate relatively important. In effect, the M-NIP variants always integrate the nutrient profiles of several species with indicator values that should not diverge beyond a threshold of confidence expressed by the rate of SC. When the species with the widest amplitude were discarded ($W = 1$), the indexes classified the ponds in trophic categories matching relatively well the classifications based on TP concentrations. However, these variants of M-NIP were not applicable to a large number of sites, mainly due to the lack of valid IV for many species. By contrast, when keeping all species, the increase in the overlap between ranges of M-NIP values belonging to adjacent trophic categories led to higher rates of misclassification and the index accuracy dropped considerably. The rate of misclassification increased particularly for the oligotrophic and hypertrophic ponds pushed, respectively, up or down by the indicator values of tolerant species, despite their lower weight in the index. The performance assessment of the M-NIP variants presented in the results and summarized in Tables 8 and 9 permits to establish a preferential order for the use of the indexes taking the rate of correct reclassification and the time investment as evaluation criteria. Knowing that the identification of Characea at the species level requires time and expertise often less available to site managers, we propose to apply a first division depending on the presence of this group of macrophytes in an assessed site:

- When Characea are observed, the surveyor records it and collects samples by quadrat. However, the M-PNIP-WC $- W > 1$ is first computed and Characea species are identified to calculate the M-PNIP $- W > 1$ only if the first index cannot be calculated or does not fulfill all requirements. If the lack of species with $W > 1$ means that both versions cannot be calculated, the less accurate but more often applicable M-PNIP-WC and M-PNIP indexes can be used instead. The results of the two latter variants must be interpreted with the limitations linked to their lower precision.
- If no Characea species are observed, the M-PNIP $- W > 1$ is used first. If it cannot be

calculated or is unreliable, the M-PNIP is used instead and interpreted with the limitations linked to the lower accuracy of this nutrient index.

Conclusions

Ecological indicators must have an ecological meaning, be easy to use and reproducible, and sensitive to moderates changes in environmental conditions. The proposed M-NIP index based on TP concentration fulfill the first three conditions: first, macrophytes are sensitive to trophic conditions, second, the index is easy to obtain with the nutrient profiles and cover of the species observed during a survey, and third, the sampling design used to define the nutrient profiles and calculate the index values is standardized and fully reproducible. The fourth condition is, however, only partly fulfilled. In effect, both the rate and the amplitude of misclassification between the physico-chemical scale and macrophytes index are relatively high for the two more applicable variants of the M-PNIP taking all species into account. Moreover, over short time intervals, the macrophyte index response to variation in nutrients concentration is measurable but limited in amplitude. This reduced response of the biotic index was observed on two ponds sampled over two subsequent years that vary by one trophic category between the two sampling occasions according to TP, but remained in the same category with the M-PNIP. By contrast, with a longer time period between two surveys, the macrophytes index seems to respond to an increase in nutrient concentration with the same amplitude as the TP scale. This latter observation was made on the single pond where such data were available, therefore, it still needs to be confirmed with other sites but, for monitoring purposes, seems promising.

Despite these limitations regarding the accuracy and delay in the response of the index, the M-NIP based on concentration of TP (M-PNIP) makes up a good indicator of the pond nutrient status that can be easily used for site assessment or monitoring. This index is, thus, a reliable metric of eutrophication to be integrated in a multimetric index to assess the water quality of Swiss ponds. Nonetheless, the index does not fulfill the requirements of the WFD to perform the assessment by a measure of the deviation from a set of reference sites considered as unimpacted. Indeed, as a pond can be naturally eutrophic the M-NIP index does not necessarily express degradations or human influences. Nevertheless, water quality is one of the aspects to be taken into account by the WFD. A biotic index assessing specifically the trophic state is a valuable complementary descriptor to an approach conducted in parallel, which is based on the comparison of composition and abundance of the macrophytes communities between references conditions and assessed site.

As stated earlier, a greater data set needs to be available in order to improve the accuracy and applicability of the index by incorporating more species and refining their IV. Moreover, a greater number of additional records would also enable the ranges of values by categories of nutrient status to be more precisely refined. Specifically, it would allow ranges of index values by biogeographic and altitudinal regions to be defined, which was unfortunately not possible with our data set as there were not enough ponds for each type of trophic categories.

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