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PLOCH: a standardized method for sampling and assessing the biodiversity in ponds

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ABSTRACT

1. As ponds are now recognized as freshwater habitats clearly distinct from lakes and running waters, there is a need for standardized tools for assessing their ecological integrity and status, and particularly their biodiversity.

2. A standardized method was developed for sampling and assessing the species richness of ponds. Experiences accumulated in previous studies, together with data gathered from 80 Swiss ponds, provided the basis of the proposed method.

3. Five taxonomic groups were chosen as complementary representatives of pond inhabitants: aquatic plants, aquatic Gastropoda, aquatic Coleoptera, adult Odonata and Amphibia.

4. To sample aquatic flora, quadrats are located along transects perpendicular to the longest axis of the pond. The number of quadrats is calculated from a relationship with pond area. A nonparametric estimator (Jackknife-1) is used to estimate the true species richness from the observed richness.

5. Aquatic invertebrates (Gastropoda, Coleoptera) are collected with a hand net. Sampling is stratified within the dominant habitats. The number of samples is calculated from a relationship with pond area. As with the vegetation, the Jackknife-1 estimator is used to estimate the true species richness.

6. The species richness of adult Odonata is assessed using a standardized field survey method combining observations from early and late summer. The species richness is corrected with an abundance-based estimator (Chao1). The species richness of Amphibia is obtained from an exhaustive inventory.

7. For the assessment of biodiversity, species richness values derived from measurements are compared to values predicted for conditions that enable a high species richness. Generalized Additive Models are used to predict species richness from environmental predictors characterizing the pond. The ratio of measured richness to predicted richness allows the allocation of a quality status to each pond. Results are divided into five biological quality classes, as recommended in the EC Water Framework Directive (WFD).

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INTRODUCTION

Ponds have only recently been recognized as important habitats for the maintenance of biodiversity. At a regional level, ponds can contribute most to freshwater biodiversity, supporting considerably more species, more unique species and more scarce species than other water body types (Williams *et al.*, 2004).

Monitoring of ecosystems is a practice now largely accepted and included in nature conservation policies, partly related to the coming into force of the Convention on Biological Diversity (Rio de Janeiro, 1992). The signatories of the Convention have the obligation to conduct identification and monitoring, through sampling and other techniques, of ecosystems and habitats, in particular those containing a high species diversity and a large number of endemic or threatened species. In this respect, the high diversity in ponds is in itself a sufficient argument for undertaking their monitoring. Nevertheless, there is little scientific knowledge of pond ecology and these water bodies have been neglected in limnological studies; for example, less than 1% of nearly 1000 communications presented at the 2004 Congress of the International Association of Limnology (SIL) were devoted to pond ecology. Limnological textbooks focusing on small water bodies are also rare (Brönmark and Hansson (2000) is an exception). The ecological basis of freshwater pond management for biodiversity is therefore poor (Gee *et al.*, 1997). Consequently, standardized tools for pond assessment are scarce (Indermuehle *et al.*, in press), and pond managers often use poorly adapted methods that were developed for streams, rivers or lakes. The only standardized method developed specifically for the assessment of ponds (the PSYM method, Biggs *et al.*, 2000b) is limited in geographic applicability to the UK.

The need for a standardized method for sampling and assessing the biodiversity in ponds was recognized by the Swiss Agency for Environment, Forest and Landscape, who financed a large research programme on ponds (Oertli *et al.*, 2000) including the development of 'PLOCH' (abbreviation from '*Plans* (PL) *d'eau* (O) *suisses* (CH)'). This new method is useful for monitoring (i) at a regional scale (to provide a baseline survey when choosing sites for conservation) or (ii) at a local scale for the long-term monitoring of a given site or for conducting an environmental impact assessment. As for the rapid assessment methods, this method is relatively easy and cheap to use by managers. It involves classical sampling tools, a relatively limited taxonomical expertise, a small number of sampling sessions with as few replicates as possible, simple data processing, and an automated assessment based upon simple computer tools. Standardization is the baseline of this new method, and is inherent not only to the sampling procedure, but also to data processing and assessment. Indeed, the assessment matches the requirements of the European Water Framework Directive (WFD; European Commission, 2000) as observed values are compared with values predicted for reference conditions.

The PLOCH method follows the philosophy of rapid bio-assessments. As it is to be used at a large spatial scale, by local reserve managers or by more global stakeholders, sampling must be economical and quick to undertake. Effort will also be made to reduce sampling bias, in particular the bias linked to heterogeneous sampling intensities, by the use of nonparametric richness estimators.

The type of quality assessment of pond biodiversity has been chosen in accordance with the WFD which stipulates that Member States must assess the ecological status of water bodies by comparing the present conditions and the expected reference conditions. Thus the assessment will be made by the comparison of the measured richness and the richness of a reference condition. However, as the PLOCH method is based on evaluating species richness, the reference conditions may not be the same as those used for WFD to evaluate water quality. The WFD describes reference conditions (i.e. high status) where there are no, or only very minor, anthropogenic alterations to the values of the hydrochemistry and hydromorphology and with biota usually associated with such undisturbed or minimally disturbed conditions. For the PLOCH method, the term 'reference conditions' is used for conditions that enable a high potential species richness; this definition does not necessarily link reference conditions to the absence of anthropogenic degradation.

The proposed method is presented here in two parts: (i) the standardized sampling of the species richness of selected taxonomic groups, and (ii) the quality assessment of species richness.

STUDY SITES

For the development of the method, the biodiversity of 80 ponds was assessed. An inventory of 8000 ponds (Borgula *et al*., 1994) provided the baseline data for choosing these 80 ponds scattered throughout Switzerland at altitudes ranging between 210 and 2757 m a.s.l. Their mean area and depth were 8817 m^2 and 1.66 m respectively. Only 31 of these ponds were known to have a natural origin with an age exceeding 4000 years (the date of the last glacial retreat). The other 49 were of various ages, and were artificial, having been created or maintained by past or present human activities (e.g. gravel or clay extraction, fish production, or nature conservation). The main pond characteristics are given in Appendix 1, and further details are available on request.

Previous studies provided information on field sampling strategies useful for the development of the PLOCH method. These included about 100 ponds situated in alluvial floodplains from the rivers Saône (Godreau *et al*., 1999) and Rhône (Castella *et al*., 1991) (France), and Lake Neuchâtel fringe wetland (Antoine *et al*., 2004; Castella-Müller, 2004) (Switzerland).

METHODS

Choice of the biological indicators

A taxonomic group which is a candidate for selection in an assessment of biodiversity should be a 'keystone', 'umbrella' or 'flagship' group. Furthermore, it must fulfil many criteria if it is to be used as a surrogate for other groups (see criteria in New (1995)). The preferred groups should in particular (i) be representative of the surveyed types of habitat, (ii) be reasonably diverse but with an established taxonomic framework, (iii) include representation of diverse ways of life, (iv) be geographically widespread, (v) be accessible and amenable to quantitative sampling by standard techniques, (vi) already be well studied with substantial knowledge of their ecology (e.g. ecological traits, conservation value), (vii) be likely to engender political sympathy and support. Five groups conforming to these criteria were selected for the PLOCH method: aquatic plants, aquatic Gastropoda, aquatic Coleoptera, Odonata and Amphibia. For Odonata, the adult stage was selected because identification and sampling are easier and less expensive than for larvae or exuviae. These five groups are ecologically complementary. In the water, plants are primary producers, Gastropoda are primary consumers, and Coleoptera are secondary consumers (predominantly predators). Adult Odonata can be considered as indicators of habitat quality in aquatic/terrestrial ecotones, especially for the structure of the shoreline vegetation (e.g. Buchwald, 1992). Amphibia are highly dependent on terrestrial habitats and landscape structure in the pond environment (e.g. Marsh and Trenham, 2001; Semlitsch, 2003). These five indicator groups also show marked differences in their dispersal strategies: passive (vegetation and Gastropoda), active terrestrial (Amphibia), and active aerial (Odonata and Coleoptera).

Plants considered here as aquatic are the 254 species listed in the highest humidity class (= 5) of Landolt (1977): this includes true hydrophytes (species submerged or with floating leaves) and most of the emergent plants. To this 'aquatic' species pool, was added a set of 22 species listed by Landolt (1977) under class 4: *Juncus effusus*, *Carex canescens*, *Carex flava*, *Carex lepidocarpa*, *Carex nigra*, *Eleocharis acicularis*, *Eleocharis quinqueflora*, *Equisetum palustre*, *Galium palustre*, *Agrostis stolonifera*, *Juncus conglomeratus*, *Scirpus sylvaticus*, *Juncus filiformis*, *Juncus inflexus*, *Lysimachia nummularia*, *Lythrum salicaria*, *Lysimachia vulgaris*, *Mentha longifolia*, *Myosotis scorpioides*, *Ranunculus repens*, *Rorippa palustris*, *Juncus articulatus*. The Characeae were considered as a single taxon.

Sampling species richness

The aim of the PLOCH method is to measure the true species richness (S_{true}) of a pond. Only an exhaustive sampling inventory can directly appraise S_{true}. Nevertheless, in practice, such sampling can rarely be managed because of the limitations of time and money. For this reason an exhaustive strategy has been selected only for Amphibia in the PLOCH method.

Species richness measured from a sampling programme (as is used here for vegetation and invertebrates) is affected by the sampling effort — as area or sample size increases the number of individuals increases and the number of species also rises (Connor and McCoy, 1979). Such sampling bias can be reduced by means of a nonparametric estimator of S_{true}, as, for example, Jackknife or Chao estimators (see Foggo *et al.*, 2003; Magurran, 2003). Such estimators were designed to overcome sample-size inadequacies and to estimate how many species are actually present in the sampled habitats (Rosenzweig *et al.*, 2003).

Vegetation

Many field techniques have already been developed for sampling vegetation (Müller-Dambois and Ellenberg, 1974). For PLOCH, the most popular and probably also the most efficient was selected: quadrats along transects. For the method development, quadrats (0.5×0.5 m) were located every 5 m along transects perpendicular to the longest axis of each of the 80 ponds. These transects were positioned at regular intervals: every 5 m for small ponds ($<650\,\mathrm{m}^2$), every 10 m for medium-size ponds and every 20 m for large ponds ($>6500\,\mathrm{m}^2$). In order to include the generally highly diverse interface between water and land, the two extreme quadrats of each transect were placed at the interface, on the water side. If there was large variation in the water level, they were placed where the average water level was thought to be. Areas deeper than 3 m were not sampled. Presence or absence of species was recorded in each quadrat; abundance information is not needed for the PLOCH method. Plants were sampled during the summer months between 1996 and 1999. Each of the 80 ponds was sampled for plants on one day. These vegetation data provide the baseline for calculating how many quadrats are needed in a new pond investigated with the PLOCH method.

Gastropoda and Coleoptera

Many field techniques for sampling aquatic invertebrates have already been developed (see Cummins, 1962; Macan, 1977; Elliott and Tullett, 1978), especially for running water. The most popular for pond managers and other stakeholders was selected here: pond-net sweeping for a fixed time period. This type of technique is already used in streams (RIVPACS; Wright *et al.*, 2000) and ponds (PSYM; Biggs *et al.*, 2000b).

The hand net has a small rectangular frame (14×10 cm, mesh size 0.5 mm) to facilitate movement within dense aquatic vegetation. One unit sample consists of the intensive sweeping of the net through the habitat (i.e. vegetation, debris and surface sediment) for 30 s. In all cases, the collected material is preserved in 70% alcohol and later comprehensively sorted in the laboratory. Gastropoda and Coleoptera are identified to species level and counted. (Counting is not required for the PLOCH method, as occurrence in the sample is sufficient.)

For the development of the method, the macroinvertebrates were sampled in the 80 ponds during the summer months between 1996 and 1999. Each pond was sampled for macroinvertebrates on one day. The number of samples collected was calculated using an exponential relationship with pond size: 4 samples for a 10 m^2 pond, 8 for 100 m^2, 16 for 1000 m^2 and 32 for 10 000 m^2. These faunistic data provide a baseline for calculating how many samples have to be collected in a new pond investigated with the PLOCH method.

As for other methods of invertebrate survey in fresh waters (e.g. AFNOR, 1992; Biggs *et al*., 2000a; Wright *et al*., 2000), sampling was stratified across the dominant mesohabitats. Sediments were not sampled, because of their low taxonomic richness for the selected taxa. When stones or gravel were sampled, they were collected in the net, and rinsed into the collection container. The list of potential mesohabitats is presented in Appendix 2.

Before sampling, a schematic map of the mesohabitats is produced. Mesohabitats are divided into two main categories: (i) those occurring between the shoreline (excluding the shoreline itself) to a depth of 2 m (deeper zones are not sampled), and (ii) shoreline aquatic mesohabitats. Only mesohabitats covering more than 1% of the total mesohabitat area are taken into account and only the pond area comprising the mesohabitats listed in Appendix 2 is considered. Two-thirds of the samples are then allocated to the first mesohabitat category and the remaining samples are allocated to the second. The samples are distributed between the mesohabitats in proportion to the coverage of each, with a minimum of one sample per mesohabitat. If the number of mesohabitats is larger than the number of samples, samples are distributed among the dominant types. If one mesohabitat is composed of scattered patches, the sampling time (30 s) is divided into shorter periods and distributed between the patches (= 1 composite sample).

Odonata

Odonata inventories are expensive because they have to include several sampling days and have to be conducted over several years (see for example the method presented by Schmidt (1985)). To limit the cost, the number of sampling days has to be restricted. Preliminary analyses were conducted on 12 ponds where inventories were available (more than 10 sampling days distributed over 3 years or more; data from the Swiss Centre for Faunal Cartography database) (Oertli *et al*., 2000). It appears that S_{true} calculated from 2 sampling days located during the flying period of Odonata, at the end of spring and at the end of summer, represented on average 66% of the species richness recorded when performing an exhaustive inventory. A third sampling date would add relatively little new information (10% more species).

Therefore, for the PLOCH method, a representative species list is gathered during two sampling days (at the end of spring and of summer). The observations are conducted through a standardized field procedure on one-third of the shore length. For details, see Oertli *et al*. (2000). The abundance of each species is recorded; however, this quantitative information is necessary only for species represented by one or two individuals, as required in the calculation of S_{true}, which is estimated from the species list by computing the estimator Chao1 (Chao, 1984). This estimator is abundance-based and has the advantage of not being dependent on sample frequencies. For the development of the method, the Odonata were sampled in the 80 ponds during the summer months between 1996 and 1999 inclusive.

Amphibia

For the PLOCH method, inventory techniques for Amphibia can be chosen by the manager (see, for example, Heyer *et al*., 1994), in order to provide an exhaustive species list. An example is the method presented by Schmidt (2004) which has been used in Switzerland for updating the national list of endangered species. In the dataset used for method development, inventories of Amphibia were recorded for each of the 80 ponds over the 10-year period ending in 1994 (Borgula *et al*., 1994; data stored in the Swiss fauna databank (CSCF-KARCH)).

Statistical methods

Species richness estimators and randomized species accumulation curves were computed using *Ws2m.exe* (Turner *et al*., 2000). Generalized Additive Models (GAMs; Hastie and Tibshirani, 1990) were chosen to model the relationship between species richness and environmental predictors. GAMs are well suited to study ecological responses because of their nonparametric characteristics. With GAMs, response curves are data-driven and defined by smoothed functions that can take any shapes, and which do not assume a linear or quadratic relationship between the dependent and the independent variables (e.g. Yee and Mitchell, 1991; Lehmann *et al*., 2002a).

Models were fitted using S-PLUS (Mathsoft) and a set of functions developed for Generalized Regression Analysis and Spatial Predictions (GRASP; Lehmann *et al*., 2002b). Species richness was assumed to follow a Poisson distribution (positive and discrete) and was therefore modelled using a log link function. A stepwise procedure was used to select significant predictors (*F*-test for quasi-Poisson distribution with a p value <0.01). A cubic-spline function with three degrees of freedom was applied to fit the partial contribution of each of the environmental predictors. Models were evaluated using the explained deviance (D^2) and cross-validated correlation between observed and cross-predicted richness (fivefold cross-validation). Comparing simple correlation ($r1$) and cross-validated correlation ($r2$) allowed assessment of model stability.

RESULTS

The PLOCH method: sampling species richness

Vegetation

For the development of the method, a mean number of 77 quadrats (min. 8, max. 460) was sampled for each pond. To assess the number of quadrats necessary for conducting the PLOCH method on a new pond, the data collected in the 80 ponds were analysed. The observed pond richness (S_{obs}) varied between 2 and 27 species (mean: 10.2). A species accumulation curve was drawn for each of the 80 ponds, and S_{true} was estimated using the first-order Jackknife richness estimator (Jackknife-1; Burnham and Overton, 1979). For example, in pond OW0167 where 83 quadrats were sampled, the species accumulation curve showed an accumulated richness at the same level as the asymptote; S_{obs} (nine species) represents 100% of S_{true} (9 ± 0 species) (Figure 1). For pond JU7000 the accumulation curve demonstrated that 121 quadrats led to an underestimation of the richness; S_{obs} (nine species) represented only 64% of S_{true} (14.0 ± 2.2 species) (Figure 1).

The 80 species accumulation curves and the associated estimations of S_{true} provided the database for estimating the mean number of samples necessary to reach a chosen proportion of S_{true}. For the PLOCH method, the strategy should ensure that the sampling will gather at least 50% of S_{true}. As this proportion is the minimum that can allow the use of the Jackknife-1 estimator of S_{true} (Krebs, 1999), it was decided to increase this proportion to 70% to improve the accuracy of the PLOCH sampling method. The relationship between the pond area and the number of quadrats necessary to collect 70% of S_{true} is presented in Figure 2(a).

For the sampling of a given pond with the PLOCH method, the number of quadrats is calculated and they are equally distributed along transects regularly spaced perpendicular to the longest axis of the pond.

Gastropoda and Coleoptera

To assess the number of samples necessary for conducting the PLOCH method on a new pond, the same procedure as for the vegetation was used. S_{obs} of Gastropoda varied between 0 and 9 species (mean: 2.8),

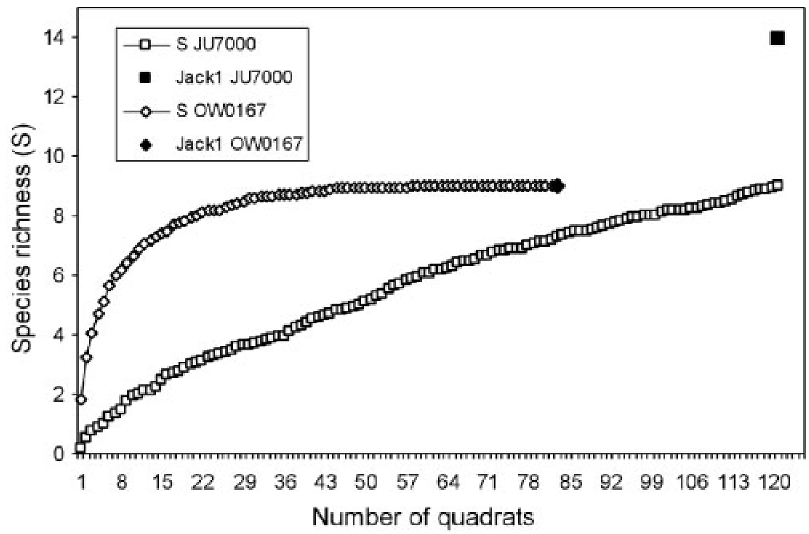

Figure 1. Randomized species accumulation curves for aquatic plant communities from two contrasting ponds (JU7000 and OW0167). First order Jackknife richness estimator (Jack1) is also indicated.

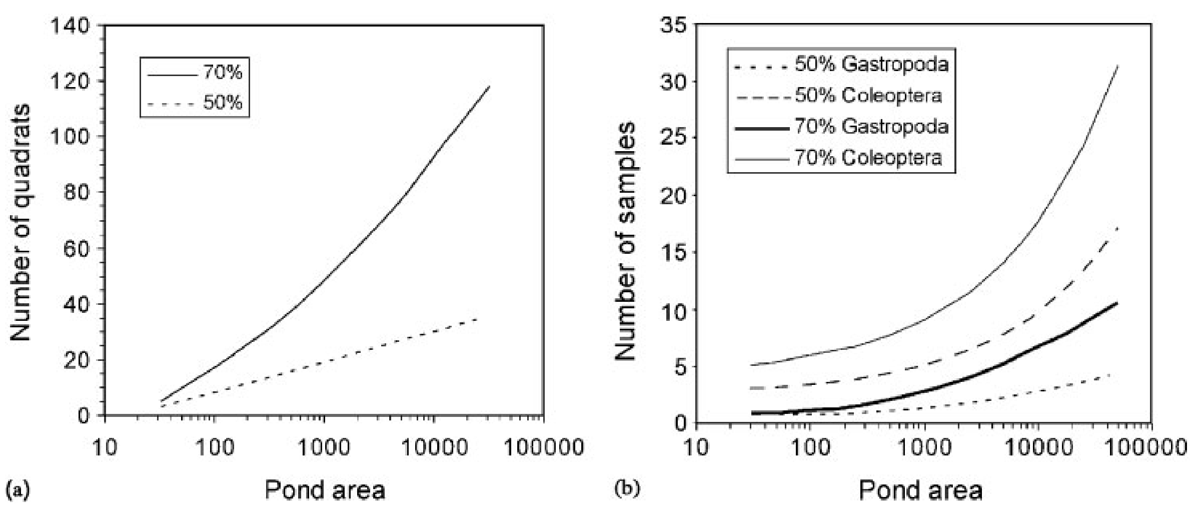

Figure 2. Mean number of samples needed to collect 70% of S_{true} as a function of pond area (in m^2). The 50% level is also indicated by the dashed line. (a) Aquatic plants. Mathematical expression of the relationship (70% level = PLOCH method choice): $n = 1.96 - 2.8 * (\log_{10}(\text{area})) + 2.6 * (\log_{10}(\text{area}))^2$. (b) Gastropoda and Coleoptera. Mathematical expression of the relationship (70% level): for the Gastropoda $n = 3.6 - 3.4 * (\log_{10}(\text{area})) + (\log_{10}(\text{area}))^2$ for the Coleoptera (= PLOCH method choice) $n = 4.01 + 0.25 * \exp(\log_{10}(\text{area}))$.

and S_{obs} of Coleoptera between 0 and 20 species (mean: 5.2). Eighty species accumulation curves were drawn and S_{true} estimated using the Jackknife-1 estimator. Such data enabled an estimation of the mean number of samples necessary to reach the chosen proportion of S_{true} (70%) for Gastropoda and for Coleoptera. Figure 2(b) presents the relationship between the pond area and the mean number of samples required to reach the expected proportion. The curves show that more samples are needed to collect 70% of S_{true} for Coleoptera than for Gastropoda; this is probably due to the greater difficulty of catching the more mobile adult Coleoptera. As the objective of the PLOCH method is to collect a minimum of 70% of S_{true} for Coleoptera *and* Gastropoda, the curve established for Coleoptera was selected as the baseline for both Gastropoda and Coleoptera. This choice enables the PLOCH method to collect 70% of Coleoptera species and a higher proportion of Gastropoda species, estimated at about 90%.

Odonata

In the 80 sampled ponds, S_{obs} of adult Odonata assessed during the 2 sampling days varied between 0 and 23 species (mean: 7.7). This richness represented on average 90% of S_{true} potentially observable during these 2 sampling days. This high proportion indicated that the proposed strategy (survey of one-third of the shore length) is largely sufficient to gather the basic information (S_{obs}) needed to estimate S_{true}.

Amphibia

In the 80 sampled ponds, species richness measured by exhaustive inventories varied between 1 and 13 species (mean: 4.4).

The PLOCH method: quality assessment of species richness

For the PLOCH assessment of biodiversity, the measured species richness (S_{true}) is compared with that predicted for conditions characteristic of high species richness (S_{ref}). The ratio S_{true}/S_{ref} allows the designation of a quality status for each pond.

Predictive models for conditions enabling a high species richness

The reference conditions for the PLOCH assessment were defined in terms of the environmental variables that significantly influence species richness (see Oertli *et al*., 2000). Their relationships with the richness (S_{true}) for the five taxonomic groups were modelled with GAM. From more than 100 local and regional variables characterizing each pond, a subset of 17 weakly correlated variables was selected as candidate predictors for the stepwise selection within the GAM procedure (Table 1). Depending on the taxonomic

Table 1. The five GAM models of the relationships between the environmental predictors and the species richness of aquatic plants, Gastropoda, Coleoptera, Odonata and Amphibia: selected predictors and validation diagnostic. The range of measured values is presented in Appendix 1. The models were evaluated using percentage of explained deviance (%D), simple validation coefficient (r_1) and cross-validation coefficient (r_2). All models were selected with threshold $p<0.01$. Seven variables proposed were not integrated in the five GAM models: percentage of shoreline shaded, trophic state as indicated by total P, presence of fish, shoreline development, connectivity (measure of pond isolation), percentage of agriculture in the catchment, the percentage of forest in the pond's surroundings (in a 50-m buffer zone)

	alt	area	mean depth	age	pH	cond	N	trans	floating veget.	subm. veget.	%D	r_1	r_2
Aquatic plants	×	×	×				×				0.49	0.51	0.70
Gastropoda	×	×						×	×		0.49	0.60	0.70
Coleoptera	×		×			×				×	0.55	0.67	0.74
Odonata	×	×			×						0.66	0.79	0.81
Amphibia	×			×							0.55	0.65	0.74

alt: altitude; area: $\log_{10}$(area); N: nitrogen trophic class (defined in Appendix 1); cond: conductivity; trans: transparency of the water; floating veget.: proportion of area covered by floating-leaved vegetation; subm. veget.: proportion of area covered by submerged vegetation.

group, GAM models integrated two to four predictors. Altitude had an important contribution in all models. Pond area was also important, but was not significant for Coleoptera and Amphibia (Oertli *et al.*, 2002). Other predictors included mean depth, conductivity, transparency, pH, trophic state as indicated by inorganic N concentration, proportion of pond area covered by floating-leaved and submerged vegetation, and pond age. Seven variables were not integrated in the five GAM models: the percentage of shoreline shaded, the trophic state as indicated by total P, the presence of fish, shoreline development, connectivity (measure of pond isolation), the percentage of agriculture in the catchment and the percentage of forest in the pond's surroundings (in a 50-m buffer zone).

In a second step, the five richness models developed in Splus were transferred into a user-friendly interface in Microsoft Excel (PREDIR v.1.1, available from http://leba.unige.ch/PLOCH/rapport/ploch1234.htm). This tool allows scenarios to be shown graphically according to changes in selected predictors. The PLOCH method uses this approach to predict the species richness of a pond under reference conditions (S_{ref}). Reference conditions are reached when modifiable predictors are set to their optimal value in order to maximize species richness (trophic state: class 1; conductivity: $8\,\mu\mathrm{S\,cm}^{-1}$; pH: class 2; transparency: 44 cm; coverage by submerged or floating-leaved vegetation: 100%), while fixed predictors stay as recorded *in situ* (altitude, area, mean depth, age).

The PLOCH assessment

The PLOCH assessment of species richness for a given pond is then made by comparison of S_{true} with S_{ref}. This comparison is based upon the ratio of these two values ($S_{\mathrm{true}}/S_{\mathrm{ref}}$) as recommended by the WFD. Values of this ratio, expressed in five classes, provide a way to assess the 'quality' of the pond species richness: bad (0 to 0.2), poor (>0.2 to 0.4), moderate (>0.4 to 0.6), good (>0.6 to 0.8) and high (>0.81). This assessment is made for each of the five taxonomic groups, the mean giving the overall value of pond biodiversity.

Choice of the taxonomic groups

An important assumption at the beginning of development of the PLOCH method was the choice of the five taxonomic groups. As they were ecologically distinct, it was expected that they would bring complementary information, in this case in terms of species richness. This could be tested with the datasets collected in the 80 ponds by examining the correlation between S_{true} of the five groups: a strong correlation between two groups would mean that they bring redundant information. The five groups all appeared significantly correlated (Table 2); however, the correlation values were relatively low (i.e. all $r<0.6$),

Table 2. Correlation matrix for S_{true} among the five selected taxonomic groups (80 ponds). All correlations (r) are significant ($p<0.05$)

	Aquatic plants	Gastropoda	Coleoptera	Odonata	Amphibia
Aquatic plants		0.52	0.45	0.53	0.29
Gastropoda	0.52		0.44	0.50	0.46
Coleoptera	0.45	0.44		0.38	0.40
Odonata	0.53	0.50	0.38		0.56
Amphibia	0.29	0.46	0.40	0.56	
Mean	0.45	0.48	0.42	0.49	0.43

indicating that each group brings different information. The weakest relationship was between aquatic plants and Amphibia ($r = 0.29$), underlining the complementary information brought by these two groups. The most strongly related groups were Odonata and Amphibia ($r = 0.56$). Mean values for comparisons between all groups showed that Coleoptera and Amphibia were the most weakly related groups (mean $r = 0.42$ and 0.43, respectively).

DISCUSSION

Choice of biological indicators

Five taxonomic groups were selected for the PLOCH method. Investigations in this study and on 30 further ponds (Auderset Joye *et al*., 2004; Menetrey *et al*., 2005) demonstrated the efficiency of the five selected taxonomic groups as biotic indicators: each of them brought complementary information, in terms of species richness and also indirectly in terms of ecosystem integrity.

Elsewhere, other assessments of the quality of ponds or of other wetland types have also successfully used plants (Melzer, 1999; Nicolet *et al*., 2004; Williams *et al*., 2004), Odonata or Coleoptera (Foster *et al*., 1989; Painter, 1999; Chovanec and Waringer, 2001; Sahlen and Ekestubbe, 2001; Chovanec *et al*., 2004) or macroinvertebrates (Biggs *et al*., 2000b; Nicolet *et al*., 2004). Nevertheless, studies rarely included more than two to three taxonomic groups. One main problem is evident with two of the groups selected for the PLOCH method: it concerns the limited scientific knowledge and availability of expertise in the taxonomy of Coleoptera and Gastropoda. This makes the method dependent upon specialists who can reliably identify these species. To overcome this problem, which also applies to the study of other freshwater habitats, enhanced efforts must be made in the training of taxonomists. It is essential to promote the organization of training courses in the taxonomy of these groups, and also of other pond inhabitants, for example at the European level.

Other groups than the five selected for the PLOCH method could also be well suited for inclusion in an assessment of pond biodiversity, but many are lacking essential qualities expected for biotic indicators. For example, diatoms can give outstanding ecological information (see Williams *et al*., 1998), but geographical distributions are still largely unknown for most species and their degree of threat is undefined; moreover, they do not represent a 'flagship' group. Birds and mammals have often been selected for assessment of large wetlands and numerous flagship species are observed in pond habitats (e.g. the great bittern *Botaurus stellaris*, the pond bat *Myotis dasycneme*, or the water shrew *Neomys fodiens*). However, these two groups are generally restricted to larger ponds making them of limited use for assessing the quality of smaller water bodies. Other invertebrate taxa, such as Ephemeroptera, Plecoptera and Trichoptera (EPT), are already used as efficient indicators in running waters, and could also bring valuable information in standing waters even if the two first groups are less diversified in ponds. Nevertheless, their sampling would require a specific strategy, as their larvae are not present in the water during the entire year.

Potentials, limitations and further developments of PLOCH

The PLOCH methodology can be applied to management and policy making, providing a useful tool for biological assessment at the local or regional scale (for baseline surveys, monitoring of changes, or environmental impact assessment).

The future user should be aware that the true richness (S_{true}) measured with the PLOCH method corresponds to an index of biodiversity and is different from the richness measured by an inventory. This highlights a human nature issue rather than a scientific problem: the managers of water bodies are often frustrated when they do not receive exhaustive species lists. Here communication skills are required rather than scientific skills, to make clear the necessity and the benefits of a non-exhaustive standardized approach. The objective of the PLOCH method is to give an evaluation of a basic element of biodiversity — species richness. This is the basic information required to carry out management and conservation of biodiversity. Other complementary approaches can then be further investigated, such as the search for rare species with high conservation values; this latter approach is much more costly and requires species inventories. Conservation value must then be assessed for the whole community. A species-rich community can be composed only of common species; conversely, a species-poor community can include many endangered species with high conservation values. As data obtained using the PLOCH approach are semi-quantitative, a useful approach for the manager is to use these standardized values for other assessments; for example, the calculation of diversity indices such as the Margalef index. This approach has been chosen for monitoring the ponds in the Swiss National Park sampled with the PLOCH method (Stoll, 2005).

To test if the species richness of a taxonomic group as measured by PLOCH could be an indicator of its conservation value (score for all species present, according to their degree of rarity; see in Oertli *et al.* (2002)), the correlation between these two variables was calculated for the 80 ponds studied. Correlations were high (r 0.76–0.99) especially for Amphibia (0.99) and also for Gastropoda (0.89) and Odonata (0.87). However, values for aquatic plants (0.76) and Coleoptera (0.80) were at the lower end of the distribution. This implies that species richness could be used as a surrogate for conservation value for Amphibia, and probably also for Odonata and Gastropoda, but should be used with more care for aquatic plants or Coleoptera because, in these cases, a species-poor pond could also include endangered species.

The geographical range of use of the PLOCH method includes the biogeographical regions with species pools similar to those of Switzerland. In other regions with larger or smaller species pools, higher or lower pond species richness might be expected. However, variation in patterns of species richness in ponds among different European biogeographical regions is still unknown, and future research should investigate this important area of knowledge. Altitude will limit use of the PLOCH method, as some of the selected groups (aquatic plants, Gastropoda, Amphibia, and Odonata) have a limited regional species pool at high altitudes (see Hinden *et al.*, 2005). Above 2000 m, these groups should be replaced by other indicators: Bryophyta, Chironomidae or Oligochaeta could be good candidates for this (Hinden, 2004; Stoll, 2005).

Even though the PLOCH method is relatively economical, it might be possible to reduce the cost even further. For example, for Amphibia, the exhaustive inventory could perhaps be replaced by an assessment made with the larvae collected in the net-sampling of the invertebrates. This approach could also be used in alpine ponds for assessing the Odonata and Amphibia, as these two groups will be present as larvae in the water owing to a slower rate of larval development at high altitude; such procedures would economize some of the travel expenditure.

The PLOCH method assesses the quality of a pond in terms of species richness. A future development and improvement of the method would be to expand it with a complementary assessment of the quality of the pond in relation to human impact (evaluation of water and environmental quality). In this regard, the metric approach used for the evaluation of water quality (Karr and Chu, 1999) seems promising and is now in development for ponds (Biggs *et al.*, 2000b; Menetrey *et al.*, 2005).

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APPENDIX 1: MEAN VALUES AND RANGES OF 10 VARIABLES CHARACTERIZING THE 80 PONDS

Variables		Units	Mean	Minimum	Maximum	Median
Morphometry	$\log_{10}$(area)		3.31	0.78	4.98	3.26
	area	m^2	8817	6	94 346	1834
	mean depth	cm	172	26	850	114.5
Physical and chemical variables	water transparency (Snellen tube)	cm	43	4	60	51
	conductivity	$\mu S\,cm^{-1}$	383	6.2	1367	396
	pH class (1: <6.5; 2: >6.5)	class	1.9	1	2	2
	trophic class N[a] (inorganic nitrogen classes, according to Wetzel (1983))	class	—	1	4	2
Coverage by aquatic vegetation	proportion of pond area covered by floating-leaved vegetation	%	30	0	100	19
	proportion of pond area covered by submerged vegetation	%	36	0	100	23
Others	age	years[b]	—	1	>4000	100
	altitude	m a.s.l.	1008	210	2757	733

[a]Trophic class N: 1 = oligotrophic, 2 = mesotrophic, 3 = eutrophic, 4 = hypertrophic.
[b]Five age classes were defined for statistical analyses: 1 (1 to 10 years), 2 (11 to 40 years), 3 (41 to 100 years), 4 (101 to 1000 years), 5 (>1000 years).

APPENDIX 2: LIST OF THE MESOHABITATS TAKEN INTO ACCOUNT FOR THE PLOCH SAMPLING METHOD

Two-thirds of the samples are allocated to the habitats occurring between the shoreline and a depth of 2 m (A); one-third of the samples are allocated to the habitats occurring at the shoreline (land–water interface) (B)

			Examples
A. Habitats occurring between the shoreline and 2 m depth (excluding the land–water interface and the sediments)			
1. Hydrophyte	1.1. Submerged	1.1.1 strongly dissected leaves	*Myriophyllum* sp., *Utricularia* sp., *Ceratophyllum* sp., *Ranunculus* sp.
		1.1.2 thread-like leaves	*Potamogeton pusillus*, *P. pectinatus*, *Zanichellia palustris*
		1.1.3 large entire leaves	*Potamogeton crispus*, *P. lucens*, *P. perfoliatus*
		1.1.4 small entire leaves	*Elodea* sp.
		1.1.5 Characeae	

			Examples
	1.2. Floating leaves	1.2.1 large leaves	Water lilies, *Trappa natans*, *Hydrocharis* sp., *Potamogeton natans*, *Polygonum amphibium*
		1.2.2 small leaves	*Lemna* sp.
	1.3. Moss		
	1.4. Algae		*Cladophora* sp.
	1.5. Other hydrophytes		*Menyanthes trifoliata*
2. Helophyte	2.1. Reed bed		*Phragmites australis*, *Typha* sp.
	2.2. Large-sized *Scirpus*		*Scirpus lacustris*
	2.3. Flooded sedge formations		*Carex elata*
	2.4. Small-sized helophytes		*Alisma* sp., *Equisetum* sp., *Eleocharis* sp., small *Scirpus* sp., *Juncus* sp.
	2.5. Other helophytes		
3. Other habitats	3.1. Leaf litter		
	3.2. Mineral substrate	3.2.1 loose	sand, gravel
	3.3. Other	3.2.2 consolidated	rock, stones
B. Habitats occurring at the shoreline (land–water interface)			
	1. Small-sized helophytes		
	2. Large-sized helophytes		
	3. Roots		
	4. Bare ground		
	5. Mineral substrate		
	6. Accumulations of CPOM (coarse particulate organic matter)		leaf litter
	7. Other		