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# A REVIEW:
# MACROPHYTES IN THE ASSESSMENT OF SPANISH LAKES ECOLOGICAL STATUS UNDER THE WATER FRAMEWORK DIRECTIVE (WFD)

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## Resumen

Basándose en que los procesos de biomonitorización aportan información muy valiosa sobre los impactos en organismos y consecuentemente en el estado ecológico de los ecosistemas, la Directiva Marco del Agua (WFD; Unión Europea, 2000) incorpora el uso de indicadores biológicos y métricas para la evaluación del estado ecológico de las masas de agua. Uno de los elementos biológicos definidos en la Directiva es "Otra flora acuática" incluyendo macrófitos y fitobentos; la composición y abundancia de macrófitos debe ser estimada y evaluada en los lagos definidos como masas de agua dentro de la WFD.

Los macrófitos tienen muchas de las características destacadas para ser un buen bioindicador; responden a fluctuaciones naturales de los regímenes hidrológicos, situación muy habitual en países mediterráneos, y además, podrían detectar cambios atribuibles a presiones e impactos antropogénicos. Existen dos tipos principales de evaluación basados en el uso de las comunidades de macrófitos; índices de diversidad (englobando diferentes variables) e índices basados en el estado trófico de las aguas. En la práctica, ambos sistemas convergen para la evaluación de estado ecológico en el sistema; sin embargo los índices tróficos no son capaces de desvelar suficiente información sobre otro tipo de perturbaciones, como por ejemplo cambios en el uso del suelo y cambios hidromorfológicos (HM). Los helófitos parecen ser una parte crucial de la evaluación y medición de impactos HM.

En este trabajo se realiza una recopilación de información y una evaluación del contexto actual sobre el uso de macrófitos como bioindicadores del estado ecológico de los lagos. Concretamente, los principales objetivos se centran en (1) el estudio de las presiones y (2) requerimientos de los lagos de la Península Ibérica y (3) las metodologías de evaluación más eficaces para su monitorización, ya que actualmente aun no existe ningún protocolo de muestreo oficial a nivel estatal.

**Palabras clave:** macrófitos, Directiva Marco del Agua, lagos españoles, métricas biológicas, estado ecológico

Abstract

Biomonitoring provides essential information on the impact of disturbances on living organisms and consequently on ecosystem health. Based on this assumption, the Water Framework Directive (WFD; DOCE, 2000) introduces the concept of biological indicators in the assessment of water bodies' ecological status. One of the Biological Quality Element defined in the Directive is named as "Other aquatic flora" which includes macrophyte communities and phytobentos; therefore, Composition and abundance of macrophyte should be assessed.

Macrophytes fit very well to many of the criteria listed for an "ideal" organism for water biomonitoring. Moreover, in Mediterranean countries, where hydrological regime strongly changes seasonally, macrophytes respond to natural fluctuations of the water level, but also detect abnormal variations that are caused by anthropogenic impacts and pressures.

There are two main types of assessment based on macrophyte communities: diversity indices (involves different variables) and trophic indices. In practice, both approaches converge and work properly together to assess eutrophication conditions in the system. However, trophic indices are not able to reveal enough information to assess other kind of stressors such land use and hydromorphological pressures. Helophytes seem to be a key part of HM impacts assessment by measuring the helophyte parameters and changes in their composition and abundance.

The work was aimed to the compilation and evaluation of the context in the use of macrophytes as bioindicator of lakes ecological status. The main objectives are the study of the disturbances and requirements of Spanish lakes and the suitability of ecological assessment methods for the monitoring of the ecological status, since, at the present, there is not an official national macrophyte sampling protocol.

Keywords: Macrophytes, Water Framework Directive, Spanish lakes, biological metrics, ecological status

## 1. INTRODUCTION

The meaning of Aquatic Macrophytes should include, in an easy way, all the water plants. However, the term "Aquatic macrophyte" is not yet determined in a proper and homogenous way.

Water plants can be grouped in three identified assemblages (Margalef, 1983):

(1) Helophytes: Water plants with roots in the sediment but with the majority of leaves and stem above ground. *Phragmites australis, Juncus spp* and *Typha spp* are some of the most representative helophytes presented in Spanish lakes.

(2) Amphyphytes: Water plants that are partly submerged, with some parts of the plant floating in the water (normally leaves floating). *Nuphar, Nymphaea* and *Ranunculus sp*.

(3) Limnophytes: Rooted plants with all the vegetative part submerged and only the flowers, if any, floating. *Potamogeton, Myriophyllum, Ruppia, Ceratophyllum* and *Najas* are some of the most representative species.

A strict definition of macrophytes only includes "true" or vascular hydrophyte (amphyphytes and limnophytes), however according to questionnaire for European macrophyte experts in 2009, most countries collect also helophyte information as macrophyte communities. Moreover, other experts have also included cyanobacteria, chlorophyta, xantophyta and rhodophyta as division of aquatic macrophytes (Chambers et al 2009).

In this review, Aquatic macrophytes is to be interpreted as all charophyta, bryophyta, pteridophyta and spermatophyta whose photosynthetically active parts are permanently or, at least, for several months each year, submerged in freshwater or floating on the water surface. Therefore, helophytes, amphyphytes and limnophytes but no algae other than charophytes are included in the definition of macrophytes used in this manuscript.

### 1.1. Macrophytes as bioindicators

Freshwater macrophytes play a very important role in aquatic ecosystems (Nurminen,, 2003). They provide, either directly or indirectly, food, shelter and a variety of habitats for a large number of organisms (Cook, 1974). Moreover, macrophytes are well known to play a significant multidimensional role in lakes (Burks et al., 2006). Littoral flora provide excellent habitats for photosynthetic and heterotrophic microbiota (Wetzel, 2001), while submerged macrophytes support a complex trophic web, very different and much more complex than a wetland without macrophyte species (Carpenter and Lodge, 1986). The main features determining the macrophyte appearance, apart from light availability, are oxygen, nutrient supply and salinity (Cirujano 2002). Having a major role in freshwater ecosystems, macrophytes may directly act as indicator of lake functioning. Both, individual species and entire types of plant communities can be used as indicators of the state of freshwater ecosystems (SEPA, 2000).

Macrophytes fulfil very well many of the criteria listed for "ideal" biomonitor organisms (Table 1). Additionally, as pollution from chemicals may be transient and unpredictable, biological monitoring often appears to be more appropriate to assess aquatic ecosystems contamination than traditional chemical evaluation of water quality (Barbour et al., 1996), macrophytes are especially good indicators in continuous, long-term monitoring.

*Table 1: Some advantages and drawbacks of using macrophytes as bioindicator of ecological status*

| Advantages | Drawbacks |
|---|---|
| They are immotile, visible to naked eye and relatively easy to collect and to handle | The term macrophyte is unclear, full scientific comparison of different national datasets is difficult. Even in Spain the terminologies used are differently used by different authors. |
| Quite easy to identify in the field due to the low number of species compared with other organisms (ie, diatoms) | Only some research about the use of macrophyte for fassessing hydromorphological disturbances |
| Some species concentrate metals and nutrients in their tissues and reflect environmental pollution | Some macrophytes are difficult to identify (ie, characeae) at the genus level. |
| They have shown changes in diversity and composition structure due to chemical changes | In many cases, there is not an official sampling and monitoring protocol to be used (sets of field methodology) |
| They have shown to have different abundance and distribution due to hydrological changes in the system | Lack of information. There are a poor or no existent data about macrophyte communities related to each type of water body, at least for most cases. |
| They provide long term information due to their long live stages | Some Spanish lakes, such as some mountain lakes, would not be suitable to be assessed by macrophyte due to the natural conditions (few or none macrophytes in high altitude lakes) |

### 1.2. The role of macrophytes in the Water Framework Directive

The main goal of the Water Framework Directive (WFD; DOCE, 2000; National law 62/2003) is "to prevent further deterioration, protect, and enhance the status of aquatic ecosystems and, with regard to their need for water, terrestrial ecosystems and wetlands that directly depend on the aquatic ecosystems by the year 2015" (Art1. 2000/60/EC). Based on the assumption that biological monitoring provides fundamental information on the impact of chemical and/or physical perturbations on living organisms and consequently on ecosystem health (Johnson et al., 1993), the WFD introduces the idea of using biological indicators to assess ecological status of water bodies. In this context, ecological status is defined as "An expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V" (Art2, definitions, 21, WFD).

One of the biological quality elements to be used is called "aquatic flora", which is defined by WFD as the macrophytes community and phytobentos. Species richness and abundance should be monitored in all the water bodies by the Member States (Annex V WFD). Assessments have to be reported and five status classes (high, good, moderate, poor, bad) have to be set. 'High status' is defined as the biological, chemical and morphological conditions associated with no or very low human pressure.

Spain has a great variety of freshwater ecosystems and some of its water bodies support valuable, rare or/and endangered plant species (i.e.; *Ruppia drepanensis* and *Lamprothamnium papulosum* in salt lakes). Nevertheless, the decline of freshwater macrophytes in Spain is going on as result, largely, of anthropogenic activities and inappropriate land use management plans (Cirujano.,1997).

The incorporation of the WFD into the Spanish law system should lend a hand to stop wetland deterioration and to improve and conserve high ecological status of freshwater bodies. Spain should use these guidelines and definitions to establish lakes typologies, to define references conditions and, in last term, to create monitoring programmes to assess macrophytes.

### 1.3. Aims of this review

Our work was aimed to the compilation and evaluation of the context in the use of macrophytes as bioindicator of l ecological status of Spanish lakes. The main goals are:

- To identify and evaluate the main disturbances affecting Spanish lakes' ecological status
- To make a compilation of the macrophyte metrics that can be used in the assessment process
- To identify the best macrophyte metrics to assess ecological status for Spanish lakes and to define a sampling protocol.

Physical-chemical and biological data used to elaborate this review were gently provided by the Agencia Vasca del Agua, Biological Data Bases of Inland Waters and Wetlands (MARM, Ministerio de Medio Ambiente, y Medio Rural y Marino), Ramsar sites information service, and River Basin Administrations of Ebro, Cantábrico, Duero, Guadiana and Júcar Rivers (see reference section).

## 2. SPANISH LAKES TYPOLOGIES UNDER THE WATER FRAMEWORK DIRECTIVE

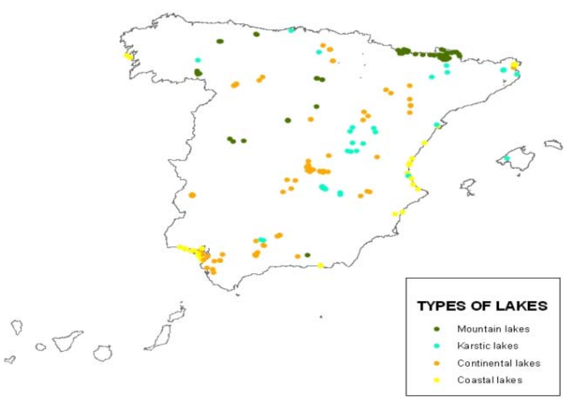
Spain shows a great diversity of aquatic ecosystems due to their natural fluctuations and environmental features. Furthermore, Spain is the country with more diverse lentic freshwater ecosystems in Europe. By 2008 around 300 lakes were already declared as water bodies under the Water Framework Directive, being the greatest number of lakes under the Mediterranean intercalibration group (where Italy included 20 and France 2 water bodies).

Different European projects (SWALE, ECOFRAME and BIOMAN) suggest that Mediterranean lakes work in a different way compared to the rest of European lakes. Some of the main differences are the fluctuation of the water layers, the size and the isolation (Bécares et al 2004) as well as the degree of salinity. Spanish freshwater bodies are very diverse due to a variability of environmental features among the country. However, they can be clustered in 4 big groups (Casado and Montes, 1995)

(1) High and medium Mountain lakes
(2) Karstic lakes on limestone and on evaporitic stone
(3) Continental lakes
(4) Coastal lakes.

For a better comprehension of the context, a map with the 4 main lakes typologies is enclosed (figure 1). This is a general approximation but this classification is not used for the WFD application, which is more specific.

***Figure 1:*** *Map with the location of Spanish lakes and main lakes' typologies (Ruiz, 2009)*



Members' states should define a list of water bodies and typologies taking into account the parameters set up in the WFD. According to WFD parameters, only lakes larger than 50 ha must, compulsorily, be identified as a water body. However, some countries, like Spain, have also considered smaller lakes. With a great lakes' diversity, a challenge is the compilation and aggregation in typologies.

WFD defines two possible ways to characterise water bodies; System A and System B. Using System A in Spain, almost no lakes and no wetlands would be considered and correctly segregated. This System proposes a lakes typology base on lake sizes, mostly bear in mind Central European water bodies. In addition, the system does not take into account important environmental parameters for Spanish water bodies such hydro period, salinity and water level fluctuation. For

these reasons, Spain adopted The official Spanish Lakes Typology follows "System B" being the most correlated to Spanish lakes features. (MARM, 2008), where different environmental variables were taken into account in the classification process (humidity index, temporality, conductivity, alkalinity, inflow regime, max. depth, size, altitude and lake origin). Only Spanish lakes which accomplish the following criteria* have been considered "water bodies" under the Water Framework Directive (table 2):

***Table 2:*** *Spanish lakes Typology and number of lakes identified in WFD, MIMAM, 2008*

| Type | Description | Nº water bodies |
|---|---|---|
| 1 | High mountain, deep, acid water | 66 |
| 2 | High mountain, deep, alkaline waters | 5 |
| 3 | High mountain, little deep, acid waters | 17 |
| 4 | High mountain, little deep, alkaline waters | 5 |
| 5 | High mountain, temporal | 2 |
| 6 | Mid mountain, deep, acid waters | 1 |
| 7 | Mid mountain, deep, alkaline waters | 1 |
| 8 | Mid mountain, little deep, alkaline waters | 1 |
| 9 | High mountain, southern, | 1 |
| 10 | Karstic, limestone rocks , feed by groundwater | 9 |
| 11 | Karstic, limestone rocks , feed by groundwater, spring type | 6 |
| 12 | Karstic, limestone rocks , mixed feeding | 17 |
| 13 | Karstic, limestone rocks , temporal | 1 |
| 14 | Karstic, evaporitic, large | 1 |
| 15 | Karstic, evaporitic, small | 10 |
| 16 | Continental, oligosaline, permanent | 2 |
| 17 | Continental, oligosaline, temporal | 11 |
| 18 | Continenral, subsaline, permanent | 2 |
| 19 | Continental, subsaline, temporal | 8 |
| 20 | Continental, hyposaline or mesosaline, permanent | 5 |
| 21 | Continental, hyposaline or mesosaline, temporal | 28 |
| 22 | Continental, hypersaline, permanent | 1 |
| 23 | Continental, hypersaline, temporal | 11 |
| 24 | Continental, fluvial origin, flood plain, oligosaline or subsaline | 3 |
| 25 | Continental, fluvial origin, flood plain, hiposaline or mesosaline | 6 |
| 26 | Continental, fluvial origin, abandoned meander | 3 |
| 27 | Continental, associated to alkaline peat moss | 1 |
| 28 | Coastal lakes without the influence of seawaters | 11 |
| 29 | Coastal lakes developed on dunes, permanent | 8 |
| 30 | Coastal lakes developed on dunes, temporal | 6 |

*The criteria involves Morphometric criteria: (size> 50 ha, or size > 8 ha and max depth > 3 m) and Environmentally meaningful: it includes also all the lakes and wetlands which are designed as RAMSAR sites.

The assignment of macrophytes species communities related to the different typologies is essential for the evaluation of disturbances and the ecological status in water bodies.

## 3. RECOGNICING THE MACROPHYTE COMMUNITIES ASSOCIATE TO THESE TYPOLOGIES

Latitude and mean temperatures are some of the factors mainly defining patterns in macrophyte richness and composition; moreover other environmental features are also affecting to regional patterns such specific physical factors, altitude and topsoil (Chambers, 2009). Spanish lakes are grouped into typologies (from T1-T30) depending on: Humidity Index, Temporality, Conductivity, Alkalinity, inflow regime, Max. Depth, Size, Altitude and Lake Origin (MIMAM, 2008). It is likely that there is a link between Lake WDF types and plant communities. However, there are not any available resources in Spain that compile this information. Autonomous regions seem to make their own lake classification and assessment protocols. In addition, for the official WFD ecological assessments it is crucial to set up reference condition in each type of lake in order to measure the deviation from the goal. Due to the lack of information, it was not possible to make definitive conclusions from a preliminary review.

Nevertheless, a primary statistical analysis with collected information from different resources about the appearances of plant species in Spanish lakes and type's typologies is done here. The main issue of this statistical approach is (1) to classify Spanish lakes attending the presence/absence of certain macrophytes species and (2) to determinate specific assemblages groups of macrophytes. The Environment Ministry Data Base is still under revision so only data * from some lakes were available. Analysing species composition and biomass data should be the best method to perform the study; however, the lack of information and the time factor make it impracticable, since data on presence/absence of macrophytes species are the only data.

Cluster analysis (CA) is a multivariate statistical method whose main purpose is to develop meaningful aggregations, or groups, of entities based on a large number of interdependent variables. We have a total of 102 different lakes (cases) from 24 WFD typologies and 179 macrophytes species (72 hydrophytes and 107 helophytes) (variables). No all the lakes have hydrophytes and helophytes information and, therefore 94 and 73 cases were run out in the system.

## Result and discussion

### 1. Clusters Analysis (CA)

Hydrophytes: The programme grouped 94 lakes in 10 clusters. The cases were added to the SPSS separately, which means no previous typologies or groups. Cluster 1 is the major group because it has 85 lakes from very different WFD typologies. For some of them the number of hydrophytes species could be too low to make significant differences among water bodies.

*Figure 2: Hydrophyte CA DendogramE*

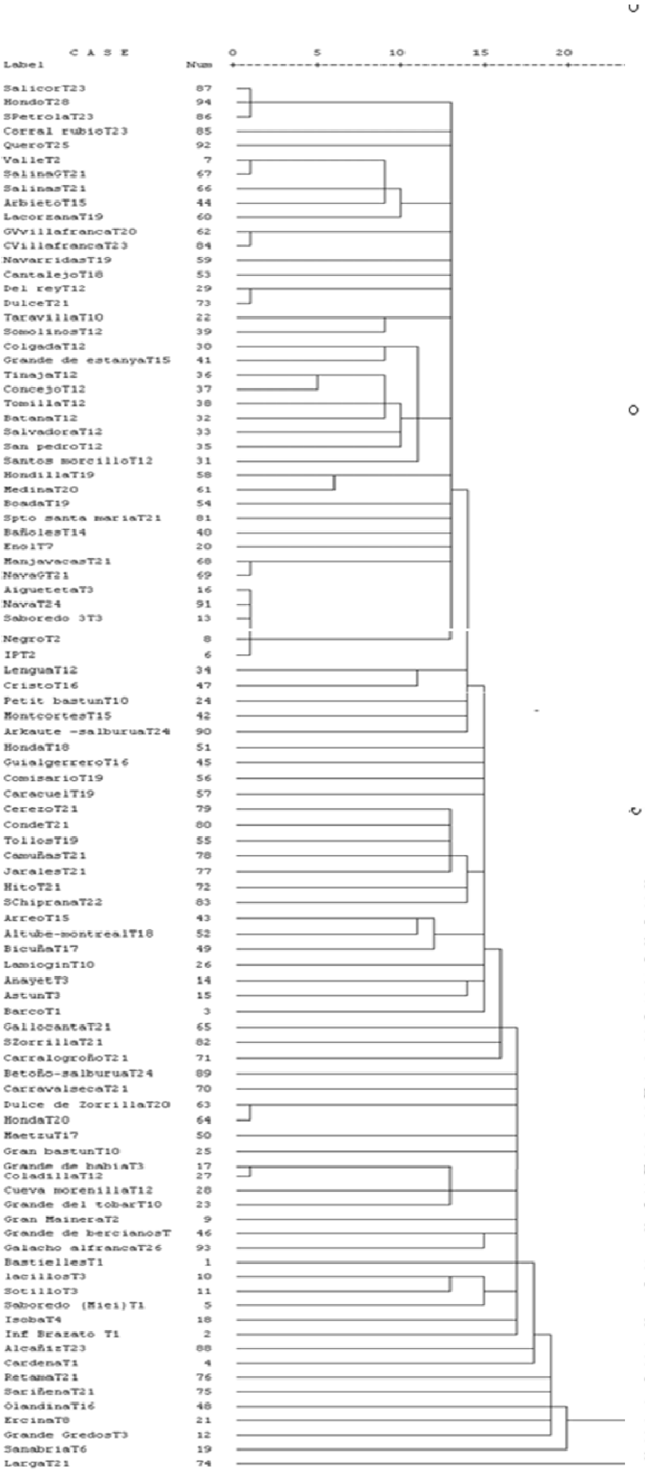
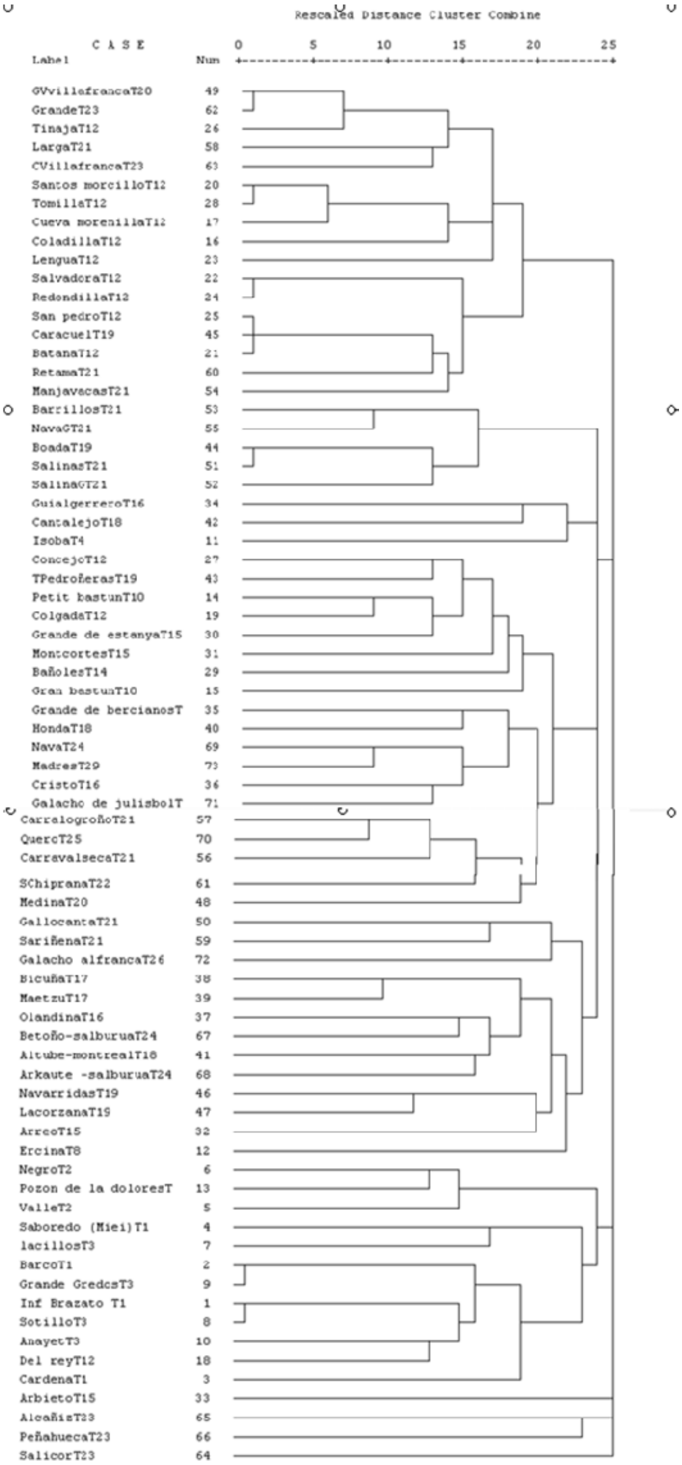



*Figure 3: Helophytes CA Dendograme*



Nevertheless, the dendrograme (figure 2) shows small groups of lakes that have a tendency to share hydrophyte communities. The cluster analysis groups together Lakes Ip T2, Lago Negro T2, Saboredo T3 and Aigueta T3 which make sense, since they are all mountain lakes and they may share hydrophyte communities. Dulce de Zorrilla T20 and Honda T 20 reflect also a tendency to be grouped. On the other hand, some water bodies appear to have very specific hydrophyte communities and they are grouped in separately clusters; Cardena T1, Grande de Gredos T3, Sanabria T 6, Ercina T5, Olandina T 16, Laguna Larga T21, Sariñena T21, Retama T21 and Alcañiz, T 23. Unexpected results show lakes from the same WFD typology bunch in different clusters.

**Helophytes** The clustering grouped 73 lakes in 10 clusters attending 107 helophytes species (figure 3). High-medium Mountain lakes are clustered in two groups together Cluster1 and Cluster 2, most likely due to the low number of helophyte species presented in these kinds of water bodies. Cluster 3, Cluster 4 and Cluster 5 appear to be a mixture of different WFD lakes typologies. On the other hand, Cluster 6 put into group all lakes from T12 with some lakes from T19-T20 and T21. Arbieto and Salicor lakes are separated alone in Cluster 7 and Cluster 9 respectively, showing specific helophyte communities and presumably different from the rest of the Spanish lakes included in this survey. Lakes of T21 are grouped all together in Cluster 8. Alcañiz and Peñahueca, both classified as T23 lakes, are put in together in Cluster 10. The dendogram shows a tendency of differences among lakes attending to the presence of helophytes (figure 3). Helophytes appear to classify Spanish lakes in smaller clusters than hydrophytes. However, it can be due to the fact that higher number of helophytes is introduced in the programme compared to hydrophytes data. Since cluster analysis is a descriptive method, therefore, the results give information of how lakes group depending on the presence of certain macrophyte species but it can not be established as testing process.

Despite the fact that some results show a tendency of differences among lakes attending the presence of helophytes, Macrophyte groups related to lakes typologies couldn't be defined properly with cluster analysis technique. Therefore, Jaccard Similarities matrixes with the macrophytes species data were run out with the purpose of reaching more accurate information. The similarities among species are there reflected in a value from 0 to 1. Values of 1 mean that species A always appear with species B; while values of 0 mean that there is no relation between species A and B appearance. We used a treshold of 0.6 (60%) as enough significant value to show relative similarities among macrophytes.

## 2. Similarity Matrix

**Hydrophytes**: The results show a tendency showing that, in Spanish lakes, *Callitriche lusitanica, Isoetes velatum and Fontinalis (antypiretica and angustifolium*) appear together in more than 70% of the cases. This could mean that they share similar environmental preferences and make assemblages. *Ceratophyllum demersun and Ceratophyllum submersun* seems to appear in relation with *Chara delicatula and Leptodictyum riparium. Potamogeon pussilus* matches with *Potamogeton lucens* and *Zannichellia pedunculata.*. Remarkably, *Chara galioides* show more affinity with *Chara canescens* than with other of *Chara* species. On the other hand, *Chara major* coincides in most cases with *Chara pedunculata.* This enhances the need of classifying charaophytes to the until species level since different species grow under very different environmental conditions.

Helophytes: Our results show a tendency of higher affinity among some helophytes species than in the case of hydrophytes. The number of species that appear in more than 0.6 (60%) of occurrence with other species is very high. Moreover, the number of matches does not make possible to define specific assemblages. Nevertheless some data information can be extracted from these results: *Agrostis stolonifera, Arundo donax and Arundo plinii* matched at 1.0 (100%) of occurrence with a high number of other helophyte species such *Phragmites australis, Carex distans, Crypsis schoenoides* and *Juncus bulbosus.* On the other hand *Baldellia ranunculoides* appeared only with *Carex ovalix* and *Carex riparia.* Other remarkable result is the fact that, as it was shown before in the case *Chara spp,* different Species of *Carex* show different affinities. *Carex nigra* appear with a 1.0 (100%) occurrence with *Carex ovalix* and *Carex hirta*.

## 4 RECOGNIZING MAIN IMPACTS AFFECTING SPANISH LAKES

Freshwaters of the world are collectively experiencing markedly accelerating rates of degradation (Wetzel, 2001). In Spain, anthropogenic activities and inadequate land use management plans increase the degradation, producing a great negative impact on Spanish wetlands, which are decreasing dramatically in number and quality (Casado and Montes, 1995; Cirujano, 1997). The assessment of ecological integrity requires the assessment of three principal elements: physical, biological and chemical Integrity (Barbour et, al; 2000).

An ideal biological quality element should respond to all the impacts in the ecosystem, however, it is possible that an ideal biological element doesn't exist for all the situations and, therefore a broad range of biological communities should be assessed in order to state a more reliable, contrast and real judgment. Pressures affecting chemical, physical and biological integrity of Spanish lakes are resumed in table 3a and 3b. Impacts such acidity processes are not included, since, although it is a great impact in other places of the world, such as Scandinavian lakes, it is lacking in interest for Spanish water bodies.

Hydrological impacts; In addition to nutrient availability and associated trophic status, the functioning of lacustrine ecosystems is controlled by the quantity and periodicity of the water resources supply, independently of lake size, depth, basin origin and climate (Coops, et al 2003). Based on Water Framework Directive, HM impacts affecting lakes are mainly those related to:

(1) Hydrological regime: water body volume, connexion with underground waters residence time.

(2) Morphological aspects: depth, quantity, structure and type of substrate in the lake bed and structure of littoral zone.

Water-level fluctuations (WLFs) emerge as the decisive element of hydrology especially in shallow lakes embedded in wetlands, (Coops et al 2003) where WLF play an important role in the aquatic-terrestrial interface processes (Leira and Cantonati 2009) making a positive contribution to the diversity and conservation value of shoreline vegetation (Schneider, 2007). Anthropogenic factors,like global climatic change and human water use may strongly alter the amplitude of hydrological regimes, whereby it becomes far higher or lower than natural. Extreme fluctuations reduce plant cover and impoverished communities (Smith et al, 1987, Hawes et al, 2003). For this reason, it is crucial and a big challenge for limnologists to simulate natural variations patterns in order to be able to asses the true human impacts, thus ,minimizing possible mistakes. So far, no report or essays have been done to establish natural variation parameters and macrophyte communities in Spanish lakes, although it should be the first step in reference values set up process.

Morphological changes in catchment areas are also considered great impacts no only on morphological aspects, but affecting the amount of water and nutrients entering the ecosystem. Areas close to the lake littoral zone changed to agriculture lands, decreasing the natural vegetation covertures and increasing nutrient loads.

Biotic Impacts include pressures from biotic elements. Apparently, biotic pressures have clear relationships with chemical and HM pressure s since they work together for ecosystem integrity.

(1) Invasive species introduction. In lakes and wetlands is a clear example of biotic impacts. As an example, the introduction of the American crayfish (*Procambarus clarkii)* in 1997 in the wetland Chozas de Arriba (León) made a change in the water properties from clear waters to a eutrophic status and it caused the reduction of 99% of water plants (Rodriguez- Villafañe et,al., ). Macrophytes can also act as invasive species causing the reduction of other species covertures. At the moment, some aquatic plants have been identified as invasive species in Spanish water bodies; such as *Azolla filiculoides, Eichhornia crassipes, Egeria densa, Elodia canadiensis* and *Ludwigia sp.*

(2) Birds and other herbivorous (cattle): There are studies reflecting the decrease of macrophyte biomass due to effects of birds and other herbivorous over aquatic plants. Herbivorous consume macrophytes that are easily assimilated by their metabolisms, producing a change in macrophyte composition (Rodriguez- et al..,). For instance, Sentiz Wetland showed a change in macrophyte composition due to high bird population feeding in the ecosystem; however, it did not show a significant change in covertures. On the other hand, Villafafila wetland shows a dramatically decrease of macrophyte covertures (Rodriguez et al.,) due to increase of bird population. Vikuña Lake and Lake of Maeztu, in the Basque Country, have been identified as impacted water bodies mainly due to the pressure of cattle practices which release nutrient in the system and changes the macrophyte diversity, mostly helophyte communities. Birds and other herbivorous sometimes maintain a balance in the food web and among ecosystems and they increase ecosystem diversity. However, when the number of herbivorous rises dramatically, the natural balance is broken causing

important impacts on the aquatic ecosystem. To identify and to set up limits and management plans is a crucial and risky dare for environmental managements in Spain.

*Table 3 a: Main pressures affecting Spanish lakes, biological elements used in the ecological assessment process and macrophytyte responses. Anexos III Main pressures affecting Spanish lakes, biological elements used in the ecological assessment of these processes and macrophytyte responses.*

| Pressure Type | Pressure indicator | Biological Quality elements | Macrophyte responses |
|---|---|---|---|
| Hydro Morphological<br>1.Morphological changes | 1.Littoral habitat alteration, changes in morphological features: depth and slope | 1.Macrophytes (mainly helophytes), benthic invertebrates | 1.Changes in the macrophyte covertures (mainly, decreasing) and species composition due to changes in their distribution patterns.<br>Changes in macrophyte community |
| 2.Inflows/outflows changes. Drainage of underground waters. Aquifers overexploitation | 2.Water level variation, changes in morphological features: slope | 2.Macrophytes, benthic invertebrates,phytoplankton, fish | 2.Cover change, species diversity variation (sensitive, tolerant and indifferent species). Changes in macrophyte community, Special attention in helophytes species. |
| 3.Hydroperiod alteration (temporal-permanent Systems). | 3.Frequency and water volume over the time | 3. Macrophytes, benthic Invertebrates, phytoplankton, fish | 3. Species composition changes. Special attention to helophyte species. Macrophyte abundance could also changes. |
| Biotic pressures<br>1.Invasive species | 1.Number and invasion potency of exotic species | 1.Macroinvertebrates Phytobento- plankton and Macrophytes | 1.Cover decreasing and/or disappearing of natural, typical macrophytes species |
| 2.Herviborous (cows and birds) | 2.Cows feed ratios. Birds population and feed ratios over the time. | 2.Macrophytes | 2.Change in macrophyte cover and species diversity (mainly Helophytes) . Decrease of most assimilated species |

*Table 3 b: Main pressures affecting Spanish lakes, biological elements used in the ecological assessment process and macrophyte responses.*

| Pressure Type | Pressure indicator | Biological Quality elements | Macrophyte responses |
|---|---|---|---|
| Chemical pressures<br>1.Organic enrichment<br>(mainly pesticides) | 1.Organic compounds concentrations | 1.Macroinvertebrates | 1. Macrophyte cover decreased. Change in community, disappearing more sensitive to pollutants species. However, macrophyte is not identified as main BQE. |
| 2.Nutrient enrichment | 2.Phosporous and Nitrogen concentrations | 2.Phytobentos, Phytoplakton Macrophytes | 2. Increase of covertures and Diversity in the beginning of the eutrophication process; then, a dramatically decrease. Increase of tolerant species and decrease of sensitive ones. Trophic Ranking Scores based on sensitive and tolerance species. Decreasing deepest macrophyte colonization area (only deep lakes). |
| 3. Pollutans charges | 3.Pollutant concentration in water ecosystem | 3.Benthic-invertebrates Macrophytes , | 3.Accumulation of pollutant in macrophytes (leafs, steam and roots ) |
| 4.Water inflows with different mineralogical characteristics | 4. Conductivity | 4.Macrophytes, benthic Invertebrates, phytoplankton, fish | 4. Macrophyte composition changes, from species adapted to a specific mineralogical characteristic to another community with different requirements |

**Chemical impacts:** External chemical compounds entering aquatic ecosystems may come from agriculture-cattle, farming practices, industrial activities and human wastes. Despite dispersion and dilution processes, bioconcentration of these substances is common, increasing toxicity exponentially (Wetzel, 2001). In Spain, a big percent of land extension also including shallow wetlands, are used as high productive agriculture fields (Casado and Montes, 1995), moreover, in Spain, around 257.595 Km$^2$ are defined as agricultural land over 505.990 Km$^2$ total Spanish land area.

Overflow of nutrients, especially phosphorus and nitrogen from urban runoff and municipal point sources lead to eutrophication (Ongley, 1996). An example is Laguna del Pueblo (Ciudad Real) where phosphorus concentration by the year 1997 was 1,15 mg P/L (Martin, 1994; Vicente et. al 1998), whereas lakes are considered as hypertrophic systems above 0.1mg P/L (Álvarez, et. al., 1991; Verdugo, 1995). Some macrophytes, such as *Lemna gibba*, are good indicadors of eutrophic waters as they live and grow in water with high nutrient loads (Cirujano, 2001) i.e; in Spain *Lemna gibba* appear in Laguna de Caracuel and Laguna de Pedro Muñoz ( Cirujano, 2002) as an example of eutrophic wetlands where this process is due to human activity.

Apart from HM, biotic impacts and chemical pressures, the pressures derived from recreational uses such as fishing, sailing and bathing should be also reflected and assessed since they can alter the chemical properties and in some cases the morphological features.

## 5 SOME EXAMPLES OF MACROPHYTE'S METRICS

A Biotic index is a scale to illustrate the quality of an environment by indicating the types of organisms it holds. Nowadays, the use of biotic indices and metrics is widespread to assess the ecological quality of rivers and lakes over the world. Following WFD, the composition and abundance of other aquatic flora (macrophyte and phytobentos) should be assessed in Spanish lakes. These methodologies must evaluate pressures affecting the aquatic ecosystems. However, it should be remarked that classification and ecological assessment of ecological status using macrophyte's reference sites is restricted to those with enough "natural" macrophyte cover, and therefore, if natural reasons for low macrophyte abundance can not be excluded, a classification based on macophyte is not possible (U.S. EPA. 2002).

Trophic indices provide information about the correlation between presence/absence of species and nutrient load, reflecting trophic condition (e-g; Trophic Index of Macrophytes, Scheneider and Melzer, 2003). Total phosphorus content (TP) in the water correlates very well with the trophic status of aquatic ecosystems (Seele, et al, 2000) since it is generally considered to be more limiting. At low P concentrations, the macrophyte community is likely to be composed of some species sensitive to P enrichment and other which are more tolerant. However, communities living in high P concentrations are likely to be dominated by tolerant or cosmopolitan low scoring species, being the intermediate situation between low and high P concentration the best for highest biodiversity. This situation is reflected in trophic indices but not in diversity indices (Thiebaut, *et al.*, 2002). For this reason diversity should be used to support the interpretation of trophic indices results (Dawson, F. et al., 2000).

Diversity indices; Macrophyte biodiversity depends on the size and on other physical characteristics of the studied site (Thiebaut, et al, 2002). Macrophye diversity of a lake can be compared with the diversity in reference conditions in order to assess the deviation from a natural condition..

Species abundance and overall biomass of submerged flora in eutrophic conditions, is mainly restructured to rather few low light tolerant species (e.g *Ceratophyllum demersum*) which form highly dominant populations….

Functional traits of species provide a useful context to investigate relationships between vegetation and environmental parameters (Abrahams, 2008). Moreover, different macrophyte life forms require nutrients from different sources and vary its tolerance to pollutants (Toivonen and Huttunen 1995). Macrophyte indices may classify aquatic macrophytes depending on their life forms (Emergent/Submerged/Floating-leaved/Pleustophyte) and ranking them from sensitive to tolerant. Rooted submerged macrophytes seem to be more sensitive to eutrophication conditions due to the light limitation promoted by the shadow effect of the increased phytoplankton biomass. On the other hand, floating macrophytes tend to develop when nutrient load increases, as they can avoid these shadow effects by having floating leaves.

As light attenuation and depth may be the most important factors explaining submerged vegetation abundance, a metric using the maximum colonization depth is usually described to light transparency in the water column and the minimum light requirements for growth (Chambers and Kalff 1985; Smith and Wallsten 1986; Blindow 1992).

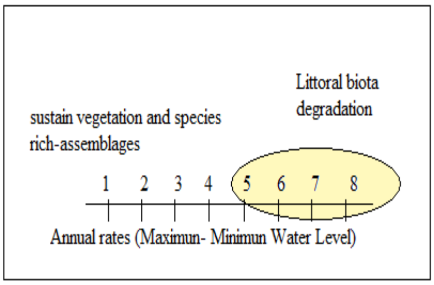
Biotic metrics are typically interpreted with respect to the expected natural status to evaluate whether a site is degraded or not. It is critical that the natural variation in biotic metrics along environmental gradients is adequately addressed, in order to quantify human disturbance induced changes. Multimetric indices combine indicators, or metrics, into a single index value. Each metric is tested and calibrated to a scale and transformed into a unit less score prior to being aggregated into a multi-metric index. Indicators such leaf N, P, overground biomass and tolerant species are included in this assessment methodology (U.S. EPA. 2002).

Macrophytes indices assessing HM impacts.

Phytoplankton and fitobentos communities together are probably the best indicator of eutrophication. (Carvalho et al, 2006). However, these communities poorly reflect hydromorphological (HM) impacts and other biological elements should be used to address them. A number of studies have been identified water level fluctuations as the key component of disturbance in terms of its influence on littoral vegetation dynamics (Gill, 1971; Nilson, 1981; Keddy, 2000). Many authors have identified an urgent requirement for continued research into the relationship between water level fluctuations and littoral vegetation (Levine, 1990, Merritt, 1994). Community composition is, consequently, a crucial information. Some evidences show that water-level fluctuations (WLF) may change macrophyte composition and species abundances., There is a trend for decreasing number of species with increasing WLF. In Pyrenean lakes, the area occupied by submersed vegetation as well as the numbers of species tend to decrease due to dam building. (Gacia,1998). Total number of sensitive large *Isoetes* may decrease when water level draw-down increasing (Hellsten, 2009). On the other hand, some species tend to increase with WLF increasing; e-g *Phragmites australis, Myriophyllum alterniflorum and Potamogeton alpinus,* while others are indifferent such *Sparganium natans* and *Potamogeton natans* (Hellsten, 2009). Total number of *Isoetes* indicates water level fluctuation only in the types of soft water lakes that hold these macrophytes and, therefore, it may only be and useful tool in oligotrophic Spanish mountain lakes.

Creating a scale of disturbance where the amplitude (by year or month) of disturbance is measured can help in the identification of ecosystems changes and water body ecological status (figure 4). Spanish wetlands are exposed to great natural Water Level Fluctuation due to annual climate variations. Water levels in natural and no regulated lakes are not often monitored and this makes very difficult to create a scale and to compare between reference values, as comparative data are largely missing.

***Figure 4***: *Scale to evaluate ecological effects of WLF (meters) (Ruiz., 2009)*




The magnitude of drawdown can be selected as the first water level indicator in hydrological status assessment, because it may explains species richness and abundance of aquatic macrophtes (Keto et al, 2006). In Spanish lakes, the drawdown should be assessed in spring and summer coinciding with the dry and hot seasons. However, studies advise that taxonomy shouldn't be used as the only indicator of water level impacts and morphological perturbations because abundance can also change associated to water level fuctuations, and could give valuable information about ecosystem quality (Nilsson 1988; Hellsten et al 1996).

## 6 DESCRIBING SOME METRICS THAT COULD BE USED IN SPANISH LAKES

Due to the high variability on freshwater ecosystems, metrics should be defined very carefully. At present, there are some metrics that have been used or proposed by different water Authorities in Spain (table 4a,b), though they are not yet official. Not only the presence and absence of macrophyte species should be recorded, but also abundance. Abundance can be measured, for instance, following a numerical scale from 0 to 5; however, features from the different values must be very well established. Moreover, dominant, reference macrophytes' communities have to be defined for each typology. However, the typologies (MIMAM, 2008) are defined according to system B following the WFD, which do not directly consider biotic features and, therefore, macrophyte communities could coincide among different lake types. Additionally. the maximum depth of macrophyte colonization seems to be a good measure for light availability and a direct measure of vegetation growth limitation.

### Sampling Protocol

In Spain, there is not yet an official and national Sampling Protocol for macrophytes in lakes. As a consequence, the recorded data are sometimes confusing and statistical analyses and monitoring programmes have handicaps to be run out. For this reason, it is crucial to define a single protocol as a national protocol to record reliable data. An ideal protocol should answer the following questions:

1. **Sampling period and regular recurrence**: The Bavarian environment agency

suggests the best period to take macrophytes samples is early July until mid August. However, having in mind that Spain is located southern Europe, the main growth season of macrophytes would be a bit earlier, in spring, and, therefore the best period for sampling should be middle spring (May-June) instead of summer (Suarez et al, 2005). In Spain, some monitoring networks are taking samples in spring and summer, some of them also in winter. Economical aspects (cost) should also be considered when defining the number of samples.

2. **Number of sampling points/area:** Commonly, it is not possible to sample all the lake surface and perimeter, for this reason, a protocol defining how to process in these cases seems also necessary. Usually, the larger and more complex is a water body, the more transects must be investigated. (Schaumburg et. al 2007), It is important to remark that sampling should not be carried out in the proximity of inflows and characteristic sections of the lake should be the main focus; to take sample of all the different "niches" in order to document possible sources of stress or nutrient inputs, transects should also cover areas of different land use (Schaumburg et. al 2007).

. **The way of sampling:** Methodology should be easy to accomplish in different typologies of water bodies and within short times and low cost of application. For deep lakes, protocols suggest to sample with a grab sampler in the case that diving it's not possible. Also, other surveys described the same methodology with rakes (Sraj-Krzic et. al, 2007).

4. **What can be measured?** Abundance is usually estimated by the use of plant abundance classes, according to Kohler (1978), with values from 1-5 (very rare, rare, common, frequent and very frequent). The depth of the lower vegetation limit must also be recorded in the field protocol (Schaumburg, et. al , 2007). It is very important the description of the shoreline and riparian zones, plant cover, use of adjacent land, shoreline morphology as well as other characteristics like slope and shading in areas of shallow water (Schaumburg et. al, 2007).

*Table 4a: Summary of the metrics use by Spanish entities for the assessment of Spanish lakes*

| Metrics | Advantages | Drawbacks |
|---|---|---|
| **1. Species Richness**<br><br>Number of species present in the water body | 1. Practical and easy to make in the field. However, it should be remarked that in some taxa to identify until the specie level is hard and tedious work<br><br>2. Species can be compared to them in a reference situation, as indicator of ecological changes in the systems<br><br>3. Nowadays, there is some data from the River Basin Administrations | 1. Lake´s size could influence the number of species presented. The same typology have water bodies with different size, reference value should take this into account<br>2. Highest number of species is related to medium values of disturbances where is supposed to be more niches<br>3. Pressures can change the macrophytes cover but in some situations, may not change the community; species richness does not reflect this situation.<br>4. The sp number must be enough high to be representative and to have statistical significance. (Mountain lakes may not have enough sps).<br>5. Period and number of samplings should be defined carefully<br>6. It is a very primary metric, it may not reflect all the pressures affecting the systems, but, today, it is almost the only possibility with the data available for Spanish lakes. |
| **2. Helophyte vegetation ring**<br><br>Percent of the littoral area occupied by helophyte vegetation. **Helophyte should be typical and exotic species are not measured | 1. Practical and easy to measure in the field<br>2. It reflects changes in land use and morphological pressures<br>3. It measures cover and abundance<br>4. It has a relationship with Water Level Fluctuation | 1. It may not directly measure eutrophic conditions<br>2. It does not describe the number of species presented in the ecosystem<br>3. Probably, it is not possible to be measured in all lakes (lakes without helophyte in a reference situation, no practical in high-medium mountain lakes) |
| **3. ECELS**<br><br>"*Índice de Valoración de Ecosistemas LenÍticos Someros*" Index to assess lenitic shallow lakes. | 1. Standard method relatively easy to measure<br>2. It measure HM and eutrophic impacts in the same index | 1. It mixes up different quality elements ( HM and Eutrophic) in the same metric, which is not allow following WFD<br>2. Mainly, it is though to be used in wetlands and no deep lakes |

*Table 4b: Summary of the metrics use by Spanish entities for the assessment of Spanish lakes*

| Metrics | Advantages | Drawbacks |
|---|---|---|
| **4. Índice de valoración de humedales (IVH, Cirujano, 1992) Evaluation Index for wetlands**<br><br>It assesses the value of wetlands with a conservational point of view | 1. It is the only index applied to Spanish lakes that incorporate composition parameters<br>2. It works with appearance/absence of species, being of easily application<br>Basque Country is using this index for a long time with good results. There is enough data to compare. | 1. It was created to assess the conservative status of specific species in aquatic ecosystems, and not for assessing the ecological status of the ecosystem itself. |
| **5.Exotic species appearance**<br><br>Presence/absence of exotic species in the aquatic ecosystem | 1. It is quite easy to measure in the field<br>2. It reflects biotic impacts (exotic-invasive introduction) | 1. It does not measure directly eutrophication and/or HM impacts<br>2. Exotic sp may no act as invasive species<br>It can be applied to all types of lakes |
| **6. ECLECTIC Index**<br>**(Camacho, 2009)**<br>Variable 1: Typical hydrophyte species cover<br>Variable 2: Community composition and helophyte and littoral species cover<br>Variable 3: Typical Species Richness. Number of species presented in the system | 1. It reflects HM impacts and eutrophic conditions<br>2. It is the only official index can be applied in Spanish lakes<br>3. The presence/absence of exotic species is also include in the metric<br>The index included submerged macrophyte as well as helophytes and littoral species. | This index is proposed in the Habitat Directive (92/43/CEE) and no for Water Framework Directive goals. |

## 7 CONCLUSIONS

From this review, it is remarkable the fact that the term "freshwater or aquatic macrophyte" is not yet well defined by all entities involving in the WFD implementation. WFD describes macrophyte as "All aquatic higher plants, mosses and characean algae" but scientists and international societies disagree in some points. It is essential to establish which species should be sampled in the monitoring programmes. Following this context, in Spain, it is also a decisive issue to set up and official sampling protocol that must be followed by all the River Basin Administrations and other entities. Moreover, in order to have an objective and correlated data and to be able to make the intercalibration processes, there is a need of sampling protocols for all European countries which assess the ecological status in a comparable way. This will provide reliable macrophyte data that should be suitable for being compared with other countries. An ideal protocol should answer (1) Sampling period and regular recurrence: The best period to take macrophytes samples should be in the main growth season of macrophytes, in spring (May-June). (2) Number of sampling points/area: The larger and more complex is a water body, the more transects must be investigated; samples should not be carried out in the proximity of inflows and characteristic sections of the lake should be the main focus. (3) Methodology of sampling which should be easy to develop in different WFD lake types and with short times and low cost of application. The best choice is to sample without rakes in all the cases except for special needs in greatest depths. (4) What can be sampled? Abundance is usually estimated by the use of plant abundance classes. The depth of the lower vegetation limit, a description of the shoreline and riparian zones, plant cover, use of adjacent land, shoreline morphology as well as other characteristics like slope and shading in areas of shallow water should also be determined.

Macrophytes play a very important role in the functioning of Spanish freshwater ecosystems and they can reflect the main impacts affecting Spanish water bodies (chemical, hydromorphological and biotic impacts). The main need is the knowledge of how to use macrophyte in order to reflect this information. Good metrics need to produce objective, repeatable and quantifiable macrophyte data to detect trends. Not only taxonomic composition but also quantitative measurements are needed in the implementation of the WFD.

Certainty,helophytes and hydrophytes may be used together for the evaluation of the ecological status; however, it seems more practical to sample and analyze them separately and then, make final conclusions. Indeed the role of helophytes in the ecological assessment of Spanish water bodies is still under study; it is recognized that they are a key part of coastal lakes ecosystems, but methods from which they can provide information are still unknown. Current research is studying the influence of WLF on littoral macrophytes, which seems to be clear; however, there is still a lack of information about the ecological effects of WLF on submerged and floating macrophytes on lakes. More research is thus needed in order to establish strong conclusions and design robust macrophyte metrics. Morphological changes of littoral zones caused by dredging or embankments are deeply disturbing vegetation development. These impacts seem to affect more helophyte species, promoting changes in the composition and abundance of these communities. Therefore, composition and abundance of the helophyte perimeter are two instruments that should be measured routinely.

The first step for the ecological assessment protocol is to set up reference conditions for biological indicators, looking for an association between lake types WFD and plant types found. Cluster analysis show that clusters with hydrophytes were very wide-ranging, grouping lakes from several typologies. although lakes appear to be better classified attending helophytes species. High-medium mountain lakes are clustered together, probably due to the low number of helophyte species presented in these kinds of water bodies. Karstic, calcareous, mixed feeding lakes are grouped together, showing similar helophyte communities. Similarities matrices with data on the presence of species such as *Callitriche lusitanica, Isoetes velatum and Fontinalis (antypiretica and angustifolium*) seem to appear as an assemblage in high-medium mountain Spanish lakes.

Data used here were provided by the River Basin Administrations and, in some cases, by local entities. The collected data set is very diverse, incomplete and scant; the number of sampled lakes per typology is very different. Despite of the low amount of data, results show that macrophyte distribution patterns do not fit exactly with the Spanish WFD typology, although this is normal as billogical data are not used for the classification of water bodies according to the WFD. More lakes need to be sampled, lakes typologies should be well defined and data must be collected following a common protocol. Reference sites should be established for each typology, then, macrophyte communities could be identified.. A further gain of a future study could be to characterize the pressures for the studied waterbodies, in order to show the response of the composition and proportion of functional groups (e.g. submerged, tall emergents, small emergents, annuals, perennials, etc) to disturbance, for each lake type. These objectives need long time and high cost consumption. Furthermore, this review is a first approximation that needs to be continued.

At the end of this work, official entities have approved new documents which include new information about metrics and referents sites under WFD for Spanish lakes (MARM 2010a, 2010b); however, since they are not yet official, they have yet not been included in this review.

## REFERENCES

Abrahams C. (2008) Climate change and lakeshore conservation: a model and review of management techniques. Hydrobiologia, 613: 33-43.

Barbour M.T; Wietlikl, W; Jackson S.K.; Courtemanch D.L; Davies S.P.; Yoder, C.O.(2000). Measuring the attainment of biological integrity in the USA. Hydrobiologia, 422-423:, 453-464.

Becares E; Conty A; Rodriguez C. and Blanco S, (2004). Funcionamiento de los lagos someros mediterráneos. Ecosistemas, 13(2): 2-16.

Blindow, I. (1988) Phosphorus toxicity in Chara. Short Aquatic Botany32, 393-395.

Blindow ,I. (1992a). Decline of charophytes during eutrophication; comparision to angiosperms. Freshwater Biology, 28, 9-14.

Camacho, A.; Borja C., Valero-Garces B; Sahuquillo M; S. Cirujano,S; Soria J. M; Rico E; De la Hera A; Santaman A. C; García De Domingo A; Chicote A and Gosálvez R. U. (2009a). Aguas continentales retenidas. Ecosistemas lenítcos. En: VV.AA., Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España. Dirección General de Medio Natural y Política Forestal, Ministerio de Medio Ambiente, y Medio Rural y Marino. Madrid, 412 pp (el capitulo), aprox. 10000 pp (la obra completa). Edición en formato DVD.

Casado, S., and Montes C, (1995). Guía de los Lagos y Humedales de España. J.M. Reyero Editores. Madrid

Chambers, C (2009). 12th EWRS International Symposium on Aquatic Weeds. Jyvaskylä, Finland.

Cirujano, S. and Medina L, (2002).Plantas acuáticas de las lagunas y humedales de Castilla - La Mancha. Real Jardin Botanico, CSIC.



Coopsl H., Beklioglu M. and Crisman T.L., (2003). The role of water-level fluctuations in shallow lake ecosystems – workshop Conclusions Hydrobiologia 506–509.2003.

Cook, C D. K; Gut B. J.; Rix, E. M; Schneller J. and Seitz M (1974). Water Plants of the World. The Hague.

DOCE (2000). Directiva 2000/60/CE del Parlamento Europeo y del Consejo, de 23 de octubre de 2000 por la que se establece el marco común de actuación en el ámbito de la política de aguas. DOCE nº L 327: 1-73, de 22 de diciembre de 2000. Bruselas.

Gacia, E and Ballesteros E (1994). Production of Isoetes lacustris in a Pyrenean lake: seasonality and ecological factors involved in the growing period. Aquatic Botany 48:77-89.

Gacia, E and Ballesteros E (1998). Effects of building up a dam in a shallow mountain lake. Oecologia Acuatica, 11:55-56.

Hellsten S. and Mjelde M (2009). Macrophyte responses to water fluctuation in fenno-scandinavian lakes- Appliying a common index. Verhandlungen Internationale Verein Limnologie 30:163.

Keto A; Tarvainen A; Marttunen M and Hellsten S. (2008). Use of water level fluctuation analysis tool in hydrological status assessment of Finnish lakes. Hydrobiologia 613: 133-142.

MARM, Ministerio de Medio Ambiente, Medio Rural y Marino, (2010a). Estado ecológico de las Aguas superficiales. Selección de métricas para la evaluación del estado ecológico de las asas de agua de la categoría "lagos" basadas en el elemento de calidad "composición, y abundancia de otro tipo de flora acuática", en aplicación de la directiva Marco del Agua. Avance, tomo único. Cedex: 44-407-1-002.

MARM (2010b). Estado ecológico de las Aguas superficiales. Establecimiento de condiciones de referencia y valores frontera entre clases de estado ecológico en masas de agua de la categoría lago para los elementos de calidad "composición, abundancia y biomasa de fitoplancton" y "composición, y abundancia de otro tipo de flora acuática", en aplicación de la directiva Marco del Agua. Avance, tomo único. Cedex: 44-407-1-002

Nurminen L.(2003). Macrophyte species composition reflecting water quality changes in adjacent water bodies of lake Hiidenvesi, SW Finland., Finnish Zoological and Botanical Publishing Board 2003. Ann. Bot. Fennici 40: 199-208.

Ongley E. (1996). Control of water pollution from agriculture - FAO irrigation and drainage paper 55. Food and Agriculture Organization of the United Nations, Rome.

Rodriguez C; Bécares E and Fernández M. Efecto de aves y cangrejos sobre la vegetación acuática de los humedales. Instituto de Medio Ambiente. Universidad de León.

Ruiz. C (2009). In which way can macrophytes be used as suitable tool to assess the ecological status of Spanish lakes. Inland Water Assessment Master of Universidad Autónoma de Madrid.

Schaumburg J; Schranz C; Stelzer D; Telzer G and Hofman F (2007). Action Instructions for the ecological evaluation of lakes for implementation of EU Water Framework Directive: Macrophytes and Phytobenthos.. Bavarian Environment Agency. April 2007. Länderarbeitsgemeinschaft Wasser (Länder Working Group) LAWA. Projekt-Nr. O 4.04.

Scheider, S. (2007). Macrophyte trophic indicator values from a European perspective. Limnologica 37; 281-289.

Seele. J; Mayr M; Staab F. and Raeder U (2000). Combination of two indication systems in pre-alpine lakes- diatom index macrophyte index. Ecological Modelling 130: 145-149

SEPA, Swedish Environmental Protection Agency, (2000). Environmental quality criteria. Lakes and watercourses. Swedish Environmental Protection Agency, Report 5050, 102.

Sraj.Krzic N; GERM M; Uuban-bercic O; U. Kuhar U; Janauer A; Gaberscik A (2007). The quality of the aquatic environment and macrophytes of karstic watercourses, Plant Ecology 192:107-118.

Suarez, M.L; A. Mellado, M; Sanchez-Montoya(2005). Propuesta de un índice de macrófitos (IM) para evaluar la calidad ecológica de los ríos de la cuenca del Segura. Limnetica, 24: 305-318.

Thiebaut G; Guerold, F. and Muller ,S; (2002). Are trophic diversity indices based on macrophyte communities' pertinent tool to monitor water quality? Water Research 36-3602-3610.

U.S. Environmental Protection Agency, EPA. (2002) Methods for Evaluating Wetland Condition: Vegetation-Based Indicators of Wetland Nutrient Enrichment. Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-R-02-0

Wantzen K. M; Rothhaupt K.O; Moetl M; Cantonati M; Toth L. G. and Fischer P (2008). Ecological effects of water level fluctuations in lakes: an urgent issue. Hydrobiologia 613:1-4.

Wetzel, R, (2001). Limnology: Lake and river ecosystems, Third edition, Elsevier Academic Press.

DATA BASES

Agencia Vasca del Agua (2008). Red de seguimiento de humedales interiores del País Vasco. Resultados del periodo 2001-2008. Agencia Vasca del Agua, Vitoria

Consejería de Medio Ambiente de la Junta de Andalucía (2006). Red de control de zonas húmedas. Resultados de análisis físico-químicos del periodo 2002-2006. Consejería de Medio Ambiente de la Junta de Andalucía, Sevilla.

MARM (2008). Base de Datos Biológicos en masas de agua. Ministerio de Medio Ambiente, y Medio Rural y Marino. Madrid.

MIMAM. (2000). Base de datos de los Humedales Españoles versión 2.0. Ministerio de Medio Ambiente. Madrid.

Ramsar (2008). Ramsar sites information service. On-line en http://www.wetlands.org/rsis/

Confederacion Hridrográfica del Ebro (2008). Red de seguimiento biológico de masas de agua de la categoría lago de la Demarcación Hidrográfica del Ebro. Resultados del periodo 2005-2008. Confederación Hidrográfica del Ebro, Zaragoza

Confederación Hidrográfica del Cantábrico (2008). Red de seguimiento biológico de masas de agua de la categoría lagos de la Demarcación Hidrográfica del Cantábrico. Resultados del 2008. Confederación Hidrográfica del Cantábrico. Oviedo

Confederación Hidrográfica del Duero (2008). Red de Seguimiento biológico de masas de agua de la categoría lago de la Demarcación Hidrográfica del Duero. Resultados del periodo 2006-2008. Confederación Hidrográfica del Duero. Valladolid

Confederación Hidrográfica del Guadiana (2008). Red de seguimiento biológico de masas de agua de la categoría lago de la Demarcación Hidrográfica del Guadiana. Resultados del periodo 2005-2008. Confederación Hidrográfica del Guadiana, Ciudad Real.

Confederación Hidrográfica del Júcar (2009). Red de seguimiento biológico de masas de agua de la categoría lagos de la Demarcación Hidrográfica del Júcar. Resultados del periodo 2006–2008. Confederación Hidrográfica del Júcar, Valencia

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