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Technical Guidelines for assessing and monitoring the condition of Annex I habitat types of the Directive 92/43/EEC

Standing water

ATECMA

DAPHNE
INŠTITÚT APLIKOVANEJ EKOLÓGIE

Institute for European Environmental Policy

EUROPEAN COMMISSION

Directorate-General for Environment
Directorate D — Biodiversity
Unit D3 — Nature Conservation

E-mail: nature@ec.europa.eu

European Commission
B-1049 Brussels

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Standing water habitats

Antonio Camacho, Daniel Morant, Alba Camacho-Santamans

University of Valencia

University of Barcelona

Geaquair S.L.

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Contents

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Glossary and definitions

Habitats

Natural habitats: terrestrial or aquatic areas distinguished by geographic, abiotic and biotic features, whether entirely natural or semi-natural" (Habitats Directive).

Habitat condition: is the quality of a natural or semi natural habitat in terms of its abiotic and biotic characteristics. Condition is assessed with respect to the habitat composition, structure and function which, in turn, underpin the integrity of the habitat. In the framework of conservation status assessment, condition corresponds to the parameter "structure and function". The condition of a habitat asset is interpreted as the ensemble of multiple relevant ecosystem characteristics, which are measured by sets of variables and indicators that in turn are used to compile the assessments.

Habitat characteristics: are the attributes of the habitat and its major abiotic and biotic components. Characteristics include the attributes of an ecosystem asset including components, structure, processes, and functionality. Ecosystems characteristics can be classified as abiotic (physical, chemical), biotic (compositional structural, functional) and landscape characteristics (SEEA-EA, UN 2021).

Species

Characteristic species: species that characterise the habitat type, are used to define the habitat (diagnostic), can be dominant or accompanying species.

Typical species*:* species that indicate good condition/quality of the habitat type concerned. They are not necessarily diagnostic, dominant or characteristic species. Their conservation status needs to be evaluated under the structure and function parameter. Usually are selected as indicators of good condition and provide additional information to that provided by other variables that are used to measure compositional, structural and functional characteristics.

Variables

Condition variables: are quantitative metrics describing individual characteristics of a habitat asset. They are related to key characteristics of the habitat that can be measured, must have clear and unambiguous definition, measurement instructions and well-defined measurement units that indicate the quantity or quality they measure. In these guidelines, the following types of condition variables are included:

- **Essential variables**: describe essential characteristics of the habitat that reflect the habitat quality or condition. These variables are selected on the basis of their relevance, validity and reliability and should be assessed in all MSs following equivalent measurement procedures.
- **Recommended variables**: are optional, additional, complementary condition variables that may be measured when relevant; these are complementary to the essential variables, can help improve the assessment and understanding or interpretation of the overall results.
- **Specific variables**: are condition variables that should be measured in some specific habitat types or habitat sub-groups; can thus be considered essential for those habitats, which need to be specified (e.g. salinity for saline grasslands, groundwater level for bog woodlands, etc.).

Descriptive or contextual variables: define environmental characteristics (e.g. climate, topography, lithology) that relate to the ecological requirements of the habitat, are useful to characterise the context in a specific location where the habitat occurs, for defining the relevant thresholds for the condition variables and interpret the results of the assessment. These variables are not included in the aggregation of the measured variables to determine the condition of the habitat.

Reference levels: Reference levels are defined for the values of the variables (or ranges) that determine whether then habitat is in good condition or not. They are set considering the distance from the reference condition (good). The value of the reference level is used to re-scale a variable to derive an individual condition indicator.

Condition indicators: are rescaled versions of condition variables. Usually, they are rescaled between a lower level that corresponds to high habitat degradation and an upper level that corresponds to the state of a reference habitat in good condition.

Aggregation: is defined in this document as a rule to integrate and summarise the information obtained from the measured variables at different spatial scales, primarily at the local scale (sampling plot, monitoring station or site).

Abbreviations

BQE: Biological Quality Elements

EQR: Ecological Quality Ratio

EU: European Union

EU Member States acronyms:

Austria	(AT)	Estonia	(EE)	Italy	(IT)	Portugal	(PT)
Belgium	(BE)	Finland	(FI)	Latvia	(LV)	Romania	(RO)
Bulgaria	(BG)	France	(FR)	Lithuania	(LT)	Slovakia	(SK)
Croatia	(HR)	Germany	(DE)	Luxembourg	(LU)	Slovenia	(SI)
Cyprus	(CY)	Greece	(EL)	Malta	(MT)	Spain	(ES)
Czechia	(CZ)	Hungary	(HU)	Netherlands	(NL)	Sweden	(SE)
Denmark	(DK)	Ireland	(IE)	Poland	(PL)		

HTCI: Habitat type of Community interest

MS: Member State

SEEA-EA: System of Environmental-Economic Accounting — Ecosystem Accounting

WFD: Water Framework Directive

Executive summary

Lentic (or Lenitic) habitats/ecosystems are the scientific terms for what we commonly call "standing waters", that is, azonal inland and coastal (non-marine) habitats/ecosystems that hold water, either permanently or temporarily, where water flow is very slow or mostly negligible for most of the water body.

Lentic habitats are characterised by a series of key features, which can be grouped as: (i) Climatic. (ii) Geological. (iii) Geomorphological and pedological. (iv) Hydrological. (v) Morphological/Physiographic. (vi) Physical and chemical characteristics of water. (vii) Components of the biological communities. (viii) Community structural factors. (ix) Biological processes. (x) Landscape exchanges with other ecosystems. Anthropic factors, including all kinds of interactions and effects of human activities, are instead considered as pressures (potentially creating impacts), which for lentic habitat types can be grouped as: (a) Hydrological. (b) Geomorphological pressures and impacts. (c) Water quality alterations. (d) Pressures on the structure of the communities. (e) Land uses in the catchment area. (f) Occupation of the lentic habitat. (g) Invasive alien species. (h) Other. (i) Climate change.

The variables selected for the assessment of lentic habitats condition would consequently need to assess the status of their key features and how these are modified by the impacts produced by specific pressures. When assessing the condition of lentic habitats, and specifically their physical characteristics, the variables most commonly used by EU Member States are those related to the hydrological pattern, variations in depth or flooded surface area, as well as water transparency and colour.

The status of chemical features is mainly addressed in the water, by examining nutrients (P and N) concentrations, pH, conductivity and dissolved oxygen concentration (or its variations along the diel cycle). The composition and abundance of biological communities is usually assessed by the number of characteristic plant species (hydrophytes, helophytes, amphiphytes) and its abundance for each habitat type, as well as by the abundance and diversity of plankton (mainly phytoplankton, but also zooplankton), and benthic macroinvertebrates, whereas vertebrates, including amphibians and fish, are barely used for the assessments.

Aquatic plant structure is the main structural variable used, followed by specific structural (physical) habitat features, whereas most functional indicators relate to trophic status and presence of invasive species. In contrast to the variables mentioned above, which cover the main aspects of the abiotic (physical and chemical) and biological (composition, structure, and functions) features of lentic habitat types, landscape variables are much less frequently used and, when included, mostly refer to spatial aspects of the lentic waterbody within its catchment.

Overall, the full set of variables used by EU MS to assess the habitat condition of lentic habitats adequately reflects their main features and how these are affected by the key pressures. Up to 33 core variables have been identified here as primary for assessment, which, along with other 12 accessory variables, can provide in-depth information on specific issues whenever needed. Since the values of some of these variables can be currently obtained by high throughput methods, such as remote sensing and e-DNA analyses, we strongly recommend exploring these approaches to improve both the coverage and quality of the assessment methods. These techniques could also be used to complement or even partially replace traditional field-based assessments laboratory sample analyses methods.

Our recommendations for the establishment of reference and threshold values for the assessment variables consider several complementary approaches, such as: (i) the

identification of reference sites, this is, those with a minimally-disturbed condition, where they exist; (ii) modelling, (iii) the use of statistical methods based on ambient distribution; (iv) the use of prescribed reference levels; (v) the use of a baseline year (with conditions preferably retrieved by paleolimnological methods), and, whenever none of these approaches works; (vi) expert judgment.

Also, concerning the aggregation at the local level, we strongly recommend the use of multimetric weighted procedures, although the "one-out all-out criterion" could also be considered, especially when the assessment is based on the widely available on WFD data for lake waterbodies. Even though some guidelines for the aggregation at the biogeographical region level have already been set by the EC, we strongly recommend reviewing its scientific foundations to better understand the rationale supporting the established thresholds.

Whereas representativeness is the main criterion we recommend for selecting monitoring sites, the natural temporal (e.g. seasonal) variations in habitat features, as well as variation due to natural and anthropogenic disturbances, should guide the choice of sampling frequency and period. Similarly, we recommend considering habitat heterogeneity when determining the appropriate number and location of sampling sites, both at the local and at the MS level, while also taking into account achieving statistical significance.

Preface

This document is inspired by the basic principles of the UN System for assessing ecosystem conditions (SEEA-EA, United Nations 2021, Edes et al., 2022) and the specific EU wide methodology for assessing ecosystem condition (Vallecillo et al., 2022), though with the term “ecosystems” here being replaced by “habitats”, particularly the “lenitic habitat types of community interest”. Ecosystem condition is the quality of an ecosystem measured in terms of its abiotic and biotic characteristics (United Nations, 2021). The System of Environmental-Economic Accounting (SEEA) “is a framework that integrates economic and environmental data to provide a more comprehensive and multipurpose view of the interrelationships between the economy and the environment, and the stocks and changes in stocks of environmental assets, as they bring benefits to humanity” (United Nations, 2021). Within this context, ecosystem accounting under the SEEA-EA “constitutes an integrated and comprehensive statistical framework for organizing data about habitats and landscapes, measuring the ecosystem services, tracking changes in ecosystem assets, and linking this information to economic and other human activity”. The SEEA-EA is spatially based and integrates a “statistical framework for organizing biophysical information about ecosystems, tracking changes in ecosystem extent and condition, measuring ecosystem services and possibly valuing ecosystem services and assets to link this information to measures of economic and human activities” (Vallecillo et al, 2022).

The SEEA-EA is built on five core accounts (Figure 1), one of which is the “Ecosystem condition”, which records the condition of ecosystem assets in terms of selected characteristics at specific points in time. Over time, they record changes to their condition and provide valuable information on the health of ecosystems”. The SEEA-EA framework has been recently applied to specific types of ecosystems (King et al., 2024).

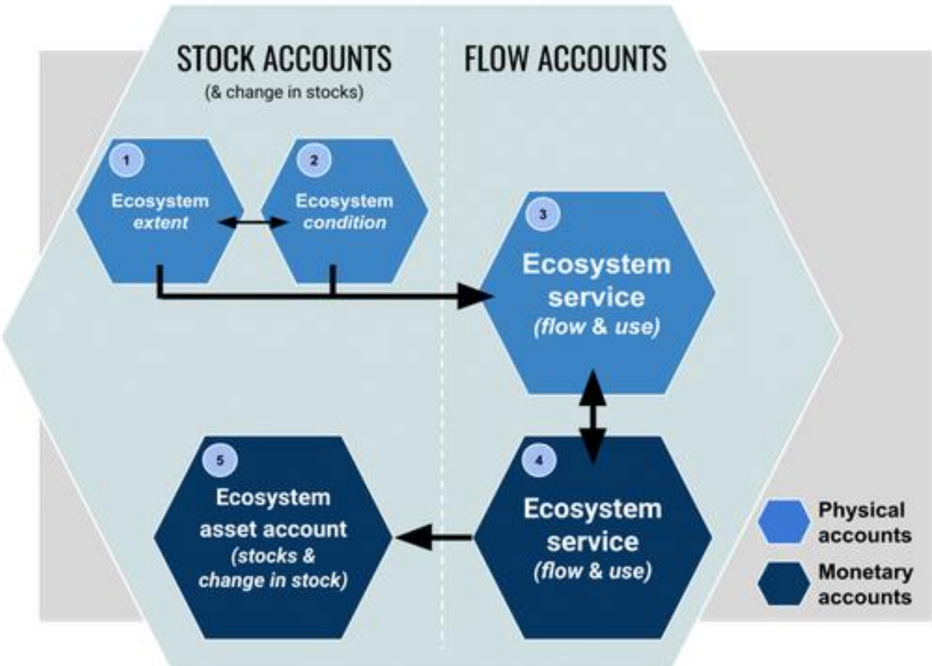
The condition (quality) of a habitat or ecosystem refers to the status of its basic and distinctive ecological characteristics (Maes et al. 2020), such as, for the lentic habitats, the status of their basic physical-chemical (e.g. water and soil features), biological (e.g. the composition of its plant communities), and functional characteristics (e.g. its trophic status), as well as landscape interactions (e.g. nutrient import/export processes).

This status is measured using series of variables selected according to specific criteria (Czúc et al, 2021), e.g. water conductivity for its salt content for aquatic habitats, which can be further aggregated at different levels (Borja et al., 2014; Langhans et al., 2014), as well as into indices (Camacho et al., 2009; Camacho et al., 2019a; Jakobsson et al, 2021).

These variables are measured at specific locations (a representative number of sites where the habitat type is present), over time (e.g., once every reporting period under Article 17 of the Habitats Directive), in such a way that, when compared to reference conditions (Camacho et al., 2009; Jakobsson et al, 2021; Vallecillo et al., 2022), namely the level or range of a variable under non-impacted conditions), “they record the changes to their condition and provide valuable information on the health of ecosystems” and help assess progress toward related targets (Rendon et al., 2019), such as achieving good conservation status under the Habitats Directive (HD).

As such, good ecosystem condition is considered to be present when a habitat exhibits good physical, chemical, and biological condition or quality, along with self-reproduction or self-restoration capability, in which species composition, ecosystem structure and ecological functions are not impaired (Vallecillo et al, 2022).

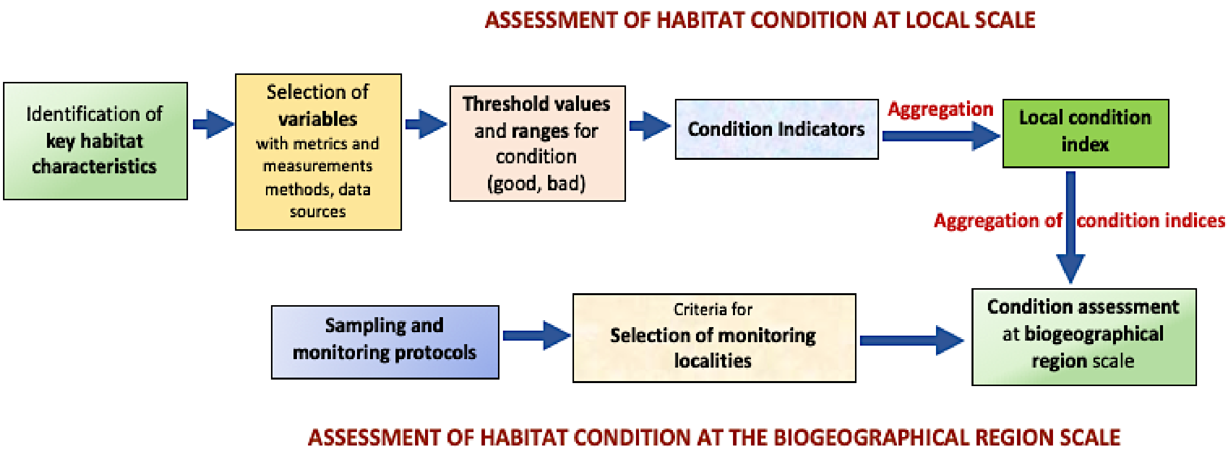
Figure 1. Connections between the ecosystem accounts



Source: https://seea.un.org/ecosystem-accounting

As with other habitat types, the guidelines for lentic habitats presented in this document aim "to support the EU Member States in producing more harmonised assessment and monitoring of the condition (structure and functions) of Annex I habitat types in the framework of the reporting on conservation status in accordance with Article 17 of the Habitats Directive", following a common sequence as shown in Figure 2.

Figure 2. Template sequence indicating the main items covered in this document.



Source: own elaboration

1. Definition and ecological characterisation

This document aims to provide guidelines for the assessment and monitoring of the lentic habitats condition, particularly in relation to the assessment of the parameter "Structure and function" within the Evaluation Matrix for the conservation status of the habitats of community interest (HCI) in Annex 1 of the Habitats Directive (HD, EC, 1992).

Section 1 of this document includes the identification and description of key abiotic (physical, chemical) and biotic (and mixed – ecosystemic –) characteristics (composition, structure and functions) of lentic habitats/ecosystems, as well as how these ecosystems are integrated into the landscape and what the main types of pressures affecting lentic habitats are. This provides the basis for the analysis of existing methodologies, which will be presented in Section 2.

1.1 Definition and interpretation of habitats covered

Lenitic or lentic habitats/ecosystems are the scientific denomination for what we commonly call "standing waters", this is, azonal inland and coastal (non-marine) habitats/ecosystems that hold water, either permanently or temporarily, where water speed is very slow or mostly negligible for most of the water body. For the purposes of the present work, the clustering is based on the above definition.

The flow regime mainly differentiates lentic habitats from the other group of habitat types of community interest (HTCI) also included in the Group 3 (Inland Waters) of Annex 1 of the Habitats Directive - Group 32 which corresponds to "running waters" (lotic habitats). Both HTCI included in Group 3 are further differentiated from the vast majority of other THIC by having water as the main abiotic component fully shaping the habitat. Thus, the THIC to which this document refers are those included in the genuine HCI group of "standing waters" (group 31), plus a few additional types that are also characterized by the presence of open water, mainly the THIC 1150 – Coastal lagoons, and, to a lesser extent, (where applicable), the flooded area of THIC 2190 – Humid dune slacks, only for the water specific variables for the later.

Among these habitat types of community interest (HTCI), the most relevant discriminant factors include natural trophic status, water mineralisation, water level (or depth), geology, soil type, alkalinity, hydroperiod, catchment geology, and water temperature. These abiotic factors, in turn, shape the biological communities that characterise each HTCI. Accordingly, these factors will be among the main elements considered for the ecological characterisation of standing water habitats in Section 1.2 of this document. Consequently, the HTCI covered in these guidelines can be described by their main distinctive factors, as presented in the list below:

3110 - Oligotrophic waters containing very few minerals of sandy plains *(Littorelletalia uniflorae*) are characterised by an oligotrophic trophic status, very low water mineralisation, and base-poor alkalinity, occurring mainly on sandy substrates in plain terrains, and supporting vegetation typical of the *Littorelletalia uniflorae* alliance, including species such as *Littorella, Lobelia dortmanna*, or *Isoetes* spp.

3120 - Oligotrophic waters containing very few minerals generally on sandy soils of the West Mediterranean, with *Isoetes* spp. is defined by an oligotrophic trophic status, very low water mineralisation, and sandy substrates, and is mainly found in the Mediterranean region, with some extensions into the thermo-Atlantic sector. It supports dwarf amphibious vegetation, primarily *Isoetes* spp., while the Annex I priority habitat type 3170 represents a specific subtype, occurring in temporary and very shallow waters.

3130 - Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or of the *Isoëto-Nanojuncetea* have an oligotrophic to mesotrophic trophic status and is characterised by aquatic to amphibious short perennial or annual vegetation, typically composed of small ephemerophytes, belonging to the *Littorelletalia uniflorae* and/or *Isoëto-Nanojuncetea syntaxa.*

3140 - Hard oligo-mesotrophic waters with benthic vegetation of Chara spp. have an oligo-mesotrophic trophic status, hard fresh to slightly saline waters, and high alkalinity, being rich in dissolved bases. It typically occurs on calcareous substrates and supports charophyte-dominated vegetation.

3150 - Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* — type vegetation is characterised by a eutrophic trophic status, hard freshwater with high alkalinity and rich in dissolved bases and typically exhibits green to blue-green water coloration. The vegetation consists of free-floating surface communities of the *Hydrocharition* or, in deeper open waters, associations of large pondweeds belonging to the *Magnopotamion*.

3160 - Natural dystrophic lakes and ponds are typical by a dystrophic trophic status, peaty soils, and acidic conditions with a pH ranging from 3 to 6. The water typically has a brown coloration, and the vegetation is dominated by communities of the *Utricularietalia* order.

3170* - Mediterranean temporary ponds are habitats characterised by a temporary hydroperiod, very shallow depth, and occurrence in the Mediterranean region. The vegetation is dominated by Mediterranean therophytic and geophytic species. The Annex I priority habitat type 3170 represents a particular subtype of HTCI 3120.

3180* - Turloughs are characterised by a temporary hydroperiod and highly variable soils and geology, including limestone bedrock, marls, peat, clay, and humus. It is primarily fed by groundwater, and the vegetation mainly belongs to the alliance *Lolio-Potentillion anserinae* Tx. 1947, but also includes communities of the *Caricion davallianae*.

3190 - Lakes of gypsum karst are habitats with a permanent hydroperiod with strong water level fluctuations and occurs on active gypsum karst geology. The waters are hard and rich in calcium sulphate, and the mixing regime is stratified when conditions allow. The vegetation includes communities of *Charetea, Lemnetea,* and *Potamogetonion*, along with the presence of sulphur bacteria.

31A0* - Transylvanian hot-spring lotus beds. This habitat is associated with geothermal waters and is geographically restricted to Petea Lake in western Romania. The vegetation is dominated by formations of *Nymphaea lotus*.

1150 - Coastal lagoons are coastal in geomorphological character, typically separated from the sea by sand banks or shingle, and less frequently by rock formations. Water mineralisation varies from brackish to hypersaline conditions. Vegetation may be absent or present, with communities from *Ruppietea maritimae, Potametea, Zosteretea,* or *Charetea*. The habitat also includes saltmarshes, and salt basins or salt ponds may be considered lagoons if they originate from transformed natural lagoons or saltmarshes and are subject to only minor exploitation impacts.

2190 - Humid dune slacks occur in humid depressions within dune systems, where it supports freshwater aquatic communities. An example is sub-type 16.31 – dune-slack pools, which include associations such as *Charetum tomentosae, Elodeetum canadense, Hippuridetum vulgaris, Hottonietum palustris,* and *Potametum pectinati*, corresponding to permanent dune-slack water bodies.

Most of these habitats can be found across many biogeographical regions of Europe, though some (e.g. 3170 - * Mediterranean temporary ponds), are mainly restricted to a specific biogeographical region, or are found in small areas of Europe (e.g., 3190 - Lakes of gypsum karst, reported so far in Lithuania and Spain); or even, in a unique case within Annex 1, limited to a single lake, such as the 31A0* - Transylvanian hot-spring lotus beds, described as the formations of *Nymphaea lotus* of geo-thermal waters of Petea Lake, in western Romania. Despite this, the clustering criterion for differentiating lentic habitats from others, that is, the permanent or temporary presence of non-flowing waters, remains consistent across all the EU biogeographical regions and ecological subtypes.

As it is widely known, Annex 1 of the Habitats Directive is not exhaustive and therefore does not include all habitat or ecosystem types, but rather those that are intended to receive specific protection under the implementation of the directive. Although many national level classifications of lentic habitats are used within EU Member States, the EUNIS classification (EEA, 2023) currently serves as the main reference at the European level. For "Standing Waters" habitats, the most recent version is updated to level 3, which represents the most detailed level currently available for these habitat types. In the updated EUNIS classification, Group P refers to Inland Waters, within which the Group P1 "Standing Waters" is nested. Group P1 includes the following habitat types:

- P11 Lowland, very shallow (unstratified), calcareous or mixed lakes non humic, often turbid
- P12 Lowland, shallow to deep (stratified), calcareous or mixed lakes, non humic
- P13 Lowland, humic lakes on calcareous or mixed bedrock
- P14 Lowland siliceous lakes, non-humic
- P15 Lowland, humic lakes on siliceous bedrock
- P16 Mid-altitude, calcareous or mixed lakes, non-humic
- P17 Mid-altitude, humic lakes on calcareous or mixed bedrock
- P18 Mid-altitude siliceous lakes, non-humic
- P19 Mid-altitude, humic lakes on siliceous bedrock
- P1A Highland, calcareous or mixed lakes, non-humic
- P1B Highland, humic lakes on calcareous or mixed bedrock
- P1C Highland siliceous lakes, non-humic
- P1D Highland, humic lakes on siliceous bedrock
- P1E Temporary calcareous lakes, including non-humic and humic lakes
- P1F Temporary siliceous lakes, including non-humic and humic lakes
- P1G Temporary saline and brackish lakes
- P1H Permanent saline and brackish lakes
- P1J Glacier fed lakes
- P1K Marl/karst lakes
- P1L Volcanic lakes
- P1M Very large lakes
- P1N Ponds, pools & very small lakes

The updated EUNIS classification for Standing Waters habitats was originally inspired by the "Broad types" classification of lake waterbodies proposed by Lyche-Solheim et al. (2019). This system differentiated lake types based on several key factors, including altitude, catchment geology, water alkalinity, water colour, calcium concentration, surface area, mean depth, stratification/mixing regime, lithology, and geographical context, the latter differentiates the Mediterranean from the rest of Europe. Some of these lake characteristics, particularly those aligned with System A of the classification scheme under the Water Framework Directive (EC, 2000) have been used to define certain habitat types in the EUNIS classification. Other factors were intended to support further differentiation of Standing Waters habitats at Level 4, which, however, has not yet been developed.

The main factors used in the updated EUNIS classification at level 3 include:

- Altitude: lowland (below 200 m), mid-altitude (200 m to the tree line), and highland (above the tree line),
- geology types: siliceous; calcareous; mixed; organic/humic,
- depth: very shallow; shallow; deep, and
- lake size: pond, pools and very small lakes, very large lakes. Only these size classes were assigned to particular groups, while other sizes are distributed across various groups).

These factors and categories primarily differentiated among EUNIS habitat types P11, P12, P13, P14, P15, P16, P17, P18, P19, P1A, P1B, P1C, P1D, P1M, and P1N. Two additional discriminant factors, temporary hydroperiod and the salinity, were widely used to define specific groups, namely P1E, P1F, P1G, and P1H. In addition, some distinct groups were created to accommodate specialized lake types, including: P1J - Glacier fed lakes; P1K - Marl/karst lakes, and P1L - Volcanic lakes. Although certain habitat types have been cross-referenced between EUNIS classification to the HTCI (EEA, 2023), this is not yet the case for standing waters habitats.

Finally, when referring the SEEA Ecosystem Type Reference Classification, based on the IUCN Global Ecosystem Typology (GET), lentic habitats can primarily be assigned to the F2-Lakes Biome within the Freshwater realm. However, several Transitional realms may also be associated with the concept of a lentic ecosystem, such as TF1 – Palustrine wetlands and, in some cases, FM – Semi-confined transitional waters and MT1 – Shoreline systems (United Nations, 2021).

1.2 Environmental and ecological characterization and selection of variables to measure habitat condition

A basic principle for the assessment of habitat condition is to evaluate if the main general characteristics (e.g. the presence of permanently or temporarily open waters), as well as the specific discriminating features for the THCI (e.g. temporary hydroperiod for HTCI 3170*) remain minimally altered. For lentic habitats, if the permanent or temporary presence of open waters no longer exists where it was previously, then the habitat type has disappeared locally. In such a case, in the evaluation matrix of Art. 17 of Habitats Directive this will be considered as a habitat loss (under the parameter “Surface”) and not as a habitat condition degradation under the parameter “Structure and function” However, even habitats have not disappeared, these key ecological characteristics might have been degraded, and thus the HTCI, even if it remains, would present unfavourable conditions. Thus, beyond the (permanent or temporary) presence of non-flowing waters, the ecological features that serve to support the differentiation of the different lentic HTCI give additional insights what are the key ecological features are. These characteristic features must be preserved, while the evaluation of these features contributes to assessing the conservation status of the HTCI. The main ecological features of each HTCI are described in the European Habitats Interpretation Manual EUR28 (EC, 2013).

As described in section 1.1, lentic HTCI are defined by specific ecological characteristics—both biotic and abiotic—that distinguish them from each other. These include their physical setting, water chemistry, and associated plant and macroalgal communities. Such features indicate which variables should be measured to assess habitat condition.

1.2.1 Ecological characterization of standing water habitats

An ecosystem (habitat) type reflects a distinct set of abiotic and biotic components and their interactions (United Nations, 2021). The main characteristics of a habitat type are those that shape its main properties and, at least some of them, distinguish it from any other. These characteristics can be broadly classified into abiotic, biotic, and landscape characteristics. In turn, abiotic characteristics can be split into physical descriptors of abiotic components of a habitat (e.g., soil texture), and the chemical composition of abiotic components (e.g., nutrient concentrations in water). Similarly, biotic features can refer to the composition of ecological communities at a given location (e.g., species inventory of helophyte), the structural aggregate properties of the whole ecosystem or its main biotic components (e.g., fish sex ratio), and functional characteristics of biological, chemical, and physical interactions between the main ecosystem compartments (e.g., phytoplankton primary production). Landscape characteristics refer to features describing the interplay of habitat/ecosystem types in a mosaic at coarse spatial scales (e.g., inter-pond connectivity through waterfowl). For lentic habitats, some of the main groups of key features are the following:

- **Climatic**: largely determining the hydrological balance.
- **Geological**: mainly rock lithology both in the lake/pond/wetland basin and its catchment.
- **Geomorphological and pedological:** including geomorphological processes underlying the configuration, structure, and functioning of each specific lentic ecosystem and soil characteristics.
- **Hydrological**: such as the type of inflow and outflow, connection to groundwater, renewal rate, hydroperiod, and water level fluctuation.
- **Morphological/Physiographic**: such as depth, surface area, extension of the coastal area, perimeter, width, and shape index.
- **Physical and chemical characteristics of water**: including mineralisation, dominant salt type, pH, alkaline reserve, water transparency, possible vertical stratification, dissolved oxygen and hydrogen sulphide concentrations, and inorganic nutrients and organic matter concentrations.
- **Components of the biological communities' characteristics of these ecosystems**: which reflect environmental conditions such as submerged or emergent macrophytes, phytoplankton, phytobenthos, planktonic and benthic invertebrates, fish, and other vertebrates.
- **Community structural factors**: both in terms of the physical structure (e.g. plant zonation) in terms of structural aspects of the community (diversity, trophic structure).
- **Biological processes**: (primary production, respiration, ecosystem metabolism, and ecological interactions).
- **Exchanges with other ecosystems**: regardless of whether they involve energy, matter or organisms.
- **Anthropic factors**: including all kinds of interactions and effects of human activities.

A detailed description of the abiotic and biotic characteristics of lentic habitats, and the ecological processes that support their occurrence and functioning, follows. Their functional interplay and roles in ecosystem dynamics are explained in the respective paragraphs.

Abiotic characteristics

These include the physical and chemical characteristics of the habitat type and the description here also explain how these factors interact with other abiotic and biotic components of the ecosystem.

Physical characteristics

Climatic: Apart from the temperature, particularly the water temperature and its regime determined by the local climate, a key ecological factor for aquatic life, the consideration of other climatic variables is also necessary to establish the water balance of the lentic ecosystem. It is therefore necessary to determine in detail the volume of water input from precipitation, as well as the amount of water that lost from the system through evaporation and evapotranspiration. There are well designed methodological approaches for these purposes (see, e.g., Allen et al., 2006), and consequently the analysis of these variables can be approached with a reasonable degree of reliability. The problem lies, most of the time, in obtaining continuous data series over time and with the necessary cadence for their processing and exploitation. Another relevant climatic factor is wind. Not only its strength, but also the location of the waterbody basin in positions more or less exposed to wind action can be relevant to the functioning of the lentic ecosystem. For example, in deep lakes, the wind action determines the depth of the thermocline and can break vertical stratification through turbulent mixing. Key aspects of this factor include the waterbody's exposure or shelter from wind, the wind's strength and dominant direction at the site, and whether this direction coincides with the waterbody's longest axis. In many cases the wind, as a wave inducer, can lead to shoreline erosion. In fluctuating systems in which complete desiccation of the basins occurs during the dry season, wind deflation processes may also be important.

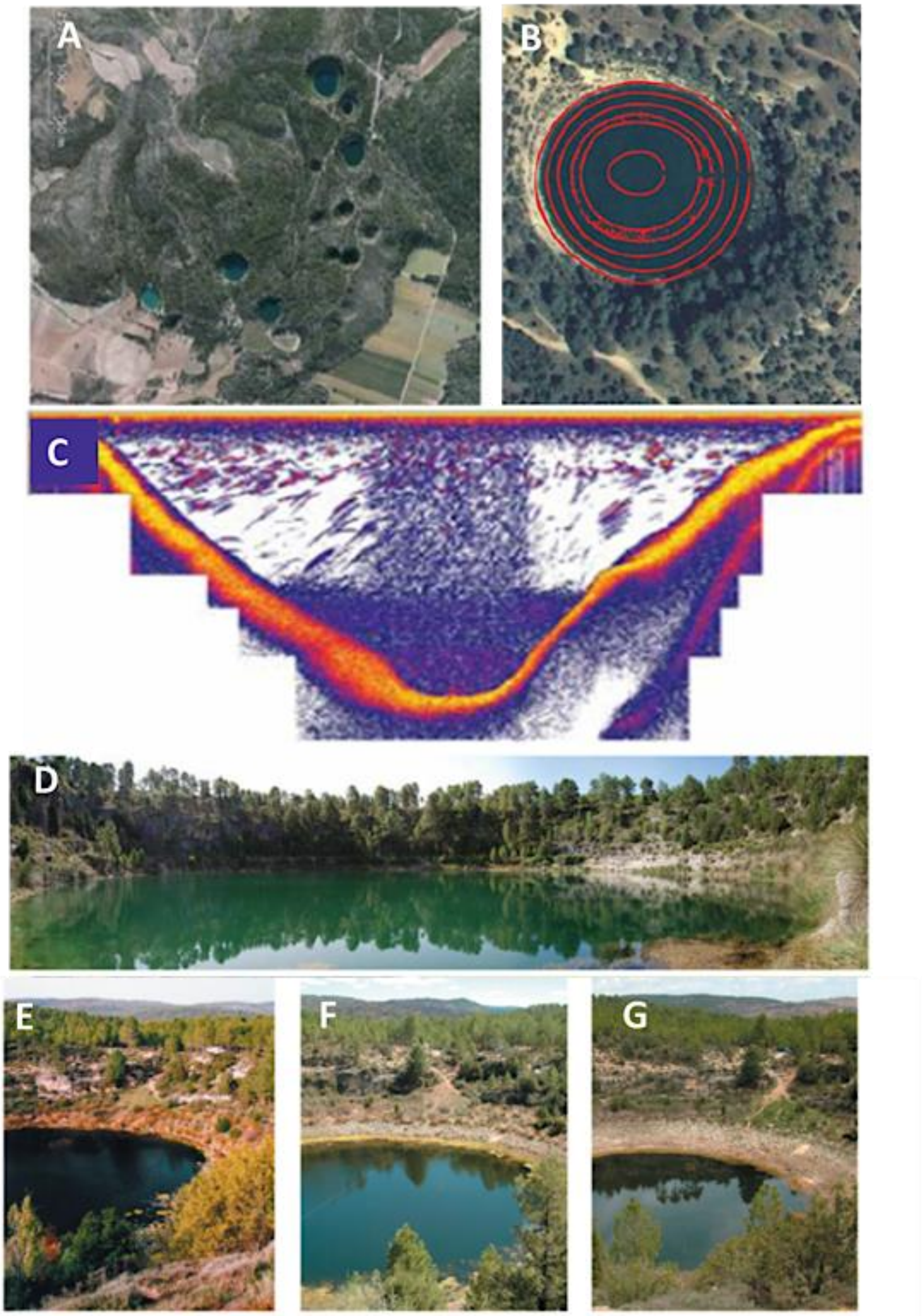
Geological: The characterization of geological factors focuses on the analysis of three fundamental elements: lithology, structural elements, and the properties of the aquifer over which the lentic ecosystem is (eventually) located.

a) Lithology: The substrate underlying the basin and its drainage catchment is a key factor for lentic ecosystems. The contributions of mineral salts come mainly from the surface and underground washing of the materials that constitute the substrate. The solubility of the rocks in the catchment is the main factor, affecting the transfer of dissolved salts to the water, whose total amount can be measured through its electrical conductivity. Additionally, aspects such as porosity, erodibility, propensity to dissolution (karstification), etc., are properties of the substrate materials that have direct consequences on the genesis and development of lentic ecosystems.
b) Structure: The conditions that favour the presence of elements of structural features in the landscape those derived from tectonic and seismic activity. The structural models that lead to the appearance of lentic ecosystems are fundamentally tectonic in origin, producing rounded depressions (pits) and elongated depressions (fractures). Additionally, recent post-orogenic readjustment processes (neotectonics) can also form shallow depressed areas that are sometimes occupied by shallow lentic ecosystems.
c) Hydrogeological (aquifer properties): The reference aquifer on which (if applicable) the lentic system is located and the relationship between the two is highly important. It can be a free, confined or semi-confined aquifer, and may have different petrographic characteristics, such as being detrital or karstic. Each of these characteristics result in differences in functioning, so lentic ecosystems related to aquifer will also display differentiated behaviour. lentic habitats can appear both in the recharge areas of the aquifer and in the discharge areas. Both ends of the gradient are controlled by the

hydraulic flow systems within each aquifer. Whether a lentic ecosystem is found in one position or another determines important differences in its hydrological functioning.

Figure 3. Physical abiotic features of a series of karstic sinkholes lakes in the complex of Cañada del Hoyo (Cuenca, Spain)

(A) Aerial view of the lake complex. (B). Hypsographic curves of Laguna de La Cruz. (C) Bathymetry of Laguna de La Cruz. (D) Photograph of Laguna de La Cruz. (E-G). Variations in the water level of Lagunillo del Tejo along three consecutive years.



Geomorphological and pedological: From the geomorphological point of view, the fundamental elements to consider are:

a) Morphogenetic system. The characterization of the spatial-temporal combination of geomorphological processes (morphogenetic system) underlying the formation of the lake basin constitutes a prerequisite for determining the genetic type of the lentic habitat. All genetic types display a positive water anomaly. The characterization of the morphogenetic system provides the framework for explaining the main natural patterns through which a lentic ecosystem is generated and evolves are explained, as it is essential for the configuration of the basin and in the distribution of surface and groundwater within the basin, as well as the responses of soils, vegetation, etc. (Borja et al., 2000).

b) Morpho-dynamic system. Within morphogenetic systems, recurrent combinations of natural mechanisms are usually recognized which determine the existence of different operating scenarios (morpho-dynamic systems). That is, within each morphogenetic system, a series of specific geomorphological processes can be identified which consistently drive and shape evolutionary trends of the lentic ecosystem, establishing also an order of priority in the processes.

c) Relief and shape formation. Modelling of basins refers to the shapes and dimensions of lentic ecosystems present, and although morphological convergence may sometimes occur, it is usually closely linked to the set of processes that govern their genesis, evolution, and dynamics (Lehner, 2024). As a result, over time, lentic ecosystems inevitably undergo alterations in their forms. Changes in the size and adjustments to the morphometry of the lake basin usually bring about modifications in the surface extent and water column, respectively. The morphology of the basins has important effects on practically all main physical, chemical and biological parameters of aquatic systems, influencing the nature of drainage, the entry of matter and energy into the system, the renewal rate, hydrodynamics, etc. (Wetzel, 2001). For example, the relationship between the volume of the waterbody and its surface, as well as other morphometric characteristics (Figure 3), can determine the eutrophic effect of a given nutrient load is different in systems with different morphological characteristics.

   The definition of the shape of the lentic ecosystem constitutes the first step in morphometric characterization. To do this, it is necessary to measure the main elements that define the shape, such as the perimeter of the lentic ecosystem, the length of the major axis and the width measured perpendicular to the major axis. These data provide information on the dimensions of the system. Likewise, the shape index or development of the coastline can be calculated to indicate the degree of irregularity of the coastal area of lentic ecosystem, based on the ration between the real length of the coastline (perimeter) and the length of a circumference whose area is equal to that of the lentic ecosystem (Håkanson, 1981).

   The total surface occupied by the lentic ecosystem is also relevant as a determinant of its functioning, since it is through this area that energy (light radiation, wind...) and material exchanges with the atmosphere occur. Measurements of the permanence and extension of the water table, the distribution of plant formations, etc., in highly fluctuating ecosystems are, in most cases, a difficult task. It is equally necessary to determine the maximum depth of the lentic ecosystem as well as the average depth and relative depth. For this, it is advisable to carry out a bathymetric survey of the system, which provides information on the shape of the lake basin. This information makes it possible to define the surface/volume relationship, which clearly differentiates the functional lake groups, shallow and deep lakes. The relationship between surface and volume determines the capacity for exchange of matter and energy with the atmosphere. Generally, the greater the relative depth (or the lower the surface/volume ratio), the greater the resistance to vertical mixing in deep systems (Wetzel, 2001), resulting in more stable stratification of lakes. Bathymetric surveys also provide information on the slope of the littoral zone and banks, which influences the distribution of submerged and emergent macrophytic communities and other benthic flora. The existence of littoral areas where depth and turbidity are not excessive allows the development of macrophytes rooted in the substrate when sufficient light reaches it. The gradient of the slope also determines a zonation of these communities (Keddy, 2023), with changes in the dominance of species as the depth varies, so that a gentle slope with gradual changes in depth is likely to support a greater diversity of rooted macrophytes.

d) Surface formations (sediment deposits and soils). Surface formations include deposits and soils, which can be understood as correlative formations resulting from a series of geomorphological processes in the first case (morphogenesis) and edaphic processes in the second (edaphogenesis).

   In lentic ecosystems, it is necessary to distinguish between deposits linked to the formation of the basins themselves (for example, alluvial lagoons where the basin is carved into a fluvial terrace-type surface formation), and accumulations at the bottom of flooded depressions, whether under a sedimentary regime of a lacustrine or palustrine nature. These are the ones that should be understood as true correlative sediments of the lake sedimentary environment, from which information regarding the functioning of the lentic ecosystem can be extracted.

   The same applies to soils. Among them, it is necessary to distinguish, on the one hand, soils that have evolved exclusively under the influence of the humid zone (generally hydric soils, exposed to saturation conditions, and/or saline soils, adopted to environmental aridity and deficient water balances); and, on the other hand, soils linked to the zonal catenas, more or less subject to the lithological nuances, typology, drainage conditions, etc., of the surfaces on which they develop, and which evolve outside the saturation conditions caused by the presence of a lentic ecosystem.

e) Siltation. The contribution of terrigenous materials has a special impact on the shallower areas of lentic ecosystems with gentle slopes, since these materials are deposited there, reducing depth, and potentially altered habitat structure. The importance of this process also depends on the type of water supply to the ecosystem, since only systems with surface feeding are, in principle, susceptible to receiving significant contributions of allochthonous particulate materials from point source influents. However, in the absence of surface water courses, diffuse surface runoff can be an important factor in contributing to siltation.

   The amount of sediments is closely related to the characteristics of the soils in the basin and their vegetation cover, semi-arid areas or areas subject to overgrazing are often a source of substantial sediment loads in runoff waters. The growth of macrophytes in coastal zones of lentic ecosystems, and the precipitation of carbonates associated with them, if high, can also produce a decrease in the depth of the coastal zone (carbonate coastal platform) due to the accumulation of materials. Even though the ultimate fate of lentic ecosystems, unless the contribution of materials to the sediment is compensated by the land subsidence, is ultimately to become clogged over time, the alteration of erosion patterns and sedimentation in the basin due to anthropogenic causes can significantly accelerate this natural clogging process. In many cases, a very significant increase in the contribution of sediments to lentic ecosystems has been observed during the last centuries – and since the mid-20th century – due to expanded agricultural activity in the drainage basin.

Hydrological: The temporary or permanent presence of water is the key factor defining a lentic ecosystem (lake, lagoon or wetland), and the alterations in hydrological patterns are the major cause of the degradation of many wetlands. The presence or absence of water determines wetland behaviour, dynamics and evolution (Keddy, 2023; Sidle & Gomi, 2024), and consequently natural hydrodynamic patterns must be preserved to achieve conservation of these ecosystems (Ramsar Convention Secretariat, 2007).

Understanding the hydrology of a lentic system requires the establishing of its water balance, that is, quantifying the water flows that enter and leave the lentic ecosystem and determining the mechanisms responsible for these inflows and outflows. However, while this is essential, it is more interesting from an ecosystem point of view to characterize the relationships between these volumes of water and the rest of the elements that make up the ecosystem.

The composition of the vegetation and fauna of a lentic ecosystem is more sensitive to factors such as depth of the water, the extent and regularity of flooding, the origin and chemical composition of the water, the waterbody feeding modes and emptying modes, and renewal rate than to the total volumes of incoming or outgoing water. In this sense, and at a detailed scale (individual lentic ecosystem), the most important hydrological control factors are the feeding mode, the emptying mode, the hydroperiod and the rate of renewal.

a) Feeding mode. The feeding mode refers, on the one hand, to the origin of the water inputs, whether from surface sources (surface runoff, direct precipitation into the basin, etc.) or groundwater; and, on the other hand, to the method of water supply, e.g., type of discharge (rain/melt, surface runoff, underground contributions from local/regional, short/medium/long-haul aquifers; etc.). Maintaining the ecological integrity of these ecosystems, including water renewal, salt content, chemical characteristics and, hydrodynamics, depends on feeding mode. Depending on the origin of the water, three types of feeding modes can be distinguished:
   - Epigenic: if the main contribution is surface water, coming from direct precipitation into the lentic ecosystem or from surface runoff (rivers, streams, channels). Epigenic lentic ecosystems often show marked water level fluctuation.
   - Hypogenic: if the main contribution is groundwater originated from local (small) or regional (large) free aquifers, or from confined or semi-confined aquifers. Combinations of the above can also occur. The type of aquifer underlying the lentic ecosystem affects the magnitude of the water flows that feed it, and this, in turn, determines the temporal variability of both the flows supplied and the mineralization of the water.
   - Mixed: refers to intermediate situations where it is difficult to determine whether the main flow is from surface or groundwater source. These cases correspond to mixed feeding systems.

b) Emptying mode. The mode of emptying or drainage of the lentic ecosystem, together with the mode of filling, defines its hydrological regime. Two basic types are can be distinguished:
   - Open drainage: lentic ecosystems that primarily lose water through liquid-phase flows, occurring either above the topographic surface (rivers, streams), or below it (recharge of the aquifer, springs) and;

- Closed drainage: lentic ecosystems that lose water in the vapor phase, either directly through evaporation, or through vegetation mediated evapotranspiration.

c) Hydroperiod pattern. It represents the frequency and persistence of the presence of water in the basin (or even in the soil in the case of crypto wetlands, seepage areas or hidden wetlands). The following main types can be recognized (except for tidal coastal wetlands whose flooding pattern is diel, depending on the tide floods): i) permanent, non-fluctuating; ii) permanent fluctuating; iii) temporary seasonal; and iv) temporary ephemeral. The climatic factor strongly influences the hydroperiod, since the rainfall/snowfall regime directly affects recharge, while evaporation losses are also strongly climate dependent.

d) Renewal rate. The renewal rate (or conversely, the residence time) can be decisive in the ecological behaviour of the system, since, among other relevant limnological characteristics, and it depends on the main mode of water supply, and also determines washing processes ("washout") in planktonic populations and the dilution/concentration of substances present in the water.

Light, water transparency and colour: Light, particularly the visible part of the spectrum (from around 400 to 700 nm of wavelength, the so called "Photosynthetically Active Radiation", PAR) is essential for photosynthesis, the key biological process fuelling most ecosystems. Light penetration in water is one of the main factors determining the capacity for photosynthetic primary production (Figure 4). The photic zone of a lentic ecosystem is defined as the layer in which primary production exceeds heterotrophic consumption, the "trophogenic" layer, whereas below the photic zone most processes are heterotrophic and consumption dominates (tropholytic layer). In the water column of deep lakes, this boundary is typically near the depth where approximately 1 % of surface irradiance penetrates.

Water transparency is primarily determined by the amount of suspended solids (inorganic e.g., soil and rock particles transported by rainfall into the lentic waterbody, or organic, e.g., detritus, phytoplankton, etc.). Water transparency can decrease due to the massive growth of phytoplankton (producing a blue or blue-green colour), the presence of suspended mineral particles (typically grey to brownish colour), or a high concentration of dissolved coloured (tea-coloured) substances, known as chromophoric organic matter or C-DOM (Rose, 2024). Changes in water transparency, may alter the functional characteristics of lentic ecosystems are, related to the massive growth of phytoplankton in surface waters giving a green or blue-green colour to the waters, often resulting from eutrophication which can greatly reduce light penetration (Camacho, 2006). On the other hand, suspended materials from surface inputs also reduces transparency.

In addition to the turbidity generated by the growth of phytoplankton or by external contributions, certain types of systems can also be characterized by high turbidity, in sometimes due to shallow depth facilitating the resuspension of sediments due to the agitation produced by the wind or, even in the case of those installed on clay substrates, due to formation of permanent turbidity from clay's capacity to create stable colloids in water. Increased turbidity reduces light availability to submerged aquatic macrophytes, and cause their decline or eventual loss, with resulting changes in the whole ecosystem structure and functioning.

Chemical characteristics

Water mineralization (salinity): Water mineralization is the total concentration of dissolved salts in water, resulting from the dissolution of the materials through which water circulates and, therefore, are related to the lithology of the catchment basin of lentic ecosystem.

The electrical conductivity (EC) of water, a physical variable, is an indirect proxy for its saline content (a chemical feature), directly related to the total concentration of dissolved salts. EC is measured in Siemens (S), the inverse unit of the Ohm per cm (distance that separates the poles of the standard electrode), although due to the range of values in water it is usually expressed in µS/cm or mS/cm.

The measurement of salinity generally involves determining the concentrations of dissolved salts and summing them to obtain the total salinity of the water, although an approximation of the total salinity can be made from conductivity values. The waters of lentic ecosystems, depending on the type of system, show a wide range of mineralization, from very little mineralized waters in mountain lakes and ponds (barely 0.01 mS/cm) to concentrated brines (more than 100 mS/cm).

Regarding seasonal dynamics, the fluctuation of mineralization throughout an annual cycle may be link the water exchanges regime. Changes in mineralization can influence the colonization of species in the ecosystem, as well as the precipitation of mineral phases. Likewise, differences in the concentration of salts in the vertical profile of deep systems, or those generated by the influents, can generate strong density gradients that make it difficult or impede the mixing of the entire mass of water, producing meromictic conditions (Wetzel, 2001), or the generation of complex gradients and/or compartments, more or less isolated, on a temporary basis. Among the classifications of athalassohaline (saline but not influenced by the sea) lentic ecosystems based on the mineralization of their waters, the most used is that of Hammer (1986).

The Hammer classification uses salinity values (g/l), but its quick measurement is usually based ion electrical conductivity. Although there are variations in the relationship between conductivity and salinity depending on the dominant ions in the saline solution, the measurement of conductivity can be used as an approximation for the classification of these ecosystems according to the mineralization of their waters. Thus, it is generally considered that fresh waters do not exceed about 1 mS/cm, subsaline waters range between 1-3 mS/cm, hyposaline waters range between 3 and 20 mS/cm, mesohaline waters between 20 and 50 mS/cm and waters exceeding 50 mS/cm are considered hypersaline, meaning more saline than average seawater salinity (35 g/L).

Inland waters do not necessarily correspond to freshwater, since, as noted, water mineralization responds to a complex interactive pattern of rock dissolution, salt precipitation or blow-out, and dissolution and evaporation processes within the waterbody.

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Type of dominant salts: The type of rock/substrate in the catchment and the climate determine the concentration and dissolved salt composition of the surface and groundwater that feed the lentic system. The main components in continental waters are salts containing calcium, magnesium, sodium or potassium as cations, and bicarbonate, sulphate or chloride as anions (Pawlowicz & Yerubandi, 2024). The chemical weathering of rocks and the washing of soils through which the water passes before reaching the lentic ecosystem supply salts to the water. Their relative abundance in both, quantity and in the type of elements, varies depending on the solubility and composition of washed materials. For example, the most soluble rocks, such as limestone, dolomite and gypsum, provide significant amounts of calcium and magnesium bicarbonates (the first two) and calcium sulphate (in the case of gypsum) in line with their mineral compositions, while less soluble siliceous rocks provide much lower amounts of salts.

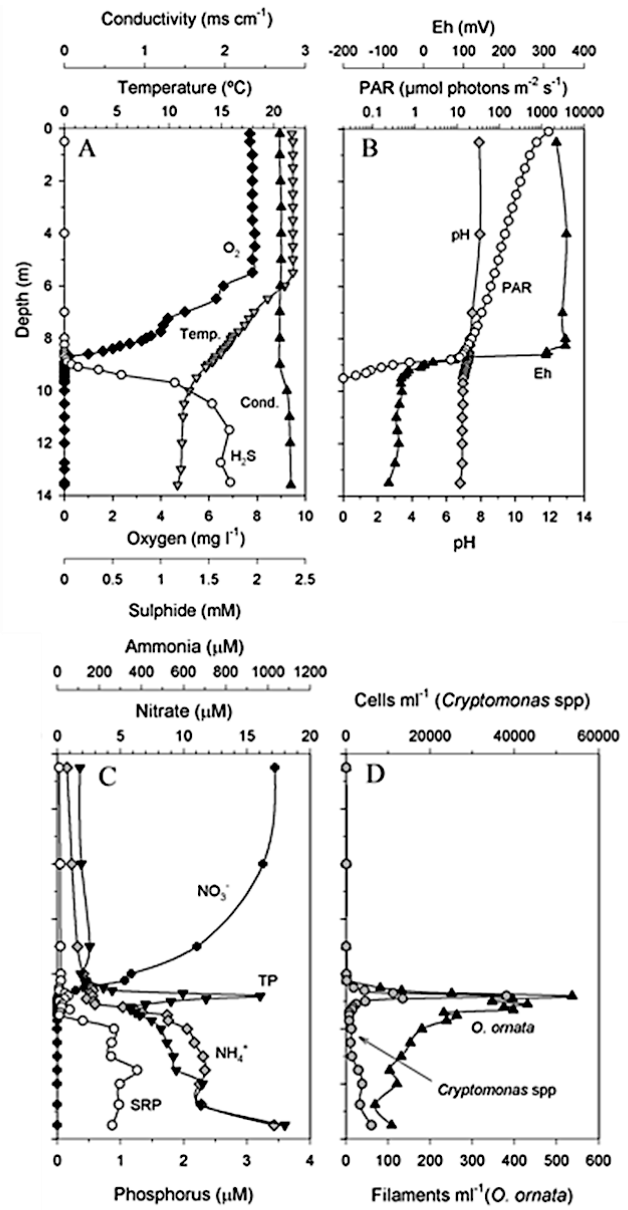
Vertical stratification: In deep-enough lentic ecosystems, certain climatic conditions, such as differential heating or cooling of surface layers, combined with the lake basin's morphometry and the surrounding topography can favour water column stratification (Kalff, 2002). Stratification occurs when the depth is great enough to resist complete mixing by wind or other physical and climatic forces, creating water masses with different physical-chemical properties, and therefor different biological communities, at different depths. Stratification of the water column due to density gradients arise from differences in temperature (MacIntyre &

Hamilton, 2024) and/or salinity between surface and deep waters. These gradients divide the lake water into vertical layers:

- Epilimnion – the well-mixed water surface layer;
- Metalimnion – the vertical transition zone with a sharp density (thermal) gradient (thermocline);
- Hypolimnion – the deep, typically cooler and less mixed water layer;
- Monimolimnion – in meromictic lakes, a permanently isolated bottom water layer that does not mix annually (Wetzel, 2001).

Key ecological gradients in stratified lakes (e.g. Figure 4) include increasing nutrient concentrations with depth, and decreasing light availability (Rose, 2024), temperature (in direct stratification), and dissolved oxygen as depth increases (Marcé et al., 2024).

Figure 4. Vertical profiles of various limnological variables in Lake Arcas, a 14 m deep gypsum karst lake (HTCI 3190)



Source: Camacho (2006)

In temperate latitudes, sufficiently deep lakes (generally more than 5-6 meters, depending on morphometry) usually develop vertical stratification of thermal origin that lasts from spring until late autumn, caused by differential heating of the upper layers of water, which reduces their density. In autumn, cooling and the weakening of the density gradient, together with meteorological factors such as wind, cause the vertical mixing of the waters (Camacho, 2006).

In lakes covered by ice in winter, such as many high mountains or polar lakes, there is also a period of winter stratification that lasts until the ice melts (see, e.g., Toro et al., 2006; Granados et al., 2020). Measuring the vertical conductivity profile in a lake can reveal vertical stratification beyond thermal layering, as salinity gradients may maintain density differences and thus prolong stratification. If deep waters have a sufficiently large salinity difference from surface waters, the resulting density gradient can sustain stratification for extended periods.

There are indices of the strength and stability of the stratification, such as the Brunt–Väisälä frequency, a measure of the stability of a fluid to vertical displacements, and the Schmidt stability index, that reflecting the resistance to mechanical mixing caused by the potential energy of the water column during stratification (Hamilton & MacIntyre, 2024). The Brunt-Väisälä frequency represents the angular frequency at which a vertically displaced parcel oscillates within a statically stable environment, whereas, the Schmidt stability index, quantifies the amount of work required by the wind, or other sources of energy, to overcome thermal stratification, providing a single value for the lake as a whole (Bertone et al., 2020).

Dissolved oxygen and hydrogen sulphide: Atmospheric oxygen dissolves in water through physical processes until it reaches a saturated concentration determined by water temperature, salinity, and the partial pressure of oxygen in the atmosphere. However, the concentration of oxygen dissolved in water is regulated by biological activities that produce and consume oxygen, through a balance between respiratory consumption, photosynthetic contributions, and dissolution from the atmosphere. The concentration of oxygen in water both influences and is influenced by the decomposition of organic matter accumulated at the bottom of lakes and ponds. Oxygen is used as an electron acceptor in respiration by aerobic organisms that consume it, obtaining energy from organic matter. The high energy yield of aerobic oxidation of organic matter makes aerobic processes the main pathway for the breakdown of organic matter in waters with sufficient dissolved oxygen.

On the other hand, oxygen is released through the photosynthetic activity of the primary producers that carry out oxygenic photosynthesis, that is, using water as an electron donor and releasing oxygen, the typical photosynthetic mode of the plants and microalgae that inhabit these ecosystems. Thus, the concentration of oxygen dissolved in water is determined by a balance between respiratory consumption, photosynthetic production, and exchange processes with the atmosphere. The effect of these exchanges largely depends on the turbulent mixing of the waterbody layers, and therefore, is reduced in the subsurface and deep layers (meta- and hypolimnion) in stratified lakes, which may remain in hypoxic or anoxic conditions after a certain period during the course of stratification (Figure 4). Likewise, the sediments of many aquatic systems, particularly the deep ones, can remain practically anoxic, as the consumption of deposited organic matter depletes oxygen, and the lack of diffusion prevents its recovery.

Regarding waters, the daily variation in concentration of dissolved oxygen in surface waters (Hutchinson, 1957) is indicative of the level of degradation in a lentic ecosystem due input of organic matter and inorganic nutrients (eutrophication). In eutrophic environments, high nutrient availability promotes the growth of primary producers (especially phytoplankton, microalgae that live suspended in the water). In extreme cases of eutrophication (hypertrophy), phytoplankton, with its great abundance and through its photosynthetic activity, can increase the oxygen concentration during the day far over the equilibrium levels with the atmosphere (well above 100% saturation). However, during the hours of darkness, when there is no photosynthetic production of oxygen, the large amount of biomass accumulated at high trophic level generates a large demand for oxygen for respiration, potentially reducing oxygen to very low concentrations, or even anoxia, causing hypoxic stress for aerobic organisms and, at severe cases, mortality from asphyxiation.

This is why the variation in oxygen concentration throughout the daily cycle can be a metabolic functional proxy for estimating the trophic state of the lentic ecosystem. On the other hand, under anoxic conditions, organic matter decomposes anaerobically through slower processes and, consequently, accumulates more easily in environments lacking electron acceptors. In these reducing environments, the sulphate ion, when abundant (as in karst lakes on gypsum, which correspond to HTCI 3190 of Annex I of the HD), is used as an electron acceptor in the anaerobic respiration of organic matter by sulphate-reducing bacteria, resulting in the production of hydrogen sulphide. This compound can accumulate in deep waters during stratification (Figure 4) and can be used by sulphur oxidizing bacteria, whether photosynthetic or not (Camacho, 2009), and can also lead to formation of metal sulphides (e.g. pyrite). The mixing of waters and variations in the oxygenation conditions at the bottom of lakes and ponds can cause the rapid release of hydrogen sulphide, with the consequent ecological impact, as well as the oxidation of part of the organic matter accumulated at the bottom.

Concentration of inorganic nutrients (N and P compounds): Phosphorus and nitrogen compounds are, among the elements necessary for the generation of biomass by primary producers, those with a relatively lower natural availability in inland waters compared to their relative demand (Kalff, 2002¸ Keddy, 2023). An increase in their availability due to anthropogenic inputs therefore allows greater growth of these producers, in a process known as **eutrophication**. Naturally, the concentrations of compounds of some elements necessary for the growth of photosynthetic organisms, such as nitrogen and phosphorus, are generally low, limiting primary production, especially that of primary planktonic production (Kalff, 2002).

Under conditions of nutrient limitation, macrophytes develop more easily, since they can obtain nutrients from the sediment, which is naturally richer in nutrients than water, while the high transparency of the water under limited phytoplankton allows enough light to reach the submerged macrophytes. An increase in the concentration of nutrients is generally caused by surface water flows or by the aquifer (relatively richer in nitrogen because of the lower solubility of phosphorus), although in certain cases nutrient deposition from the atmosphere can be relevant. This promotes greater growth of phytoplankton, with the consequent increase in turbidity and reduction in light penetration (Rose, 2024), placing macrophytes at a disadvantage (Talling, 2003). Then, macrophytes, and consequently the habitats they create, are damaged or even disappear, leading to consequent decrease in biodiversity in the ecosystem.

The same process can occur if, for any other reason, macrophytes disappear from the system, eliminating their competition for nutrients with phytoplankton, and allowing the latter to monopolise the available nutrients, thus promoting stronger phytoplankton growth. On the other hand, nutrients accumulate in the sediment, constituting the so-called internal load, which may or may not be progressively released depending on the biogeochemical processes taking place in the sediment (Corrales-Gonzalez et al., 2019). In stratified lakes, these nutrients are depleted in surface waters throughout the stratification period (unless there is a sustained external contribution that compensates for consumption), while accumulating in deep waters (Figure 4) as result of mineralization of organic matter that is brought to the bottom by the action of gravity.

Organic matter: In waters loaded with easily biodegradable organic matter, its mineralization generates a demand for dissolved oxygen, which reduces the availability of this gas, which is necessary for the respiration of aerobic aquatic organisms. The most easily biodegradable organic matter often comes from anthropogenic inputs, such as wastewater. In contrast, the organic remains from the vegetation of the basin have a higher proportion of recalcitrant organic matter (Hotchkiss & DelSontro, 2024), making it less useful for biological consumption, implying a lower demand for oxygen and causing fewer hypoxic problems.

The accumulation of this recalcitrant organic matter confers dystrophic characteristics to these waters (Mladenov et al., 2011), including the characteristic brown colour that reveals the presence of humic substances, a feature typical of dystrophic lakes (THIC 3160) in the Habitats Directive. Further, the sediments such as accumulate organic matter in excess, both from autochthonous primary production and external inputs not degraded within the water column and or in surface sediments, as the rates of organic matter degradation strongly decrease after burial because it is more refractory (since easily degradable fractions has already been consumed) and due to lower availability of electron acceptors (or those available being energetically less productive). However, this excess organic matter can be a source of nutrients through mineralization and may promote new oxygen demand when it is released from these restrictive conditions.

pH and alkaline reserve (acid neutralizing capacity), and redox potential: The pH of water is determined by both dissolved salts and the physical-chemical and biological processes occurring in the waterbody. The waters of calcareous areas are rich in bicarbonates, which give them a slightly alkaline pH (7.5 – 8.5). In basins formed from siliceous materials, however, the input of these salts is small, and consequently the pH of the water can be neutral or, in some cases, slightly acidic.

Biological activity can considerably modify the pH of water, especially in poorly buffered systems (low alkalinity), since the release of CO_2 during respiration or its uptake during photosynthesis can increase or decrease dissolved CO_2 concentrations. Dissolved CO_2 forms carbonic acid and participate in an acid-base balance with bicarbonates and carbonates, the main component of the alkaline reserve (Cole & Prairie, 2024). In this way, photosynthesis tends to increase pH, while respiration tends to reduce it. Additionally, the release of acidic substances, resulting from the metabolism and degradation of organic matter or the accumulation of humic acids, can cause acidification of the water, as occurs in some of the dystrophic systems characteristic of HTCI 3160.

On the other hand, anthropogenic activity can also cause acidification of aquatic ecosystems. Acid deposition is mainly a consequence of atmospheric deposition arising from nitrogen and sulphur oxides that, when mixed with cloud water, produce strong acids that fall with rain, acidifying the environments where they precipitate. Acidification, in addition to its direct effects on living organisms, has other indirect effects on the biota that are equally or even more important, associated with changes in the solubility of compounds relevant to life, either by serving as nutrients, or by increasing the effects of toxic elements, whose bioavailability can be altered by variations in pH (Molot & Dillon, 2024).

Acidification has especially serious effects on the soils and waters of land formed by poorly soluble rocks, such as siliceous rocks, compared to calcareous rocks, because the latter provide bicarbonates to the water, which have a neutralizing function against the acidification. On the other hand, redox potential refers to the tendency of a chemical species to be reduced by accepting electrons or oxidized by donating electrons. Inorganic oxidants include oxygen, nitrate, nitrite, manganese, iron, sulphate, and CO_2, while the reductants include various organic substrates and reduced inorganic compounds (DeLaune & Reddy 2005). Redox potential is a parameter used to describe a system's overall reducing or oxidizing capacity (Søndergaard, 2009) and is therefore being highly informative about the biogeochemical status of waters and sediments in any aquatic ecosystem.

Biotic characteristics

This section describes the main biotic components and characteristics of the habitat type, including how these factors interact with other abiotic and biotic components of the ecosystem. Although grouped here under biotic characteristics to align with the project’s logical structure, many of the structural and, especially functional characteristics, as well as most of the variables used to assess ecosystem conditions, are actually ecosystem-level in nature and therefore encompass both biotic and abiotic interactions.

Compositional characteristics

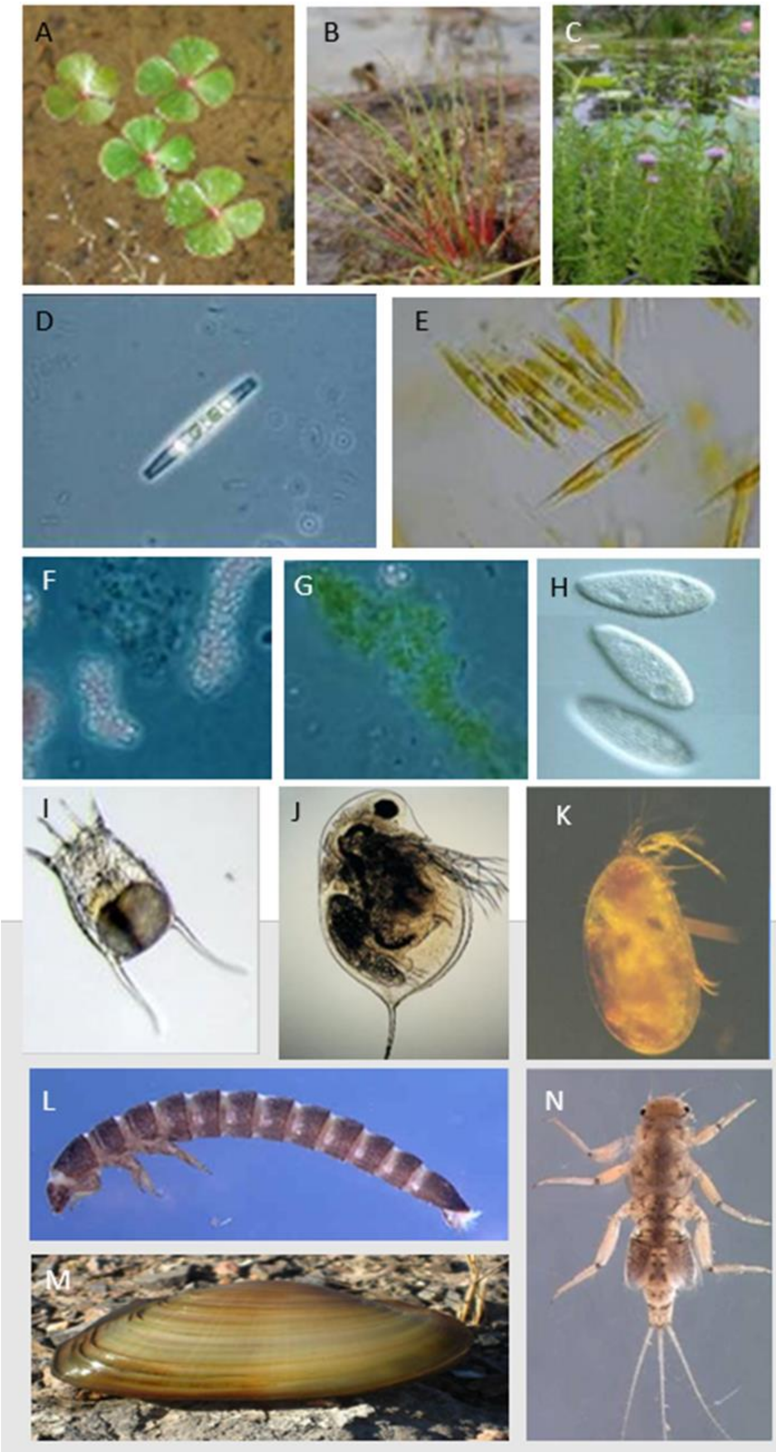
This section describes the main biological components of lentic ecosystems, grouped either by taxonomy or by life modes or functional groups. The components of the biological community’s characteristics of these ecosystems, such as submerged or emerging macrophytes, phytoplankton, phytobenthos, planktonic and benthic invertebrates, fish, and other vertebrates, respond to environmental characteristics, while also shaping the habitats features.

Macrophytes: Aquatic macrophytes (aquatic plants and macroalgae) can thrive in areas where sufficient light reaches the depths et which grow. Macrophytes increase habitat heterogeneity, offering spaces for colonization, food and shelter to numerous species of plankton and nekton, thereby enabling the ecosystem to host a more diverse biological community (Stefanidis et al., 2019). In this way, macrophytes act as structuring species in the areas of the lentic ecosystem that host them, but also respond to environmental changes in the habitat condition, providing insights into these processes (e.g. hydrology, Camacho et al., 2016). Macrophytes, can in principle, occupy the entire basin in shallow systems and typically only the littoral zone in deeper ones.

Plants associated with lentic aquatic ecosystems include two basic types: those with leaves submerged or floating (**hydrophytes**) and those that grow in the saturated zone of the shores, with both leaves and inflorescences emergent but rooted underwater (**helophytes**). Hydrophytes can represent different biological forms, some rooted and others free-floating. Helophytes, or at least some of them, do not necessarily require the existence of flooding; in many cases, a water table close to the surface is sufficient for their development, as in case of rushes, grasses, and sedges.

Some plants have an amphibious character, flowering usually during the emergence period but capable of completing their life cycles submerged thanks to adaptations to these conditions. This is the case of *Littorella uniflora*, a taxon characteristic of HTCI 3110. In general, many aquatic plants, especially those from environments with temporary flooding, present ecological characteristics that allow them to survive the unfavourable season (Figure 5). Therefore, the hydroperiod determines the development of these communities (Fernández-Aláez et al., 1999), and macrophytes can serve as indicators of hydrological alterations.

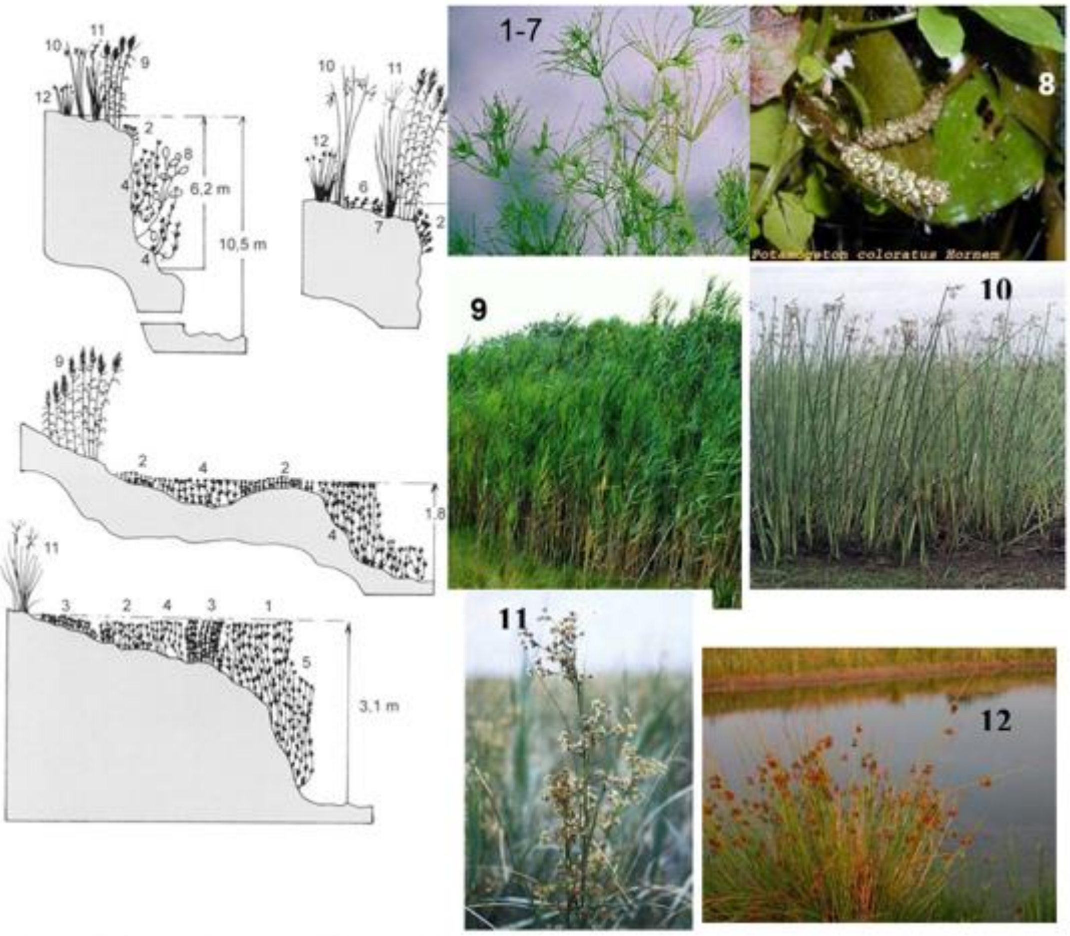
Figure 5. Some taxa characteristic of lentic ecosystems



A-C) Characteristic plant species of HTCI 3170 – Mediterranean temporary ponds: (A*) Marsilea strigosa, (B) Juncus pygmaeus, C) Mentha cervina; (D-E) Diatoms: (D) planktonic and (E) benthic; (F-G) Photosynthetic sulfur bacteria: (F) purple and (G) Green; (H-J) Zooplankton: (H) –Paramecium sp. (ciliated protozoan), (I) Keratella sp. (rotifer), (J) Daphnia sp. (cladoceran); (K-N) Zoobenthos: (K) Ostracod, (L) Coleopteran larvae, (M) Bivalve, and (N) Ephemeropteran larvae*

Sessile macrophytes are gradually distributed along the depth gradient around the banks according to their ecological characteristics (Figure 6), including helophytes (emerged), which grow on the banks, and the hydrophytes (submerged or floating), which colonize the flooded areas, rooted up to several meters deep. The distribution of the main helophytes in a lentic ecosystem is a clear landscape-forming feature and must be considered in relation to habitat alterations. The connectivity of plant formations support interactions between different biological components of the ecosystem (invertebrates, birds, etc.). Therefore, it is important to characterize the degree of fragmentation of helophyte communities commonly dominated by plants from genera like *Phragmites, Typha, Cladium,* and *Scirpus*. In addition, to the specific aquatic plants linked to the lentic ecosystem itself, shrubs and trees forming riparian communities along lake and ponds banks can also play important roles. For example, they can help reduce the input of contaminants and nutrients from runoff into lentic ecosystems.

Figure 6. Scheme of the spatial distribution in several lakes within the Arcas complex, HTCI 3190



1. *Chara aspera*, 2. *Chara aspera* var. *curta*, 3. *Chara canescens*, 4. *Chara hispida* var. *major*, 5. *Chara hispida* var. *major* f. *crassicaulis*, 6. *Chara vulgaris*, 7. *Chara vulgaris* var. *longibracteata*, 8. *Potamogeton coloratus*, 9. *Phragmites australis*, 10. *Scirpus lacustris* subps. *tabernaemontanii* 11. *Cladium mariscus*, 12. *Schoenus nigricans*.

Source: Modified from Cirujano and Medina (2002)

In shallow systems, the balance maintained by the presence of submerged macrophytes (hydrophytes) is unstable within certain ranges of ecological conditions. Within these ranges, the ecosystem can shift from phases with macrophyte coverage to others in which they disappear, and planktonic primary producers dominate (turbid phase) once thresholds of specific ecological factors are exceeding (Scheffer & Carpenter, 2003): The long-term maintenance of turbidity conditions due to phytoplankton dominance and the absence of hydrophytes can generally be considered a sign of degradation, usually implying a reduction in the biological diversity of the system.

The causes of shifts between clear and turbid phases can be diverse. In addition to alterations in nutrient availability caused by changes in external inputs, such shifts can also result from changes in community structure mediated by invasions of non-native species. As previously mentioned, in the HTCI under the Habitats Directive, and particularly in lentic HTCI, plant communities receive special consideration, since some aquatic habitats in group 31 are defined, among other factors, by the macrophyte communities they host. For example, the HTCI 3140 is characterized by the presence of charophyte meadows (macroalgae in this case) in its macrophyte assemblages.

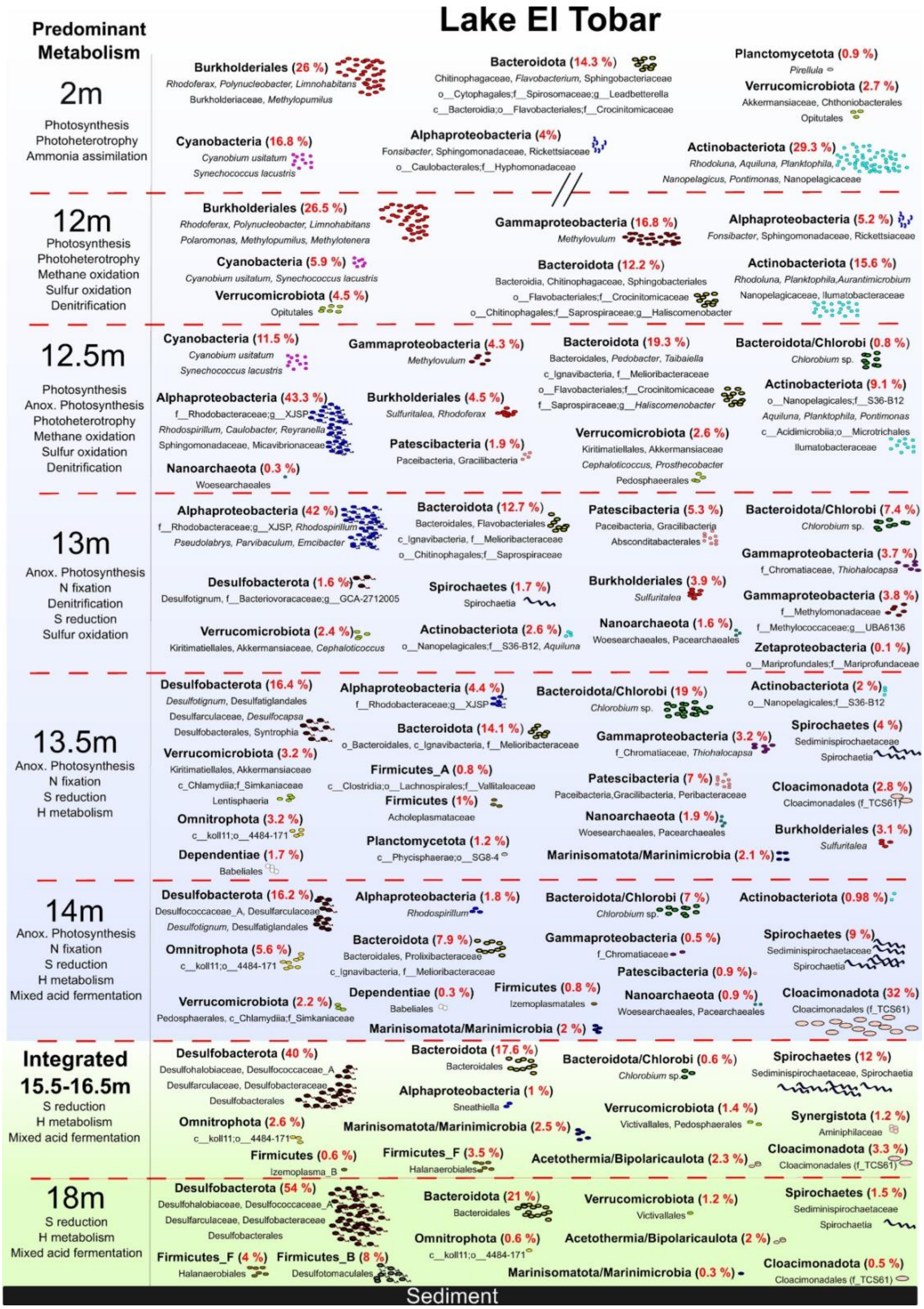
Bacteria: Planktonic and benthic bacteria (and Archaea) in aquatic environments are responsible for an important part of both energy and matter fluxes. Because of their small size, extreme diversity, and largely indistinguishable morphological features, the mechanisms of their activities and interactions with other organism remained largely unknown for decades (McMahon & Newton, 2024). However, the new –omic techniques have made it possible to reveal their huge role in the biogeochemistry of lentic ecosystems, shaping the environment and water features, among others, along horizontal and vertical gradients (e.g., Figure 7, Cabello-Yeves et al., 2023).

Phytoplankton: Plankton refers to a set of organisms that live suspended in water. Within it, phytoplankton (Figure 5) constitutes the photosynthetic fraction and includes photosynthetic algal protists and cyanobacteria (Litchman & de Tezanos Pinto, 2024). The composition of phytoplankton can be decisive for ecosystem functioning because it is the trophic support for the consumer community and influences the structure of the pelagic community, due to factors such as the palatability of the dominant species and the production of allelopathic substances or toxins (Bláha et al., 2009).

The growth of phytoplankton is controlled by the availability of inorganic nutrients (Istvánovics, 2009), especially inorganic compounds of phosphorus (orthophosphate) and nitrogen (ammonium, nitrate, nitrite), so that the enrichment of waters in these compounds provides these nutrients and promotes excessive growth of phytoplankton, in a process called eutrophication. In deep lakes the formation of deep maxima of phytoplankton is common (Camacho, 2006). These deep maxima can accumulate a large amount of biomass throughout the summer period in the vicinity of the lower part of the metalimnion or the meta-hypolimnion interface, under conditions of very low illumination (Figure 4). Since all microalgae and cyanobacteria contain chlorophyll- *a* as its main photosynthetic pigment harvesting light for photosynthesis, the concentration of chlorophyll- *a*, which follows, is used as a main proxy to assess the phytoplankton abundance, thereby the trophic level and its possible alteration.

Phytobenthos: In general terms, benthos refers to aquatic organisms that live in or on a solid substrate, whether mobile or sessile. In case of photosynthetic microorganisms, in addition to being part of the planktonic communities of microalgae (phytoplankton), many microscopic primary producers (Figure 4) can grow attached to solid substrates - on stones (epiliton), on sediments (episammon) or on the vegetation (epiphyton), together forming the so-called phytobenthos. Although in pelagic systems, phytoplankton generally plays more important role in primary production than phytobenthos, in shallow systems and those dominated by littoral zones, this relationship is often reversed. However, in such systems, when macrophytes (also sessile organisms rooted to the substrate, thus benthic) are present, they typically contribute more to primary production than phytobenthos.

Figure 7. Microbial spatial distribution and abundance across the different layers of Lake El Tobar



For each sample, the most prevalent metabolic pathways, identified in metagenomic assembled genomes (MAGs), are shown. Each microbial group, the % of 16S rRNA gene DNA associated with each sample is indicated in red.

Source: Cabello-Yeves et al. (2023)

Photosynthetic microorganisms, along with others, may also form benthic communities known as microbial mats. These are multistratified assemblages of microbial populations that can develop in the shallow sediments of lentic ecosystems with certain characteristics, such as types of saline lakes. Within microbial mats, the most characteristic primary producers are filamentous cyanobacteria, often accompanied by pennate diatoms and/or purple photosynthetic sulphur bacteria.

Photosynthetic sulphur bacteria: These primary producers, like algal protist can develop both planktonic and benthic populations. Although they are not commonly visible, as they typically develop in anoxic layers well below the water or sediment surface, photosynthetic sulphur bacteria (Figure 4) are characteristic organisms of a specific THIC, the “Karstic lakes on gypsum” (3190).

The production of hydrogen sulphide occurs either through the desulfurization of sulphur-containing amino acids during the proteolysis (generally in oligosulphidophilous lakes) or via anaerobic respiration that uses sulphate as an electron acceptor (sulphate reduction). These processes lead to the accumulation of relatively high concentrations of this substance during stratification periods in the deeper layers of lentic ecosystems deep enough for stratification to occur (Camacho, 2009), where populations of photosynthetic sulphur bacteria develop. Furthermore, benthic populations of these organisms may also appear in intermediate depth layers of microbial mats in saline shallow lakes.

In both the hypolimnion of deep lakes and in microbial mats, photosynthetic sulphur bacteria, which use hydrogen sulphide as an electron donor in their anoxygenic photosynthesis, thrive in deep zones rich in hydrogen sulphide, particularly in the upper layers where sufficient light still penetrates for photosynthesis. Additionally, they play a biological filter role, preventing both hydrogen sulphide (a toxic substance for aerobic organisms) and inorganic nutrients diffusing into upper layers.

Planktonic and benthic micro and macroinvertebrates: Microanimals that live suspended in water are known as zooplankton. Zooplankton (Figure 5) includes heterotrophic protists (flagellated and ciliated protozoans) and metazoans, mainly rotifers and microcrustaceans such as cladocerans and copepods. Protists are part of the microbial loop, which includes bacteria, flagellates, ciliates.

Planktonic microinvertebrates are mostly filter feeders, feeding mainly on phytoplankton, through some species also consume bacterioplankton to a lesser extent. Predatory species that consume individuals of other zooplankton are also present (Sterner, 2009). Zooplankton serve as food for planktivorous fish, benthic invertebrates, and some aquatic birds.

In most lentic ecosystems in a healthy ecological state, a higher relative abundance (compared to rotifers) and species richness of cladocerans and calanoid copepods is typically observed. In contrast, eutrophic ecosystems with fish populations and without vegetation are dominated by rotifers and cyclopoid copepods.

The presence of large, filter-feeding zooplankton contributes to maintaining water transparency, as they reduce the turbidity created by the phytoplankton. However, in lakes dominated by planktivorous fish and lacking piscivores, smaller zooplankton tend to dominate due to predation pressure. Many zooplankton species find refuge within submerged vegetation (hydrophytes), forming diverse littoral communities in deep lakes, or more widely distributed communities in shallow lakes with extensive hydrophyte cover.

In temporary lagoons and ponds, different species of large branchiopods are especially characteristic, although they are neither so small nor strictly planktonic (Miracle et al, 2008). Large branchiopods are adapted to temporary waters or extreme environments (e.g., in hypersaline lakes). Due to their lack of defences against predation, their success depends on colonizing ephemeral waters of unpredictable duration, typically in the initial stages of ecological succession or in extreme conditions, thus lacking visual predators like fishes.

The zoobenthos (Figure 3) consists of conspicuous invertebrates, usually visible to the naked eye, that live on or within the substrate (benthos). In addition to the larvae and, in some cases, also adult individuals of numerous insect taxa (*Ephemeroptera, Plecoptera, Trichoptera, Odonata, Diptera, Coleoptera*...), it includes molluscs, annelids, flatworms, macro- and microcrustaceans, and hydracarids, among others (Strayer, 2024; Bonada & Mogan, 2024).

Benthic microcrustaceans are mainly represented by ostracods, but also include species of branchiopods. In shallow lentic ecosystems, where the main habitats for these organisms dominate, zoobenthos has a greater relative importance compared to pelagic microanimals. In contrast, their distribution in the deep sediments of lakes is highly influenced by oxygen availability, as all zoobenthic organisms are aerobic. However, some (e.g. certain taxa of oligochaetes and chironomid taxa) possess adaptations that enable them to survive in microaerobic aquatic environments, such as having high concentrations of haemoglobin.

The diverse ecological requirements of macroinvertebrate taxa allowed them to be used as bioindicators. For example, river biological quality is often assessed based on the presence or absence of specific macroinvertebrate groups, using indices such as the BMWP (Biological Monitoring Working Party). However, these indices were primarily developed for use in running waters (Hellawell, 1986) and are not well suited for application in lentic ecosystems.

Nekton (particularly fish) and other ichthyofauna: Nekton refers to organisms that actively swim in the water, with fish being the most ecologically significant group in lentic ecosystems (Jeppesen et al., 2024). From a trophic perspective, a distinction can be made between planktivorous fish, which feed on planktonic organisms, and piscivorous fish, which feed on other fish, are thus positioned at a higher level in the food web.

Further fish, big crustaceans such as crayfish species, and molluscs also act as important consumers in aquatic habitats.

In many European lentic ecosystems, as in other regions of the world, these communities have been extremely modified by invasions by exotic species. Most of these invasions have had significant impacts, leading to alterations in the native biological communities, although in some cases, ecosystems have reorganized to accommodate the new colonizers. Typically, the successful establishment of exotic species results in the displacement of native species, making the relative abundance of native species—those characteristics of a particular lentic habitat type—a potentially useful indicator of the conservation status of the ichthyofaunal community. In Europe, most introduced species have a Holarctic distribution, primarily originating from Palearctic and Nearctic. In some cases, the mere presence of fish in some lentic ecosystems where they naturally do not occur, such as temporary ponds characteristic of HTCI 3170*, constitutes a major ecological disturbance itself.

Other vertebrates: Vertebrate species, such as waterfowl and amphibians, find their primary habitat in lentic ecosystems and can play a significant role in aquatic ecosystems. Waterfowl species are among the most visible components of animal biodiversity (Hale, 2023). They can act as important consumers of aquatic productivity, but also, particularly in the case of species that feed in terrestrial environments and use lentic ecosystems for roosting, such as cranes or seagulls, they may become a source of nutrients which can negatively affect the trophic status these ecosystems (guano-driven eutrophication; Martín-Vélez et al., 2019; Laguna et al., 2021).

Amphibians, another taxonomic groups characteristic of inland waters, are currently experiencing a marked decline (Luedtke et al., 2023), partly due intrapopulation population factors (e.g., chytridiomycetes infections) and the degradation of the aquatic habitats that host them. However, additional causes also appear to contribute to their decline.

In addition to birds, amphibians and fish, other vertebrates, such as certain reptiles and mammals are also characteristic of lentic ecosystems. However, their species diversity is much more limited, and only a few species delve in standing waters. Examples include aquatic snakes, such as *Natrix* spp., aquatic turtles such as the European (*Emys orbicularis*) and the

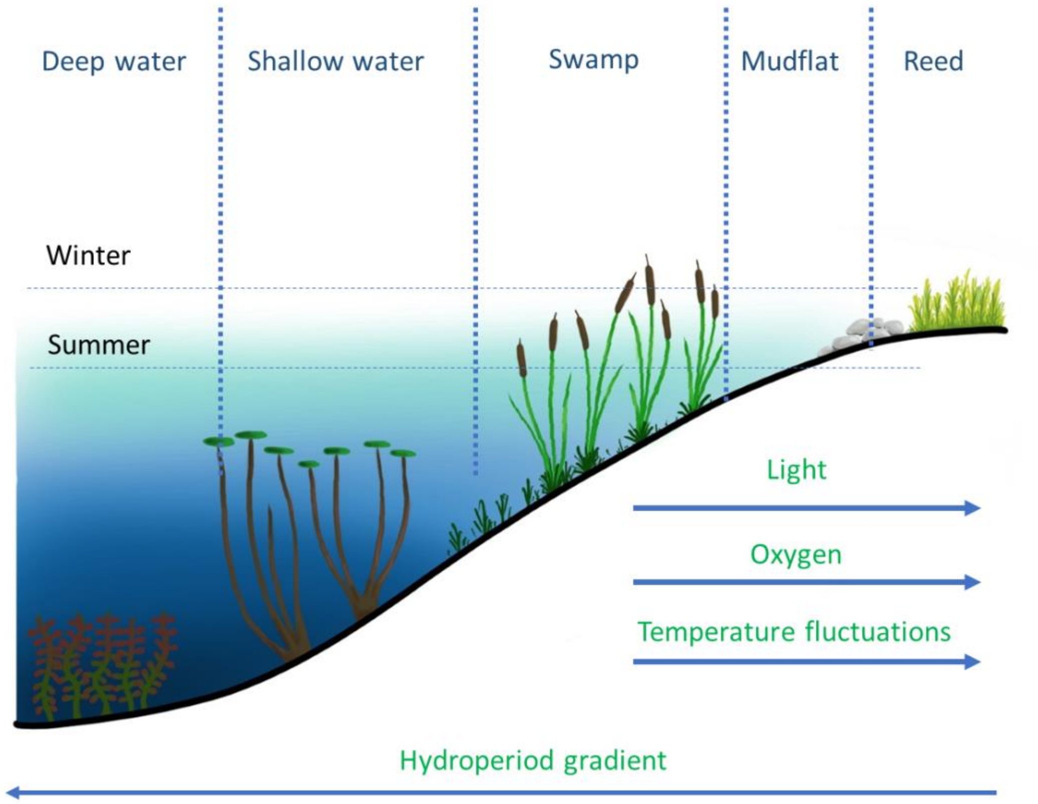
Mediterranean (*Mauremis leprosa*) pond turtles, and mammals such as the Eurasian otter (*Lutra lutra*) and the talpid (*Galemys pyrenaicus*).

Structural characteristics

The structural characteristics of lentic ecosystems refer not only to the spatial structure of the biota, but also to other biotic structural factors, such as population structure, community diversity and food web structure), among others. The main types of these biotic structural features can be grouped into the following categories of factors.

Physical biotic structure: The physical structural characteristics of biotic communities refer not only to how the organisms are distributed in the space, but also to the shaping role that the biota itself plays in the habitat (Figure 8). Examples from lentic ecosystems include plant zonation (Rosbakh et al., 2020; Campbell & Keddy, 2022; Keddy, 2023), which occurs from the banks, through emerged to submerged shores, down the lake slopes to deep waters, where each plant species find specific spatial niches in which it can grow. These plat species also contribute to the creation of spatial niches for other species (e.g., zooplankton). Another example is the vertical distribution of planktonic species, where different phytoplankton taxa may thrive at different depths, depending on their pigment composition or nutrient requirements.

Figure 8. Wetland vegetation zonation along a hydroperiod gradient in a permanent shallow lake



The arrows in the lower right corner indicate a decrease in light availability, oxygen concentrations and temperature fluctuations. The dashed lines represent seasonal differences in water levels, specifically between winter and summer.

Source: Modified from Rosbakh et al. (2020)

Population structure: A biological population is a group of individuals of the same species that coexist in a specific spatial context and can potentially interact among them (Begon et al., 2005). Some specific features of the population can be considered as structural characteristic. For example, in lentic habitats, the age structure of a fish population (Jeppessen et al., 2024), or the spatial preferences of different individuals within a fish population depending on their life stage (e.g., juveniles vs. adults), are considered structural features.

Diversity (and complexity): One of the most relevant structural features of a biological community is its level of diversity (Keddy, 2023). Parameters used to quantify biological diversity usually combine both, the number of species and how the total number of individuals is distributed among the species in the community (Magurran, 2021). The simplest index is the number of species in a community (n), known as species richness, where richer communities contain more species.

However, diversity involve not only the number of species but also relative abundance of each species. Several diversity indices combine species richness with abundance, such as the Shannon's diversity index or the Simpson dominance index, which are primarily applied to individual communities at a given locality.

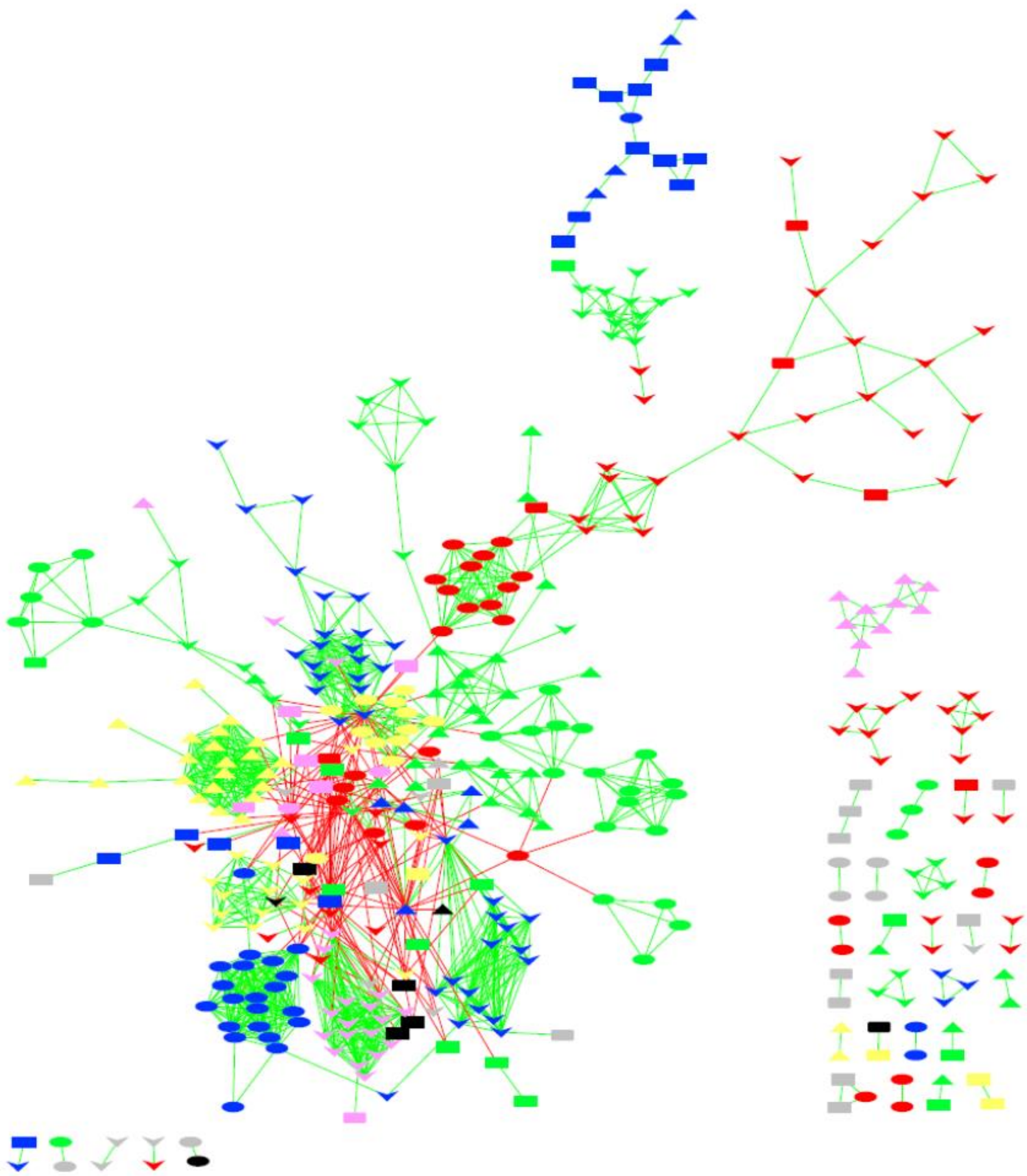
Diversity indices can be applied to the entire biological community or, depending on the needs, to a part of it (e.g. a taxonomic or functional group). The results obtained at the different scales are usually comparable and scalable, as the complexity of the biological structure is linked throughout the different organization levels. In addition to local diversity, known as alpha-diversity (α-diversity), diversity can be compared among sites at narrower (beta-diversity, β-diversity) and regional (gamma-diversity, γ-diversity) scales, which indicate how communities differ among geographic and environmental gradients. All these considerations apply to all habitat types, and therefore the use of diversity indices to describe the main structural features of biological communities in lentic ecosystems is highly recommended.

Food web structure: In an ecosystem, the food web is a complex network (Figure 9) of interconnected and overlapping food chains that represent feeding relationships within a biological community (Sottosanti, 2024). Although food web dynamics will be described later as a functional feature, a basic distinction can be made between organisms that produce organic matter, the "primary producers", and those that consume it - the "consumers" (De Ruiter et al., 2005).

While primary producers shape the food web through resource availability (bottom-up control), in lentic ecosystems, as in other ecosystem types, consumers, such as fish, benthic invertebrates, and other heterotrophic organisms, are not only influenced by the resource availability, but they can also shape biological community through predation (top-down control).

The effects of predation can propagate throughout the food web, leading to trophic cascades in the sense of Carpenter et al. (1985), who originally described this phenomenon for lentic ecosystems. These effects spread throughout the community. Although a particular species may be situated in a certain position of the food web, its diet may vary depending on factors such as age (from juveniles to adults) or the seasonal variation of resource availability (Smith & Smith, 2001).

Figure 9. Examples of a complex (left) and a simpler (right) microbial network



Functional characteristics

The functional characteristics of ecosystems refer to the processes involving the flux of materials and energy, as well as biotic interactions among the components of the biological community, occurring at across various temporal and spatial scales. The functional characteristics of any ecosystem include not only biotic processes, but also their interplay with abiotic processes, therefore strict compartmentalization in their description challenging.

In this document, which deals with lentic ecosystems, most abiotically driven physical and chemical processes have been described in the respective sections of the abiotic characteristics. Consequently, this describes the main processes primarily driven by the biotic components of lentic ecosystems (e.g., organic matter consumption), or those where both abiotic and biotic components play equally important roles (e.g. biogeochemical cycles).

Primary production: Aquatic plants (hydro- and helophytes), macroalgae, phytoplankton and phytobenthos are the main primary producers in lentic ecosystems. Primary production, together with allochthonous inputs of organic matter, determines the trophic status of a lentic ecosystem, with low productivity systems being classified as oligotrophic, those with intermediate productivity as mesotrophic, high productivity systems as eutrophic, and hypertrophic those with extraordinarily high productivity due to the high availability of inorganic nutrients that allow higher rates of primary production (Kalff, 2002). Photosynthetic biosynthesis, in which organic matter is produced from the inorganic components using light energy, is the basis of primary production in ecosystems, and it also supports the secondary production of consumers. Through the action of decomposers (mainly bacteria), element recycling and re-mineralization of essential nutrients.

High productivity, often supported by allochthonous contributions (eutrophication), provide a greater quantity of food to the system, but not necessarily a diversification of food resources. Instead, this often favour species that are more efficient in exploiting of those resources, relegating others to rarity or local disappearance, thereby decreasing the diversity of the biological community (Margalef, 1983; Begon et al, 2005).The degree of eutrophication can be determined by some variables that will be described later, as the concentration of chlorophyll- *a* in water, but also the intensity of functional processes based on variations in chemical variables, such as the concentration of dissolved oxygen, whose diel variations largely depend on biological activity. This activity is related to the amount of biomass, both that of photosynthetic organisms, which produce oxygen via the photolysis of water, and of aerobic consumers, which drive aerobic respiration, the main type of organic matter respiration. In relation to the increased rates of respiration that accompany higher organic matter input and trophic status, variables such as chemical oxygen demand (COD) and biochemical oxygen demand (BOD) can also be useful to detecting alterations linked to an increased trophic status.

Consumption and food webs: In addition to the structural effects of consumers on the food web, the functional aspects of energy and matter transfer derived from the consumption of organic matter generated within the ecosystem (primary production) are key ecosystem characteristics (Gaedke, 2009; Keddy, 2023). Organic matter is used by consumers to obtain energy through aerobic respiration (with oxygen as the electron acceptor) or anaerobic respiration (with other electron acceptors, such as nitrate, sulphate, CO_2 or organic compounds). Although the efficiency of energy transfer among different types of lakes and ponds, aquatic food webs tend to be more efficient than terrestrial ones due to several factors (e.g., the higher palatability and lower biomass/necromass ratio of aquatic organisms). However, this pattern can be altered in some cases, for example by low palatability of filamentous cyanobacteria for filter feeders like branchiopods, especially when these can produce cyanotoxins. Beyond energy, consumers also obtain nutrients needed for growth from the organic matter in their diets, excreting excess or non-assimilable fractions.

Interspecific interactions and colonization processes: Although most interactions among the various components of the biota have a trophic basis, specific ecosystem functional features emerge when the classically recognized ecological interactions are considered (Begon et al., 2005). For instance, competition among individuals of the same or different species is usually viewed in terms of resource use, but other types of competition can also occur (e.g., competition for mating). Similarly, although predation is undoubtedly a trophic interaction, the way it is exerted can have different functional effects (e.g., browsers vs. killers, with have different impacts on individual preys). Mutualistic interactions, which increase the ecological efficiency of the partners, are another key type of interaction. These can explain, for instance, niche exploitation that would otherwise be unavailable for the individual partners (e.g., anaerobic ciliates thriving in anoxic areas of lakes and ponds due to their association with anaerobic bacteria Macek et al., 2020). A particularly relevant type of biotic process, increasingly common, is the invasion by exotic species, a colonization process (Bundschuh et al, 2023) that not only strongly impacts the structure of biological communities, but can also cause significant functional changes in ecosystem (LIFE INVASAQUA Project, 2023).

Biogeochemical cycles: Biogeochemical cycles describe the circulation of chemical elements through the interplay of abiotic and biotic processes (Schlesinger & Bernhardt, 2020). The importance of biota in these cycles depends largely on the contribution of each element to the organic matter content of living organisms. Carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorus, iron and calcium are the main elements forming the cells and tissues of living beings approximately in this order, though it may vary among taxa. Consequently, the role of organisms in the cycling of these elements increases with their relative proportion in biomass.

Specific processes distinguish how biogeochemical cycles operate in terrestrial versus aquatic systems. In lentic ecosystems, for example, the low solubility of inorganic phosphorus compounds at the near-neutral pH typical of natural waters makes phosphorus relatively scarce for aquatic primary producers (Dillon & Molot, 2024). By contrast, inorganic nitrogen compounds such as nitrate and ammonium are highly soluble. Their bioavailability in water is not limited by solubility but rather by their lower abundance in the biosphere, since most nitrogen occurs as atmospheric N_2, a form unavailable to plants, macroalgae and protist algae (Schlesinger & Bernhardt, 2020). As outlined in the section on chemical features, these dynamics explain why nitrogen and phosphorus are the main limiting nutrients for primary production in inland waters (Kalff, 2002), and why artificial enrichment drives eutrophication.

Planktonic chlorophyll- *a* concentration: Although chlorophyll-*a* represents only one component of lentic systems – specifically a photosynthetic pigment within phytoplankton) – its widespread use for assessment purposes merits particular attention. in evaluating water quality to controlling eutrophication, planktonic chlorophyll-a concentration is a fundamental quantitative variable (OECD, 1982). Because chlorophyll-a is common to all phytoplankton and relatively straightforward to measure (Picazo et al., 2013), its concentration is directly proportional to phytoplankton abundance. Excessive phytoplankton growth is the primary symptom of eutrophication, thus chlorophyll-a concentration provides the most reliable indicator of trophic status and the likelihood of eutrophication in lentic waterbodies. It reflects both the occurrence and the severity of this problem, which constitutes the most widespread alteration of inland lentic ecosystems, typically of anthropogenic origin.

Landscape characteristics

According to the SEEA EA framework, "in concept, an ecosystem asset is differentiated from neighbouring ecosystem assets – integrated within a landscape – by the extent to which the interactions between its biotic and abiotic components are stronger than the interactions with components outside the ecosystem asset" (United Nations, 2021). Nonetheless, exchanges may occur between different types of ecosystems, or between similar ecosystem assets, when they are physically or ecologically connected.

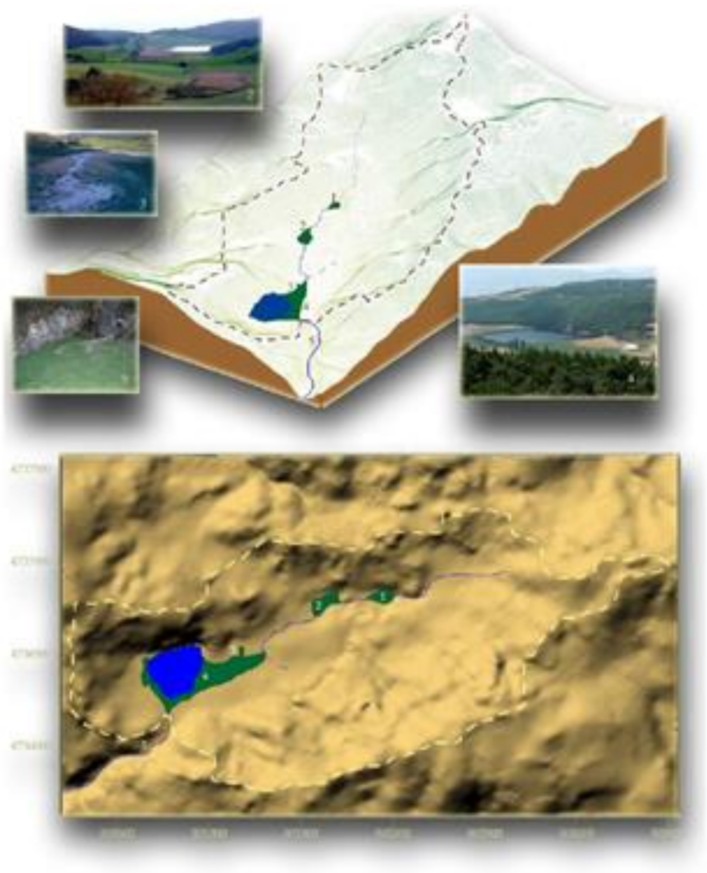
In ecological terms, a landscape is understood as a set of different ecosystems located in close geographical proximity that exchange organisms, energy and/or materials (Turner & Gardner, 2015). The rates of exchange depend on the degree of connectivity among ecosystems, which is influenced by factors such as the distance and shape of ecosystem assets, as well as the intensity of abiotic processes (e.g. water flux) and biotic processes (e.g.

emigration) that sustain these interactions.

Several types of landscape interactions can be distinguished, most of them involving exchanges of individuals, materials or energy. Examples include immigration–emigration processes, such as the transport of plant seeds or zooplankton eggs (propagules) by waterfowl between lakes; fluxes of materials, such as lake siltation caused by inorganic particles delivered through riverine inlets; and energy transfers, such as shoreline modification of a lake due to erosion from inflowing rivers. The fluxes may be unidirectional (e.g. from a catchment to a lake) or bidirectional (e.g. fish migrations between lakes connected by a river).

Figure 10. Tridimensional model of the catchment basin of Lake Arreo (Álava, Spain)

A karstic lake on gypsum (3190), showing the lake (blue) and palustrine wetlands (green)



Source: Chicote (2004)

For lentic ecosystems, the concept of landscape can be broadly assimilated to that of the catchment area (Figure 10). Water is the main transporting element in aquatic systems, and it both collects and is collected within the catchment. This applies not only to the visible surface catchment, but also to the aquifers with which a lentic ecosystem may be associated.

In addition to water-mediated connectivity, which facilitates exchanges between ecosystems, other types of connectivity may also play a role. These include exchanges between ecosystems that are neither spatially contiguous nor linked by abiotic vectors such as water. One example is biological connectivity, as in the dispersal of propagules between distant lakes.

1.2.2 Main ecological characteristics and identification of variables to measure habitat condition

Ecosystem condition refers to the quality of an ecosystem, expressed through its abiotic and biotic characteristics (United Nations, 2021). To assess habitat condition, these key features must be evaluated using representative variables that reflect their quality. Condition variables are quantitative metrics that describe individual characteristics of a habitat asset; they must be measurable and linked to relevant ecological attributes of the habitat.

The main ecological characteristics of lentic ecosystems were outlined in section 1.2.1. Building on that, this section illustrates how to propose variables suitable for measuring habitat condition. The approach follows the framework for ecosystem condition assessment developed in the EU-wide methodology for mapping and assessing ecosystems, as well as the UN System of Environmental-Economic Accounting— Ecosystem Accounting (SEEA EA; United Nations, 2021), as set out in the Technical Specifications of this project.

The main aim of this document is to identify variables that can be used to measure the condition of lentic habitats, in relation to their key characteristics and ecological processes described above. Section 1 provides the basis for the analysis of existing methodologies presented in Section 2, as well as the proposal of key variables set out in Section 3.

Condition variables should assess how key habitat features are influenced by the pressures acting on the habitat or ecosystem, and how these pressures affect its ecological integrity (Kristensen, 2004). Accordingly, the document also describes and lists the main pressures affecting lentic ecosystems, and analyses the extent to which these pressures are addressed for lentic habitats by the variables currently used in EU Member States.

Condition variables are understood here as indicators of both the characteristics and dependency relationships among the components and structure of an ecosystem or habitat type, as well as the features and magnitudes of its dynamics and processes (functions). As a general rule, condition variables should have a clear and unambiguous definition, explicit measurement instructions, and well-defined units that express the quantity or quality being measured. They should also include reference values against which observed values can be compared to evaluate condition.

Since the main ecological characteristics of lentic ecosystems and habitat types were described in section 1.2.1 following this framework, the present section sequentially considers variables for assessing the status of abiotic (physical and chemical), biotic (compositional, structural, and functional) and landscape features, consistent with the approach applied in this project for other habitat types. these variables are summarized in Table 1.

As noted above, in lentic ecosystems it is necessary to assess not only the status of the waterbody itself, but also, for certain features (e.g. water mineralisation), the corresponding conditions in the catchment. This is because the characteristics of the water largely depend on catchment features (e.g. lithology in the case of mineralisation).

Among abiotic characteristics, the most relevant are those related to climate, geology, soil properties, morphology and physiography, hydrology, and the physical variables of the water, including transparency, colour, as well as temperature and conductivity – the latter two determining water density and thus the potential for water column stratification in sufficiently deep systems. As these are basic features of lentic ecosystems, their maintenance within reference values should be monitored using measurable variables. Table 1 provides examples of variables that can be used to assess the status of these fundamental physical features.

In addition, several chemical characteristics are essential to lentic ecosystem and should be included in habitat condition assessment. At a minimum, these comprise water mineralisation and the type of dominant salts, pH, alkalinity, dissolved oxygen and sulphide concentrations, concentrations of inorganic nutrients (N and P compounds), and organic matter concentrations in both water and sediments (Table 1).

With respect to biotic features—compositional, structural, and functional— the condition of selected key aspects should also be assessed.

For **composition**, the main components of the biological community of lentic ecosystems should be included in the assessment, namely macrophytes, phytoplankton, phytobenthos, photosynthetic sulphur bacteria, planktonic and benthic micro- and macroinvertebrates, nekton (particularly fish and other ichthyofauna), and other vertebrates. For **structure**, attention should be given to the to the physical structure of the ecosystem (arising from interactions between biotic and abiotic components), the population structure of key species, and the diversity and complexity of the biological community, including its food-web organisation.

For **functions**, which also depend on interactions between biotic and abiotic factors, the following processes should at minimum be assessed: primary production, consumption and food webs, interspecific interactions, colonisation processes, biogeochemical cycles, and planktonic chlorophyll-a concentration – the latter being a particularly sensitive indicator for assessing eutrophication in lentic systems.

Table 1 summarises these characteristics and provides examples of variables that can be used to assess their status in the evaluation of lentic ecosystem condition. It also presents examples of the main processes occurring at the landscape level. These include the exchange of individuals (immigration-emigration processes), fluxes of materials and energy, and the parametrisation of connectivity, among other relevant landscape processes.

3160 Natural dystrophic lake. Marquesado, Spain
© A. Camacho

Table 1. Ecological characterisation and selection of variables to measure the condition of standing water habitats

Ecological characteristics	Category	Description	Examples of variables (measurements methods)
Abiotic characteristics	**Physical state characteristics**	Physical descriptors of the abiotic components of the ecosystem, including: 1. Climatic: largely determining the hydrological balance. 2. Geological: rock lithology in the basin and catchment, influencing the transfer of salts to runoff water. 3. Geomorphological and pedological: factors shaping the configuration, structuration, and functioning of each specific lentic ecosystem, as well as soil characteristics. 4. Hydrological: type of inflow and outflow, groundwater connection, renewal rate, hydroperiod, and water level fluctuation. 5. Morphological/physiographic: depth, surface area, extent of the coastal zone, perimeter, width, and shape index. 6. Water physical features: transparency, temperature, density, and related parameters.	1. Change in average annual evapotranspiration (mm/year). 2. Water electrical conductivity (μS/cm). 3. Siltation (mm/year). 4. Hydroperiod length (for temporary systems, e.g. HTCI 3170*) (days/year). 5. Shoreline length (m). 6. Secchi disk depth.
	Chemical state characteristics	Descriptors of the chemical composition of the abiotic components of the lentic ecosystem (water and sediments), including: 1. Water mineralisation and dominant salt types. 2. pH. 3. Alkaline reserve. 4. Dissolved oxygen concentration. 5. Hydrogen sulphide concentration. 6. Inorganic nutrients. 7. Organic matter concentration.	1. Ca^{2+}/Na^{+} ratio. 2. Deviation from the reference pH range. 3. Bicarbonate concentration (mg/l). 4. Oxygen concentration (mg/l). 5. Absence of dissolved hydrogen sulphide. 6. Soluble reactive phosphorus (SRP, as orthophosphate) concentration (mg/l). 7. Organic matter content in the sediments (%).
Biotic characteristics	**Compositional state characteristics**	Composition and number of species in ecological communities at a given location and time, including at least the following groups: 1. Macrophytes. 2. Bacteria. 3. Phytoplankton. 4. Phytobenthos. 5. Photosynthetic sulphur bacteria. 6. Planktonic and benthic micro- and macroinvertebrates.	1. Number of the HTCI characteristic plant species (n). 2. Potential pathogenic bacterial taxa. 3. Number of desmid species (n). 4. Number of diatom species (n). 5. Bacteriochlorophyll (Bchl-a/c/d/e) concentration (mg/m^3). 6. Number of species of large branchiopods (n).

Ecological characteristics	Category	Description	Examples of variables (measurements methods)
		7. Nekton (particularly fish) and other ichthyofauna. 8. Other vertebrates.	7. Presence of native crayfish species (presence/absence). 8. Number of amphibian species (n).
	Structural state characteristics	Aggregate properties of the ecosystem or its main biotic components, at least the following: 1. Physical biotic structure. 2. Population structure. 3. Diversity (and complexity). 4. Food web structure. 5. Exotic species.	1. Number of plant strata (n). 2. Age structure of a characteristic fish population (average age). 3. Diversity of zooplankton assemblages (Shannon index, bits). 4. Ratio between nodes (species) and interactions (links) in the food web. 5. Proportion of individuals of native vs. exotic species (%)
	Functional state characteristics	Summary statistics (e.g. frequency, intensity) of the biological, chemical, and physical interactions between the main ecosystem compartments, including at least: 1. Primary production. 2. Consumption and food webs. 3. Interspecific interactions. 4. Colonisation processes. 5. Biogeochemical cycles. 6. Planktonic chlorophyll-a concentration.	1. Average Chl-a concentration in the photic zone (mg/m3). 2. Average energy transfer between trophic levels (%). 3. Colonisable area by characteristic plant species (m^2). 4. Number of seeds per gram of crane faeces (n). 5. Ratio SRP to total phosphorus. 6. Epilimnetic Chl-a concentration (mg/m^3).
Landscape characteristics		Metrics describing mosaics of ecosystem types at coarse (landscape) spatial scales (e.g. landscape diversity, connectivity, fragmentation), including at least: 1. Exchange of individuals (immigration–emigration processes). 2. Fluxes of materials. 3. Fluxes of energy. 4. Connectivity.	1. Net balance of individual exchange for a mobile characteristic species (positive vs. negative). 2. Amount of sediments provided by a riverine inlet (tones/year). 3. Wind mixing forcing (W/m^2). 4. Landscape percolation index (%).

1.3 Selecting of typical species for condition assessment

The evaluation of the *structure and functions* parameter within the assessment of the conservation status of habitat types of Community interest (HTCI) also requires consideration of the conservation status of their typical species. According to the Habitats Directive, a habitat type can only be regarded as having a favourable conservation status if both its structure and functions and its typical species are in favourable status (Art. 1e of the Habitats Directive; EC, 1992, 2017, 2023). The Directive uses the term "typical species" but does not define it, including for reporting purposes (Bonari et al, 2021). The guidelines for reporting the status of habitats and species under to Article 17 provide recommendations on the selection of typical species for monitoring and reporting the conservation status of habitats.

This section offers considerations to support the definition of criteria and procedures for selecting typical species, in general and specifically for lentic habitats, within the framework of HTCI condition assessment. To align with the project approach and the updated Guidelines for Article 17 reporting (EC, 2023), as well as earlier EC guidance on typical species (Evans & Arvela, 2011), we analyse in more detail the suitability of the following features as selection criteria: (i) species occurring regularly with high constancy (in a habitat, or at least in a major subtype or variant of a habitat type); (ii) species that are reliable indicators of favourable habitat quality; and (iii) species sensitive to changes in habitat condition (early warning indicator species). Dominant species, however, may not be suitable as typical species if they provide no additional information beyond that already captured in the assessment of structure and functions. The Article 17 Guidelines also note that different species may occur in different parts of a habitat's range or in different subtypes. Given the variability of habitat types across their range, it is unlikely that all typical species will be present in every instance of a given habitat type, particularly in larger Member States. Moreover, a species may be typical of several habitats and not dependent on a single Annex I habitat type.

The use of the typical species concept in assessing the structure and functions of HTCI has generally relied on expert-identified species. For plants, Bonari et al. (2021) discussed this approach, while Gigante et al. (2016) proposed criteria for selecting typical species sensu the Habitats Directive, which may serve as a basis for further reflection.

Additional concepts may also be considered when designating typical species for HTCI. Diagnostic and characteristic species – those relatively specific to an HTCI and/or consistently present wherever the HTCI occurs – correspond to the criterion of "occurring regularly at a high constancy in a habitat or at least in a major subtype or variant of a habitat type" (EC, 2023). For lentic HTCI, an example would be charophytes (*Chara* spp.) for HTCI 3140. However, diagnostic and characteristic species may not always be good indicators of habitat quality or of changes in the habitat condition. In the example above, charophytes are not only characteristics of HTCI 3140 but also of other lentic HTCI in group 31 (standing waters), such as HTCI 3190, which may coexist with HTCI 3140 in the same waterbody. Overall, characteristic species can be considered typical species – and used for habitat monitoring – only when their relationship with habitat structure and functions is established (Bonari et al., 2021; EC, 2023). Nevertheless, assessing the status of each typical species remains a specific requirement of the Article 17 reporting guidelines under the Habitats Directive.

Contrastingly, some species associated with specific HTCI may serve as good indicators of the status of key habitat features but are not diagnostic species (i.e., they do not co-define the habitat type and are not exclusive to it). Instead, they can act as indicators of habitat quality. An example is certain species of the genus *Potamogeton*, which can be diagnostic for HTCI 3150 (Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition*-type vegetation), but may also thrive in other HTCI, such as 3190 (Lakes of gypsum karst). In these habitats they contribute to structural diversity, providing habitat for small animals and food for herbivorous waterfowl, while not serving as diagnostic species for HTCI 3190. This example illustrates that a strict application of the three criteria set out in the guidelines could make it difficult to identify typical species suitable for condition assessment, or worse, lead to inconsistencies in the assessment itself.

According to the Guidelines for Article 17 reporting, a typical species does not necessarily have to occur exclusively in one HTCI; it may also be shared with other HTCI, provided it appears regularly in the habitat type being assessed. This implies that several HTCI may share typical species, as long as they also meet the criteria of being indicators of favourable habitat quality and of being sensitive to changes in habitat condition. The question, however, is whether this is necessary, since other indicator taxa could be used in condition assessments without forcing them into the category of typical species. We suggest that such indicators – variables based on non-diagnostic or non-characteristic taxa, or their assemblages – can indeed be applied in condition assessment, but they should not be regarded as typical species of an HTCI for which they are neither diagnostic nor characteristic. Moreover, although alien species may in some cases act as indicators (e.g., by reflecting processes of habitat degradation, since invasive exotics can strongly affect local condition), they would not be considered typical species of an HTCI.

Referring to the essential characteristics of HTCI, the concept of keystone species can be linked to the assessment of the favourable habitat quality. Some keystone species (or taxa types) may be used as condition indicators for lentic HTCI. Keystone species are usually defined as those that play critical ecological roles disproportionate to their abundance or biomass (Paine, 1966, 1995; Power et al. 1996). When such species decline or disappear, this triggers strong, even catastrophic, effects on the community composition and ecosystem functioning (Primack, 2018). If a diagnostic or characteristic species of an HTCI is also a keystone species, then damage to it would indicate that the habitat is not in good condition. If this species is also sensitive to changes in habitat condition, it could be considered a typical species whose status directly reflects the condition of the habitat, thus meeting all requirements. In any case, the use of diagnostic and characteristic species for assessing conservation status will depend on the ecological traits of the species in question and the specific HTCI in which they occur.

To illustrate the situation described above, we can take the example of HTCI 3140 (Hard oligo-mesotrophic waters with benthic vegetation of Chara spp.) developing in inland brackish or saline waters (Waiser & Robarts, 2009). As noted in the European Habitats Interpretation Manual EUR28 (EC, 2013), charophytes are diagnostic and characteristic species for HTCI 3140. If these species in inland brackish waters are sensitive to changes in salinity, a decrease in salinity would reduce their dominance or even cause their disappearance. Salt-tolerant Characeae include some species of *Chara, Tolypella,* and all *Lamprothamnium* species. The most salt-tolerant species respond to salinity changes through complete turgor regulation, adjusting vacuolar concentrations of K^+, Cl^- and sometimes Na^+ or sucrose (Beilby, 2015).

Although inland saline lakes are not widespread in Europe, they are common in some areas, such as the endorheic basins of the Iberian Peninsula. Many of these lakes have received freshwater inputs from wastewater, which have reduced salinity (García-Ferrer et al., 2003; Corrales-González et al., 2019). These inputs not only increased trophic status—already unfavourable for submerged macrophytes such as characeans—but also lowered salinity, allowing less salt-tolerant hydrophytes to replace the more tolerant characeans.

Characeans, which are diagnostic and characteristic species for HTCI 3140, also act as keystone species and ecosystem engineers (Jones et al., 1994; Wright & Jones, 2006; de Visser et al., 2012). They shape the physical habitat and provide key resources such as shelter and spawning grounds for small animals. At the same time, they are sensitive to changes in a defining feature of HTCI 3140 in inland saline lakes: moderate to high salinity. This example illustrates how typical species can be defined and identified in lentic habitats, and how their own status can be assessed as part of habitat condition.

Most characteristic species reported for HTCI are plants (EC, 2013). However, when examining the common plant species of a specific HTCI, the situation is not straightforward. Even if several species are shared among EU Member States (MSs), or at least within each biogeographical region, many taxa common in some MSs are absent in others, and many are not even listed in the EU Interpretation Manual of European Habitats. For example, Camacho et al. (2009) reported that, of the 41 plant species listed in the Interpretation Manual for HTCI 3170* (Mediterranean temporary ponds), only 18 were found in Spain. At the same time, experts of the Spanish Society for Plant Conservation identified 26 additional species not listed in the Manual. In this case, as reflected in the localities where HTCI 3170 occurs (Table 2), up to 44 potentially diagnostic plant species could be expected across the full set of Spanish sites. Such a list would allow assessments of HTCI status to be based on, for instance, changes in diversity indices. Yet the question remains: can the status of specific, supposedly characteristic species also be used for condition assessment, for example in this lentic HTCI?

As described above, for the HTCI 3170*, as a representative example of standing water habitats (group 31 of Annex 1 of the Habitats Directive), some characteristic species occur in certain MSs but not in others. In addition, some MSs record potentially characteristic in the HTCI that are not listed in the EU Interpretation Manual (EC, 2013). A comparative example of plant species lists for MSs within the Mediterranean biogeographical region for the priority lentic HTCI 3170* (Mediterranean temporary ponds, MTP) is presented in Table 3.

Among these plant species, some are present in HTCI 3170* across most surveyed MSs, such as *Lythrum hyssopifolia* and *Crassula vaillantii*, and could therefore be considered diagnostic species for this HTCI throughout the Mediterranean biogeographical region. Others occur only in one or a few MSs, where they may also be considered diagnostic, but their populations are particularly sensitive to ecological impoverishment, making them useful indicators of changes in habitat quality. In this context, Camacho (2020) proposed a set of characteristic species for HTCI 3170* in the Iberian Peninsula that could also serve as typical species for condition assessment. This is because the ecological requirements of the selected species align closely with the need for high ecological quality in this habitat type. Each of these species thus provides information both on the condition of the habitat (as they are demanding species tightly linked to the maintenance of its features) and on their own conservation status as typical species.

- Some of the species proposed by Camacho (2020) include: *Marsilea strigosa* Willd. This specialist species of HTCI 3170* has a circum-Mediterranean potential distribution, although its current range is more restricted. Its role as a habitat specialist, combined with its sensitivity to ecological change, makes it a strong candidate for use as a typical species in the assessment of habitat condition. It is also included in Annexes II and IV of the Habitats Directive, which highlights its own conservation importance and provides another potentially distinctive feature of a typical species. In a survey of Spanish Mediterranean temporary ponds (MTP*), M. strigosa* was recorded in 87 of 137 ponds larger than 1 ha (63.5% occurrence) and was absent from all surveyed localities showing any degree of alteration.

- *Juncus pygmaeus* Rich. This species is almost exclusive to HTCI 3170*, with a distribution across western and southern Europe, Anatolia and North Africa. It rapidly disappears when the main features of this HTCI temporality —water mineralization, etc.— are altered.
- *Mentha (Preslia) cervina* L. This characteristic species, almost exclusive to HTCI 3170*, plays an important structural role in the habitat. It is distributed in the Iberian Peninsula, southern France, and North Africa. Because of its role in shaping habitat structure, it can be regarded as a keystone species for this HTCI and could therefore be used in habitat condition assessment. When it is damaged, the habitat loses part of its structural and functional characteristics.
- *Lythrum flexuosum* Lag. This diagnostic species for HTCI 3170* is highly sensitive to the hydrology of the Mediterranean temporary ponds, as it requires both flooding and dry periods to complete its development. It is also included in Annexes II and IV of the Habitats Directive, making it a conservation target in its own right and a candidate for assessment as a typical species.

Table 2. Plant species listed for HTCI 3170 in the EUR28 - Interpretation Manual of European Habitats (EC 2013). The table indicates species from the Manual reported in Spain and additional species recorded in surveyed localities of HTCI 3170 in Spain.

HTCI 3170* characteristic plant species (EUR28, EC 2013)	HTCI 3170* characteristic plant species from the EUR28 reported for Spain (Camacho et al, 2009)	Plant species not included in the EUR28 but repeatedly reported in localities of HTCI 3170* in Spain
*Agrostis pourretii, Centaurium spicatum, Chaetopogon fasciculatus, Cicendia filiformis, Crypsis aculeata, C. alopecuroides, C. schoenoides, Cyperus flavescens, C. fuscus, C. michelianus, Damasonium alisma, Elatine macropoda, Eryngium corniculatum, E. galioides, Exaculum pusillum, Fimbristylis bisumbellata, Glinus lotoides, Gnaphalium uliginosum, Illecebrum verticillatum, #Isoetes boryana, I. delilei, I. durieui, I. heldreichii, I. histrix, #I. malinverniana, I. velatum, Juncus bufonius, J. capitatus, J. pygmaeus, J. tenageia, Lythrum castellanum (= L. baeticum), *L. flexuosum, L. tribracteatum, #Marsilea batardae, #M. strigosa, Mentha cervina, Ranunculus dichotomiflorus, R. lateriflorus, Serapias lingua, S. neglecta, S. vomeracea.*	*Agrostis pourretii, Cicendia filiformis, Eryngium corniculatum, Illecebrum verticillatum, Isoetes durieui, I. histrix, I. velatum, Juncus bufonius, J. capitatus, J. pygmaeus, J. tenageia, Lythrum castellanum (= L. baeticum), *L. flexuosum, L. tribracteatum, #Marsilea batardae, #M. strigosa, Mentha cervina, Ranunculus lateriflorus*	*Antinoria agrostidea subsp. annua, Baldellia ranunculoides, Blackstonia perfoliata, Briza minor, Centaurium pulchellum, Crassula vaillantii, Damasonium polyspermum, Gnaphalium luteo-album, Hypericum humifusum, Illecebrum verticillatum, Isoetes setacea, Isolepis setacea, Lotus subbiflorus, Lythrum acutangulum, L. borysthenicum, L. hyssopifolia, L. thymifolia, Mentha pulegium, Myosurus minimus, Polypogon maritimus, Ranunculus batrachioides subsp. brachypodus, R. longipes, Sedum lagascae, Silene laeta, Solenopsis laurentia, Verbena supina.*

Table 3. Occurrence of plant species in the priority lentic HTCI 3170* (Mediterranean temporary ponds) across EU Member States of the Mediterranean region

- 5 MS
- 4 MS
- 3 MS
- 2 MS
- 1 MS

3170* typical species	CY	ES	FR	GR	MT	PT	IT
Agrostis castellana		X					
Agrostis pourretii		X					
Alopecurus aequalis		X					
Anagallis minima		X					
Antinoria agrostidea		X					
Antinoria insularis				X			
Artemisia molinieri			X				
Asteriscus aquaticus				X			
Baldellia ranunculoides		X	X				
Baldellia repens		X					
Callitriche brutia	X			X			
Callitriche pulchra				X			
Callitriche truncata					X		
Centaurium maritimum		X					
Centaurium pulchellum	X			X			
Chaetonychia cymosa		X					
Chaetopogon fasciculatus						X	
Chara spp.	X				X		
Cicendia filiformis		X		X		X	
Coronopus navasii		X					
Coronopus squamatus	X	X					
Corrigiola litoralis				X			
Corrigiola spp.		X					
Crassula alata	X						
Crassula tillaea				X			
Crassula vaillantii	X	X	X	X	X		
Crepis pusilla				X			
Crypsis aculeata			X				
Crypsis schoenoides			X				
Crypsis spp.		X					
Damasonium alisma	X						
Damasonium bourgaei					X		
Damasonium polyspermum		X					
Elatine alsinastrum				X			
Elatine gussonei					X		
Elatine macropoda	X		X	X			
Elatine spp.		X					
Eleocharis acicularis		X					
Eleocharis multicaulis		X					
Eleocharis palustris	X						
Erophila praecox				X			
Eryngium corniculatum		X				X	
Eryngium galioides		X				X	
Eryngium viviparum		X					
Exacullum pusillum		X					
Exaculum pusillum			X				
Fimbristylis bisumbellata				X			
Gypsophila muralis				X			
Heliotropium supinum		X	X				
Illecebrum verticillatum		X				X	
Isoetes duriaei		X	X			X	
Isoetes histrix		X		X			
Isoetes setaceum		X				X	
Isoetes spp.		X					
Isoetes velata			X				
Isoetes velatum		X				X	
Isoeto-Nanojuncetea	X						
Isolepis cernua	X			X			
Isolepis setacea	X						
Isolepis pseudosetacea						X	
Isolepis spp.		X					
Juncus bufonius	X	X		X		X	
Juncus capitatus		X				X	
Juncus hybridus	X	X		X			
Juncus pygmaeus		X		X		X	
Juncus spp.						X	
Juncus tenageia		X					
Lepidium spinosum				X			
Limosella aquatica	X						
Lotus angustissimus	X						
Lotus hispidus						X	
Lotus parviflorus						X	
Lotus spp.						X	
Lythrum baeticum		X					
Lythrum borysthenicum		X	X	X		X	
Lythrum flexuosum		X					
Lythrum hyssopifolia	X	X		X	X	X	
Lythrum portula		X					
Lythrum spp.		X					
Lythrum thymifolia		X					
Lythrum tribracteatum	X	X	X	X			
Marsilea batardae		X				X	
Marsilea strigosa		X					
Mentha cervina		X	X			X	
Mentha pulegium		X			X		
Montia arvensis				X			
Myosotis sicula		X	X				
Myosurus heldreichii				X			
Myosurus minimus	X	X		X			
Ophioglossum azoricum		X					
Ophioglossum lusitanicum	X	X		X			
Pilularia minuta			X				
Plantago cretica	X						
Plantago weldenii				X			
Poa infirma				X			
Polypogon maritimus				X			
Pulicaria paludosa		X				X	
Pycreus flavescens				X			
Radiola linoides		X				X	
Ranunculus batrachiodes		X					
Ranunculus lateriflorus		X		X			
Ranunculus longipes		X					
Ranunculus marginatus				X			
Ranunculus nodiflorus		X					
Ranunculus peltatus	X				X		
Ranunculus revellierii			X				
Riella notarisii			X				
Schoenoplectus supinus		X					
Sedum eriocarpum subsp. porphyreum	X						
Sedum caeruleum					X		
Sedum maireanum		X					
Sedum microcarpum	X						
Sedum nevadense		X					
Sisymbrella aspera		X				X	
Solenopsis laurentia		X					
Spergula purpurea		X					
Spergularia bocconei				X			
Spergularia capillacea						X	
Trifolium cernuum		X					
Trifolium micranthum		X					
Trifolium ornithopodiodes		X					
Trifolium retusum		X					
Trifolium suffocatum				X			
Verbena supina	X	X					
Veronica anagalloides		X				X	
Zannichellia melitensis					X		
Zannichellia palustris	X			X			

Source: Camacho, 2020

Even though definitions of HTCI biological communities usually focus on plant components, typical and characteristic species can belong to any species group (Tsiripidis et al., 2018). Vegetation databases may serve as a starting point for compiling lists of typical species, but they should not be the only source.

Fish are often considered the most characteristic aquatic organisms in inland waters. However, few fish species are exclusive to lakes and ponds, meaning there are limited candidates among them for diagnostic species, and it is therefore difficult to identify typical species that are strictly lacustrine. By contrast, amphibians appear to be better candidates. Some species are characteristic of lake shores and small ponds, and they are highly sensitive to environmental quality. As such, several amphibian species have been proposed by Spanish experts as candidates for typical species in condition assessments: *Alytes cisternasii, Bufo calamita, Bufo viridis, Discoglossus jeanneae, Discoglossus galganoi, Discoglossus pictus, Hyla arborea, Hyla meridionalis, Lissotriton boscai, Pelobates cultripes, Pelodytes ibericus, Pelodytes punctatus, Pleurodeles waltl, Pelophylax perezi, Salamandra salamandra, Triturus marmoratus,* and *Triturus pygmaeus* (Camacho et al., 2009).

Beyond amphibians, other taxa are also associated with specific types of lentic HTCI. For example, fairy shrimps (Anostraca, from the Greek “without shell”), the only order of the subclass Sarsostraca of branchiopod crustaceans, are regularly found in well-preserved temporary ponds, in some cases corresponding to HTCI 3170* (Alonso & García de Lomas, 2009). Certain anostracans, such as species of the family Chirocephalidae, may be considered typical species for condition assessment and even characteristic species for freshwater Mediterranean temporary ponds (HTCI 3170*). They are, however, extremely sensitive to changes in key habitat features such as hydroperiod. Ponds converted to permanent hydroperiods can sustain fish populations, which in turn may eliminate these small crustaceans through predation. The status of such species, when considered typical, would therefore also fulfil the requirements for assessing the status of typical species under the Article 17 guidelines.

The use of specific species as surrogates for conserving biodiversity in lentic habitats must be approached with caution. Stewart et al. (2017) compiled occurrence data on 72 species of freshwater fishes, amphibians, mussels, and aquatic reptiles across a large region of North America. They found that the landscape-scale factors shaping species distributions differed among groups, with low congruence in conservation priorities (<20%). As a result, surrogate priority areas based on one taxonomic group did not protect the best habitats of others. While common, wide-ranging aquatic species were included in taxa-specific priority areas, rare freshwater species were largely excluded. The authors concluded that conservation priorities developed from a single aquatic taxonomic group would not adequately protect all other aquatic taxa.

This does not mean, however, that particular typical species cannot serve as indicators of habitat condition. When used in this way, typical species can also function as umbrella species—species whose habitat or ecological requirements are broad enough that protecting them ensures the protection of other species in the same ecosystem. Alternatively, their status may reflect whether or not their own ecological requirements are being met by the contemporary habitat.

1.4 Main pressures affecting lentic habitats and their influence on habitat condition

Freshwater ecosystems are at constant risk of irreversible damage from human pressures that threaten biodiversity, ecosystem services , and human well-being (Langhans et al., 2019). The alteration of natural habitats usually results from pressures acting on them, which in turn cause major biodiversity loss (Visconti et al., 2024; Tickner et al., 2020).

Significant human-induced alterations of freshwater systems include changes to soil erosion–deposition dynamics, modifications of hydrological regimes through impoundment and diversion, land-use conversion, chemical and nutrient pollution, and the spread of invasive species (EC, 2015; Flitcroft et al., 2019). In lentic ecosystems, many pressures do not act directly on the ecosystem itself but rather on its catchment. Throughout history, such alterations have led to major transformations in the structure and functioning of lentic ecosystems, and in some cases even to their physical disappearance.

These pressures[1], which are diverse but mostly of human origin, materialise in a wide range of impacts, including: modification of surface drainage networks in the surroundings of the ecosystem; drainage or, conversely, damming of the water body; overexploitation of aquifers on which they depend; introduction of phreatophytic species that increase water consumption and evapotranspiration; ploughing of lake basins, especially those with fluctuating hydrology; implementation of intensive agriculture systems in receiving basins; contamination of surface and groundwater; increased sedimentation and clogging rates; destruction of ecosystem morphology; and substitution of morphodynamic processes.

Basically, pressures cause impacts that alter the essential ecological features of lentic ecosystem, as reported in section 1.2.1. Therefore, a robust assessment system for evaluating habitat condition should not only examine the status of these key ecological features, but also consider the types of impacts potentially generated by different pressures.

In the Water Framework Directive (EC, 2000), the indicative variables (metrics) used for assessing the ecological status of water bodies are required to respond proportionally to the gradient of pressures (Bolpagni et al., 2017). This provides a complementary approach for evaluating the completeness of the currently applied methods.

Following Camacho et al (2019b), we define below the main groups of pressures affecting lentic habitats. These groups are later used in Section 3.1 to analyse which pressures are actually covered by the variables applied in the assessment of structure and function by EU Member States, and to inform the proposal of key variables to be measured for assessing the condition status of lentic habitats. The main groups of pressures considered here, both direct and indirect, are:

A. Hydrological pressures and impacts
B. Geomorphological pressures and impacts
C. Pressures and impacts altering water quality
D. Pressures and impacts on the structure of communities
E. Pressures and impacts due to land use
F. Pressures and impacts related to occupation and area shifts of lentic habitats
G. Pressures and impacts due to the presence of invasive alien species

[1] For an overview of major pressure types, see: http://fis.freshwatertools.eu/index.php/infolib/pressures.html, and https://tableau-public.discomap.eea.europa.eu/views/sonpressuresandthreats/Pressuresandthreats?%3Adisplaycount=n&%3Aembed=y&%3AisGuestRedirectFromVizportal=y&%3Aorigin=vizsharelink&%3AshowAppBanner=false&%3AshowVizHome=n

H. Other pressures and impacts
I. Climate change.

Each of these groups of pressures can be further divided into specific types. As mentioned, these will be used to verify whether the current condition assessment systems applied in the EU adequately cover all possible effects of pressures, or whether additional variables should be proposed to assess the effects of specific pressures on the main habitat features. Section 3 of this document also include the analysis on how these pressures are addressed by the respective variables, and will identify the corresponding gaps.

Box 1. Parameters for evaluating pressures on lentic habitats, described and updated by Camacho et al. (2009, 2019b).

A. Hydrological pressures and impacts

- A1. Direct water abstraction
- A2. Alteration of the natural flooding regime and water flow patterns (e.g., drainage, external flow inputs, flow variations due to exploitation or non-natural inputs)
- A3. Flow regulation in tributaries
- A4. Presence of drainage infrastructure
- A5. Extraction of water from the associated aquifer (where applicable).

B. Geomorphological pressures and impacts

- B1. Alteration of morphometry or substrate characteristics affecting the structure, function, or surface of the wetland (e.g., infilling, slope modification, construction of structures)
- B2. Extraction of materials
- B3 – Disposal of rubble disposal

C. Pressures and impacts altering water quality

- C1. Intermittent spills of urban and/or industrial wastewater
- C2. Spills of specific pollutants (e.g., heavy metals and priority substances)
- C3. Presence of diffuse pollution sources in the catchment
- C4. Inflows with mineralogical characteristics different from the natural ones
- C5. Thermal discharges (e.g., from cooling processes)
- C6. Alteration of the natural chemical quality of the associated aquifer (where applicable)

D. Pressures and impacts on the structure of communities

- D1. Connectivity with adjacent natural ecosystems
- D2. Exploitation or other pressures on the biological community
- D3. Aquaculture activities

E. Pressures and impacts due to land use

- E1. Land use for road and residential infrastructure
- E2. Presence of electrical lines

F. Pressures and impacts related to occupation and area shifts of lentic habitats

- F1. Reduction of the area occupied by the habitat type at the local scale
- F2. Occupation of the wetland basin or its banks

G. Pressures and impacts due to the presence of invasive alien species

- G1. Presence of exotic species included in catalogues of invasive alien species
- G2. Presence of exotic (alien) species of the habitat type not included in such catalogues

H. Other pressures and impacts

- H1. Solid waste
- H2. Livestock overgrazing
- H3- Recreational activities
- H4- Other pressures and impacts (e.g., periodic vegetation burning)

I. Climate change

2. Analysis of existing methodologies for the assessment and monitoring of habitat condition

This section presents the results of the review and analysis of existing methodologies and procedures for assessing and monitoring the condition of running water habitats in the EU Member States. A screening of methodologies used by up to 2/3 (18 from 27) of the EU MSs has been carried out, identifying the variables and metrics used and other relevant aspects of the monitoring procedures applied (see Annexes 1 and 2 for complete information).

2.1 Variables used, metrics and measurement methods, existing data sources

This section reviews and analyses the variables used to measure habitat condition and the extent to which they reflect the key features identified in the ecological characterisation of lentic habitats described in Section 1. The analysis is based on a classification of variables according to the framework defined in Table 1, grouping them into abiotic (physical, chemical), biotic (compositional, structural, functional), and landscape characteristics with their associated variables. The variables used in the reviewed methodologies include 25 variables assessing physical abiotic characteristics, 17 for chemical abiotic characteristics, 12 for compositional characteristics, 19 for structural characteristics, 27 for functional characteristics, and 7 related to landscape characteristics.

Although some variables are applied in a generalised way by most MSs, the methodologies used to apply them may differ. In addition, some variables are specific to only one or a few MSs, while others are specific to certain lentic habitat types.

Following the classification of variables in Table 1, the variables used for each type of feature are analysed separately, assessing their consistency with the ecological characterisation of lentic habitats detailed in the previous section. An overview of the main groups of variables used by EU MSs in the assessment of lentic habitats, with respect to their main abiotic, biotic, and landscape characteristics, together with a summary description of these variables is presented below. Further details on the use of these variables for specific habitat types, with references to the corresponding methodologies, are provided in Annex 1.

Abiotic physical characteristics

The set of physical abiotic variables identified could be grouped into hydrological, morphological, soil or sediment-related, and water-related variables. Among these, **hydrological** variables are the most widely used across EU MSs. They are key for assessing one of the determinant features of lentic ecosystems – the water regime – and its possible alterations. Variables related to the hydrological regime and water level fluctuations are the most frequently applied in the analysed methodologies (in 9 and 7 MSs, respectively), not only in the Mediterranean biogeographic region, where seasonality is a characteristic factor of certain HTCIs, but also in other regions.

Methodological approaches differ among MSs. For example, the maximum extent of water or maximum depth may be measured, and these measurements may be taken at different times or with different periodicities. In some cases, hydrological alterations linked to anthropogenic inflows or water abstractions are also considered. Other hydrological variables capture more specific aspects such as drainage (IE, DK, LT, PL), evaluation of the emptying mode (ES), the nature of the water entering the system (BE), or its availability (HR).

Morphological variables are also used to assess the physical structure of lentic habitats, since the basin and shores of lakes or ponds have a specific morphology under natural conditions that should be preserved for the habitat to remain in good condition. However, morphological variables are used less frequently than hydrological ones, and no single variable predominates. Approaches also vary among MSs: some apply indicators for siltation and clogging processes (DE, ES, FR), shoreline and depth characteristics and their possible alterations (DE, DK, IE), basin shaping processes (ES), or erosion processes (HU).

Variables addressing **soil and sediment** physical features are not widely used by MSs in the assessment of lentic habitat types. Only two MSs apply a related variable, namely the type of bottom substrate (HR, RO). In contrast, variables describing the status of the **physical characteristics of the water** are commonly applied. The main indicators are water transparency (11 MSs) and colour (9 MSs). The former is mainly linked to phytoplankton growth and the assessment of eutrophication. Additional variables – such as the depth of the photic zone, water temperature, suspended solids, and water turbidity (the latter also related to water transparency) – are used in only one or two MSs each.

Table 4. Abiotic physical characteristics - Variables used by the screened Member States (see complete information in Annex 1)

Group of variables	Variables used in national methodologies	MS Nr.	BE	BG	CZ	DE	DK	ES	FR	GR	HR	HU	IE	IT	LT	NL	PL	RO
Hydrology	Hydroperiod/hydrological regime	9	■	■		■		■		■		■	■	■		■		
	Water level fluctuation/flooding	7						■		■	■		■	■		■		■
	Drainage	4					■						■		■		■	
	Emptying system	1						■										
	Nature of the water supply	1	■															
	Contact with karstic rock	1				■												
	Use for production of still water	1																■
	Water availability	1																
Morphology	Siltation and clogging	3				■		■	■									
	Shoreline characteristics	2				■							■					
	Depth ratio	1					■											
	Mean lake depth	1					■											■
	Maximum depth	1					■											
	Secchi depth	1					■											
	Relief modelling	1						■										
	Bank profile	1	■															
	Erosion	1	■									■						
	Crust cover (%)	1											■					
Soil & sediment	Type of bottom substrate	2									■							■
Physical water characteristics	Water transparency	11	■	■	■		■	■			■		■		■	■	■	■
	Suspended solids	1	■															
	Water colour	9	■				■	■	■	■			■		■		■	■
	Photic/euphotic zone depth	2											■				■	
	Water temperature	2		■										■				■
	Water turbidity	2							■									■

Abiotic chemical characteristics

Some **basic water chemistry parameters** are widely used as variables in lentic habitat assessments. These include pH (acidity, 10 MSs), and electrical conductivity (water salinity, 7 MSs). pH is used to assess variations linked to acidification (e.g., by acid rain, pyrite mining, industrial activities), while conductivity reflects mineral content, indicating salinisation (e.g., by salt-rich inflows such as leather industry wastewaters or road salt use) or desalination (usually by excessive agricultural freshwater runoff). Other parameters are less widely applied, such as oxygen concentration and saturation (BE, BG, DK, ES, IT, RO). Although highly informative about metabolic processes within the ecosystem, these could also be considered functional variables. Alkalinity (BE, DK, IE), which illustrates the buffering capacity of lake waters, is likewise applied in only a few Member States.

Additional indicators rarely used, include the hydrochemical dystrophy index (PL) and redox potential (ORP) (BG). In aquatic ecosystems, **nitrogen (N) and phosphorus (P) compounds (nutrients),** which are key for assessing trophic status (eutrophication), are among the most commonly used chemical variables. The main indicators are total P (10 MSs) and total N (8 MSs). In addition to these two indicators, BE also uses ammonium, nitrite, and inorganic nitrogen in its assessments, which are informative, among other aspects, about the sources of this chemical alteration. Another group of variables relates to the analysis of **major ions**, such as chloride, sodium, sulphate, and calcium, also applied by BE. By contrast, the study of **sediment chemistry** is marginal in status assessments: only the composition and organic content of the substrate are analysed as trophic indicators (DE, FR, GR), and substrate maturity is considered by BE.

Table 5. Abiotic chemical characteristics - Variables used by the screened Member States (see complete information in Annex 1).

Group of variables	Variables used in national methodologies	MS Nr.	BE	BG	CZ	DE	DK	ES	FR	GR	HR	HU	IE	IT	LT	NL	PL	RO
Abiotic Chemical Characteristicsnr																		
Inorganic nutrients	Total P	10	■	■		■	■	■	■				■			■	■	■
	Total N	8	■	■		■	■		■							■	■	■
	Ammonia / ammonium	1	■															
	Nitrite nitrogen	1	■															
	Inorganic nitrogen	1	■															
Main ions	Chloride	2	■													■		
	Sodium	1	■															
	Sulphate	1	■															
	Calcium	1	■															
Soil / Sediment chemistry	Organic sediments (and N & P) on lake bottom/substrate composition	3				■			■	■								
	Maturity of the substrate	1	■															
Water chemistry basic features	pH, acidity	10	■	■			■	■					■	■	■	■	■	■
	Water electrical conductivity (salinity)	7	■	■				■						■		■	■	■
	Oxygen concentration and saturation	6	■	■		■	■							■				■
	Alkalinity	3	■				■						■					
	Hydrochemical Dystrophy Index	1															■	
	Redox potential	1		■														
	Suspended solids	1	■															

In general, laboratory methodologies do not vary among MSs, as these are standard chemical analyses (many of them included in APHA, 2005). However, sampling strategies can differ both spatially and temporally among MSs and between HTCI, depending on their ecological characteristics.

Biotic compositional characteristics

With respect to biotic variables, one of the most widely used in the general assessment of HTCI – and particularly of lentic habitats – is the composition, as it provides direct results on habitat status through the presence of communities or species linked to that habitat.

The **vegetation composition** of hydrophytes, helophytes, and sometimes halophytes, is the main type of variable applied almost unanimously by most Member States (MSs). The presence of characteristic, typical, or key species of each lenitic habitat is the preferred variable. In some methodologies, a checklist of species that must be present for each habitat type is used; in others, the richness and number of identified species are scored.

At least 5 MSs (HR, IE, LT, PL, RO) additionally apply variables associated with plant communities and their presence, similar to the variables assessed by species. In PL, a variable assessing the dominant species of the habitat in question is also included. Since characteristic vegetation is often part of the definition of the HTCI (EU, 2013), assessing the status of characteristic species is a key aspect in evaluating habitat condition. For this reason, the assessment of characteristic vegetation is carried out in all MSs for lenitic habitats, as all HTCIs of group 31 (Standing waters) include the characteristic vegetation in their definition. In addition to plants directly associated with water, HU also assesses weeds, shrubs, and tree species.

Analysing other indicators collected from different MSs, and grouped according to trophic level, the study of **phytoplankton community** composition is applied by six MSs (DK, ES, IE, IT, PL, RO). In this case, the methodology focuses on identifying and describing the community, sometimes grouping algal taxa to calculate indices, following procedures similar to those used in the assessment of ecological status the Water Framework Directive (EC, 2000). This approach is based on the indicative value of algal species and/or groups for describing processes such as eutrophication and acidification. Beyond compositional or structural variables, many MSs also apply methods related to phytoplankton that address ecosystem functioning, which will be described below.

Three MS (ES, LT, PL) also include **zooplankton** as status assessment variables for lenitic habitats, either through community studies and group-level analysis or by recording the presence of certain indicator taxa that reflect habitat status. Similarly, **benthic aquatic macroinvertebrates** are used, though only marginally, by five MSs (DK, DE, ES, FR, IE), usually through presence/absence of selected taxa; in two cases (DE, ES), the number of taxa is also recorded. Invertebrates have an indicative value for habitat condition as they respond to pressures such as organic pollution and the presence of invasive species.

Regarding vertebrates, ES and GR include compositional studies of **ichthyofauna**, while ES also assesses **amphibians and reptiles**. GR and IT additionally assess other faunal groups, such as **birds or mammals**. In all these cases, the presence of autochthonous (native) species is analysed as a relevant compositional variable for evaluating the conservation status of the habitat, whereas invasive alien species are analysed as a separate type of biotic variable and will be described below.

Table 6. Biotic compositional characteristics - Variables used by the screened Member States (see complete information in Annex 1).

Group of variables	Variables used in national methodologies	MS Nr.	BE	BG	CZ	DE	DK	ES	FR	GR	HR	HU	IE	IT	LT	NL	PL	RO	SL	SK
Biotic compositional characteristics																				
Phytoplankton	Phytoplankton / algal communities' composition	6																		
Vegetation	Characteristic, typical plant species (presence, number)	18																		
	Characteristic plant communities (presence)	5																		
	Dominant species	1																		
	Shrub and tree species	1																		
	Weed species	1																		
Zooplankton	Zooplankton	3																		
Benthic aquatic macro-invertebrates	Presence/absence of selected aquatic invertebrate taxa	5																		
	Benthic aquatic macro-invertebrates (number of taxa)	2																		
Ichthyofauna	Proportion of individuals of native ichthyofauna	2																		
Amphibians and reptiles	Number of species of native amphibians and reptiles	1																		
Other fauna (e.g., birds, mammals)	Presence of animal species	2																		

Biotic structural characteristics

Beyond composition, different biotic and abiotic structural variables are used by MSs. Some are applied more generally to vegetation, while others are used by only a few MSs for soil or habitat structure. Structural features of habitats are often an interplay of biotic and abiotic components, and are therefore considered together here.

Vegetation structure, as in the case of compositional characteristics, is the most commonly used feature for assessing the structural elements of lentic habitats. More than half of the MSs analysed use variables related to the extent of cover of aquatic vegetation (hydrophytes), often linked to methods that also address composition. Methodological differences are observed, with many MSs specifying the communities or species to be considered when assessing these variables, generally focusing on the characteristic species of the habitat whose condition is being evaluated. Spatial and/or temporal specifications may also differ between MSs in the application protocols of their methodologies.

In parallel with aquatic vegetation (hydrophytes) cover, most countries that apply this variable to helophyte cover. Aquatic plant communities thus represent a common element in assessing the status of lentic habitats, given the ecology of these ecosystems, where both hydrophytes and helophytes not only determine to the physical structure of the habitat itself, but also contribute to other structural aspects such as community structure and diversity.

Table 7. Biotic structural characteristics - Variables used by the screened Member States (see complete information in Annex 1).

Group of variables	Variables used in national methodologies	MS Nr.	BE	BG	CZ	DE	DK	ES	FR	GR	HR	HU	IE	IT	LT	NL	PL	RO	SK
Biotic structural characteristics																			
Vegetation structure	Coverage of aquatic vegetation / vegetation structure	12	■		■	■	■	■	■	■	■			■	■	■		■	
	Coverage of helophytes	8	■		■			■	■					■	■	■		■	
	Cover of other plants (shrubs, trees, herbaceous plants…) and open surfaces. Width and zonation of riparian vegetation	6							■	■		■				■	■		■
	Absence of specific biotic forms (e.g., tall halophytes)	1								■									
	Canopy heterogeneity	1									■								
	Density of phytobenthos (IPS index)	1					■												
	Charophyte and cyanobacterial crust score (C&K)	1											■						
	Depth zonation	1											■						
Soil structure	Extent of bare soil	2							■							■			
	Characteristics of the bottom and banks	2			■											■			
Habitat structure	Habitat area / extent	5						■				■					■	■	■
	Habitat degradation indicators, fragmentation	5		■	■	■									■		■		
	Water body area	4									■			■		■		■	
	Habitat complexity / mosaic / heterogeneity / patches	4				■			■			■					■		
	Bottom surface area	1										■							
	Shading	1										■							
	Exposure	1																■	
Animal taxa	Fish abundance and size distribution CPUE nr. & weight)	1					■												
	Benthic fauna (Macro-invertebrate Index)	1					■												

Other types of vegetation cover not specifically linked to aquatic environments—such as shrubs and trees, herbaceous plants, stoneworts, and open surfaces—are also analysed by six Member States (MSs), with each applying the assessment to one or several of these covers. More rarely, specific variables address particular aspects of vegetation structure, such as the absence of specific biotic forms (GR), canopy heterogeneity (HU), or depth zonation (IE).

When compared to the quality elements used to assess the ecological status of lakes in the WFD, higher plants and macroalgae are the biological quality element (BQE) predominantly used in determining the condition of the HTCIs, whereas phytobenthos — the BQE corresponding to “other aquatic flora”— is used as a condition indicator only by DK.

Soil structural variables are not widely used in habitat condition assessment. The extent of bare soil is applied only by FR and NL, while the characteristics of the bottom and banks are assessed by CZ and NL.

Some variables related to **habitat structure** are used by several MSs, although they are not generalised or widely applied. Both habitat extent (area) and habitat fragmentation or degradation are used as independent variables by five MSs. The area of the water body, often measured in parallel with habitat extent, is applied by up to four MSs, the same number that assess habitat complexity or heterogeneity. Other specific variables are applied by only one MS, such as the surface area of the bottom or shading that may affect the habitat (HU), or habitat exposure (RO). Finally, only DK assesses structural variables related to biota other than vegetation, such as the size structure of fish populations.

Biotic functional characteristics

Variables linked to the functional characteristics of ecosystems—usually describing processes where both biotic and abiotic components interact—are often closely related to compositional, structural, and abiotic variables. However, functional variables are the least commonly shared among EU Member States. Although the list is larger (27 variables) than in other groups, there is little standardisation, and only a couple of variables are applied by more than six MSs.

Functional variables are therefore not widely shared in the assessment of lentic habitats condition across MSs. Those related to **invasive alien species** are the most frequently used, with nine MSs applying them (plus one MS recording their absence). Invasive alien species can potentially alter many habitat characteristics of the habitats, but their impacts on ecosystem functioning are considered the most significant, and for this reason they are categorised here as functional variables. Other functional variables—such as those related to acidification, faunal functions, or organic loads—are applied by only one MS, mostly BE.

The functional variables assessing the trophic status, resulting from multiple interactions are widely used among MSs. Up to 11 variables are applied, using different methodologies, including indicators such as the growth of filamentous algae, phytoplankton biomass, nutrient load, and daily oxygen saturation, among others. All of these variables respond to the need to assess the trophic status of lentic habitats, which represents the main problem in many aquatic ecosystems.

Other processes are assessed by some Member States using specific indicators or indices. These include the number of plant species with particular traits (eutrophilous, destructuring, woody, wasteland, stoneworts, negative-indicator species, hydromorphological functioning, etc.), which can be applied to different functional characteristics. Depending on the MS and HTCI, these species differ, and in many cases, they are listed in checklists to confirm their presence.

Some MSs also apply variables considered functional but which are in fact associated with specific **pressures and impacts**, such as land use, management and its influences, or other anthropogenic activities. These pressure-related variables should properly be associated with the parameter **Future prospects** in the evaluation matrix for the conservation status of HTCIs in Annex I of the Habitats Directive for Article 17 reporting. In fact, **Structure and function** indicators should assess how the habitat's characteristic ecological features are altered (and the extent of alteration) by the pressures and impacts, rather than the pressures themselves.

Table 8. Biotic functional characteristics - Variables used by the screened Member States (see complete information in Annex 1).

Group of variables	Variables used in national methodologies	MS Nr.	BE	BG	CZ	DE	DK	ES	FR	GR	HR	HU	IE	IT	LT	NL	PL	RO	SK
Acid status	Cover of species indicative of acidification	1																	
Invasive species	Presence (and cover) of invasive alien species	9																	
	Absence of alien and/or invasive species	1																	
Faunal functions	Imbalance in fish populations	1																	
	Impacts of animals	1																	
Organic loads	Biological oxygen demand	1																	
	Chemical oxygen demand	1																	
Trophic status	Strong algal growth (filamentous algae or blooms)	3																	
	Phytoplankton biomass/ chlorophyll-a	3																	
	Nutrient richness/nutrient loading	3																	
	Crust chlorophyll-a	1																	
	Water trophic state index	1																	
	Nitrogen deposition	1																	
	Daily oxygen saturation variation	1																	
	List of species indicative of eutrophication	1																	
	Eutrophication of the habitat	2																	
	Deep chlorophyll maxima and photosynthetic bacterial populations in summer	1																	
	Zooplankton/phytoplankton trophic ratio (shallow, non-saline lakes)	1																	
Diverse processes	Number of plant species with specific traits/negative indicators (eutrophilous, etc.)	6																	
	Dynamics	1																	
	Leaf litter	1																	
	Hydro-morphological functioning	1																	
	Index total phosphorous x Water colour	1																	
	Maximal depth of stoneworts meadows	1																	
Pressures and threats	Land use and human disturbance	3																	
	Management influences	2																	

In Section 3.1 and Annex 2 we analyse how the variables used by EU MSs assess the pressures described in section 1.3. This involves evaluating not only whether the main habitat features are covered by the variables currently applied, but also whether the possible impacts on these key habitats' features, caused by pressures listed in Section 1.3, are adequately captured. This joint analysis and the identification of gaps will guide the selection of the recommended variables presented in Section 3.1 and Annex 2.

Landscape characteristics

In ecology, the concept of landscape is linked to physical proximity or to the possibility of interactions mediated by abiotic or biotic elements, and requires the existence of strong connectivity between two or more ecosystems (Turner & Gardner, 2016). This connectivity may be physical (adjacent territories), abiotic (e.g., through water in a hydrological basin), or biological (through individuals of one or more species moving from one habitat to another). Variables related to the assessment of habitat condition at the landscape level are compiled in Table 9. These variables complement those addressed by abiotic and biotic factors, providing a broader perspective and relating the habitat to its wider environment.

This group of variables is rarely used in the condition assessment of lentic habitats, and only by a handful of Member States (MSs). Six MSs apply landscape variables, each using a different one, without harmonisation. These include: the evolution of water bodies where the habitat is present (FR); environment, isolation, or distance from other similar habitats (HU); metrics related to the shape and contact between landscape patches (IT); habitat mapping and analysis (NL); legal protection status of the habitat (LT); and fragmentation of landscape patches (PL).

Table 9. Landscape characteristics - Variables used by the screened Member States (see complete information in Annex 1).

Group of variables	Variables used in national methodologies	MS Nr.	FR	HU	IT	LT	NL	PL
Landscape	Evolution of the number of water bodies where the habitat is present	1	■					
	Landscape environment	1		■				
	Landscape metrics	1			■			
	Spatial conditions	1					■	
	Vegetation mapping	1					■	
	Protected area	1				■		
	Structure of habitat patches (fragmentation)	1						■

2.2 Definition of ranges and thresholds to obtain condition indicators

This section reviews and analyses the methodologies used by EU Member States (MSs) to establish reference values and thresholds among state classes for the indicators applied in assessing the habitat (HTCI) condition. Overall, the compiled information provides only limited insights into how these class ranges or boundaries are defined. Where limits are provided, the origin or method of deriving them is rarely explained explicitly, and in many cases, the values are not directly identified.

An important concept here is that of **reference conditions**. These are the values of an indicator variable in sites where no significant alteration of the ecological feature of the habitat has occurred, as applied, for example, in the Water Framework Directive (WFD; EC, 2000).

This does not necessarily mean pristine sites, but rather sites where there are no significant effects of pressures on the specific habitat feature and its interaction with other habitat or ecosystem components. Measured values of a given variable at a site are compared with those under reference conditions, and a ratio is established (Ecological Quality Ratio, EQR, in the case of the WFD). Depending on where this ratio falls, a state class is then assigned.

For abiotic factors, most MSs for which detailed information is available define statistical values that differentiate between state classes. For example, BE, ES, IE and PL define threshold values for physic-chemical parameters, probably derived from statistics based on reference site data, although the source of these thresholds is not specified. ES defines classes of hydromorphological variables by indicating the absence or presence of elements or modifications of patterns related to these variables. HU and IE for some variables also provide classifications or groupings of values, but without specifying how class limits are determined; in many cases, assessments are made by expert opinion. Other MSs, such as GR, HU, IT, LT, and RO, do not provide detailed information on class thresholds for physico-chemical parameters in the documents examined.

For compositional variables, each MS defines its own threshold values, which may vary between habitats within the same country. In most cases, thresholds are based on the number of species recorded during the assessment (BE, DE, ES, FR, HU, IE, NL, PL, RO, SK), with favourable status assigned above a certain number of characteristic and/or typical species—usually between three and seven for plants—and unfavourable status assigned below that number. In ES and FR, for example, these thresholds are used to score variables within an index rather than to define status classes directly. In HR, and in PL for certain compositional variables, status classes are defined on the basis of the dominance or abundance of characteristic and/or typical species, as determined by expert opinion, without reference to the number of taxa. Most identification of these species is based on expert visual assessment. In MSs such as GR, IT, LT, and SI, no information on these issues could be retrieved.

The ranges and class limits of structural variables are mostly determined on the basis of the percentage cover of characteristic and/or typical species or communities (BE, DE, ES, PL) or the current area they occupy (BE). Thresholds may also include the **number of different, typically formed vegetation structure elements**, which can vary depending on lake or pond morphology. Examples include submerged vascular plant vegetation, floating vegetation, willow or alder scrub, alder riparian forest, marsh, and reed bed (DE).

In other cases, as for HR, composition is combined with structure, with classification defined by the number of characteristic or typical taxa and their cover. In HU, the heterogeneity of the assessed area is evaluated, while in NL the number of structural elements present is determined. In all these cases, thresholds are defined on the basis of quantitative estimates that are often derived from expert opinion. No information was retrieved for GR or RO regarding thresholds for structural variables.

Information on threshold values for functional variables is even scarcer. For invasive alien species or for other taxa indicative of specific processes, species numbers or cover are sometimes defined as quantifying values for class changes. Regarding eutrophication, threshold values for chlorophyll-a (ES, IE) or the presence of certain filamentous algal groups (IE) are specified in some cases. In others, no information is available in the examined documents, or it is only stated that the progression or evolution of filamentous algae is evaluated (FR). For other functional variables, threshold values are not specifically provided.

For some landscape variables, categorical classifications are made on the basis of expert opinion, while for others no information is specified.

In summary, considering the general trends in the analysis, only for some abiotic variables and for the presence and cover of plant species is there relative consensus or sufficient information on threshold values, which are mostly defined either statistically or by expert opinion. Many variables lack defined thresholds or clear information on the classification adopted, which prevents the methodologies established by different EU Member States from being comparable in terms of reference conditions and threshold setting.

2.3 Aggregation methods at the local scale

This section examines the approaches used by EU Member States (MSs) for aggregating indicators at the local scale (i.e., assessing the condition of lentic habitats at the level of the monitoring locality), including how variables are weighted. In general, local-scale assessments are carried out at the site or monitoring plot level, integrating abiotic and biotic variables through aggregation methods to obtain a quantitative or categorical value that define the conservation status. based on the information available from the MSs, data on individual variables within each group (abiotic, compositional, structural, and functional) are combined using various methods—quantitative, conditional, or categorical rules. Examples of how different MSs apply these rules are described below.

Quantitative rules apply arithmetic operators or, in some cases, multivariate analyses to the values estimated for each variable. Some multimetric indices aggregate and integrate information of several variables, weighting the different categories. From the resulting value, thresholds are set to classify the conservation status, usually for the parameter Structure and function'.

In ES, a multilevel and flexible index called ECLECTIC (Camacho et al, 2009) was developed. This index enables assessment of the conservation status in terms of habitat structure and function, as well as the status of typical species, at the site scale. It incorporates variables on biological communities, hydrogeomorphological and physico-chemical factors, and their response to pressures and impacts at the local scale. These variables are grouped into four categories, scored according to defined metrics, and weighted so that each category contributes equally. The resulting index value is then translated into conservation status classes using defined threshold values.

In FR, habitat status at the local scale is determined by comparing field-derived indicator values with threshold values obtained from bibliographic research, expert opinion, or field tests. Each indicator is assigned a score, and the sum of indicator scores is subtracted from a baseline of 100 to produce a final score, which is then positioned along a conservation status gradient.

In IE, scores for biological response indicators (i.e., algal communities, vegetation, Rumex cover, vascular plant indicators, and aquatic invertebrate indicators) are summed to give a total score for biological responses. The same is done for hydrological function and water chemistry. The final integration class is then defined as: favourable (no more than one intermediate and no bad scores); inadequate (any other combination); or bad (two or more bad scores, or at least one bad combined with one not good).

In HU, the condition of a single sampled habitat plot is determined from 16 main variables. An aggregated score reflects the local structure and function. Three condition classes are established: good, non-satisfactory and bad. Thresholds are set based on unpublished inter-assessments of thousands of plots.

A detailed example of aggregation methods at the local scale is provided by DK. For the structural indicators, points are assigned to each category by which the indicator can be characterised. The maximum value an indicator can assume is 1, assigned to the category that represents the most optimal state. This optimal condition differs between habitat types. Other categories are assigned progressively lower point values between 0 and 1, depending on their distance from the optimal condition.

Indicators are weighted according to their importance. Because they are organised in a tiered system, weighting is applied at each hierarchical level. The weights are normalised so that their sum equals 1. Ideally, weights are based on robust data; however, in the absence of such data, they are determined using expert judgement, informed by experiences from well-studied sites.

The total structure index is calculated as the corrected sum of the weighted point values. Each indicator is weighted partly by the weight of its own level and partly by the share of the higher levels in the total structure index. Similarly, indicators are weighted within each indicator group, with group weights also summing to 1. In habitat types where all groups contribute equally to the structure index, an indicator has a weight of 0 if it is not relevant, while a weight 1 indicates that it constitutes the full contribution of that hierarchical level to the structure index.

Categorical rules define habitat condition based on pre-established combinations of ordinal condition categories. Some MSs apply assessment matrices when using these methods. In DE, for example, the procedures for assessing the specific structure and function of habitat types is based mainly on inspections of sufficiently large individual occurrences (e.g., plots). The parameter of specific structure and function is divided into several criteria. For each criterion, one or more features are recorded in the field and assessed individually by assigning one of three scaled levels: A = excellent conservation status, B = good conservation status, C = medium to poor conservation status. The evaluations of individual features are then combined in an accounting matrix to produce overall assessment of the parameter specific structure and function for each sample plot.

In LT, the values of parameter indicators for Structure and Functions collected from all monitoring sites are organised, and the habitat status for this parameter is determined using two approaches: i) multidimensional data matrices of indicators (e.g., characteristic/typical species, invasive species, expansive species, projection coverage, amount of dead wood), or ii) descriptive statistics (e.g., averages, modes, frequencies), which are applied to indicators described by linear numerical sequences or binary estimates.

Not all EU MSs combine different variables; some rely solely on typical species to define the conservation status. In GR, the conservation degree of typical species at the sampling locality is assessed using the AFOR index. This index evaluates conservation status based on the distribution of species values (abundant, frequent, occasional, or rare) and their vitality. In HR, the methodology does not specify in detail how condition indicators should be aggregated at the local level. Instead, the composition of characteristic/typical species and changes in their abundance are used to indicate negative trends in habitat condition. Their abundance and condition serve as a reference for assessing habitat status through objective observation and interpretation of external factors, as well as by comparing changes over time.

Conditional rules define the final status by deriving it from the state values of the aggregated variables. These rules usually give more weight to non-favourable condition. In practice, this means that non-favourable habitat condition is assigned unless all variables are favourable — the "one-out, all-out" approach used in the WFD (EC 2000).

In BE, for example, the BioHab/EBONE method is applied. This integrates the conservation status according to specific criteria across different habitat sites, based on relative area or number of monitoring points. The final statement (favourable/unfavourable) for local conservation status at a habitat location is determined by the worst score among criteria groups (structure, disturbance, vegetation, and spatial context), in line with the “one-out, all-out” principle.

Variations of this approach include applying a majority rule, whereby the final status reflects whether the majority of indicators are favourable or unfavourable. In such cases, it is preferable to assign weights to the indicators according to their relative importance, in order to obtain a more nuanced final assessment.

In PL, the condition index at the local level is assessed using the parameters Area, Structure and Function, and Future Prospect, also applying the “one-out, all-out” rule. No further methodological detail is provided.

In summary, EU Member States apply different approaches to aggregating habitat condition at the local scale. Most use a multivariable index that integrates individual variables—typically with different weighting schemes and methods (e.g., indices, specific statistics). If the selection of indicators is appropriate, and the weighting and combination criteria (or algorithms) are sound, this represents a robust approach, as it bases the assessment on all key components of the habitat according to their relative relevance. Such an approach assesses the overall habitat status, which is its main purpose, although it may overlook poor status in specific components that could, over time, lead to overall degradation.

By contrast, conditional approaches such as the “one-out, all-out” rule give more weight to individual components. If even one component is not in good condition, this signals that something is not functioning properly and, although it may not yet degrade the overall habitat status, it could do so in the future. These conditional approaches are less integrative and less robust, but they can serve as early warning systems, allowing action to be taken before the degradation of a single component spreads and compromises the habitat as a whole.

2.4 Aggregation at biogeographical scale

This section reviews and analyses the methodologies used to aggregate local condition indices to the biogeographical region scale, and assesses whether the general rule introduced in the Article 17 reporting guidelines for the 2013-2018 period has been applied.

According to the Article 17 guidelines (EC, 2023), conservation status is reported at the national biogeographical region level as follows:

- If the area (or number of localities) in bad (unfavourable-bad) condition exceeds 25% of the region, the status of structure and function is classified as unfavourable-bad.
- If the area (or number of localities) in good (favourable) condition exceeds 90%, the status is classified as favourable.
- All other cases are classified as unfavourable-inadequate.

This rule was applied by most EU MSs when integrating parameters, particularly for the Structure and function assessment at the regional level. While the scientific basis for these thresholds could be better explained, the adoption of this common approach facilitates the targeting of conservation objectives not only at MS level, but also among MSs sharing Habitat Types of Community Interest (HTCI) within a biogeographical region. This, in turn, supports the establishment of priorities linked to EU biodiversity conservation policies and the identification of critical HTCI to be prioritised in the implementation of the EU Nature Restoration Law.

Other methods differ from those established in the Article 17 reporting guidelines for 2013-2018. In GR, for example, extrapolation was carried out at the 10 x 10 km2 grid cell level and then upscaled to the national level. The thresholds applied were less stringent than those of Article 17 when classifying favourable condition for Structure and function. Specifically:

- If ≥ 75% of the sampling localities within a grid cell were in favourable (FV) condition for Structure and function, the grid cell was classified as favourable (FV).
- If > 25% of the sampling localities were in bad (U2) condition, the grid cell was classified as bad (U2).
- Any other combination resulted in a classification of inadequate (U1).
- If > 25% of the sampling localities were in unknown condition but < 25% were in U2, the grid cell was classified as unknown.

At the national level, the aggregation was as follows:

- Favourable (FV) if > 50% of grid cells were classified as FV,
- Inadequate (U1) if > 50% were classified as U1,
- Bad (U2) if > 50% were classified as U2,
- Unknown if > 50% were classified as unknown.

A particular feature of the IE approach is that the median scores for lentic HTCI—calculated separately for hydrological functions, water total phosphorus (TP), and biological responses—were used to define Good, Inadequate, and Bad scores, as at the individual site level. In addition, the qualifier Very Good was applied to identify exceptionally good sites. For Article 17 reporting, however, both Very Good and Good are considered equivalent to Favourable.

In BE, site-level integration was achieved by combining different criteria, including the one-out, all-out rule, weighting of indicators, and/or majority rule. This approach was applied to the Article 17 reporting on the conservation status of habitat types for 2013–2018, specifically for the Structure and function component. Weighting of indicators was carried out by classifying them as important or very important. Site-level statements were then calculated based on the proportional share of favourable and unfavourable statuses. Finally, to reach a consolidated judgement per habitat type, the indicators were integrated according to a defined decision framework.

In summary, most EU MSs followed the aggregation rules forest out in the Article 17 reporting guidelines (EC, 2023), which makes the results at the biogeographical level interoperable. However, the scientific basis of the thresholds established in the guidelines would benefit from further explanation.

2.5 Selection of localities

This section reviews and analyses the methods and criteria used by MS to identify the number and distribution of localities for condition assessment within monitoring programmes, with the aim of ensuring statistical representation for each HTCI. When selecting localities for assessing the condition of lentic HTCI, all biogeographical regions within each MS should be represented. Representativeness should also be ensured through adequate geographical distribution, covering the diversity and extent of all lentic HTCI and their ecological variability.

In most of the examined documents, explicit information on the criteria used for locality selection is lacking. However, some MSs provide detailed information on statistical representativeness and on the ecological, environmental, geographic, and logistic criteria applied. In general, the aim is to maximise the sampling coverage across plots and sites, in order to capture the variety of conditions and provide strong statistical support for the assessment.

It should be noted, however, that most MSs do not apply specific criteria for azonal HTCI, such as lentic habitats, even though these have particular characteristics that may differ significantly from the more commonly assessed zonal HTCI.

Mapping analysis based on different criteria—usually combining expert opinion with statistical approaches—is the most common method reported by MSs for selecting localities. Some MSs note that monitoring methodologies are applied mainly in protected natural areas where target habitats have been identified. However, several MSs emphasise that monitoring could, and ideally should, also extend outside protected areas.

Different criteria are applied to select localities. In ES, for example, statistical significance, ecological representativeness, and accessibility are used to meet sample size requirements (Camacho et al, 2019c). For sample distribution, multivariate ordination and clustering techniques are applied to identify a reasonable number of homogeneous geo-environmental strata where the habitat type occurs. In HU, the number of necessary plots is determined by several factors, including distribution, rarity, importance in nature conservation, and the role of Hungary in preservation. Assessments are carried out not only on "typical" habitat plots but also on secondary or degraded localities, although no further details on the selection process are provided.

Also with an ecological approach, though without the additional criteria applied in ES or HU, in IE the selection of sites for the specific Habitat type 3180 – Turloughs is primarily driven by the need to represent the range of hydrogeological variation among turloughs. Site selection is initially based on a specific range of ecological features, with no attempt to predetermine the number of each type. Instead, sites are identified where groundwater flow can be reliably inferred as conduit or epikarst flow. Once the minimum number of localities has been defined and selected, additional sites are chosen to expand the dataset and strengthen statistical representativeness. Secondary criteria may include the availability of groundwater tracing data and information on deposits and swallow holes.

In BE, a specific criterion requires that at least 75% of the potentially present fauna species are covered within the selected localities, with at least 50% of the potentially present fauna species supported by sufficient area. The process begins with functional habitat clustering, based on an overview of vegetation types that are functionally related to faunal species. This is followed by a critical mapping analysis, which considers potential fragmentation and clusters near boundary values, usually carried out by an expert.

In FR, for instance, site selection should ideally include area data, maps, and information on activities and their impacts across the site. Polygons (sites) under different conservation statuses are considered to calibrate the method across all possible categories, while also accounting for variation in plant communities within the same habitat (e.g., associations, alliances). As in the ES methodology, the accessibility of habitat polygons is taken into account to maximise efficiency, aiming to optimise the information gathered per visit and to ensure broad geographical coverage for assessing habitat condition, within the limits of available human and material resources.

In HR, mapping is likewise the primary tool for site selection. The methodology is based on grid distribution maps that define key broad areas to be included in monitoring. Monitoring sites are selected once habitat mapping has been finalised, in agreement with the national expert institution for nature protection. Beyond this, no detailed methods for identifying monitoring localities are provides in the consulted documents.

Some MSs build on existing resources, as in GR, where monitoring localities may be added from previous projects (e.g., the IDHTACI project 1999–2001). In this way, the need to assess the condition of habitat types can be addressed even when the number of existing sampling locations is otherwise insufficient.

In LV, the number of monitoring sites for each type of habitat is established in proportion to the total number of polygons allocated for the year when the national habitat inventory and assessment is scheduled. Sites are also selected in relative proportion to the number of habitat localities situated in protected (including Natura 2000) and non-protected areas.

Overall, although detailed information on how localities are selected for condition assessment is scarce—and even more so for specific criteria applied to azonal HTCI such as lentic habitats—the main criteria reported by MSs include representativeness (geographical coverage, relative relevance of the HTCI in the overall assessment, ecological subtypes, and inclusion of sites in protected and unprotected areas), use of mapped data, and statistical significance. These main criteria are complemented in some MSs by ancillary considerations such as accessibility, the presence of particular taxa, or the availability of additional information relevant to the assessment.

2.6 General monitoring and sampling methods

This section outlines the main elements of existing monitoring schemes for assessing the condition of lentic HTCI in EU Member States, focusing on sampling protocols, monitoring frequency, selection of localities, and the use of existing data sources or information from other EU reporting schemes. Each MS applies its own sampling protocols, which differ in both the spatial and the temporal coverage. Differences are also observed between habitat types within some MSs, for example between temporary and permanent ecosystems, largely due to ecological—mainly hydrological—factors.

MSs use either statistical or mapping-based methods for selecting sampling points. Some apply permanent plots monitored throughout the six-year reporting period to ensure long-term consistency. However, there is no Pan-European methodological harmonisation, partly due to these ecological differences, although most MSs use scientifically standardised analytical methods (e.g., APHA, 2005, for physical and chemical analyses). Furthermore, not all MSs provide methodological information, so the patterns derived from the available material are necessarily partial.

Nevertheless, a general pattern can be observed in how MSs define methods for selecting sampling points or transects. Depending on the variables and protocols, in-situ measurements and/or questionnaires are used to evaluate the relevant parameters. Although the frequency and number of transects vary between the cases analysed, most MSs that provide detailed information establish sampling dates during months of ecological optimum—when vegetation is at maximum growth or in bloom—while also considering hydrological characteristics. A short description of the protocols applied in some MSs is provided below.

In CZ and HU, sampling is carried out in permanent plots to enable long-term assessment. In CZ, these are so-called permanent monitoring plots (PMP), defined polygons where a specific habitat occurs, generally homogeneous in character and under consistent management. Similarly, in HU, sampling is conducted within fixed permanent monitoring localities and plots. In both cases, the main aim is to monitor long-term changes in natural habitats. Most other MSs do not explicitly state whether their sampling plots are permanent or temporary.

In BE, in the Flemish Region, sampling plots are identified through mapping combined with specific algorithms, using Generalized Random Tessellation Stratified (GRTS) sampling. In this approach, habitat maps are superimposed on master samples, and each point is assigned a rank number. Based on these ranks, a sample of the desired size can be selected. The master sample thus provides the basis from which samples are drawn for the different habitat (sub)types. The desired number of sample points within the polygons of a given habitat type can then be selected. Additional criteria are applied for plot selection and for determining the appropriate assessment scale, based primarily on ecological relevance rather than solely on practical feasibility.

In FR, general methodological principles are applied to all standing waters. The assessment is initially carried out at the scale of the habitat polygon, defined as a continuous and delimited habitat area, where several variables are assessed using indicators. These indicators can be recorded either at the polygon scale or, more often, at the smaller scale of a plot that must be representative of the polygon. A plot is considered representative when it includes all major characteristics observable at the polygon scale (e.g., floristic species, site conditions).

In GR, sampling is standardised according to guidelines prepared for each habitat type. Field researchers apply these standard conservation status assessment protocols, recording information on typical species and indicators of structure and function at the local scale on field sheets.

In HR, monitoring is implemented across all three biogeographical regions using the same methodology, which combines data collection on habitats and on species. The first step consists of mapping suitable water bodies. Following mapping, monitoring locations (water bodies) are selected. Within each monitored water body, one sampling plot is chosen; for larger water bodies, three sampling plots are randomly selected, while an entire small water body may constitute a single plot.

Sampling for some variables is conducted through visual assessment at the water body or plot level and is repeated every three years, between the second half of June and the end of August, when aquatic vegetation is fully developed. All monitoring plots should be visited once every three years, although visits do not need to be completed in the same year. Depending on the region, some plots may be visited earlier in the year and others later, but specific plots must always be visited during the same period to ensure consistency.

In LT, monitoring operator or field researcher completes four questionnaires at each monitoring location for the assessment of structure and functions: (i) a general questionnaire of the monitoring site, where some information may be obtained from auxiliary sources and databases; (ii) an impact and threat assessment questionnaire; (iii) a questionnaire on the floristic phytosociological characteristics of the habitat; and (iv) a habitat structure and function monitoring questionnaire.

The monitoring calendar is prepared for a 12-year period, covering two reporting cycles for the assessment of habitat protection status under Article 17 of the Habitats Directive. During this period, each monitoring plot and location is observed at least three times. In total, observations are conducted in 16 monitoring plots every twelve years.

In IE, transects rather than plots are sampled within the same lake basin. For each basin, all transects are evaluated separately, and the most favourable transect is used. In lakes with a simple structure comprising a single basin, the best transect is likewise taken as the basis for the assessment.

For water chemistry and algal biomass, monthly water samples are collected from the shore to an area of open water, avoiding locations near springs and swallow holes. To study spatial variation within Habitat type 3180 – Turloughs, monthly samples are taken in four turloughs from the onset of flooding until they empty. Filamentous algae are collected near the edge of the water body by placing them into vials filled with turlough water.

In LV, transects are also used. Monitoring of habitat structure and functions is carried out along a transect selected in a typical location of the monitoring site. If the polygon is smaller than the required transect, observations are made using transects adapted to the polygon’s size. Normally, the transect follows a straight line, but where this is not possible due to irregular configuration of the polygon, a broken-line transect is used.

If the site is little impacted, it is preferable to survey at least 20-30% of the polygons in the area. If the site is impacted or potentially impacted, 50-70% of the polygons should be surveyed, including both impacted and non-impacted polygons. The survey route is normally followed by boat in a zigzag pattern across the polygon, except for HTCI 3160 – Natural dystrophic lakes and ponds, which can also be surveyed from the shore, and in some cases HTCI 3130 – Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or of the *Isoëto-Nanojuncetea*, which can also be surveyed by wading.

Polygons (surveyed sites) and routes must be selected and mapped prior to departure, ensuring that routes pass through areas where the largest populations of species typical of the HTCI are located. The optimum monitoring period is July and August. At each polygon, experts record features listed in the specific questionnaire. For Habitat type 3130, questionnaires are completed at sites where characteristic plant species of the biotope occur.

In PL, monitoring includes a description of the entire lake together with a transect placed perpendicular to the shoreline. The monitoring point is established either over the deepest part of the lake or pond, along the lake shore covered by relevant vegetation, or at locations where clusters of relevant vegetation patches are found.

In RO, the monitoring guidelines recommend selective sampling, with a minimum of six surveys/transects (seven in the case of Habitat type 31A0 – Transylvanian hot-spring lotus beds) distributed both within and outside the targeted protected natural areas where the habitats occur. It is not specified whether this minimum number refers to a local, regional, or national distribution of vegetation samples. A field sheet must be completed for each sample area surveyed. Correspondence between vegetation types (vegetation associations) is established using the national vegetation inventory and descriptive monographs.

In ES, a combination of plots and transects is used to asses different abiotic and biotic variables, with the choice depending on habitat size. Sampling dates also vary: in temporary systems they are scheduled during spring at maximum flooding level, while in permanent systems they are carried out in late spring and early summer.

In SL, monitoring relies on available data and existing habitat maps (which are highly fragmented), complemented by information databases, phytocoenological studies, and the personal knowledge and experience of field experts.

In summary, EU Member States apply a variety of approaches to sampling, differing in protocols, monitoring frequency, locality selection, and the use of existing data sources or other EU reporting schemes—the latter being relatively scarce among the described methodologies. A common pattern, however, is that most Member States carry out sampling during the ecological optimum, thereby minimising bias linked to seasonal variability. Nevertheless, interannual variability is not well captured in those MSs where the assessment is conducted only once during the Article 17 reporting period.

2.7 Other relevant methodologies

In addition to the System of Environmental Economic Accounting – Ecosystem Accounting (SEEA EA) (United Nations, 2021; Vallecillo et al, 2022), whose approach underpins this work, a number of other methodologies have been developed in applied research projects and under other EU directives that are relevant for the assessment of lentic habitats condition. Among these, the **Water Framework Directive** (WFD; EC, 2000) is the most important for lentic habitats. Under the WFD, the “ecological status” of a water body – defined as a measure of its ecological quality – is broadly analogous to the concepts of structure and function or habitat condition used in the assessment of HTCI.

The WFD applies **biological quality elements** (BQE) to assess the ecological status of water bodies, complemented by variables addressing chemical status and hydro-morphological conditions. Assessment variables must respond to pressures: the greater the pressure, the stronger the impact, and the lower the ecological status. For lentic ecosystems (corresponding to the "lake" water body type) the BQE are: (i) phytoplankton, usually considered the best indicator of eutrophication pressures; (ii) other aquatic flora, including phytobenthos and aquatic macrophytes. While both have often been used to assess eutrophication pressures, phytobenthos is also a good indicator of acidification, whereas aquatic macrophytes (hydrophytes and helophytes) are in some cases —such as in ES—used to indicate hydro-morphological pressures; (iii) benthic invertebrate fauna, both micro- and macroinvertebrates, responding directly to pressures such as organic pollution and toxic substances, and; (iv) fish fauna. These BQE are assessed using indicator variables (metrics), the results of which are compared against reference conditions—that is, the normal value or range observed in non-impacted sites. From this, the **Ecological Quality Ratio (EQR)** is calculated. The EQR, ranging from 0 to 1, is then compared to thresholds that distinguish five status classes: very good and good (meeting WFD requirements), and moderate, poor, or bad (requiring action to achieve or restore good ecological status). Higher EQR values correspond to better ecological status.

Similar to the procedure used by some EU MSs for local-level aggregation, the WFD applies the "one–out, all-out" principle: if any BQE fails, the overall ecological status is classified as worse than good. Reference (non-impacted) sites are preferred for setting reference values, but other approaches—including statistical methods, paleolimnology, or expert opinion —are also used.

EuropaBON (https://europabon.org/) was a Horizon 2020 research and innovation action funded by the European Commission. Its aim was to co-design a European Biodiversity Observation Network to bridge the gap between the biodiversity data needs of policymakers and authorities, and the existing reporting streams and available data sources, addressing both current obligations and emerging policy requirements (Lumbierres et al., 2024).

A central concept of EuropaBON is the use of **Essential Biodiversity Variables** (EBVs), which provide a standardised framework for biodiversity monitoring and reporting. For freshwater ecosystems, the following EBVs have been selected (Lumbierres et al., 2024):

- Genetic diversity of selected freshwater taxa
- Species abundance of wetland birds
- Species distributions of freshwater fishes
- Species distributions of amphibians and freshwater reptiles
- Species distributions of freshwater mammals
- Species distributions of freshwater invertebrates
- Species distributions of freshwater macrophytes
- Species distributions of invasive alien freshwater taxa of European concern
- Phenology of wetland bird migration
- Ecological Quality Ratio (EQR) of phytoplankton in lakes
- Ecological Quality Ratio (EQR) of freshwater macrophytes
- Ecological Quality Ratio (EQR) of freshwater phytobenthos
- Ecological Quality Ratio (EQR) of benthic freshwater invertebrates
- Ecological Quality Ratio (EQR) of freshwater fish
- Ecological Quality Ratio (EQR) of freshwater zooplankton
- River Connectivity / free river flow
- Ecosystem distribution of freshwater EUNIS habitats
- Structural complexity of riparian habitats
- Harmful freshwater algal blooms
- Freshwater primary productivity

Even though some of these variables are specific to rivers (e.g., River Connectivity / Free River flow), most can be applied to all types of freshwater habitats, including lentic habitats, and some are particularly relevant to lentic systems (e.g., Ecological Quality Ratio of freshwater zooplankton). Several variables —especially those related to the EQR, which expresses the relative value of assessed metrics compared to reference conditions—are derived from the WFD. Most of these variables could be suitable for use in assessing habitat condition.

Among the biotic variables, compositional variables are usually determined through morphotaxonomic identification of individuals, resulting in species inventories. Identifications can be direct, or may require technical resources such as microscopes for small planktonic organisms. Morphotaxonomic species determination demands strong taxonomic expertise, and skilled experts remain indispensable for ecological studies requiring accurate species identification.

In recent decades, however, genomic techniques have increasingly been applied. DNA barcoding — using taxon-specific sequences of taxonomically conservative genes (e.g., 12S rRNA, 16S rRNA, 18S rRNA, rbcL, COI, COX) — is now used to identify biological taxa in environmental samples. In lentic habitats, DNA-based approaches are employed not only to describe the composition of prokaryotic assemblages (metabarcoding) but more recently also to investigate functional ecosystem processes (e.g., Camacho et al., 2022). Over the last decade, DNA analysis has also been used to identify eukaryotic organisms, including some applied in biotic indices for aquatic environments.

Thus, the recent development of DNA barcoding (for individuals) and metabarcoding (for communities) has the potential to alleviate taxonomic limitations by replacing morphological identification with DNA sequences to characterise the biota of a given ecosystem (Pawlowski et al., 2018; Ruppert et al., 2019). These techniques have emerged as among the most promising tools for improving biomonitoring in aquatic habitats (Filipe et al., 2018). Sequences can be obtained from bulk samples or from environmental DNA (eDNA)—that is, genetic material left by organisms in the environment (Múrria et al., 2024).

Although the use of metabarcoding and eDNA is not yet widespread for describing communities and assessing lentic habitats based in biological indicators, rapid methodological advances suggest these techniques will become widely used within just a few years. At present, they primarily provide qualitative (presence/absence) data, but under certain approaches they can also yield quantitative (abundance) information.

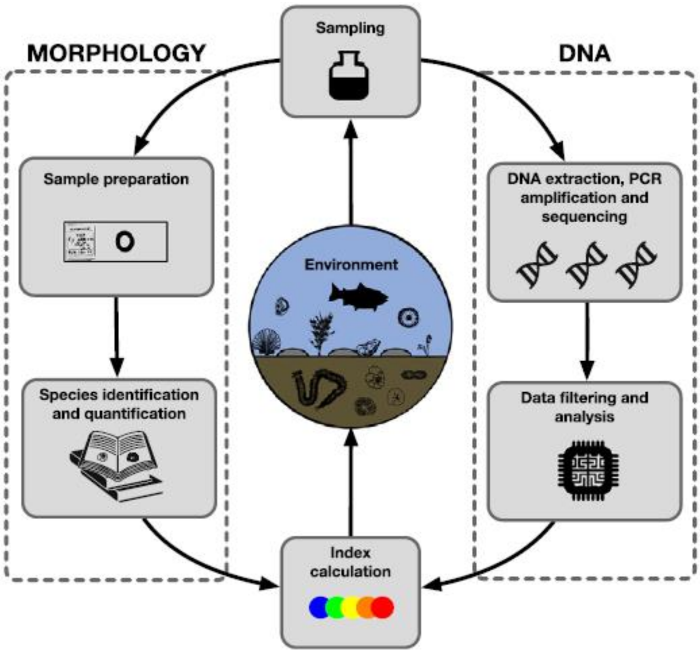
Among new methodologies, remote sensing techniques have probably achieved the greatest progress in their applicability to the assessment of habitat and ecosystem condition. In Europe, the **Copernicus** Earth Observation Programme (https://www.copernicus.eu) provides satellite data characterising water with high spatial and temporal resolution (Filipe et al., 2018). The U.S. **Landsat** programme, launched decades ago, also delivers regular remote sensing data, offering a continuous record spanning the last four decades.

The availability of these systems has enabled the development of specific applications for lentic ecosystems that provide high-resolution information (both temporal and spatial). Notable among these is the EU Framework Research project **SWOS (Satellite Wetland Observation System**, https://www.etc.uma.es/swos/), which established a monitoring and information service for wetland ecosystems based on the European Space Agency's Sentinel satellites.

Satellite-based remote sensing techniques are useful not only for measuring changes in the extent of lentic ecosystems (Camacho et al., 2019d), but also for assessing habitat condition

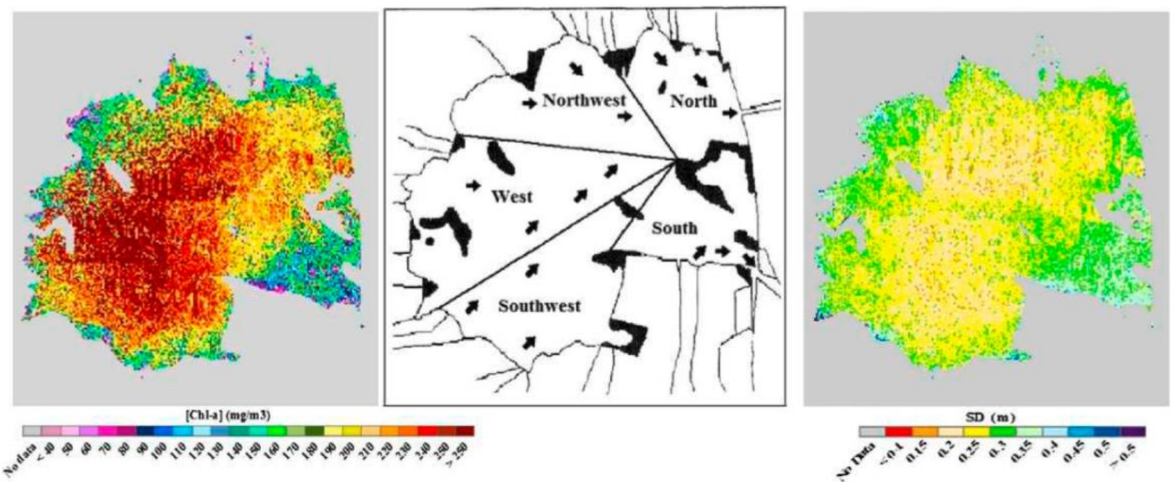
(Swedish Environmental Protection Agency, 2015; Camacho et al., 2019e). Hydrological variables (e.g., Doña et al., 2016) and their temporal changes (e.g., Doña et al., 2021), as well as trophic status and water transparency variables (e.g., Doña et al., 2015), are among the main variables described in Section 2.1 of this document that can be derived through remote sensing methods.

Figure 11. Alternative approaches for species classification in the use of biotic indices



Source: Pawlowski et al. (2018)

Figure 12. Chlorophyll a concentration map (left), water transparency map (right), and lake sectors defined in Lake Albufera (Spain).



Source: Doña et al., (2015).

Finally, **ecological modelling** (Jørgensen, 2011).), using both mathematical tools and conceptual approaches, has expanded as valuable method in ecological monitoring for multiple purposes (e.g., setting reference conditions, evaluating the performance of variables, assessing ecological processes). Modelling methods can be applied across a wide spectrum of issues ranging, from basic ecology to human ecology to socio-ecological systems.

2.8 Conclusions

The most relevant conclusions of the analysis of existing methodologies for the assessment and monitoring of standing water habitat condition are summarised below:

- The most widespread variable used by EU Member States (MSs) is vegetation composition. Lists of characteristic/typical taxa of each habitat type are established as a reference to evaluate presence and species richness.
- Vegetation structure, based on the percentage cover of characteristic/typical species (both aquatic plants and, to a lesser extent, helophytes), is another widely used variables among MSs.
- Abiotic factors related to hydrology (water regime, flooding pattern) and the main physical properties of water (transparency and colour) are also widely used indicators. For chemical variables, the most common are total phosphorus and pH.
- The composition of other biological groups—such as phytoplankton, zooplankton, benthic invertebrates, amphibians, reptiles, and fish—is used by only a few MSs as a status indicator.
- There is no uniformity in the use of functional variables, apart from the common assessment of invasive alien species. Different processes are analysed without a single trend, except for eutrophication, for which MS propose or apply different indicator variables.
- Landscape-level variables are rarely used by MS.
- Thresholds for class definitions are established using different methods, though in many cases they are not specified in MS methodologies. For abiotic factors, statistical approaches are the most common. For compositional and structural variables, thresholds usually involve minimum numbers of taxa or percentage cover. Expert opinion also remains widely used.
- Different approaches are applied to aggregate variables at the local level. Quantitative rules quantify, integrate, and weight values into to a single score. Conditional rules are mostly based on the “one-out, all-out” principle, giving greater weight to variables in poor condition, which determine the final outcome. Categorical rules assign classes based on defined thresholds. These rules can also be combined. There is no uniformity: all three approaches are used interchangeably in different MSs.
- Extrapolation at the biogeographical level should follow the Article 17 rule, based on the percentage of each status class. However, some MSs do not apply these percentages. Overall, there is limited information available on this subject.
- The methodology for site selection is also not homogeneous across MSs, although various ecological, environmental, statistical, and logistical criteria are used. Another approach is to ensure that a minimum percentage of characteristic/typical habitat taxa is represented among the selected monitoring locations, although this method is less widespread.

- For sampling the selected locations, there is no common approach between MSs or between habitat types. Sampling points or transects are chosen according to different criteria, including expert opinion, mapping and statistical methods. The frequency, timing, number, and size of sampling points or transects vary among MS.
- New approaches, based on ecological modelling, e-DNA, and especially remote sensing, are increasingly applied as powerful tools to enhance the reliability of habitat condition monitoring, although their development and potential applications are still rapidly evolving.

3110 Oligotrophic waters containing very few minerals of sandy plains, Llebreta, Spain.
© A. Camacho.

3. Guidance for the harmonisation of methodologies for assessment and monitoring of habitat condition

3.1 Selection of condition variables, metrics and measurement methods

The selection of variables presented in this section is based on a critical analysis of the variables used by EU MS, which evaluated how effectively they reflect the condition of the most relevant characteristics of lentic habitat types (as reported and discussed in Section 1.2.1), as well as the main pressures affecting these habitats (described in Section 1.3). Based on this analysis, a common set of variables has been selected for assessing the condition of lentic habitats.

Following the Ecosystem Condition Typology defined in the SEEA-EA (United Nations et al., 2021), the variables are grouped into abiotic (physical and chemical), biotic (composition, structure, and function), and landscape categories.

As described in section 2, only 18 MS provided sufficient information for analysis. Considering the variables used by MSs, a gap analysis was performed to identify which variables, jointly, would cover all main ecological features of lentic habitat types and allow assessment of the effects of the main pressures affecting them. This dual approach ensures that both the status of the main ecological characteristics and the influence of key pressures are adequately assessed when essential variables are applied.

Depending on their relevance for assessing the condition of key habitat features and their sensitivity to different types of pressures, variables were evaluated and ranked according to adequacy, coverage, accuracy, feasibility, and overall appropriateness. The most suitable variables are here designated as essential (though some of them can be alternative as informing for similar habitat features, see Annexes 1 and 2 for selection details), while those that just provide additional information are classified as recommended. Variables with low coverage or limited appropriateness, although used by some MS, are not included.

The tables included in this section (Tables 10–15), and the description of the proposed variables provide information on the justification and rationale for the selection, the main standardised measurement procedures, and the recommended metrics. Further details on the analysis and the rationale for variable selection are provided in Annex 2, where each variable is related to the main ecological features of lentic habitats and the pressures they can cover.

As a result, a total of 33 essential variables, 12 recommended variables and 2 specific variables have been selected to comprehensively assess the condition of lentic habitats, covering all habitat characteristics as well as all main types and subtypes of pressures:

Even though the set of selected variables is considered sufficiently comprehensive, additional needs, if identified, could be addressed by including variables from other sources, such as Vallecillo et al (2021), Lumbierres et al (2024), or from Member State methods for ecological status under the WFD (EC, 2000).

Abiotic physical variables

The selected essential and recommended physical variables cover the hydromorphological features and the physical water and sediment properties characteristic of the lentic HTCI. They also address, to a large extent, the potential effects of the main hydrological and morphological pressures, as well as some effects of climate change and, to a lesser degree, other specific pressures. These selected physical variables are presented in table the following:

Table 10. Proposed variables for assessing abiotic physical characteristics, indicating the number of EU Member States that use them (from 18 EU MSs examined)

(E) Essential, (R) recommended, (S) Specific

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
1. Abiotic characteristics				
1.1 Physical characteristics				
Hydrology	Hydroperiod / hydrological regime (E)	9	Integrative; covers hydrological features and the effects of hydrological pressures. Widely used.	Limnological gauges / remote sensing – water indices (type of hydroperiod: permanent, semipermanent, temporary; number of flooding months for temporary systems).
	Fluctuation of water level / flooding (E)	7	Similar to the above, but more quantitative.	Limnological gauges (maximum-minimum depth difference divided by the maximum depth).
	Draining / emptying system (R)	5	Together with the variable below, assesses whether natural processes are responsible for water losses and related hydrological alterations.	Water balances / visual inspection or automatic registers (% of water drained through non-natural processes).
Morphology	Siltation and clogging (E)	3	Assesses main morpho-dynamical processes and the pressures increasing erosion in the catchment.	Sedimentation traps (g sediment m^{-2} yr^{-1}).
	Shoreline features / profile of the banks (E)	3	Provides information on shoreline physical features and habitat suitability, and their possible alteration. Represents well the horizontal morphometric features and pressures affecting them.	Remote sensing and GIS tools (% of shoreline modified).
	Mean lake depth (E)	1	Integrates morphometric and hydrological features and the related pressures.	GIS and bathymetry (mean depth, m).
Soil and sediment	Type of the bottom surface (E)	2	Useful to assess physical features of soil and sediment and their changes	Granulometry / visual inspection.
Physical water characteristics	Water transparency / turbidity (E)	13	Integrates physical and biological processes but is also a functional variable, as it influences the productivity of the lentic habitat through light availability for photosynthesis. Water turbidity can be used as a surrogate for water transparency in very shallow systems.	Secchi disk / light extinction coefficient (measured by underwater radiometry).
	Suspended solids (R)	1	Can be used as a surrogate for water transparency in very shallow systems	

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Abiotic characteristics				
1.2 Physical characteristics				
	Water colour (S)	9	Key feature for defining some lentic habitat types, particularly dystrophic waters (HTCI 3160).	Visual inspection / colourimetry.
	Photic (euphotic) zone depth (E)	2	Although a physical variable, it describes functional processes such as planktonic primary production, which is altered by eutrophication (nutrient) pressures.	Underwater radiometry (approximately 1 % of surface irradiance).
	Water temperature (E)	2	Temperature is a key functional parameter, relevant for both physical (e.g., stratification) and biological (e.g., metabolic rates) activity, and can also indicate thermal pollution pressures	Thermometry.

Abiotic chemical variables

The selected essential and recommended abiotic chemical variables (Table 11) mainly describe the chemical properties of water and sediments, but they also reflect functional processes such as primary production and respiration. In addition, some of these variables provide information on the effects of climate change and other pressures, particularly those related to human land use and its influence on water quality.

Table 11. Proposed variables for assessing abiotic chemical characteristics, indicating the number of EU Member States that use them (from 18 MSs examined)

(E) Essential, (R) recommended

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
1. Abiotic characteristics				
1.2 Chemical characteristics				
Inorganic nutrients	Total P (E)	10	Phosphorus is one of the two main elements limiting primary production, thus determining ecosystem functioning, and it describes the level of eutrophication pressures.	Digestion and spectrophotometric determination (Total P, mg/L).
	Total N (E)	8	Nitrogen is one of the two main elements limiting primary production, thus determining ecosystem functioning, and it describes the level of eutrophication pressures.	Digestion and spectrophotometric determination (Total N, mg/L).
	Ammonia (R)	1	The relative amount of ammonium compared to total nitrogen (TN) reflects N-cycle processes and reveals the level of organic pollution pressures.	Spectrophotometric determination (ammonia, mg/L).

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
1. Abiotic characteristics				
1.2 Chemical characteristics				
Main ions	Chloride (E)	2	Dissolved chloride salts originate from catchment lithology or from marine (in coastal lentic ecosystems). This also covers salinisation pressures resulting from climate change and/or industrial processes or seawater intrusion, the later in coastal areas.	Argentometric titration (chloride concentration, mg/L).
	Sulphate (R)	1	Sulphate is an indicator for saline inland lakes, as it is the main anion of non-hypersaline saline lakes.	Precipitation with barium and gravimetric/ turbidimetric determination (sulphate concentration, mg/L).
Soil and sediment chemistry	Organic sediments (and N & P) on the lake bottom/ composition of the substrate (E)	2	The relative amount of organic matter in sediments indicates the level of productivity and/or external organic loads (e.g., sewage pollution)	Loss on ignition (mg/g or % of dry weight).
Basic water chemistry	pH (E)	10	Natural water pH depends on catchment lithology. However, shifts are caused by pollution and acid rain, as well as by increases in trophic level, the later particularly in poorly buffered, low mineralisation waters.	Potentiometry (pH, log scale).
	Electrical conductivity of water (salinity) (E)	7	Salinity is one of the main ecological features of lentic ecosystems and distinguishes between ecological types. It is influenced by salinisation pressures resulting from climate change and/or industrial processes or seawater intrusion, the later in coastal areas.	Potentiometry (electrical conductivity, mS/cm).
	Dissolved oxygen concentration and saturation (E)	6	Dissolved oxygen concentration in natural waters depends on atmospheric pressure, salinity and temperature, reaching equilibrium at 100 % saturation. Pressures such as organic pollution and eutrophication alter this physico-chemical equilibrium through enhanced biological processes, such as primary production and respiration.	Polarography (dissolved oxygen concentration, mg/L; saturation, %).
	Alkalinity (R)	3	Water alkalinity is mainly due to the solubility of calcareous rocks, which provide bicarbonate and thus pH buffering capacity (acid neutralising capacity, ANC). This functional chemical parameter allows the assessment of lentic ecosystems 'sensitivity to acidification pressures.	Titration (alkalinity, meq/L, or $CaCO_3$ in mg/L).

Biotic compositional characteristics

The selected essential and recommended biotic compositional variables (Table 12) assess both the composition of biological communities and the effects of pressures influencing species number and the presence or absence of characteristic or typical species. Most pressures described for lentic habitats affect the composition of biological communities of these habitats: from those altering the abiotic environment (e.g., hydrological, morphological or water quality pressures) to those reducing habitat availability through land reclamation or land use, as well as invasive species and, more broadly, climate change.

Table 12. Proposed variables for assessing biotic compositional characteristic), indicating the number of EU Member States that use them (from 18 MSs examined)

(E) Essential, (R) recommended

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Biotic characteristics				
2.1 Compositional characteristics				
Phytoplankton	Phytoplankton community composition; algal communities (E)	6	Phytoplankton is the main group of pelagic primary producers, and its composition integrates multiple environmental features, mainly indicating the level of eutrophication pressures.	Utermöhl sedimentation method and microscopy (phytoplankton indices e.g., TPI)
Vegetation	Characteristic species richness (presence, number of species) (E)	18	Aquatic plants (hydrophytes, helophytes, halophytes, amphibious species) are characteristic for most lentic HTCI (Group 31, standing waters). The status of those considered typical species can also be assessed. They are sensitive to almost all types of pressures.	Taxonomic determination (number of species, n)
Zooplankton	Zooplankton (R)	3	Zooplankton is a key component of aquatic food webs, and its composition and species richness in the main groups (rotifers, microcrustaceans) reflect food-web diversity and complexity. Zooplankton is sensitive to pressures such as organic pollution, eutrophication, and specific chemicals.	Microscopy (number of species, n)
Benthic aquatic macro-invertebrates	Presence / absence of selected aquatic invertebrate taxa (R)	5	Sensitive invertebrate taxa (EPT) are indicative of environmental conditions. They are particularly sensitive to organic pollution and specific pollutant pressures.	Microscopy (presence of sensitive taxa; presence/ absence of EPT)
	Benthic aquatic macro-invertebrates (number of taxa) (E)	2	The number of macroinvertebrate taxa can be used as a proxy for habitat suitability and ecological niche availability. They are sensitive to organic pollution and specific pollutants pressures.	Microscopy (number of species, n)
Ichthyofauna	Proportion of autochthonous	2	Although there are not many specific lacustrine fish species,	Electrofishing and minimum-damage handling

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Biotic characteristics				
2.1 Compositional characteristics				
	ichthyofauna species (R)		the presence of exotic fish species replacing autochthonous fish has a strong impact on community composition. This variable is therefore suitable for assessing pressures from exotic species	for taxonomic assessment (proportion of autochthonous fish species, %)
Amphibians and reptiles	Number of species of native aquatic amphibians and reptiles (E)	1	Amphibians are among the most endangered aquatic animal groups and are highly sensitive to pressures related to chemical pollution and diseases introduced by exotic species vectors.	Funnel traps with minimum-damage handling (number of species, n; using a reference list for the habitat type and region/locality)
Other fauna (e.g., birds and mammals)	Presence of other animal species (E)	2	The greater the number of animal groups, the greater the number of trophic niches in an ecosystem. In lentic habitats, waterfowl in particular is indicative of habitat suitability and niche diversity	Visual inventories (e.g., number of other animal species, or percentage of wetland bird species with increasing or stable populations, using a reference list)

Biotic (and abiotic) structural characteristics

The selected essential and recommended biotic (and abiotic) structural variables (Table 13) assess the status of the main habitat features related to the configuration of both physical habitat structure and overall community structure, as well as that of individual components. Community structure is influenced by many types of pressures that affect the structuring capacity of the biotic component, mainly plants. These pressures also influence the structure of biological communities as a whole.

Table 13. Proposal of variables for assessing biotic (and abiotic) structural characteristics, indicating the number of EU Member States that use them (from 18 MSs examined)

(E) Essential, (R) recommended

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Biotic characteristics				
2.2. Structural characteristics				
Vegetation structure	Coverage of aquatic vegetation/ vegetation structure (E)	12	Aquatic plants are the main physically structuring biotic component in lentic habitats. Widely used among MSs.	Visual inspection (scuba diving); remote sensing / Google Earth (coverage by species/types – hydrophytes, helophytes, amphibious species; %)
	Coverage of helophytes and other plants; width and zonation of riparian vegetation (R)	8	Helophytes are physically structuring components of shallow water areas and emergent shorelines. Other plants are the main biotic structuring components of bank areas	Visual inspection and identification of other plants (e.g. halophytes, shrubs and trees, herbaceous plants, stoneworts); remote sensing / Google Earth (coverage by species, %)

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Biotic characteristics				
2.2. Structural characteristics				
	Density of phytobenthos (index IPS) (R)	1	Phytobenthos (also a compositional and functional component) covers benthic areas and provides food to some macroinvertebrate feeding groups, which are selectively associated with phytobenthos-rich habitats.	Stones/plants brushing and microscopic determination (IPS index, expressed as EQR)
	Depth zonation (E)	1	Aquatic plants are distributed along a gradient of humidity, flooding, and depth, the so-called zonation. This reflects the incidence of environmental factors and species interactions in determining habitat suitability for aquatic plants and associated fauna. Although not assessed by most MSs, this indicator is very appropriate for describing the horizontal and vertical structures determined by biotic components.	Visual determination (number of plant strata, n)

Biotic functional characteristics

The selected essential and recommended biotic functional variables (Table 14) describe the status of the most relevant functional characteristics of lentic habitats, where the abiotic and biotic components of the ecosystem interact. Their status reflects the effects of major pressures altering ecosystem functioning, whether directly through pollution and biological invasions, or indirectly through pressures such as climate change.

Table 14. Proposed variables for assessing biotic functional characteristics, indicating the number of EU Member States that use them (from 18 MSs examined)

(E) Essential, (R) recommended, (S) Specific

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Biotic characteristics				
2.3 Functional characteristics				
Acid status	Cover of species indicating acidification (E)	1	Acidification is usually caused by industrial activities (e.g., mining) and atmospheric deposition (acid rain) and can be traced by the presence, absence or cover of acid-tolerant/ intolerant taxa.	Microscopy (number of acid-tolerant algal taxa, e.g., number of phytoplankton/ phytobenthos acid-tolerant species (such as some desmids).

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Biotic characteristics				
2.3 Functional characteristics				
Invasive species	Presence (and cover) of invasive alien species (E)	9	Invasive exotic species can replace or damage habitat-characteristic taxa (through predation, diseases vectors, etc.). This variable therefore describes a functional process (invasion by alien species) that affects both community structure and ecosystem functioning.	Visual determination of coverage (for macro-organisms) in transects or plots (area covered by exotic plant species vs total habitat area, %)
Organic loads	Biological oxygen demand (BOD) (E)	1	The BOD indicates the intensity of respiratory metabolic processes within the ecosystem, which increases with the organic load (both internal and external).	BOD_5 (mg O_2 /L)
	Chemical oxygen demand (COD) (E)	1	The COD measures the total amount of organic matter in water, resulting either from autochthonous primary production (biomass) or from external detritus inputs.	COD (mg O_2 /L)
Trophic status	Presence of strong algal growth (filamentous algae or blooms) (E)	3	The occurrence of algal blooms (usually cyanobacteria) and massive growth of filamentous algae is commonly the consequence of extreme eutrophication.	Visual inspection (surface of open waters covered by filamentous algae, %)
	Phytoplankton biomass / chlorophyll-a (E)	3	Eutrophication is the main pollution process in lentic ecosystems, resulting from increased nutrient loads from point or non-point sources, which promote excessive phytoplankton growth.	Pigment concentration after water filtration and extraction, followed by spectrophotometric measurement (chlorophyll-a concentration, mg/m^3)
	Daily oxygen saturation variation (E)	1	The greater the fluctuation of dissolved oxygen concentration across the diel cycle, the more active the ecosystem metabolism. This is a clear signal of functional stress. It is a particularly relevant variable that provides information on system metabolism (Hutchinson, 1957).	Polarography (diel range of dissolved oxygen saturation (% min *vs.* % max)
	Formation of deep chlorophyll maxima and photosynthetic bacterial populations in summer (S)	1	Typical feature of the HTCI 3190	Pigment extraction from waters immediately below the oxycline/oxic-anoxic interface (concentration of bacteriochlorophylls, mg/m3)

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
2. Biotic characteristics				
2.3 Functional characteristics				
Other functional processes	Number of plant species with specific traits (eutrophilous, destructuring, woody, wasteland, stoneworts, negative indicator species, etc.) (E)	6	Uses the indicator value of certain plant species based on specific traits.	Visual identification or plant sampling and determination (e.g., number of plant species that are indicators of ecosystem dysfunction from a given list, n)

Landscape characteristics

The selected essential and recommended landscape variables (Table 15) represent the most relevant landscape characteristics in terms of biological exchanges (movement of individuals) and assess the potential impacts of pressures that interfere with these exchanges. For lentic habitats, most of the suggested metrics are widely used in landscape ecology; however, among those currently applied by Member States, there is a lack of specific metrics tailored to lentic habitat types.

Table 15. Proposal of variables for assessing landscape characteristics, indicating the number of EU Member States that use it (from 18 MSs examined)

(E) Essential, (R) recommended

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
3 Landscape characteristics				
	Habitat area (extent) (E)	5	Although total habitat area is addressed as a separate parameter ("area") in the Article 17 (HD) reporting matrix, assessing variation in habitat extent itself at appropriate scales remains essential.	Remote sensing (extent, ha or m^2)
	Evolution in the number of water bodies where the habitat is present (E)	1	A higher number of water bodies that maintain some form of connectivity (hydrological or via waterfowl flyways, for instance) facilitates exchanges among habitat fragments. A clear spatial scale should therefore be defined (e.g., surface sub-catchment).	Remote sensing and field testing (number of water bodies in a sub-catchment)
	Vegetation mapping in the catchment area (E)	1	Vegetation mapping in the catchment area helps to explain import processes affecting the habitat.	Copernicus Land Cover products, remote sensing and field testing (% cover of main vegetation types and subtypes in the catchment)

Group of variables	Variable	Nr. of MSs	Selection rationale	Measurement procedures (recommended metrics)
3 Landscape characteristics				
	Structure of habitat patches (fragmentation) (E)	1	The distance between habitats patches and the characteristics of the surrounding matrix (other ecosystems or land uses) are key factors facilitating exchanges, particularly for individuals (Turner & Gardner, 2015).	Distance among patches (lakes, ponds) holding the HTCI (distance, m)
	Indicators of habitat degradation and fragmentation (R)	5	Habitat degradation may result from multiple causes, while fragmentation in lentic ecosystems is generally less common.	Depends on the indicator used (further refinement is required to select appropriate metrics for specific degradation or fragmentation causes)
	Habitat complexes (mosaic, heterogeneity, patchiness) (R)	4	Overall, environmental heterogeneity tends to favour biodiversity.	Depends on the indicator used

3.2 Guidelines for the establishment of reference and threshold values, and obtaining condition indicators for the variables measured

This section includes some guidance on how to obtain reference values of good condition for the establishment of thresholds or ranges for the selected variables to determine whether they indicate good or not good condition.

This guidance is based on the SEEA-EA recommendations and other available literature (United Nations, 2021; Vallecillo et al., 2022) on how to obtain reference condition and reference levels for the variables used in the assessment of habitat condition. In the SEEA-EA framework, a reference condition is defined as “a condition against which past, present and future ecosystem condition is compared to, in order to measure relative changes over time. It represents the condition of an ecosystem that is used for setting the upper reference level (‘optimal’ endpoint) of ecosystem variables, reflecting high ecological integrity”.

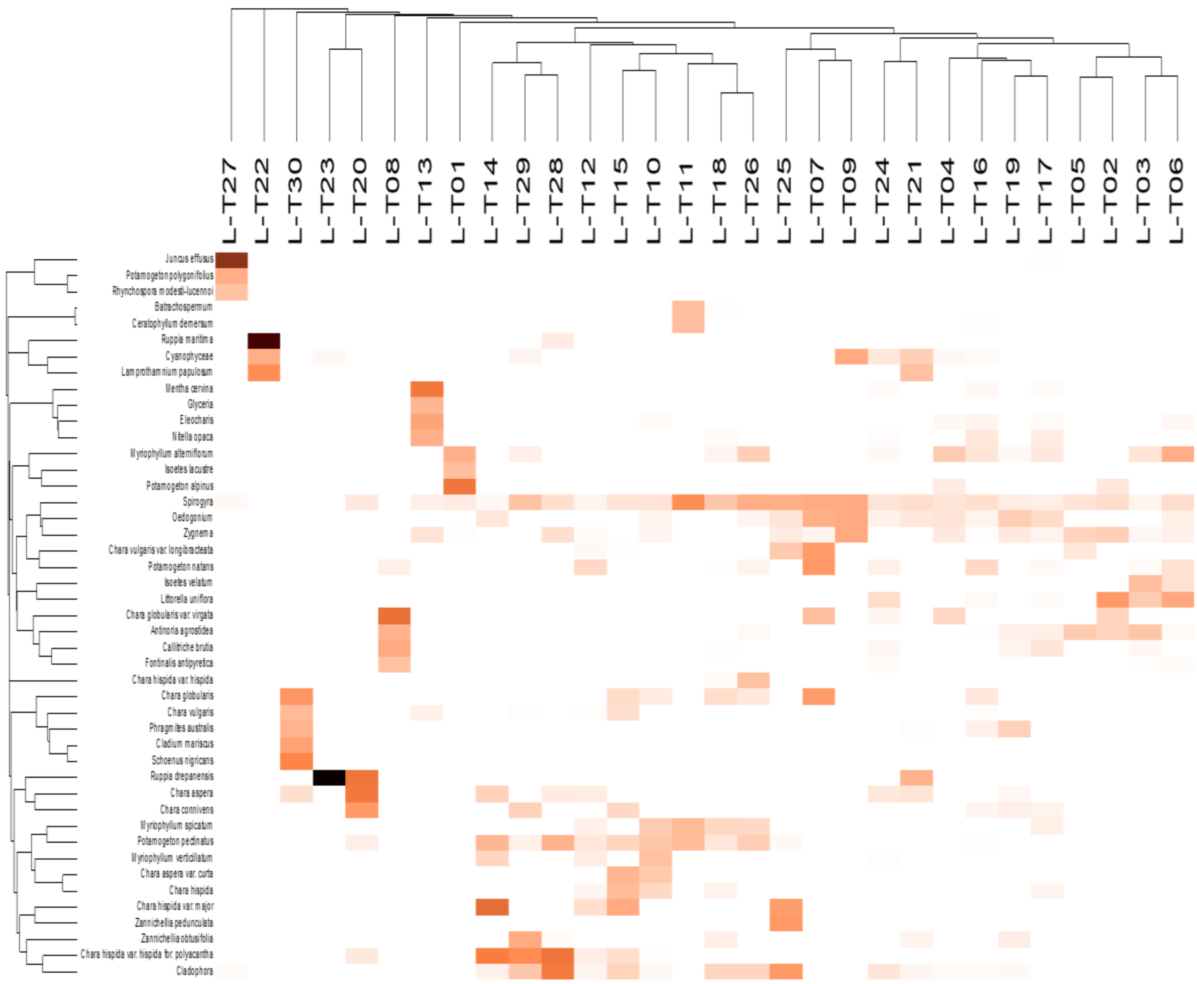
Meanwhile, the reference level “is the value of a variable against which it is meaningful to compare past, present or future measured values of the variable (as required to determine status under the HD, and also under the WFD and MSFD)”. In the following sections, we briefly describe and analyse possible approaches and provide examples on how these can be applied to lentic habitat types, as, for example, in the monitoring system of the WFD (EC, 2000).

1. Identification of reference sites: minimally-disturbed condition

The condition found in these areas is used to define the upper reference level (e.g. the level describing the very good ecological status in the WFD scoring system), scored for each variable to identify which areas are closer to the natural state ('pristine' ecosystems with no or minimal human disturbance), where the level of anthropogenic disturbances do not have a significant impact on ecosystem integrity.

This approach is especially suitable for natural ecosystems when enough “reference sites or assets” are available for each type of lentic habitat. However, the number of “reference sites” may be not enough for the habitat types whose assets are mainly located in highly populated areas, or those with strong human activity, particularly for the most disturbing activities, and then this approach must be complemented/replaced by one or some of those listed below. An example for community composition and abundance of the main taxa of plant species in “lake waterbody types” in Spain is given in Figure 13.

Figure 13. Main macrophyte species (and accompanying taxa) for the different lake waterbodies types according to the national classification (LT-XX) in Spain



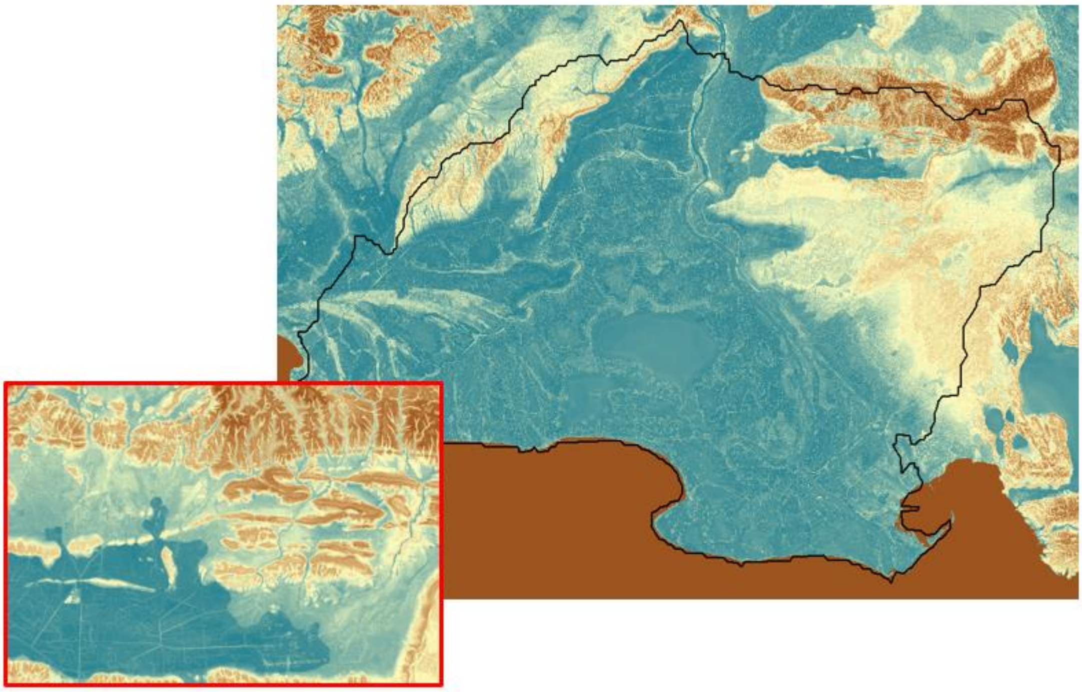
The intensity of the colour accounts for the relative abundance of each taxon per type

2. Modelled condition

This approach consists in forecasting where the habitat type could be located. This has been often used determining the potential occupation areas of habitat types classically defined by their vegetation (potential vegetation maps), such as certain types of forests, shrub formations, and grasslands.

However, for lentic habitat types, the distinctive and key environmental feature is the presence of water, either permanently or temporarily, thus the primary feature to be modelled is the likelihood of flooding, as it was developed in the Potential Wetland Areas Index, which is based on: (i) remote sensing spectral images; (ii) topographic (e.g. DEM); (iii) hydrological; and (iv) climatic variables (Figure 14).

Figure 14. Map of potential wetland areas in the Camargue (France)

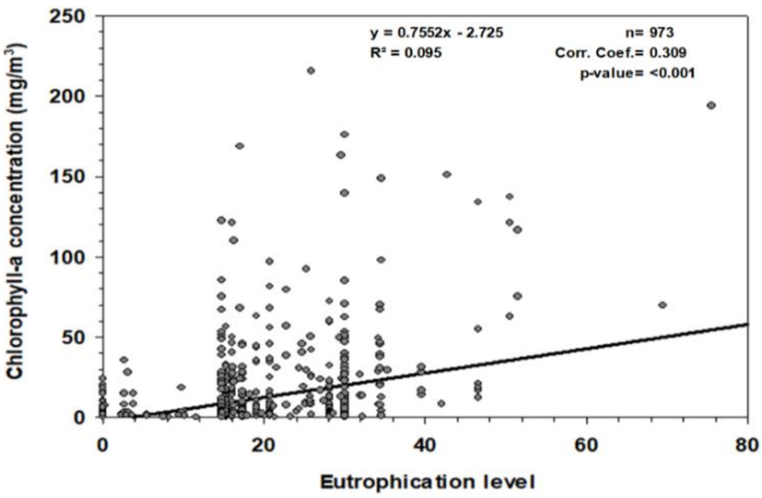


The intensity of the blue colour is a proxy to the probability of flooding.
Source: Guelmamii, TdV, reproduced with permission from the author

3. Statistical methods based on ambient distribution

These methods are based on the measurement of a variable (metrics) in a high number of locations, then using statistical approaches to correlate the value of the metrics with the level of pressures. The value of the metrics when the level of pressures is considered as non-significant is considered the reference value. A typical example of the correlation of the concentration of chlorophyll-a related to the level of eutrophication pressures is given in Figure 15.

Figure 15. Correlation between concentration of chlorophyll-a as the response variable in European lakes and eutrophication level, as determined by the LUPLES method



Source: Morant et al., (2021)

4. Prescribed reference levels

In the framework of EU-wide methodologies related to the European environmental Directives, different types of prescribed reference levels can be established using various criteria, such as those based on: (i) scientific criteria (e.g. minimum viable population size for an aquatic typical species living in a lentic HTCI for which its status is assessed); (ii) policy targets/thresholds (e.g. maximum tolerable exposure to a pesticide by waterfowl), and (iii) absolute physical boundaries (e.g. zero net surface loss for a HTCI in a biogeographical region).

5. Contemporary condition: making use of a baseline year (recent history)

This approach sets a fixed time/period baseline when the value of the variable was supposed to be that of non-disturbed conditions. The value of the variable at this temporal reference can be obtained by historical register or, mainly, by palaeoecological methods (Figure 16).

Figure 16. Varved sediment core

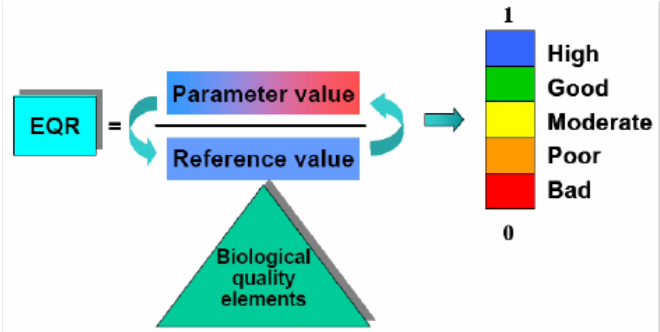
Each varve (white band) represents a year, and the black layer in between contain the chemical and biological proxies that can be used to infer the conditions at the desired year

6. Expert opinion

This consists in consulting a number of experts (the biggest the number, the more accurate the value obtained) about thresholds for reference levels of the metrics used. Although it has been widely used, the expert opinion is the last of the alternatives, because subjectivity cannot be excluded.

The reference levels obtained for each metric by any of the above methods, or by a combination of them, are then referred to the level of pressures, in order to determine its response and predictive capacity to the type of pressure for which the metric is sensitive. In the assessment, the values of the variable (metrics) obtained in a certain site are referred to the reference condition. The best example of harmonized and normalized index in Europe is the so-called “Ecological Quality Ratio” (EQR) used to standardize the values of any variable between 0 (the worse status) and 1 (the reference level (Figure 17) in the WFD. The boundary limits among classes are then set following statistical approaches.

Figure 17. Concept of Ecological Quality Ratio (EQR) and its relationship to the five status classes defined by the WFD (EC, 2000)



Source: WFD, EC, 2025.

The WFD system, based on the EQR calculation in comparison to the reference levels obtained by one or several of the above-described approaches, has demonstrated its efficiency for aquatic habitats, and particularly for standing waters ("lake type waterbodies"). We therefore propose using this system to set the reference levels for the proposed variables.

3.3 Guidelines for the aggregation of variables at the local level

Ecological assessments require the integration of physical, chemical, and biological quality elements. The choice of an aggregation method for combining these partial assessments into an overall evaluation has been widely discussed within the scientific community, as it can significantly influence the final outcome. Various approaches can be used to integrate the values of measured variables into an overall index reflecting the condition of habitat types at the local scale (e.g., monitoring plot, station, or site). Applying appropriate aggregation approaches is essential for categorising condition at the local scale as good or not good, since the proportions of habitat type area in good/not good condition is the key information needed for evaluating the conservation status of structure and functions at the biogeographical level. This section provides guidance on methods for aggregation of a set of condition variables or indicators to assess the condition of the habitat type at the local scale.

As described in section 2.3, most EU MS use a **multivariable index**, usually applying weights and different approaches (indices, specific statistics, etc.) to integrate the values obtained in the measurement of individual variables. An appropriate selection of indicators, weighting and combination criteria (or algorithms) guarantees a robust assessment that accounts for the status of the main habitat characteristics as described in section 1.2.1, especially if the variables used are covering the potential effects of all types of pressures. The relevance of the different components of the habitat, the metrics used, and how they also represent the impacts of the main pressures on these components, provide the relative weighting according to their relative relevance.

An example of this type of multivariable indices is the ECLECTIC index (Table 16), used to assess the ecological status of the lentic HTCI in Spain. The index, ranking from 0 (worse status) to 100 (best status) consists of four evaluation blocks, where each block can take a value comprised between 0 and 25 points, and each of the variables has a different weighting. These blocks are the following:

- Block 1: Characteristic Vegetation - This block evaluates the status of the typical vegetation of the HTCI, represented by the characteristic (and, when convenient, typical) species. The approach used includes the determination of both the percentage cover of each species as well as their abundance and number of taxa.

- Block 2: Other biological factors - This includes the evaluation of variables that indicate the status of the biological community (except vegetation, which is evaluated in Block 1), with indicator taxa (or aggregate variables thereof) commonly used to evaluate the ecological status of lentic habitat types or ecosystems. These are phytoplankton, zooplankton and benthic invertebrates, fish and amphibians. In addition, other animal taxa (especially vertebrates) and plants included in Annexes II and IV of the Habitats Directive are considered. Additionally, and as a penalizing factor, the presence of exotic species, especially those with invasive behaviour, is accounted in this block.
- Block 3: Hydro geomorphological factors - This includes variables related to the physical environment where the habitat type is located, as well as the hydrological characteristics of the lentic ecosystem.
- Block 4: Physical and chemical factors - This block includes variables commonly used to evaluate the physical and chemical water quality, such as the concentration of inorganic nutrients, pH, water salt content, water transparency and oxygen concentration. It also includes the chemical status of the aquifer (if any) associated with the lentic ecosystem

As previously mentioned, each block includes a series of indicators and their corresponding metrics (table 16), whose observed values are ranked, then weighted within each of the blocks.

Table 16. Groups of variables and specific variables included in the assessment of the ECLECTIC index

Compulsory variables are highlighted in bold. Source: Camacho et al, (2019a)

Biological factors	
Typical vegetation (Block 1)	**Coverage of characteristic/typical hydrophyte's species (submerged or floating plants)**
	Community composition and coverage of helophytes and riparian vegetation
	Diversity (species richness) of typical or characteristic species representative of submerged, helophytic, and riparian vegetation of the habitat type
Composition, abundance and biomass of phytoplankton (Block 2)	**Phytoplankton biomass (chlorophyll-a concentration)**
	Composition of the phytoplankton community
	Appearance of a deep chlorophyll maximum and presence of photosynthetic bacteria populations in anoxic layers in summer
Composition and abundance of invertebrate fauna (Block 2)	Number of branchiopods and copepods taxa
	Zooplankton/phytoplankton trophic ratio
	Number of benthic invertebrate taxa in the littoral area
Composition, abundance and age structure of fish fauna (Block 2)	Proportion of individuals of autochthonous vs allochthonous species
Diversity of amphibians and reptiles (Block 2)	Number of autochthonous species
Other aquatic fauna and flora (rare, threatened, protected, exotic species) (Block 2)	**Number of taxa from Annexes II and IV of the Habitats Directive, and exotic species, weighted by their indicator value**

Hydrogeomorphological factor	
Surface area (Block 3)	**Habitat type surface area**
Hydrological regime (Block 3)	**Feeding system**
	Emptying system
	Hydroperiod
Geomorphological characteristics (Block 3)	Dynamic status
	Geomorphological setup
	Siltation
Physical and chemical factors	
General (Block 4)	**Water transparency**
	Daily variation of oxygen saturation (%)
	Water electrical conductivity range
	pH
	Total phosphorus concentration
	Water colour
	Salinity of the associated aquifer

The **conditional approaches**, such as the “one out all out” rule, commonly used in the Water Framework Directive monitoring, give significant importance to individual components, since when at least one of them is not in good condition this is a signal that something is not working perfectly, and, even if this does not currently degrade the overall habitat condition, it could do it in the future. These conditional approaches are not so integrative neither robust as the weighting approaches, however they may act as early warning systems allowing to take actions before the degradation of the whole habitat condition is apparent.

Given the positive aspects of both approaches, but since the multivariable index appear to be more robust, **we recommend using multivariable weighted indices** for the overall assessment of lentic habitats’ condition at local level for reporting purposes. It allows assessing the overall habitat condition, which is the main purpose, although it can miss the possible bad status of specific components that can further result in an impoverishment of the habitat condition over time. Because of that reason, and given the own indicative value of each of the individual variables, we propose that, additionally to the reporting use of multivariate weighted indices, individual variables can be used to indicate malfunctions on specific habitat components and/or specific impacts caused by particular pressures, and thus we support its use for refinement of specific management/restoration actions that could tackle with these specific degrade components /degrading pressures.

3.4 Guidelines for aggregation at the biogeographical region scale

The condition indices obtained at the local scale must be scaled up to the biogeographical region. According to the Article 17 reporting guidelines for the latest reporting period (2019-2024), It is recommended to use an indicative value of 90 % of the habitat type area in ‘good’ condition as the threshold to conclude on ‘favourable’ Structure and functions. If Member State uses a different value, this should be noted and explained. This indicative value could, for example, be adapted according to the rarity/abundance of the habitat type: closer to 100 % for rare habitat types.

If more than 25 % of the habitat type area in the region being assessed is not in good condition, then the status of Structure and functions is 'unfavourable-bad'.

The need to properly determine the proportion of habitat area in good or not good condition highlights the importance of implementing a proper selection of localities and a statistically robust estimate of sample size that can provide reliable and representative information about habitat condition across its distribution area (see Section 3.6 below).

3.5 Guidelines on general sampling methods and protocols

Considerations on how to select monitoring localities are presented in Section 3.6, including how many sites need to be assessed depending on the desired statistical reliability. Instead, this section provides insights into monitoring frequency, the preferred period(s) of the year for sampling, the minimum surface area per sampling plot and how this may depend on the habitat element to be sampled, the number of sampling areas, etc.

In lentic habitats, the required monitoring frequency depends on natural variability and the frequency and magnitude of major disturbances, both natural and anthropogenic, as the condition assessment needs to capture this variability in order to be representative. As water-dependent ecosystems, lentic habitats are fully dependent on hydrological conditions, which derive mainly from climatic patterns and their variability, in determining habitat characteristics (e.g., the hydroperiod). Even if climatic patterns are relatively stable and can be incorporated into the HTCI definition (e.g., temporary flooding for HTCI 3170*, Mediterranean temporary ponds – MTP), interannual variability can alter hydrological conditions, setting specific ecosystem characteristics from year to year (e.g., the same MTP site may be ephemeral in a dry year while almost semi-permanent in a wet year). Considering this meteorological variability alone, it is advisable to increase sampling frequency conditions fluctuate strongly from year to year. For example, following this criterion, the monitoring frequency for lentic habitats should be higher in the Mediterranean biogeographical region, with less predictable extreme meteorological events, than in the Atlantic biogeographical region, where rainfall is distributed more regularly over time.

Further, the frequency of monitoring should also be adjusted to the relative functional role of species, and to their temporal and spatial variability, in ecosystem function. Generally, while it is well established that these impacts can be geographically and temporally nuanced, most assessments of species' contributions to ecosystems assume that species traits are temporally and spatially fixed; however, the relative functional contributions species make to ecosystems can be temporally or spatially transient and may therefore diverge from expectations based on contemporary functional group typologies, highlighting the need for the development of dynamic ecological assessment and management approaches that account for individual as well as species responses to changing environments (Sanders et al., 2025). For keystone species (even if only temporarily), temporal changes and population divergences must also be considered when setting the frequency of habitat functional parameter assessments.

Not only the natural temporal variability of environmental conditions (e.g., precipitation) and of the main biotic ecosystem players, but also the temporal distribution of the main disturbances experienced by the HTCI (both in general and at each site), whether natural or anthropogenic, must be considered when setting the sampling frequency and its temporal distribution, and even when selecting sites. For instance, the relative natural frequency and alteration of dry and wet periods, or changes in these patterns because of climate change, need to be considered. Similar examples can be given for anthropogenic disturbances, e.g., seasonal crop production patterns such as the timing of seeding, fertilisation, or pest control treatments, in lentic habitat sites located within agricultural landscapes.

On the other hand, since the results of the assessment are compared with reference conditions for the measured variables, the sampling periods must match those for which the reference conditions were set. Generally, reference conditions are established for the period of ecological optimum. For lentic habitats, this is usually in spring (earlier or later depending on latitude/altitude – the higher the latitude/altitude, the later the date) for temporary systems, and in summer (also earlier or later depending on latitude/altitude) for permanent systems. Consequently, the sampling period must be adjusted to the periods when the reference conditions were set, and autumn and winter are usually avoided.

With respect to the minimum surface area to be assessed per sampling site, and the number and size of sampling areas, representativeness and the desired relative error are the main criteria used for designing the sampling. However, this may depend on the habitat element to be assessed. In some cases, a volume (for water and plankton) or a surface (for sediments and benthic organisms, including vegetation) needs to be sampled for the specific variable to be measured. On the other hand, depending on whether maximising areal coverage or, instead, capturing ecological variability is preferred, the priority could be to focus on sampling larger sites and setting more plots (in the former case), or to use just a single sampling area per site and then sample more sites (in the latter case). These selections must then be combined with statistical significance requirements as described in Section 3.6.

3.6 Criteria to select a minimum number of localities

The selection of sampling localities – along with the sample size (number of plots) and power – is essential to ensure that the results of assessment and monitoring are representative of each habitat type at the biogeographical scale.

Identifying and selecting localities for sampling requires a systematic approach to ensure that the chosen sites provide comprehensive and representative data on habitat condition within the biogeographical region. Sampling localities should reflect the full range of habitat diversity, as well as environmental gradients, including variations in elevation, soil types, and climate. Moreover, sites should be selected both inside and outside protected areas. This requires a sound understanding of the distribution and variability of each habitat across its range, including the identification of ecotypes or subtypes, where relevant.

This section proposes **methods and criteria for the identification of localities** to carry out the assessment and monitoring of lentic HTCI. In order to select a representative number of sites for the assessment of the habitat condition status among all the sites identified as holding a specific HTCI, specific criteria can be used to avoid biases in the assessment and extrapolation of the conservation status.

The minimum number of statistically representative sites (n) can be obtained from the total population (total number of localities where the habitat type is found within a biogeographical region of a MS) using the following formula:

$$n = \frac{Z_\alpha^2 Npq}{e^2(N-1) + Z_\alpha^2 pq}$$

where:

N = population size (total number of sites of the HTCI in the biogeographical region of the MS).

Zα= confidence level, expressed in values relative to the desired confidence percentage. The most used values are: 1.28 for a confidence level of 80%, 1.44 for 85%, 1.65 for 90%, 1.69 for 91%, 1.75 for 92%, 1.81 for 93%, 1.88 for 94%, 1.96 for 95% and 2.58 for 99%.

p = probability of success. When study characteristics for success are not defined, by default it is usually assumed that p=q=0.5.

q = proportion of localities that do not have the probability of success, q=1-p.

e = desired sampling error (in percentage).

Once the minimum number of sites to be assessed has been determined, then specific criteria to select the sites can be set (Camacho et al. 2019c), using several criteria, or a combination of them, such as the following:

- Extent, covering the largest area possible per site for a habitat type, avoiding evaluating a greater number of different sites.
- Representativeness in the Natura 2000 Network and other protection figures at the international (e.g. Ramsar), national or regional level, also considering sites not officially classified as protected areas.
- Threat status (or danger of disappearance), with a selection of sites in, a priori, both good and poor condition, indistinctively.
- Environmental-ecological diversity subtypes, e.g., shallow and deeper lakes, small ponds and bigger lakes.
- Existing information, both on the selected variables or other complementary, e.g. WFD monitoring metrics matching those of the condition assessment system, which can provide directly the required information or other supplementary information.
- Environmental and geographic variability.
- Accessibility and representativeness of the localities.

Each of these criteria need to be valued and weighted to obtain the final sites to be included in the assessment as representative of the habitat type. Alternatively, they can be selected randomly, which would reduce selection bias but also the representativeness, or making a stratification sampling, where population is divided into subgroups (strata), with each stratum representing a specific characteristic of the population (e.g. a subtype, or sites within and outside protected areas). This allows each stratum to be sampled independently, leading to a more accurate representation of the overall population (all sites).

3190 Karstic lake on gypsum. Montcortès, Spain. © A. Camacho

3.7 Use of available data sources, open data bases, new technologies and modelling

Although this section is foreseen to address support for HTCI condition assessment through available data sources and databases, new methodological approaches, and modelling, it mainly deals with the first aspect – data sources, particularly those located in repositories and databases – since the other aspects have already been considered in Section 2.7.

There is a vast amount of information on biodiversity, both generic and specific to particular HTCI and/or geographic areas, that can be used for assessing the condition of European habitats. For example, this information can help set reference conditions for specific metrics and parameters, based on data available from non-impacted instances of the HTCI.

The available information from the WFD reference sites network could, at least for "lake type" (standing waters) reference water bodies resembling lentic HTCI, be a valuable source of information, given that many condition variables used for the lentic HTCI condition assessment are also shared with the WFD according to waterbody type. Equally, information on the shared variables (biotic and abiotic) gathered in the assessment of the ecological status of lake-type water bodies during regular WFD monitoring campaigns can be useful, when applying the HTCI condition assessment workflows, by contributing to the data pool for lentic HTCI condition assessment.

At the European level, probably the most complete database, which includes both processed and raw data, is the WISE (Water Information System for Europe) – Water Framework Directive Database (https://www.eea.europa.eu/data-and-maps/data/wise-wfd-4/wise-wfd-database-1), which collects data from several sources, but particularly from national reports of ecological status assessment under Article 13 of the Water Framework Directive (WFD). "The database includes information about surface water bodies (number and size, water body category, ecological status or potential, chemical status, significant pressures and impacts, and exemptions) and about groundwater bodies (number and size, quantitative status, chemical status, significant pressures and impacts, and exemptions). The information is presented by country, river basin district (RBD) and river basin district sub-unit (where applicable)."

Linking to water planning, with particular relevance for HTCI conservation, and associated with WISE, is **WISE WFD Protected Areas under the Water Framework Directive**, which contains, among other elements, the location of areas designated as requiring special protection for the conservation of habitats and species directly dependent on water. According to Article 6 of the Water Framework Directive (WFD, Directive 2000/60/EC), Member States shall ensure the establishment of a register of all areas within each River Basin District that have been designated as requiring special protection under specific Community legislation for the protection of surface water and groundwater, or for the conservation of habitats and species directly dependent on water, including the protection of Natura 2000 sites and economically significant aquatic species.

Also providing biodiversity data that can be used for HTCI condition assessment is **WISE WFD Quality Elements Status reported under the Water Framework Directive**, which contains information on the ecological status or potential of European surface water bodies, delineated for the River Basin Management Plans (RBMP) under the WFD. This includes the Quality Element status, and particularly the assessment of the Biological Quality Elements (phytoplankton, other aquatic flora, benthic invertebrate fauna, and fish), many of which are used in HTCI condition status assessments carried out in many EU Member States.

Also at the European level, and concerning taxonomic assignments for a wide variety of life groups, the **Pan-European Species-directories Infrastructure** (PESI, https://www.eu-nomen.eu/portal/) provides a mechanism for delivering an integrated, annotated checklist of species occurring in 'geographic Europe', aiming to cover the Western Palearctic biogeographic region (de Jong et al., 2015). This is an authoritative taxonomic checklist of European species, including higher taxonomy, synonyms, vernacular names and European distribution (PESI, 2025). The PESI checklist (also called EU-nomen) aims to serve as a taxonomic standard and backbone for Europe. For particular life groups, there are also specific European-level databases useful for biodiversity assessment purposes, such as, for animals, Fauna Europaea.

Moreover, the EU-funded projects **EuropaBON** (https://europabon.org/) and **MAMBO** (https://mambo-project.eu/) have recently proposed the creation of the European Biodiversity Observation Coordination Centre (EBOCC), aimed at coordinating biodiversity monitoring efforts in Europe and establishing a shared European biodiversity monitoring framework (https://knowledge4policy.ec.europa.eu/news/proposal-eu-biodiversity-observation-coordination-centre-eboccen; https://cordis.europa.eu/article/id/454278-improving-europe-s-biodiversity-monitoring).

Currently, the existing **Biodiversity Information System for Europe** (BISE, https://biodiversity.europa.eu/) serves as the European reference gateway for accessing data, information and knowledge regarding the status and progress towards EU biodiversity targets, derived from data collected through key nature-related policy instruments. For habitat condition assessment, the information on habitats displayed in that portal is of particular relevance (https://biodiversity.europa.eu/europes-biodiversity/habitats), more specifically, among others, the expert dashboard, which provides information on distribution (maps) and areas (distribution and surface) of habitat types from Annex I of the Habitats Directive.

In a global context, the **Global Biodiversity Information Facility** (GBIF, www.gbif.org) is one of the main sources for general biodiversity data and can be useful for specific targets of HTCI condition assessment. GBIF is an international network and data infrastructure funded by the world's governments and aimed at providing anyone, anywhere, with open access to data on all types of life on Earth. Other databases also provide biodiversity information on all taxa, some of which can be accessed at https://biodiversitytools.com/databases/. One of the most exhaustive is the **Catalogue of Life** (CoL, https://www.catalogueoflife.org/), an authoritative list of the world's species maintained by hundreds of global taxonomists, which includes mostly animals (e.g., 1,566,695 species in the January 2025 version), plants (386,117 species), and fungi (155,869 species). Also of particular interest as a source of general biodiversity information is the **Encyclopedia of Life** (EOL, https://eol.org/), mainly managed by the Smithsonian Institution's National Museum of Natural History and linked to the **Biodiversity Heritage Library** (BHL, https://www.biodiversitylibrary.org/), which collaboratively makes biodiversity literature openly available to the world as part of a global biodiversity community.

Other information portals are fed by citizen science contributions, such as **iSpot** (https://www.ispotnature.org/), a website developed and hosted by the Open University where users upload images of wildlife observations, identify species, and discuss their findings with other members, thereby providing a database of observations that is made available for scientific analysis.

Further, the **IBAT Alliance** (https://www.ibat-alliance.org/) provides data, tools and guidance that help organizations address biodiversity-related risks and opportunities, while generating sustainable funding to support biodiversity datasets. IBAT is a biodiversity data provider licensing commercial access to global biodiversity datasets and derived data layers, including the IUCN Red List of Threatened Species™, the World Database on Protected Areas (WDPA), and the World Database of Key Biodiversity Areas (WDKBA).

In addition to the databases covering biodiversity across all taxonomic groups, there are also databases providing specific information on aquatic fauna. Examples include **FishBase** (https://fishbase.mnhn.fr/) and **FishNet2** (https://www.fishnet2.net), which provide open access to data housed in fish collections in natural history museums, universities, and other institutions, for fish species; **HerpNET** for amphibians and reptiles; and resources on freshwater habitats such as the **Freshwater Ecoregions of the World** (FEOW, https://www.feow.org/).

Some biodiversity databases or portals dealing with freshwater biodiversity were created by collaborative EU research projects. For instance, the EU-funded research project **BioFresh** established an internet platform bringing together information and data on freshwater biodiversity (http://www.freshwaterplatform.eu/). The Freshwater Information Platform (FIP), focused on Europe but with a global perspective, provided information on freshwater science as well as a range of research resources and tools for the assessment and management of freshwater ecosystems. The portal gives access to authoritative global species lists through the Freshwater Animal Diversity Assessment (FADA) project (Lévêque et al., 2005; Balian et al., 2008), an informal network of scientists specializing in freshwater biodiversity (http://fada.biodiversity.be/). It also provides access to occurrence data mobilized during the BioFresh project, and harvested from the **Global Biodiversity Information Facility** (GBIF), with results displayed either as occurrence points or in density grids.

Since 2014, most developments of this portal have focused on publishing and attracting new data, which have been released through the FIP Integrated Publishing Toolkit (IPT) and are now included in the GBIF network, while the database underlying this website has not been updated. On the other hand, most EU Member States have national biodiversity databases, some of them associated with monitoring for reporting under the WFD and/or the Habitats Directive, which are particularly useful for national assessments of habitat condition and its typical species, as they are geographically focused.

In general, not only monitoring networks are a main source of biodiversity and other habitat information that can be used for assessing habitat condition, but another source, non-yet well developed, is research projects providing biodiversity survey data. At the pan-European level, these include projects funded by the **Biodiversa** and **Biodiversa+** calls, or specific calls within EU research programmes, such as some included in Cluster 6 of the EU Horizon Europe Research Programme. In addition, the growing practice of publishing data papers in public repositories (e.g., Zenodo https://zenodo.org/, linked to the EU Open Research Repository, https://research-and-innovation.ec.europa.eu) is increasing the availability of raw and elaborated data provided by projects dealing with the study of biodiversity and habitats.

Most of the above-mentioned databases are based on taxonomic assignments that rely on morphological identifications. However, next generation biomonitoring systems provide improved methods for more accurate biodiversity monitoring (Bohan et al., 2018), which are also applicable to habitat condition assessment. In line with these new approaches to biodiversity study and assessment, some of the databases also include genetic (barcode) data, or are even specifically focused on DNA sequence data (Leese et al., 2018).

As reported in Section 2.7 (see there for further details on the metabarcoding approach), DNA metabarcoding provides a rapid means of biodiversity assessment by identifying multiple taxa simultaneously in a sample using DNA sequencing, obtained from samples of bulk tissue from whole organisms or environmental samples of water or soil with residual or bound DNA, the so-called Environmental DNA (e-DNA) (Deiner et al., 2017; Compson et al, 2020).

Some genomic databases are not curated, and therefore the taxonomic assignments they provide for DNA sequences are less accurate (e.g., nucleotide databases such as GeneBank-NCBI or ENA-EMBL). Others are quality-controlled or even curated, and can therefore provide reliable taxonomic assignments. Among the quality-controlled databases, the Barcode of Life Data Systems (BOLD, https://v3.boldsystems.org/) is probably the most comprehensive for eukaryotic sequences based on specific marker genes (e.g., COI, ITS2). BOLD is a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada. It comprises four modules: a data portal, an educational portal, a registry of BINs (putative species), and a data collection and analysis workbench offering an online platform for analysing DNA sequences.

Some DNA databases are not only quality-checked but also curated, such as SILVA (https://www.arb-silva.de), which focuses mainly on RNA genes and is the most widely used for prokaryotes. Other curated databases specialize in particular groups of aquatic organisms. An important example for lentic ecosystems is R-Syst: diatom (http://www.rsyst.inra.fr/), a DNA sequences of diatoms, which are highly valuable for biomonitoring in aquatic environments (Rimet et al. 2017). This can be complemented, for both taxonomic and ecological purposes, by Diatombase (https://www.diatombase.org/), maintained by LifeWatch Belgium (Kociolek et al, 2025).

For other taxa, both complementary approaches – when well harmonized – can synergistically contribute to accurate taxonomic annotation of species presence in biodiversity databases (Pereira et al., 2021). With respect to DNA metabarcoding, Table 17 summarizes the main marker genes currently used for the taxonomic annotation of the main life forms in lentic habitats.

Table 17. Main marker genes used for taxonomic assignments of major life forms in inland aquatic habitats

Taxonomic group	Sampling method	Main environmental matrices sampled	Marker gene(s)
Bacteria and Archaea	Water filtration (0.2 µm)	Water and sediments	16S rRNA
Photosynthetic protists	Water filtration (3 µm)	Water	rcbl, 18S rRNA
Non-photosynthetic protists	Water filtration (3 µm)	Water	COI, 18S rRNA, 23S rRNA
Fungi	Surface scraping	Plant remains	ITS2, 28S LSU rRNA
Plants and macroalgae (charophytes)	Bulk sample of plant leaves or stems	Plant material	rbcl, ITS2
ZooplanKton	Plankton net	Water	COI
Macrozoobenthos	Hand-net (20 kicks)	Sediments	COI
Fish	Non-invasive electrofishing	Fish scales	12S rRNA
Amphibians	Non-invasive trapping	Buccal swabs	12S rRNA
e-DNA eukaryotes	Water filtration (0.2 and 3 µm)	Water	rcbl, 18S rRNA, ITS2, COI, 12S rRNA

As previously reported in Section 2.7, genomic approaches based on DNA barcodes have been the most developed functionality of DNA-based technologies for biodiversity description and taxonomic assignment. Their current status is close to reaching sufficient maturity to complement, or even replace, some classical morphotaxonomic approaches for taxonomic assignment (Pawlowski et al, 2018), including the estimation of biotic indices used for habitat condition assessment.

However, recent developments in –omic approaches (metagenomics, metatranscriptomics, proteomics, metabolomics, etc.) appear even more promising for describing ecosystem function (e.g., Camacho et al., 2022; Cabello-Yeves et al., 2023), which is probably the weakest component – together with landscape processes – of current habitat condition assessment procedures. These molecular methods for assessing ecosystem (habitat) processes and functions offer the prospect of evaluating functional processes in ecosystems, particularly in lentic habitats, in much greater detail.

As also described in Section 2.7 (see there for further details), remote sensing methods – i.e., methodologies acquiring data from a distance – are among the main tools that can support habitat condition assessment through detection instruments mounted on satellites, aircraft, or drones (NV5, 2025). When appropriately processed, these methods can provide data not only on the extent of coverage of an HTCI (e.g., wetland mapping; Perennou et al., 2016; Rapinel et al., 2023), but also on the status of key features of lentic habitats. Examples include the hydrological pattern (e.g., water coverage measured by the NDWI index; Doña et al., 2016, 2021), vegetation extent and status (e.g., NDVI index), trophic status (e.g., Chl-a concentration; Doña et al., 2015), and soil moisture (e.g., thermal imagery; Loizeau-Woollgar et al., 2025), all at high temporal and spatial resolution.

Remote sensing results can be incorporated into a Geographical Information System (GIS), where information layers can be combined to perform geospatial analyses, habitat condition assessments, and data-driven land-use planning (Wegmann et al., 2016). There are basically three main types of sensors:

- **Optical sensors** are so far the most widely used type of remote sensing sensor. They passively capture light of different wavelengths within and around the visible part of the electromagnetic spectrum, reflected or emitted from the Earth's surface. Multispectral sensors collect information in distinct, separated wavelength regions, whereas hyperspectral sensors collect data across a wide array of contiguous spectral bands, ranging from the visible to the longwave infrared regions.
- **LiDAR (Light Detection and Ranging)** actively uses laser pulses to measure distances by recording the return time. It provides precise elevation data that can be used to generate Digital Elevation Models (DEMs), which are highly valuable for delineating the catchments of lentic ecosystems. High-resolution DEMs obtained during the dry season can also provide bathymetric information for temporary lentic ecosystems.
- **Synthetic Aperture Radar (SAR)** is another active sensor. It emits microwave radiation towards the Earth's surface and records the reflected signal to create detailed images containing information about amplitude (the strength of the backscattered signal) and phase. For lentic HTCI assessments, SAR can provide, for example, detailed information on vegetation structure, biomass estimation, carbon stocks, and plant and soil moisture.

Additionally, beyond providing images and raw remote sensing data, elaborated products derived from remote sensing approaches can also be very useful for habitat condition assessments. Among the most relevant for multiple purposes, particularly for the determination of landscape indicators (e.g., Morant et al., 2021), is the Coordination of information on the Environment (CORINE), an inventory of European land cover classified into 44 different land cover classes (EEA, 2024). Data are available from 1990 onwards and can be downloaded free of charge from the Copernicus website (https://www.copernicus.eu) or the EEA Datahub (https://www.eea.europa.eu/en/datahub).

The vast amount of biodiversity data and habitat condition assessment variables that can be retrieved from databases, or obtained directly at large scale through next-generation monitoring techniques (e.g., e-DNA, remote sensing), can also be used to calibrate and validate models for habitat condition assessment and conservation planning. Some specific models and tools are available as open informatics resources, such as R (https://www.r-project.org/) and the widely used conservation planning software Marxan (https://marxansolutions.org/).

In addition, species-specific models (Elith & Franklin, 2013; Feng et al., 2019), such as species distribution models (predictive habitat distribution models), use ecological modelling to predict species distributions across geographic space and time using environmental data. These models can be applied to compare potential reference conditions with observed data on species-related habitat condition variables, or even to assess the status of typical species. Predictive habitat distribution models can also be combined with climate models (Wiens et al., 2009), allowing forecasts of future geographical suitability for the different HTCI under various climate scenarios by means of climate envelope modelling.

This is particularly relevant for lentic ecosystems, whose hydrological regime depends heavily on meteorological and climate variables such as temperature, precipitation, and evapotranspiration (Doña et al., 2021).

For example, remote sensing – particularly data from the Copernicus Sentinel satellites – plays a crucial role in monitoring and managing wetlands in Europe. Projects such as SWOS (Satellite-based Wetlands Observation Service) make use of this technology to track wetland extent, water dynamics, and vegetation changes, thereby supporting policy frameworks such as Ramsar and the EU's Nature Directives.

3140 Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp. Carabelas, Spain.
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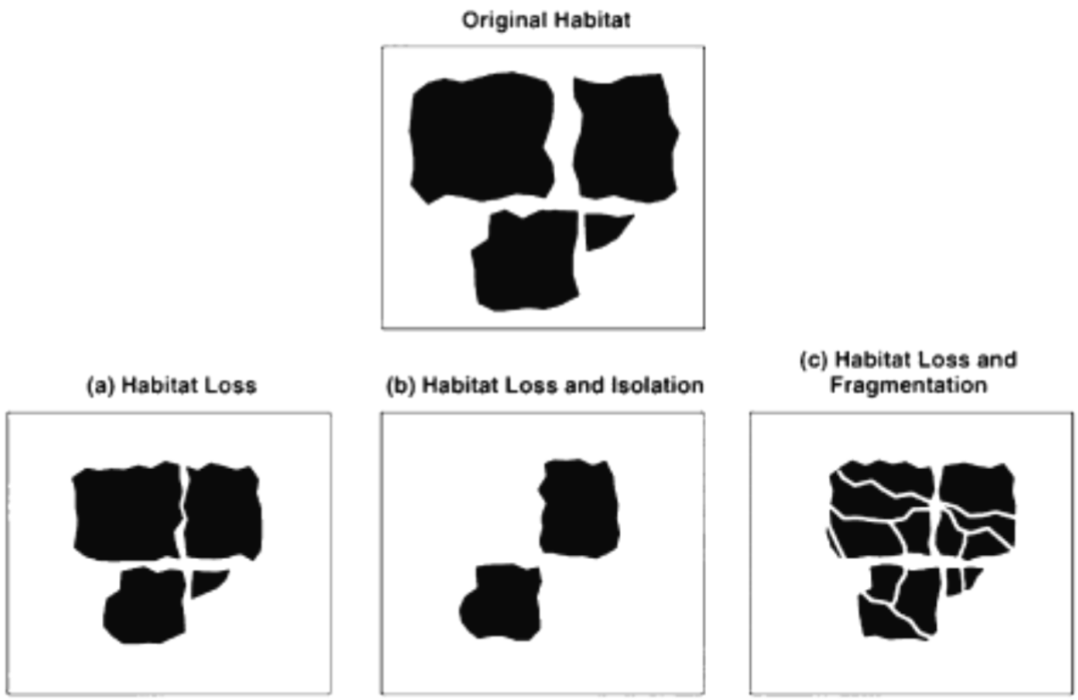
4. Guidelines to assess fragmentation at appropriate scales

This section briefly addresses habitat fragmentation for lentic habitat types. For these habitats, fragmentation has specific characteristics and scale effects that may differ from other habitat types. As stated in the Guidelines for assessing fragmentation of habitat types (see separate volume in this collection of technical guidelines), fragmentation refers to spatial properties of the distribution of a habitat type that can affect its functionality and persistence. From this perspective, fragmentation is considered a component of the habitat ecological condition, along with its status and trends.

Since the concept of fragmentation and the methodologies to assess it have been developed mostly for zonal habitats (e.g., forests), the objective of this section is not to provide detailed guidance on the implementation of standard methods and procedures for assessing the degree of fragmentation of lentic habitats, but rather to highlight the particularities of applying the fragmentation concept to these habitats, and to consider the implications for approaches and methodologies addressing spatial effects and landscape fluxes in these ecosystems.

A common characteristic of fragmentation processes in both azonal and zonal habitats is their frequent association with habitat loss and isolation (Figure 18). Overall, these processes enhance biodiversity loss and ecosystem dysfunction through various effects.

Figure 18. Geometric effects of fragmentation, usually associated with both habitat loss and isolation



Source: Van Dyke, 2008

Lentic habitats, as azonal ecosystems, are naturally fragmented, as they occupy only their specific basins. Therefore, rather than focusing on the concept of fragmentation alone, it is more appropriate to consider the combined effects of fragmentation, habitat loss, and isolation through the lens of connectivity, since this modulates the flows of organisms, matter (particularly water), and energy both among lentic ecosystems and between lentic and other ecosystem types.

The key element of lentic ecosystems is water, and thus the relevant spatial context is the lake or pond catchment, where water flows follow physically determined patterns. In this sense, the most relevant aspects relate to connectivity between lentic sites through surface (e.g., rivers) or groundwater connections. These dynamics vary across different types of lentic ecosystems. For instance, floodplains are strongly influenced by river dynamics, and fragmentation processes may be more intense within the water body itself, for example due to impacts of river water abstraction. Similarly, in groundwater-fed lentic sites, aquifer overexploitation can lead not only to fragmentation but also to increased isolation and habitat loss (Green et al., 2024a).

Physical connectivity among lentic ecosystems is therefore regulated both by surface connections through other aquatic ecosystem types (e.g., rivers, streams, channels, tidal connections in coastal systems) and, when present, by groundwater through aquifers. In both cases, water acts as the main vector for transporting propagules and individuals (hydrochory) as well as materials. Additionally, wind dispersal (anemochory) is also a relevant mechanism for the movement of aquatic organisms between lentic water bodies (Vanschoenwinkel et al., 2008), particularly in non-forested areas.

Apart from physical connectivity, biological connectivity – either alone (Green et al., 2023) or in combination with the former (Navarro-Ramos et al., 2024) – is the other main spatial linking mechanism in lentic habitats. It is mainly mediated by the transport of propagules by waterfowl (a type of zoochory). Waterbirds are well documented as a major vector for the dispersal of viable propagules such as seeds (García-Álvarez et al., 2015; Green et al., 2024b) and invertebrates (e.g., resistant eggs; van Leeuwen et al., 2012; Santamaria et al, 2023,).

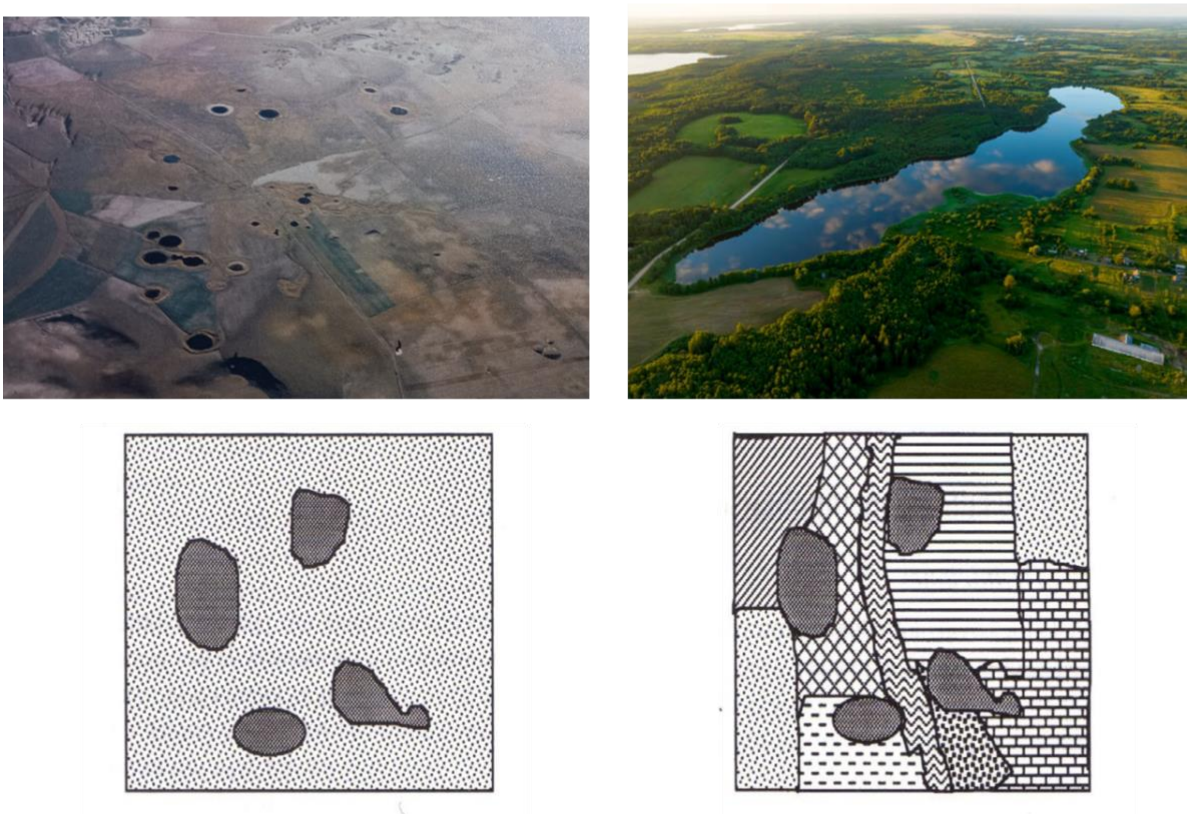
They contribute substantially to biological connectivity and to colonization and exchange processes, mediated not only by the ecology of waterfowl themselves (Green et al., 2002) and of the dispersed organisms (De Bie et al., 2012), but also by spatial factors, such as the distance between water bodies (Castillo-Escrivá et al., 2016). Zoochory-based dispersal is relevant even at the continental scale (Viana et al. 2013).

Methodological approaches used to assess the spatial contribution of fragmentation and connectivity to the shaping of biological communities – such as variance partitioning and related calculations (Peres-Neto et al., 2006; Dray et al, 2012; Dray, 2013; Castillo-Escriva et al, 2017) – can thus be applied to lentic habitats. These methods provide valuable insights into how spatial effects influence biological communities (Leibold et al., 2004; Heino, 2013; Heino et al., 2015).

Concerning the transport of materials from the catchment into lentic ecosystems, physical processes such as erosion and dissolution make an obvious contribution. Examples include sediment transport leading to siltation, or inorganic nutrient transport through runoff increasing the trophic level. Animals – mainly waterfowl – can also be a major nutrient source, driving the so-called guano-eutrophication (Adhurya et al., 2021). This is especially relevant for species that feed outside the water bodies (e.g., cranes, seagulls) but rest there at night, depositing large amounts of organic droppings that enrich the lakes.

As with zonal habitat types, the characteristics of the matrix within which habitat patches (lentic water bodies) are embedded are highly relevant for connectivity (Figure 19) These characteristics determine the percolation threshold, which is often used as a proxy for connectivity.

Figure 19. Examples of lakes surrounded by a homogeneous (left) and a variegated (right) landscape matrix



In summary, although lentic habitats – azonal systems whose occurrence is determined by geomorphological and hydrological context – differ from zonal habitats in the applicability of concepts such as fragmentation, spatial factors remain equally relevant for their structure and functions. Both physical (water, wind) and biological (fauna, mainly though waterfowl movements) processes are key determinants of lentic habitat connectivity and exchanges within the landscape. For lentic habitats, the most appropriate geographical context is the catchment of the waterbody (lake, pond, wetland).

Additional spatial factors, such as the distance between water bodies and the characteristics of the matrix in which they are embedded (e.g., land use), also influence habitat condition. The development of specific landscape indicators for lentic habitats therefore remains a pending need.

5. Next steps to address future needs

This section briefly outlines the main future needs for assessing the condition of lentic habitats. These can be summarized as follows:

Research needs

Expanding knowledge on the structure and functioning of lentic habitats remains a specific priority, although the scientific understanding already available provides a solid basis for applying science in habitat management and restoration. A key challenge is how this knowledge — together with data from extensive monitoring systems such as those under the WFD and the Habitats Directive — can be effectively used for the conservation and restoration of lentic habitats.

Related to habitat conservation status, and although not specifically addressing habitat condition assessment, two important questions remain to be solved, requiring new knowledge: (i) How much habitat area is needed to avoid disappearance (i.e.,, what is the Favourable Reference Area)? and (ii) How does habitat condition (structure and function in the Art.17 assessment matrix) interact with the other components of the HTCI assessment matrix, particularly the area of occurrence and the future prospects (pressures and threats)? (iii) Strengthen knowledge of the links between specific metrics and the impacts of specific pressures. The latter would enable the design of targeted conservation and restoration actions to reduce these impacts on lentic habitats.

In addition, expanding scientific knowledge on HTCI, especially those currently in unfavourable conservation status, is necessary to better inform their recovery.

Promote Harmonisation and methodological development

Methodologies for habitat condition assessment should be harmonised and further developed wherever needed. Since the information provided in the six-yearly reporting of HTCI conservation status is a key diagnostic tool, assessment methods must be comparable to ensure similar reliability. In order to ensure consistency and comparability of mire habitat assessments, the following main steps are suggested:

- **Test the proposed set of variables with agreed measurement procedures and methods**, including common protocols for sampling, while considering the particularities of different habitats and the existing contextual factors at local and country level; this testing would be useful to identify gaps of knowledge, flaws of applicability and robustness and reliability of results. The evaluation should provide recommendations to be further integrated in the harmonised procedures, as needed.

  In addition, specific methodological requirements for habitat condition assessment include developing approaches to:

- Improve the **setting of reference conditions** for the variables measured and define **threshold values** to determine good condition.
- **Aggregation methods**. Harmonise and improve methods for local-level aggregation of condition assessments, and provide a stronger scientific basis for the criteria currently used for aggregation at the biogeographical region level.
- Improve methodologies for **selecting monitoring sites** for each HTCI and for sampling design to ensure a sufficiently representative sample that ensures proper aggregation of results at the biogeographical region level.

- **Typical species**. Clarify the concept of "typical species" and develop methods to assess their status and integrate the results into the overall condition assessment for each habitat type.
- Finally, the coverage provided by the variables and metrics currently used should be expanded by incorporating new **landscape indicators**.

Other relevant aspect to address

- **Coordination and synergies with the WFD reporting.** For lentic habitats in particular, reporting under the WFD on the ecological status of "lake water bodies" provides additional assessment tools and results. Harmonising and jointly using the information generated by these parallel monitoring networks could greatly enhance the suitability of a common management framework for lentic habitats. This is especially important given that water planning and management are carried out within River Basin Administrations, and the conservation and restoration of lentic ecosystems must be aligned with river basin management plans.
- **Integration into policies and planning**. Strengthen the integration of habitat condition assessment into sectoral policies and territorial planning.
- **Optimisation of data use.** Improve the use of available data sources and monitoring programmes to address multi-sectoral needs related to lentic habitats.
- **High-throughput monitoring technologies**. Effectively implement, and improve where necessary, available high-throughput monitoring technologies such as remote sensing and –omics (e.g., e-DNA), along with their related products (e.g., Copernicus LULUCF products, DNA-based biodiversity databases).
- **Link to restoration needs**. Ensure habitat condition assessment is closely linked to restoration requirements, particularly within the existing framework of the EU Nature Restoration Law.
- **Climate change context**. Consider the ongoing context of climate change, and apply adaptive approaches to all aspects of habitat condition assessment, conservation, and restoration.

As can be seen, most of the recommended next steps that are similar across all HTCI. However, in the case of lentic habitats, additional requirements arise from their specific characteristics as water-dependent ecosystem types. This reinforces the need for integration, harmonisation, and linkage of lentic habitat condition assessment, management, and restoration with the development of the WFD and its derived programmes (e.g., the river basin management plans) and assessment systems. Further efforts will be required from both the EU and its Member States to improve assessment methods, strengthen coordination procedures, and ultimately enhance the condition of lentic habitats in Europe.

A European framework is needed to jointly address these requirements and to achieve results that are consistently implemented across the EU.

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Annex 1. Examples of variables used in the assessment of standing water habitats by EU Member States

Classification of variables related to the assessment of the status of habitat characteristics, indicating the EU Member States (MS) that included them in their methodologies for assessing the condition of the lentic habitat types that are identifiged. The tables indicate the number of EU Member States (MS) that uses the variables for the assessment of the habitats ‘condition, but it must be noted that this is not an exhaustive list.

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
Abiotic physical characteristics				
Hydrology	Hydroperiod/ hydrological regime	9	**BE** (3150), **BG** (1150, 2190, 3130, 3140, 3150, 3160), **DE** (3110, 3130, 3140, 3150, 3160, 3180), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **GR** (3130, 3140, 3150, 3170), **HU** (3130), **IE** (3130, 3140, 3180), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **NL** (3140)	Van Calster et al, 2020 Tabel 23; MOEW, 2013. annex 6; BfN 2017, p 40; Camacho et al, 2019ª, p.14; Dimopoulos, 2018, p54; Horváth et al. 2021; Roden et al, 2021, p 53 and 57; Angelini et al. 2016, p. 68-82; BIJ12, p. 26
	Fluctuation of water level / flooding	7	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **GR** (3130, 3140, 3150, 3170), **HR** (3150), **IE** (3140), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **NL** (3110, 3130, 3160), **RO** (3130, 3140, 3150, 3160, 31A0)	Camacho et al, 2019a, p.14; Dimopoulos, 2018, p. 54; BIJ12, p. 54, 57: Roden et al, 2020, p26 and 28; Alegro, 2013 p 9; Angelini et al. 2016. p. 68-82; Trif et al. 2015, p. 44;
	Draining	4	**DK** (2190), **IE** (3180), **LT** (2190), **PL** (3160)	Fredshavn et al., 2016; Waldren, 2015, p. 652; Rašomavičius, 2015, chapter 1.2.2; Wilk-Woźniak 2012, Tab.1, Tab.2
	Emptying system	1	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190)	Camacho et al, 2019a, p.14
	Nature of the water supply	1	**BE** (3130, 3140, 3150, 3160)	Delmarche et al., 2023, p. 30
	Contact of the water body in relation to karstic rock	1	**DE** (3190)	BfN 2017, p. 42
	Use for production of still water (lakes, ponds).	1	**RO** (3130, 3140, 3150, 3160, 31A0)	Trif et al. 2015, p. 44-46, 129. (Actually, this is not a physical attribute of the habitat, though it has been demonstrated that the source protection can also help protecting aquatic biodiversity, see Abell et al., 2018)

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
	Water availability	1	**HR** (3150)	Alegro, 2013, p. 9
Morphology	Siltation and clogging	3	**DE** (3130, 3140), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **FR** (3140)	BfN 2017 27, p. 31; Camacho et al, 2019ª, p.14; Mistarz et Latour, 2019, p. 161
	Shoreline features	2	**DE** (3110, 3130, 3140, 3150, 3160, 3180), **IE** (3140)	BfN 2017, p. 24, 27, 31, 34, 37, 40; Waldren, 2015, p. 652; Rašomavičius, p. 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27.
	Depth ratio	1	**DK** (1150, 3110, 3130, 3140, 3150, 3160)	Miljøstyrelsen 2022, p.43
	Lake mean depth	1	**DK** (1150, 3110, 3130, 3140, 3150, 3160), **RO** (1150)	Johansson, 2024, p. 58, Zaharia et al., 2013, p. 73
	Maximum dybde	1	**DK** (1150, 3110, 3130, 3140, 3150, 3160)	Johansson, 2024, p. 50
	Sieve depth	1	**DK** (1150, 3110, 3130, 3140, 3150, 3160)	Johansson, 202,4 p. 50
	Relief modeling	1	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190)	Camacho et al. 2019a, p.14
	Profile of the banks	1	**BE** (3130, 3140, 3150, 3160)	Delmarche et al. 2023, p.30
	Erosion	1	**HU** (3130)	Horváth et al. 2021, Tab. 20
	Crust cover (%)	1	**IE** (3140)	Roden et al. 2020, p.26
Soil/Sediment physical features	Type of the bottom substrate	2	**HR** (3150), **RO** (1150)	Alegro, 2013, p 9, Zaharia et al. 2013, p. 73
Water physical features	Water transparency	11	**BE** (3110, 3130, 3140, 3150), **BG** (3130, 3140, 3150, 3160), **CZ** (3130, 3140, 3150, 3160), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **HR** (3150), **IE** (3130), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **NL** (3140), **PL** (3110, 3150, 3160), **RO** (1150)	Van Calster et al, 2020, Table 19, 20, 22, 23; MOEW, 2013, annex 6; Vydrová & Lustyk 2014, p. 10-11; Miljøstyrelsen 2022, pg.43; Camacho et al, 2019a, p.14; Alegro, 2013, p 9; Roden et al, 2021, p. 58; Waldren, 2015, p. 652; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27; BIJ12, p. 26; Wilk-Woźnak et al. 2012, Tab. 1, Tab. 2; Zaharia et al. 2013, p. 73

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
	Suspended solids	1	**BE** (3110, 3130, 3140, 3150)	Van Calster et al, 2020, Tab 19, 20, 22, 23
	Water color	9	**BE** (3130, 3140, 3150), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **FR** (3150), **GR** (3130, 3140, 3150, 3170), **IE** (3130, 3140), **LT** (1150, 3130, 3140, 3150), **PL** (3110, 3150, 3160), **RO** (3130, 3140, 3150, 3160, 31A0),	Delmarche et al., 2023 30; Johansson 2024, p. 50; Camacho et al, 2019a, p.14; Mistarz & Latour, 2019, p. 172; Dimopoulos, 2018, p. 54; Roden et al. 2021, p. 53; Roden et al. 2020, p. 18; Waldren, 2015; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27; Wilk-Woźniak, 2012, Tab.1, Tab.2; Trif et al. 2015, p. 44
	Photic zone depth/ Euphotic depth	2	**IE** (3130, 3140), **PL** (3140)	Roden et al, 2021, p. 53 and 56; Roden et al, 2020 p.27; Gąbka et al. 2015, Tab. 1, Tab. 2
	Water temperature	2	**BG** (3130, 3140, 3150, 3160), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170)	MOEW, 2013, annex 6; Angelini et al. 2016, p. 68-82
	Water turbidity	2	**FR** (3140), **BG** (3130, 3140, 3150, 3160)	Mistarz et Latour, 2019, p. 162; MOEW, 2013, annex 6
Abiotic chemical characteristics				
Inorganic nutrients	Total P	10	**BE** (3110, 3130, 3140, 3150), **BG** (3140), **DE** (1150), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **FR** (1150), **IE** (3130, 3140, 3180), **NL** (3140), **PL** (1150), **RO** (1150)	Van Calster et al. 2020, Tab. 19, 20, 22, 23; MOEW, 2013, annex 6; Krause et al, 2008, p. 17; Johansson 2024, p 50; Camacho et al, 2019a, p.14; Lepareur et al. 2018, p. 30; 37-38, Waldren, 2015, p. 652; Roden et al. 2021, p. 53, 56, 57; Roden et al. 2020, p. 26 and 29; BIJ12, p. 26; Zalewska-Gałosz 2010, p. 40-41; Zaharia et al. 2013, p. 73
	Total N	8	**BE** (3110, 3130, 3140), **BG** (3140), **DE** (1150), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **FR** (1150), **NL** (3140), **PL** (1150), **RO** (1150)	Van Calster et al, 2020 Tab. 19, 20, 22; MOEW, 2013, annex 6; Krause et al. 2008, p. 17, Johansson 2024, p 50; Lepareur et al. 2018, p. 30; 37-38; BIJ12, p. 26, Zalewska-Gałosz 2010 p. 40-41, Zaharia et al., 2013, p. 73
	Ammonia/ Ammoniak	1	**BE** (3110, 3130, 3140, 3150, 3160)	Van Calster et al. 2020, Tab. 19, 20, 22, 23, 24
	Nitrite nitrogen	1	**BE** (3110, 3130, 3140, 3150, 3160)	Van Calster et al. 2020, Tab. 19, 20, 22, 23, 24
	Inorganic nitrogen	1	**BE** (3110, 3130)	Van Calster et al. 2020, Tab. 19, 20, 22, 23, 24

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
Main ions	Chloride	2	**BE** (3110, 3130, 3140, 3150, 3160), **NL** (3140)	Van Calster et al. 2020, Tab. 19, 20, 22, 23, 24; BIJ12, p. 26
	Sodium	1	**BE** (3110)	Van Calster et al. 2020, Tab. 19
	Sulfate	1	**BE** (3110, 3130, 3140, 3150, 3160)	Van Calster et al. 2020, Tab. 19, 20, 22, 23, 24
	Calcium	1	**BE** (3110)	Van Calster et al, 2020 Tab. 19
Soil/Sediment chemistry	Organic sediments (and N&P) on lake bottom /Composition of the substrate	3	**DE** (3110), **FR** (1150)**, GR** (3130, 3140, 3150, 3170)	BfN 2017, p. 24, Lepareur et al. 2018, p. 30; 37-38, Dimopoulos, 2018, p.54
	Maturity of the substrate	1	**BE** (3130, 3140, 3150, 3160)	Delmarche et al., 2023, p. 30
Water chemistry basic features	pH, acidity	10	**BE** (3110, 3130, 3140, 3150, 3160), **BG** (3130, 3140, 3150, 3160), **DK** (2190), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **IE** (3130), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **NL** (3110, 3130, 3140, 3160), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **PL** (1150, 3110, 3150, 3160), **RO** (1150)	Van Calster et al. 2020, Tab.19, 20, 22, 23, 24; MOEW, 2013, annex 6; Fredshavn et al. 2016; Camacho et al. 2019a, p.14; Roden et al. 2021, p. 58; Angelini et al. 2016, p. 68-82; BIJ12 p. 26, Waldren, 2015; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27; Wilk-Woźnak et al. 2012, Tab. 1, Tab. 2; Zalewska-Gałosz 2010 p. 40-41, Zaharia et al. 2013, p. 73
	Water electrical conductivity (salinity)	7	**BE** (3110, 3130, 3140, 3150, 3160), **BG** (3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **PL** (3110, 3140, 3150, 3160), **LT** (1150, 3130, 3140, 3150, 3160, 3190); **RO** (1150)	Van Calster et al. 2020, Tab. 19, 20, 22, 23, 24; MOEW, 2013, annex 6; Camacho et al. 2019[a], p.14; Angelini et al. 2016, p. 68-82; Wilk-Woźniak 2012, Tab.1, Tab.2; Zaharia et al. 2013, p. 73
	Oxygen concentration and its saturation	6	**BE** (3110, 3130, 3140, 3150, 3160), **BG** (3130, 3140, 3150, 3160), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **RO** (1150)	Van Calster et al, 2020, Tab. 19, 20, 22, 23, 24; MOEW, 2013. annex 6; Miljostyrelsen 2022, p. 43; Camacho et al. 2019[a], p.14; Angelini et al. 2016, p. 68-82, Zaharia et al. 2013, p. 73
	Alkalinity	3	**BE** (3110, 3130), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **IE** (3130)	Van Calster et al. 2020, Tab.19, 20; Johansson 2024, p. 50; Roden et al. 2021, p. 58

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
	Hydrochemical Dystrophy Index	1	**PL** (3160)	Wilk-Woźniak 2012, Tab.1, Tab.2
	Redox potential	1	**BG** (3140)	MOEW, 2013, annex 6
	Suspended solids	1	**BE** (3110, 3130, 3140, 3150)	Van Calster et al. 2020, Tab. 19, 20, 22, 23
Biotic compositional characteristics				
Phytoplankton	Phytoplankton community composition, algal communities	6	**DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **IE** (3140, 3180), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **PL** (3110, 3140, 3150, 3160), **RO** (1150)	Miljøstyrelsen 2022, p. 50; Camacho et al. 2019a, p.14; Roden et al, 2020, p. 31, Waldren, 2015 653; Rašomavičius, 2015, Table 12 and 14; Wilk-Woźnak et al. 2012; Gąbka et al. 2015, Tab. 1, Tab. 2; Zaharia et al. 2013, p. 73
Vegetation	Characteristic/ typical/ key plant species (presence, nº species)	18	**BE** (3110, 3130, 3140, 3150, 3160), **BG** (1150, 3130, 3140, 3150, 3160), **CZ** (3130, 3140, 3150, 3160), **DE** (1150, 3110, 3130, 3140, 3150, 3160, 3180), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **FR** (1150, 3160, 3170), **GR**, **HR** (1150, 3150), **HU** (3130, 3150, 3160), **IE** (3130, 3140, 3180), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **NL** (3110, 3130, 3140, 3160), **PL** (1150, 3130, 3160), **RO** (1150, 3130, 3140, 3150, 3160, 31A0), **SK** (3130, 3140, 3150, 3160), **SL** (1150, 3140, 3150, 3160, 3180),	Oosterlynck et al. 2020, p. 110, 114, 117, 122; MOEW, 2013, annex 1150_Species_104; BfN 2017, 31-33, 34, 36 (annex). 27-29, 37 and 39; Krause et al. 2008, p. 16; Miljøstyrelsen 2022, p. 50: Waldren, 2015, p. 653 and 655; Delmarche et al. 2023, p. 31; Camacho et al. 2019a, p.14; Lepareur et al. 2018, p. 26, 37-38; Mistarz et Latour, 2019, p. 227, 265 and 273; Dimopoulos, et al. 2018, p. 60; Bakran-Petricioli, 2016, p. 30-36; Alegro, 2013, p 8; BIJ12, p. 25, 53, 56; Angelini et al. 2016, p. 68-82; Annex D p. 45 and onward; Lustyk 2023, p. 21–23, 43–45; Vydrová & Lustyk 2014, p. 11–12; Zalewska-Gałosz 2015, Tab. 1, Tab. 2; Wilk-Woźniak 2012, Tab.1, Tab.2; Zaharia et al. 2013, p. 73; Horváth et al. 2021, Tab. 2; Waldren, 2015; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27; Gafta et Mountford (eds.) 2008, p. 27-33; Roden et al. 2021, p. 53-54, Kačičnik Jančar 2011, p. 6; Šeffer et Lasák, 2022.
	Characteristic plant communities (presence)	5	**HR**, **IE** (3130), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **PL** (1150, 3110, 3150), **RO** (3130, 3140, 3150, 3160, 31A0)	Topić et al. 2006, annexed field forms; Roden et al. 2021, p. 53-54; Waldren, 2015; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 20, 24, 26, 27; Wilk-Woźnak et al. 2012; Zalewska-Gałosz 2010 p. 40-41, Gafta et Mountford (eds.) 2008, p. 27-33

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
	Dominating species	1	**PL** (3130)	Zalewska-Gałosz 2015, Tab. 1, Tab. 2
	Shrub and tree species	1	**HU** (3130)	Horváth et al. 2021, Tab 3., 13
	Weeds	1	**HU** (3130)	Horváth et al. 2021, Tab. 9, 10
Zooplankton	Zooplankton	3	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **PL** (3110, 3140, 3150),	Camacho et al, 2019a, p.14; Waldren, 2015; Rašomavičius, 2015, Table 12 and 14; Wilk-Woźnak et al. 2012, Tab. 1, Tab. 2; Gąbka et al. 2015, Tab. 1, Tab. 2
Benthic aquatic macroinvertebrates	Presence/absence of certain aquatic invertebrates taxa	5	**DE** (3160), **DK** (1150), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **FR** (1150, 3160, 3170), **IE** (3180)	BfN 2017, p. 37 and 39; Miljøstyrelsen, 2022 Chapter 2.3.2, p. 18. Chapter 2.4.2, table 2.2, pages 24-25; Camacho et al, 2019a, p.14; Lepareur et al. 2018, p. 30; 37-38, Mistarz et Latour, 2019, p. 231, 266; Waldren, 2015, p. 656
	Benthic aquatic macroinvertebrates (Number of taxa)	2	**DE** (1150); **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190)	Krause et al. 2008, p.16; Camacho et al. 2019[a], p.14
Ichthyofauna	Proportion of individuals of autochthonous species of ichthyofauna	2	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **GR** (3130, 3140, 3150, 3170)	Camacho et al. 2019[a], p.14, Dimopoulos et al. 2018, p. 54
Amphibians and reptiles	Number of species of native aquatic amphibians and reptiles	1	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190)	Camacho et al, 2019[a], p.14
Others (e.g. birds and mammals)	Presence of animal species	2	**GR** (3130, 3140, 3150, 3170), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170)	Dimopoulos et al. 2018, p.54; Angelini et al. 2016, p. 68-82
Biotic structural characteristics				
Vegetation structure	Coverage of aquatic vegetation/ vegetation structure	12	**BE** (3110, 3130, 3140, 3150), **CZ** (3130, 3140, 3150, 3160), **DE** (3110, 3130, 3140, 3150, 3160, 3180), **DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **FR** (3140, 3150), **GR** (3130, 3140, 3150, 3170), **HR** (1150) **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **LT** (1150, 3130, 3140,	Oosterlynck et al. 2020, p. 120, 111, 114, 118; Lustyk 2023, p. 15–16; BfN 2017, p. 24, 27, 31. 34, 37, 40; Miljostyrelsen 2022, p. 43; Johansson 2024, p. 50.; Camacho et al, 2019, p.14; Mistarz et Latour, 2019, p. 164; Dimopoulos et al. 2018, p. 54; Bakran-Petricioli, 2016, p. 30-36; Angelini et al. 2016, p. 68-82; Waldren, 2015; Rašomavičius, 2015, Tables 12, 14,

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
			3150, 3160, 3190), **NL** (3110, 3130, 3140), **RO** (1150, 3130, 3140, 3150, 3160, 31A0)	16, 18, 20, 22, 24, 26, 27; BIJ12, p. 25, 53; Trif et al. 2015, p. 44-46, Zaharia et al. 2013, p. 73
	Coverage of helophytes	8	**BE** (3110, 3130, 3140, 3150), **CZ** (3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **FR** (3120), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **NL** (3110, 3130, 3140), **RO** (3130, 3140, 3150, 3160, 31A0)	Oosterlynck et al. 2020, p. 120, 111, 114, 118; Lustyk 2023, p. 23; Camacho et al, 2019a, p.14; Mistarz et Latour, 2019, p. 107; Angelini et al. 2016. p. 68-82; Waldren, 2015; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27; BIJ12, p. 25, 53; Trif et al. 2015, p. 44
	Other plants cover (e.g. halophytes, shrubs and trees, herbaceous plants, stoneworts) and open surfaces. Width and zonation of riparian vegetation	6	**FR** (1150, 3120, 3170), **GR** (3130, 3140, 3150, 3170), **HU** (3130), **NL** (3110, 3130, 3140), **PL** (3130, 3140, 3160), **SK** (3130, 3140, 3150, 3160)	Lepareur et al. 2018, p. 30; 37-38, Mistarz et Latour, 2019, p. 103, 263; Dimopoulos et al. 2018, p. 54; Horváth et al. 2021, Tab 15, 16; BIJ12, p. 25, 53; Gąbka et al. 2015, Tab. 1, Tab. 2; Šeffer & Lasák, 2022;
	Absence of specific biotic form (e.g. tall halophytes)	1	**GR** (3130, 3140, 3150, 3170)	Dimopoulos et al. 2018, p. 54
	Heterogeneity	1	**HU** (3130)	Horváth et al. 2021, Tab. 17, 18
	Density of phytobenthos (index IPS)	1	**DK** (1150, 3110, 3130, 3140, 3150, 3160),	Miljøstyrelsen 2022, p. 43;
	Charophyte and cyanobacterial crust score (C&K)	1	**IE** (3140)	Roden et al. 2020, p. 26 and 28
	Depth zonation	1	**IE** (3140)	Roden et al. 2020, p. 27
Soil structure	Extent of bare soil	2	**FR** (3130), **NL** (3110, 3130, 3140)	Mistarz & Latour, 2019, p. 135; BIJ12, p. 25, 53
	Characteristics of the bottom and banks	2	**CZ** (3130, 3140, 3150, 3160), **NL** (3110, 3130, 3140)	Vydrová & Lustyk 2014, p. 11–12; BIJ12, p. 25, 53

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
Habitat structure	Habitat area/extent	5	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **HU** (3130), **PL** (3130, 3140, 3160), **RO** (1150, 3130, 3140, 3150, 3160, 31A0), **SK** (3130, 3140, 3150, 3160)	Camacho et al, 2019a, p.14; Horváth et al. 2021; Wilk-Woźniak 2012, Tab.1, Tab. 2; Zalewska-Gałosz, 2015, Expert assessment; Trif et al. 2015, p. 44, 129; Zaharia et al. 2013, p. 73; Šeffer & Lasák, 2022; Gąbka et al. 2015, Tab.1, Tab. 2.
	Habitat degeneration indicators, fragmentation	5	**BG** (1150, 3130, 3140, 3150, 3160), **CZ** (3130, 3140, 3150, 3160), **DE** (3190), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **PL** (3110, 3150)	MOEW, 2013, Annex Species_104; Lustyk 2023, p. 17–19, 38–42; BfN 2017, p. 42; Waldren, 2015; Rašomavičius, 2015, Table 27; Wilk-Woźnak et al. 2012, Tab. 1, Tab. 2
	Water body surface area	4	**HR** (3150), **IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170), **NL** (3140, 3150), **RO** (3130, 3140, 3150, 3160, 31A0)	Alegro, 2013, p 9; Angelini et al. 2016, p. 68-82; Alegro, 2013, p 9; Annex D p. 35; Trif et al. 2015, p. 44
	Habitat complex/ Mosaic/heterogeneity/ patches/	4	**DE** (3190), **FR** (1150), **HU** (3150, 3160), **PL** (3130)	BfN 2017, p. 42; Csiky et al. 2021, Tab 33; Lepareur et al. 2018, p. 30; 37-38; Zalewska-Gałosz 2015, Tab. 1, Tab. 2.
	Surface of the bottom	1	**HU** (3150, 3160)	Csiky et al. 2021, Tab. 33, p.18.
	Shading	1	**HU** (3150, 3160)	Csiky et al. 2021, Tab 33, p.18.
	Exposure	1	**RO** (3130, 3140, 3150, 3160, 31A0)	Trif et al. 2015, p. 44.
Animal taxa	Ichthyofauna (Fish abundance and size distribution, CPUE number, CPUE weight)	1	**DK** (1150, 3110, 3130, 3140, 3150, 3160),	Miljøstyrelsen 2022 p. 43, Johansson 2024 p. 50.
	Bottom fauna (Danish Littoral Zone Macroinvertebrate Index, (DLMI))	1	**DK** (1150, 3110, 3130, 3140, 3150, 3160),	Miljøstyrelsen 2022, p. 43
Biotic functional characteristics				
Acid status	Cover of species indicative of acidification	1	**BE** (3110, 3130, 3160)	Delmarche et al. 2023, p. 32

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
Invasive species	Presence (and cover) of invasive alien/exotic species	9	**BE** (3130, 3140, 3150, 3160), **BG** (1150), **DK** (1150), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **HU** (3130, 3150, 3160), **IE** (3130, 3140), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **PL** (3130, 3140, 3160), **RO** (3130, 3140, 3150, 3160, 31A0)	Lustyk 2023, p. 17–19, 23, 38–42; MOEW, 2013, annex 6, p. 3; Aarhus Univerisitet – DCE, 2021; Camacho et al. 2019a, p.14; Horváth et al. 2021, Tab. 11, 12; Csiky et al. 2021, Tab 33; Roden et al. 2021, p. 53 and 55-56; Wilk-Woźniak 2012, Tab.1, Tab.2; Zalewska-Gałosz 2015, Tab. 1, Tab. 2; Waldren, 2015; Rašomavičius, 2015, Tables 26 and 27; Trif et al. 2015, p. 46, 129
	Absence of alien and/or invasive species	1	**GR** (3130, 3140, 3150, 3170)	Dimopoulos et al. 2018, p. 54
Fauna functioning	Imbalance in fish populations	1	**BE** (3130, 3140, 3150, 3160)	Lustyk 2023.
	Impacts of animals	1	**HU** (3130, 3150, 3160)	Horváth et al. 2021, Tab 6; Csiky et al. 2021, Tab 33.
Organic loads	Biological Oxygen Demand	1	**BE** (3110, 3130, 3140, 3150, 3160)	Van Calster et al. 2020, Table 19, 20, 22, 23, 24.
	Chemical Oxygen Demand	1	**BE** (3130, 3140, 3150, 3160)	Van Calster et al. 2020, Table 19, 20, 22, 23, 24.
Trophic status	Presence of strong algal growth (filamentous algae or "blooms")	3	**BE** (3130, 3140, 3150, 3160), **FR** (3110, 3130, 3140, 3150, 3160), **IE** (3180)	Delmarche et al. 2023, p. 32; MOEW, 2013, annex 6; Vydrová & Lustyk 2014, p. 13–14; Mistarz & Latour, 2019, p. 74, 137, 177, 229.
	Phytoplankton biomass / Chlorophyll-a	3	**DK** (1150, 3110, 3130, 3140, 3150, 3160), **ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **IE** (3180)	Miljøstyrelsen 2022, p. 43; Johansson 2024, p. 50; Camacho et al. 2019a; Waldren, 2015, p. 653.
	Nutrient richness, Nutrient loading	3	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190), **IE** (3140), **NL** (3110, 3130, 3160)	Camacho et al, 2019a; Roden et al. 2020, p. 31; BIJ12, p. 54, 57.
	Crust chlorophyll a	1	**IE** (3140)	Roden et al. 2020, p. 26, 27.
	Water trophic state index	1	**RO** (3130, 3140, 3150, 3160, 31A0)	Trif et al. 2015, p. 44.
	Nitrogen deposition	1	**NL** (3110, 3140, 3130, 3160)	BIJ12, p. 26, 54, 57.

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
	Daily oxygen saturation variation	1	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190)	Camacho et al, 2019a.
	List of species indicators of eutrophication	1	**BE** (3110, 3130, 3140, 3150, 3160)	Delmarche et al. 2023, p. 32.
	Eutrophication of the habitat	2	**BG** (3130, 3140, 3150, 3160), **DK** (1150)	MOEW, 2013, annex 6; Oosterlynck et al. 2020, p. 111, 114, 118, 120, 121; Aarhus Univerisitet- DCE, 2021.
	Formation of deep chlorophyll maxima and presence of photosynthetic bacterial populations in summer	1	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190)	Camacho et al. 2019a.
	Zooplankton /phytoplankton trophic ratio (shallow, non-saline lakes)	1	**ES** (1150, 3110, 3140, 3150, 3160, 3170, 3190)	Camacho et al. 2019a.
Diverse processes	Number of plant species with specific traits /indicators (eutrophilous, destructuring, woordy, wasteland, stoneworts, pessima species, etc.)	6	**DE** (3110, 3130, 3140, 3150, 3160, 3180); **FR** (3110, 3120, 3130, 3140, 3160, 3170), **GR** (3130, 3140, 3150, 3170), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **PL** (3140), **SK** (3130, 3140, 3150, 3160),	BfN 2017, p. 24, 27, 31, 34, 37, 40; Mistarz & Latour, 2019, p. 72, 73, 105, 171,173, 235, 263; Dimopoulos et al. 2018, p. 54; Waldren, 2015; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27; Gąbka et al. 2015, Tab. 1, Tab. 2; Šeffer & Lasák, 2022.
	Dynamics	1	**HU** (3130)	Horváth et al. 2021, Tab. 22.
	Leaf litter	1	**HU** (3130)	Horváth et al. 2021, Tab. 23, 24, 25.
	Hydro-morphological functioning	1	**FR** (1150)	Lepareur et al. 2018, p. 30.
	Index total phosphorous x Water colour	1	**IE** (3140)	Roden et al. 2020, p. 29.
	Maximal depth of stoneworts meadows	1	**PL** (3140)	Gąbka et al. 2015, Tab. 1, Tab. 2.

Group of variables	Variables	Nº MS	Member states and THIC	References (section and /or pages of citation)
Pressures and threats	Land use and (human) disturbances	3	**HU** (3130, 3150, 3160), **LT** (1150, 3130, 3140, 3150, 3160, 3190), **RO** (1150, 3130, 3140, 3150, 3160, 31A0)	Horváth et al. 2021, Tab. 26; Waldren, 2015; Rašomavičius, 2015, Tables 12, 14, 16, 18, 20, 22, 24, 26, 27; Trif et al. 2015, p. 44-46, 129; Zaharia et al. 2013, p. 73
	Influences and management	2	**CZ** (3130, 3140, 3150, 3160), **SK** (3130, 3140, 3150, 3160	Vydrová & Lustyk 2014, p. 13–14; Šeffer & Lasák 2022.
Landscape characteristics				
Landscape	Evolution of the number of water bodies where the habitat is present	1	**FR** (3150)	Mistarz & Latour, 2019, p. 170.
	Landscape environment	1	**HU** (3130, 3150, 3160)	Horváth et al. 2021, Tab 27-30; Csiky et al. 2021, Tab 33.
	Landscape metrics	1	**IT** (3110, 3120, 3130, 3140, 3150, 3160, 3170)	Angelini et al. 2016, p. 68-82
	Spatial conditions	1	**NL** (3110, 3130, 3160)	BIJ12, p. 26, 55, 57.
	Vegetation mapping	1	**NL** (3110, 3130, 3160)	BIJ12, p. 26, 55, 57.
	Protected area	1	**LT** (1150, 3130, 3140, 3150, 3160, 3190)	Waldren, 2015; Rašomavičius, 2015, Tables 12, p. 14, 16, 18, 20, 22, 24, 26, 27.
	Structure of habitat patches (fragmentation)	1	**PL** (3130)	Zalewska-Gałosz, 2015.

Annex 2. Selection of variables for assessing and monitoring the condition of standing water habitats

The tables included in this Annex provide information on the proposed variables and their links to the main groups of habitat features and pressures, which have been analysed for their selection.

The main groups of key features for lentic habitats indicated for each variable are the following (see Section 1.2.1 of the document for further details):

- **F1. Climatic:** Temperature and precipitations regime, evapotranspiration, insolation, wind
- **F2. Geological**: Lithology, structure, hydrogeological properties (aquifer characteristics)
- **F3. Geomorphological and pedological:** Morphogenetic system, morpho-dynamic system, relief and shape formation, surface formations (sediment deposits and soils), siltation
- **F4. Hydrological**: Feeding mode, emptying mode, hydroperiod pattern, renewal rate
- **F5. Morphological/physiographic**: Depth, surface area, relative extent of the coastal area and perimeter, width, shape index
- **F6. Physical and chemical characteristics of water:** Vertical stratification (from water density mediated by temperature and salinity), light, water transparency and colour, total mineralisation (salinity), type of dominant salts, dissolved oxygen and hydrogen sulphide, concentration of inorganic nutrients (N and P compounds), organic matter, pH and alkaline reserve (acid-neutralising capacity), redox potential
- **F7. Components of biological communities (characteristic taxa):** Submerged and emergent macrophytes, bacteria, phytoplankton, phytobenthos, photosynthetic sulphur bacteria, planktonic and benthic micro- and macroinvertebrates, nekton (particularly fish) and other ichthyofauna, other vertebrates
- **F8. Community structural factors:** Physical biotic structure (e.g., plant zonation, vertical distribution of plankton), population structure, community structure (diversity and complexity, trophic structure)
- **F9. Biological processes:** Primary production, consumption and food webs (energy and matter transfers), interspecific interactions, biogeochemical cycles, planktonic chlorophyll-a concentration
- **F10. Landscape processes of exchange with other ecosystems:** Exchange of individuals (immigration-emigration), fluxes of materials, fluxes of energy (unidirectional or bidirectional)
- **Anthropic factors** (listed separately, as their effects are linked to pressures rather than intrinsic ecological features)

On the other hand, the main groups of **pressures with potential direct and indirect impacts on lentic HTCI** are the following (see Section 1.4 of the document for further details on the subgroups used in the tables included below).

A. Hydrological pressures and impacts

- A1. Direct water abstraction
- A2. Alteration of the natural flooding regime and water flow patterns (e.g., drainage, external flow inputs, flow variations due to exploitation or non-natural inputs)
- A3. Flow regulation in tributaries
- A4. Presence of drainage infrastructure
- A5. Extraction of water from the associated aquifer (where applicable).

B. Geomorphological pressures and impacts

- B1. Alteration of morphometry or substrate characteristics affecting the structure, function, or surface of the wetland (e.g., infilling, slope modification, construction of structures)
- B2. Extraction of materials
- B3 – Disposal of rubble disposal

C. Pressures and impacts altering water quality

- C1. Intermittent spills of urban and/or industrial wastewater
- C2. Spills of specific pollutants (e.g., heavy metals and priority substances)
- C3. Presence of diffuse pollution sources in the catchment
- C4. Inflows with mineralogical characteristics different from the natural ones
- C5. Thermal discharges (e.g., from cooling processes)
- C6. Alteration of the natural chemical quality of the associated aquifer (where applicable)

D. Pressures and impacts on the structure of communities

- D1. Connectivity with adjacent natural ecosystems
- D2. Exploitation or other pressures on the biological community
- D3. Aquaculture activities

E. Pressures and impacts due to land use

- E1. Land use for road and residential infrastructure
- E2. Presence of electrical lines

F. Pressures and impacts related to occupation and area shifts of lentic habitats

- F1. Reduction of the area occupied by the habitat type at the local scale
- F2. Occupation of the wetland basin or its banks

G. Pressures and impacts due to the presence of invasive alien species

- G1. Presence of exotic species included in catalogues of invasive alien species
- G2. Presence of exotic (alien) species of the habitat type not included in such catalogues

H. Other pressures and impacts

- H1. Solid waste
- H2. Livestock overgrazing
- H3- Recreational activities
- H4- Other pressures and impacts (e.g., periodic vegetation burning)

I. Climate change

The following tables present the variables measured by EU Member States (MSs), indicating those selected and proposed in these Guidelines as essential (E), Recommended (R) and Specific (S). Those variables that were not selected although being used by some Member States are also indicated. The tables also provide the justification and rationale for the selection of the proposed variables, the main standardised measurement procedures, and the recommended metrics.

The table below indicates the total number of EU Member States (MS) that use each variable (from 18 EU MS examined), the main types of ecological features whose status they can describe (in **bold** the main types of ecological features addressed), as well as the types and subtypes of pressures potentially affecting the lentic habitats of community interest, whose impacts on the habitat condition they may cover.

(E) inidicates essential variables, (R) recommended variables and (S) specific variables. The reasoning for the selection of the variable and the main standardised measurement procedures with the recommended metrics, are given in the right columns

Table. Examples of variables used by EU Member States (MS) for assessment and monitoring o standing water habitats

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
Physical characteristics						
Hydrology	Hydroperiod/ hydrological regime (E)	9	F1, F2, F3, **F4**, F5, F10	A1, A2, A3, A4, A5, I	Integrative; covers hydrological features and the effects of hydrological pressures. Widely used.	Limnological gauges / remote sensing – water indices (type of hydroperiod: permanent, semi-permanent, temporary; number of flooding months for temporary systems).
	Fluctuation of water level / flooding (E)	7	F1, F2, F3, **F4**, F5, F10	A1, A2, A3, A4, A5, I	Similar to the above, but more quantitative.	Limnological gauges (maximum-minimum depth difference divided by the maximum depth).
	Draining (R)	4	F1, F2, F3, **F4**, F5, F10	A1, A2, A4	Together with the variable below, assesses whether natural processes are responsible for water losses and related hydrological alterations.	Water balances / visual inspection or automatic registers (% of water drained through non-natural processes).
	Emptying system (R)	1	F1, F2, F3, **F4**, F5, F10	A1, A2, A4	Same as above	Same as above
	Nature of the water supply	1	F1, F2, **F4**, F6, F10	A2, A3	NOT SELECTED	
	Contact of the water body in relation to karstic rock	1	**F2**, F3, F4	A5	NOT SELECTED	
	Use for production of still water (lakes, ponds)	1	None (anthropic)	A1	NOT SELECTED	
	Water availability	1	F1, F2, F3, **F4**, F5, F10	A2, I	NOT SELECTED	

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
Morphology	Siltation and clogging (E)	3	F2, **F3**, F5, F10	B1, I	Assesses main morphodynamical processes and the pressures increasing erosion in the catchment	Sedimentation traps (g sediment m^{-2} yr^{-1})
	Shoreline features (E)	2	F2, **F5**, F10	B1, B2, B3, E1, F1, F2, H3, I	Gives a good representation of the horizontal morphometric features and pressures affecting them	Remote sensing and GIS tools (% of shoreline modified)
	Depth ratio	1	F1, F2, F3, **F5**, F10	A1, A2, A3, A4, A5, B1, B2, B3, F2, I		
	Mean lake depth (E)	1	F1, F2, F3, **F5**, F10	A1, A2, A3, A4, A5, B1, B2, B3, F2, I	Integrates well morphometric with hydrological features ad the related pressures	GIS and Bathymetry (Mean depth, in m)
	Maximum depth	1	F1, F2, F3, **F5**, F10	A1, A2, A3, A4, A5, B1, B2, B3, F2, I	NOT SELECTED	
	Sieve depth	1	F2, F3, **F5**, F10	A1, A2, A3, A4, A5, B1, B2, B3, F2, I	NOT SELECTED	
	Relief modelling	1	F2, **F3**, F5, F10	B1, B2, B3, E1, F1, F2, H3, I	NOT SELECTED	
	Profile of the banks (R)	1	F2, **F3**, F5, F10	B1, B2, B3, E1, F1, F2, H3	Provides additional information about shoreline physical features and habitat suitability, and its alteration.	Remote sensing and GIS tools
	Erosion	1	F2, **F3**, F5, F10	B1, B2, B3, E1, F1, F2, H3, I	NOT SELECTED	
	Crust cover (%)	1	F2, **F3**	B1	NOT SELECTED	
Soil/Sediment physical features	Type of the bottom substrate (E)	2	F2, **F3**, F5, F10	B2, B3		Granulometry /Visual inspection
Water physical features	Water transparency (E)	11	F1, F2, F4, **F6**, F9, F10	C1, C2, C3, C4, D3, H1, H2, I	Integrates physical and biological processes but is also a functional variable as it influences the productivity of the lentic habitat though light availability for photosynthesis	Secchi disk / Light extinction coefficient (measured by underwater radiometry)

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
Water physical features	Suspended solids (R)	1	F3, **F6**, F9, F10	B1, B2, B3, C1, C4, E1, F2, H1, I	Can be a surrogate of water transparency for very shallow systems	
	Water colour (S)	9	**F6**, F9, F10	C1, C2, C3, C4, H1, H2, I	Is a key specific feature in defining some habitat types, particularly dystrophic waters (HTCI 3160)	Visual inspection / Colorimetry
	Photic / euphotic zone depth (E)	2	F5, **F6**, F7, F8, F9, F10	C1, C2, C3, C4, D3, H1, H2, I	Being a physical variable, describes well functional processes such as planktonic primary production, which is altered by eutrophication (nutrient) pressures.	Approximately 1 % of surface irradiance, measured by underwater radiometry
Water physical features	Water temperature (E)	2	**F1**, F5	C5, I	Temperature is a key functional parameter both for physical (e.g. stratification) and biological (metabolic rates) activity, and can describe thermic water spill pressures	Thermometry
	Water turbidity (R)	2	F3, **F6**, F9, F10	C1, C2, C3, C4, D3, H1, H2, I	Can be a surrogate of water transparency for very shallow systems	
Chemical characteristics						
Inorganic nutrients	Total P (E)	10	F1, F2, F4, **F6**, F9, F10	C1, C3, D3, E1, H1, H2, I	Phosphorus is one of the two main elements limiting primary production, thus determining ecosystem functioning, and describes the level of eutrophication pressures	Digestion and spectrophotometric determination (Total P, in mg/l).
	Total N (E)	8	F1, F4, **F6**, F9, F10	C1, C3, C6, D3, E1, H1, H2, I	Nitrogen is one of the two main elements limiting primary production, thus determining ecosystem functioning, and describes the level of eutrophication pressures	Digestion and spectrophotometric determination (Total N, in mg/l).
	Ammonia (R)	1	**F6**, F9, F10	C1, C3, C4, D3, E1, H1, H2, I	The relative amount of ammonium compared to TN describes N-cycle	Spectrophotometric determination (Total N, in mg/l).

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
					processes and unveils the level of organic pollution pressures	
	Nitrite nitrogen	1	**F6**, F9, F10	C1, C3, C6, D3, H1, H2	NOT SELECTED	
	Inorganic nitrogen	1	**F6**, F9, F10	C1, C3, C6, D3, H1, H2	NOT SELECTED	
Main ions	Chloride (E)	2	F2, F4, **F6**, F10	C4, C6	Chloride dissolved salts originally result from the catchment lithology or from the influence of the sea (in coastal lentic ecosystems). This also covers salinization pressures resulting from climate change and/ or industrial processes or seawater intrusion, the later in coastal areas.	Argentometric titration (Chloride concentration, in mg/l)
	Sodium	1	F2, F4, **F6**, F10	C4, C6	NOT SELECTED	
	Sulphate (R)	1	F2, F4, **F6**, F10	C4, C6	This is an indicator for saline inland lakes, as it is the main anion of not hypersaline saline lakes	Precipitation with barium and gravimetric/turbidimetric determina-tion. (Sulphate concentration, mg/l)
	Calcium	1	F2, F4, **F6**, F10	C4, C6	NOT SELECTED	
Soil/Sediment chemistry	Organic sediments (and N&P) on lake bottom /Composition of the substrate (E)	2	F2, F4, **F6**, F9, F10	C1, C3, D3, E1, H1, H2, I	The relative amount of organic matter in the sediments indicates the level of productivity and or external organic loads (e.g. sewage pollution)	Loss on Ignition (in mg/g, or % of dry weight)
	Maturity of the substrate	1	**F6**, F9, F10	C1, C3, D3, H1, H2	NOT SELECTED	
Water chemistry basic features	pH, acidity (E)	10	F1, F2, **F6**, F9, F10	C2, I	Natural water pH depends on rock lithology in the catchment. However, shifts are caused by pollution processes and acid rain, as well as by increases in the trophic	Potentiometry (pH, log scale)

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
					level, the later particularly for scarcely buffered low mineralization waters	
	Water electrical conductivity (salinity) (E)	7	F1, F2, **F6**, F10	C3, C4, C6, I	Salinity is one of the main ecological features of lentic ecosystems and can distinguish among different ecological types. It is affected by salinization pressures resulting from climate change and/ or industrial processes or seawater intrusion, the later in coastal areas.	Potentiometric. (Conductivity, in mS/cm)
	Dissolved oxygen concentration and its saturation (E)	6	**F6, F9**, F10	C1, C3, D3, E1, H1, H2, I	The dissolved oxygen concentration in water under natural conditions depend on the atmospheric pressure, salinity and temperature, reaching an equilibrium resulting in 100 % saturation. Pressures such as organic pollution and eutrophication alter this physical-chemical equilibrium mediated by the enhancement of biological processes, such as primary production and respiration	Polarographic (Oxygen concentration, in mg/l, and saturation, in %)
	Alkalinity (R)	3	**F2**, F6, F9, F10	C1, C2, C3, C4, C6	Water alkalinity is mainly due to the solubility of calcareous rocks providing bicarbonate to the water, thus pH buffering capacity. This is a functional chemical parameter that allows to assess the sensitivity of lentic ecosystems to acidification pressures.	Titration (Alkalinity, in meq/l, or $CaCO_3$ in mg/l)
	Hydrochemical Dystrophy Index	1	**F6**	C1, C2, C3, C4, C6, I	NOT SELECTED	
	Redox potential	1	**F6**, F9, F10	C1, C2, C3, D3, E1, H1, H2, I	NOT SELECTED	
Compositional characteristics						

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
Phytoplankton	Phytoplankton community composition, algal communities (E)	6	F1, F2, F6, **F7**, F9, F10	A2, C1, C2, C3, C4, C5, D3, H2, I	Phytoplankton is the main group of pelagic primary producers and its composition integrates a number of environmental features and mainly indicates the level of eutrophication pressures	Utermöhl sedimentation method & microscopy (Phytoplankton indices e.g. TPI)
Vegetation	Characteristic/ typical/ key plant species (presence, nº species) (E)	18	F1, F2, F3, F4, F5, F6, **F7**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, D3, E1, F1, F2, G1, G2, H1, H2, H4, I	Aquatic plants (hydrophytes, helophytes, halophytes, amphibious) are characteristic species for most lentic (Group 31, standing waters) HTCI, and the status of those that can be considered as typical species can also be assessed. They are sensitive to almost all types of pressures.	Taxonomic determination (number of species, n)
	Characteristic plant communities (presence)	5	F1, F2, F3, F4, F5, F6, **F7**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, D3, E1, F1, F2, G1, G2, H1, H2, H4, I	NOT SELECTED	
	Dominant species	1	F1, F2, F3, F4, F5, F6, **F7**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, D3, E1, F1, F2, G1, G2, H1, H2, H4, I	NOT SELECTED	
	Shrub and tree species	1	F5, **F7**	A2, B1, B2, B3, D2, E1, F1, F2, G1, G2, H1, H2, H4, I	NOT SELECTED	
	Weeds	1	F5, **F7**	A2, B1, B2, B3, C1, C2, C3, C4, D2, E1, F1, F2, G1, G2, H1, H2, H4, I	NOT SELECTED	
Zooplankton	Zooplankton (R)	3	F1, F5, F6, **F7**, F9, F10	A2, C1, C2, C3, C4, C5, C6, D1, D2, D3, F1, F2, G1, G2, H1, H2, H3, H4, I	Zooplankton is a key component of the aquatic food webs, and its composition and number of species of the main groups (rotifers, microcrustaceans) illustrates on	Microscopy (number of species, n)

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
					the diversity and complexity of these food webs. They are sensitive to pressures such as organic pollution, eutrophication, and specific chemicals	
Benthic aquatic macroinvertebrates	Presence/absence of certain aquatic invertebrate taxa (R)	5	F1, F4, F6, **F7**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D1, D2, D3, E1, F1. F2, G1, G2, H1, H2, H3, H4, I	Fastidious invertebrate taxa are indicative of environmental conditions. All these are particularly sensitive to organic pollution and specific pollutants pressures	Microscopy (presence of fastidious taxa, presence-absence)
	Benthic aquatic macroinvertebrates (Number of taxa) (E)	2	F1, F4, F6, **F7**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D1, D2, D3, E1, F1. F2, G1, G2, H1, H2, H3, H4, I	The number of taxa of macroinvertebrate can be used as a proxy of habitat suitability and availability of ecological niches. Macroinvertebrates are sensitive to organic pollution and specific pollutants pressures	Microscopy (number of species, n)
Ichthyofauna	Proportion of autochthonous species of ichthyofauna (R)	2	F1, F4, F6, **F7**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, C5, D1, D2, D3, E1, G1, G2, H1, H2, H3, H4, I	Although there are not many specific lacustrine fish species, the presence of exotic fish species replacing the native species has a deep impact on the community composition of most aquatic assemblages. This variable is consequently capable of assessing pressures by exotic species	Electrofishing and minimum damage manipulations for taxonomic assessment (Proportions of individuals of autochthonous fish species, %)
Amphibians and reptiles	Number of species of native aquatic amphibians and reptiles (E)	1	F1, F4, F6, **F7**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D1, D2, D3, E1, F1, F2, G1, G2, H1, H2, H3, H4, I	Amphibians are among the most endangered aquatic animal groups and are highly sensitive to pressures related to chemical pollution and the diseases brough by exotic species vectors.	Funnel traps with minimum damage manipulations (number of species, n)
Others (e.g. birds and mammals)	Presence of other animal species (E)	2	F1, F4, F6, **F7**, F9, F10	A2, B1, C1, C2, C3, D1, D2, D3, E1, E2,	The more animal groups, the higher number of trophic niches in an ecosystem. In lentic habitats, particularly waterfowl is	Visual inventories (e.g. number of other animal species, percentage of

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
				F1, F2, G1, G2, H1, H2, H3, H4, I	indicative of habitat suitability and niche diversity	wetland bird species with increasing or stable population trends -short term)
Structural characteristics						
Vegetation structure	Coverage of aquatic vegetation/ vegetation structure (E)	12	F1, F2, F3, F4, F5, F6, **F8**, F9, F10	A1, A2, A3, A4, A5, B2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, D3, E1, F1, F2, G1, G2, H1, H2, H3, H4, I	Aquatic plants are the main physically structuring biotic component in lentic habitats. Widely used among MS.	Visual inspection (scuba diving). Remote sensing / Google Earth -helophytes. (coverage by species / types – hydrophytes-helophytes-amphiphytes; %)
	Coverage of helophytes (R)	8	F1, F2, F3, F4, F5, F6, **F8**, F9, F10	A1, A2, A3, A4, A5, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, E1, F1, F2, G1, G2, H1, H2, H3, H4, I	Helophytes are physically structuring components of shallow water areas and emerged shorelines	Visual inspection plus Remote sensing / Google Earth (coverage by species %)
	Other plants cover (e.g. halophytes, shrubs and trees, herbaceous plants, stoneworts) and open surfaces. Width and zonation of riparian vegetation (R)	6	F1, F2, F3, F4, F5, F6, **F8**, F9, F10	A1, A2, A3, A4, A5, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, E1, F1, F2, G1, G2, H1, H2, H3, H4, I	These are the main biotic structuring component of the bank areas	Visual inspection plus Remote sensing / Google Earth. (coverage by species %)
	Absence of specific biotic form (e.g. tall halophytes)	1	F4, F6, **F8**, F9	A1, A2, A3, A4, A5, B1, B2, B3, C1, C2, C3,C4, C5, C6, D2, E1, F1,F2, G1, G2, H1, H2, H3,H4	NOT SELECTED	
	Heterogeneity	1	F5, **F8**	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6,	NOT SELECTED	

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
				D2, E1, F1, F2, G1, G2, H1, H2, H3, H4		
Vegetation structure	Density of phytobenthos (index IPS) (R)	1	F4, F5, F6, **F8**, F9	C1, C2, C3, D3, H1, H2, I	Phytobenthos (also a compositional and functional component) covers benthic areas and provides food to some macroinvertebrate feeding groups, which are selectively associated to phytobenthos rich habitats	Stones/plants brushing and microscopic determination. (IPS index, referred to EQR)
	Charophyte and cyanobacterial crust score (C&K)	1	F2, F3, F4, F5, F6, **F8**, F9	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, G1, G2	NOT SELECTED	
	Depth zonation (E)	1	F3, F4, F5, F6, **F8**, F9	A1, A2, A3, A4, A5, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, F1, F2, G1, G2, H1, H2, H3, H4	Aquatic plants distribute along a gradient of humidity-flooding-depth, the so-called zonation, which represents well the incidence of environmental factors and species interaction in determining the habitat suitability for aquatic plants and associated fauna This indicator, even not been determined by most MS, is very appropriate to describe the horizontal vs vertical structure determined by biotic components	Visual determination (number of plant strata, n)
Soil / bottom	Extent of bare soil	2	F2, F3, F4, F5, F6, **F8**, F10	A2, B1, B2, B3, D2, D3, E1, H3	NOT SELECTED	
	Characteristics of the bottom and banks	2	F2, F3, F4, **F5**, F8, F10	A2, B1, B2, B3, D2, D3, E1, F2, H3	NOT SELECTED	
	Surface of the bottom	1	F2, **F5**	A2, B1, B2, B3, D2, E1	NOT SELECTED	
	Shading	1	F2, F6, **F8**	A2, B1, D2, E1, E2, F1, F2, G1, G2, H2, H3, H4	NOT SELECTED	

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
	Exposure	1	F1, **F5**	A2, B1, D2, E1, F1, F2, G1, G2, H1, H2, H3, H4	NOT SELECTED	
Animal taxa	Bottom fauna (Danish Littoral Zone Macroinvertebrate Index, (DLMI) (R)	1	F5, F6, **F8**, F9	A1, A2, A3, A4, B1, B2, B3, C1, C2, C3, C4, D1, D3, E1, F2, G1, G2, H1, H2, I I	NOT SELECTED	Handnet kicking capture and binocular microscopic determination (Macroinvertebrate indices, e.g. DLMI)
	Ichthyofauna (Fish abundance and size distribution, CPUE number, CPUE weight) (R)	1	F6, **F8**, F9, F10	A1, A2, A3, A4, B3, C1, C2, C3, C4, C5, D1, D2, D3, E1, F1, G1, G2, H1, H2, H3, H4, I	NOT SELECTED	Electrofishing (e.g. Size classes, in % of the total population)
Functional characteristics						
Acid status	Cover of species indicating acidification (E)	1	F1, F2. **F6, F9**, F10	C1, C2, C3, E1, I	Acidification is a process usually deriving from industrial (e.g. mining) and atmospheric deposition (acid rain) that can be traced by the presence/absence or extent of coverage of acid-tolerant/intolerant taxa	Microscopy (Number of acid tolerant algal taxa, (e.g. number of phytoplankton/ phytobenthos acid-tolerant species, e.g. some desmids)
Invasive species	Presence (and cover) of invasive alien/exotic species (E)	9	F7, F8, **F9, F10**	A2, B1, D3, G1, G2, I	Invasive exotic species can replace or damage (by predation, as diseases vectors, etc.) the owns habitat characteristic taxa. Thus, this variable describes a functional process (invasion by alien species) that impacts both community structure and ecosystem functioning	Visual determinations of coverage (for macro-organisms) in transects or plots (Area covered by exotic plant species vs total habitat area, in %
	Absence of alien and/or invasive species	1	F7, F8, **F9, F10**	A2, B1, D3, G1, G2, I	NOT SELECTED	

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
Fauna functioning	Imbalance in fish populations	1	F7, F8, **F9, F10**	A1, A2, A3, A4, C1, C2, C3, C4, C5, C6, D2, D3, G1, G2, I	NOT SELECTED	
	Impacts of animals	1	F7, F8, **F9**, F10	B1, D3, G1, G2, H2	NOT SELECTED	
Organic loads	Biological Oxygen Demand (E)	1	F6, **F9**, F10	A2, C1, C2, C3, D3, H1, H2, I	The biochemical oxygen demand gives an idea of the intensity of respiratory metabolic processes within the ecosystem, which increases with the organic load (both internal and external)	BOD_5 (BOD_5 in mg O_2 /l)
	Chemical Oxygen Demand (E)	1	**F6**, F9, F10	A2, C1, C2, C3, D3, H1, H2, I	As capable of oxidising chemically all organic matter in the sample, this variable can measure the total amount of organic matter in water, which results from autochthonous primary production (biomass) or for external detritus inputs	COD (COD in mg O_2 /l)
Trophic status	Presence of strong algal growth (filamentous algae or "blooms") (E)	3	F6, **F9**, F10	A2, C1, C2, C3, D3, H2	The occurrence of algal (usually cyanobacterial) blooms and massive growth of filamentous algae is commonly the consequence of extreme eutrophication	Visual inspection (Open waters surface covered by filamentous algae, in %)
	Phytoplankton biomass / Chlorophyll-a (E)	3	F1, F2, **F6, F9,** F10	A1, A2, C1, C2, C3, D3, H2, G2, I	Eutrophication is the main pollution process in lentic ecosystem, as a consequence of increased nutrient loads from pint or non-point sources, promoting the exacerbated growth of phytoplankton	Pigment concentration after water filtration and pigment extraction, plus spectrophotometric measurement. (Chlorophyll-a concentration, in mg/m^3)
	Nutrient richness, Nutrient loading	3	F1, F2, **F6**, F9, F10	A2, C1, C2, C3, D3, H2, I	Concentrations already covered in the chemical variables. Total loads reflected in other eutrophication indicators	
	Crust chlorophyll-a (	1	F1, F2, F6, **F9**, F10	A2, C1, C2, C3, D3, H2	NOT SELECTED	

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
	Water trophic state index	1	F6, **F9**, F10	C1, C2, C3, D3, H2, I	NOT SELECTED	
	Nitrogen deposition	1	F1, F6, **F9**, F10	C3, I	NOT SELECTED	
	Daily oxygen saturation variation (E)	1	F6, **F9**, F10	A2, C1, C2, C3, D3, H2, I	The wider the fluctuation of dissolved oxygen concentration along the diel cycle, the most active ecosystem metabolism, a signal of the habitat functional stress	Polarography (Diel range of variation of dissolved oxygen saturation (%Min vs % Max)
	List of species indicators of eutrophication	1	F1, F6, F7, F8, **F9**,	A1, A2, C1, C2, C3, D1, D3, H2, G2	NOT SELECTED	
	Eutrophication of the habitat	2	F6, **F9,** F10	A1, A2, C1, C2, C3, D1,D3, H2, G2	NOT SELECTED	
	Formation of deep chlorophyll maxima and presence of photo-synthetic bacterial populations in summer (S)	1	F1, F5, F6, F7, **F9**	A1, A2, B1, C1, C2, C3, D1, D3, H2	Typical feature of the HTCI 3190	Pigment extraction from waters immediately below the oxygen-anoxic interface. (Concentration of Bacteriochlorophylls, in mg/m^3)
	Zooplankton/phytoplankton trophic ratio (shallow, non-saline lakes)	1	**F9**	A1, A2, C1, C2, C3, D1, D3, H2	NOT SELECTED	
Diverse processes	Number of plant species with specific traits /indicators (eutrophilous, destructuring, woordy, wasteland, stoneworts, negative species) (E)	6	F8, **F9**	A2, B1, B2, B3, C1, C2, C3, C4, C5, C6, D2, D3, E1, F1, F2, G1, G2, H2, H4, I	Uses the indicator value of certain plant species based on specific traits.	Visual identification or plant sampling and determination (e.g. number of plant species which are indicators of ecosystem dysfunction from a given list, n))
	Dynamics	1	**F9**	A2, B1, E1, F2, I	NOT SELECTED	

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
	Leaf litter (	1	**F9**	A2, F2, I	NOT SELECTED	
	Hydro-morphological functioning	1	**F9**	A1, A2, A3, A4, B1, D1 ,D3, I	NOT SELECTED	
	Index total phosphorous x Water colour	1	F2, **F6**, F9	C1, C2, C3, C4, D3, H2	NOT SELECTED	
	Maximal depth of stoneworts meadows	1	F5, **F9**	A2, B1, C3, D3	NOT SELECTED	
Pressures and threats	Land use and human disturbance	3	These are pressures, not habitat features	ALL	NOT SELECTED	
	Influences and management	2	These are not habitat features	ALL	NOT SELECTED	
	Naturalness	1	F8, **F9**, F10	ALL	NOT SELECTED	
Landscape characteristics						
Habitat	Habitat area/extent (E)	5	F2, F3, F4, F5, F8, **F10**	A1, A2, A3, A4, A5, B1, B2, B3, D2, E1, F1, F2, G1, G2, H1, H2, H3, H4, I	Although total habitat area is addressed as a separate parameter ("area") in the Article 17 (HD) reporting matrix, assessing variation in habitat extent itself at appropriate scales remains essential.	Remote sensing (extent, ha or m^2)
	Evolution of the number of water bodies where the habitat is present (E)	1	F10	A2, A3, B1, D1, F1, F2, I	The higher the number of water bodies that can keep some connectivity (hydrological, or through waterfowl flyways, for instance) facilitates exchanges among habitat fragments. A spatial scale should be defined (e.g. surface sub-catchment)	Remote sensing and field testing (number of waterbodies in a sub-catchment).
	Vegetation mapping in the catchment area (E)	1	F10	A2, A3, B1, D1, E1,F1, F2, G1, G2, H2, H3, H4, I	Vegetation mapping in the catchment area helps to explain import processes affecting the habitat.	Copernicus Land Cover products, remote sensing and field testing (% cover of main vegetation types and subtypes in the catchment)

Group of variables	Variables	Nr. of MSs	Main ecological features addressed	Pressures and effects assessed	Selection rationale	Main standardised measurement procedures (recommended metrics)
	Structure of habitat patches (fragmentation) (E)	1	**F7, F10**	A2, A3, B1, C1, C3, C6, D1, E1, E2, F1, F2, H2, H3, H4, I	The distance among habitats patches and the matrix (other ecosystems /land uses) characteristics between patches are key for the facilitation of exchanges, mainly for individuals (Turner & Gardner (2015).)	Distance among patches - lakes, ponds- holding the HTCI (Distance, m)
	Indicators of habitat degradation and fragmentation (R)	5	F1, F2, F3, F4, F5, **F8**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, D2, D3, E1, F1, F2, G1, G2, H1, H2, H3, H4	Habitat degradation may result from multiple causes, while fragmentation in lentic ecosystems is generally less common.	Depends on the indicator used (further refinement is required to select appropriate metrics for specific degradation or fragmentation causes)
	Habitat complexes (mosaic, heterogeneity, patchiness) (R)	4	F1, F2, F3, F4, F5, **F8**, F9, F10	A2, B1, B2, B3, C1, C2, C3, C4, D2, D3, E1, F1, F2, G1, G2, H1, H2, H3, H4, I	Overall, environmental heterogeneity tends to favour biodiversity.	Depends on the indicator used
	Water body surface area	4	F2, F3, **F4**, F5, F8, F10	A1, A2, A3, A4, A5, B1, B2, B3, D2, E1, F1, F2, H3, H4, I	NOT SELECTED	
	Landscape environment (Landscape metrics & Spatial conditions)	3	F10	A2, A3, B1, C1, C3, C6, D1, E1, E2, F1, F2, G1, G2, H2, H3, H4, I	NOT SELECTED	
	Protected area	1	This not a habitat feature	D1	NOT SELECTED	

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