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Project INHABIT - LIFE08 ENV/IT/000413

Local hydro-morphology, habitat and RBMPs: new measures to improve ecological quality in South European rivers and lakes

ACTION GROUP I3: Proposal of innovative measures for river basin management plans

- Action I3_IRSA (month 19-36) Proposal of innovative measures for river basin management plans by IRSA
- Action I3_ISE (month 19-36) *Proposal of innovative measures for river basin management plans by ISE*
- Action I3_PI (month 19-36) *Proposal of innovative measures for river basin management plans by I ARPA Piemonte*
- Action I3_SA (month 19-36) *Proposal of innovative measures for river basin management plans by RAS*

Deliverable I3d4

Guidelines on where and how the new set of measures should be adopted to aid the achievement of good ecological status by 2015

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Guidelines on where and how the new set of measures should be adopted to aid the achievement of good ecological status by 2015

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Summary

Short abstract

These guidelines present INHABIT project main outcomes, listed as key points. In particular, 28 points were identified, as listed in table of contents; each of the points represents a crucial aspect of ecological status assessment or implementation of River Basin Management Plans (RBMPs). Some points should be considered when river restoration measures have to be applied and when these have to be evaluated in terms of efficacy. Each point was briefly developed as an operational indication to face a specific problem, as carried out in INHABIT project. For each point the reference Deliverable, where all the details about the issues in question can be found, is reported. The points forming these guidelines include the main INHABIT results obtained for both rivers and lakes, as well as aspects related to nutrients dynamics. In summary, the points can be grouped into different themes, as presented here below. The document opens with a description of the importance of river habitats and alterations, and the possible aims of their description (points 1. and 2.). Subsequently issues related to the lakes are presented, focusing in particular on the definition of reference conditions in such environments and the modeling approaches used for this purpose, the method for the description and assessment of lake habitats, the dynamics of atmospheric nitrogen and the impact of nitrogen compounds on biological communities and, finally, the relationship between habitat characteristics and ecological classification (points 3. - 10.) . The following two points (11. and 12.) deal with two aspects closely related to national environmental legislation: the validation of reference sites for rivers and the discussion about river typology and its criticality in the Mediterranean area. The next issue is the quantification, for rivers, of natural variability to improve the accuracy of classification systems, introducing the crucial role played in this context by the lentic - lotic character (points 13. - 17.). The paper continues with the discussion about nutrients retention and its efficiency in river basins in relation with habitat features (points 18. - 19.), the discussion on the factors influencing biological metrics used for classification, the evaluation of effects of water abstraction and problems associated with the minimum acceptable flows (points 20. - 23.). Following points (24. - 28.) deal with the suggestions for the improvement of RBMPs, how to manage river morphological information over different spatial scales, the issue of Heavily Modified water Bodies and the evaluation of the effectiveness of restoration measures. The guidelines conclude with a presentation of the practical tools developed by INHABIT (29.) and a note on possible connections between WFD and HABITAT Directive (30.).

Guidelines have been prepared both in Italian and English.

1. Why and how to describe river habitats (Pd3, D1d5)

Streams are characterized by a complex mosaic of habitats, defined by a number of factors, such as: hydrological dynamics, sediments characteristics, lithology, geomorphological processes, climatic factors, direct and indirect effects of human activities, etc. Habitats are one of the most explicit expression of physical features of river ecosystems, representing the interface between biological organisms inhabiting the river - and its surroundings - and the river itself. The description of river habitats by means of appropriate features, allows to assess both the status of aquatic and riparian ecosystems and the potential of the river to support biological communities or particular animals and plants species. These two aspects also permeate the contents of two important EU environmental directives, respectively, the Water Framework Directive (WFD, 2000/60/EC) and the Habitat Directive (HD, 92/43/EEC).

INHABIT project has addressed in detail the investigation of the relationships between habitat conditions and biological communities structure. In order to do this, particular attention was paid to the selection of the procedures of collection and description of habitat characteristics.

In the European context, several methods are dedicated to the description and evaluation of stream habitats. The CARAVAGGIO method (Core Assessment of River hAbitat VAlue and hydromorpholoGIcal cOndition), as the British River Habitat Survey (RHS), fits into this scenario, where different definitions of 'habitats' are given, but without going into the heart of the matter. The CARAVAGGIO, widely used in INHABIT, offers a collection as objective as possible of information for an extensive characterization of the river, so that collected features can be used for the evaluation of a large number of specific habitats, as well as of the "character" and the general "quality" of the investigated river reach, as expressed through a set of descriptors (e.g. HQA , LRD , LUI, HMS). No method could represent alone a complete system to map and to evaluate all possible habitats for all species or populations. Furthermore, the river environments and, consequently, their associated habitat features, show often a strong variability , strictly related to river dynamism, and many different approaches exist to the definition of the possible pieces of the complex mosaic river. With particular reference to the purposes of the two Directives (WFD and HD), during the project INHABIT, habitat data collected were used independently or in combination with other information, for a variety of purposes including:

- River habitat description and quality classification.
- Reference sites selection and description of type-specific reference conditions.
- Support to the interpretation of Biological Quality Elements (BQEs) data, *sensu* WFD.
- Protection of biodiversity in river systems.
- Collection of information for the evaluation of natural capacity of nutrient retention.
- Collection of elements to support a better definition of river types according to D.M. 131/2008.
- Definition of lentic-lotic character in rivers.
- Evaluation of pressures and impacts in river water bodies.
- Quantification of the impact of water abstraction on habitat and on river biota.
- Risk estimation of failing quality objectives and Good Ecological Status.
- Habitat characterization in Heavily Modified and Artificial Water Bodies.
- Definition of protection and restoration measures.

- Support in the drafting of management and protection plans.

In addition, the same data can be used for:

- Habitat characterization for fish fauna management and fishing activities.
- Identification of valuable habitat to support Habitat Directive and other environmental protection regulations.
- Environmental education in river ecology.
- Procedures for Environmental Impact Assessment (EIA) and Strategic Environmental Assessment (SEA), affecting riverine environments.
- Assessment of the impacts of hydroelectric plants.
- Support to the definition of ecologically acceptable flow for the aquatic biocoenosis.

2. Why and how to quantify river habitat alterations (I3d2, D1d5).

In the general framework of Water Framework Directive implementation, it is crucial to consider that alteration of river habitats and their characteristics play a key role in river ecosystems. Monitoring the effects of habitat changes has recently received increasing interest.

The WFD has also recognized the importance of habitat and hydromorphological elements in the understanding of the processes structuring biological communities. River restoration , if we exclude restoration of water quality, is largely based, in general, on the possibility of obtaining better habitat conditions, including habitat practice management for improving biocoenosis or restore fluvial processes in order to mitigate the effects of anthropogenic disturbance.

In this context, it is important to describe and quantify habitat alterations in order to relate collected information to the aquatic biocoenosis, that represent both the primary tool for providing quality judgments and the object of protection.

The synthetic descriptors HMS (for the presence of artificial structures), LUI (for land use in riverine and perifluvial areas) and HQA (for habitat diversification and quality), that can be derived from the application of CARAVAGGIO method, are well suited to quantify these changes. Their use can easily combine the tools related to, for example, the estimation of risk and hydro-morphological characterization, already in use by environmental agencies and other public bodies responsible for environmental management and protection.

In general, INHABIT has allowed to determine how the quantity and quality of aquatic and riparian habitats have a direct influence on the ability of aquatic communities to tolerate pollution, reductions in flow and hydromorphological alterations. For example, it has been shown how the simultaneous presence of optimal conditions for different habitat factors (lentic lotic character and general diversification) can limit the negative effects of disturbance e.g. related to water pollution and morphological alteration.

3. Is the "Lake Habitat Survey" method suitable to evaluate the hydromorphological quality of Italian Lakes and reservoirs? (Pd3 - I3d2)

Annex V to the European Directive 2000/60/EC (Water Framework Directive, WFD) states that two elements should be used to evaluate lake hydromorphological status:

(i) hydrological regime: quantity and dynamics of water flow, residence time and connection to the groundwater body;

(ii) morphological conditions: lake depth variation, quantity, structure

and substrate of the lake bed and structure of the lake shore.

In Europe, different methods and approaches to evaluate lake hydromorphological quality were developed, with different ability to identify deviations from reference conditions.

In 2004, a group of British researchers developed a method purposely to fulfill WFD requirements, namely the Lake Habitat Survey (LHS). It was applied to the large majority of the lakes in the United Kingdom, using field activity and aerial photographs together to have a more accurate picture of shore alteration.

LHS was designed to evaluate and characterize lake (or reservoir) physical habitat, and can be applied to any standing water.

Within LHS, a field survey form is used to list habitat features and human pressures affecting the whole lake or the riparian, shore and littoral zones. A series of guidance sheets includes code legends, plots and photographs helping method application.

During the INHABIT project, we applied LHS to 5 lakes and 6 reservoirs, during summer months (June to September), i.e. during lake stratification. The original field survey form was translated in Italian, updated and extended in order to include habitat features and pressures found in the lakes evaluated during the project and in other lakes evaluated in a preparatory phase.

Survey data were then included in a database and used to calculate the Lake Habitat Quality Assessment (LHQA) index and the Lake Habitat Modification Score (LHMS) index.

After applying the LHS to a number of Italian lakes and reservoirs we can assert that:

- LHS can be applied to Italian lakes and reservoirs, as it considers their main features and pressures;
- field survey is not too complex or costly, and it is more effective if aerial photographs are used to define hab-plots positions and to identify the main alterations in the riparian zone;
- collected information fully describes morphological alteration due to recreational activities, land use and modification of the riparian and littoral habitats;
- beside the synthetic indices (LHQA and LHMS), individual collected data can be used to evaluate relationships between the features of lake habitat and of biological quality elements (BQEs);
- to better identify such relationships, it is important to locate the hab-plots close to the areas and transects used for sampling the BQEs: this allowed for example to identify relationships between elements of the riparian zone and macrophyte species composition. Further data are needed to better define the influence of habitat alteration on the BQEs which usually react more evidently to lake trophic status than to habitat alterations;
- we consider that in reservoirs it would be necessary to apply LHS twice, at the highest and lowest water level. The comparison of the two periods may give a better picture of the hydromorphological quality of the reservoir.

Concluding, LHS gives useful information on lake and reservoir habitat quality, can be used to quantify pressures on the riparian, shore and littoral zones and to identify habitats that need particular protection. It can also help in designing mitigation measures. It would then be useful to consider the application of the LHS during the definition of the River Basin Management Plans to obtain a better comprehension of the ecological status of lakes and reservoirs.

4. Is it possible to infer the environmental conditions that natural lakes showed before the recent strong human impact and use them as reference conditions? (D1d3)

Reference conditions are frequently defined used the spatial method, i.e. grouping waterbodies in types and selecting for each type a statistically significant number of reference sites.
However, when the number of waterbodies is small, and most of them are affected by strong human pressures, this method is not reliable. This is the case of Italian lakes and reservoirs.
When the spatial approach is not possible, an alternative method is represented by modeling.
It is possible to use simple statistical models to infer the concentration of total phosphorus in reference conditions for all relevant Italian lakes and reservoirs. Using regression equations, modeling results can be used to infer reference conditions for chlorophyll *a* concentration and for phytoplankton composition indices.
The modeling exercise carried out during the INHABIT project showed that:

- in the case of phytoplankton, the spatial approach used to define the class boundaries in the Italian rules can be considered reliable for most Italian lakes. Our results also suggest that PTI_{ot} index may be used for all Italian lakes, and the use of a specific index ($PTI_{species}$) for the deepest lakes is not necessary;
- for shallow Mediterranean lakes (types ME-1 and ME-2) it seems to be necessary to verify reference conditions case by case, using more complex models or palaeolimnological approaches, to avoid to set too strict quality targets;
- the lack of a simple and direct relationship between reference total phosphorus concentration and quality indices based on the other BQEs does not allow to extend this exercise to all the quality indices.

5. Atmospheric deposition of pollutants: a path that does not follow the concepts at the basis of the Water Framework Directive (I2d5)

Directive 2000/60/EC represents a marked step forward in the management of European surface, round and coastal water because it underlines the need to take measures at the scale of river basin, considering all hydraulic connections among waterbodies and human pressures in the whole river basin.
However, this concept does not consider the fate of pollutants that can be emitted into the atmosphere outside the river basin, and that can be carried to the rive basin itself by atmospheric deposition.
This path is quantitatively important for nitrogen compounds, emitted into the atmosphere as nitrogen oxides (by traffic and high temperature combustions) and ammonia (by agriculture, farming and in smaller quantity by traffic). Together with nitrogen compounds, long-range transport of atmospheric pollutants also concern heavy metals, such as mercury, and persistent organic pollutants.
In the INHABIT project, the quantification of the atmospheric deposition of nitrogen compounds to lakes and reservoirs allowed to define some key aspects which need to be considered in the updating of the River Basin Management Plans (RBMPs):

- in Piedmont, ammonium and nitrate deposition is large, mainly in the prealpine hills where most natural lakes are located. In the Alps, deposition are markedly lower. Nitrogen compounds are emitted into the atmosphere within the River Po basin, but

mostly in the plain, outside the basin of each waterbody;
- in Sardinia, ammonium and nitrate deposition is low, and comparable with the values measured in the Alps;
- the amount of nitrogen carried by atmospheric deposition is important for waterbodies not affected by direct pollution and not having strong agricultural pressures in the catchment. This is the case for Lago Maggiore, which receives every year from its tributaries 5760 tons of nitrogen, but only 2659 derives from human activities within the watershed. Atmospheric deposition carries every year to the Lago Maggiore watershed more than 11000 tons of nitrogen, mostly retained by forest and agriculture soils.

6. The amount of nitrogen compounds carried by atmospheric deposition is particularly relevant for references lakes (I2d6)

To evaluate the relevance of nitrogen deposition to rivers and lakes, it is possible to use numerical models estimating the amount of nitrogen compounds retained in soils and cultivations. These models can be more or less complex, depending if deposition amounts can be considered steady or not.
Examining time series of nitrogen concentration in atmospheric deposition no general significant trend was detected. Steady-state models were then used to estimate the fate of nitrogen compounds.
We assumed that atmospheric deposition should be more relevant in sites receiving less direct pollution, such reference sites, and that reference sites should have undisturbed catchments, we simplified the model assuming fully forested watersheds, which was the natural condition of most Italian low and mid altitude areas.
Using steady-state models of forest soils, it was possible to estimate that in Piedmont most soils are N-saturated and should release relatively large amounts of nitrogen during all seasons, while in Sardinia N-saturation is slighter and soils should release smaller amounts of nitrogen, only in specific seasons. These findings were confirmed by the examination of long time series of nitrogen concentration in relatively undisturbed rives and streams in both regions, substantially validating the selected model and assumptions.
The results of this modeling exercise showed that:
- in reference lakes, nitrogen carried by atmospheric deposition can be larger than in-watershed load by one order of magnitude;
- reference lakes are selected because of the slight intensity of human pressures, and are assumed to host reference communities. If any biological element is affected by high nitrogen concentration, it can deviate from reference condition in reference lakes receiving large amounts of nitrogen through atmospheric deposition. In this case, the use of this lake for assessing reference conditions and of the Ecological Quality Ratio to define the ecological quality of other lakes may lead to misclassification.

7. Not all biological quality elements are affected by high nitrogen concentration (I2d7)

Annex V to the Directive 2000/60/EC states that the ecological quality of the waterbodies should be evaluated on the basis of four biological quality elements (BQEs): phytoplankton, macrophytes and other aquatic flora, macroinvertebrates and fish fauna. In the INHABIT project we tried to disentangle the effect of increased nitrogen concentration and

other human pressures (such as eutrophication and hydromorphological alteration) on each BQE.

Only phytoplankton showed a distinct relationship between species composition and nitrogen levels. In the case of the other BQEs, the effect of the trophic status was evident, but a specific response to nitrogen alone was not detectable. For example, macroinvertebrate respond to increased nutrient levels by a community shift towards carnivorous species. Fish biomass and average length are also correlated with nutrient levels, and in eutrophic lakes the proportion of tolerant and omnivorous fishes and of fishes feeding on benthos also increases. However, the effects of increased nitrogen and phosphorous levels cannot be distinguished.

Among macrophytes, which were only found in natural lakes, there are evident differences between Lago Sirio and Lago di Candia, which show the lowest and highest ammonium concentration, respectively. *Lemna minor* and *Myriophyllum verticillatum* were only found in the former, *Nymphoides peltata* in the latter, which also shows high cover of *Trapa natans, Nelumbo nucifera, Nuphar luteus* and of species of the genus *Najas*. However, the small number of lakes does not allow a statistical treatment of these data.

The results of the INHABIT project suggest possible improvements in the methods for evaluating the ecological quality of lakes:

- most biological methods for the evaluation of the ecological quality of the lakes included in the Commission Decision of 30 October 2008 establishing the values of the Member State monitoring system classifications as a result of the intercalibration exercise were designed to evaluate eutrophication as the most significant human pressure, and the trophic status was mainly summarized by total phosphorus concentration; however, the response of the BQEs to increasing trophic status is more complex: it depends on the levels of different nutrients and on their ratios.
- for waterbody management, reducing the load of both nutrients is important, but a specific effort to reduce nitrogen load alone is not justified.

8. There are marked differences in phytoplankton composition in lakes slightly affected by human pressures but receiving different amounts of nitrogen deposition (I2d8)

As the relative importance of the atmospheric load of nitrogen compound is larger in lakes close to reference conditions, we looked with more details to the waterbodies affected by the slighter human pressures in both Sardinia and Piedmont, where atmospheric nitrogen load is slight and strong, respectively.

We compared two lakes in Piedmont and two reservoirs in Sardinia with low average concentration of total phosphorus (lower than 25 µg L^{-1}), but with different N:P ratio (200 in Piedmont and 30 in Sardinia). Lakes in Piedmont were strongly dominated by diatoms (Bacillariophyceae), while reservoirs in Sardinia were dominated by Dynophyceae. The latter can use different metabolisms, coupling algal autotrophy with the ability to heterotrophically ingest food particles, that can represent nitrogen sources.

On the contrary, two reservoirs in Piedmont were dominated by diatoms.

Even if we take in account the small number of studied waterbodies, it is interesting to note that in the two regions the phytoplankton communities in relatively oligotrophic lakes are dominated by different algal classes.

These differences may be important within the RBMPs:

- these results underline the necessity to better define reference conditions, in

particular in the Mediterranean ecoregion, because they suggest that lakes with the same trophic pressure may host completely different algal assemblages, depending on nitrogen levels;

- the results of the measures intended for the improvement of waterbody ecological quality should be evaluated by monitoring the progressive reduction of differences between actual ecological status and reference ecological status. It is important to verify that other ecological factors, such as nitrogen atmospheric load, will not lead the biotic communities towards a status different from the one which is assumed to be the reference status for that specific waterbody type.

9. How large is the natural variability of lake ecological conditions and how much it can affect lake quality assessment? (D1d4)

Within the WFD, BQEs role in waterbody quality assessment is fundamental. For this reason, the WFD states that BQE-based quality assessment should include an estimate of its precision and reliability.

Quality assessment is performed using the Ecological Quality Ratios (EQRs), by dividing the present-day value of the biotic index by its reference value: the precision of the EQR depend then by both the precision of the present-day estimate of the biotic index and the precision of the estimated reference conditions.

Some BQEs, such as phytoplankton and other aquatic flora, are directly affected by lake trophic status, and it is then possible to estimate their variability on the basis the variability of the trophic status.

The latter can be inferred on large time scales (decades or centuries) using palaeolimnological techniques.

The results of the INHABIT project show that:

- phytoplankton-based indices are sensitive to meteorological conditions, and it is important to activate a network of frequently sampled reference sites, to be used to correct EQRs on the basis of the annual conditions, in order to avoid that natural fluctuations in the trophic status would lead to fluctuations in waterbody quality classification;
- the index based on macrophytes seems to be less sensitive to trophic natural variability, but the boundaries defined during the European intercalibration exercise seems to be too strict, if compared to the values expected on the basis of the natural trophic status. The slow response of macrophyte assemblages to the trophic variability also suggests the need of a second method, based on phytobenthos, to assess the "macrophyte and other aquatic flora" BQE defined by Annex V to the WFD.

10. Does lake habitat features affect lake quality classification? (I3d1)

Waterbody quality assessment is based on the properties of the BQEs, which can be influenced by habitat quality. To understand if habitat features may influence quality assessment, we looked for relationships between quality classes and the habitat characteristics evaluated by means of the LHS, such as littoral and sublittoral substrate characteristics, shore alterations, human pressures around the lake and in the riparian zone.

Summarizing all the statistical analyses performed within the INHABIT project:

- human pressure along the shore does not significantly affect macroinvertebrate assemblages, even if a relationship can be found between the BQIES index and some hydromorphological parameters;
- no significant relationship was found between fish fauna distribution and habitat characteristics, but estimated abundance was correlated with the morphology of the littoral zone. Fish fauna composition is more affected by chemical (trophic) aspects than by lake hydromorphology;
- for macrophytes, hydromorphological aspects does not seem to affect lake quality assessment, but it is necessary to sample a large number of transects, paying particular attention to submerged macrophytes, which frequently inhabit small habitats, to correctly assess lake quality;
- however, macrophyte species composition is related to substrate and shore features, such as trees or buildings that can shadow lake surface;
- phytoplankton, being pelagic, is not affected by shore morphology, but its biomass may be influenced by hydrological events, both directly and by the interaction with lake watershed. For example, rainy winters may cause a larger runoff of nutrient from arable land and then larger biomass in the following spring, leading to higher algal biovolumes and chlorophyll a concentration, i.e. lower ecological quality.

11. How to proceed for river reference sites validation (I1d1, I1d4)

According to WFD indications the selection and testing of reference sites is a key step in all the processes involving a comparison between observed and expected conditions. The activity carried on by INHABIT project has played an important role in the verification based on abiotic features of reference status of sites in the two investigated areas. Within INHABIT project potential reference sites selected in Sardinia and Piedmont has been verified according to the national procedure. Such procedure consists in a set of questions or 'criteria' to be addressed. Questions are related to pressures acting on sites at different spatial scale and are organized in a table ('check table'). Validation process consists in the completion of the check table that includes 57 criteria, dedicated to the quantification of a specific anthropic pressure. To each criterion a different weight is assigned according to its relevance: Necessary (IR: weight 1), Important (IM: weight 0.6) and Ancillary (AC: weight 0.2). To each criterion a reference threshold and a rejection threshold are set.

The first step of the testing process must assess if:

- more than 2 IR criteria fall above the rejection threshold
- more than 4 IR criteria fall above the reference threshold
- more than 3 IM criteria fall above the rejection threshold
- more than 6 IM criteria fall above the reference threshold

if one or more of the four statements are met, then the site cannot be considered as reference. If more than one criteria fall above any threshold, then the maximum allowed is 6 criteria above any threshold or 3 criteria above rejection threshold.

A score of 1, 0.5 or 0 is then assigned to each question if it falls, respectively, below the reference threshold, between the reference and the rejection threshold or above the rejection threshold. The assigned score is then multiplied by the relative weight. According to the type of alteration identified the questions are distinguished in 7 categories: Point source pollution (score A); Diffuse source pollution

(score B); Riparian vegetation (score C); Morphological alterations (score D); Hydrological alterations (score E and F); Biological pressures (score G); Other pressures (score H). Partial scores obtained by summing up the questions within the same category are divided by the maximum possible score. Scores obtained from the single category are then mediated (weighted mean considering the number of questions per category) to obtain the final score. The total score of 0.9 is set as threshold for rejection.

Once the compliance with the table criteria has been established, the reference status must be confirmed by habitat or hydromorphological evaluation (e.g. IDRAIM method - hydromorphological assessment method developed by ISPRA – has to be used for hydromorphological evaluation according to Italian Decree for ecological classification). In the case of INHABIT project in the process of reference validation the habitat was evaluated by means of CARAVAGGIO method that has allowed the calculation of descriptors HQA, HMS and LUI, together contributing to the definition of habitat quality (IQH - Index of Habitat Quality). The verification of IQH high status constitutes the element of reference site validation.

12. Adequacy of regionalization and critical issues in Mediterranean rivers: the importance of river types definition (I1d4)

When setting up management plans, one of the needed prerequisites for providing accurate ecological classifications, in accordance with the WFD provisions, is the designation of river types to assign water bodies subject to monitoring.
Among the options outlined by WFD, Italian typology (i.e. a cluster of all types in a given area) refers to System B. In particular, the process of establishing river types is divided in three levels; the first stage requires the allocation of the water body to a specific Hydroecoregion (HER). The second level involves the verification of pre-defined descriptors (leading to the real typological attribution) and the third (optional) allows detail insights, especially related to local specificities and data availability. In the definition of river types, mainly because of the rigidity of the formal system (the second level), some regions may face difficulties in types allocation due to some peculiar features of their territory. A critical issue in this sense is represented by the definition of water persistence. Lastly, type definition, carried out at the moment in all Italian regions, should be verified on a biological basis. Analyses carried out in the context of INHABIT project have identified the following key issues, particularly relevant for the preparation of River Basin Management Plans.

- For Piedmont, and large areas of Northern Italy the general validity of the biological (macrobenthic community) clusters obtained by level 1 typization is confirmed, mainly for the high number of HERs and types. Not clear evidence is available in order to confirm or reject the validity of level 2 typization (e.g. at the moment no differences were highlighted for water bodies size). However, in Piedmont, where the gradient of water persistence is usually less evident if compared to Sardinia, the quantification of the lentic-lotic character would be desirable (see below).
- In some areas, e.g. Sardinia, characterized by strong Mediterranean water regime, the typological definition can prove particularly awkward, especially from the point of view of the definition of the degree of water persistence and the biological significance of the types defined

according to level 2. In fact these issues may be properly addressed by level 3 of Italian river typological system, in particular by quantifying the lentic - lotic character , as defined by LRD descriptor. Lentic-lotic character plays a primary role in the characterization of water ecosystem, as main factor discriminating macrobenthic communities groups and observed gradients, representing a synthesis of the effects of hydrologic / hydraulic features on biotic communities.

As also shown in the following, it is therefore important, when evaluating the ecological status, to assess the elements concurring in defining lentic – lotic character, in order to add site-specific insights to a typological system that cannot always be sufficient for effective quality classification.

13. Why and how to quantify natural variability in rivers (I1d4, I3d2)

Temporal and spatial variability of Mediterranean environments, coupled with a lack of predictability, can make particularly problematic the development of ecological status assessment systems. Some hydrological and habitat features related to the reach scale in Mediterranean rivers, may exert a considerable influence on biological metrics commonly used for quality classification. The differences related to the habitat availability may therefore be interpreted as changes in the ecological status, although no source of anthropogenic alteration is present. In particular, one of the challenging aspects in Mediterranean area is the definition of appropriate reference conditions. A proper definition of reference conditions must take into account the natural variability that should be quantified through adequate habitat survey techniques.

In this context, INHABIT project has provided a key to the reading of the natural variability in rivers, in terms of habitat and in particular in terms of lentic-lotic character (LRD), i.e. the relative presence of lentic and lotic areas. The method adopted by INHABIT as effective tool for river habitats characterization is the CARAVAGGIO method, which allows to derive synthetic descriptors, found to be strongly related to the variability of aquatic biocoenoses. .

In this respect, it is confirmed that the natural variability observed in reference - or not significantly altered - river stretches may be explained in terms of variability of synthetic descriptors derived from the CARAVAGGIO method. So collected information can allow a correction of the classification systems, addressed to increase methods accuracy.

14. The Lentic-lotic character

Since a few years ago, in European context, it was difficult to easily summarize habitat aspects directly related to ‘water availability’ in a given river stretch. In order to fill this gap, LRD (Lentic-lotic River Descriptor) was recently developed. LRD allows the characterization of a river reach in terms of lentic-lotic character, that depends on river morphology, sediment transport and deposition and water level. Among the habitat features, lentic-lotic proportion has resulted as one of the most important in defining aquatic macroinvertebrates community structure in Mediterranean area. For LRD calculation information related to presence and variety of flow types, substrates, channel vegetation, bars, artificial structures etc. are considered. LRD descriptor, through a synthesis of hydraulic conditions and habitat, provides an overall picture of the ratio between lentic and lotic habitats, of great relevance in supporting biological data. Lentic lotic character is a key aspect, for instance, in the evaluation of the comparability of the different areas of the river in terms of expected biocoenoses, in order to verify applicability and accuracy of several

biological assessment methods and to quantify the impact of water abstraction.

15. Uncertainty of ecological quality assessment systems in rivers: which components do you need to consider (I3d1)?

WFD requires uncertainty of classification to be defined for ecological status classification. The overall uncertainty and potential error in estimating the "real" ecological status of a water body are determined by the combined effect of numerous factors, including:
i) the spatial variability of the biological community within the water body;
ii) the temporal variability of the community;
iii) the intrinsic characteristics of the considered sampling method;
iv) the characteristics of the considered sorting, transport and preservation methods;
v) the available methods and expertise for identification of sampled organisms;
vi) an inaccurate definition of reference values, caused by limited availability of reference sites and / or uncertainty in the definition (predictive modeling) of biota - environment relations in such sites;
vii) in general, the characteristics of the considered classification method (e.g. the choice of metrics and indices, their conversion into EQR, class boundaries etc.).

By using appropriate coefficients (some may be found in literature), defining uncertainty in the reference conditions and the variability associated with the sampling and sample processing, it is possible to quantify the probability of assignment of a given sample – the water body - to a certain quality class, in terms of method precision, for the considered aspects. This does not guarantee that the value obtained is also accurate (and, for example, there will be no systematic errors) and close to an hypothetical "true value " (although, in the case of biological elements, the concept is questionable). .
In general terms, metrics characterized by high precision, i.e. showing limited variability between replicate samples, may not necessarily represent a reliable indicator of the real quality and ecological status of a water body. Results obtained in INHABIT project show, however, how the effects of the precision of the method related to the aspects of identification, sorting and sampling are only a small part of the overall "uncertainty", as the largest share of the variability is related to habitat aspects, and in particular to lentic-lotic character (LRD) (see the next point). In order to increase what is the “accuracy” of the classification, beyond a higher or lower precision - not very relevant to the final judgment, it is appropriate to assess - especially in the Mediterranean area - some habitat aspects.

16. How to improve overall accuracy of assessment systems for rivers (benthic macroinvertebrates) (I3d1)

INHABIT has demonstrated how a significant portion of the variability observed in reference sites, or in any not significantly altered river stretch, is associated with habitat factors, that are detectable and measurable. It was possible to confirm that the lentic - lotic character (LRD) plays a key role in structuring aquatic invertebrates communities. INHABIT has defined a general model that relates the variability of STAR_ICMi its component metrics (used for the assessment of the ecological status of rivers based on invertebrates) with the lentic - lotic character. Considering river stretches not affected by water pollution nor by significant hydromorphological alterations and land use, the relationship between STAR_ICMi and LRD - that follows a bell-shaped curve - is significant, with the maximum values of STAR_ICMi obtained at intermediate values of LRD (i.e. not too much lotic nor too lentic). In general, the derived models indicate that the

values of the biological metrics are negatively influenced by very lotic or lentic LRD values, and get, instead, optimal values for intermediate values of the LRD. The overall accuracy of classification methods can then be poor when lentic – lotic conditions are far from neutrality. When values greater than LRD 50-60 are recorded, river stretches that do not involve significant anthropogenic disturbance may be misclassified as in good, or even moderate, condition. The same underestimation of ecological status could be achieved when LRD values score <-20 . In very lentic or lotic conditions, when the absence of significant water abstraction is confirmed, it is necessary to provide "refinements" to the expected values, in terms of reference conditions for the STAR_ICMi and its component metrics. Starting from the river type (e.g. for the use of appropriate class limits and the "optimal" value of the metric), it is useful to operate a site – specific refinement, based on the lentic - lotic character observed in the river stretch at the moment of sampling. This can be easily done when the quantification of the lentic - lotic character of the river stretch at the time (or similar periods) of the biological sampling is available. The needed information can be easily obtained by applying the CARAVAGGIO protocol, involving about 3 hours work in total, including field survey, data input and calculation of descriptors. The collected data will allow the estimation of a correction factor for official reference conditions in terms of accuracy, therefore in line with WFD requirements. The application of this correction factor is, in our opinion, essential in the Mediterranean area, in order to limit the underestimation of the ecological status in periods characterized by water scarcity. About this, models defined during INHABIT project, immediately available for Sardinia, can be easily adapted to other contexts, where a sufficient data base is available (i.e. CARAVAGGIO survey in reference sites, in different character lentic - lotic). In other words, values of the biological metrics used for classification should be accompanied by a quantification of the lentic - lotic character, related to period of the sampling. With reference to the observed LRD value, it will be possible to verify if hydraulic conditions and local habitats are optimal or not and, therefore, whether or not a correction is necessary - in terms of the best estimate - for reference values actually obtained in those conditions.

INHABIT project highlighted that the effects of a lack of accuracy on the classification, i.e. if site - specific correction is NOT used, there may be some ≈ 30 % of water bodies in Mediterranean area, whose ecological status will be underestimated. The approach here summarized allows, in many cases, to identify and reduce the occurrence of " false positives", for example, in the relatively frequent cases when the risk analysis does not reveal significant pressures, while the biological judgment indicates obvious deviations from the expected conditions.

17. Which solutions to obtain a good river habitat quality? (I3d2)

INHABIT has provided guidelines on what aspects have to be considered for habitat improvement, in terms of selection of potential reference sites and features to be preserved when river management actions are planned. In general, it was considered that any measures related to banks are more applicable than interventions affecting the territory beyond the banktop. On this basis, and considering the actual feasibility of the measure, the best option to determine a significant improvement in the diversification of habitats (e.g. as quantified by HQA descriptor) involves the removal of non-natural features related to land use coupled with the insertion of the typical features of reference conditions. Among the possible suggested actions, reduction of bank and channel resectioning, if accompanied by

recovery of naturalness (which could occur as a passive restoration), is a measure that would have a significant effect in reducing morphological alteration (reduction HMS) and increasing habitat diversity (increased values of HQA), effective to improve the ecological status, as detected by macrobenthic biotic communities.

18. Why is it important to quantify nutrients retention in river environments?

The term "nutrient retention" refers to all the processes by which nutrients are removed from the water column, but also stored and transformed. It is an important functional property of the aquatic ecosystem influencing the ecological status of a river and it can be used as an indicator of stream ecological condition. In Europe, the achievement of good ecological status, as indicated by the WFD before 2015, requires the rapid approval of effective and verifiable measures to reduce nutrient loading to surface waters and groundwater. Many studies demonstrated that suitable conditions for nutrient recycling naturally occur in non-altered basins. As a result of various processes, both biotic (e.g. denitrification, biological uptake) and physical (e.g. adsorption on sediments), the nutrients are nearly completely consumed in natural rivers.

The assessment of nutrient retention processes, the identification of the functional units of the river ecosystem where the processes are most active and the identification of environmental factors limiting the processes are crucial in the development of management strategies for the protection of aquatic ecosystems.

The existing methods to measure nutrient retention require the application of rather laborious (experimental addition of nutrients) and/or very expensive (the use of stable isotopes) experimental protocols, relatively unfeasible as routine procedures and not adoptable by the management and monitoring agencies. For these reasons, among INHABIT results, the observed relationship between storage zones and the ratio of channel width vs water depth acquires considerable importance. These morphological features are easily measurable and detected by the Caravaggio method.

That ratio, for some river types, may be considered a proxy for the nutrient retention, i.e. provides indications, although general, to the potential of river stretches in retaining nutrients.

19. What actions to increase nutrient retention efficiency in river catchments ?

INHABIT project showed very clearly the importance of transient storage zones, that are specific river habitats defined by current velocity depending features, although they are actually more complex systems characterized by multiple attributes, both physical and biological. In other words, it is clear that many characteristics defining river habitats may represent the crucial factors that can control the extent of transient storage. Habitat features seem to deeply influence, not only the biological community, but also the nutrient dynamics, particularly ammonia and orthophosphate. River stretches with high diversity and richness of habitats are favorite because the chance to find the specific habitats influencing the storage zones increases. In altered rivers with a low habitat quality, the nutrient retention efficiency may be improved by a river channel management leading to i) higher topographic complexity, ii) higher surface/volume ratio (between water column and sediments) and iii) higher hydrological retention in order to allow a greater contact between water and benthic organisms. Excluding the hyporheic, which represents a very complex system, even the mere presence

of surface structures within the channel may contribute to transient storage. For example, debris dams, woody debris, as well as leaf litter, contribute to locally increase water residence time in the river bed, favoring not only hydrological retention, but also the contact with the biological communities, and then nutrients assimilation and / or processing.

A very important result of INHABIT for management purposes is the observed relation between the retention efficiency of NH_4 and the ratio of channel width vs water depth. The hypothesis that the dimensional features of the river reach are crucial in nutrient dynamics is supported by many studies; the role played by headwaters and, generally, by low order rivers, in mitigating loads of N and P is widely recognized. In these water bodies, low water depth and high surface/volume ratios enhance the influence of biogeochemical processes to the water quality. Compared to larger rivers that are fed by upstream networks and affected by cumulative upstream stressors, the small drainage areas of headwater streams give these systems high levels of hydrologic independence and ecological autonomy.

In relation to the river management and restoration on a large scale, the headwaters and low order streams – often not included in the WFD and thus not monitored – represent fundamental functional units that have to be protected to preserve a number of ecosystem services provided by the hydrographic basins as whole. Because of the close terrestrial–aquatic linkage, these water bodies may easily receive nutrients and toxic compounds and so they tend to be very sensitive to natural and anthropogenic disturbance of surrounding lands. For these reasons too, it is crucial to include their protection and maintenance within the River Basin Management Plans.

20. What are the main environmental factors influencing biological metrics used for rivers ecological classification (D1d5)?

The first important consideration that can be obtained from the analysis carried out in the Mediterranean area, is that it is difficult to separate the individual effects of the various factors that contribute to the definition of environmental quality gradient, in terms of impact on aquatic organisms. Regarding biological metrics, one of the most important factors in determining the variability of the metrics can be associated to the gradient of anthropogenic alteration, although it cannot be separated in the individual factors determining the overall anthropogenic alteration. As already highlighted, the lentic – lotic character (LRD) also clearly affects biological metrics and, in this case, its effect is easily discernible from that of other environmental descriptors expressing more specifically anthropogenic alteration (water quality, morphology, land use, etc.). By comparing the response of different biological metrics (i.e. over 50 selected) to the considered disturbance factors, it was possible to identify two main groups of metrics. First group is strongly related to alteration gradient, the second is mostly related to lentic - lotic (LRD) gradient. The group of metrics related to the quality gradient confirms literature information and includes (among others) the following metrics: ASPT, N_EPT, EPTD , GOLD (all included into STAR_ICMi index, formally used for quality assessment). Other metrics are: Sel OLICHI_SA , DipAb , sel_TRI_GN , and LEPab (details on individual metrics can be found in INHABIT Deliverable).

Using more detailed regression approaches, for pool and riffle mesohabitats separately, some metrics responding to specific impacts have been identified, such as: habitat alterations 1 - GOLD (pool), log(SelEPTD) (pool) , DIPB_Siph_G

(pool) and MTS (riffle); water pollution Sel_OLICHI_SA (pool) and MTS (pool).
Among the biological metrics potentially useful to detect problems related to the water level, number of Odonata, Coleoptera and Heteroptera (nOCH, positively correlated to LRD), LIFE index, ratio LIFE Baetis / BAETIDAE (Baetis_BAE, negatively correlated with LRD) have been identified with particular reference to the pool mesohabitat. Pool mesohabitats seem more adequate in separating alteration gradient from LRD gradient. If in presence of water abstraction it is also possible the evaluation of its effects by means of LRD.

Is it true that, at least in mountain areas, biological methods are not able to detect morphological changes and flow reduction due to water abstraction? No. It is true that the indices commonly used, interpreted in the ordinary way, are not able to do so. However, the use of dedicated metrics (for morphological alteration) coupled with habitat information (for the impact of water abstraction) can provide an efficient evaluation of possible adverse effects on biotic communities.

21. Notes on monitoring and classification using benthic macroinvertebrates in temporary rivers

The intrinsic nature of temporary rivers, that includes extreme seasonal and interannual variability, put serious difficulties in planning monitoring activities. In particular, there are considerable difficulties in defining appropriate sampling periods. In this regard, it is possible to provide some general guidelines, that, if implemented, will reduce variability associated to sampling of biological elements in non-optimal sampling periods (see also guidelines to be published prepared in collaboration with ISPRA, which include some of the points listed below).

1. A water body of a temporary type should be sampled during the periods of eurheic Aquatic State (AS) (Gallart et al. , 2012). The flow rate should be be high enough to allow the presence of all aquatic habitats normally found in the river stretch, including the presence of abundant riffles, and to enable optimal hydraulic connectivity between different habitats. As a general rule, alternating riffle and pool areas should be evident, with significant differences in the conditions of microhabitats between the two areas.
2. If the considered water body is subject to upstream water abstraction, it is necessary to refer to other bodies of the same type in order to assess whether conditions are suitable for the sampling. Such 'comparing' water bodies should be possibly located in the same river basin and present similar general characteristics, but lacking significant water abstraction.
3. For the evaluation of these conditions - and , in general, to define the most appropriate sampling season - it is suggested to take pictured (at least 3) of the sampling sites at each visit. Photos will be able to support the interpretation of aquatic state (depending on water conditions). For example, photographs can be taken during water sampling for chemical and physical analysis, often performed monthly.
4 . The temporary rivers should not be sampled when - in its natural hydrological conditions (see point 2) – isolated, i.e. disconnected, pools are present, or when pools are dominant in the water body together with a low frequency of riffle areas (e.g. <10 %).
5. Water bodies subject to significant abstraction can be regularly sampled, if expected eurheic conditions have been verified (points 1 and 2), even if the conditions observed in the water body in question deviate from the eurheic aquatic state.
6. In general, following periods of drought, an adequate recolonization should be allowed. In

order to do so, sampling activities should be planned at least 2 months after the reappearance of the water in the river bed; in areas adjacent to water bodies having not undergone a dry period and that are therefore able to support rapid recolonization , this period - after verification - may be reduced up to a minimum of 4 weeks .

If the above recommendations cannot be followed, it will be essential to apply - where appropriate - the accuracy correction based on LRD values, in the estimation of reference conditions (see § 14 and 16): in water bodies showing no water abstraction (full application of the model) and in water bodies with moderate abstraction (compensation). If this "best estimate" is not applied, a high probability to derive a seriously inaccurate classification of ecological status will be obtained, underestimating the actual quality of the water body.

22. Do we have innovative elements to evaluate the effects of water abstraction on river biocoenoses (D1d5, I3d2)?

As a premise, it is important to remember that one of the guiding principles of the WFD to operate the ecological status classification on a biological basis (Annex V 1.2.1) is to quantify the deviation from "undisturbed" conditions expected for the type.
Although for moderate status the absence of major taxonomic groups is mentioned, together with the possible occurrence of different taxa, WFD refers in general to "composition", "abundance", "ratio" and "diversity", for which the degree of change from the "type-specific level" is observed. The guiding principle is the distance, that does not necessarily involve a "decrease" in biological metrics, abundance, ratio or diversity. At least some important aspects should be noted:
1) not always an increase in the number of taxa results in an increase in the overall biodiversity; sometimes it only corresponds to a greater overall uniformity;
2) the classification of the ecological status and the extent of the deviation from expected conditions are defined through selected-on-purpose biological metrics, meaning that metrics are conventional tools; as known, some of them show increasing values as quality improves, some others show decreasing values. It is how the different metrics are combined that can provide an overall "judgment".

In some cases, it is obvious, and quite normal, to expect some metrics (i.e. biological responses) to vary in response to increasing disturbance, and vice versa. It is how we will read information given by biological data that will allow us to understand its meaning.

Annex V, 1.2.1 – Rivers, Benthic invertebrate fauna

High status:
The taxonomic composition and abundance correspond totally or nearly totally to undisturbed conditions. The ratio of disturbance sensitive taxa to insensitive taxa shows no signs of alteration from undisturbed levels. The level of diversity of invertebrate taxa shows no sign of alteration from undisturbed levels.

Good status:
There are slight changes in the composition and abundance of invertebrate taxa from the type-specific communities. The ratio of disturbance-sensitive taxa to insensitive taxa shows slight alteration from type-specific levels. The level of diversity of invertebrate taxa shows slight signs of alteration from type specific levels

Moderate status:

The composition and abundance of invertebrate taxa differ moderately from the type-specific communities. Major taxonomic groups of the type-specific community are absent. The ratio of disturbance-sensitive taxa to insensitive taxa, and the level of diversity, are substantially lower than the type-specific level and significantly lower than for good status.

Some additional aspects must be considered, related to how to select and combine metrics, in order to associate their biological response to environmental changes.

1) Not necessarily metrics will perform equally well in all the cases. In particular, metrics and classification systems selected and calibrated to derive a classification of overall quality, are unlikely to provide an adequate interpretation of extreme detail aspects. The same "direction" of the metric response can be opposite in the presence of different alteration factors.

2) For particular types of alteration, such as those associated with decreasing flow rate, - dedicated metrics should be selected, or the used metrics should be adapted.

3) In the case of disturbances that are also observed when the same situation occurs for natural reasons (e.g. reduction of flow rate) the selection of indicators should be performed towards those as possible “indifferent” to that natural factor.

In the case of water abstraction, it is appropriate to refer again to the sensitivity of the macrobenthic communities to lentic - lotic character. When abstraction is known for the considered water body, the adaptation of aquatic biocoenosis to the resulting lentic - lotic character can, in fact, be used to interpret the change from the expected conditions in the absence of abstraction. The general relation describing the benthic communities response to the lentic - lotic character can in fact be used not only to estimate the best reference conditions expected when abstraction is present, but also to estimate the changes in the community as a result related to abstraction. When water abstraction is present, the deviation from the optimal values of the biological metrics derived from the adaptation of communities to lentic - lotic character arising e.g. by a decrease of flow, can be successfully used to quantify the effect of such abstraction. Generally, in the Mediterranean area, the lentic lotic character - as a result of water abstraction - changes from neutral / slightly positive to strongly positive, with obvious detrimental effects on communities. These effects cause the classification metrics value to decrease; so, no problem: STAR_ICMi can already adequately detect the response of benthic organisms to discharge reduction.

However, INHABIT has also shown that, for example, in the Alps, the reduction in flow could result in an increase of LRD values (e.g. from negative to neutral), resulting in an apparent "improvement" of the ecological quality, in fact corresponding to a strong alteration of biotic communities, clearly detectable in the presence of information on the lentic - lotic character. In the latter case, many biological metrics show an increase moving from high stress environment (very negative values, i.e. lotic and strong stress on aquatic communities) for many aquatic organisms to a more favorable environment, with a higher number of taxa (including not only taxa colonizing high turbulence areas). The biological response is therefore present, and it is evident. Simply such response moves in a direction that we, conventionally, are used to associate with an "improvement" of the state of the environment. In this specific case, however, retaining a "conventional" vision of the problem we are overlooking WFD conceptual framework, requiring the evaluation of a “change”, not necessarily an increase or a decrease.

This view of the problem, rather simple to manage from a technical standpoint , allows the identification and reduction of "false

negative" results, for example when river stretches affected by water abstraction fall into good or high biological conditions, according to the results provided by the mere application of the generic classification system. In this case, given the peculiarities of the impact - whose effects mimic natural situations - it is always necessary to carry out further investigations. They can also be considered for investigative monitoring, in order to highlight the effects of a cause only hypothetically known that resulted in a deviation from the conditions actually expected.
The answer to the question given in the title is therefore: “Yes, we do have innovative elements to evaluate the effects of water abstraction” .

23. Notes on “minimum acceptable flows” and e-flows.

In the previous point it has been shown how does it exists, and it is sometimes relatively simple, the possibility to highlight and quantify the effects of water abstraction on biotic communities, combining biological and habitat information. This is just one side of the coin. The other one, which is its logical consequence, is that the same type of information can be used to set quality objectives in terms, at least, of " minimum flow". When it is possible to quantify the variation of the biological metrics in response to flow variations, as seen through level changes mediated by the conformation of the riverbed, i.e. with the lentic - lotic character, it is also possible to estimate the effects of flow reduction due to water abstraction. We have anticipated (§ 22) that, at least in the Mediterranean area, reduction in flow is associated with a decrease in many biological metrics used for the classification of ecological status. In this case, the quantification of the effects is very simple and direct. Accordingly, it will be easy to define the lentic - lotic character corresponding to a given quality objective (e.g. good ecological status), for example, in the most critical season for the biocoenosis. We are talking about e -flows and, more precisely, about the aspects related to the modulation of water releases to get the (at least) good environmental status. The definition of ecologically acceptable flows to ensure ecosystem structure and functions as a whole is certainly not an easy task and should go beyond the "simple" achievement of good ecological status. Nevertheless, the WFD requires us to pursue at least this objective, in the hope of dealing with this issue more completely in a second time. Moreover, far from holistic approaches to the topic, often we are content to define the so-called minimum acceptable flows viable (DMV), which should ensure a basic level in supporting aquatic biocoenoses. However, the experimentations supporting the validity of flow releases, are often biased by the selection of ineffective indicators, compromising the results of the whole activity. This is very common in the Alps, as well as already highlighted on the "direction" of response of many biological metrics. We hope that in future studies of evaluation or validation of the shares of the DMV will take into account such aspects, in order to highlight what is in some cases obvious. For example, the effects of a permanent reduction in flow force the river stretch to seek a new equilibrium, where quality and quantity of riverine habitats vary significantly compared to the initial state. In these cases, it is relatively simple to highlight the effects on habitat (through appropriate indicators) and on the possible biological communities (with dedicated in depth analyses). In terms of lentic - lotic character, the changes may modify conditions of river stretch so that its type will differ from that the water body was originally allocated to, resulting in a response of biotic communities (and the creation of significant, apparent false negatives) .

In summary, the relationships defined by INHABIT between biological metrics and lentic-

lotic character can be used in a relatively simple way to define quality objectives related to the so-called “minimum acceptable flows”, in terms of achievement of good ecological status. At least in the Alps, in the presence and in the absence of significant water abstraction, the ecological status should, however, be assessed by quantifying the deviation from the expected conditions (with increasing and / or decreasing metric values), otherwise “false negatives” may occur.

24. Are the sampling protocols presently in use suitable for both lakes and reservoirs in the Alpine and Mediterranean ecoregions? (I1d1 - I1d5)

The WFD requires an evaluation of the ecological quality of the waterbodies based on BQEs. However the biotic components used for waterbody quality assessment show a marked natural variability, including seasonality, which strongly affect their biomass and composition. The choice of sampling frequency and sampling temporal distribution during the year may then represent a critical aspect in the waterbody quality assessment.

Three out of four BQEs (phytoplankton, macrophytes and macroinvertebrates) used for classification show marked seasonal and/or spatial variability. Sampling protocol presently in use consider this aspect, but they require a relatively large number of samples, increasing sampling and analysis costs.

The analyses carried out during the INHABIT project show that:

- metrics used to define phytoplankton indices reflect the trophic gradient among lakes and the variability to the seasonal fluctuations is relatively small. A reduction in the number of samples from 6 to 4 per year can be accepted, but their distribution among the seasons should be respected, avoiding the concentration of the sampling in one season;
- for macrophytes, it is not possible to significantly reduce the number of transects without a marked decrease in classification reliability;
- as expected, macrophytes were virtually absent in all reservoirs, because of the large fluctuations in water level;
- for macroinvetebrates, the littoral area host the largest diversity in habitat features, and an improvement in classification reliability would require a larger sampling effort in this area;
- in reservoirs, macroinvertebrate sampling in the littoral area is not useful, as this area is very sensitive to water level fluctuation. Quality assessment is possible using sublittoral and deepwater samples.

25. Concerning lakes and reservoirs, which are the main gaps in the Italian River Basin Management Plans and how can they be filled? (Pd2 – I3d2)

The River Basin Management Plans (RBMPs) developed to fulfill WFD requirements were analyzed to evaluate approaches, methods and programs of measures intended to protect and recover waterbody quality. In particular we examined the River Po and Sardinia Region RBMPs, where INHABIT lakes and reservoirs are included.

We verified the ecological quality of the INHABIT lakes and reservoir, their management target and any derogation to the achievement of the good ecological status and we concluded that:

- in the Piedmont Region, improvement measures were planned for all study

lakes, mainly based on rules and prescriptions. Shared actions were also planned for water and landscape management. Apart from the Morasco reservoir, specific studies are planned for all lakes in order to define strategies to improve basin management and lake quality.

- however, the RBMPs do not explicitly quote the hydromorphological quality of the lakes and its relationship with the ecological quality of the waterbodies;
- in the Region Sardinia RBMP, specific measures are planned for improving the ecological quality of the reservoirs, including their hydrological management and problems related to the accumulation of sediments in the reservoirs. The improvement of the ecological status and of water quality is also considered, also because most reservoirs are used for drinking water storage;
- also in this case, it is not clear from the RBMP if the planned measures consider the relationship between the biological elements and the alterations of the local habitat features and no measures specifically aimed to the improvement of lake habitat quality are proposed.

All RBMPs submitted by Member States to the European Commission, were evaluated following the Article 18 of the WFD and a synthetic report was prepared for all of them, including recommendations and proposing ameliorations. For what concerns these specific RBMPs, the EC evaluation underlined the need of:

- filling the monitoring gaps for both BQEs and priority hazardous substances in order to improve basin management plans;
- better identifying monitored substances, monitoring sites and possible levels above the limits, and explaining how the levels of hazardous substances are used in defining the ecological status of the waterbodies;
- ameliorating the procedures for the identification of heavily modified waterbodies, clearly defining the negative effects of the modifications on waterbody quality and on the larger environment and the possible management options;
- including measures clearly aimed to the improvement of the ecological quality of each waterbody, based on the results of monitoring;
- better and more clearly justifying any derogation to the target of good ecological status, defining specific targets for all waterbodies;
- including agriculture among the human pressures affecting water quality and develop further measures together with the farmers and within the rural development plans;
- including the foreseen (or further) quality targets in all working documents dealing with water resource;
- evaluating costs recovery including the individual cost of all water services, such as abstraction, treatment, storage and transport of surface water, collection and treatment of urban, industrial and rural wastewater; and of quality recovery; all the costs should be detailed at the user level.

In conclusion, to fill the gaps in the RBMPs concerning lakes and reservoirs, it is necessary to complete the monitoring activities required by the national law for all BQEs and to analyze the chemical and hydromorphological quality of the waterbodies. It is also necessary to better define the impacts of chemical and hydromorphological pressures on pelagic and littoral biocoenoses.

The INHABIT project has shown that the Lake Habitat Survey can be successfully applied to Italian lakes and reservoirs, on both the Alpine and Mediterranean ecoregions, and some

relationships between habitat and hydrological alterations and the ecological quality of lakes and reservoirs were identified. Its results can thus be used as a basis to improve the knowledge of other waterbodies and the overall quality of the RBMPs.

26. How to manage river morphological information over different spatial scales (I3d2)

In general, in Italy, with the partial exception of the Po River Management Plan, it has become apparent as one of the major flaws in the first draft of the River Basin Management Plans is the lack of knowledge related to morphological conditions of streams and, in particular, to alteration processes and fluvial dynamics (Pd1). As noted in the European Commission assessment, systematic measures for river morphology regarding both river basins and water bodies are not generally provided, if excluded generic guidelines. As well, no measures related to protection and enhancement of habitats are covered.

Through the DM (Ministerial Decree) 260/2010, technical tools to record, catalog and evaluate morphological information in rivers have been made officially available in Italy, in a consistent and WFD-standardized way. Such tools, that are survey methods, operate distinctly on two spatial and temporal scales, i.e. i) waterbody / watershed (MQI method) and ii) river stretch / habitat (CARAVAGGIO method). The two methods can quantify morphological and habitat conditions supporting the assessment of ecological status, allowing the collection of information needed for the definition of measures for improvement and restoration of ecological quality.

Since the two methods work with different, although parallel, purposes it may be useful to find a way to successfully transfer the processed information from one system to another, in order to both optimize resources and, above all, to create a pathway for the integrated analysis procedures that allow managers to implement measures for the benefit of the river system as a whole, single elements of riverine ecosystems and related biological communities.

INHABIT has addressed the issue by comparing studies carried out at different scales, - investigated in the two region investigated by the project, Piedmont and Sardinia - with habitat characterization, that has been one of the main themes of the project. The study performed by Cantabria University is based on predictive spatial models on real data. The assessment of longitudinal continuity and sediment transport in relation to man-made transverse structures, together with the analysis of the condition of riparian buffer strips in relation to bank stability, allowed the definition of key and critical areas, also in relation to habitats, along examined river stretches and basins. As performed in Sardinia with modeling approach, the application of MQI (Morphological Quality Index) on a set of water bodies in the Piedmont region has highlighted river stretches subject to morphological changes in an otherwise good or high natural condition.

The performed comparison, i.e. between the two procedures based on large-scale on one side and the CARAVAGGIO on the other, to understand the potential of a down-scaling/up-scaling process, has highlighted the comparability of the two scales, although outlying their limitations, mainly due to the complexity of a territorial mosaic influenced by anthropogenic impacts that may prevent both the methods to get all the aspects of river environment, in a comprehensive manner. For these reasons, the need for an integration of the two scales has become apparent. It is therefore important to use the same evaluation method, over different spatial scales, to define and properly consider collected information in order to translate them into effective action

and reach their goals. The parallelism between the methodologies set up in INHABIT wants to be a starting point to suggest possible pathways for implementing water bodies critical morphological and habitat issues into river management plans.

27.HMWB, habitat and measures

In the context of the WFD, heavily modified water bodies (HMWB) are a complex, still open issue, primarily - but not only - from the management point of view. Also due to the limited knowledge of the effects of hydromorphological alterations on biotic communities, the definition of quality objectives for HMWB is still matter of discussion. INHABIT project has addressed this issue, in particular with regard to the identification of the physical and habitat variables that, in such contexts, could be strongly correlated with macrobenthic biotic communities. Similarly, we wanted to test the response of biological metrics to such alterations. During the INHABIT project, an external activity has considered the study and the characterization of HMWBs in a densely inhabited plain watershed, using an analytical approach very similar to the one developed in INHABIT. In this context it has been observed how, even in situations so impaired from the physical point of view, biotic communities respond primarily to habitat modifications. Data analyses showed that the differences in quality between the different groups of water bodies (reference -> natural -> heavily modified -> artificial) do correspond to differences in the macrobenthic communities, confirming the validity of the separation of HMWB from the natural water bodies. However, a partial overlap between natural and heavily modified water bodies is maintained, due to the fact that even among the non-HMWB alterations can be very relevant. In particular, the variables that predominantly exert an effect on the biological communities (macrobenthos) were: the quality and the development of a riparian buffer strip, the presence of non-artificialized banks, absence of embankment leaning on the channel and the type of land use close to the channel. The spatial scale resulted as more relevant for macroinvertebrate community has been the 500m stretch and, more in general, the land strip close to the channel has resulted as important. This confirms the importance of aquatic and riparian habitats for macroinvertebrates communities, also in streams whose ecosystems have been permanently and extensively compromised from the physical point of view. It is also confirmed that spatial scale of habitat characterization (INHABIT approach) can be, even in the context of HMWB, the spatial scale most appropriate to identify, apply and test restoration measures designed to improve ecological quality through specific interventions on identified components of habitat (e.g. condition of the riparian zone and banks).

Obtained results also showed that HMWB characterization based on ‘specified uses’ - that in plains is mostly defined according to the local context - and on the dominant hydromorphological alterations, may also provide an important base of information where differentiated management measures can be based on. If the alterations that identify the heavily modified character are non-removable elements, then land use and local context define the room for intervention on the same disturbing factors or, better yet, on the elements that are not essential for maintaining the use.

28. How can we use biological metrics information to evaluate restoration measures efficacy in rivers (D1d5)?

To prevent the deterioration of ecological status in surface water bodies, protecting and improving them, has always been considered a central theme in European environmental policy. The evaluation of pollution and the resulting effects on ecosystems are not new in the European scenario. Since the 70ies we have dealt in various ways with the development of assessment systems able to detect the effects of anthropogenic disturbance on aquatic ecosystems and specific policy proposals have been issued since then to improve the status of water bodies.
In this context, the issue of Directive 2000/60/EC - WFD has set new approaches for the assessment of ecological status, also establishing the centrality of the Biological Quality Elements for this purpose. The WFD has also recognized the importance of habitat and hydromorphological elements in the interpretation of the processes structuring biological communities.

To meet WFD requirements it is therefore first necessary to obtain elements allowing the assessment of ecological status, in order to set up management plans and dedicated measures.
In Italy, the transposition of the WFD has determined, the adoption of STAR_ICMi for the assessment of macrobenthic component in rivers. STAR_ICMi is a multimetric index, developed in the European context and suitable to assess general degradation. Being STAR_ICMi formed by 6 different metrics, the assessment of individual metrics can provide an indication of the different pressures acting on a given water body. Effects of specific pressures and specific measures, can then be quantified in relation to the individual metrics forming STAR_ICMi. Each of them may have different sensitivity to various forms of impact, as put in evidence by INHABIT project. In addition, INHABIT activities led to the selection of additional metrics, specifically dedicated to highlight specific impacts or environmental factors, through these additional metrics it will be possible to assess the effectiveness of restoration measures. They are suited, as well, to be used in surveillance and investigation monitoring and, in any case, to provide better understandings within the general framework of operational monitoring.
In Sardinia, and more in general in Mediterranean area, adequate metrics for the evaluation of overall alterations are:
STAR_ICMi, ASPT, NEPT, Shannon diversity, LEPab (Leptophlebidae Abundance), DIPab (Diptera abundance), SelTRI_GN (Abundance of Odontoceridae, Limnephilidae, Polycentropodidae); for the evaluation of habitat alterations: log(SelEPTD), DIPB (Abundance of Ceratopogonidae, Culicidae e Syrphidae), % shredders, MTS (in riffle), 1-GOLD and Ab. of *Dugesia* & *Lymnaea*; for the evaluation of water quality: SelOLIGHI_SA (Abundance of Naididae, Tubificidae and Chironomidae), MTS (in pool), TRIab (Abundance of Trichopera), SelTri_SA (Abundance Leptoceridae, Rhyacophilidae, Glossosomatidae), *Leuctra*&*Calopteryx*, SelEpheGN (Abundance of *Procloeon, Centroptilum, Ecdyonurus*); lastly for the evaluation of effect of water abstraction (lentic-lotic character), in addition to what previously mentioned: nOCH (Odonata, Coeloptera and Heteroptera), *Baetis*/BAETIDAE, SelEpheM (Abundance of *B. cfr. rhodani, Ecdyonurus, Habrophlebia*).

29. Practical tools developed and distributed within INHABIT

MacrOper.ICM - Software MacrOper.ICM allows for the classification of ecological quality

based on benthic macroinvertebrates in all Italian river types. It has been improved during INHABIT project, in partnership with DEB University of Tuscia. Classification provided is compliant with Water Framework Directive (WFD : EC 2000/60), DM 260/2010 ("Classification Decree"), DM 56/2009 ("Monitoring Decree") and DM 131/2008 ("Typization Decree") requirements, for the monitoring of Italian watercourses.
Software MacrOper.ICM, represents the calculation tool combined with MacrOper Classification System. It allows to:

- Easily and automatically calculate metrics requested for rivers classification, based on macrobenthic invertebrates.
- Classify water bodies of all Italian river types according to the WFD on the basis of benthic macroinvertebrates.
- Obtain quality classes directly comparable with those obtained in other European countries.
- Classify both individual samples and sites including different samples.
- Import (taxalist) and export (metric, quality classes and calculation options) information in a simple and intuitive way.
- Provide, if necessary , taxonomic adjustment of input taxalists, saving the new version of the data.

The software, is available for download at INHABIT website (www.life-inhabit.it), after login (different login procedures are considered for agencies, private etc.).

CARAVAGGIOsoft - All the information collected with the CARAVAGGIO method can be archived in the software CARAVAGGIOsoft. The software, developed on MS Access 2000, allows for the storing of all data recorded on the field form. It also allows the calculation of some of the descriptors available for the CARAVAGGIO method: LRD, HMS, HQA and LUI and the export of raw and processed data. During INHABIT, the software - in collaboration with ITC-CNR, who developed it from the technical point of view - has been improved and updated. CARAVAGGIOsoft is distributed through INHABIT website (www.life-inhabit.it).

Guide to the survey and description of river habitats - Application Manual of the CARAVAGGIO method - Within INHABIT, the Manual of CARAVAGGIO method, was completed and published as first volume of the CNR – IRSA Monographs series, under the patronage of the Ministry of Environment and Protection of Land and Sea. The book describes the application in the field of the CARAVAGGIO method - Core Assessment of River hAbitat VAlue and hydromorpholoGIcal cOndition - dedicated to the characterization of river habitats.
The manual, presenting the method, is provided as support for those who have already attended, or is about to attend, a dedicated training course, that is considered indispensable for the correct application of the protocol. The manual describes in detail each part of the method, providing definitions and information for application; it contains useful details to understand and decode the field form in every aspect, section by section. The manual of CARAVAGGIO method is also distributed through the website of INHABIT (www.life-inhabit.it).

30. WFD and HABITAT Directive

INHABIT has highlighted how an harmonization between the WFD and the HABITAT Directive (HD 92/43/EEC) is needed. It may happen, e.g. in areas rich in endemic and / or rare species, such as Sardinia, that the management of water bodies only devoted to WFD quality objectives could neglect the protection of species at risk of extinction, thus determining the overall failure of biodiversity conservation strategies at regional level. In planning the

integration between the two Directives, it is crucial that not only the habitats and species included in the Annex to Directive HABITAT are considered, but also the endemic species at risk not included in those Annexes. For aquatic invertebrates, insects in particular, it must be noted that they deserve more attention in biodiversity conservation plans. On converse, the few aquatic species - and their associated habitats - directly included in various environmental regulations, represent only a small part of the elements worthy of protection. Therefore, despite the presence of the HD and the WFD, important gaps remain for species protection, largely related to the lack of knowledge of their autoecological preferences, especially in the Mediterranean area. Such lack of knowledge is also likely to be the reason for the limited presence of aquatic insects in the HD Annexes. Moreover, the achievement of the WFD objectives falls only apparently beyond the taxonomical, distributional and ecological issues of many biological groups - with the result that these aspects are neglected - although it is precisely the variability related to such issues that determines the difficulties in interpreting quality results. We are referring, in this case, to a so-called 'grey zone', related to those species whose protection would be necessary or desirable but that, in fact, are not included in any environmental legislation, due to the fragmentary or total unavailability of information. It will be necessary for the foreseeable future, to aim to fill some of these gaps, focusing on organisms considered to be of particular interest for biodiversity protection in Southern Europe .

As conclusion of INHABIT project activities, it seems appropriate to reassert how habitat conditions and local hydro-morphology play a crucial role in rivers and lakes ecosystems functioning, as well as in determining the structure of biological communities. Therefore, approaches and methods used for monitoring and classification of ecological status should take in great consideration such features, in order to identify and quantify in detail their influence on biota and on environmental processes.