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"The response of benthic macroinvertebrates to lentic-lotic conditions and hydro-morphological alteration in rivers: the role of habitat"

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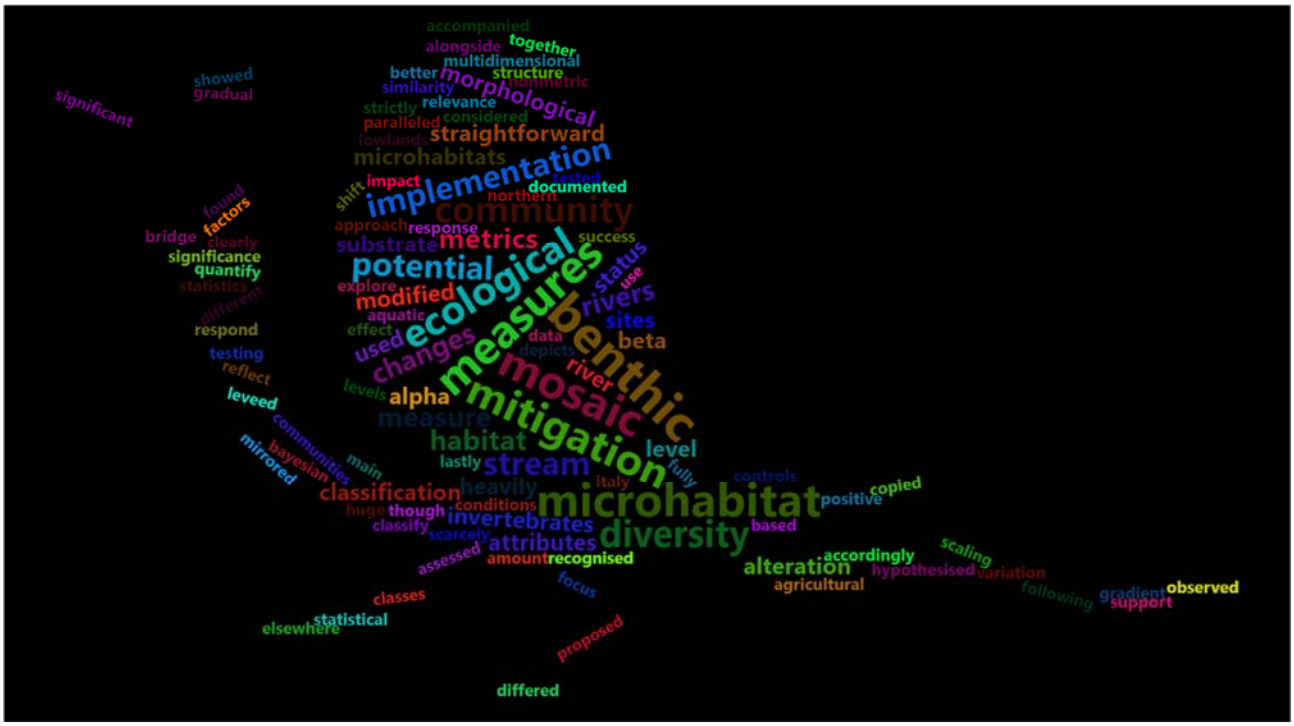
This work is dedicated to Romano Pagnotta and Rita Casula, who are pleasantly present in my thoughts, and to all those who believe an open mind can make dreams real.

‘The response of benthic macroinvertebrates to lentic-lotic conditions and hydro-morphological alteration in rivers: the role of habitat’.

“La risposta dei macroinvertebrati bentonici alle condizioni lentico-lotiche e alle alterazioni morfologiche nei fiumi: il ruolo dell’habitat**”**



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Preface

This PhD work was supported by the National Research Council (CNR) and was made possible by the availability of the Tuscia University, which agreed a flexible programme to let me continue with my institutional activities at the Water Research Institute (CNR-IRSA), when needed. For this, I am very grateful to both Institutions.

The research activity at the basis of this thesis was carried out mainly at CNR-IRSA (Brugherio and Bari locations). However, most of the data used derive from previous projects, whose reference can be found in the acknowledgement section of individual papers. A particular mention should anyway be given to two projects that provided most data for flow-related analyses, the LIFE+ INHABIT project (European Union) and the MICARI project (MIUR). In the same way, the activities on heavily modified rivers were partly funded by the Veneto Region and were made possible by the joint cooperation of the Environmental Protection Agency of Veneto (ARPAV).

Apart from the much-loved co-authors of papers/manuscripts presented in this dissertation - I will not mention here - I want to remember the great pleasure to work and exchange ideas with colleagues from two Research Institutes I visited for a while in the PhD period. I am grateful to Sonja Jähnig, Karan Kakouei, Jens Kiesel, Alain Maasri, Sami Domisch, Markus Venohr and other colleagues from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB - Berlin, Germany) for the long and fruitful discussions, especially on methodological prospects and joint exploration of flow-related subjects for further research. I am equally indebt to colleagues from the Institute of Marine Biological Resources and Inland Waters (IMBRIW - Athens, Greece) where I've been hosted for quite a long and fruitful period. I am particularly grateful to Nikolaos Skoulikidis, Christina Papadaki, Christos Theodoropoulos, Elias Dimitriou, Anastasia Lampou, Ioannis Karaouzas, Stamatis Zogaris, Sofia Laschou and many others, especially for the time devoted to facilitate my entry into their own research activities and for the detailed discussions we had afterwards on a series of flow-related issues relevant for our imperilled Mediterranean rivers.

All my love to Raffaella, who is with me, always.

Vimercate (Italy), January 2020.

Andrea Buffagni

GENERAL ABSTRACT

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The study of habitat features in rivers is crucial to understand the responses of organisms to natural variability and environmental alteration due to anthropic activities. In this dissertation, a range of hydromorphological characteristics that contribute to shaping aquatic and riparian habitats are related to benthic communities.

In more detail, the response of benthic organisms to the changes of flow-related habitat features was investigated, with emphasis on temporary, Mediterranean rivers and on low-flow conditions. In a first paper based on Sardinian river data, the response of benthic taxa to the lentic-lotic character of rivers was investigated i.e., the main flow-related habitat conditions observed at the time of sampling were under focus. For more than 60 taxa a significant response to such conditions was apparent and an optimal range of presence was described. Taxa and the main taxonomic groups showed a heterogeneous distribution along the lentic-lotic gradient, which determined characteristic responses of benthic metrics, investigated in the second paper. In this paper, macroinvertebrate metrics and indices used in South Europe to classify ecological status were related to the same lentic-lotic gradient. Emphasis was set on the need for site-specific adjustments to reference conditions, so that the expectd habitat situation at the time of sampling is taken into account. Finally, a third paper related to the Candelaro basin (Puglia) investigated the effects of expected climate changes on a range of indicators of hydrological alteration. Potential consequences on aquatic communities were discussed, with emphasis on the lentic-lotic river character and on the opportunity of refining reference conditions to account for a changing climate.

A second line of research, equally related to the role of habitat information in interpreting biological data, centred on the relationship between the benthic community and the degree of morphological alteration of rivers. A first paper investigated the response of macroinvertebrate metrics in use for the classification of ecological status in Italy and elsewhere to morphological modification of river channel and banks. The study based on data from five river types ranging north to south in Italy and the presence of artificial features was especially under focus. A second paper dealt with heavily modified rivers of northern Italy lowlands, where habitat alteration can be massive. Criteria and approaches to define maximum ecological potential – a concept similar to reference conditions – were proposed and discussed in relation to habitat features. Finally, in the same context, a third paper highlighted the importance of in-stream microhabitat mosaic to link macroinvertebrate response to morphological alteration and implementation of mitigation measures.

The results of the aforementioned papers support the conclusion that habitat analysis, modulated according to study aims, is crucial for the interpretation of associated biological data. Biological responses to anthropic pressures and/or natural variability connected to e.g. seasonal, climatic or typological factors, can be correctly understood only if habitat information is incorporated. As well, habitat analysis emerged as a key element to clarify and properly assess the classification of ecological status, as required by existing environmental legislation.

Hereafter, abstracts of the six papers are presented.

Paper 1: "**The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers**".

The importance of flow-related factors to benthic organisms, as well as the role of habitat conditions in shaping aquatic communities during low-flow periods, have been recognized. However, the responses of macroinvertebrates to the overall conditions of lentic and lotic habitats at the reach scale are poorly known.

We investigated aquatic invertebrates and habitat features in a range of temporary rivers in Sardinia. The investigation focused on the flow-related characteristics that contribute to defining the lentic-lotic condition of the river reaches. We related habitat features to benthic taxa distributions using multidimensional scaling. We then quantified the responses of taxa to the different lentic and lotic habitat conditions by applying hierarchical logistic regressions. Finally, we aligned taxon optima along the lentic-lotic gradient and compared the responses of different taxonomic groups.

Unbroken waves and not perceptible flow were related to benthic taxa variability, suggesting local hydraulics and turbulence have a major role in regulating communities. The overall lentic-lotic character of the river sites was also clearly related to the benthic taxa distribution. More than 80% of taxa were significantly related to the lentic-lotic gradient, and an asymmetrical response curve was the predominant model.

Benthic groups showed taxon optima clustered in different ranges of the lentic-lotic gradient. Odonata, Coleoptera, Hemiptera and Mollusca preferred clearly lentic conditions. Diptera mainly ranged on the lotic side of the gradient, while Trichoptera was relatively uniformly stretched across the gradient. Ephemeroptera taxa clustered in intermediate lentic-lotic conditions, with two species preferring extremely lentic habitats. In general, optima converged at intermediate and extremely lentic conditions, presumably due, respectively, to the coexistence of different lentic and lotic features and to the high diversification of environmental characteristics in extremely lentic situations.

The uneven distribution of optima of different taxonomic groups along the lentic-lotic gradient might concern the use of benthic metrics when, e.g., there is a focus on the water quality or ecological status or establishing reference conditions under variable climatic conditions.

Paper 2: "**The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification**".

Biological quality in rivers based on benthic macroinvertebrates is often assessed by comparison with the expected reference conditions, which represent relatively undisturbed situations. Commonly, reference conditions are set in agreement with river typologies to handle major ecological differences and to limit natural variability. Even if natural variations can be highly influential, site-specific tuning of reference conditions is rare in Mediterranean countries.

River flow and local hydraulics change continuously over time, determining changes in the occurrence of lentic and lotic habitat features. Thus, biological reference conditions might require a site-specific adjustment in function of the ratio of lentic to lotic habitat features expected at the time of sampling. This would help reducing systematic bias in ecological assessments, interpreting benthic invertebrates responses to pressures and diminishing the amount of unexplained biological variability.

The responses to the lentic-lotic character of river reaches were assessed for nineteen macroinvertebrate metrics and indices commonly used for the classification of ecological status in South European rivers, by piecewise spline regression analysis. The study sites, with a prevalent temporary character, were located in Sardinia, South Western Italy.

Most metrics were significantly related to the lentic-lotic habitat conditions, both in pool and riffle mesohabitats, and their response trends were parabolic or decreasing lotic to lentic. Taxonomic richness, score-based metrics, ovoviviparous taxa and multi-metric indices related well to the lentic-lotic conditions, while abundance metrics correlated less.

The potential impact on ecological status classification was tested for the method formally in use in Italy, which had a major role in comparing and inter-calibrating European assessment methods for the Water Framework Directive. After adjusting for bias due to the ratio of lentic to lotic habitat features, quality classification shifted towards better ecological status for ≈ 23% samples. This exemplified the likely impact of ignoring lentic-lotic information when defining reference conditions for assessing ecological status. The potential for considering the responses of invertebrate metrics to the lentic-lotic conditions in relation to climate change and e-flows setting was also briefly outlined.

Paper 3: "**Hydrology under climate change in a temporary river system: Potential impact on water balance and flow regime**".

The potential impacts of future climate scenarios on water balance and flow regime are presented and discussed for a temporary river system in southern Italy. Different climate projections for the future (2030–2059) and the recent conditions (1980–2009) were investigated. A hydrological model (Soil and Water Assessment Tool) was used to simulate water balance at the basin scale and streamflow in a number of river sections under various climate change scenarios, based on different combinations of global and regional models (global circulation models and regional climate models). The impact on water balance components was quantified at the basin and subbasin levels as deviation from the baseline (1980–2009), and the flow regime alteration under changing climate was estimated using a number of hydrological indicators.

An increase in mean temperature for all months between 0.5–2.4 °C and a reduction in precipitation (by 4–7%) was predicted for the future. As a consequence, a decline of blue water (7–18%) and total water yield (11–28%) was estimated. Although the river type classification remains unvaried, the flow regime distinctly moves towards drier conditions and the divergence from the current status increases in future scenarios, especially for those reaches classified as I-D (ie, intermittent-dry) and E (ephemeral). Hydrological indicators showed a decrease in both high flow and low flow magnitudes for various time durations, an extension of the dry season and an exacerbation of extreme low flow conditions. A reduction of snowfall in the mountainous part of the basin and an increase in potential evapotranspiration was also estimated (4–4.4%).

Finally, the paper analyses the implications of the climate change for river ecosystems and for River Basin Management Planning. The defined quantitative estimates of water balance alteration could support the identification of priorities that should be addressed in upcoming years to set water-saving actions.

Paper 4: “**Macroinvertebrate metrics responses to morphological alteration in Italian rivers**”.

The responses of river macroinvertebrates to hydromorphological alteration are often considered weak or unclear. It is therefore crucial to verify if and how existing invertebrate-based approaches can reveal the effects of hydromorphological modification.

We analysed the responses of benthic metrics to morphological impairment, with emphasis on the STAR_ICM index, legally required for macroinvertebrate-based ecological status assessment in Italy. A Principal Component Analysis (PCA) was run to condense information on morphological impairment.

The major gradient (Component 1) expressed a combination of bank and channel modifications opposed to tree-related features indicating the presence of comparatively unmodified habitats. Jointly, habitat descriptors including Habitat Modification Score (HMS) derived from the application of a habitat survey method were calculated. Spearman rank Correlations between biological metrics and morphological impairment indicators (PCA scores and HMS) were significant. A linear mixed-effects regression approach was applied to relate HMS and STAR_ICMi across a wide geographical context. HMS explained ≈ 60% of STAR_ICMi variability, in the absence of apparent water pollution.

Results demonstrated that morphological information resumed with habitat survey methods is meaningful for the biological community and that HMS can support the interpretation of ecological status across rivers types and in different environmental settings.

Paper 5: “**Defining Maximum Ecological Potential for heavily modified lowland streams of Northern Italy**”.

In relation to hydromorphological alteration the Water Framework Directive (WFD), a major piece of European legislation, has introduced the concept of Heavily Modified Water Bodies (HMWB). In water bodies falling in this category, hydromorphological modifications are permanent, significantly alter the character of the river and cannot be removed without compromising the use of the water body. In HMWBs a dedicated approach to the evaluation of their status is set, and their Ecological Potential must be assessed. Crucial to the process is the definition of Maximum Ecological Potential (MEP) as the reference conditions for HMWB.

In the present paper we aim to define MEP conditions for Italian heavily modified lowland rivers, affected by strong bank protection (i.e. levees or bank reinforcement) in reason of flood protection and land drainage uses. The approach applied to identify MEP conditions follows the one considered for natural (not heavily modified) rivers in Italy and large part of Europe and bases on the identification of ‘reference sites’ representative for the river category and alteration.

For the selection of MEP sites environmental features representing mitigation measures and/or expected natural features were considered. The ability of such features in discriminating MEP and disturbed sites was verified by multivariate analyses run on abiotic features (Principal Component Analysis) and biological communities (non-metric multidimensional scaling).

We demostrated differences both in terms of invertebrate community and biological metrics used to assess ecological status (and potential) between MEP and impaired river stretches. Finally, we recognised relevant habitat features able to clearly separate MEP reaches from non-MEP reaches with indication on the type and quantity of measures significant for benthic invertebrates and applicable in lowland heavily modified water bodies.

Paper 6: “**In-stream microhabitat mosaic depicts the success of mitigation measures and controls the Ecological Potential of benthic communities in heavily modified rivers**”.

The positive effect of mitigation measures on in-stream habitat conditions and the benthic community is recognised. In heavily modified rivers, though, the response of aquatic invertebrates to mitigation measures and habitat mosaic changes is scarcely documented.

We used non-metric multidimensional scaling to explore the benthic community of leveed rivers in the agricultural lowlands of Northern Italy. The relevance of in-stream substrate microhabitat for the benthic community was assessed, together with the impact of mitigation measures. We proposed a straightforward approach to quantify similarity of microhabitat mosaic between sites testing its statistical significance based on Bayesian statistics. We hypothesised that changes of microhabitat mosaic would reflect the level of implementation of mitigation measures and benthic invertebrates would respond accordingly. Alpha, beta diversity and benthic metrics used to classify ecological status/potential were considered and their variation tested against different levels of measure implementation. Lastly, ecological potential classification was paralleled to both the level of measure implementation and habitat attributes.

The microhabitats found at sites where measures were fully implemented differed from those observed elsewhere and they clearly mirrored morphological alteration and mitigation measures. Moreover, alongside morphological alteration, microhabitat diversity and mosaic were the main factors for benthic community structure. While benthic beta diversity strictly reflected microhabitat diversity, alpha diversity and ecological status metrics copied the mosaic gradient. Microhabitat attributes and most benthic metrics showed significant changes following measure implementation and they were accompanied by a gradual shift in ecological potential classes.

We demonstrated the importance of in-stream substrate microhabitats as a bridge between mitigation measures and the benthic community. Particularly when ecological classification is under focus, microhabitat mosaic should be evaluated for achieving a better understanding of biological responses. The huge amount of data available worldwide could support a straightforward use of river mosaic information for river management.

CHAPTER 1

GENERAL INTRODUCTION

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This dissertation is articulated in four parts and contains nine chapters. The first part (chapter 1) provides a general introduction to the macroinvertebrate and habitat issues relevant for the study. A few cross-cutting themes are illustrated, which will be useful as background information while reading the following chapters and papers. The second part (chapter two) presents the objectives of the papers/manuscripts included in the dissertation, which are reported in the subsequent section (chapters 3 to 8). Finally, the fourth part of the thesis includes the main conclusions (chapter 9).

1.1 Organisms and their habitat

The term 'habitat' derives from the Latin habitāre: 'to inhabit'. It was originally used in ecology to indicate 'all the physical and chemical factors that operate upon the community' (Clements & Shelford, 1939). Among other definitions, Odum (1971) suggested an organism's (or population's) habitat includes the physical place where it lives or can be found, literally its 'address'. Over the years, the word 'habitat' and its related terminology have been used in the scientific literature to express ecological concepts in an excessively generic manner, often causing miscommunication. This misunderstanding is particularly noticeable in the fields of natural resources protection and 'habitat' management. A generally agreed upon concept of habitat links the presence of a given species to the physical and biological features of its environment (Morrison et al., 1992). Illustrative of the difficulty of providing a simple, sufficiently comprehensive and rigorous definition of habitat is the question raised by some authors regarding the role of human perception. These authors argue that assigning the term 'habitat' to connections between species, populations and environmental features could simply be a conceptual device used by man to pigeonhole life forms within the environment (MacMahon et al., 1981).

More recently, a comprehensive proposal that embraces different terms and concepts to overcome such ambiguities has been formulated (Hall et al. 1997). This concept of habitat includes the set of resources and conditions present in an area that favours settlement by a given organism, while also ensuring its ability to survive and reproduce. Such resources include food, shelter, water and all other factors a species needs for survival and reproductive success. Habitat is any place where an organism can find the set of resources that enables it to survive. In this regard, migration and dispersal corridors and the territory that a species occupies only during the reproductive season are habitats. Habitats are physical places with spatial dimensions; they also have a temporal component, as their use may be tied to an organism's different life stages. Habitats have resources that could be cyclically connected to a given species; and, at the same time, habitats may be used on an annual, seasonal, or cyclic basis and by different species at different times. Organisms select habitat through an active process that is influenced by the presence or absence of resources and other limiting factors, such as competition and predation; habitat availability is defined by access to the set of physical and biological resources upon which a species relies. Habitat quality can be viewed as the ability of the environment to provide the necessary conditions for persistence of an individual or population of a species. Quality ranges from a minimum level that ensures only individual survival, to higher levels that can sustain a population.

In a nutshell, the habitat of an organism - or population - can be conceived as the physical place where it lives or where it may be found. In terms of scale, an entire riverine ecosystem may itself be a 'habitat', within which a number of smaller-scale habitats can be recognized.

Within the EU, the Habitat Directive (EC, 1992) provides a normative definition of habitat for a species' habitat as an environment defined by specific abiotic and biotic factors, in which the species lives at any stage of its biological cycle.

For the aims of this thesis, the complexities created by multiple definitions of habitat and habitat scenarios were kept in mind as background information. Within each of the presented papers, different habitat aspects – in relation to the aims and scale of investigation – were specifically explored.

1.2 Benthic macroinvertebrates and flow-related habitat conditions

Especially in Mediterranean regions, where a large proportion of rivers already show non-perennial characters (Skoulikidis et al., 2017), a consistent decrease in water resources is anticipated under all major future climate scenarios (e.g., De Girolamo et al., 2017). Reduced flows in the dry season are expected, which may hasten the degree of temporariness of streams and rivers (Larned, Datry, Arscott & Tockner, 2010). In addition, Mediterranean rivers are increasingly exposed to multiple stressors, which affect their freshwater biodiversity and ecological status and also limit ecosystem services (Skoulikidis et al., 2017). Consequently, the responses of biota to hydrological and flow-related habitat changes are a central issue for future projections of river ecology (Kakouei et al., 2018), which should be preferably based on quantitative relationships between flow-related indices and biological responses (e.g., Dolédec et al., 2007; Kakouei et al., 2017).

The distribution of suitable habitat finally controls the distribution of freshwater organisms, which are, by definition, confined to aquatic habitats. Physical habitat in rivers is largely determined by flow-related processes, whose variability is a central feature of river systems and their ecological functioning (Poff et al. 1997). Intrinsically, the flow of a river varies on diverse time scales e.g. hours, days, seasons, years, decades and longer, as a function of river size, climate, geology, topography, vegetation cover and other factors, which ultimately determine its flow regime. Five key components of flow variability, i.e., magnitude, frequency, duration, timing, and rate of change are recognized as central in defining river regime (Poff et al. 1997). When they do not differ significantly from the expectation for natural conditions, habitat, biodiversity and ecosystem integrity can be adequately sustained (Poff & Ward 1989). Thus, habitat structure in rivers is largely defined by flow-related physical processes and sediment movements. Such factors continuously determine the pattern of local hydraulics, how substrate microhabitats and organic matter dispose in the channel, if and how features of erosion and deposition are found (Zeiringer et al., 2018). However, especially in low flow periods, environmental settings can exacerbate habitat conditions and various factors can potentially act as strong environmental filters for aquatic organisms (e.g. Graeber et al., 2013). As well, when low flow conditions become harsher, a range of benthic organisms can actively move between habitats, thus making habitats interconnection relevant (e.g., Suren & Jowett, 2006). Connectivity among habitat patches becomes important, supporting aggregations of local communities, especially when disconnected pools appear in the river channel (Larned et al., 2010) and pools may become controlled by local rather than longitudinal processes (Lake, 2000). Hence, habitat conditions observed at the time of organism collection may be crucial in interpreting biological communities, because species composition changes with time after disturbance (Lake, 2000).

As stated above, hydrological variations over time and the overall regime characteristics of river reaches are crucial for aquatic taxa (Poff et al., 2010). However, their representation is not necessarily exhaustive in illustrating the flow-related conditions experienced in real time by aquatic communities and the resulting overall scenario. To account for local scale factors, and focused on the substrate where invertebrates are collected, the hydraulic preferences of benthic macroinvertebrates were derived for a range of European streams (Dolédec et al., 2007) based on bed shear stress estimations. Yet, the use of thresholds based on simple variables e.g. water velocity, to differentiate lentic and lotic freshwater environments may lead to inconsistent results across systems and do not appear to be easily reconcilable (Jones et al., 2017). Even at the same site and without anthropogenic disturbance, river flow and the resulting local hydraulics change continuously over time. Such changes are obvious in Mediterranean regions, where dramatic floods in high flow periods contrast potential droughts in the dry season (Cid et al., 2017). Therefore, the ratio of lentic to lotic habitats found in the river fluctuates accordingly, following supra-seasonal, seasonal and even shorter time-scale flow variations. With emphasis on the continuous nature of transitions between dissimilar flow and habitat environments, the concept of rivers with a lentic-lotic character has been proposed (Buffagni, 2004; Buffagni, Erba & Armanini, 2010). According to this approach, the overall lentic-lotic attitude of a river can be quantified at the reach scale on the basis of a selection of habitat features connected to the local hydraulic conditions, e.g., flow types, substrate size, and the presence of macrophytes. Such a concept was suggested to derive habitat information useful in the interpretation of responses of biological communities, and its relevance for benthic invertebrates has been demonstrated in European rivers (e.g., Buffagni, Armanini & Erba, 2009; Buffagni et al., 2010; Lobera et al., 2019).

1.3 Defining reference conditions in Mediterranean rivers and in Heavily Modified Water Bodies

To monitor the effects of water pollution and other impacts on aquatic ecosystems, biological and ecological approaches were introduced in the last decades. Legislation to protect rivers worldwide now requires the assessment of 'ecological and chemical status' e.g. for the Water Framework Directive in Europe (WFD; EC, 2000), 'chemical, physical, and biological integrity' in the U.S.A. (Hawkins, 2015), or similar evaluations in other geographical areas (e.g. Chile, Australia; Dallas, 2013). Often, the quality classification of a river site is provided by comparing e.g. its biological attributes to the expected 'reference' conditions, representing relatively undisturbed situations (see Stoddard et al, 2006). Any numerical value chosen to quantify the reference conditions has therefore a crucial role in the whole classification process and in subsequent management plans (Nijboer et al., 2004). In fact, such value supports the calculation of Ecological Quality Ratios (EQRs, for the WFD) used to assign the river site/sample to a quality class.

However, a high intrinsic and/or unexplained biological variability has been considered a major limitation, due to the consequent uncertainty in environmental evaluations (e.g. Chessman, Thurtell & Royal, 2006; Àlvarez-Cabria, Barquín, & Juanes, 2010). All riverine ecosystems are inherently dynamic and show a range of abiotic and biotic conditions varying over time. Hence, a generic adjustment of reference conditions to e.g. river type or season might not be sufficient to encompass the natural habitat variation acting on invertebrate communities (De Girolamo et al., 2017). Benthic macroinvertebrate presence and distribution likely respond continuously to changing habitat conditions as they result from concomitant flow events, morphological features, biological interactions and ecological processes. Simply, there is no reason why we should expect a single value to represent efficiently the expected biological reference condition if the environment is continuously changing and/or is highly heterogeneous (Vander Laan & Hawkins, 2014). Predictive modelling is a common way to obtain site-specific estimates. Its application can lead to improvements of the accuracy and precision of reference condition setting and its use should be encouraged (Hawkins, Olson & Hill, 2010). However, data scarcity in low flow rivers is still a relevant issue (Bangash et al., 2012) and the official use of basic, typology-based assessment systems is common e.g. in southern Europe. In general, the adoption of fixed reference conditions i.e., an 'average' situation that should correspond to typical habitat conditions, may likely determine noticeably wrong quality attributions when habitat diverges from common situations (Hawkins, Cao & Roper, 2010). On this regard, accounting for lentic-lotic habitat conditions may be appropriate to adjust for natural factors on a site-specific basis when describing reference conditions and assessing ecological status.

Hydromorphological modification is one of the major problems affecting river health (EEA, 2018; Vaughan et al., 2009). In relation to hydromorphological alteration, the WFD has introduced the concept of Heavily Modified Water Body (HMWB). Such concept, which exists in reason of the WFD, is strictly linked to the presence of major physical alterations, established to allow for a range of water uses. In HMWBs, hydromorphological modifications are permanent and significantly alter the morphological and hydrological characteristics of the water body (Halleraker et al., 2016). By definition, the removal of major physical modifications from a HMWB compromises the use of the water body. When a water body is designated as heavily modified, its environmental objective changes from Good Ecological Status (GES) to Good Ecological Potential (GEP). The GEP is conceptually very similar to the GES, although it takes into consideration the limitations imposed by the water body use.

As for natural water bodies, and regardless of the adopted classification approach, ecological potential in HMWBs has to be based on the assessment of biological conditions. The definition of Maximum Ecological Potential (MEP), i.e. the reference condition for HMWB, is crucial in the process of assessing ecological potential. Such condition is described as the best approximation to a natural aquatic ecosystem that could be achieved given the hydromorphological alterations that cannot be removed without significant adverse effects on the specified use or the wider environment (CIS, 2003). Selection criteria for sites at MEP conditions are central to the assessment of ecological potential in the same way as reference conditions and reference site selection principles are for ecological status definition (Feio et al., 2014; Pardo et al, 2012). Because in HMWB entirely unaltered conditions cannot be attained, the MEP can be obtained by applying all the mitigation measures compatible with the specified use (CIS, 2003). MEP conditions can be defined and assessed on the basis of actual characteristics found in a specific river reach where the environmental conditions are the best available, provided that all possible mitigation measures are implemented.

As far as habitat characteristics are concerned, a direct assessment of habitat conditions e.g. by contrasting the number and proportion of in-stream benthic habitats between sites in conjunction with invertebrate assessments, may provide useful information for both managing rivers and better understanding biological responses to pressures. This is likely to be particularly relevant in HMWBs, where disturbance of physical habitat can shape homogeneous channel units (Rabeni et al., 2002) and substrate microhabitats can be of extreme importance for the local distribution and abundance of benthic invertebrates (Wohl et al., 1995).

In general terms, the reference condition concept (Nijboer et al., 2004) is central for any evaluation of ecological status (Stoddard et al, 2006) and it directly relates to the main aims of the papers produced for this dissertation or is a relevant background theme.

1.4 Benthic macroinvertebrates and morphological alteration HMS

Hydromorphological degradation is one of the most important anthropogenic pressures affecting European rivers and streams (Feld, 2004; Vaughan et al., 2009; EEA, 2018). The term 'hydromorphological degradation' describes an assortment of impacts acting at different spatial scales (Feld & Hering, 2007). This often entails that research in this field mainly focuses on specific impact types such as channel resectioning (Wyżga et al., 2014), river impoundment (Krajenbrink et al., 2019), or fine sediments accumulation (Doretto et al., 2018), and does not refer to the overall degree of morphological alteration.

In Europe, the achievement of good ecological status is a legally binding objective according to the Water Framework Directive (WFD EC, 2000) and this implies monitoring biological quality elements sensitive to anthropogenic impacts. However, invertebrate assemblages response to hydromorphological pressures is considered weak or unclear by many authors (e.g. Feld et al., 2014; Villeneuve et al., 2015). In addition to the complexity in defining hydromorphological impacts and their heterogeneous biological responses, rivers are usually affected by a suite of multiple stressors, which makes the quantification and the ranking of the different pressures influencing biological communities difficult (Turunen et al., 2016). Due to such complexity, some authors tend to conclude that macroinvertebrates do not respond to such alterations or invertebrate-based assessment methods currently in use are insensitive to morphological impairment (e.g. Friberg et al., 2009; 2011).

Especially because the link between biological elements and morphological impairment can be uncertain, particularly when other pressures are simultaneously acting, there is no scientific consensus on which river features are the best to be considered and which methods are most suitable for long-term monitoring. Many countries have adopted physical habitat assessment protocols to evaluate river hydromorphology for the WFD objectives (Belletti et al., 2015; Wiatkowski & Tomczyk, 2018). Physical habitat is traditionally recognized as important in explaining the composition and structure of biological communities (Fernández et al., 2011) but assessment methods focused exclusively on physical habitat can be not exhaustive for overall hydromorphological evaluation. By contrary, hydromorphological assessment methods with a stronger emphasis on river dynamics and processes are considered the most comprehensive (Wiatkowski & Tomczyk, 2018), but can be unsuccessful in establishing a direct link with biological components. Hydromorphological processes focus on features that are not necessarily significant for biological communities and their assessment may fail to consider key biological habitats (Verdonschot et al., 2016). According to the WFD, to measure the difference between altered and natural condition is only possible when biologically relevant variables are considered (Gieswein et al. 2017; Boon et al., 2019). Outlining the relationships between biotic community assemblages and morphological alteration is crucial to plan specific measures for rivers and achieve WFD environmental goals.

1.5 The Normative level: macroinvertebrates and habitats in the Italian environmental legislation

The Water Framework Directive (WFD, EC 2000/60– 'Establishing a Framework for Community Action in the Field of Water Policy'), published in October 2000, is a major piece of European environmental legislation aimed at protecting and enhancing surface waters and groundwaters and defines innovative criteria and actions for the classification of ecological status of European water bodies. The implementation of the WFD has been a long and complex process, centred on the key objective of achieving good ecological status for all water bodies by 2015 (Carré et al., 2017). The WFD implementation required Member States to adopt important scientific and technical adjustments, to make national classification systems compliant with the new normative regulation. Although four years have passed from this ambitious deadline, the WFD implementation remains far from being concluded. Critical reviews on achievements and open challenges related to WFD topics were recently made available from many authors (e.g. Carvalho et al., 2019; Hering et al., 2010; 2018; Reyjol et al., 2014).

The complex system of inter-connected rules synchronising the formal adoption of the WFD in Italy was promulgated in 2006 by the Italian government (DL n. 152). After that date, a series of ministerial Decrees have been prepared and approved to regulate specific aspects of the WFD implementation (Table 1.1).

Table 1.1. Main Italian decrees adopted for the WFD implementation relevant for this dissertation. MATTM: Ministero dell'ambiente e della Tutela del Territorio e del Mare (Italian Ministry of Environment, land and sea); DL: Governmental Decree; DM: Ministerial Decree; DD: Water Director Decree.

Authority	Decree reference	Main subject	Status
Italian Government	DL April 6th , 2006, n. 152	Adoption of the WFD in Italy	Implemented
MATTM, 2008	DM June 16th , 2008, n. 131	River typology	Implemented
MATTM, 2009	DM April 14th , 2009, n. 56	Reference conditions and monitoring criteria	Implemented
MATTM, 2010	DM November 8th , 2010, n. 260	Classification of Ecological and Chemical status	Implemented (updates under evaluation)
MATTM, 2014	DM November 27th, 2013, n. 156	Criteria for the designation of HMWBs	Implemented
MATTM, 2016	DD May 30th , 2016, n. 341/STA	Classification of Ecological potential (HMWB)	Validation phase
MATTM, 2017	DD February 13th, 2017, n. 30/STA	Technical guidelines for e-flows definition	Validation phase

For the aims of this dissertation, a central role is played by the so-called 'classification decree' (MATTM, 2010), where methods for the classification of ecological status based on aquatic macroinvertebrates are reported, and reference values for all Italian rivers provided. On this regard, the STAR_ICM multi-metric index (Buffagni et al., 2006; 2007), which is included in the MacrOper classification system, is selected as official method for the assessment of ecological status based on macroinvertebrate fauna in rivers. As well as being national assessment method in Italy, it is equally used in Cyprus and Greece (for very large rivers), and have been used throughout Europe for the WFD Intercalibration of ecological status (Bennet et al., 2011). Any evaluation of the performance of this index and its component metrics, especially when factors other than overall degradation are considered, is therefore valuable to support a better interpretation of monitoring results and future planning of dedicated research.

Other decrees focus on environmental issues that were brought to the general attention at a second stage of implementation of the WFD e.g. heavily modified water bodies (MATTM, 2014; 2016) and e-flows definition (MATTM, 2017), which directly connect to some of the subjects discussed in this dissertation. In the same way, criteria and approaches to define reference conditions and select least disturbed sites (MATTM, 2009) or the analogous for maximum ecological potential i.e., in heavily modified situations (MATTM, 2016), gained a direct or indirect focus in some of the papers presented hereafter. Finally, the same river habitat information as formalised for some analysis in this dissertation (Buffagni et al., 2013) can be directly used to confirm high ecological status at reference sites (MATTM, 2010).

CHAPTER 2

OBJECTIVES

CHAPTER 2

OBJECTIVES

The main goal of this dissertation is to study the response of benthic macroinvertebrates of rivers to the variation of habitat features linked to hydromorphological factors. As introduced in chapter one, two main aspects are studied.

On one side, flow-related habitat conditions are considered (chapters 3 to 5), by studying the influence of the ratio of lentic to lotic habitat features on macroinvertebrates. Focus is placed on the response of both individual benthic taxa (chapter 3) and macroinvertebrate metrics and indices (chapter 4) to the lentic-lotic gradient in Mediterranean rivers of Sardinia. Additionally, the potential impact of climate changes on flow regime in a river catchment in the Puglia region, together with how the biological expectations connected to the lentic-lotic character of rivers might vary, are investigated (chapter four). From this perspective, the key hypothesis in this dissertation is that the relative proportions of lentic and lotic habitat features should play an important role in defining macroinvertebrate presence and distribution, influence invertebrate metrics and affect the classification of ecological status. Accordingly, it is presumed that they should be considered when setting reference conditions. In fact, macroinvertebrates are known to react to flow-related habitat conditions, and this should have significant consequences on most benthic invertebrate metrics and indices.

On the other side, the response of macroinvertebrates to morphological alteration (chapters 6 to 8) is analysed. An overall evaluation of benthic response to gradients of morphological alteration defined on the basis of in-field habitat analysis is performed for a few Italian river types ranging north to south (chapter 6). In turn, the arduous subject of defining biological 'reference conditions' in heavily modified water bodies (*sensu* WFD) is approached for lowland rivers of northern Italy (chapter 7), where important habitat alteration is linked to flood defence and land drainage works. Finally, in the same context, the response of macroinvertebrates to the implementation of mitigation measures and to the level of morphological alteration is studied (chapter 8). This latter study gives the opportunity to investigate the role of in-stream micro-habitats i.e., here substrate mosaic, as a link between mitigation measures/morphological alterations and invertebrate responses. From this point of view, the central hypothesis in this dissertation is that habitat features e.g. tree-related characteristics, substrate, and their deviation from relatively undisturbed situations should play an important role in regulating macroinvertebrate distribution, especially in environmental contexts where habitats are over-simplified. A correct record of these features in the field and the use of the resulting information might support a wider comprehension of biological response and ecological classification results based on benthic macroinvertebrates.

Chapter 3 to 5 – Macroinvertebrate response to flow-related habitat features

The third chapter, "**The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers**" aims to study whether the benthic invertebrates inhabiting unpolluted Mediterranean rivers respond to the lentic-lotic character of river reaches. I focus predominantly on the non-perennial rivers and streams of Sardinia, where water scarcity and climate changes will presumably amplify the current threats to aquatic biodiversity and ecosystems. Emphasis is placed on flow-related habitat features, such as flow types and channel substrates, that have experienced previous flow conditions and the influences of hydraulics. Altogether, the variables used to define the lentic–lotic character of rivers, which also include instream vegetation, organic debris and water depth, are considered. How individual pieces of habitat information relate to the overall lentic-lotic character of river stretches and how this character acts on benthic communities have not been investigated. Additionally, there is no information on how invertebrate taxa respond to the lentic-lotic gradient, i.e., the gradient from extremely lotic to extremely lentic conditions.

The specific objectives of this study are:

- i) to examine the relevance for invertebrates of a range of flow-related habitat features influencing the lentic-lotic character of rivers;
- ii) when a biological answer is evident, to quantitatively outline the association of aquatic invertebrates to the lentic-lotic range by defining the best-fitting response model;
- iii) to explore the distribution of taxon optima, overall and for the main benthic groups, along the lentic-lotic gradient.

The fourth chapter, "**The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification**" aims to study if biological attributes of the benthic community relate to the ratio of lentic to lotic habitats found at the time of sampling. As for chapter three, this study was conducted in Sardinia (South Western Italy). Likely, multi-metric indices sum up the effects noticed on individual macroinvertebrate metrics. Hence, checking their performance in function of the ratio of lentic to lotic habitats is highly relevant for classification and legally binding concerns. The key idea behind this paper is that biological reference conditions used in most assessment systems might require a site-specific adjustment in function of the lentic-lotic habitat conditions observed at the time of sampling. This would help reducing systematic bias in ecological assessments, interpreting benthic index responses and diminishing the amount of unexplained biological variability. The macroinvertebrate metrics considered cover a large part of South Europe, from western (Portugal) to eastern (Cyprus) limits, and are all included in official National methods.

The specific objectives of this study are:

i) to assess if a range of macroinvertebrate metrics commonly used for the classification of ecological status respond to the lentic-lotic character in Mediterranean rivers;
ii) if a response is evident, to describe the overall relationship between benthic invertebrate metrics and the ratio of lentic to lotic habitat features, for both pool and riffle mesohabitats;
iii) to provide an example of the impact of paper results on classification issues, additional focus will be placed on the STAR_ICM index, which is in use in Italy and elsewhere to classify ecological status for the WFD.

The fifth chapter, "**Hydrology under climate change in a temporary river system: Potential impact on water balance and flow regime**" aims to analyse the potential impacts of future climate scenarios on water balance and flow regime in a temporary river system. Additionally, we aim to briefly outline and discuss the possible implications of these changes for the assessment of ecosystem health, bearing in mind some of the WFD central assumptions and overall lentic-lotic conditions observed at river sites at the time of sampling. The study area is the Candelaro river basin, located in the Puglia region (SE Italy).

The specific objectives of this study are:

i) to give a contribution to the evaluation of climate change impact on water resources in the Mediterranean Basin;
ii) to provide information to support long-term WFD-based water resources management and planning in the study area and in similar basins.

Chapter 6 to 8 – Macroinvertebrate response to morphological alteration

The sixth chapter, "**Macroinvertebrate metrics responses to morphological alteration in Italian rivers**" aims to study whether benthic invertebrate metrics and indices respond to morphological alteration, based on a river habitat survey approach. In fact, invertebrate assemblages response to hydromorphological pressures is still considered weak or unclear by many authors. Five Italian stream types included in three environmental contexts i.e., Lowland streams (small and medium sized), Mediterranean mountain streams (small and medium sized) and Mediterranean temporary streams, across a wide geographical context, are considered. The chapter specifically focuses on the use of the Habitat Modification Score (HMS), which provides an indication of artificial modification to the physical structure of a river reach and considers a variety of modifications of river banks and channel. To maintain the emphasis on the evaluation of ecological status, biological metrics legally required by the Italian assessment method i.e. the STAR_ICMi and its component metrics, are considered.

More specifically, the paper aimed at answering the following questions:

i. are benthic invertebrate metrics able to reveal physical habitat alteration?
ii. Is HMS able to quantify morphological alteration in relation to the evaluation of ecological status for the WFD?

The seventh chapter, "**Defining Maximum Ecological Potential for heavily modified lowland streams of Northern Italy**" aims to describe Maximum Ecological Potential (MEP) conditions as defined for Italian heavily modified lowland rivers in relation to flood protection and land drainage. The WFD has introduced the concept of Heavily Modified Water Body (HMWB), which is strictly linked to the presence of major physical alterations, introduced to allow for a range of water uses. When a water body is designated as heavily modified its environmental objective changes from Good Ecological Status (GES) to Good Ecological Potential (GEP). As for natural water bodies, and regardless of the adopted classification approach, ecological potential in HMWBs has to be based on the assessment of biological conditions. The definition of Maximum Ecological Potential i.e., the reference condition for HMWBs, can be crucial in the process of assessing ecological potential. Such condition is described as the best approximation to a natural aquatic ecosystem that could be achieved given the hydromorphological alterations that cannot be removed without significant adverse effects on the specified use or the wider environment. The studied context consists of river reaches bearing levees or bank reinforcement both constructed to preserve the use of designation. Starting from some of the mitigation measures there defined, which implied gentle riparian vegetation management with a passive restoration approach for in-stream habitats, this chapter discusses in detail which measures and/or expected natural features are the most important in determining macroinvertebrate and sites ordinations and how these measures are related to MEP definition.

The specific objectives of this study are:

- i) to select appropriate MEP sites for the studied context;
- ii) to explore the variation of a range of morphological and habitat characteristics to verify if they can discriminate between MEP and non-MEP sites;
- iii) to examine if such distinction is supported by differences in the macroinvertebrate community.

The eighth chapter, "**In-stream microhabitat mosaic depicts the success of mitigation measures and controls the Ecological Potential of benthic communities in heavily modified rivers**" aims to explore the response of benthic invertebrates to different levels of mitigation measures, while focusing on the role of the in-stream microhabitat mosaic. Aspects of alpha and beta diversity are analysed, together with benthic metrics used in Europe to classify ecological status and potential. The context of the study is that described in chapter five, with focus on leveed rivers, where habitat conditions are inevitably linked to the degree of morphological alteration. In a general habitat shortage, in-stream substrate microhabitats are expected to play a central role in controlling aquatic invertebrates.

The specific objectives of this study are:

- i) describing a simple approach to quantify similarity between in-stream microhabitat mosaics;
- ii) assessing if and how the level of implementation of mitigation measures is reflected by benthic metrics and river microhabitats;
- iii) investigating the relationships between in-stream microhabitats, mitigation measures, the benthic community and ecological classification;
- iv) briefly discussing the potential of microhabitat mosaic to support the interpretation of macroinvertebrate data.

CHAPTER 3

The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers

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The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers

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ABSTRACT

1. The importance of flow-related factors to benthic organisms, as well as the role of habitat conditions in shaping aquatic communities during low-flow periods, have been recognized. However, the responses of macroinvertebrates to the overall conditions of lentic and lotic habitats at the reach scale are poorly known.

2. We investigated aquatic invertebrates and habitat features in a range of temporary rivers in Sardinia. The investigation focused on the flow-related characteristics that contribute to defining the lentic-lotic condition of the river reaches. We related habitat features to benthic taxa distributions using multidimensional scaling. We then quantified the responses of taxa to the different lentic and lotic habitat conditions by applying hierarchical logistic regressions. Finally, we aligned taxon optima along the lentic-lotic gradient and compared the responses of different taxonomic groups.

3. Unbroken waves and not perceptible flow were related to benthic taxa variability, suggesting local hydraulics and turbulence have a major role in regulating communities. The overall lentic-lotic character of the river sites was also clearly related to the benthic taxa distribution. More than 80% of taxa were significantly related to the lentic-lotic gradient, and an asymmetrical response curve was the predominant model.

4. Benthic groups showed taxon optima clustered in different ranges of the lentic-lotic gradient. Odonata, Coleoptera, Hemiptera and Mollusca preferred clearly lentic conditions. Diptera mainly ranged on the lotic side of the gradient, while Trichoptera was relatively uniformly stretched across the gradient. Ephemeroptera taxa clustered in intermediate lentic-lotic conditions, with two species preferring extremely lentic habitats. In general, optima converged at intermediate and extremely lentic conditions, presumably due, respectively, to the coexistence of different lentic and lotic features and to the high diversification of environmental characteristics in extremely lentic situations.

5. The uneven distribution of optima of different taxonomic groups along the lentic-lotic gradient might concern the use of benthic metrics when, e.g., there is a focus on the water quality or ecological status or establishing reference conditions under variable climatic conditions.

1 INTRODUCTION

In Mediterranean regions, where a large proportion of rivers already show non-perennial characters (Skoulikidis et al., 2017), a consistent decrease in water resources is anticipated under all major future climate scenarios (e.g., De Girolamo et al., 2017). Reduced flows in the dry season are expected, which may hasten the degree of temporariness of streams and rivers (Larned, Datry, Arscott & Tockner, 2010). In addition, Mediterranean rivers are increasingly exposed to multiple stressors, which affect their freshwater biodiversity and ecological status and also limit ecosystem services (Skoulikidis et al., 2017). Hydrological variations over time and the overall regime characteristics of river reaches are crucial for aquatic taxa (Poff et al., 2010). However, their representation is not necessarily exhaustive in illustrating the flow-related conditions experienced in real time by aquatic communities. In this regard, depending on the hydrological conditions, Gallart et al. (2012) defined six 'aquatic states', each describing a transient mesohabitat condition potentially observed at a given river reach at a particular moment; these states range from 'hyperrheic' (i.e., flooding events), to 'edaphic' (no surface water and no active hyporheic life observed). At a larger scale, an approach to classify lentic and lotic environments, including tidal habitats and 'oscillic' systems (i.e., systems that switch between lentic and lotic behaviours over time), has been proposed based on water resident time (Jones et al., 2017). Most Mediterranean rivers presumably fit in the oscillic category and require a focus on subtle habitat features and dissimilarities that may affect benthic communities (Buffagni et al., 2016; Karaouzas et al., 2019). A series of studies proposed the use of water velocity thresholds to differentiate lentic and lotic freshwater environments, but these quantitative definitions are often inconsistent across systems and do not appear to be reconcilable (Jones et al., 2017). Regarding the scale of organisms, the usefulness of measuring and modelling local bottom shear stress in streams based on easily obtained input variables has been demonstrated, especially where channel complexity increases and/or channel size decreases (Lamouroux et al., 1992). Accordingly, the hydraulic preferences of benthic macroinvertebrates were derived for a range of European streams (Dolédec, Lamouroux, Fuchs & Mérigoux, 2007) based on bed shear stress estimated from FliesswasserStammTisch hemisphere numbers. Dolédec et al. (2007) focused on the substrate where invertebrates are collected, determining near-bed local flow conditions to describe the microhabitat of benthic invertebrates. Additionally, coexisting areas with different hydraulic characteristics should be inspected for their potential as refuges for benthic taxa, especially under low flows (Brooks & Haeusler, 2016). With emphasis on the continuous nature of transitions between dissimilar flow and habitat environments, the concept of rivers with a lentic-lotic character has been proposed (Buffagni, Erba & Armanini, 2010). According to this approach, on the basis of a selection of habitat features connected to the local hydraulic conditions, e.g., flow types, substrate size, and the presence of macrophytes, the overall lentic-lotic attitude of a river can be quantified at the reach scale. Such a concept was suggested to derive habitat information useful in the interpretation of biological communities, and its relevance for benthic invertebrates has been demonstrated in European rivers (e.g., Buffagni, Armanini & Erba, 2009; Buffagni et al., 2010; Lobera, Pardo, García & García, 2019). In riffle-pool sequences of Mediterranean rivers, pool habitats increase and riffle habitats decrease gradually as drought advances, with pools often disconnecting from each other in the driest period (Bonada, Rieradevall, Prat & Resh, 2006). Biological tools and metrics to differentiate among the main aquatic states have been proposed (Bonada et al., 2006; Cid et al., 2016). Additionally, attempts have been made to assess habitat loss due to the effects of droughts, focusing on the sequential loss of aquatic invertebrates to changing biotic and abiotic conditions (Chadd et al., 2017). However, how individual pieces of habitat information relate to the overall lentic-lotic character of river stretches and how this character acts on benthic communities have not been investigated. Additionally, there is no information on how invertebrate taxa respond to the lentic-lotic gradient, i.e., the gradient from extremely lotic to extremely lentic conditions. To infer the real potential of applying the lentic-lotic concept in relation to benthic communities, the links between flow-related habitat information and the responses of aquatic invertebrates should be further clarified. Indeed, the responses of biota to hydrological and flow-related habitat changes are a central issue for future projections of river ecology (Kakouei et al., 2018), which should be preferably based on quantitative relationships between flow-related indices and biological responses (e.g., Dolédec et al., 2007; Kakouei et al., 2017).

Overall, I aim to test whether the benthic invertebrates inhabiting unpolluted Mediterranean rivers respond to the lentic-lotic character of river reaches. I focus predominantly on the non-perennial rivers and streams of Sardinia, where water scarcity and climate changes will presumably amplify the current threats to aquatic biodiversity and ecosystems. Emphasis is placed on flow-related habitat features, such as flow types (*sensu* Padmore, 1998) and channel substrates, that have experienced previous flow conditions and the influences of hydraulics. Altogether, the variables used to define the lentic–lotic character of rivers (Buffagni et al., 2010), which also include instream vegetation, organic debris and water depth, are considered. More specifically, I aim to i) examine the relevance for invertebrates of a range of flow-related habitat features influencing the lentic-lotic character of rivers; ii) when a biological answer is evident, quantitatively outline the association of aquatic invertebrates to the lentic-lotic range by defining the best-fitting response model; iii) explore the distribution of taxon optima, overall and for the main benthic groups, along the lentic-lotic gradient

2 METHODS

2.1. Study area and sampling sites

All sampling sites are located in Sardinia (South Western Italy), which is the second largest island in the Mediterranean basin and has a Mediterranean climate. Summers are dry and hot, while winters are mild and relatively rainy. Rivers typically show strong seasonal hydrological variations (Mulas et al., 2009; De Waele et al., 2010). Reduced water quality can have a relevant impact in the area and often derives from partly treated civil sewage, farming and agricultural practices (RAS, 2005). Locally, morphological alteration is also relevant (Buffagni et al., 2016). Especially in the low flow season, reductions in river discharge for water storage and irrigation largely affect river hydrology (RAS, 2005). The investigated river reaches are located across Sardinia, mainly on its eastern side, and show a prevalent temporary character. However, they also show high supra-seasonal variability and belong to various river types (Mulas et al., 2009; RAS, 2009). A list of the investigated sites is shown in Table S1, with site coordinates and the overall reach characterization. The studied rivers are mainly located in mountainous or hilly settings at low altitudes, i.e., all sites but one are located below 450 m a.s.l. and are mid- or small-sized. The total channel width varies between 2 and ≈ 80 m, with the wetted portion at the time of sampling occupying on average ≈ 50% of the channel and flowing discharge ranging from ≈ 0 to ≈ 0.6 m^3s^{-1}. The most represented flow types are 'rippled' and 'smooth' flows (see Table 1 and section 2.2). The observed water temperature ranged from 8 to 25°C. The selected sites all have good water quality, are a non-heavily modified water body (*sensu* Water Framework Directive - WFD, European Commission, 2000), have no intense upstream ponding, experience no potential tidal influence on local hydrology, have experienced no recent disturbances from maintenance works, and have a comparable river type. In a few situations, more than one site was assessed in the same river. In addition, for a few sites, samples were collected in two different seasons at adjacent locations to increase the range of observed hydraulic habitats. However, the majority of sites and/or dates were well disconnected. Therefore, because of the main role of local river morphology in influencing the main variables of interest, accounts of the lentic-lotic features were considered independent. The LIM_{eco} descriptor (MATTM, 2010), formally used in Italy for chemistry-based classifications of ecological status, was used to check the water quality i.e., sites with a status below good were rejected. Finally, 62 river reaches were selected for the study (Table S1). Further details on most sites can be found in Buffagni et al. (2016).

2.2 Invertebrate sampling, environmental variables and the reach-scale survey

2.2.1 Aquatic communities

In the studied rivers, pool-riffle sequences usually characterize the stream reach type (Bisson, Montgomery & Buffington, 2007) and reaches show a high spatial heterogeneity (Buffington & Montgomery 1999). In this situation, the large variability of habitat conditions suggests that, for purposes of comparison of taxa lists and abundances, invertebrate samples are collected from

Table 3.1 List of habitat features and environmental variables used to interpret the variation in benthic taxa abundances as revealed by NMDS analysis. For each variable, the scores for NMDS axes 1 and 2 are provided, together with the r^2 value describing its association to the axes. p-values are shown and were directly obtained by applying the vector fitting technique and after-FDR correction ((*)p < 0.1; *p < 0.05; **p < 0.01; ***p < 0.001). For variables involved in the estimation of the lentic-lotic character (LRD), the assigned score (after Buffagni et al., 2010) is reported.

Variable category	Variable description	NMDS1	NMDS2	r^2	Pr(>r)		Pr(>r) FDR		Acronym	Score in LRD
Overall reach characterization	Altitude a.s.l. (m)	0.38	0.92	0.16	0.004	**	0.058	(*)	alt	-
	Distance to source (km)	0.13	-0.99	0.10	0.046	*	0.430		dist_fin	-
	Slope of thalweg (%)	0.39	-0.92	0.01	0.782		1.000		slope_th	-
	Discharge (field measure)(m^3/s)	-0.78	-0.62	0.20	0.002	**	0.031	*	Q	-
	Mean channel width (m)	-0.29	-0.96	0.29	0.001	***	0.023	*	ch_width	-
	Mean water width of the main channel (m)	-0.57	-0.82	0.09	0.049	*	0.436		w_chl	-
	Habitat Modification Score (Raven et al., 1998)	-0.68	-0.74	0.02	0.562		1.000		HMS	-
	Habitat Quality Assessment score (Raven et al., 1998)	-0.91	-0.41	0.04	0.346		1.000		HQA	-
	Land Use Index (Erba et al., 2016)	-0.93	0.38	0.00	0.948		1.000		LUI	-
Lentic-lotic feature	Lentic-lotic River Descriptor (Buffagni et al., 2010)	0.86	0.51	0.36	0.001	***	0.023	*	LRD	total
	Thalweg water depth in the main channel (reach average)(m)	-0.86	-0.51	0.04	0.295		1.000		w_de_chl	0-1
	Sections with over-depeened channel (count)	-0.78	0.63	0.18	0.002	**	0.031	*	over_deep	(1)
	'Free-fall' flow type (%). Poorly represented at the study sites	0.03	-1.00	0.00	0.893		1.000		FP_FF	-2
	'Chute' flow type (%)	-0.89	0.46	0.13	0.02	*	0.208		FP_CH	0
	'Broken waves' flow type (%)	-0.72	-0.70	0.29	0.001	***	0.023	*	FP_BW	-2
	'Unbroken waves' flow type (%)	-0.93	-0.36	0.55	0.001	***	0.023	*	FP_UW	-1
	'Chaotic' flow type (%)	-0.74	-0.68	0.16	0.008	**	0.094	(*)	FP_CF	-2
	'Rippled' flow type (%)	-0.99	-0.13	0.39	0.001	***	0.023	*	FP_RP	-0.5
	'Upwelling' flow type (%)	-0.66	0.75	0.04	0.263		1.000		FP_UP	0
	'Smooth' flow type (%)	-0.50	0.87	0.09	0.059	(*)	0.502		FP_SM	0
	'Not perceptible' flow type (%)	0.95	0.32	0.58	0.001	***	0.023	*	FP_NP	2
	Number of dry sections (flow interruption) in the river channel	0.97	-0.23	0.27	0.001	***	0.023	*	nDRY	8
	'Algae' habitat (%)	1.00	0.05	0.16	0.005	**	0.067	(*)	OP_AL	0
	'CPOM/FPOM/XY' habitat (%)	0.90	0.44	0.22	0.002	**	0.031	*	OP_CFXY	1-3
	'Submerged macrophytes' habitat (%)	-0.20	-0.98	0.03	0.464		1.000		OP_SUB	0
	'Emergent macrophytes' habitat (%)	0.05	1.00	0.00	0.934		1.000		OP_EMER	1-3
	'Living parts of terrestrial plants' habitat (%)	0.41	0.91	0.02	0.645		1.000		OP_TP	0
	'Liverworts/mosses/lichens'' habitat (%). Aquatic forms quite rare in the studied rivers	-0.09	1.00	0.00	0.862		1.000		OP_LML	-1 to -3
	'Silt' substrate (%)	0.06	1.00	0.11	0.039	*	0.384		SP_SI	1
	'Sand' substrate (%)	-0.09	1.00	0.02	0.496		1.000		SP_SA	1
	'Pebble (with some gravel)' substrate (%)	0.88	-0.47	0.05	0.18		1.000		SP_PG	0
	'Gravel (with some pebble)' substrate (%)	-0.63	0.77	0.14	0.011	*	0.121		SP_GP	0
	'Pebble' substrate (%)	0.80	-0.61	0.08	0.086	(*)	0.670		SP_PP	0
	'Cobble' substrate (%)	-0.08	-1.00	0.05	0.246		1.000		SP_CO	-1
	'Boulder' substrate (%). Poorly represented at the study sites	-0.60	-0.80	0.05	0.203		1.000		SP_BO	-1
	'Bedrock' substrate (%)	0.96	-0.30	0.05	0.207		1.000		SP_BE	0
Water physio-chemistry	Oxygen saturation (%)	-0.92	0.39	0.17	0.007	**	0.087	(*)	O2	-
	$N\text{-}NH_4$ concentration (mg/l)	0.69	0.72	0.05	0.203		1.000		NNH	-
	$N\text{-}NO_3$ concentration (mg/l)	-0.93	0.36	0.08	0.081	(*)	0.659		NNO	-
	P-PO4 concentration (µg/l)	0.51	0.86	0.06	0.17		1.000		PPO	-
	pH	0.96	0.27	0.01	0.771		1.000		pH	-
	Conductivity (µS/cm)	0.65	0.76	0.23	0.001	***	0.023	*	cond	-
	Water temperature (°C)	0.98	0.19	0.21	0.001	**	0.023	*	T	-

like physical units (e.g. Bisson et al., 2007). When sites are compared, the physical characteristics of sampled areas should be as similar as possible (Carter, Resh, Hannaford & Myers, 2007). In the study area, the 'pool' sub-unit, i.e., the slow water unit (sensu Hawkins et al., 1993) that includes scour and/or dammed pools when the river is in an eurheic phase (Gallart et al., 2012), is usually largely dominant. On the contrary, 'riffles' i.e., fast water units, often cover a small percentage of the reach, especially in the low flow season, when fast water habitats tend to disappear. Prior to sampling, the pool-riffle sequence was recognized, and the 'pool' sub-unit of the reach was then selected for sampling, in adherence to the Italian WFD-compliant sampling protocol for invertebrates (Buffagni et al., 2001). The sampling followed a multi-habitat, proportional approach (Hering, Moog, Sandin & Verdonschot, 2004). According to such approach, the most representative habitats and habitat patches (e.g. Lake, 2000) present in the river section, e.g. middle of the channel, behind boulders, channel margins, 'pools' (*stricto sensu*), were sampled; microhabitats with turbulent flow, if significantly present in the reach sub-unit, were also sampled. Benthic invertebrates were collected–from ten Surber sample units at each site (0.05 m^2 each, mesh size 0.5 mm). The identification level for invertebrates varied from species (e.g., for most taxa of Ephemeroptera, Plecoptera and Odonata) to family (e.g., Diptera and Trichoptera) (more details in Table S1).

2.2.2 Water physiochemistry

The water concentrations of O_2, N-NO_3, N-NH_4, P-PO_4 plus pH and conductivity (µs/s) were determined to characterize the water quality. With the exclusion of the last two variables, these variables were used to calculate the LIM_{eco} descriptor to classify water quality (MATTM, 2010). For further data analysis, physio-chemical parameters, apart from pH, were log-transformed. Variables expressed as percentages were arcsin√-transformed.

2.2.3 Reach-scale survey

For each river reach, habitat data were collected with the CARAVAGGIO method (Buffagni, Demartini & Terranova, 2013) based on an extended RHS-derived protocol (Raven et al., 1997) to cope with the variety of habitats of Mediterranean rivers (Buffagni & Kemp, 2002). This method includes a double evaluation of river habitats. The first evaluation considers habitat features at a detailed scale along ten ≈ equally spaced crosswise transects (every 50 m) over a river segment ≈ 500 m long. Flow types and substrates, together with aquatic macrophytes and woody debris, were assessed in the channel by visual recording at each transect. Water, channel width, and water depth at thalweg were measured. Both primary and secondary channels, if present, were assessed. Data collected at each transect, e.g., flow type and substrate information, were finally merged to derive the overall river character at the reach scale using weighting based on the actual water widths. This provided a quantitative estimate of the main habitat features present at the reach. A second level of field inspection was performed by exploring habitat features at a larger scale; for example, land use and habitat characteristics of banks and adjacent areas over the river bank-top were investigated. In particular, in this review, I used data on flow discontinuities, i.e., interruptions. Data from both levels of inspection (i.e., transects and general) were used to calculate a few environmental indices that were useful for characterizing river reaches in terms of their overall degree of habitat modification and quality (HMS and HQA, respectively: Raven et al., 1998) and land use degradation (LUI: Erba et al., 2015). Additionally, other variables were investigated for overall reach characterization. The full list of recorded variables is shown in Table 1.

2.2.4 Quantification of the lentic-lotic river character

For each crosswise transect, dominant and co-dominant flow types and substrates, as well as the type and proportional presence of detritus and macrophytes, were recorded. Together with water depth and the number of flow interruptions, each of these features were assigned a score of < 0 for lotic and > 0 for lentic (a feature in-between lotic and lentic was given no score). The more lotic a feature, the lower is its negative score, e.g., -2 for ‘broken standing waves’ as the primary flow type. More lentic features were associated with a higher positive score, e.g., +1 when the ‘silt’ substrate was dominant or +3 for an extended presence of ‘emergent reeds’. The highest score (+8) was assigned for each flow interruption observed at the ≈ 500 m reach. These scores were finally added up, and the lentic-lotic river descriptor (LRD) was obtained (Buffagni et al., 2010). Ideally, LRD can vary from ≈ -80 to ≈ +100.

2.3 Relating the benthic community to habitat features

To investigate the relevance of variables involved in LRD calculation and its significance for the benthic community, I used non-metric multidimensional scaling (NMDS): metaMDS in the vegan R package (Oksanen et al., 2018). Based on the Bray-Curtis distance, NMDS was performed on log-transformed abundance data. For the flow-related and other environmental variables described above, vectors were fitted onto NMDS axes 1 and 2 (vector fitting technique in the vegan R library), based on 999 permutations to see how they were related to the ordination. Vectors' significance was used to highlight the most relevant variables. Due to the relatively high number of correlated habitat variables tested, a false discovery rate (FDR) correction was performed by adjusting the obtained p-values with the Benjamini & Yekutieli (2001) approach (function ‘p.adjust’ of the stats package, R Core Team, 2018).

2.4 Lentic-lotic preferences of aquatic invertebrates

Hierarchical logistic regression modelling was used to assess the response of individual taxa to the LRD gradient and quantify the corresponding relationship, where the fitted values were the probabilities of occurrence. An extended version of the Huisman-Olff-Fresco models (Huisman, Olff & Fresco, 1993), the eHOF (Jansen & Oksanen, 2013), was applied to log-transformed benthic abundance data. This logistic regression approach offers a reliable theoretical background for univariate ecological interpretation and efficiently fits taxa response data (Jansen & Oksanen, 2013). The original five unimodal models (Huisman et al. 1993) were considered in fitting; these models can be ordered by increasing complexity of their biological information, as follows. I: no significant trend; II: a monotone increasing or decreasing trend with the maximum at one end of the observed range; III: an increasing (or decreasing) trend towards (or from) a plateau representing the optimal range; IV: a symmetrical response curve, with an increase and decrease of the same rate around the optimum; V: a skewed response curve, with an increase and decrease of different rates i.e., a steeper slope on one of the two sides of the optimum. Further details on HOF models can be found in Huisman et al. (1993) and Kakouei et al. (2017). During the analysis, all models were fitted to the data by maximum likelihood estimation. Models were then compared with a penalization factor derived from the number of model parameters based on the Akaike information criterion corrected for small data sets (AICc). For models with the same number of parameters (i.e., III, IV and V), model selection only depended on data deviance (Jansen & Oksanen, 2013). To cope with the relative flexibility in estimating the best model (e.g., Kakouei et al. 2017) and to assess the predictive ability of the selected model, for each taxon, I randomly split (49 times) the database into training (2/3) and testing (1/3) sub-datasets. Each time, model stability was searched based on a bootstrap approach (100 re-sampling). A model was selected by combining the results across all of the model selections (a majority vote in classification), with the most frequent model chosen by the software. In general, except for when no relation was found with LRD, a particular model was straightforwardly accepted when it was selected more than 75% times. The area ender ROC curve (AUC) approach was then employed to evaluate the aptitude of the models to discriminate between true and false positives in a multiple class situation (Hand & Till, 2001). The area under the receiver operating characteristic curve (ROC) was calculated ("multiclass. ROC" function, in the R package pROC, ver. 1.13.0; Robin et al., 2011) for the 49 test datasets and then averaged for each taxon (Table SM1). AUC values ranged from 0.5 (model is ≈ random) to 1 (exact discrimination), with values ≥ 0.7 indicative of a suitable discrimination (Hosmer et al., 2013). Last, a model fit was performed on the whole dataset (bootstrap approach, 100 re-sampling). The results were then compared with those of the 49 runs described above, and after a visual check of the curve for each taxon, a model was finally selected. In some circumstances, the visual inspection of models fit to the whole dataset guided the final model selection. For a few taxa, the consideration of more than one model was the best option, as an unambiguous interpretation of the results was not achievable using just one model.

The widely used space-for-time substitution approach (e.g. Banet & Trexler, 2013), supports the general assumption that the spatial relationship between the observed lentic-lotic conditions and invertebrate response would similarly represent possible changes over time and season of a given site.

2.5 Distribution of taxon optima along the lentic-lotic gradient

A kernel density estimate for univariate observations of the distribution of optima of benthic taxa along the lentic-lotic gradient was performed by means of the R package stats (R Core Team, 2018). The fitted kernels (Gaussian) were scaled such that the smoothing bandwidth was the standard deviation of the smoothing kernel. The fitting was completed for the whole community and for individual benthic taxonomic groups that had a sufficient number (≥ 5) of significant taxon optima for LRD.

3 RESULTS

3.1 The lentic-lotic gradient

The observed lentic-lotic gradient, quantified by means of the LRD descriptor, ranged from -38.82 to 85.5 (Table SM1), with 50% records assigned LRD values between -10 and 25 (median 3.6). Four typical situations illustrating the observed gradient are shown in Fig. 1 and are arranged from low to high LRD values: lotic → intermediate → lentic → extremely lentic. Refer to the INHABIT project website for images illustrating a broader lentic-lotic gradient for Mediterranean to Alpine rivers (http://www.life-inhabit.it/it/photogallery).

3.2 Flow-related habitat features and the benthic community

The full list of variables considered in the paper, including flow-related ones, is presented in Table 1. The variable scores, which were obtained in relation to the NMDS analysis of benthic abundances, are also shown, with an indication of their significance before and after FDR correction. More than 94,000 invertebrates representing 115 taxa were identified and considered in the analysis. NMDS ordination (3D stress 0.172, non-metric fit R^2 0.970) on taxa abundances was used to aid interpretation of the benthic response to flow-related variables (Fig. 2).

After FDR correction, the significant flow-related variables include i) a range of flow types (namely, broken and unbroken standing waves and rippled or not perceptible flow), ii) the number of dry stretches along the river reach, and iii) the number of over-deepened zones in the wetted channel (information on water depth). These were accompanied by CPOM, FPOM and xylal scoring the LRD. The relatively weak relationship of other substrates (i.e., silt, gravel and pebbles) to benthic axes is not statistically significant after FDR correction. The LRD descriptor is among the variables best correlated to the benthic axes. Among the other variables, channel width, conductivity, water temperature and river discharge are related to the NMDS axes.

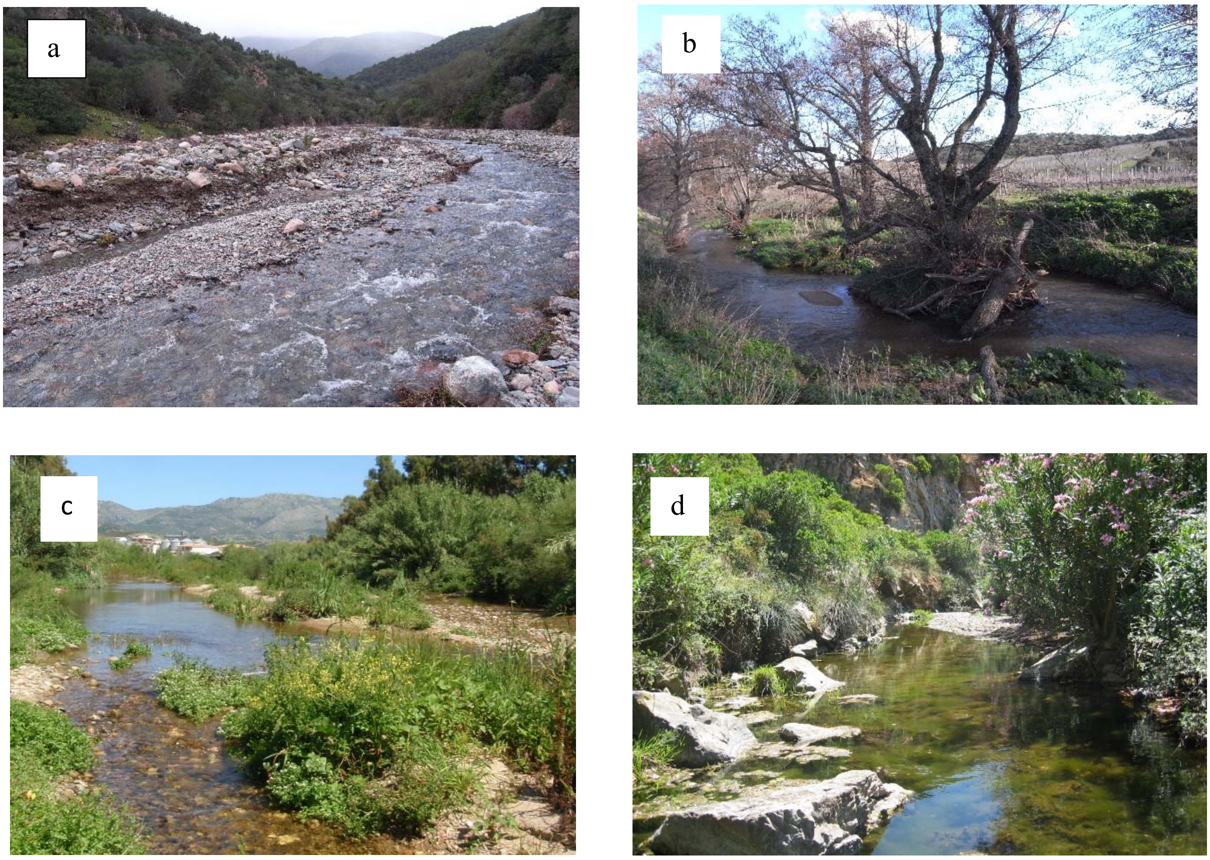


Fig. 3.1 River stretches showing different lentic-lotic characters. a) Lotic, LRD ≈ -28 (S29: Torrente Mortorinci, NU). b) Intermediate, LRD = -6.5 (S5: Fiume Baldu, SS). c) Lentic, LRD ≈ 21 (S10: Fiume Cedrino. NU). d) Extremely lentic, LRD = 64.5 (S35: Rio Mulargia, SU).

Concerning the relative positioning of variables in NMDS space, lentic features (right side of the diagram) are clearly opposed to lotic features, which are aligned in the opposite direction (Fig. 2). The river discharge vector lays in the area delimitated by lotic variables and is somewhat associated with channel width. The orientation of water conductivity, possibly related to the closeness of some sites to the sea, is opposite to that of discharge. Finally, the over-deepened zones in the river stretch are almost orthogonal to the main axis described by flow-type variables, which depict a lentic-lotic gradient. The LRD vector is opposite to the area delimitated by the two more turbulent flow types, i.e., broken and unbroken standing waves. To visually show how sites with different lentic and lotic habitat characteristics are perceived by benthic invertebrates, polygons encompassing sites belonging to the same lentic-lotic class (based on LRD values, Buffagni et al., 2010) are plotted on the NMDS diagram. These polygons are lined up with the LRD vector, and the very lotic and extremely lentic classes, representing the most extreme conditions observed in the area, are clearly separated from the other classes. The other four LRD classes show a more complex positioning in NMDS space, with an evident overlap in the central part of the diagram. Sites with very lentic conditions show taxa abundances that reflect the gradient defined by the number of dry zones. The location of sites belonging to the intermediate LRD class seems to reflect a gradient in the number of over-deepened zones. Lentic sites largely overlap with very lentic sites and partly overlap with intermediate and lotic sites.

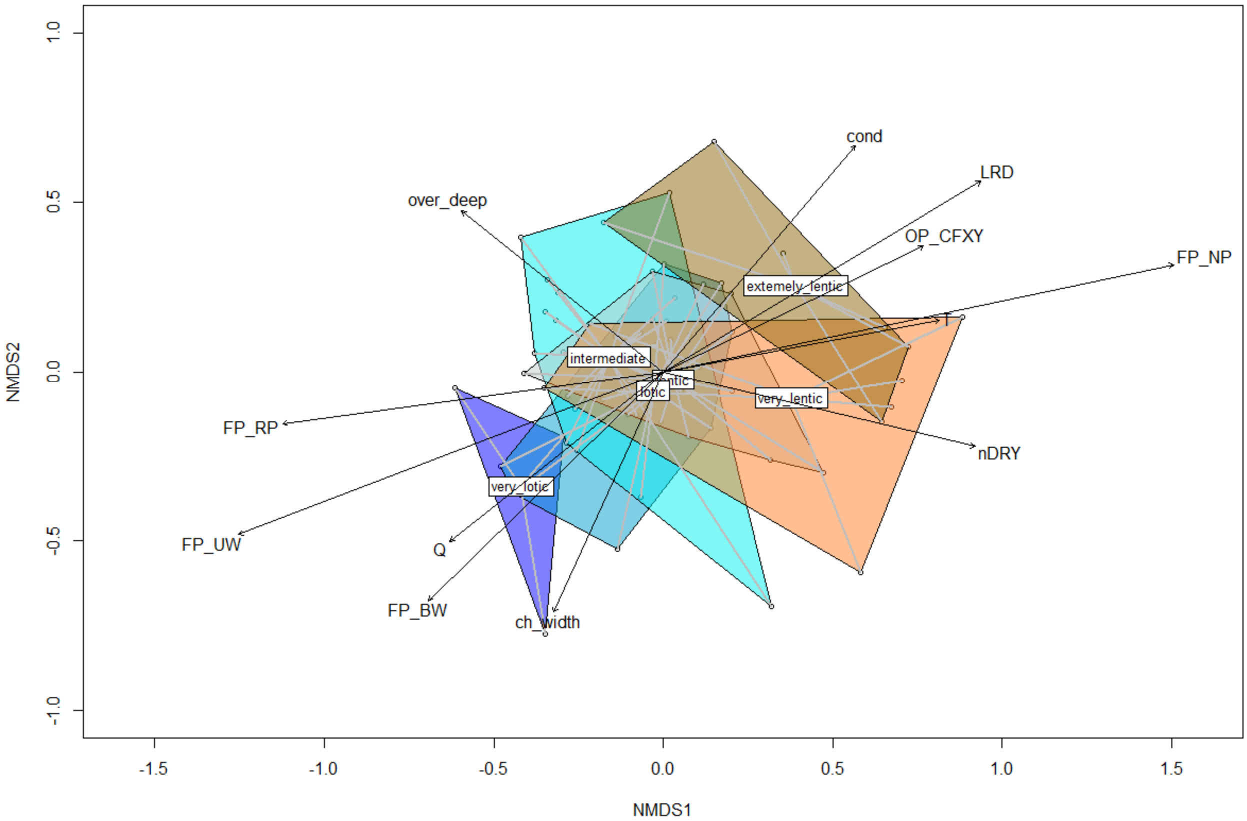


Fig. 3.2 NMDS diagram based on benthic taxa abundances. Significant vectors of reach-scale habitat features and physio-chemical variables are shown. Polygons encompass the sites belonging to the same LRD class i.e., sites within one polygon show a comparable lentic-lotic character, ranging from 'very lotic' to 'extremely lentic'. The longer the arrows, the better the concordance between the NMDS axes and variable projection (i.e., vectors are scaled by their correlation with the ordination results). Please refer to Table 1 for acronyms.

3.3 Association of aquatic invertebrates to the lentic-lotic gradient

For more than 80% taxa, a single response model to LRD was found, with model V (skewed response curve) representing 50% of all taxa (see also Table S2). The second most represented individual model is model II (> 13%), which corresponds to a regular increase or decrease in abundance. A total of 16.7% of the taxa were assigned combined models, frequently including model V. Approximately 13% of taxa show no response to LRD variation. Visual inspection of the response curves indicated that 11 taxa did not show a real optimum, even statistically assigned a model (different from model I) (Table 2). In general, out of the 84 taxa that had an appropriate abundance for model fitting, for 67 taxa, the same logistic model was attained > 50% times during the model validation phase or the combination of similar models seemed realistic (Table S2). Finally, an optimum value was defined for 64 taxa.

The dominance of model V is apparent for most taxonomic groups, transversally to identification level (Table S3). For Diptera, Hemiptera and Plecoptera, no model type was clearly dominant over the other model types. For Coleoptera, a comparatively large proportion (≈ 30%) of taxa was attributed to a combination of possible models, i.e., the attribution to a prevalent model type was not considered unequivocal. Model V curves are usually less steep towards lentic conditions if the optimum is lotic or are close to the equilibrium between lotic and lentic habitats. In contrast, the curves are gentler towards lotic values when the optimum is in the lentic range. As an example of curve profiles, the models and respective curves obtained for Ephemeroptera species are shown in Fig. 3. There, for the two models considered mixed (Table 2), the most frequently fitted model (V) is shown.

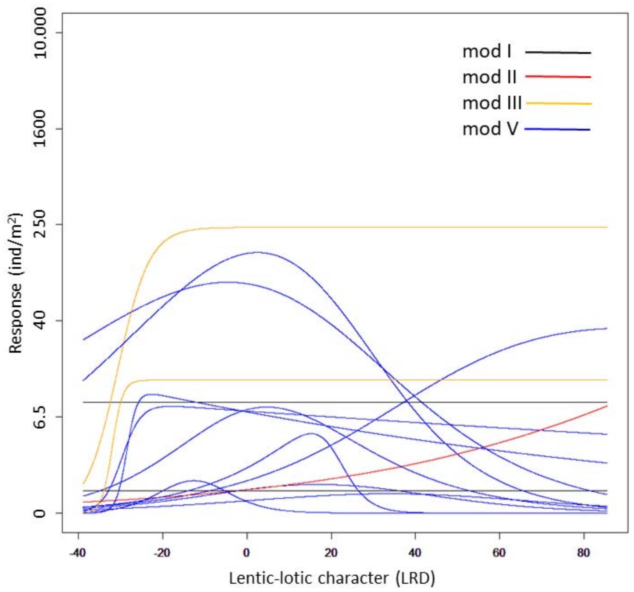


Fig. 3.3 Response curves of the lentic-lotic character (LRD) of rivers for Ephemeroptera species. The y-axis displays the actual abundance range (logarithmic scale) observed in the samples for individual species, up to a maximum of ≈ 8000 ind/m2 (Caenis macrura-Gr).

3.4 Distribution of taxon optima along the lentic-lotic gradient

Based on the optimum values of individual taxa (Table 2), the kernel densities of optima for the whole community and for each main taxonomic group were derived (Fig. 4). The full list of taxa showing an optimum (Fig. 4 a) covered the gradient of habitat conditions from lotic to lentic almost uniformly. Intermediate conditions (slightly above 0) showed a general peak, and the lotic/very lotic (< -20) range was less represented than the intermediate range. In general terms, compared to the kernel density of the sampling effort (Fig. 4 a, dashed line), optimum values were more frequent in lentic conditions than in other conditions. It was clear that most individual taxonomic groups showed a distinct positioning of taxon optima in restricted spans of the LRD range. Odonata, Hemiptera, Coleoptera and Mollusca exhibited optima in areas where lentic habitat features were dominant (i.e., positive LRD values), while the majority of Diptera families showed optima in the intermediate to lotic side of the gradient. Trichoptera taxa showed optima across the gradient of lotic and lentic river reaches, with the exclusion of the most extreme lentic conditions. Ephemeroptera showed a bimodal distribution of optima, with the main peak located in intermediate conditions and where lotic habitats prevailed (i.e., -20 to +10); there was a further peak (two *Cloeon* species) in extremely lentic environments.

A numerical summary of the optima distribution along the lentic-lotic gradient is shown in Table 3, where optimum values are attributed to LRD classes, and the families that are or that host representative taxa are specified. In a few cases (e.g., Leuctridae, Leptophlebiidae), other taxa belonging to the same family showed no class preference or had an optimum falling in another class (Table 2).

A numerical summary of the optima distribution along the lentic-lotic gradient is shown in Table 3, where optimum values are attributed to LRD classes, and the families that are or that host representative taxa are specified. In a few cases (e.g., Leuctridae, Leptophlebiidae), other taxa belonging to the same family showed no class preference or had an optimum falling in another class (Table 2). Globally, few taxa exhibited an optimum for very lotic (4) or lotic (6) conditions. Many taxa favour an intermediate situation, i.e., where lentic, transitional and lotic habitats can usually be found. The number of taxa exhibiting a preference decreased gradually towards very lentic situations. Interestingly, the lentic-lotic condition that contained the highest number of taxon optima corresponded to extremely lentic river reaches (18 out of 64 taxa showed an optimum under this condition).

Table 3.2 Models describing the quantitative response of benthic taxa to different lentic and lotic habitat features (LRD). The optimum value (if any), with confirmation after inspection (see text), and related curve parameters are shown. The AUC column expresses the aptitude of the model to discriminate between true and false positives (0.5: model is ≈ random; 1: exact discrimination; ≥ 0.7 suitable discrimination). Last column: information on optimum use in kernel density estimations.

Taxonomic group	Taxon	Final model	AUC	Estimated optimum	Actual optimum	Start/end optimum	Max slope	Inflection 1	Inflection 2	Optimum used in kernel density
Anellida	LUMBRICIDAE	I	0.50							-
Anellida	LUMBRICULIDAE	V	0.82	8.00	y		26.20	-8.31	25.32	y
Anellida	NAIDIDAE	V	0.89	11.50	y		17.48	-25.35	50.18	y
Anellidae	TUBIFICIDAE	V	0.90	-7.16	y		68.31	-19.56	6.84	y
Coleoptera	Dryopidae Gen. sp. Adult	V	0.74	15.34	y		52.70	11.06	19.47	y
Coleoptera	Dryopidae Gen. sp. Larvae	II-III-V	0.81	-38.03	y		4.00	<NA>	-9.93	y
Coleoptera	Dytiscidae Gen. sp. Ad.	I	0.50		n					-
Coleoptera	Dytiscidae Gen. sp. Lv.	V	0.87	1.35	y		22.16	-36.80	46.04	y
Coleoptera	Elmidae Gen. sp. Ad.	V-IV	0.82	29.21	y		12.86	-5.30	63.71	y
Coleoptera	Elmidae Gen. sp. Lv.	V	0.87	25.17	y		22.77	-0.29	52.88	y
Coleoptera	Gyrinidae Gen. sp. Ad.	II	0.87		n		0.23			n
Coleoptera	Gyrinidae Gen. sp. Lv.	V	0.85	37.85	y		11.65	21.05	54.60	y
Coleoptera	Haliplidae Gen. sp. Ad.	II-III-V	0.84		n	33.62	14.90	11.42		n
Coleoptera	Haliplidae Gen. sp. Lv.	V	0.81	63.01	y		92.02	57.94	67.62	y
Coleoptera	Helophoridae Gen. sp. Ad.	no	-		-					n
Coleoptera	Hydraenidae Gen. sp. Ad.	V	0.95	47.70	y		90.47	43.41	51.54	y
Coleoptera	Hydrophilidae Gen. sp. Ad.	II	0.80	85.50	y		10.19			y
Coleoptera	Hydrophilidae Gen. sp. Lv.	II-III	0.78		n		5.00			n
Crustacea	ASELLIDAE	V	0.81	52.42	y		131.33	47.40	56.98	y
Crustacea	ATYIDAE	no	-		-					n
Crustacea	GAMMARIDAE	no	-		-					n
Diptera	ATHERICIDAE	II	0.87	-38.82	y		4.97			y
Diptera	CERATOPOGONIDAE	V	0.89	1.46	y		39.93	-24.93	33.94	y
Diptera	CHIRONOMIDAE	II	0.98	85.50	y		6.35	-18.71		y
Diptera	DIXIDAE	I	0.50		n		3.77			-
Diptera	EMPIDIDAE	no	-		-					-
Diptera	LIMONIIDAE	II-V	0.89		n					-
Diptera	PSYCHOMYIIDAE	V	0.86	-10.20	y		37.47	-23.79	4.25	y
Diptera	SIMULIIDAE	II	0.91	-38.82	y		11.58			y
Diptera	STRATIOMYIIDAE	V	0.69	7.48	y		1.98	-12.84	27.62	y
Diptera	TABANIDAE	III	0.78		n	-22.54	69.91	-25.27		n
Diptera	TIPULIDAE	V-IV	0.86	-16.88	y		9.28			y
Ephemeroptera	*Baetis cyrneus* Thomas & Gazagnes, 1984	V	0.88	-9.64	y		21.05	-16.42	-2.98	y
Ephemeroptera	*Baetis fuscatus* (Linnaeus, 1761)	V	0.90	15.04	y		68.94	7.72	21.91	y
Ephemeroptera	*Baetis ingridae* Thomas & Soldan, 1987	V	0.95	-3.73	y		32.58	<NA>	32.93	y
Ephemeroptera	*Baetis cfr. muticus* (Linnaeus, 1758)	I	0.50		n					n
Ephemeroptera	*Caenis macrura-Gr.*	III	0.99		n	-20.85	152.93	-29.95		n
Ephemeroptera	*Centroptilum luteolum* (Muller, 1776)	I	0.87							-
Ephemeroptera	*Cloeon dipterum* (Linnaeus, 1761)	II-III-V	0.93	85.50	y		23.50	36.92	<NA>	y
Ephemeroptera	*Cloeon simile* Eaton, 1870	II	0.90	85.50	y		12.99			y
Ephemeroptera	*Electrogena fallax* (Hagen, 1864)	V	0.80	43.06	y		66.45	38.57	47.36	y
Ephemeroptera	*Electrogena zebrata* (Hagen, 1864)	III	0.89		n	-28.65	216.96	-31.38		n
Ephemeroptera	*Serratella ignita* (Poda, 1761)	V	0.97	2.69	y		51.48	-27.33	30.20	y
Ephemeroptera	*Habrophlebia consiglioi* Biancheri, 1959	V	0.92	4.70	y		26.10	-13.89	23.34	y
Ephemeroptera	*Habrophlebia eldae* Jacob & Sartori, 1984	V	0.91		n	85.50	195.55	-28.58	-15.89	n

Ephemeroptera	*Procloeon bifidum* (Bengtsson, 1912)	V-III	0.87	-17.99	y		104.40	-29.09	-5.75	y
Ephemeroptera	*Siphlonurus lacustris* (Eaton, 1870)	V	0.81	-0.40	y		19.96	-10.35	9.93	y
Heteroptera	CORIXIDAE	V	0.85	63.49	y		43.11	46.54	78.76	y
Heteroptera	NAUCORIDAE	III	0.86		n	29.31	7.96		6.89	n
Heteroptera	*Nepa sp.*	I	0.50		n					-
Heteroptera	NOTONECTIDAE	V	0.86	25.36	y		8.89	-20.10	74.90	y
Heteroptera	*Plea sp.*	II	0.89	85.50	y		9.81			y
Hirudinea	*Dina lineata* (O.F. Muller, 1774)	V	0.83	6.29	y		22.09	-14.15	28.59	y
Hirudinea	*Helobdella stagnalis* (Linnaeus, 1758)	V	0.84	44.99	y		39.71	32.97	56.24	y
Mollusca	*Ancylus fluviatilis* O.F. Muller, 1774	I	0.50		n					-
Mollusca	PISIDIIDAE	V	0.86	58.09	y		89.70	52.97	62.36	y
Mollusca	HYDROBIIDAE	V	0.84	30.66	y		9.77	1.30	59.78	y
Mollusca	LYMNAEIDAE	V	0.74	13.95	y		13.81	3.99	23.46	y
Mollusca	*Physella acuta* (Draparnaud, 1805)	V-II	0.90	85.50	y		9.90			y
Mollusca	PLANORBIDAE	II	0.84	85.50	y		4.35			y
Odonata	*Anax sp.*	II	0.88	85.50	y		19.09			y
Odonata	*Boyeria irene* (Fonscolombe, 1838)	I	0.50		n					-
Odonata	*Calopteryx sp.*	V	0.84	36.38	y		17.33	9.51	62.11	y
Odonata	*Ceriagrion tenellum* (de Villers, 1789)	V	0.89	70.13	y		126.69	64.71	74.91	y
Odonata	*Coenagrion sp.*	V	0.84	39.12	y		8.42	20.82	57.47	y
Odonata	*Lestes sp.*	V	0.90	13.84	y		17.06	-1.13	29.51	y
Odonata	*Orthetrum sp.*	V-II	0.82	69.19	y		151.14	63.16	74.04	y
Odonata	*Pyrrhosoma nymphula* (Sulzer, 1776)	V-II	0.89	65.29	y		172.49	59.36	70.22	y
Odonata	*Sympetrum sp.*	II	0.84	85.50	y		4.00			y
Plecoptera	*Isoperla insularis* (Morton, 1930)	V	0.79	2.13	y		77.23	-2.71	6.64	y
Plecoptera	*Leuctra sp.*	III	0.79		n	-40	137.39	35.07	48.44	n
Plecoptera	*Tyrrhenoleuctra zavattarii* (Consiglio, 1956)	V-II	0.92	-24.08	y		156.28	-27.82	-20.05	y
Trichoptera	*Agapetus cyrnensis* Mosely, 1930	V	0.88	17.77	y		54.61	3.74	30.72	y
Trichoptera	BERAEIDAE	V	0.88		-					n
Trichoptera	GOERIDAE	V	0.86	-7.70	y		8.67	-37.04	21.58	y
Trichoptera	HYDROPSYCHIDAE	V	0.86	25.00	y		114.20	19.34	30.31	y
Trichoptera	HYDROPTILIDAE	V	0.85	-9.87	y		19.88	-28.56	9.17	y
Trichoptera	LEPTOCERIDAE	V-II	0.90	45.37	y		11.97	13.85	76.01	y
Trichoptera	LIMNEPHILIDAE	V	0.82	-23.43	y		143.85	-28.40	-17.56	y
Trichoptera	PHILOPOTAMIDAE	V	0.73	-20.04	y		4.63	<NA>	-1.55	y
Trichoptera	POLYCENTROPODIDAE	V-III	0.70	45.49	y		18.91	38.08	52.72	y
Trichoptera	RHYACOPHILIDAE	II	0.89	-38.82	y		2.07			y
Trichoptera	SERICOSTOMATIDAE	V	0.82	28.30	y		87.73	23.24	33.09	y
Tricladida	DUGESIIDAE	V	0.90	24.52	y		32.08	3.27	44.48	y

4 DISCUSSION

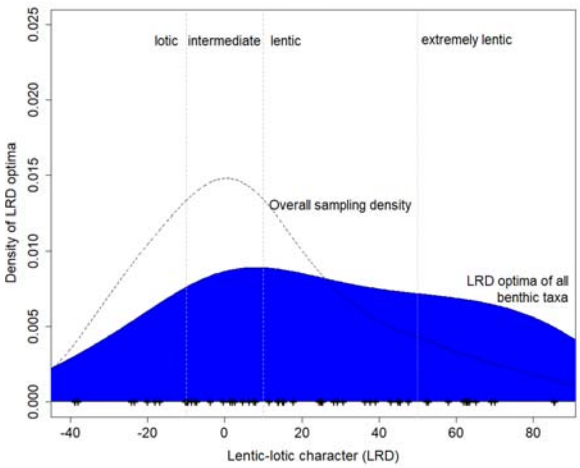
4.1 Response of benthic taxa to flow-related habitat factors

In this research, I studied habitat attributes observed at the time of benthic invertebrate sampling by collecting information on representative river transects and deriving reach-scale prospects. Next, I related the mean reach habitat conditions to the abundance of benthic taxa at the microhabitat scale. Among my goals, I aimed to identify the flow-related habitat factors that influenced the benthic community. More particularly, I focused on those features shaping the lentic-lotic character of rivers, i.e., the overall reach condition defined by the lentic and lotic habitat characteristics (Buffagni et al., 2010).

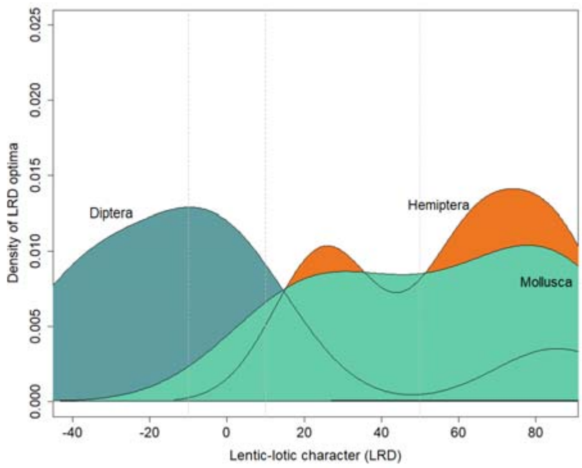
Table 3.3 Distribution of optimum values of benthic taxa across lentic-lotic classes. Families bearing representative taxa are shown for each class.

Lentic-lotic class	LRD range	Number of taxon optima in the class	%	Families bearing taxa representative of the lentic-lotic class	Overall lentic-lotic range	%
Extremely lotic	LRD ≤ -50	na	na	(not covered by the study)		
Very lotic	-50 ≤ LRD < -30	4	6.3	DRYOPIDAE, RHYACOPHILIDAE, ATHERICIDAE, SIMULIIDAE	Lotic	15.6
Lotic	-30 ≤ LRD < -10	6	9.4	BAETIDAE (*partim*), LEUCTRIDAE, LIMNEPHILIDAE, PHILOPOTAMIDAE, PSYCHOMYIIDAE, TIPULIDAE		
Intermediate	-10 ≤ LRD < 10	15	23.4	SIPHLONURIDAE, BAETIDAE (*partim*), EPHEMERELLIDAE, LEPTOPHLEBIIDAE, PERLODIDAE, DYTISCIDAE, HYDROPTILIDAE, GOERIDAE, CERATOPOGONIDAE, STRATIOMYIIDAE, LUMBRICULIDAE, TUBIFICIDAE, ERPOBDELLIDAE	Intermediate	23.4
Lentic	10 ≤ LRD < 30	12	18.8	LESTIDAE, NOTONECTIDAE, DRYOPIDAE, ELMIDAE, SERICOSTOMATIDAE, LYMNAEIDAE, NAIDIDAE	Lentic	60.9
Very lentic	30 ≤ LRD < 50	9	14.1	HEPTAGENIIDAE, CALOPTERYXIDAE, COENAGRIONIDAE (*partim*), GYRINIDAE, HYDRAENIDAE, LEPTOCERIDAE, POLYCENTROPODIDAE, HYDROBIIDAE, GLOSSIPHONIIDAE		
Extremely lentic	LRD ≥ 50	18	28.1	BAETIDAE (partim), AESCHNIDAE, COENAGRIONIDAE (*partim*), LIBELLULIDAE, CORIXIDAE, NEPIDAE, PLEIDAE, HALIPLIDAE, HYDROPHILIDAE, CHIRONOMIDAE, ASELLIDAE, PISIDIIDAE, PLANORBIDAE		

a



c



b

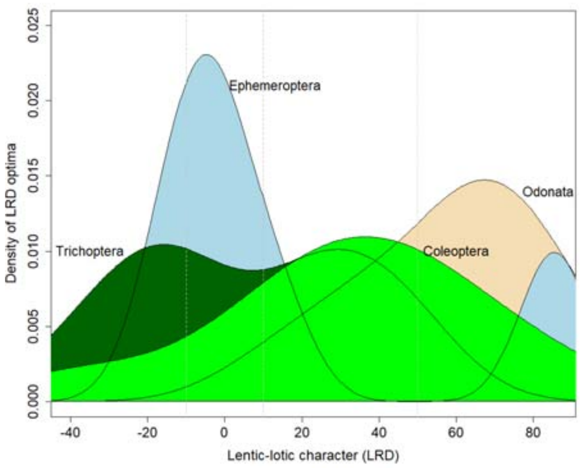


Fig. 3.4 Density of taxa' optima along the lentic-lotic (LRD) gradient. Distribution (kernel density estimate) for the whole community and for individual benthic taxonomic groups: a) taxa from all groups combined. Individual optima are indicated as plus-signs along the x-axis. The overall density of sampled lentic-lotic conditions (i.e., sampling effort) is also shown (dashed line). b) Ephemeroptera, Trichoptera, Odonata and Coleoptera. c) Diptera, Hemiptera and Mollusca. Vertical dashed lines delimitate the main lentic-lotic ranges

The taxon distribution was strongly related to the relative presence of distinctive flow types (*sensu* Padmore,1998), with unbroken waves and not perceptible flow representing the flow types most associated with benthic differences. Broken waves and rippled flow were also correlated with the taxon distribution.

The significance of these flow types for invertebrates is not surprising. In fact, flow types are interrelated with the degree of turbulence and provide information on the most important physical habitat zone provided by the flow: the boundary layer (Newson et al., 1998). Flow types are the outcome of a combination of flow velocity, flow depth and substrate roughness and are expected to adequately characterize the near-bed flows occurring within the microhabitats of benthic invertebrates (Davis & Barmuta, 1989). Numerous invertebrate taxa are affected by near-bed turbulence regimes (Dolédec et al., 2007), which are partially controlled by upstream roughness elements that cause flow separation (Hart, Clark & Jasentuliyana, 1996). The presence of plant detritus, such as xylal, FPOM and CPOM, was also important for the distribution of benthic taxa and was mainly associated with not perceptible flow and higher water temperatures. This result was not unexpected because lower flow velocity and turbulence can increase the particle sedimentation rate, and organic accumulations are important habitats and food sources for invertebrates. In these conditions, the benthic respiration rate increases, and dissolved oxygen decreases, which leads to anoxic conditions in extreme cases, thus potentially acting as a strong environmental filter for aquatic organisms (Graeber, Pusch, Lorenz & Brauns, 2013). When low flow conditions become harsher, a range of benthic organisms can actively drift between habitats, thus making adjacent habitats relevant (e.g., Suren & Jowett, 2006). Connectivity among habitat patches is important and supports aggregations of local communities, especially when disconnected pools appear in the river channel (Larned et al., 2010). These factors sustain the relevance of different habitat scales, e.g., the micro, meso and reach scales, in defining invertebrate assemblages and species distributions. Accordingly, I found that flow types, as well as other habitat features studied at the reach scale, related well with benthic invertebrates collected at the microhabitat scale. Zones where the channel was over-deepened also influenced benthic taxa. This may be due to their potential as refugia for benthic taxa during low-flow periods, when pools often remain connected by subsurface flow through the hyporheic zone (Boulton, 2003). When portions of the channel dry up, the ahreic aquatic state (Gallart et al., 2012) is reached, and disconnected pools become the dominant habitat. Their presence largely contributes to the spread of taxa among sites from lotic to very lentic conditions. High variability among macroinvertebrate assemblages in the disconnected-pool habitat at different sites has already been observed (Bonada et al., 2006) and can be explained by a series of factors, including the duration of the disconnection, differential colonization and predation, or other environmental factors that vary among sites and pools (Bonada et al., 2006). The overall evaluation of the lentic-lotic character of rivers, here expressed through the LRD descriptor, includes all the individual pieces of information discussed above. Its highly significant association with benthic taxa ordination is therefore not surprising, nor is its alignment to the major axis of benthic variation.

4.2 Association of taxa to specific lentic-lotic conditions

In the studied rivers, most benthic taxa exhibited a significant quantitative relationship with the gradual shift along the lentic-lotic gradient. This attests that the existing lentic and lotic habitat characteristics are relevant not only when droughts are approaching but also across the range of lentic-lotic conditions and aquatic states. In fact, more than 85% of taxa showed a significant response to LRD variation. With the same methodological approach used here, Kakouei et al. (2017) found that 23–41% of benthic taxa showed preferences over the ranges of seven hydrological metrics. Many studies have reported that high flow conditions have a strong impact on benthic invertebrate taxa (e.g., Suren and Jowett, 2006; Kakouei et al., 2017), and hydrological preferences have been defined for

German rivers (Kakouei et al., 2017). Conversely, the authors are unaware of studies that quantified the responses of benthic taxa to a combination of lentic and lotic habitat conditions, with the exception of Buffagni et al. (2010), who proposed preliminary optimal ranges for some taxa in Italian rivers. However, the quantitative response of taxa was not investigated. In the present research, I conducted modelling for each taxon individually, and when the response was significant, I determined the optimum and range of presence along the lentic-lotic gradient. Buffagni et al. (2010) reported the lentic–lotic character (LRD) preferences of 36 taxa, 16 of which were also considered in the present research. For the majority of these taxa (≈ 60%), the results are comparable, while two taxa (*Orthetrum*, *Physella acuta*) fit in terms of the overall lentic attitude. In a few circumstances, the suggested optima were different. This is presumably linked to the presence of coexisting endemic species in Sardinia, e.g., *Baetis cfr. muticus* and *B. albinatii* Sartori & Thomas, 1989 and/or to a dissimilar species composition in the studied areas (e.g., for *Leuctra* sp. and *Caenis macrura*-Gr.). Even though a preference had been previously assigned to *Centroptilum luteolum* and *B. cfr. muticus* (Buffagni et al., 2010), the logistic fit performed here selected model I (no response) for these taxa. Furthermore, even though a model (V) was formally fitted for *Habrophlebia eldae*, the obtained optimum was rejected due to the species' widespread distribution, and the optimal range provided in Buffagni et al. (2010) could not be confirmed as a general statement. The observed differences are presumably linked to the extended dataset and to the different and more accurate statistical approaches used here. In most cases, the optima outlined for the lentic-lotic conditions matched the common classifications of taxa into rheophilic and lentic groups (e.g., Extence, Balbi & Chadd, 1999; Graf et al., 2008; Buffagni et al., 2009; England et al., 2019).

When an optimum value/range was obtained, the taxon response, i.e., the curve expressing the probabilities of taxon occurrence, was often skewed, with a steeper response on one of the two sides of the optimum. An undoubtedly symmetrical response was not found for any taxon. The distributions of lentic taxa were mostly lentic-skewed, whereas those of lotic taxa were mostly lotic-skewed. In other words, the expected decrease in abundance for taxa with a lentic optimum was steeper towards more lentic values and gentler towards more lotic values. The reverse was true for lotic taxa on the other side of the lentic-lotic range. The observed skewness profile might be an adaptation (Waldock et al., 2019) to favour the coexistence of lentic and lotic taxa at the same site. In addition, the larger availability of diversified habitats in more balanced lentic-lotic conditions might justify the observed curve skewness. How an organism's performance changes along gradients in environmental conditions i.e., here, the lentic-lotic range, is a central issue in ecology. An approximately symmetrical bell-shaped curve is the basis for niche theory (e.g., Swan, 1970), but it is now accepted that many factors such as disturbances, fine-scale environmental heterogeneity, local adaptation and species interactions (Santika & Hutchinson 2009; Waldock et al., 2019) can affect species' distribution. These factors would lead the curve to change from symmetrical to skewed in the realized niche (Santika & Hutchinson 2009).

4.3 Taxonomic groups and the lentic-lotic gradient

I found that most taxonomic groups exhibited a clustered positioning of optima along the lentic-lotic gradient and that the highest optimum densities were often located in different ranges of lentic-lotic conditions for different groups. Odonata, Coleoptera, Hemiptera (i.e., OCH) and Mollusca displayed the highest optimum densities in the lentic range. This agrees with a number of studies focusing on Mediterranean rivers. For instance, Bonada, Rieradevall & Prat (2007) investigated how flow permanence constrains macroinvertebrate community structure and observed that the number of OCH taxa differed among sites. These authors found more OCH taxa at ephemeral reaches, i.e., reaches expected to show very lentic LRD values, than at perennial sites, i.e., sites with intermediate or lotic LRD values. Additionally, Bonada et al. (2006) and Stubbington et al. (2017) reported OCH and Molluscs as typical of lentic pools in intermittent rivers and ephemeral streams (IRES). Air breathers (e.g., CH) are favoured when pools tend to disconnect from each other and the oxygen deficit increases (Cid et al., 2016), conditions mirrored by the presence of very lentic habitats. In addition, the dispersal ability and high fecundity of large OCH species partly explains their limited sensitivity to habitat reduction and fragmentation (Datry, Larned and Tockner, 2014). As far as Mollusca are concerned, even if desiccation during low flow conditions is an important stressor, their desiccation tolerance can be quite high (Collas et al., 2014). Diptera (e.g., Athericidae, Ceratopogonidae, Psychomyiidae, Simuliidae, Tipulidae) optima were located in the intermediate to lotic side of the LRD gradient, with only Chironomidae showing increasing abundances towards the lentic edge. This Dipteran family includes species bearing trait states that promote resistance and/or resilience to intermittence (Stubbington et al., 2017) and are thus able to colonize river stretches with extremely lentic conditions. Simuliidae, which is well known as a running flow taxon, has been associated with connected flow conditions (Cid et al., 2016). All Trichoptera families showed a significant relationship with the lentic-lotic character, and their optima and distribution ranged over the LRD gradient, with a lower representation at the more lentic end. Notwithstanding such relationships, the expected abundance distribution of most Trichoptera taxa along the gradient was quite disperse. This is not surprising because of the likely presence of different species in each family with potentially different ecological needs and because of the wide ecological spectrum of this taxonomic group (Graf et al., 2008). The optima of Ephemeroptera species were clustered in two distinct ranges of the LRD gradient. Most species optima were centred in intermediate lentic-lotic conditions, where a variety of lentic and lotic habitats usually co-occur and may sustain species' differentiated ecological needs (Buffagni et al., 2009). No species exhibited a preference for very lotic conditions. In this regard, it must be noted that only a few Heptageniidae species were investigated. Genera expected to show a truly lotic preference, such as *Epeorus* and *Rhithrogena*, are not present in Sardinia and are confined to colder waters of upper river stretches and thus not covered by this research. In contrast, the two studied *Electrogena* species not surprisingly (e.g., Belfiore, 1995; Buffagni et al., 2009) had an association with lentic conditions. The same is true for species of the *Cloeon* genus that are known as typically lentic taxa (e.g., Sowa, 1980). Even if the more frequent sampling corresponded to intermediate lentic-lotic conditions, no taxonomic groups showed a high optima density in such a range, with the exclusion of mayflies, which showed a bimodal distribution.

4.4 Overall distribution of optima across lotic, intermediate and lentic conditions

Only a small proportion of taxa (≈ 15%) are expected to preferentially occur in lotic conditions. The low number of these taxa might be related to the nestedness of intermittent river stretches (showing more extreme lentic conditions in the low flow period), where lotic taxa are generally a subset of taxa from the least intermittent and perennial sections (Datry et al., 2014). Accordingly, many communities of intermittent reaches are often dominated by ubiquitous species with a high dispersal ability (Arscott, Larned, Scarsbrook & Lambert, 2010; Datry et al., 2014). Indeed, some of the taxa associated with lotic conditions showed a decreasing abundance trend from lotic to lentic habitats (e.g., Athericidae, Simuliidae) and no distinct preference for a specific lotic range, i.e., they were widespread taxa at the study sites. The decreasing proportion of habitats where flowing water persists likely acts as the main limiting factor (Lancaster & Belyea, 2006). In addition, in this study, I focused on the 'pool' sub-unit of the reach in the collection of benthic invertebrates. This might have added emphasis on lentic taxa compared to lotic taxa. In addition, the study focused on downstream river stretches, and truly lotic environments were marginally covered, with a minimum observed LRD value of ≈ -39 in a potential range reaching values of < -80. Thus, the opportunity to collect taxa specifically adapted to lotic conditions with a sufficient abundance was limited.

A relatively high number of taxa exhibited an LRD optimum in balanced lentic-lotic conditions, which were often sampled. In this lentic-lotic range, i.e., from -10 to +10 LRD, the number of dissimilar flow-related local environments increases as an outcome of the coexistence and summation of distinctive lotic, transitional and lentic features and habitats (Buffagni et al., 2010). This habitat diversification can generate suitable or advantageous conditions for a range of taxa, thus increasing the prospect that the optima of many taxa' will be detected. In this research, the largest span covered by taxa variation was observed around these conditions (Fig. SM1). Additionally, sites in this lentic-lotic class (i.e., intermediate) largely overlap with sites attributed to the lotic and lentic/very lentic classes due to a fraction of shared taxa. Taxonomic richness was not the focus of this study, but according to the intermediate disturbance hypothesis (Townsend, Scarsbrook & Dolédec, 1997; Mérigoux & Dolédec, 2004; Bendix, Wiley & Commons, 2017), we should expect the highest alpha diversity near this lentic-lotic range .

The majority of taxon optima (≈ 60%) fell in the lentic range. In particular, many OCH taxa were associated with extremely lentic conditions. This is not surprising because I worked in Mediterranean, potentially intermittent river reaches, and OCH taxa can be dominant in this type of river (Dolédec, Tilbian & Bonada, 2017). It is therefore likely that a high number of OCH taxa associated with habitats with not perceptible flow (Lobera et al., 2019), which are always observed when rivers approach their dry phase (Gallart et al., 2012), will be found here. In fact, in the early stages of drought, reduced flow and altered flow types result in a decrease of rheophilous taxa counterbalanced by an increase of taxa typical of lentic habitats (Rose, Metzeling & Catzikiris, 2008). These latter taxa are thus expected to show a clear association with river stretches where habitats tend to become lentic recurrently, such as the river systems I investigated in Sardinia. Mostly, the numerous taxa linked to extremely lentic conditions are expected to be present alternatively to each other. While the balance between flow types defined the main gradient of variation for benthic taxa, a range of other factors was related to the communities. The degree of flow disconnection, the water physiochemistry and the presence of organic detritus were significantly associated with the distribution of taxa and were the main disarranged factors in the studied system. All these factors are highly influential for benthic communities in Mediterranean and intermittent rivers (e.g., Bonada et al., 2006; Karaouzas et al., 2019; Lake, 2003; Lobera et al., 2019). For instance, the disconnection of flow, here quantified as the number of dry zones in a river stretch and significantly associated with the benthic taxa distribution, is expected to be highly relevant in shaping benthic communities (Arscott et al., 2010; Gallart et al., 2012; Rose et al., 2008). In such situations, extreme habitat variability regulates the presence and distribution of taxa. This helps clarify why extremely lentic conditions show the highest number of associated taxa. In addition, here, we might expect a high beta diversity (Hawkins, Mykrä, Oksanen & Vander Laan, 2015). However, the importance of biological factors will have to be considered in an assessment of more detailed taxa preferences under extreme conditions because variations in the spatial distribution of individual species may have knock-on effects on interspecific interactions (Lancaster & Downes, 2010).

5 CONCLUSION

The interpretation of the results in this paper supports a wide understanding of the following points:

i) The main factor governing the distribution and optima of taxa along the lentic-lotic gradient is the range of flow types at the site. This determines the main taxonomic variations from the very lotic end of the gradient to the end where the water velocity and turbulence are null, resulting in flow cessation. In this range, near-bed hydraulics and the resulting benthic forces act intensively on benthic communities.

ii) Along the continuum of flow-type variation (i), where diverse lotic, lentic and transitional features coexist in the river stretch i.e., under intermediate lentic-lotic conditions, high habitat diversification is observed, and many taxa can find suitable conditions to colonize the river and survive. We expect the highest alpha diversity (e.g., taxonomic richness) under these conditions.

iii) In the low flow season and/or when a drought is approaching, if/when the flow becomes predominantly or totally not perceptible, the degree of connectivity among aquatic habitats, the physio-chemical properties of the water and biological interactions strongly influence the presence of taxa. In these extremely lentic conditions, a large variety of different, alternative habitat conditions can be observed. Correspondingly, taxa associated with extremely lentic habitats are selectively present, depending on a range of environmental features that are no longer dependent on surface flow, e.g., temperature, water physio-chemical characteristics, the presence of organic detritus, and vertical connectivity. River reaches under these conditions (oligorheic or arheic aquatic state) are expected to show high levels of beta diversity.

As far as the applied issues are concerned, most invertebrate-based assessment systems involve the use of metrics and indices that assign progressive indicator values (and scores) to the presence of taxa belonging to different taxonomic groups. Most benthic metrics, when used to support an evaluation of the ecological status, likely incorporate a relevant reflection of the variation related to the lentic-lotic character of rivers that is not necessarily linked to the degree of habitat alteration and water quality variation. Additionally, the definition of biological reference conditions, both for taxonomy- and trait-based metrics, will presumably need adaptation to the actual lentic-lotic conditions found at different river types and sites. Especially under a changing climate scenario, the lentic and lotic characteristics of habitats should be regularly assessed to strengthen the biological understanding of river ecosystems.

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Data Availability

The data that support the findings of this study are partly shown in the supporting information section. Other data are available from the author, upon reasonable request.

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CHAPTER 4

The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification

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The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification

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ABSTRACT

1. Biological quality in rivers based on benthic macroinvertebrates is often assessed by comparison with the expected reference conditions, which represent relatively undisturbed situations. Commonly, reference conditions are set in agreement with river typologies to handle major ecological differences and to limit natural variability. Even if natural variations can be highly influential, site-specific tuning of reference conditions is rare in Mediterranean countries.

2. River flow and local hydraulics change continuously over time, determining changes in the occurrence of lentic and lotic habitat features. Thus, biological reference conditions might require a site-specific adjustment in function of the ratio of lentic to lotic habitat features expected at the time of sampling. This would help reducing systematic bias in ecological assessments, interpreting benthic invertebrates responses to pressures and diminishing the amount of unexplained biological variability.

3. The responses to the lentic-lotic character of river reaches were assessed for nineteen macroinvertebrate metrics and indices commonly used for the classification of ecological status in South European rivers, by piecewise spline regression analysis. The study sites, with a prevalent temporary character, were located in Sardinia, South Western Italy.

4. Most metrics were significantly related to the lentic-lotic habitat conditions, both in pool and riffle mesohabitats, and their response trends were parabolic or decreasing lotic to lentic. Taxonomic richness, score-based metrics, ovoviviparous taxa and multi-metric indices related well to the lentic-lotic conditions, while abundance metrics correlated less.

5. The potential impact on ecological status classification was tested for the method formally in use in Italy, which had a major role in comparing and inter-calibrating European assessment methods for the Water Framework Directive. After adjusting for bias due to the ratio of lentic to lotic habitat features, quality classification shifted towards better ecological status for ≈ 23% samples. This exemplified the likely impact of ignoring lentic-lotic information when defining reference conditions for assessing ecological status. The potential for considering the responses of invertebrate metrics to the lentic-lotic conditions in relation to climate change and e-flows setting was also briefly outlined.

1. INTRODUCTION

Water pollution has been a major concern for more than one century and is still highly relevant in many geographical areas and anthropized contexts (UNESCO, 2009; Schwarzenbach et al., 2010). To monitor the effects of water pollution and other impacts on aquatic ecosystems, biological and ecological approaches were introduced in the last decades. Traditionally, a high intrinsic and/or unexplained biological variability has been considered a major limitation, due to the consequent uncertainty in environmental evaluations (e.g. Chessman, Thurtell & Royal, 2006; Àlvarez-Cabria, Barquín, & Juanes, 2010). However, legislation to protect rivers worldwide now requires the assessment of 'ecological and chemical status' e.g. for the Water Framework Directive in Europe (WFD; EC, 2000), 'chemical, physical, and biological integrity' in the U.S.A. (Hawkins, 2015), or similar evaluations in other geographical areas (e.g. Chile, Australia; Dallas, 2013). Often, the quality classification of a river site is provided by comparing e.g. its biological attributes to the expected 'reference' conditions, representing relatively undisturbed situations. Any numerical value chosen to quantify the reference conditions has therefore a crucial role in the whole classification process and in subsequent management plans (Nijboer et al., 2004). In fact, such value supports the calculation of Ecological Quality Ratios (EQRs, for the WFD) used to assign the river site/sample to a quality class. The core biological attributes are often summarized by means of metrics illustrating different aspects of the community e.g. alpha diversity, abundance, biological traits, and providing an insight on potential causes of degradation. As far as benthic invertebrates are concerned, common examples of such metrics are the total number of taxa collected, the number of taxa belonging to the Insect orders of Ephemeroptera, Plecoptera and Trichoptera (EPT) or the Shannon-Wiener index. Different metrics are used in different contexts and they are typically often combined into multi-metric indices. Mostly, biological reference conditions are differently set between river macro-types to handle major ecological differences, with regionalization, typologies and special a priori classification schemes habitually used to improve the estimates of reference conditions (Hawkins, Olson & Hill, 2010). As well, seasonal adjustments of the expected values for metrics and indices should possibly be provided (e.g. Linke et al., 1999; Feio, Reinoldson & Graça, 2006), to account for biological cycles of aquatic organisms, which might not be present in the river throughout the year (Johnson et al., 2012). For instance, Chen et al. (2014) demonstrated that multi-season and multi-year adjustments are an effective way to diminish natural seasonal variation in multi-metric evaluations. In addition, reference conditions are sometimes distinct for pool and riffle mesohabitats (Brabec et al., 2004; Buffagni et al., 2004), to represent the most important differences found within river stretches for the combined effects of river discharge and channel morphology. However, even at the same site and without anthropogenic disturbance, river flow and the resulting local hydraulics change continuously over time. Such changes are obvious in Mediterranean regions, where dramatic floods in high flow periods contrast potential droughts in the dry season (Cid et al., 2017). Therefore, the ratio of lentic to lotic habitats found in the river fluctuates accordingly, following supra-seasonal, seasonal and even shorter time-scale flow variations. Hence, a generic adjustment of reference conditions to e.g. river type or season might not be sufficient to encompass the natural habitat variation acting on invertebrate communities (De Girolamo et al., 2017). Benthic macroinvertebrate presence and distribution likely respond continuously to changing habitat conditions as they result from concomitant flow events, morphological features, biological interactions and ecological processes. All riverine ecosystems are inherently dynamic and show a range of abiotic and biotic conditions varying over time. Simply, there is no reason why we should expect a single value to represent efficiently the expected biological reference condition if the environment is continuously changing and/or is highly heterogeneous (Vander Laan & Hawkins, 2014). Predictive modelling is a common way to obtain site-specific estimates. Its application can lead to improvements of the accuracy and precision of reference condition setting and its use should be encouraged (Hawkins, Olson & Hill, 2010). However, data scarcity in low flow rivers is still a relevant issue (Bangash et al., 2012) and the official use of basic, typology-based assessment systems is common e.g. in southern Europe. We expect that the adoption of fixed reference conditions i.e., an 'average' situation that should correspond to typical habitat conditions, might determine noticeably wrong quality attributions in the absence of adjustments for natural factors when habitat diverges from common situations (Hawkins, Cao & Roper, 2010). This circumstance might conceal the evaluation of the effects of policies aimed at reducing the impact of water pollution or confound restoration strategies for improving river habitats.

In this paper, we test if biological attributes of the benthic community relate to the ratio of lentic to lotic habitats found at the time of sampling. Such proportion results from the combined effects of all the natural and anthropogenic influences acting on river hydrology, hydraulics and morphology (Frissell et al., 1986). Likely, multi-metric indices sum up the effects noticed on individual macroinvertebrate metrics. Hence, checking their performance in function of the ratio of lentic to lotic habitats is highly relevant for classification and legally binding concerns (Buffagni et al., 2009). The key idea behind this paper is that biological reference conditions used in most assessment systems might require a site-specific adjustment in function of the lentic-lotic habitat conditions observed at the time of sampling. This would help reducing systematic bias in ecological assessments, interpreting benthic index responses and diminishing the amount of unexplained biological variability. Our main objectives are: i) to assess if a range of macroinvertebrate metrics commonly used for the classification of ecological status respond to the lentic-lotic character in Mediterranean rivers; ii) if a response is evident, to describe the overall relationship between benthic invertebrate metrics and the ratio of lentic to lotic habitat features, for both pool and riffle mesohabitats. The macroinvertebrate metrics considered cover a large part of South Europe, from western (Portugal) to eastern (Cyprus) limits, and are all included in official National methods. To provide an example of the impact of paper results on classification issues, additional focus will be placed on the STAR_ICM index (Buffagni et al., 2006; 2007), which is in use in Italy and elsewhere to classify ecological status for the WFD (MATTM, 2010; WDD, 2016; Lazaridou et al., 2018). The relationships that describe the response of the STAR_ICMi to lentic-lotic conditions will be used to approximate site-specific, habitat-adjusted reference conditions. We believe that the proposed approach is appropriate for Mediterranean rivers, and we expect a potentially high impact on the large scale.

2. MATERIAL AND METHODS

2.1 Study area and site selection

This study was conducted in Sardinia (South Western Italy), the second largest island in the Mediterranean Sea. The territory exhibits a Mediterranean climate with dry and hot summers and mild and relatively rainy winters (Mulas et al., 2009; De Waele et al., 2010). Investigated river reaches have temporary character showing high seasonal and supra-seasonal variability (Mulas et al., 2009). Such variability leads to significant differences among studied river stretches in terms of habitat availability as defined by lentic and lotic conditions (Buffagni et al., 2009; 2010; Buffagni, 2020). In a territory showing a large proportion of natural or semi-natural land uses (≈60%, Cilloccu et al., 2003), Sardinian rivers may be affected by an assortment of anthropic alterations that include poor water quality (RAS, 2005) and local morphological impairment (Buffagni et al., 2016).

We sought to achieve a sufficiently extended dataset to ensure statistical robustness. We therefore included a conspicuous number of sites (20) in reference conditions (sensu Feio et al., 2014 and MATTM, 2010) together with non-reference sites exhibiting some degree of morphological impairment, nonetheless avoiding as much as possible pervasive alterations and severe water pollution. Through this approach we considered a gradient of occurrence of lentic and lotic habitats without taking into account the role of morphological alteration in determining the lentic-lotic character. For easier comprehension, sites were qualitatively grouped in three categories of morphological alteration (Table S1). Regarding water quality, all sites are classified in 'high' or 'good' status – except for one in 'moderate status' – according to LIM_{eco} descriptor, formally used in the national Italian legislation (DM 260/2010, MATTM, 2010) for classifying rivers on the basis of nutrients concentration and oxygen saturation. Lastly, we verified the absence of the following pressures (see also Buffagni, 2020): i) intense upstream ponding, ii) tidal influence on local hydrology, iii) recent disturbance from maintenance works. In addition, comparability among stream types was checked considering that according to Italian legislation investigated sites share the same biological reference conditions for benthic invertebrates. Aggregated information on measured physiochemical variables – i.e. water concentration of O_2, N-NO_3, N-NH_4, P-PO_4, plus Cl^-, pH and conductivity – geographical data and a brief description of morphological conditions is provided in table S1. In total 59 river reaches were eventually selected. Biological and environmental data were simultaneously collected in February–August 2004 (22 sites), May 2011 (24 sites) and March 2013 (13 sites). The list of studied sites including geographic coordinates is reported in table S2.

2.2 Reach-scale survey and quantification of the lentic-lotic character

Habitat data were collected with the CARAVAGGIO method (Buffagni et al., 2013), based on an RHS-derived protocol (Raven et al., 1997) extended to record the variety of habitats of Mediterranean rivers (Buffagni & Kemp, 2002). The survey is performed on ten transversal sections along a standard ≈500 m river stretch and includes visual assessment of features (e.g. flow types, substrates), measures (e.g. water maximum depth, wetted channel width) and counts (e.g. riffles, pools, bars) (see also Buffagni et al., 2016 for survey description). Data from CARAVAGGIO was used to assess the lentic-lotic character in the reach i.e., by calculating the Lentic-lotic River Descriptor (LRD, Buffagni et al., 2009; 2010), which summarizes the ratio of lentic to lotic habitat features. Features considered for calculation get a score < 0 if lotic and > 0 if lentic and include dominant and co-dominant flow type, channel substrate type, channel vegetation type/organic debris. The higher the lotic character, the higher is its negative score, e.g. 'Rippled' as primary flow type scores -0.5, 'Unbroken standing waves' scores -1. Conversely, high positive scores correspond to lentic features, e.g. +3 for extended presence of CPOM. The highest score (+8) is assigned for each flow interruption within the reach. Scores of the single features are summed up to obtain the descriptor. LRD values commonly range from ≈ -80 to ≈ +100. Full list of variables included in LRD calculation is available in Buffagni et al. (2010). Reference sites included in the dataset cover a broad range of LRD values between ≈ -28 and ≈ +85; a comparable, wide LRD gradient was observed for non-reference sites (see Tab. S2).

2.3 Benthic invertebrate survey

A riffle-pool sequence was identified as first step (Buffagni et al., 2001). The sequence is recognized as two adjacent areas characterized by comparatively different turbulence, depth, substrate size. Within each area, benthic invertebrates were collected following a multi-habitat approach where mineral and organic substrates are sampled proportionally according to their occurrence. Providing the same sampling effort, ten sampling units were gathered in each of the two areas. Each sampling unit is a 0.05 m^2 Surber sample (500 μm mesh) resulting in two 0.5 m^2 samples, one in pool and one in riffle. As acknowledged for Mediterranean river types, where water scarcity may alter habitat availability and riffle-pool sequence features (Bonada et al., 2007), we observed a strong variability in the intrinsic features of riffle and pool areas. For instance, at the time of sampling riffles may cover a limited area within the channel and pools exhibit wide variability in depth and turbulence in different reaches, although they are usually dominated by low to null flow water velocities. According to the Italian national standard (MATTM, 2010), only samples collected from the pool mesohabitats are required for WFD ecological status classification for operational monitoring in

Table 4.1. Benthic metrics tested for response to the reach scale ratio of lentic to lotic habitat features.

Metric group	Code	Description	South Europe CY	FR	GR	IT	PT	SP	Other EU countries	Outside EU	Original metric description
Richness	n_families[1]	Total Number of benthic invertebrate families	✓	✓		✓	✓	✓	✓	✓	Plafkin et al. (1988)
	EPT_families[1]	Number of families of Ephemeroptera, Plecoptera and Trichoptera	✓			✓	✓	✓	✓	✓	Plafkin et al. (1988)
Diversity	Evenness	E = D/ln S (with D: Shannon-Wiener Diversity index; S: total number of taxa)					✓			✓	Pielou (1966)
	Shannon[1]	Shannon-Wiener Diversity index $H' = -\sum_{n=1}^{n}(p_i * \ln p_i)$ where p_i is the proportion of each taxon in the sample	✓	✓		✓			✓	✓	Shannon & Weaver (1949)
Biological trait	Ovoviviparous	Relative abundance of ovoviviparous taxa		✓							Dolédec et al. (1999)
	Polyvoltine	Relative abundance of polyvoltine taxa		✓							Dolédec et al. (1999)
Scored indices	IBMWP	Biological Monitoring Working Party score - Iberian version						✓			Alba-Tercedor & Sánchez-Ortega (1988)
	HES	Hellenic Evaluation Score			✓						Artemiadou & Lazaridou (2005)
	ASPT[1]	Average Score Per Taxon	✓	✓		✓			✓	✓	Armitage et al. (1983)
	IASPT	Average Score Per Taxon - Iberian version					✓	✓			Rodriguez & Wright (1988)
	AHES	Average Hellenic Evaluation Score			✓						Artemiadou & Lazaridou (2005)

Table 1. Continued

Metric group	Code	Description	South Europe						Other EU countries	Outside EU	Original metric description
			CY	FR	GR	IT	PT	SP			
Abundance	Sel_EPTD[1]	$\log_{10}$ (sum of abundance of Heptageniidae, Ephemeridae, Leptophlebiidae, Nemouridae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratiomyidae, Dixidae, Empididae & Athericidae +1)	✓			✓			✓		Buffagni et al. (2006; 2007)
	Sel_EPTCD	$\log_{10}$ (sum of abundance of Leptophlebiidae, Ephemerellidae, Chloroperlidae, Nemouridae, Leuctridae, Philopotamidae, Limnephilidae, Psychomyiidae, Sericostomatidae, Elmidae, Dryopidae & Athericidae +1)					✓	✓			INAG (2009); Munné & Prat (2009)
	Sel_ETD	$\log_{10}$ (sum of abundance of Heptageniidae, Ephemeridae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratiomyidae, Dixidae, Empididae & Athericidae+1)					✓				INAG (2009)
	1-GOLD[1]	(1 - relative abundance of Gastropoda, Oligochaeta, and Diptera)	✓			✓			✓		Pinto et al. (2004)
Multi-metric indices	IMMi-T	Iberian Mediterranean Multimetric Index						✓			Munné & Prat (2009)
	$IPtI_N$	Invertebrates Portuguese Index North					✓				INAG (2009)
	$IPtI_S$	Invertebrates Portuguese Index South					✓				INAG (2009)
	STAR_ICMi	STAR Intercalibration Common Metric Index	✓			✓			✓		Buffagni et al. (2006; 2007)

[1]Component metric of the STAR_ICM index.

Mediterranean rivers. In our analyses, to better depict the overall site conditions, we focused on samples from both pool and riffle mesohabitats, for a total of 118 samples (2x59). The identification level for invertebrates varied from species (e.g., for most taxa of Ephemeroptera, Plecoptera and Odonata) to family (e.g., Diptera and Trichoptera) (more details in Buffagni, 2020). Benthic indices and metrics were calculated on family level taxa lists.

2.4 Benthic metrics

Metrics here explored (Table 1) have been selected as part of multi-metric indices officially in use in South European countries for river ecological assessment (EU, 2018; Stubbington et al., 2018). Additional criteria for metric selection were the ease of retrieving information about their calculation and the identification based on family level. A portion of the considered metrics are also included in official assessment methods for a variety of river types throughout Europe (Birk et al., 2012) and used in combination with other metrics for invertebrates-based ecological quality methods worldwide, in original or adapted version. Some metrics have a long tradition of application (e.g. Armitage et al., 1983; Plafkin, 1989) but are still in use (e.g. Mondy et al., 2012) and are often included in the development of new assessment systems (e.g. Arman et al., 2019; Mao et al., 2019). Additionally, four official multi-metric indices were embraced: STAR_ICMi (Buffagni et al., 2006; 2007), IMMi-T (Munné & Prat, 2009), $IPtI_N$ and $IPtI_S$ (INAG, 2009). As well as being national assessment method in Italy, Cyprus and Greece (for very large rivers), the STAR_ICM index and its component metrics have been used throughout Europe for the WFD Intercalibration of ecological status (Bennet et al., 2011). Composition and calculation of multi-metric indices and metrics are reported in Table S3.

Based on their features, we grouped metrics into the following categories: 'Richness', i.e. the number of total or selected taxa in the community; 'Diversity', measuring the diversification of taxa composition; 'Biological traits', based on taxa biological qualities (e.g. voltinism); 'Scored indices' (raw or averaged), cumulative score assigned to taxa according to their tolerance to a given pressure that may be averaged by the number of taxa; 'Abundance', considering absolute or relative abundance of selected taxa and 'Multi-metric indices', combining multiple metrics from different categories. In some groups a 'guiding' metric has inspired the others of the same category. It is the case of scored indices, all derived from the BMWP/ASPT approach (Armitage et al., 1983). Similarly, abundance metrics Sel_EPTD, Sel_EPTCD and Sel_ETD are all based on the same concept of selecting taxa on the basis of their trophic/habitat value (Buffagni et al., 2004).

Response to lentic-lotic character was verified for all the selected metrics and multi-metric indices while classification of ecological status was performed according to the STAR_ICMi only.

2.5 Statistical analysis

2.5.1. Responses of invertebrate metrics to lentic-lotic habitat features

For finding the right balance between an accurate representation of the data and a direct interpretation of the resulting response curve, lowess smoothing (i.e. too flexible) and a direct fit of classical polynomial regression (i.e. potentially too rigid) were excluded. For its inherent fitting accuracy, piecewise spline regression provides the best characteristics in terms of representation goodness and interpretability (James et al., 2013). We fitted natural splines ('ns' function in R package 'splines'), that use additional boundary constraints, so that the function is required to be linear at the boundary (i.e. where the LRD value < smallest knot, or > largest knot). This generally produces more stable estimates at the boundaries (James et al., 2013). As well, to favour the curves continuity within the domain, we fitted them under the constraint that they must be derivable at knots. The predictor, namely the LRD variable, is transformed in a piecewise polynomial whose pieces are joined each other by points called knots. The knots selection is a critical stage of the fitting process that might heavily influence the accuracy of the resulting curve (Perperoglou et al., 2019). For the present case, knots selection was data driven. Fixed percentiles routinely selected by the software were tested. Likewise, the transition values that separate the MRT clusters (please, see § 2.5.2) were also used. For each metric, a range of possible solutions were compared, namely from no knots (i.e. linear fit) to up to 4 knots. Fitted models based on fixed percentiles and on MRT values as knots were compared. The models with all terms significant (i.e. intercept plus all individual piecewise segments) and the lowest p value were finally selected. These and further analyses were performed in the R v.3.6.1 software environment (R Core Team, 2019).

2.5.2 Discontinuities in invertebrate assemblages

For the identification of potential discontinuities in invertebrate assemblages, which may influence the delineation of relationships between the extent of lentic-lotic habitats and invertebrate metrics, we applied sequential clustering of benthic samples along the lentic-lotic gradient (Borcard, Gillet & -Legendre, 2018), by means of multivariate regression trees (MRT: De'ath, 2002). This technique creates a hierarchical tree with successive dichotomies, here in function of the LRD descriptor, with related invertebrate assemblages for each tree partition. No assumptions about the form of the relationship between taxa and LRD are made. Due to the relatively small number of observations, we used as many cross validation groups as rows in the data set, and 100 iterations. The minimum number of cases for each terminal node was set at four samples. Prior to analysis, benthic (log) abundances were chord transformed. Criteria for solution selection were lower CVRE and higher frequency of clusters number (Borcard et al., 2018). The analysis was performed on samples collected in the pool mesohabitat, which is here considered more representative for Mediterranean rivers. MRT was carried out by using the 'mvpart' package (Therneau et al., 2014). Between-clusters LRD separation values were tested as potential knots in spline regression analysis. These values were considered both individually (for one-knot fits) and in combination (for the two-knot fits).

2.5.3. Reference conditions and classification of benthic samples

To calculate the standard STAR_ICM index, the legally-binding reference values were used (MATTM, 2010). However, to derive site-specific reference values adjusted for the ratio of lentic to lotic habitat features, the basis spline functions and the piecewise polynomial coefficients of the best fitting models (§ 2.5.1) were used. The obtained curves represent the reference conditions expected for varying lentic-lotic (LRD) conditions and support a classification of ecological status arranged according to the LRD value observed at the time of sampling i.e. by calculating LRD-adjusted EQRs (named STAR_LRD). For illustrative purposes, the shape of the curve was considered generally representative for reference conditions even if samples from non-reference sites were included in the analysis (please see § 2.1). Finally, both the standard STAR_ICMi values and those obtained with the procedure above were compared to EU-harmonized class boundaries (MATTM, 2010; EC, 2018) for the R-M5 IC Italian macro-type: High-Good: 0.970; Good-Moderate: 0.729; Moderate-Poor: 0.490; Poor-Bad: 0.240. To estimate the magnitude of the difference between standard STAR_ICMi and STAR_LRD values, the Hodges-Lehmann estimator was used (Hollander and Wolfe, 1973), which is the median of all possible pairwise averages of the differences. A few dispersion statistics were used for exploring if the LRD-adjusted values tend to show different ranges and dispersion, compared to the original ones.

Table 4.2. Association of benthic invertebrate metrics to the ratio of lentic to lotic habitat features observed at the river reach scale. Models were accepted as statistically significant when all individual segments of the piecewise regression were significant.

		Pool mesohabitat					Riffle mesohabitat				
Metric category	Benthic metric	trend	p	R^2	LRD max-y	knot	trend	p	R^2	LRD max-y	knot
Richness	n_families	parabolic	0.0029	0.16	23.25	24.3	ns[1] (parabolic)	0.0402			
	EPT_families	straight	0.0000	0.25	lotic	-	straight	0.0146	0.08	lotic	-
Diversity	Evenness	straight	0.0018	0.14	lotic	-	straight	0.0473	0.05	lotic	-
	Shannon	straight	0.0061	0.11	lotic	-	straight	0.0922	0.03	lotic	-
Biological trait	Ovoviviparous	straight	0.0001	0.23	lentic	-	straight	0.0040	0.12	lentic	-
	Polyvoltine	ns	0.0966				ns	0.2402			
Score indices	IBMWP	parabolic	0.0054	0.14	11.25	-27.68	parabolic	0.0201	0.10	10.25	-27.68
	HES	parabolic	0.0133	0.11	14.25	3.49	parabolic	0.0293	0.09	11.25	-27.68
	ASPT	parabolic	0.0016	0.18	-38.82	24.3	straight	0.0015	0.15	lotic	-
	IASPT	parabolic	0.0006	0.21	-38.82	24.3	straight	0.0009	0.16	lotic	-
	AHES	straight	0.0013	0.15	lotic	-	straight	0.0007	0.17	lotic	-
Abundance	Sel_EPTD	ns[1] (parabolic)	0.0406				parabolic	0.0180	0.10	23.25	24.3
	Sel_EPTCD	straight[2]	0.0385	0.06	lotic	-	parabolic	0.0002	0.24	10.25	-27.68
	Sel_ETD	ns	0.2594				ns	0.0757			
	1-GOLD	parabolic	0.0146	0.11	21.44	24.3	ns[1]	0.0321			
Multi-metric indices	IMMiT	straight[2]	0.0013	0.15	lotic	-	parabolic	0.0000	0.28	4.5	-27.68
	$IPtI_N$	ns[1] (parabolic)	0.0027				ns[1] (parabolic)	0.0109			
	$IPtI_S$	parabolic	0.0001	0.26	4.5	-27.68	parabolic	0.0005	0.21	8.37	-27.68
	STAR_ICMi	parabolic	0.0005	0.21	8.51	-27.68	parabolic	0.0034	0.15	10.25	-27.68

[1]not all segments *
[2]parabolic fit similarly *

3. RESULTS

3.1. Benthic discontinuities in the lentic-lotic range

The multivariate regression tree analysis selected the highest number of times a three-group solution as the best one during the cross-validation iterations (Fig. S1). Globally, the one-variable analysis based on the lentic-lotic character explained 13% of the overall biological variance (RE = 0.87) in the MRT model, with a standard error of 0.085. The points indicating the discontinuities among groups were computed as the mean value between maximum and minimum observed in sites groups of the two branches of the regression tree. Accordingly, the interruptions in the lentic-lotic range were defined at 24.3 (first MRT split: R2 6.98%) and -27.78 (second MRT split: R2 5.65 %) LRD values. The value of ≈ 24 define cluster 3 whose sites are characterized by the presence of e.g. *Cloeon dipterum* (Linnaeus, 1761), *Anax spp., Orthetrum spp., Helobdella stagnalis* (Linnaeus, 1758), *Corixidae.* The LRD value of ≈ -28 differentiates the four sites (cluster 1) with the most extreme lotic character from the remaining sites, which show either lotic, intermediate or lentic conditions (cluster 2). The more clearly lotic sites are characterized by the presence of e.g. Rhyacophilidae nymphs.

3.2. Macroinvertebrate metric responses to the lentic-lotic gradient

Nearly 80% of invertebrate metrics displayed a significant association to the lentic-lotic gradient in pools and ≈more than 70% in riffles (Tab. 2). Natural splines values used in piecewise regressions and statistics of the final models selected for pools and riffles are reported in Table S4.

The proportion of ovoviviparous taxa was significant in both pools and riffles, while voltinism did not show an association to LRD. A patchy pattern was observed for abundance metrics, with Sel_ETD never significant, SelEPTD and 1-GOLD only significant in riffles and in pools, respectively. Benthic metrics representing all the other information categories resulted significant in both mesohabitats, apart from IPtIN and n_families in riffles, for which the association with LRD was not depicted clearly. The amount of variation explained by the share of lentic and lotic habitats (Tab. 2) for significant metrics goes from ≈ 3% (Shannon diversity) to more than 28% for the IMMiT index in riffles and from ≈ 6 % (Sel_EPTCD) to around 26% for the IPtIS index in pools. The average explained variance (adjusted R2) is higher in pools (0.16) than in riffles (0.14), and the same is true for metrics based on scores. For richness and diversity metrics, the association to the lentic-lotic gradient is notably higher in pools than in riffles. Multi-metric indices comprehensively show a relatively high (mean adjusted R2 0.21) and comparable level of variance explained in the two mesohabitats. Examples of the fitted models are reported in Figure 1. Overall, most metrics (58%; see Tab. 2) showed a parabolic response to the ratio of lentic to lotic habitat features (e.g. Fig. 1 a), while the remaining ones exhibited a linear response (e.g. Fig. 1 b). All the finally selected curves are reported in the Supplementary material (Fig. S2). A straight pattern was the best fit for diversity metrics, decreasing at increasing lentic conditions (Figure 1 c), and for few other metrics belonging to different categories (Figure 1 b; Tab. 2). In general, a parabolic curve fitted well the response of score-based metrics and multi-metric indices (Figure 1 e, f). When the parabolic fit was selected, usually the trend was nearly symmetrical, for both pools and riffles. The ASPT and IASPT metrics are an exception, because in pools the parabolic fit showed a gentle decrease in the lotic and intermediate range, while decreasing more sharply towards lentic conditions (Fig. 1 d). This response is then quite similar to the straight trend observed in riffles for the same metrics. The Sel_EPTCD and IMMiT indices showed a straight decreasing trend in pools and a parabolic response in riffles. However, further data should be analysed to define if this discrepancy deserves an ecological interpretation. Possibly, for these two indices, the information to be saved here is their clear response to

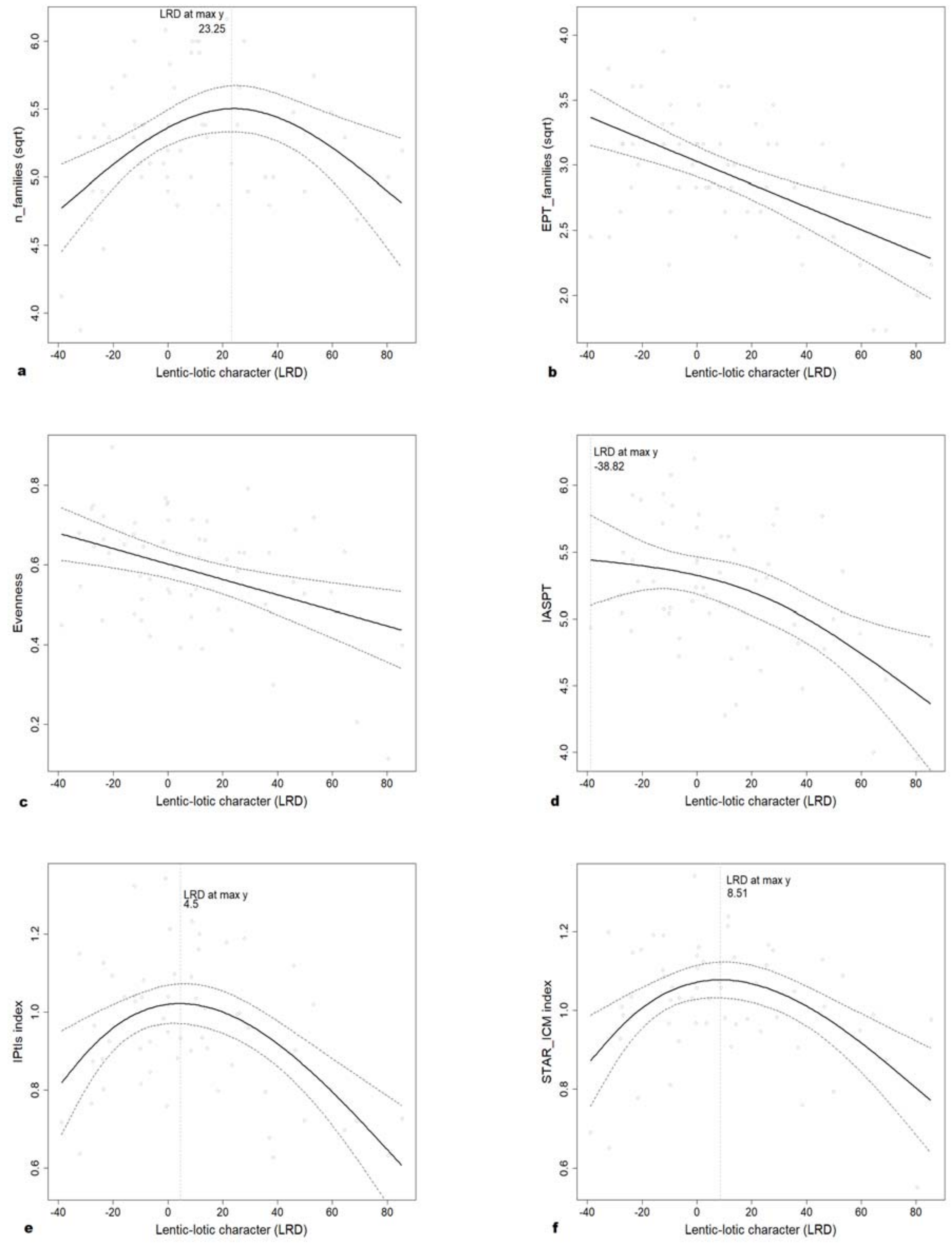

Figure 4.1. Models representing the variation of benthic invertebrate metrics as a function of the ratio of lentic to lotic habitat features (LRD). a) Total number of families; b) Total number of Ephemeroptera, Plecoptera and Trichoptera families; c) Evenness; d) Iberian Average Score Per Taxon; e) Invertebrate Portuguese Index South; f) STAR Intercalibration Common Metric index. The curves refer to the pool mesohabitat. Parabolic trends are based on natural cubic spline functions and vertical dashed lines show the LRD value at maximum-y in the model. Lotic conditions: left side of diagram, values < 0; lentic conditions: right side, values > 0.

the share of lentic and lotic habitats, leaving behind the assessment of the response shape.

When a non-straight model was selected, one knot always supported the best fit i.e. a single discontinuity in the lentic-lotic range was highlighted by the piecewise natural spline fitting. In all parabolic cases but one, one of the two values defined by the MRT analysis was selected as knot (Tab. 2). The only exception is the HES score metric, for which the split of the data based on the median dataset LRD value (3.49) supported the best fit. The discontinuity in the lotic range (-27.68) was always appropriate for multi-metric indices and IBMWP, while that on the lentic side (24.3) was applicable for the total number of families. In general, the lentic thresholds (knots) were more relevant in pools than in riffles, where the lotic knot predominated.

Both in pools and riffles, maximum-y values of the fitted curves were comprised between 4.5 (after excluding the ASPT and IASPT metrics in pools, see above) and 23.25 LRD values. The average maximum-y LRD value was, respectively, 13.9 and 8.9 for pools and riffles. These values are very close to the limit between intermediate and lentic conditions (sensu Buffagni et al., 2010) yet corresponding to rather balanced conditions between lentic and lotic habitats. This is especially the case for multi-metric indices, whose maximum-y values ranged between 4.5 and 10.25. Conversely, a few benthic attributes showed a distinct y-maximum in lentic conditions i.e. the total number of families, 1-GOLD (pools) and Sel_EPTD (riffles).

Models were derived from a dataset including reference sites together with sites at a certain degree of alteration. Despite this, median values of most metrics calculated from the dataset in pool mesohabitat were higher than those reported in the Italian legislation (DM 260/2010) for reference conditions, e.g. ASPT (DM 260) = 5.667, dataset median = 5.947; Sel_EPTD (DM 260) = 1.785, dataset median = 1.826). In riffles, obtained median values were in line or only slightly lower than the Italian official values indicated for EQR calculation. Likely, the overall situation depicted by the dataset used here approximated reasonably reference conditions as defined for the purposes of WFD classification. All metrics were highly variable, ranging well above and below their median value.

3.3. The impact of lentic-lotic conditions on ecological status classification

STAR_ICMi standard (i.e. official) values (STAR, in Fig. 2), calculated from fixed reference conditions i.e., the legally-binding value, were compared to STAR_ICMi values adjusted on a site-specific basis i.e., calculated as a function of the share of lentic and lotic habitats (STAR_LRD, in Fig. 2).

To calculate the STAR_LRD values it was assumed that the fitted model (described in 3.2: Figure 1 f; Table S4) represents hypothetical reference conditions. The difference (Hodges-Lehmann estimator) between the values obtained with the two STAR_ICMi versions is ≈ 0.05 in both reference and non-reference sites, for pool and riffle mesohabitats. Further results are reported in Table S5. Ecological status classification obtained from standard and LRD-adjusted STAR_ICMi values is shown in Fig. 2 (see also Table S6). Respectively, 30% and 25% of pool and riffle reference samples got a better status classification when site-specific, LRD-adjusted reference conditions were used. Among non-reference sites, the percentage of samples improving ecological status was respectively ≈ 28% in pool and ≈ 13% in riffle.

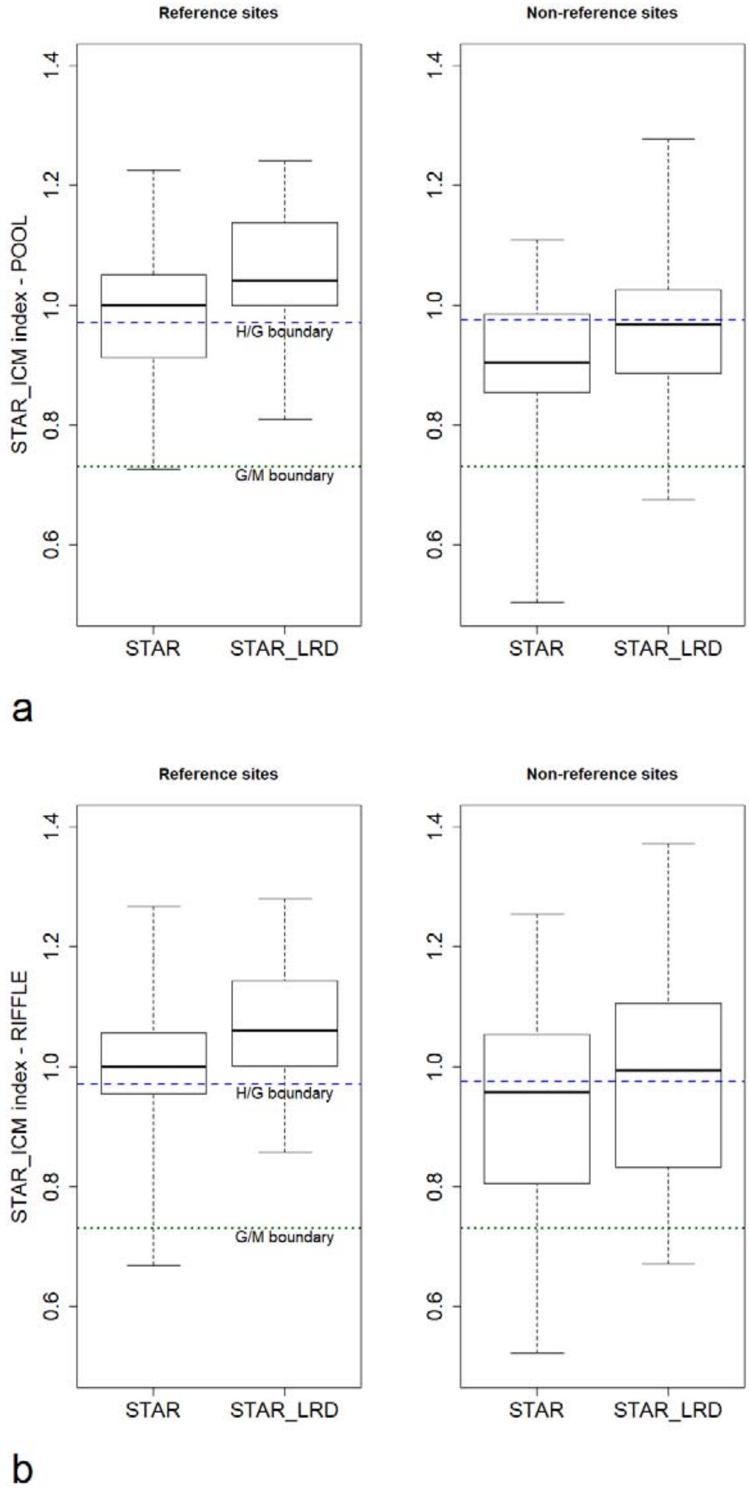

Figure 4.2. Distribution of STAR_ICM index values at reference and non-reference sites, for pool (a) and riffle (b) mesohabitats. Standard STAR_ICMi values (STAR) are paralleled by those obtained after habitat-adjusting the index based on the ratio of lentic to lotic habitat features (STAR_LRD). Horizontal lines represent the legally-binding class boundaries for the classification of ecological status (dashed/blue line: High-Good boundary; dotted/green line: Good-Moderate boundary).

4. DISCUSSION

4.1. Do macroinvertebrate metrics respond to the lentic-lotic habitat conditions?

The ratio of lentic to lotic habitat characteristics observed at the time of sampling rises from how river discharge occupies the channel, determining the presence and proportion of flow-related features that characterize the river bottom (Buffagni et al., 2010). The actual lentic-lotic conditions firstly depend on natural, seasonal and supra-seasonal i.e., climatic, factors. Indeed, the proportion of such habitats is also influenced by the history of the river site for e.g. channel modification and resectioning, and by larger scale factors e.g. regime alteration and water intakes. The focus here was the overall habitat conditions at the time of sampling, which result from all the above-mentioned factors and might influence the structure of the benthic invertebrate community (Chessman, Thurtell & Royal, 2006). We found a direct and significant association between lentic-lotic habitat conditions (i.e., LRD) and almost all the biological metrics tested. This result strengthens the conclusion of Buffagni et al. (2009), who recognized the value of the lentic-lotic character of river reaches for explaining the variability of benthic metrics in eleven datasets ranging from lowland to alpine streams across Europe. Ecological status classification can depend on microhabitat composition and character (Buffagni et al., 2019), especially because different taxa may show preference for different microhabitats (e.g. García-Roger et al., 2011). On this regard, the prominence of lentic and lotic habitat conditions for a range of benthic taxa inhabiting Mediterranean rivers and the scattering of taxa optima along the LRD gradient have been recently explored (Buffagni, 2020). That study demonstrated which habitat variables, among those used to calculate LRD, mostly related to the assembly of the benthic community. Among these, no perceptible flow (mostly associated to lentic conditions) and unbroken waves flow (linked to lotic situations) had a critical role, suggesting local hydraulics and turbulence were important factors in regulating communities in relation to the proportion of lentic and lotic habitat units. However, the relevance of the linkages among lentic-lotic conditions and invertebrates was established beyond the hydraulic concern (Buffagni, 2020). Overall, more than 80% of benthic taxa significantly related to the lentic-lotic gradient, which is expected to correspondingly influence the response of benthic metrics. All metric categories showed a significant response to the LRD gradient, even if abundance metrics seem to correlate less than other metrics. Biological traits are expected to react promptly to important habitat changes (e.g. Feio & Dolédec, 2012). Accordingly, one of the two trait-based metrics presently included in south European assessment systems and tested here i.e., the relative proportion of ovoviviparous taxa, was significantly related to LRD. This is possibly related to the aptitude of this trait to depict the degree of temporariness of the studied rivers. In fact, when drought approaches, extremely lentic habitats tend to predominate. Voltinism was not related to the lentic-lotic conditions, probably because the most abundant species in the studied area are multivoltine. The response of metrics based solely on abundance was in general weak, and this might be partly related to the intrinsic high variability of taxonomic abundance (Cid et al., 2017), which also possibly increased uncertainty in statistical estimations. However, abundance of benthic taxa is expected to react to flow changes in the range of those governing lentic-lotic conditions (Mèrigoux et al., 2015) and further analysis is needed.

The method we used to quantify the ratio of lentic to lotic habitats i.e., the LRD descriptor, is based on a range of individual pieces of information, picked up at different scales and summed up at the reach level (≈ 500 m). However, it accounts for a specific habitat aspect in the gamut of potentially relevant natural factors that can affect benthic invertebrates. The high percentage of explained variance observed here for a range of metrics e.g. multi-metric indices (up to 28%), is thus somewhat surprising, because based on one variable only.

Consequently, when amendments to assessment systems are to be founded on reach scale habitat information, we think the ratio of lentic to lotic habitats should be considered in what Hawkins, Cao & Roper (2010) called ‘the challenge of adjusting for natural variability’.

4.2. What kind of response to lentic-lotic conditions?

Taxonomic richness, averaged scores and multi-metric indices related to the ratio of lentic to lotic habitats. This result was not surprising because different taxa and taxonomic groups present optimum values or ranges in correspondence to distinctive lentic-lotic conditions (Buffagni, 2020). Thus, the differences in optima location likely influence the value obtained for these metrics, whose calculation is explicitly founded in most cases on different weighting of taxa presence. The occurrence of many taxa can be limited during or after high flow events that stress macroinvertebrates (e.g. Kakouei et al., 2017) by a combination of current velocity (Holomuzki and Biggs, 2000), local shear stress conditions (Dolédec, Lamouroux, Fuchs & Mérigoux, 2007), hydraulic stress (Mèrigoux & Dolédec, 2004) and substratum stability (Schwendel, Death & Fuller, 2010). In addition, the efficiency in collecting samples in those conditions can be limited. On the other hand, when flow decreases, habitat space is generally diminished and effects on invertebrates are expected especially in relation to the presence of habitat refugia and low flow period duration (Gjerlov et al., 2003; Suren & Jowett, 2006). In low flow periods, the degree of connectivity among aquatic habitats, the physio-chemical properties of the water and biological interactions (Walters, 2011; Buffagni, 2020) might have strongly influenced the presence of taxa in the studied river systems. Moreover, during extreme lotic or lentic conditions specific in-stream habitats are lost, causing a lowering in biological metric values due to a loss in taxonomic richness and diversity (Lake, 2000). Conversely, when lotic, lentic and transitional features co-occur in the river reach i.e., under intermediate lentic-lotic conditions, high habitat diversification is noticed, and many taxa can find appropriate conditions to coexist and survive (Buffagni, 2020). Frequently, the maximum value of the benthic metric was here found in the proximity of the intermediate/lentic boundary i.e., under such intermediate lentic-lotic conditions. In general, aquatic organisms inhabiting rivers have to tackle either habitats with low hydraulic forces and less oxygen supply, or more stressful hydraulic habitats with higher oxygen supply (e.g. Maasri et al., 2019). According to the intermediate disturbance hypothesis (Townsend, Scarsbrook & Dolédec, 1997), a dome-shaped relationship might be expected between richness-based metrics and hydraulic forces (Mèrigoux & Dolédec, 2004). A combination of the abovementioned causes can explain the parabolic response to the ratio of lentic to lotic habitats showed by most metrics. However, for EPT_families, which includes rheophilous invertebrates, and for other metrics a linear response was observed with decreasing values from lotic to lentic ranges. Frequently, macroinvertebrate richness is negatively associated to increasing hydrological harshness (Rolls et al., 2018). Very lentic conditions and/or flow intermittency associated to minimum flow variables are expected to deplete richness (Belmar et al., 2013) and abundances of EPT sensitive taxa (Datry et al., 2014). The linear trend observed here in response to lentic-lotic conditions, as opposed to a parabolic trend, can be explained by the relatively short gradient covered by this study on the lotic side, with truly lotic environments marginally covered. Hence, the opportunity to depict a likely decrease of these metrics on the lotic side was limited. To describe exhaustively the shape of the response curve for EPT_families and for the other metrics that showed a decreasing linear response, a gradient including more extreme lotic conditions should be explored.

The relationship between biological metrics and the lentic-lotic conditions assessed at the reach scale (i.e., LRD) was evident both in pools and riffles. However, for ovoviviparous taxa, richness and diversity metrics the response in pools generally explained a higher proportion of biological variation. The observed differences can be explained by the physical features of the two mesohabitats. For example, richness metrics calculated from samples collected in riffle were less sensitive to the overall lentic-lotic condition. When present, riffles usually bear mostly flowing water habitats (Bonada, Rieradevall & Prat, 2007) and minor differences are observed between riffles of reaches showing prevailing lentic or truly lotic conditions. On the contrary, pool mesohabitats found in lotic reaches often bear both flowing and still water habitat units whereas when the dry season advances only still water habitats are found (Bonada et al., 2007). The harsher physio-chemical conditions found in pools when drought is approaching (Graeber, Pusch, Lorenz & Brauns, 2013), together with a limited habitat availability and stronger biological interactions (Lancaster & Downes, 2010), also support the higher association to lentic-lotic conditions observed in pools for the proportion of ovoviviparous taxa, richness and diversity metrics. However, both metrics based on averaged scores and multi-metric indices i.e., those most frequently used to assessing ecological status, showed a comparable average level of explained variance in pools and riffles. Therefore, the natural variability of the ratio of lentic to lotic habitats should be contemplated when using such indices, independently from what mesohabitat is chosen for the collection of macroinvertebrate samples.

4.3. Does the ratio of lentic to lotic habitats influence ecological status classification?

Hawkins, Olson & Hill (2010) clearly described how sampling variability i.e., the variation that would occur among replicate samples collected at a site at the same time, is usually confounded with other types of variation in ecological assessments. In addition to differences due to life-history traits of invertebrate taxa, floods, short- and mid-term temporal variation related to climate and drought periods can lead to biased predictions of reference conditions and incorrect inferences. At least disturbed sites, these latter factors control the ratio of lentic to lotic habitat characteristics (Buffagni et al., 2010; Buffagni, 2020). However, because of the resulting variation in invertebrate assemblages, evaluations based on comparisons of samples taken with specific lentic-lotic conditions with reference expectations derived from other lentic-lotic conditions will introduce a systematic bias in assessing ecological status. In fact, the observed bias between values of the STAR_ICM index found at optimal conditions i.e., where lentic and lotic features are rather balanced, and values expected at the extreme LRD ranges is up to > 20% (lentic side). Even if the relationships between the STAR_ICMi and the lentic-lotic conditions described in this study are not solely based on samples from reference sites (see Stoddard et al., 2006), the fitted curves exemplify the general shape of response for this index. Based on this response curves, we investigated how the classification of the studied rivers was affected by the ratio of lentic to lotic conditions. The modelled STAR_ICMi was used to estimate the habitat-adjusted expectation for the index along the lentic-lotic gradient and to calculate EQR values corrected for environmental variability. The average shift of values of the STAR_ICMi was around 5% for both reference and test sites, at both pools and riffles. For demonstrative purposes, flow regime was assumed undisturbed also at test sites. In terms of classification, the effect of the lentic-lotic conditions was more apparent in pools, where ≈ 28% and 30% samples, for non-reference and reference sites respectively, improved their ecological status class after refining the expectation based on LRD values observed at the time of sampling. In riffles, where the association of the STAR_ICMi with lentic-lotic conditions is weaker, the analogous shift in classification is ≈ 13% and 25%. Overall, adjusting the bias due to the ratio of lentic to lotic features, a classification shift towards better ecological status of ≈ 23% samples was noticed. This exemplifies the potential impact of ignoring lentic-lotic conditions – as largely done in Mediterranean regions – when assessing ecological status. Because the relationship with lentic-lotic conditions was significant for most of the invertebrate metrics used in the EU and outside to assess ecological status, we presume the impact on classification is widespread. We expect more important effects in the Mediterranean area that is characterized by high flow variability and heterogeneity (Feio et al., 2014), with many rivers naturally exhibiting a non-perennial flow regime (Skoulikidis et al., 2017) and frequently experiencing limiting lentic conditions.

Predictive modelling approaches for biomonitoring are recognised as advanced systems to improve the accuracy of ecological classification and disentangle the confounding effect of natural variability in relation to time, climate and hydrology (e.g. Hawkins, Cao & Roper, 2010; Hawkins, Olson & Hill, 2010). New predictive systems may comprehend an estimate of the lentic-lotic conditions. At present, few predictive models are in use in Europe for assessing ecological status based on benthic invertebrates (e.g. Clarke, Wright & Furse, 2003; Kokeš et al., 2006; Poquet et al., 2009). However, most Mediterranean countries use formally a multi-metric method that relies on a typological approach and usually does not depend on site-specific, abiotic data for its implementation (Hering et al., 2004). The infrequent availability of widely applicable, predictive models is due to a range of factors, which include data scarcity, large environmental variability, partly unknown taxonomy and complex biogeography. Likely, the same reasons will slow the development of predictive systems in the near future, at least in many south European countries. Therefore, approaches oriented at developing habitat-adjusted reference conditions for existing methods, like that proposed in this paper, can represent a ready-to-use solution for improving ecological assessment. In addition, Europe has recently completed the harmonization of official methods – largely multi-metric - used for classifying ecological status (EC, 2008; 2018). The comparison of invertebrate methods across the EU has been largely based on the STAR_ICMi (Buffagni & Furse, 2006; Bennet et al., 2011). Due to STAR_ICMi response to the lentic-lotic conditions, the results of such evaluations might need a revision focused on habitat discrepancies among rivers and site-specific tuning.

4.4. Needs, opportunities, and challenges using lentic-lotic condition approach in ecological assessment

A relevant outcome of this study concerns the description of biological reference conditions for ecological classification. A strict definition of sampling periods (Gallart et al., 2017) or the use of biological tools i.e., traits or specific metrics, to predict hydrological conditions (Cid et al., 2016) are sometimes used in south Europe to limit the variability observed at reference sites. However, when single or inflexible, seasonal-adjusted values are designated to calculate EQRs and sampling conditions for reference sites fall within a restricted lentic-lotic range, reference values would fail to characterize the actual range of potential situations (Vander Laan & Hawkins, 2014). Likely, they would result in scarce representativeness, causing a relevant bias for all other sampling circumstances (Hawkins, Olson & Hill, 2010). Quite the reverse, when the lentic-lotic gradient of the site is fully covered, a large and seemingly unexplained biological variability is incorporated i.e., scarce classification precision is associated to the method, for any assessment conditions (Buffagni et al., 2009). For both situations, the use of biological reference conditions modelled on the basis of the ratio of lentic to lotic features would support a site-specific tuning. This would increase classification performance and interpretability of results.

Apart from direct anthropic factors, climatic conditions eventually control the relative proportion of lentic and lotic habitat characteristics found in a river channel. Therefore, a combined use of climate-related information and macroinvertebrates can be helpful when assessing the effect of climate changes on river ecology. Large scale studies, e.g. Dhungel et al., (2016), emphasized that climate-driven changes in ecologically relevant flow regimes are expected to act strongly on variables related to low flows, which are those controlling the lentic-lotic characteristics most significant for invertebrate communities (Belmar et al., 2013; Buffagni, 2020). The same trend is expected for Mediterranean rivers in south Europe (e.g. De Girolamo et al., 2017).

The consequences of ignoring the lentic-lotic character of river sites being a potential confounding factor for ecological status assignment has been discussed above and elsewhere (Buffagni et al., 2009). Concurrently, the LRD was found as the most significant factor affecting invertebrate communities in Mediterranean mountain rivers and a relevant factor for benthic metrics in Alpine and central European mountain rivers (Buffagni et al., 2009). Additionally, an alteration of the ratio of lentic to lotic features per se can be useful to assess and quantify the effect of discharge reduction at river sites (Buffagni et al., 2009), because the lentic lotic conditions are affected by altered hydrology. Low or extremely low flow conditions are expected to lead to loss of benthic alpha diversity and biomass, due to declines or heavy modification of riffle and pool habitats (Poff et al., 2010), to exacerbated physiochemical water conditions and to the presence of a variety of alternative habitat characteristics (Buffagni, 2020). Long-term hydrological and ecological data are often required to apply existing e-flows methods (Poff et al., 2010). Unfortunately, such data are not promptly available for many south European river catchments and field information on the ratio of lentic to lotic habitat conditions, joined with macroinvertebrate data, may provide support in developing environmental flow standards.

In conclusion, the responses of benthic invertebrate metrics to the ratio of lentic to lotic habitat features observed at the time of sampling should not be ignored. Consequences would imply the difficulty of understanding biological responses to pressures (best scenario) and/or a largely biased classification of ecological status in many circumstances (worst scenario). A site-specific adjustment of reference conditions and classification based on the proportion of the lentic and lotic habitat characteristics should be conceived for future assessment programs based on invertebrate metrics in the Mediterranean area.

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I directly covered all main aspects of the manuscript. Co-authors provided support for field sampling, taxonomic identification, data curation, statistical setting and review & editing.

CHAPTER 5

Hydrology under climate change in a temporary river system: potential impact on water balance and flow regime

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Hydrology under climate change in a temporary river system: potential impact on water balance and flow regime

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ABSTRACT

The potential impacts of future climate scenarios on water balance and flow regime are presented and discussed for a temporary river system in southern Italy. Different climate projections for the future (2030–2059) and the recent conditions (1980–2009) were investigated. A hydrological model (SWAT) was used to simulate water balance at the basin scale and streamflow in a number of river sections under various climate change scenarios, based on different combinations of global and regional models (GCMs and RCMs). The impact on water balance components was quantified at the basin and sub-basin levels as deviation from the baseline (1980–2009), and the flow regime alteration under changing climate was estimated using a number of hydrological indicators (IHA). An increase in mean temperature for all months between 0.5–2.4°C and a reduction in precipitation (by 4–7%) was predicted for the future. As a consequence, a decline of blue water (7–18%) and total water yield (11–28%) was estimated. Although the river type classification remains unvaried, the flow regime distinctly moves towards drier conditions and the divergence from the current status increases in future scenarios, especially for those reaches classified as I-D (i.e. Intermittent-Dry) and E (Ephemeral). Hydrological indicators showed a decrease in both high flow and low flow magnitudes for various time durations, an extension of the dry season and an exacerbation of extreme low flow conditions. A reduction of snowfall in the mountainous part of the basin and an increase in potential evapotranspiration was also estimated (4–4.4%). Finally, the paper analyses the implications of the climate change for river ecosystems and for River Basin Management Planning. The defined quantitative estimates of water balance alteration could support the identification of priorities that should be addressed in upcoming years to set water-saving actions.

1. INTRODUCTION

In recent decades, changes in climate have caused impacts on natural and human systems on all continents (IPCC, 2014). The IPCC Report (2014) pointed out that climate changes (CC) are affecting the quantity and quality of water resources at global, regional and local scale. Around the world, several authors have analysed the effects of climate change on water resources focusing on trends over recent decades (Lorenzo-Lacruz et al., 2012) and how the predicted changes might affect hydrological regime, water quantity and ecosystems (Dhungel et al., 2016; Salmoral et al., 2015; Tang et al., 2015; Gallart et al., 2014; Barkhordarian et al., 2013; VijayaVenkataRaman, 2012). In Mediterranean climate regions (S-Australia, central Chile, coastal California, S-Africa, Mediterranean Basin) a warmed climate is predicted by all climate scenarios and due to the close relationship between precipitation and river discharge a consistent decrease in water resources is expected. Key results of some relevant studies are summarized in Table 1. The most common tools used in these studies to predict future climate are Global Circulation Models (GCMs) and Regional Climate Models (RCMs), which are based on the Special Report on Emission Scenarios developed by IPCC. Although these studies differ with respect to the methods used or variables analysed, all of them report significant changes in hydrological variables due to climate evolution. In particular, Poff et al. (1997) and Gibson et al. (2005) highlighted that decreasing rainfall amount produces alterations of river regime components such as timing, magnitude and duration of annual extremes, peak flows, and zero flow days and consequently an increase in pressures to aquatic ecosystems (e.g. flow reduction). However, knowledge gaps still exist about the direct and indirect effects of CC on non-perennial river systems and on resulting problems in their management. Non-perennial rivers cover more than 50% of the global river network, and diminishing water resources will increase their exposure to multiple stressors (Skoulikidis et al., 2017, Garcia et al., 2017b). The European Commission, in its document "Adapting to climate change: Towards a European framework for action" (EC, 2009 COM/2009/147), calls for a strategic approach to integrate climate change adaptation into the implementation of the EU water policy (Water Framework Directive, Floods Directive, EU Water Scarcity and Droughts strategy). Actually, climate change may restrain a straightforward use of fixed river typologies (i.e. a perennial river could be classified as an intermittent river in the near future) and affect ecological status classification (e.g. Nõges et al., 2007). Reference conditions for Biological Quality Elements (BQEs) will change and need to be evaluated periodically and, after that, quality assessment systems should be updated accordingly (Nõges et al., 2007; Buffagni et al., 2009). All these aspects have not been sufficiently studied until now, especially for temporary rivers, although some relevant aspects concerning the river basin management in a changing climate have been analysed in the Common Implementation Strategy for the WFD (Technical report 2009-040 Guidance Document N. 24; Nikolaidis et al., 2013, Prat et al., 2014).

The main objective of this paper is to analyse the potential impacts of future climate scenarios on water balance and flow regime in a temporary river system. Additionally, we aim to briefly outline and discuss the possible implications of these changes for the assessment of ecosystem health, bearing in mind some of the WFD central assumptions. The study area is the Candelaro river basin (2200 km2), located in the Puglia region (SE Italy). Our aim is on the one hand to give a contribution to the evaluation of climate change impact on water resources in the Mediterranean Basin and, on the other hand, to provide information to support long-term WFD-based water resources management and planning in the study area and in other similar basins.

MATERIALS AND METHODS

2.1. Study area: Candelaro river basin

The study was performed in the Candelaro river basin (CRB), located in the Puglia region in southern Italy (Figure 1). The basin is characterised by a mean elevation of 300 m above sea level, ranging from 0–1142 m. The drainage area is about 2200 km2 and the main river course has a length of 67 km. Water quality and water quantity problems are spread throughout the river basin, especially in the plain area where the main economic activity is intensive agriculture. In particular, an elevated nutrient level were found in surface waters, which can be attributed to the use of fertilizers, to the spreading of animal manure and point sources discharges (urbane sewage). Water abstractions and a dam which was built in 2000 for agricultural use purpose are the main hydrological pressures in the basin. The main farm products are durum wheat, tomatoes, olives, and vineyard. In the mountainous part of the basin, natural and man-made forest lands are present and pasture and cereal crops are quite frequent. In the hilly and mountainous areas, agriculture is not intensive and crops are not irrigated.

The Candelaro and its tributaries show a temporary character, with an absence of flow or extreme low flow conditions in most of the river segments during summer. The main tributaries of the Candelaro river are three: Triolo (Qave= 0.19 m3s-1, observed at m2 station in Fig. 1), Salsola (Qave= 1.26 m3s-1, obs. at m3 station in Fig. 1) and Celone (Qave= 0.48 m3s-1, obs. at m8 station in Fig. 1). The natural flow pattern includes a wetter season from the end of December to April and a low flow period from May till December. Generally, the highest monthly flows were recorded in March and April. The dry season can be very long, with a mean of about 140 days for no flow (zero days)

Table 5.1. A selection of relevant studies performed in the Mediterranean climatic region dealing with water balance and flow regime alteration in a changing climate. The current paper is added for completeness.

Related case studies	Study area	Data for current and future scenarios	Key results
Giorgi and Lionello, 2008	Mediterranean Region	Current: 20th century Future: 21st century	A pronounced decrease in precipitation and warming is projected, especially in the warm season. Precipitation change signal produced by the regional models shows substantial orographically-induced fine scale structure absent in the global models.
Abouabdillah et al., 2010	Tunisia	Current: 1986-2005 Future: 2010–2039 2070–2099	Significant reduction in maxima streamflow for different time duration are predicted in the Merguellil basin.
Moran-Tejeda et al., 2011	Spain	Trends over the period 1961-2006	A marked reduction in river discharge in winter and spring, leading to significant changes in both the magnitude and timing of flows was found in Duero basin (Spain) over the study period. These results indicate that river regimes have changed in the direction of reduced flow volumes and shifts in peak flows.
Erol and Randhir, 2012	Mediterranean Region	Current: 1950-1999 Future: 2100	A review of studies in Mediterranean countries shows that impacts of CC on surface flow, Et_0, water quantity and quality, river ecology and socio-economic aspects can be significant.
Schneider et al., 2013	Europe	Current: 1971-2000 Future: 2040–2070	CC will compromise river ecosystems and their vital ecosystem services. CC will modify hydrological regime in unequal way depending on climate zones. Flow magnitudes will be predominantly altered in the Mediterranean Region.
Demaria et al., 2013	Chile	Current: 1960-1999 Future: 2100	A drier and warmer future will shift the location of snow line to higher elevations and reduce the number of days with precipitation falling as snow and low flow conditions will intensify during the warm months.
Moyle et al., 2013	California	Current: 2002-2012 Future: 2100	Fifty percent of California's native fish fauna was assessed as having critical vulnerability to extinction whereas all alien species were classified as being less or least vulnerable.
Nerantzaki et al., 2015	Greece	Current: 1973-2010 Future: 2010–2049 2050–2089	In a complex and intensively managed watershed, an assessment of the future ecological flows revealed that the frequency of minimum flow events increases over the years. During the same periods, spring flow and the surface sediment export is decreasing (54.5%).
Liu, Q., 2016	California	Future: 21st century	CC increased water stresses from drought, reduced ecosystem services and affected the water and energy nexus and agricultural food production, as well as fish and wildlife habitats in California. The author developed a conceptual framework interlinking climate change with water, energy, food, and related ecosystem processes and provided a road map for long term planning.
Garcia et al., 2017a	Mallorca	Trend 1977-2009	Long-term reduction in number of days with flow in temporary streams; a decreasing trend of streamflow in spring and summer was found. Forest expansion and temperature increase caused less available water for the streams.
This study	Italy	Current: 1980-2009 Future: 2030–2059	River type classification remains unvaried, even if flow regime moves towards drier conditions. Hydrological indicators showed a decrease in both high flow and low flow magnitudes for various time durations, an extension of the period with an absence of flow and an exacerbation of extreme low flow conditions.

recorded from 1965 to 1994 along the main course of the Celone river, varying from 0 to 236 days. Similarly, 82 days with absence of flow were recorded over the same period along the Salsola, varying from 0 to 223 days.
The soils show a texture varying from sandy-clay-loam to clay-loam or clay and generally are related to the lithology. The average annual precipitation recorded in the catchment from 1990–2009 was 611 mm. Orographic factors have a great influence on rainfall amount and the rainfall patterns. The rainfall is mostly concentrated in autumn and winter; generally it has a great spatial variability and often occurs with high intensities of short duration.
At the European level, water resource experts and stakeholders discuss the need to incorporate climate change issues into the implementation of EU water policy (Quevauviller et al., 2012), but at local level the River Basin Authorities do not yet identify specific policies or recommendations that could be taken within the forthcoming years on various water-related aspects concerning climate change.

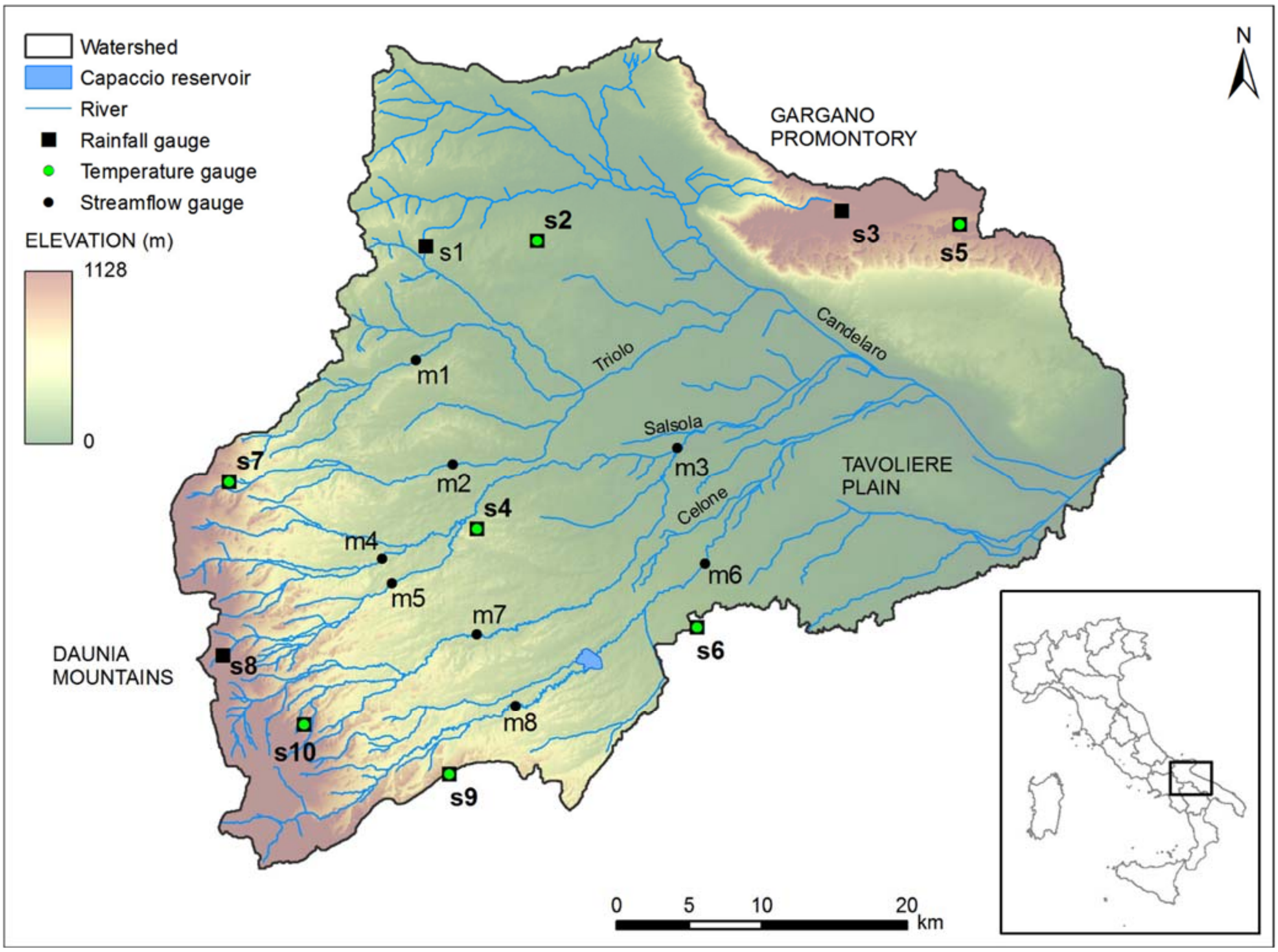

Figure 5.1. Study area. Candelaro river basin (Apulia, Italy)

2.3 Future climate scenarios and data

The most common tools used to predict future climate conditions are Global Circulation Models (GCMs) and Regional Climate Models (RCMs). Large scale GCMs have a resolution of around few 100s km, which makes them unable to represent the local and regional variability that is quite marked in the Mediterranean region (Quintana Segui et al., 2010). To represent this sub-grid variability, downscaling can be performed using Regional Climate Models (RCM). These RCMs of a few 10s km resolution can capture local variability, while boundary conditions are still driven by GCMs.

This downscaling can add to the uncertainties embedded in the GCM. These uncertainties could be reduced by spanning a broad range of GCM-RCM combinations, and further reduced by performing a bias correction (Christensen et al., 2008). In the present study a GCM [ECHAM5 (Roeckner, 2003)] and two RCMs [RACMO2 (van Meijgaard et al., 2008); RCA (Kjellstrom et al., 2005)] were used to derive the combinations for the climate changes scenarios. Data were obtained from FP6 ENSEMBLES Project. The scenarios are based on the A1B storyline. The A1 family of scenarios is based on a rapid economic growth, an increase in population until the Mid 21st Century, a decrease thereafter, and the introduction of new and more efficient technologies. The scenario A1B puts a balanced emphasis on all energy sources (IPCC, 2000). The combination we used, called hereafter KNMI, SMHI and MPI, is as follows:

KNMI_RACMO_ECHAM5

SMHI_RCA_ECHAM5

MPI_REMO_ECHAM5.

Each of the combinations has a 25 km resolution. Daily maximum and minimum temperature data as well as daily precipitation corresponding to these scenarios were extracted as a grid covering the study area for two periods, 1980–2009 and 2030–2059. We selected the period from 1980–2009 as a reference baseline from which to assess changes in climate. This period is representative of the recent average climate in the study region and encompasses a range of climatic variations, including severe droughts and cool seasons.

The most important part of statistical downscaling is to create a realistic model from the observed data (Maurer et al., 2010). This is a crucial point in future projection evaluations, especially in the Mediterranean Basin where precipitation shows high spatial and temporal variability. In this area, because of rainfall events being affected by orographic effects, a large difference in rainfall depths is often recorded in nearby gauging stations. This is also the case for the study area, where a large difference in the observed mean annual rainfall was found in nearby stations (i.e. from 758 mm to 487 mm, for two stations that are 15 km apart). To keep this peculiarity of local climate also in the dataset for the future scenarios, we used an approach that downscales GCM-RCM output directly to specific weather stations. Hence, we compared observed monthly data (precipitation and temperature), representing the current climate condition, to those resulting from the GCM-RCM in order to fit the monthly empirical relationships between modelled data and observed data for each weather station. The period over which observed daily climatic data were available (1980–2009) was divided into two periods. The relationships were derived for the first period (1980–2000) and validated in the second (2001–2009). For the precipitation, we used a simple ratio method where for each i-month we divided the average observed data by the average GCM-RCMs data (fi) and then multiplied the daily GCM-RCMs data (2030-2059) by the corresponding monthly factor (fi) to obtain future daily precipitation data (Abbaspour et al., 2009). We elaborated a dataset for each weather station (10 gauging stations). For temperature data, future daily data were generated by applying a non-linear model to the future GCM-RCMs data for each scenario on a monthly basis (Wilby et al., 1998; Abouabdillah et al., 2010); seven stations were used. In particular, for each month and each station, the following forth-order polynomial regression equation (Eq.1) was applied to fit the monthly empirical relationship between the GCM-RCMs and the observed hystorical data (minimum and maximum daily temperature):

$$Temp_D = a_0 + a_1 Temp_{GCM} + a_2(Temp_{GCM})^2 + a_3(Temp_{GCM})^3 + (Temp_{GCM})^4$$

Where TempD is the downscaled temperature, TempGCM is the temperature predicted by the GCM-RCMs, and a0, a1, a2, a3, a4 are the regression coefficients.

For future climate scenarios, we used downscaled rainfall and temperature datasets in the Soil and Water Assessment Tool model to predict water balance and hydrological variables. Figure 2 represents a schematic overview of the methodology we used.

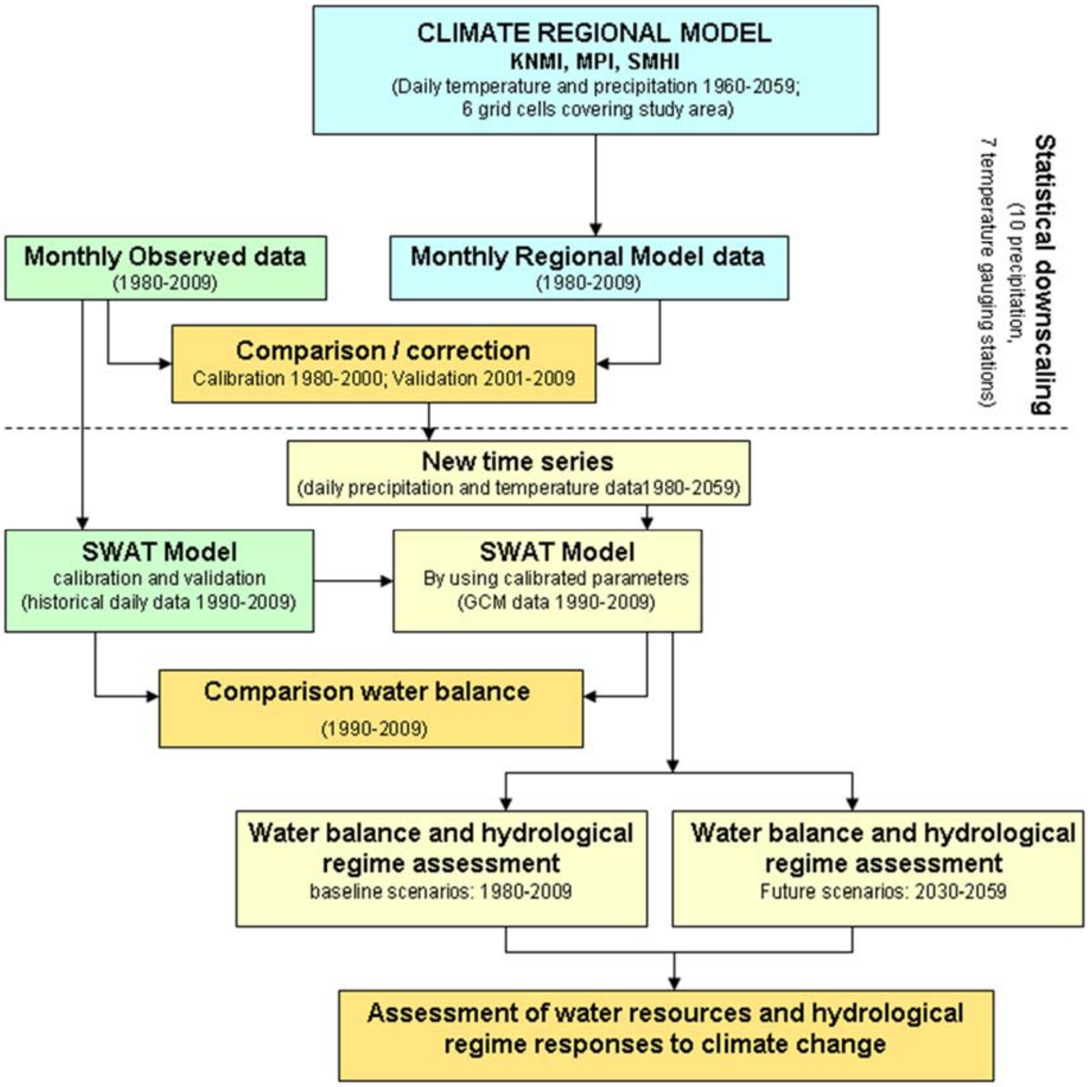

Figure 5.2. Schematic overview of methodology.

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2.3 Modelling streamflow and water balance

The Soil and Water Assessment Tool, SWAT2005 version with Arcgis interface (Arnold et al., 1998; Neitsch et al., 2002; Winchell et al, 2007), was used in this study to evaluate current and predicted future water balance and streamflow regimes. SWAT is a continuous model that is able to simulate hydrology, water quality and best management practices in agricultural basins (Srinivasan et al., 1998; De Girolamo and Lo Porto, 2012; Krysanova and White, 2015; Volk et al., 2016). It is a semi-distributed ecohydrological model suitable for applications in modelling framework for assessing the effects of pressures on river habitat (Kail et al., 2015). Several articles can be found in the literature that report the model applications in climate change impact assessment (e.g. Abouabdillah et al., 2010; Jeong et al., 2014; Kundzewicz et al., 2015; Glavan et al., 2015).

The SWAT model maintains a daily water balance and requires information about the soil texture, land use and land management, and climatic data. In SWAT, the basin is divided in subbasins, which are further divided in hydrological response units (HRUs) that consists of homogeneous land use, management, and soil characteristics. The hydrologic cycle is based on water balance equation (Neitsch et al., 2001), where evapotranspiration and runoff are predicted for each HRU. The Hargreaves-Samani method (Hargreaves and Samani, 1985) was chosen to evaluate evapotranspiration, since only daily temperature (Tmax and Tmin) and solar radiation were available for the study area, and the SCS Curve Number Method (USDA Soil Conservation Service, 1972) was selected to calculate surface runoff.

Before calibrating the model the sensitivity analysis (SA) developed by van Griensven et al. (2002) was conducted to assess the most sensitive hydrological parameters that can influence hydrological processes. The calibration and uncertainty analysis was performed using the SWAT - CUP (Abbaspour et al., 2011). We selected the Sequential Uncertainty Fitting version 2 (SUFI 2) procedure to perform the uncertainty analysis. The SWAT hydrologic cycle components (evapotranspiration, surface runoff, percolation, lateral flow, groundwater and total flow) were determined at basin and subbasins scale. A complete description of the model setup, including input data, and calibration and validation results can be found in De Girolamo et al. (2015) and De Girolamo et al. (2017).

In the future scenarios simulation, we run the SWAT model in consideration of the current land uses, management practices and fertiliser plans currently adopted in the study area, while an increase of irrigation equal to the increase in reference potential evapotranspiration was considered.

2.4 Streamflow regime alteration analysis

In our study, river segments showing near-natural or slightly altered hydrological conditions (De Girolamo et al., 2015) were selected for comparing future flow regime to the regime of the present. A two-step procedure was used. In the first step, two metrics developed by Gallart et al. (2012) were used in order to obtain an overall evaluation of the flow regime and its change. These metrics: Mf (number of months with flow) and Sd6 (predictability of dry conditions) are evaluated using monthly streamflow data. The predictability of dry conditions is computed with the equation 2:

$$Sd6 = 1 - \left(\sum_{1}^{6} Fd_i / \sum_{1}^{6} Fd_j \right)$$

where: Fdi is the multiannual frequency of no-flow months for the six contiguous wetter months per year and Fdj is the multiannual frequency of no-flow months for the six drier months). These metrics can be plotted to show a river classification based on the degree of temporariness of streams. Representing in the same plot a river segment for current and future conditions, it is possible to show the expected variation in degree of temporariness and whether the river reach changes its classification due to climate change (Tzoraki et al., 2015).

In the second step, we applied the IHA method (The Nature Conservancy, 2009) to calculate a large number of parameters (33) that are able to describe different aspects of flow regime (such as magnitude of monthly discharge, magnitude and duration of peak discharge, timing of annual peak discharge, frequency and duration of high and low discharge, and rate and frequency of discharge changes). The IHA indices are evaluated using daily flow data. Comparing these hydrological indicators, calculated for current and the future scenarios, it is possible to assess changes in the river flow regime caused by anthropogenic pressures and climate change (The Nature Conservancy, 2009). Before comparing the HIs for current and future scenarios, we verified the ability of statistical downscaled GCMs to predict hydrological indicators. For analysing the change between the two time periods, we implemented the Range of Variability Approach (RVA) described in Richter et al. (1997). The RVA uses the pre-impact natural variation of IHA parameter values as a reference for defining the extent to which flow regimes will be altered in future scenarios. In particular, the RVA divides the full range of pre-impact data for each parameter into three different categories. We adopted the RVA category boundaries recommended by Richter et al. (1997) in the absence of ecological information selecting the mean (M) plus/minus one standard deviation (SD). Hence, an automatic delineation of three categories is identified: the lowest category contains all values less than or equal to the M-SD; the middle category contains all values falling in the range of the M-SD to M+SD; and the highest category contains all values greater than the M+SD. Then, the software (IHA version 7.1) computes the frequency with which the "post-impact" values of the IHA parameters fall within each category, quantifying their degree of alteration as follows: (post-impact frequency – pre-impact frequency) / pre-impact frequency. A positive IHA factor means that the frequency of values in the category has increased from the pre-impact to the post-impact period, while a negative value means that the frequency of values has decreased. After the estimated changes in streamflow regime due to climate change were assessed, we explored the potential implications for river ecosystems.

3. RESULTS

3.1 Downscaling climate variables

The correlation between the downscaled variables and recorded historical data was evaluated. All the seven temperature stations had R2 values in the range of 0.97–0.99 during the validation period (2001–2009). Figure 3 shows a comparison between downscaled maximum daily temperature and measured data for the validation period at a hilly station (Troia gauge, S9 in Figure 1). We used a forth-order polynomial regression equation, which fits measured data better than the linear equation.

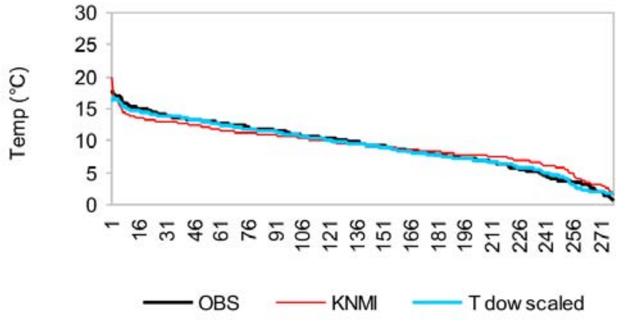

Figure 5.3. Measured and KNMI downscaled temperature daily data (Tmax) for the months of January over the validation period (2000–2009) at Troia gauge, S9, (R2=0.996).

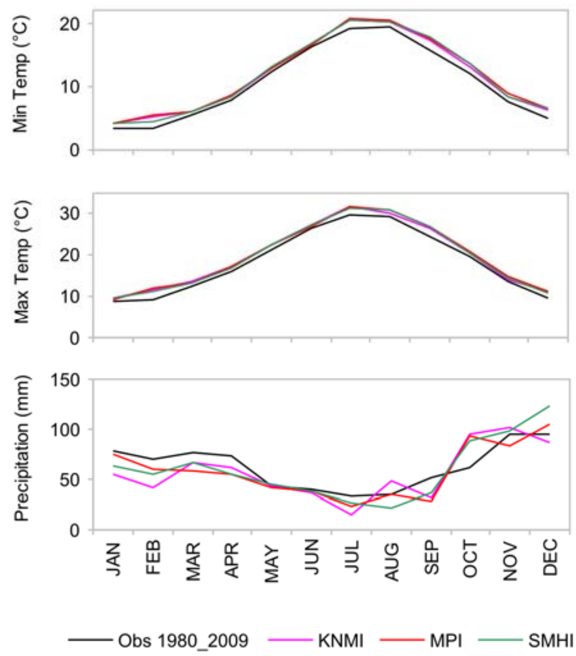

Figure 5.4. Comparison of the historical measured data and downscaled average monthly maximum and minimum temperature for the future scenarios (2030–2059). Comparison of historical measured precipitations (1980–2009) and downscaled precipitation for the future scenarios (2030–2059) in a wet station (S10).

Figure 4 shows a comparison of average monthly temperature (maximum and minimum) for historical data (measured from 1980 to 2009) and for the downscaled scenarios (2030–2059) at a hilly station of the basin (Biccari, S10 in Figure 1). All the scenarios show an increase of temperature for all months: maximum temperature varies between 0.5–2.4°C in different scenarios, with the highest in September for the MPI scenario. The difference of minimum temperature varies between 0.3–2.1°C, with the highest in the wet area in February. This is a very interesting result as the increase in minimum temperature, which is forecasted for the winter months, will bring a different type of precipitation with a reduction of snowfall in the mountainous part of the basin during the period 2030–2059.

Figure 4 also shows a comparison of the downscaled precipitation for the future scenarios and historical data on a monthly basis for a wet station. All the scenarios predict for the period 2030–2059 a decrease in annual precipitation and a different distribution through the year with a major decrease from January to May.

3.2 Water balance

In the Candelaro river basin, the water balance computed with measured climatic data from 1990 to 2009 is dominated by the evapotranspiration. At basin scale, the potential evapotranspiration estimated with the Hargreaves and Samani method ranges from 975 mm to 1102 mm.

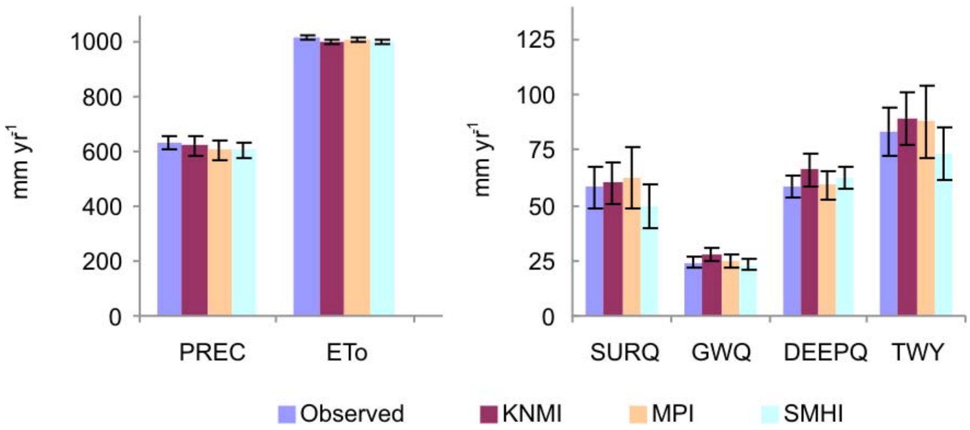

Figure 5.5. Comparison of water balance components determined with measured and downscaled GCMs climatic data for a common time period (1990–2009) at basin scale. PREC is for precipitation; SURQ for surface runoff; GWQ for baseflow; DEEPQ for aquifer recharge; Et0 for potential evapotranspiration; TWY for total water yield. Error bars show standard errors.

The average annual value of the actual evapotranspiration is estimated in 495 mm, it varies from 424 to 579 mm and constitutes about 78% of precipitation (635 mm, ranging from 440 to 787 mm). Groundwater recharge is about 10% of rainfall. The mean annual surface runoff is quantified in about 58 annual variability in runoff is a peculiarity of streamflow regime in the Mediterranean Region (D'Ambrosio et al., computed for the study period for the entire basin are shown in Figure 5.

3.3 Impact of climate change on water balance

The model calibration and validation results in addition to the uncertainty analysis for the current hydrological and climate conditions can be found in De Girolamo et al., 2015 and De Girolamo et al., 2017. We assessed the ability of statistical downscaled GCMs to predict water mm, it varies from 8 mm in a severe drought year to 135 mm in a very wet year. Surface runoff constitutes about 9% of annual precipitation. Annual value of total water yield varies significantly in the study period (18÷167 mm) and it constitute 13% of annual precipitation. This result was expected as the inter-

2016; Oueslati et al., 2015). Mean annual values of the water balance components

balance comparing the components simulated with observed climatic data (1990–2009) to those simulated with GCM downscaled data for the period 1990–2009. As the downscaling was performed over a different time period (calibration: 1990-2000; validation 2001-2009), the predicted water balance components with GCMs data do not replicate exactly

Table 5.2. Average effect of climate change on the blue and green water at basin scale.

	Current scenarios GCMs (baseline: 1980-2009)			Future scenarios GCMs-RCMs (2030-2059)		
	KNMI	MPI	SMHI	KNMI	MPI	SMHI
Rainfall (mm yr^{-1})	658	614	627	631	568	590
Diff. in rain (%)				-4	-7	-6
Blue water (mm yr^{-1})	172	146	137	160	120	127
Diff. blue water (%)				-7	-18	-7
Green water (mm yr^{-1})	485	475	494	476	459	470
Diff. Green water (%)				-2	3	-5

those with measured data. However, a good agreement was found for all the variables (Figure 5). We defined acceptable change in a modelled factor if the difference from the observed variable is less than the absolute sum of standard errors of the modelled factor and observed variable.

The impact of climate change on the green and blue water was evaluated at the basin scale (Table II) and sub-basin level. Blue water includes fresh surface (TWY) and groundwater (DEEPQ), while green water flow is the actual evapotranspiration (Et). A decrease of blue water was forecasted for all analysed scenarios over the period 2030–2059, with a maximum decrease (percentage) predicted for MPI scenarios (17.9%). Although an increase of potential evapotranspiration is expected for all the studied scenarios (4%), only a modest change in green water flow was forecast for the KNMI scenario (-2%) while a reduction of 3% and 5% is predicted for MPI and SMHI scenarios, respectively. This is due to the fact that green water flow depends not only on the air temperature but also on water into soil. In our study, no land use changes were assumed for the future and an increase of 4% in irrigation was considered only for the areas where crops are actually irrigated in the basin. As a result of the increase in temperature, the model simulates a reduction of snowfall that is 59% for KNMI and MPI and 63% for SMHI.

Blue water change varies among the sub-basins, the highest variation in percentage is predicted in sub-basins located in the central part of the basin towards the confluence of the most important tributaries into the Candelaro stream. Overall, we predict a reduction of the total inflow into the “Capaccio” reservoir ranging from 7.9% (KNMI) to 9.4% (MPI).

3.4 Assessment of climate change impact on the flow regime

Before assessing the climate change impact on the flow regime we verified the ability of statistical downscaled GCMs to predict hydrological indicators. As the downscaling of climatic variables was calibrated from 1990 to 2000 and validated from 2001 to 2009, we compared for the entire period 1990–2009 the hydrological indicators computed with observed climatic data and GCM downscaled data. A good agreement was found for the indicators, especially for those which are considered relevant for temporary rivers (1-, 3-, 7-, 30-day minimum flow, Mf and Sd6). Figure 6 shows the comparison for a sub-basin (Sub 30).

In order to evaluate only the effects of climate change, independently of anthropogenic pressures, we selected some reaches that are currently in near-natural conditions, and we represented them in the Temporary Streams Plot (Sd6 versus Mf: Gallart et al., 2012) for the baseline and for the worst scenario (MPI). As the Figure 7 shows, the points representing the flow regime moved from the right to the left towards more dry conditions for the future scenarios. Even if their classification (sensu Gallart et al., 2002) is not expected to change in the future, it is interesting to note that the divergence from the current status increases in future scenarios, especially for those reaches classified as I-D (i.e. Intermittent-Dry) and E (Ephemeral).

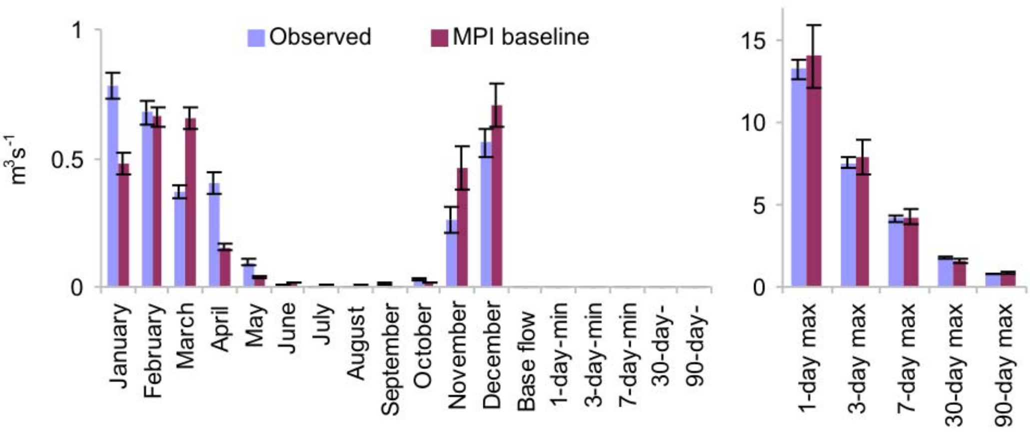

Figure 5.6. Hydrological indicators computed with measured and downscaled GCMs climatic data for a common time period (1990–2009). Error bars show standard errors.

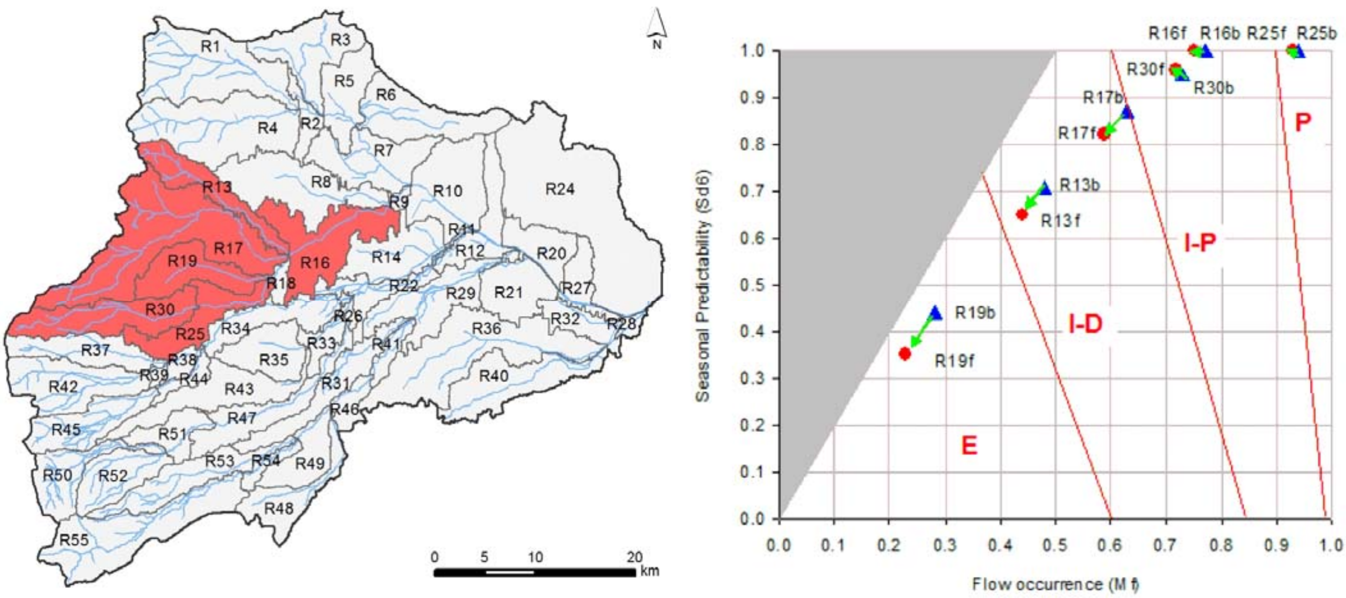

Figure 5.7. Temporary Rivers Plot. Classification of river regime for the baseline (blue triangles) and future scenarios (MPI; red circles) of the river reaches (13, 16, 17, 19, 25, 30) in red in the picture. P is for perennial, I-P is for intermittent Pool, I-D is for intermittent dry and E is for ephemeral.

By using simulated daily streamflow as input in the IHA software (IHA version 7.1) the changes in streamflow regime and Environmental Flow Components (EFCs) were evaluated. Actually, the general reliability of the hydrological indicators evaluated by using simulated data instead of measured flow data was found (De Girolamo et al., 2016). We implemented the Range of Variability Approach (RVA) to quantify flow regime alterations, as suggested by Richter et al. (1997). The results, which concern to the worst climate scenarios (MPI) applied to a near-natural reach (R 19 in Figure 7), are presented in Figure 8. A positive HA value in the high, middle and low categories means that the frequency of values in that category has increased from the baseline to the post-impact period. The graph shows a high level of alteration for mean monthly flow: the negative values of the High-RVA category recorded from October to May means a reduction in the occurrence of high values in Mean Monthly Flow, while the positive values (i.e. July Mean Flow, in the graph) represent an increase in the frequency of high values for Mean Monthly Flow. This is due to an increase of flash flood events. Generally, streamflow during the dry months is sustained by runoff only, as the baseflow is absent in most reaches. For river segments R13, R19 and R25, no changes are expected for hydrological variables such as 1-, 3-, 7-, 30-day minimum flow (recorded over consecutive days), as both baseline and future values are zero. A significant reduction in the magnitude of 90-day minimum was also predicted, which is <0.5 L s-1 not able to sustain a continuous flow. Hence, only pools connected or disconnected can be found along the stream course. For the river segments: R16; R17; R30, the 30-day minimum flow became zero. A reduction is also predicted for 90- and 30-day maximum flow. Concerning the date of minimum flow, in some river reaches an earlier data of minimum was predicted (R13, R19, R30), while in the other river segments it remains more or less the same.

4. DISCUSSION

In agreement with results already reported in the literature (Table I) concerning regional climate change projections over the Mediterranean climatic zone, an overall increase of drier and warmer conditions is predicted in the future climate scenarios over the Candelaro river basin. However, the range of the possible climate change is still disputed and the uncertainty associated with projections is very large. Uncertainties are not only due to scientific and methodological deficiencies, but also depend on economic and social development or land use changes (Glavan et al., 2012). In addition, a crucial point in climate change impact analysis at the basin scale is the spatial and temporal downscaling of the Global Circulation Models results (Dibike and Coulibaly, 2005). In fact, off-line hydrological models driven directly by GCM-RCM outputs have been found to perform poorly, and results vary considerably between models (Wilby et al, 1999). The quality of GCM outputs could preclude their direct use for hydrological impact studies (Prudhomme et al., 2002). Hence, to estimate the differences between measured and baseline datasets (CGM over the same time period of the measured data) has recently become an important step in scenario development because these differences can have an important effect on the results obtained in climate change impact assessments. In recent years, several downscaling techniques have been developed for hydrological modelling (Fowler et al., 2007).

In our study, we found a large discrepancy between GCM-RCMs data and historical measured data, especially in the upper part of the basin. In the Candelaro, as in most of the Mediterranean basins, rainfall regime shows a high spatial variation (Oueslati et al., 2015, D'Ambrosio et al., 2016). This is an important factor to take into account when selecting both the scenario resolution and the downscaling techniques.

We did not address issues such as extreme rainfall events, while we focus on the prediction of changes in the characteristics of extreme low flow, which should be considered in an ecological perspective (Larned et al., 2010). Further studies are needed to analyse the effects of extreme rainfall events on hydrology and ecology of this river system, which has a temporary character.

The results of our study show that a drier and warmer future will reduce the precipitation falling as snow and low flow conditions will intensify during the dry season. Analogous results were found by Demaria et al. (2013) in Chile. Although we did not analyse the simulated water quality, we can say that decreasing precipitation and the consequent predicted exacerbation of the extreme low flow conditions may increase the risk of water source contamination from sewage and nonpoint source pollutants to water courses.

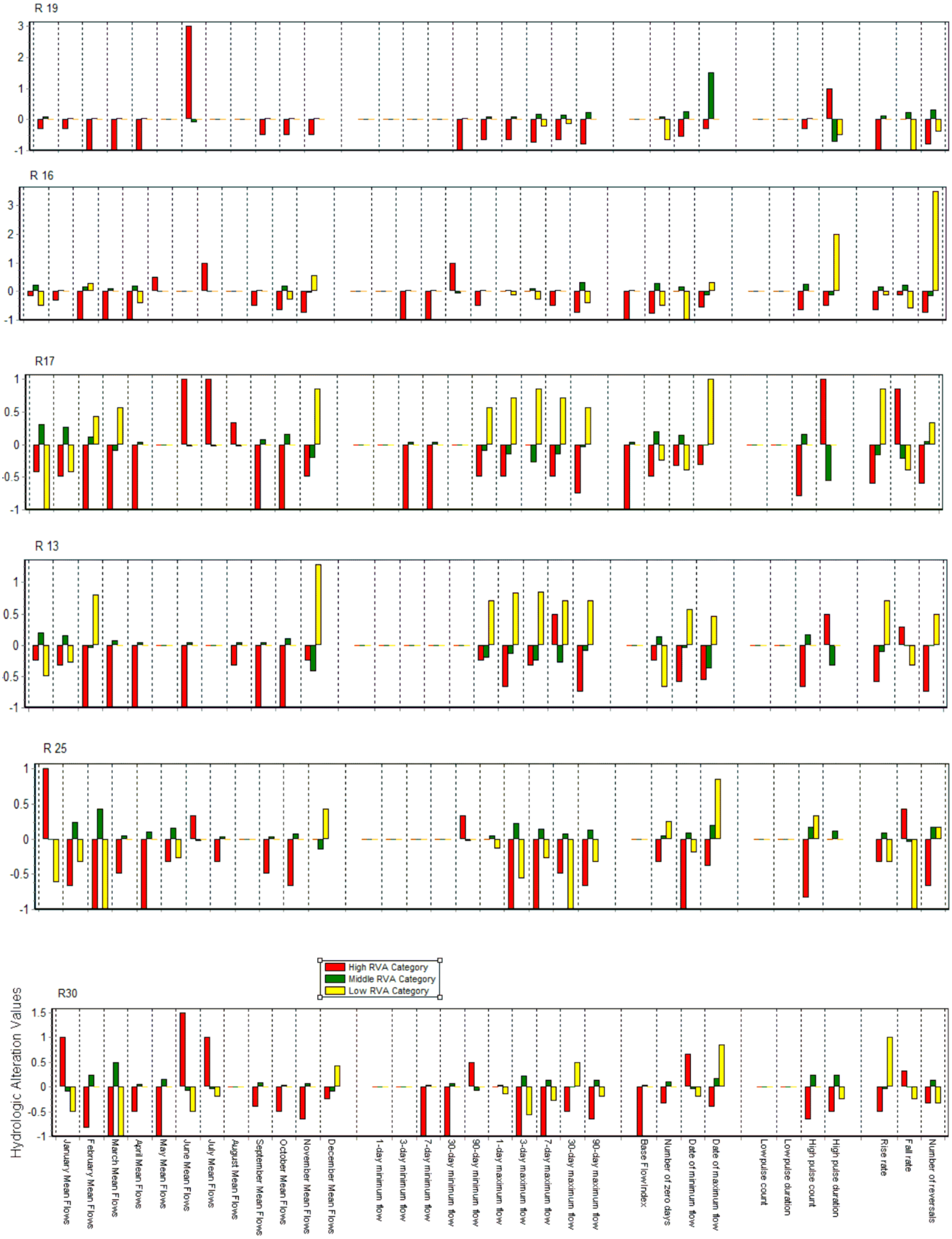

Figure 5.8. Hydrological alteration values evaluated with the Range of Variability Approach in the selected near-natural reaches (R19, R13, R17, R30, R16, R25 in Figure 7). Current period: 1990-2009; future scenario: MPI_REMO_ECHAM5 2030-2059. The degree of alteration in each category is computed as: (post-impact frequency – pre-impact frequency) / pre-impact frequency. A positive IHA factor means that the frequency of values in the category has increased from the pre-impact to the post-impact period, while a negative value means that the frequency of values has decreased.

Some of the river reaches, which have a natural intermittent character, became perennial (Reach 22) due to WWTPs discharges, but this could have consequences on water quality, for instance some chemical parameters could be over the fixed thresholds for “good” water quality due to a restricted dilution effect (De Girolamo et al., 2015). In fact, even if wastewater effluents play a relevant role in stream restoration under a Mediterranean climate (Luthy et al., 2015), obvious consequences of pollution (e.g. Brooks et al. 2006) are still to be expected in the study area in the next few decades.

4.1 Implications for river ecosystems

Hydrological alterations due to climate change predicted in the near-natural reaches were evaluated using a number of hydrological indicators, which can be used to infer potential implications for river ecosystems. Several methods have been developed to display and interpret indicators results. We selected the range of variability approach (RVA) that takes into account the natural inter-annual variability present in the indicator values in the baseline period and in a future period. In this way, most relevant changes in flow regime can be easily highlighted. Considering threshold values from the literature and the uncertainty of such studies, Schneider et al. (2013) assumed that the impact on river ecosystems is relevant, when the indicator difference is outside a range of ±30%. They also assumed for the mean timing indicators a threshold of ±30 days. In this study, the indicators assessment described above was carried out separately for each sub-basin at the outlet. Based on these criteria, we found that the majority of indicators showed a relevant alteration. Quite obviously, such alterations can be relevant for different biological elements and ecosystem functions. When flow regime changes, peculiar adaptations may be required to endure the new situation, which involve life histories, behaviours and morphologies of plants and animals (Lytle and Poff, 2004). An overview of advantages and disadvantages of different adaptations to flooding and drought for a range of organisms can be found in Lytle and Poff (2004).

In arid and semi-arid regions, such as the Candelaro basin, the combined effects of climate change and water abstraction are expected to reduce flows and maybe hasten the degree of temporariness of streams and rivers (Larned et al., 2010). Besides the specific indicators listed in Figure 6, the variation in the degree of temporariness due to climate change for the investigated reaches can be visually evaluated from Figures 7 and 8, where current and modelled, future conditions are shown. This is an important piece of information as, in a WFD context, the assessment of river ecosystem’s health implies a comparison to type- or site-specific reference conditions. Of course, reference conditions will have to be redefined if a water body changes its type e.g. shifting from perennial to intermittent. This was not the case for the study reaches. Nonetheless, in general terms, it is worth noting that reference conditions cannot be considered as static and will change, echoing the effect of climate change on the physical and chemical conditions of water bodies (Nõges et al., 2007). If, in the future, a river reach i.e. the point in Figure 7 representing its flow regime, moves toward more temporary conditions, the biological reference conditions are realistically expected to be different, at least in the low-flow period (Feio et al., 2014). In fact, biological assemblages observed in phases close to the dry season can show quite different attributes to those of the other time periods (García-Roger et al., 2013). This reflects the overall balance between lentic and lotic habitats present in a river reach that, especially in Mediterranean rivers, can be the most important factor affecting e.g. invertebrate communities (Buffagni et al., 2010). In fact, though overall biological traits may change weakly over regional scales (Bonada et al., 2007a), they are mediated by habitat characteristics and interrelated to flow permanence in Mediterranean rivers (Bonada et al., 2007b). Therefore, flow-related habitat features, and especially the lentic-lotic character, should be taken into account when assessing the ecological quality of rivers, because they can greatly affect the assignment of ecological status and the interpretation of biological data (Buffagni et al., 2009).

4.2 Implications for River Basin Management Planning

As shown before, the expected climate change will have an impact not only on the water balance of the Candelaro river basin, but also on some of the characteristics of the flow behaviour, including an increase of flash floods, decrease of the 90-day minimum flow, and 90- and 30-day maximum flow. These changes will call for improved management of a scarce resource, whose availability will decrease with time and whose quality might degrade with strong implications on the ecosystem functioning, and strong impacts on the environmental, social and economic sectors. New management options and mitigation measures are needed urgently, as warming is taking place at greater rates than previously anticipated (Filipe et al., 2013), and competition for water will be exacerbated by an increased demand for human consumption, irrigation and lower availability.

The reduction of current water inflow in the Capaccio reservoir (8÷10%) will need a revision of the water release downstream the dam and adaptive adjustments of the environmental flow including hydrological, ecological and social processes. Innovative measures will be required as some of the river segments might change in flow regime, even though we did not predict any flow regime change in our analysis (we did not consider that the extent of irrigated areas, and human demand will possibly increase in the near future, leading to increased water stress in many river segments). Daring management and new governance are needed to face the tremendous challenge of maintaining or restoring ephemeral streams while meeting the growing demand for freshwater resources (Grantham et al., 2013). Climate change will exacerbate the competition for water resources; however, it must be stressed that socio-economic factors might play a key role in the future decrease of water abundance, thus the management of water resources should not be water-centric but must also include environmental and socio-economic factors in view of sustainable development and use of an already scarce resource.

5. CONCLUSIONS

The main purpose of the present study was to assess the climate change impact on flow regime in a Mediterranean basin characterised by a river system with an intermittent flow. Our assessment is based on the availability of recent climate change simulations on a global and regional scale. However, predictions of the hydrological response of a river basin under climate change conditions are affected by several sources of uncertainties that depend both on the used hydrological models and on the climate change scenarios. In addition, some of the assumptions made (i.e. that land use does not change in the future) could be incorrect as climate change could also result in a significant alteration of land cover. Hence, we have to consider projections not as a predictive method, but as a tool that may be used to assess changes in process dynamics.

The results of our study show that climate change will bring a reduction of water resource availability and some alterations in the hydrological regime. The SWAT model, which proved to be a valuable operational tool for evaluating the potential impact of climate change on water resources, estimates a reduction of blue water and total water yield and a shift of the flow regime towards drier conditions, although the river type classification will probably remain essentially unvaried. In particular, the results show that the divergence from the current status increases in future scenarios, especially for those reaches classified as I-D (i.e. Intermittent-Dry) and E (Ephemeral). Hydrological Indicators (IHAs) showed a decrease in both high flow and low flow magnitudes for various time duration, an extension of the period with an absence of flow (zero flow days) and an exacerbation of extreme low flow conditions.

Like most Mediterranean basins, the Candelaro River is currently characterised by water shortage and is undergoing a continuous process of agricultural intensification. In this condition, we can assume that in the next few decades climate change will exacerbate the competition for water for human demand and irrigation. This probably implies an increase of anthropogenic pressures on water systems. Hence, River Basin management planning will play a crucial role in facing socio-economic and environmental problems. A continuous upgrade will be needed to assess the river ecosystem health, which is based on the type- or site-specific reference conditions that cannot be considered static. Hence, if hydrological and habitat characteristics relevant for type attribution vary, or a water body even shifts among types (i.e. from perennial to intermittent), biological communities will be greatly affected and the reference conditions will have to be methodically redefined. New water resource management options are needed to mitigate the impact of climate change on water resources, especially for intermittent and ephemeral streams, for which specific measures have to be identified.

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I contributed to paper conceptualization, I wrote the parts of the manuscript dealing with its ecological aspects; I provided critical review of the whole paper and detailed commentary on all aspects (apart from climate modelling).

CHAPTER 6

"Macroinvertebrate metrics responses to morphological alteration in Italian rivers".

Erba et al., submitted to Hydrobiologia

Macroinvertebrate metrics responses to morphological alteration in Italian rivers

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ABSTRACT

The responses of river macroinvertebrates to hydromorphological alteration are often considered weak or unclear. It is therefore crucial to verify if and how existing invertebrate-based approaches can reveal the effects of hydromorphological modification. We analysed the response of benthic metrics to morphological impairment, with emphasis on the STAR_ICM index, legally required for macroinvertebrate-based ecological status assessment in Italy. A Principal Component Analysis (PCA) was run to condense information on morphological impairment. The major gradient (Component 1) expressed a combination of bank and channel modifications opposed to tree-related features indicating the presence of comparatively unmodified habitats. Jointly, habitat descriptors including Habitat Modification Score (HMS) derived from the application of a habitat survey method were calculated. Spearman rank Correlations between biological metrics and morphological impairment indicators (PCA scores and HMS) were significant. A linear mixed-effect regression approach was applied to relate HMS and STAR_ICMi across a wide geographical context. HMS explained ≈ 60%of STAR_ICMi variability, in the absence of apparent water pollution. Results demonstrated that morphological information resumed with habitat survey methods is meaningful for the biological community and that HMS can support the interpretation of ecological status across rivers types and in different environmental settings.

INTRODUCTION

Hydromorphological degradation is one of the most important anthropogenic pressures affecting European rivers and streams (Feld, 2004; Vaughan et al., 2009; EEA, 2018). The term 'hydromorphological degradation' describes an assortment of impacts acting at different spatial scales (Feld & Hering, 2007). This often entails that research in this field mainly focuses on specific impact types such as channel resectioning (Wyżga et al., 2014), river impoundment (Krajenbrink et al., 2019), or fine sediments accumulation (Doretto et al., 2018), and does not refer to the overall degree of morphological alteration.

In Europe, the achievement of good ecological status is a legally binding objective according to the Water Framework Directive (WFD EC, 2000) and this implies monitoring biological quality elements sensitive to anthropogenic impacts. The interest in developing specific assessment tools based on invertebrates to detect and quantify morphological alteration is apparent (Lorenz et al., 2004; Ofenböck et al., 2004; Urbanič, 2014). However, invertebrate assemblages response to hydromorphological pressures is considered weak or unclear by many authors (e.g. Feld et al., 2014; Villeneuve et al., 2015).

In addition to the complexity in defining hydromorphological impacts and their heterogeneous biological responses, rivers are usually affected by a suite of multiple stressors, which makes difficult to quantify and rank the different pressures influencing biological communities (Turunen et al., 2016). Due to such complexity, some authors tend to conclude that invertebratesbased assessment methods currently in use are insensitive to morphological impairment or that macroinvertebrates do not respond to such alterations (e.g. Friberg et al., 2009; 2011). Moreover, the scale used to measure hydromorphological habitat conditions can interfere with the capacity of detecting the impact on biological communities (Stoll et al., 2016).

Especially because the link between biological elements and morphological impairment can be uncertain in particular when other pressures are simultaneously acting, there is no scientific consensus on which methods are most suitable for long-term monitoring and which river features are the best to be considered. Many countries have adopted physical habitat assessment protocols to evaluate river hydromorphology for the WFD objectives (Belletti et al., 2015; Wiatkowski & Tomczyk, 2018). Physical habitat is traditionally recognized as important in explaining the composition and structure of biological communities (Fernández et al., 2011) but assessment methods focused exclusively on physical habitat can be not exhaustive for overall hydromorphological evaluation. By contrary, hydromorphological assessment methods with a stronger emphasis on river dynamics and processes are considered the most comprehensive (Wiatkowski & Tomczyk, 2018), but can be unsuccessful in establishing a direct link with biological components. Hydromorphological processes focus on features that are not necessarily significant for biological communities and their assessment may fail to consider key biological habitats (Verdonschot et al., 2016). According to the WFD, to measure the difference between altered and natural condition is only possible when biologically relevant variables are considered (Gieswein et al. 2017; Boon et al., 2019), independently from the water body type. Also, outlining the relationship between biotic community assemblages and morphological alteration is crucial to plan specific measures to achieve environmental goals.

In Italy, the present legislation (DM 260/2010) requires the application of the Morphological Quality Index (MQI – Rinaldi et al., 2013) for the evaluation of the hydromorphological status. River habitat information (Buffagni et al., 2013) must also be assessed to confirm reference conditions for a river reach for the purpose of benthic invertebrates monitoring (DM 260/2010). The same regulation , requires the use of the STAR_ICMi (Buffagni et al., 2006; 2007), included in the MacrOper classification system (MATTM, 2010), as official method for the assessment of ecological status based on macroinvertebrate fauna.

Conceptual framework and methods are already available and in use in the Italian national territory as they are in other European Countries. What is still missing is an explicit link between morphological alteration and biological methods for the assessment of ecological status.

We selected CARAVAGGIO river habitat survey method (Buffagni et al., 2013) for collecting data on morphological alteration at the river reach, which is the scale generally known to have direct influence on biological communities (González del Tanágo et al., 2016). Among others available, river habitat-based surveys (e.g. RHS and CARAVAGGIO) have been extensively applied at European level (e.g. Raven et al., 1997; Balestrini et al., 2004; Szoszkiewicz et al., 2006; Ioannou et al., 2009; Tavzes & Urbanič, 2009; Vaughan, 2010; Belmar et al., 2013; Bruno et al., 2014; Dresti et al., 2016) and outside the EU (e.g. Kijowska-Strugała et al., 2017). Moreover, river habitat survey data are profitably used to develop broadly applicable habitat-based tools for impact assessment, diagnostics and management planning (e.g. Naura et al., 2016; Extence et al., 2017). We specifically focused on the Habitat Modification Score (HMS) summarizing the impact of different specific alterations. The HMS provides an indication of artificial modification to the physical structure of a river reach considering a variety of modifications on river banks and channel (Raven et al., 1998).

Therefore, this paper considered morphological impairment mainly in terms of bank and channel modifications which include reinforcement, resectioning, presence of embankment, presence of bridges, weirs, culverts and often determine changes in the occurrence of natural features (e.g. underwater tree or shrub roots, exposed bankside roots, channel shading) linked with the presence of trees (Buffagni et al., 2016; 2019). To maintain the emphasis on the evaluation of ecological status, the focus was addressed on response to morphological impairment of benthic community as expressed by biological metrics legally required in the Italian assessment method. Testing macroinvertebrate response to morphological impairment to develop new specific metrics was beyond the scope of the present paper, consequentially we centered on the STAR_ICMi and its component metrics.

In detail, the paper aimed at answering the following questions: i. Are benthic invertebrate metrics able to reveal physical habitat alteration? ii. How can we quantify morphological alteration in relation to the evaluation of ecological status for the WFD?

METHODS

Study sites

Data were collected in five Italian stream types included in three environmental contexts: Lowland streams - small and medium sized; Mediterranean mountain streams - small and medium sized and Mediterranean temporary streams. (Fig. 1; Table 1). In total 95 sites located in 66 rivers were investigated (Appendix 1). Map-based geographical data were collected for each site (see Table 1).

Sites included in the study cover a gradient in morphological alteration mainly linked with the presence of artificial structures, including reinforcement of channel and banks as well as the presence of transversal structures and comprise an overall amount of 29 reference sites according to Feio et al. (2014) and MATTM (2010). Sites were carefully checked for not being affected by heavy pollution and, when available, historical data were considered to confirm the absence of relevant water pollution (e.g. ARPAC, 2003; RER, 2005; Lamaddalena et al., 2008; RAS, 2009; ARPAS, 2014). Samples included in the analysis belong to water bodies classified from High to Moderate quality according to the LIMeco descriptor, formally used in National Italian legislation (DM 260/2010, MATTM, 2010) for classifying rivers on the basis of nutrients concentration and oxygen saturation. Information on organic pollution expressed by means of E. coli and BOD5 was also checked: all samples have a minimum-maximum range respectively between 1-3400 (UFC/100 ml) and 0.22-4.3 (mg/l). Sites downstream water intakes were also excluded.

Morphological impairment quantification

Habitat features and river modifications were collected at reach scale by means of the CARAVAGGIO method (Buffagni & Kemp, 2002; Buffagni et al., 2013), developed on the basis of the River Habitat Survey protocol (RHS, Raven et al., 1997). The method founds on a comprehensive survey, with data collected along a 500 m channel length, independently from the stream order or discharge. This length is selected to be representative of a wider portion of rivers and significant for macroinvertebrates, and follows a long history in application of the method. Survey is undertaken on channel, banks and peri-fluvial areas following a two-section protocol. In the first part, bank and channel features are recorded every 50 m on 10 ≈ equally spaced spot-checks perpendicular to river flow; in the second section (sweep-up), features are recorded along the whole 500 m stretch. Qualitative, quantitative and categorical information is collected, depending on the kind of feature.

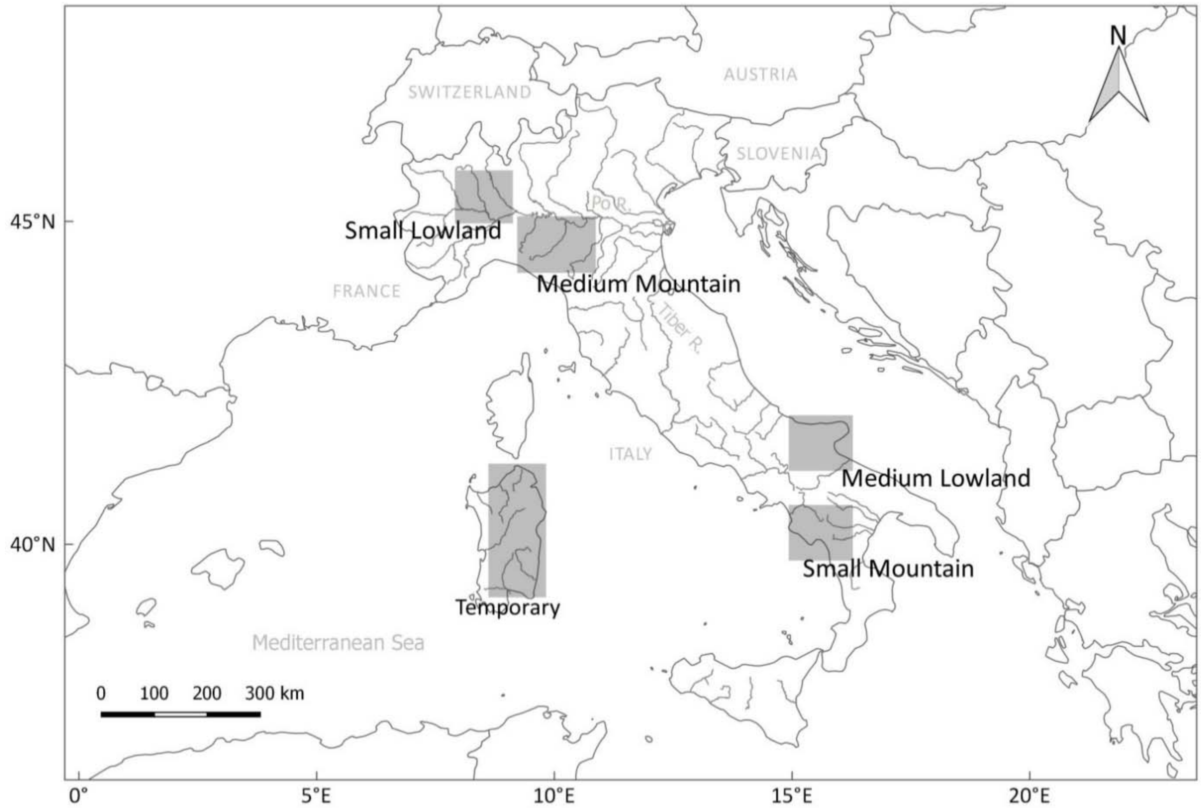


Fig. 6.1 Southern Europe map with location of sampling areas (grey squares). The full list of the studied sites is reported in Appendix 1.

In addition to single habitat features, based on the collected information, the following habitat descriptors were calculated and included in the data analysis: HMS (Habitat Modification Score – presence and quantity of artificial features along river channel and banks) and HQA (Habitat Quality Assessment Score – measure of habitat richness and diversification) (Raven et al., 1998); LUIr (Land Use Index (reach scale) – quantification of land use modification at the reach scale) (Erba et al., 2015); LRD (Lentic-lotic River Descriptor) – that quantifies the ratio between lentic and lotic habitats present in the channel (Buffagni et al., 2010). In particular, for the calculation of the HMS the scoring system was not updated to the most recent UK Environment Agency rules (UK EA, 2003), since the Italian legislation provides for the old scoring system (UK EA, 1998). CARAVAGGIO survey was undertaken simultaneously to benthic invertebrate collection. The 500 m reach extends ≈400 m upstream and ≈100 m downstream the macroinvertebrate sampling site. CARAVAGGIO application was usually repeated when the site was sampled in different seasons. No variation in bank and channel modification is expected from season to season, but other features, e.g. flow types, may change. On the basis of literature information (Feld et al., 2014; Buffagni et al., 2016; González del Tanágo et al., 2016), variables listed in Table 2, collected through CARAVAGGIO survey, were considered relevant for the quantification of morphological impairment and habitat degradation.

Table 6.1 Typological features of the considered datasets. Sampling season and year are also reported. Location of sampling sites is reported in Appendix 1.

			Distance from Source (km)	Altitude (m a.s.l.)	Slope of the valley (%)	Hardness (mmol/l $CaCO_3$)	Substrate median dimension (cm)	Channel width (m)	Season / Year	Sites / Samples
Lowland	Small size	Min.	0.1	119	0.1	0.68	0.02	2.2	Spring, and Autumn 2000; Winter 2001	15/24
		Median	0.5	124	0.2	1.19	0.28	3.4		
		Max.	4	151	0.4	1.48	1.92	6.2		
	Medium size	Min.	3.9	4	0.07	1.15	<0.01	1.3	Spring and Winter 2006	18/18
		Median	26.1	176	0.47	3.6	0.02	2.9		
		Max.	81.3	535	11	4.4	9.16	7		
Mediterranean mountain	Small size	Min.	5	250	0.25	1.55	0.78	2	Spring, and Autumn 2000; Winter 2001	8/19
		Median	7.7	470	2.22	2.64	9.16	6.1		
		Max.	37	620	4.44	4.17	34.29	11.9		
	Medium size	Min.	20.4	190	0.43	0.96	0.48	6.6	Spring, and Autumn 2000; Winter 2001	10/27
		Median	41.3	380	0.67	1.59	9.16	21.9		
		Max.	79.7	566	1.25	2.18	34.29	44.2		
Mediterranean	Temporary	Min.	2.5	12	0.1	0.26	0.75	1.9	Spring and Winter 2004; Spring 2011; Winter 2013	44/44
		Median	9.5	85	1.2	0.7	9.67	4.8		
		Max.	28	770	13.89	0.7	34.91	12.3		

Biological and water quality data

At each site, an invertebrate standard sample was collected according to the Italian national legislation (MATTM, 2010). Each sample consists of 10 sampling units collected with a Surber net (area 0.05 m2; mesh size 0.5 mm) according to a multihabitat sampling procedure (Buffagni et al., 2004). All stream types were sampled in spring (different years). In some cases, the same site was sampled in additional seasons (winter and autumn) (Table 1), making a total of 132 biological samples available for the 95 sites. Data were collected during different projects activities: AQEM project (EVK1-CT1999-00027) for Small Lowland, Small and Medium Mediterraenan Mountain river types; APQ Candelaro (Settore Tutela e Gestione Integrata delle Risorse Idriche in Puglia) for Medium sized Lowland type and MiCaRi project (MIUR, D.M. 408 Ric.20.03.2002 – Settore “Risorse Idriche”) and INHABIT (LIFE08 ENV/IT/000413) for temporary rivers. Invertebrates were sorted in the field and preserved in alcohol (90%), counted in laboratory and identified at family level as required by national Italian standard and in order to guarantee a wide geographical application.

Table 6.2 List of variables obtained from the CARAVAGGIO protocol for the quantification of morphological impairment and habitat degradation. Variables were selected on the basis of Feld et al. (2014); Buffagni et al. (2016) and González del Tanágo et al. (2016).

Characteristic	Code	Description	Measure
Weir, Sluices and Bridges	WSB	Transversal structure having an impact on the longitudinal section of the river. Bridges causing narrowing and or artificialization of river bed and banks.	Number in 500m
Culverts	CV	Closed conduits generally used to convey water for stream crossing or run-off management	
Artificial Straightening	AS	Channel realignment, with consequent relevant modification of natural form	% over 500m Absent, Present: 30%, Extensive: 70%
Artificial Stagnation	ST	Zones where ponding caused by artificial structures are observed. To be recorded as present, 'ponded' portion should extend for at least 50m within the reach (i.e. ≥ 10%)	
Tree extent	ET	Continuity of woody vegetation along banks (mainly trees, ≥ 2 m high)	Arithmetic mean of extent on left and right bank, after numeric conversion (None: 0, Isolated/scattered: 1, Regularly spaced-single: 2, Occasional clumps: 3, Semi-continuous: 4, Continuous: 5)
Tree related channel habitats	TC	Includes extent of direct, overhead, tree canopy shade and shade created by any natural feature; Uprooted or collapsed tree(s), either alive or dead; Large trunks and branches swept downstream and lodged in the channel or on the banks; Underwater tree or shrub roots (e.g. alder and willow roots)	Average % over 500m Absent, Present: 30%, Extensive: 70%
Tree related bank habitats	TB	Includes Large (forearm-size or larger) tree boughs which arc over, or dip close to, the water surface and Large exposed roots and associated habitats	
Channel Reinforcement	cRI	Includes channel armouring	% of Occurrence at 10 ≈ equidistant transects
Channel Resectioning	cRS	Includes channel reprofiling	
Bank Reinforcement	bRI	Includes bank armouring	
Bank Resectioning	bRS	Includes bank reprofiling and embankment	

The following biological metrics (see also Buffagni et al., 2016) were calculated to family level for each sample: ASPT (Average Score per Taxon); N_families (Total Number of Families); EPT (number of Ephemeroptera, Plecoptera and Trichoptera taxa); Sel_EPTD (log abundance of selected taxa of Ephemeroptera, Plecoptera, Trichoptera and Diptera); Shannon (Shannon-Wiener Diversity index); 1-GOLD (1 - relative abundance of Gastropoda, Oligochaeta and Diptera). These six metrics are combined (weighted average) in the STAR_ICM index (STAR Intercalibration Common Metric index), designed to assess general degradation (Erba et al., 2009) and officially adopted for ecological status classification according to macroinvertebrate fauna in Italy (MATTM, 2010). Abovementioned metrics were calculated by means of MacrOper.ICM software (ver. 1.0.5, Buffagni & Belfiore, 2013).

Water samples were collected simultaneously to biological sampling (number of water quality samples used for benthic community analysis: 132) and the following water quality variables were measured: O_2 (mg/l), N-NH_4 (mg/l), N-NO_3 (mg/l) and TP (μg/l) or P-PO_4 in temporary rivers. Such variables were used to support environmental gradients interpretation and to calculate the LIMeco descriptor.

Data analysis

A Principal Component Analysis (PCA) was applied to condense the variability of morphological impairment variables (Table 2) observed at the investigated rivers into an ordination reduced space (Legendre & Legendre, 1998). Prior to analysis, all variables were arc-sin square root transformed excluding Weir, Sluices and Bridges (WSB) and Tree Extent (ET) that were log transformed. Eleven morphological impairment variables were kept in the analysis independently from their autocorrelation because considered to provide important and different information on the type of morphological alteration.
The PCA results were represented as a distance bi-plot (Legendre & Legendre, 1998). Multivariate analysis was run by means of the PAST software (Hammer et al., 2001). PCA components were interpreted looking at Spearman rank correlation (Legendre & Legendre, 1998, eq 5.1) between axes and water quality variables and CARAVAGGIO derived habitat descriptors (i.e. HMS, HQA, LUIr, LRD). Spearman rank correlation was also applied to describe biological metrics response to morphological alteration as expressed by CARAVAGGIO habitat descriptors, multivariate components and other variables indicating water quality. Biological metrics values were transformed in Ecological Quality Ratios (EQRs), by dividing raw values by type-specific reference values reported in MATTM (2010) to allow comparison among river types for correlation analysis. For Spearman correlation calculations STATISTICA 7 software (StatSoft, Inc., 2004) was used.

To evaluate the strength of the relationship between ecological assessment (i.e. STAR_ICMi) and morphological impairment (i.e. HMS), a linear regression approach was adopted.
For both raw values and model residuals, a Lilliefors (Kolmogorov-Smirnov) test for the assumption of normality was used (R package "nortest": Gross & Ligges, 2015) and residual qq-plots were inspected. Assumption of normality was met ($p = 0.2767$, and $p = 0.09649$, respectively, according to Lilliefors test).
We then applied linear mixed-effects models, using the "lme4" package (Bates et al., 2017), with HMS set as the fixed factor. We refer to HMS because representing a comprehensive way to quantify morphological alteration. Because a few lowland sites showed a slight nutrient contamination, nitrate concentration and LIMeco were explored as fixed factors together with HMS. Stream type was fitted as random effect variable to account for spatial autocorrelation within areas and for the possible presence of dissimilar types of morphological alteration across types. Because the predictor variables are on differing scales, they were standardized prior to analysis according to the formula (x-mean(x))/sd(x) to limit numerical instability during model fitting. In the models, the parameters were estimated using maximum likelihood. Pseudo R squared for linear mixed-effect models was calculated using the "MuMIn" package (Bartoń, 2018). The selection of the regression model (i.e. including or excluding random effects and one or more fixed factors) was based on the AIC (Akaike's Information Criterion) values (package "stats"). The non-parametric Fligner-Killeen's test was performed on the residual values (R base package "stats") of the final model to check the assumption of homogeneity of variance across the five stream types. The assumption of variance homogeneity was not violated ($p = 0.1127$). These analyses were conducted in the R v.3.6.1 statistical software environment (R Core Development Team, 2019).

RESULTS

Investigated alteration gradient

Studied sites do not present heavy alteration in water quality (Table 3). In few cases comparatively higher concentration of single chemical parameters can be observed, in particular N-NO3 for 10 sites in lowland streams.

Table 6.3 Variability (min, median and max values) of morphological and water quality variables in the dataset studied. The variability of CARAVAGGIO-derived descriptors is also reported.

			Variables included in PCA analysis											Variables used to interpret PCA gradients								
			#Culverts	#Weir,Sluices &Bridges	% Artificial Straightening	% artificial STagnation	Tree extent	% Tree Related bank	% Tree Related channel	% Bank Resectioning	% Bank Reinforcement	% Channel Resectioning	% Channel Reinforcement	HMS	HQA	LUIr	LRD	LIMeco	O_2 (mg/l)	$N\text{-}NH_4$ (mg/l)	$N\text{-}NO_3$ (mg/l)	TP[1] (□g/l)
Lowland	Small sized	Min.	0	0	0	0	1	0	0.08	0.1	0	0	0	0	28	0.91	-8.5	0.4	4.6	0.003	0.88	4
		Median	0	1	0	0	3.5	0.23	0.25	0.49	0	0.75	0	14	45	0.95	28.4	0.7	8	0.03	3.77	50
		Max.	1	4	0.6	0.7	5	0.3	0.43	1	0.1	1	1	59	62	1	53.8	0.9	9.7	0.09	16.27	140
	Medium sized	Min.	0	0	0	0	0	0	0	0	0	0	0	0	11	0.52	-12	0.1	6.4	0.04	0.5	1
		Median	0	1	0	0	0.5	0	0.11	0.96	0	0	0	22	31	0.72	26.9	0.5	9.6	0.075	7.4	1
		Max.	1	2	0.9	0	5	0.7	0.43	1	0.3	1	0.1	47	59	1	101.8	0.8	18.7	1.53	38.8	880
Mediterranean mountain	Small sized	Min.	0	0	0	0	0	0	0	0	0	0	0	0	38	0.98	-55	0.5	7.8	0	0.01	3
		Median	0	0.5	0	0	0	0	0	0.13	0.05	0.1	0	14	59	0.99	-23.3	0.9	10	0.016	0.38	43.5
		Max.	0	4	0	0.3	5	0.35	0.5	0.98	0.23	1	0.25	71	77	1	34.5	1	11	0.143	2.1	451
	Medium sized	Min.	0	0	0	0	0	0	0	0	0	0	0	0	24	0.98	-35.7	0.5	7.9	0	0.01	0
		Median	0	1.5	0	0	4	0	0.08	0.13	0.14	0	0	25	63	0.98	-15.5	0.9	9.4	0.008	0.24	4
		Max.	0	3	0	0.7	5	0.7	0.5	0.53	0.6	0.9	0.5	44	72	1	11.3	1	12.6	0.16	0.32	81
Mediterranean	Temporary	Min.	0	0	0	0	0	0	0	0	0	0	0	0	29	0.58	-32.1	0.2	7.8	0.001	0	1
		Median	0	0	0	0	4	0.15	0.25	0.05	0	0	0	11	55	0.97	0.4	0.9	9.4	0.026	0.5	46.5
		Max.	1	10	1	0.7	5	0.7	0.7	1	0.6	1	0.9	88	84	1	80.3	1	12.5	0.291	6.91	247

[1]For Temporary streams, $P\text{-}PO_4$ values were available instead of TP.

The gradient of morphological alteration quantified by HMS is well covered: HMS values range from 0 (unaltered river sites) to >40 (highly modified) (these latter corresponding e.g. to 60% of channel modification, 100% bank modification and 2 bridges). In all river types, sites showing at least 90% of bank and/or channel modification (re-sectioning and/or reinforcing) are present. A wide variability in riparian vegetation modification is also observed, ranging from no tree present on banks to continuous tree vegetation and extensive presence of tree-related features (e.g. coarse woody debris, shading of channel).

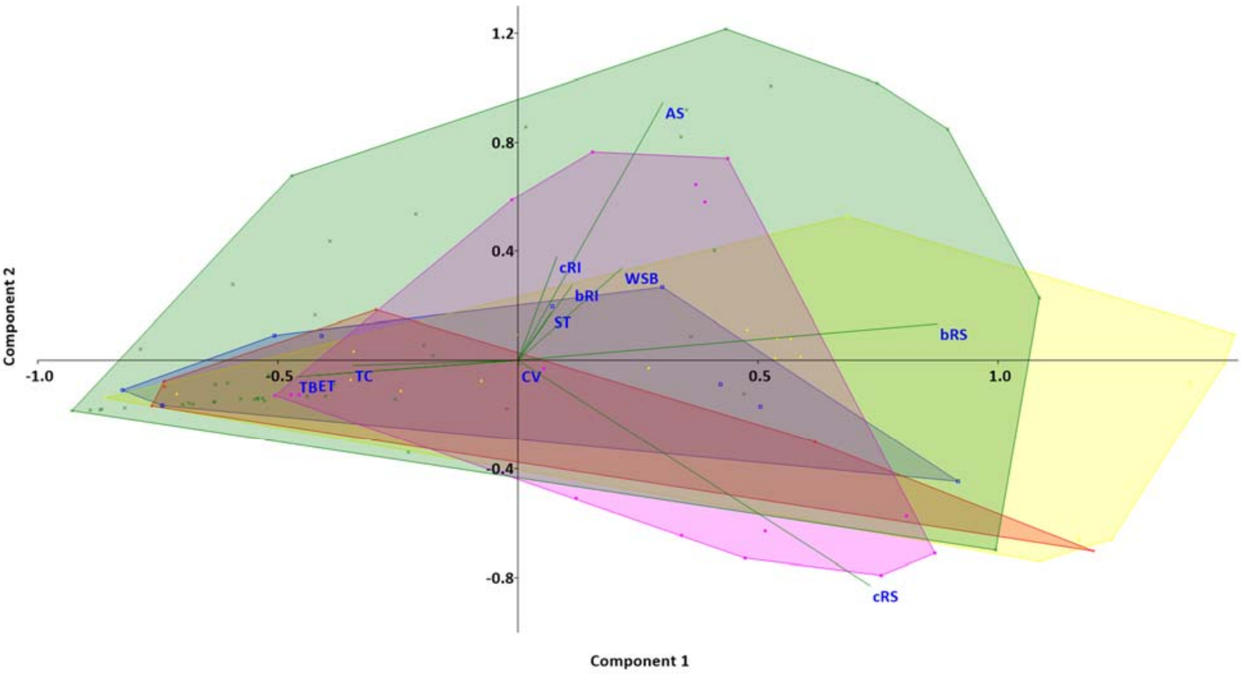


Fig. 6.2 Plot of PCA analysis (components 1 and 2) on morphological impairment variables. WSB: Weir, Sluices and Bridges; CV: Culverts; AS: Artificial Straightening; ST: Artificial Stagnation; ET: Tree extent; TC: Tree related channel habitats; TB: Tree related bank habitats; cRI: Channel reinforcement; cRS: Channel resectioning; bRI: Bank reinforcement; bRS: Bank resectioning. Red: Small Mountain; Blue: medium Mountain; Dark green: temporary streams; Yellow: medium lowland; Pink: small lowland.

The eleven morphological impairment variables considered in PCA showed autocorrelation lower than 0.50. Channel and bank reinforcement as well as tree-related features showed $\rho > 0.50$ among each other but they were not considered redundant because providing information on different kinds of impacts and/or different river portions. The PCA (Fig. 2) revealed a major gradient (Component 1) explaining ≈ 43% of variance. Along this axis, rivers with unmodified channel and banks and characterized by high percentage of tree related features are separated from modified river stretches. This first axis is thus representing morphological impairment (Table 4a) expressed as a combination of presence of artificial structures like e.g. bridges, weirs and sluices ($\rho = 0.50$), channel resectioning ($\rho = 0.66$) and bank modification ($\rho = 0.90$ for bank resectioning) and tree-related features indicating the presence of natural habitats. These latter features generally have a lower correlation with Component 1 axis than features representing an alteration. The first axis (Table 4b) is also highly correlated to the CARAVAGGIO/RHS descriptors of morphological impairment (HMS) and habitat quality (HQA). Although with values lower than other summary descriptors, this axis is also correlated to land use features (LUIr) and water quality (LIMeco). Correlations with chemical parameters (Table 4b) are weak and only significant for the first axis.

Table 6.4a Spearman correlation values among features considered in PCA analysis, resulting components and impairment descriptors. Only significant correlation values are reported. * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

	CV	WSB	AS	ST	ET	TB	TC	bRS	bRI	cRS	cRI
#Culverts	--										
#Weir, Sluices & Bridges		--									
% Artificial Straightening		0.32 ***	--								
% Stagnation		0.53 ***		--							
Tree extent		-0.23 *			--						
% Tree Related bank	0.15 *	-0.22 *			0.68 ***	--					
% Tree Related channel	0.22 *				0.72 ***	0.79 ***	--				
% Bank Resectioning		0.52 ***	0.34 ***		-0.47 ***	-0.39 ***	-0.38 ***	--			
% Bank Reinforcement	-0.22 *	0.40 ***		0.21 *	-0.3 ***		-0.24 *	0.21 *	--		
% Channel Resectioning		0.26 *			-0.39 ***	-0.32 ***	-0.32 ***	0.51 ***	0.22 *	--	
% Channel Reinforcement		0.33 ***	0.19 *	0.21					0.56 ***		--
Component 1		0.50 ***	0.34 ***	0.19 *	-0.54 ***	-0.55 ***	-0.5 ***	0.9 ***	0.29 **	0.66 ***	
Component 2		0.45 ***	0.60 ***	0.18 *					0.28 **	-0.51 ***	0.34 ***
HMS		0.61 ***	0.42 ***	0.32 ***	-0.48 ***	-0.39 ***	-0.38 ***	0.8 ***	0.52 ***	0.46 ***	0.40 ***
HQA		-0.40 ***	-0.35 ***		0.49 ***	0.47 ***	0.47 ***	-0.73 ***	-0.20 *	-0.41 ***	
LUIr		-0.38 ***	-0.35 ***		0.40 ***	0.28 **	0.27 **	-0.59 ***	-0.22 *	-0.24 *	-0.19 *
LRD		0.29 **	0.31 ***	0.23 **				0.53 ***			
LIMeco		-0.27 **	-0.25 **		0.27 **	0.21 *		-0.62 ***		-0.34 ***	
O_2	-0.21 *							-0.41 ***		-0.30 ***	
$N\text{-}NH_4$			0.21 *		-0.27 **	-0.19 *		0.48 ***		0.31 ***	
$N\text{-}NO_3$	0.26 **							0.52 ***	-0.19 *	0.25 **	
TP[1]	0.23 *				-0.23 *			0.44 ***	-0.25 *	0.45 ***	

[1] For Temporary streams, $P\text{-}PO_4$ values were available instead of TP.

Table 6.4b Spearman correlation values among abiotic impairment descriptors including the first two PCA components and chemical compounds. Only significant correlation values are reported. * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

	Component 1	Component 2	HMS	HQA	LUIr	LRD	LIMeco	O_2	$N\text{-}NH_4$
HMS	0.81 ***	0.27 **	--						
HQA	-0.82 ***		-0.63 ***	--					
LUIr	-0.58 ***	-0.23 **	-0.59 ***	0.61 ***	--				
LRD	0.46 ***		0.32 ***	-0.48 ***	-0.4 ***	--			
LIMeco	-0.59 ***		-0.47 ***	0.58 ***	0.56 ***	-0.52 ***	--		
O_2	-0.39 ***		-0.24 **	0.34 ***		-0.53 ***	0.45 ***	--	
$N\text{-}NH_4$	0.46 ***		0.38 ***	-0.5 ***	-0.45 ***	0.37 ***	-0.64 ***		--
$N\text{-}NO_3$	0.40 ***		0.2 *	-0.4 ***	-0.44 ***	0.55 ***	-0.8 ***	-0.37 ***	0.55 ***
TP[1]	0.41 ***	-0.3 ***	0.3 **	-0.31 ***		0.31 ***	-0.5 ***	-0.51 ***	0.34 ***

[1] For Temporary streams, $P\text{-}PO_4$ values were available instead of TP.

Table 6.5 Spearman rank correlation results among biological metrics and abiotic impairment variables including the first two PCA components. Only significant correlation values are reported. * $p<0.05$ ** $p<0.01$ *** $p<0.001$. In bold: the highest ρ value each biological metric gets with respect to variables indicating anthropic impact.

	ASPT		1-GOLD		Sel_EPTD		N_Families		EPT		Shannon		STAR_ICMi	
#Culverts											-0.18	*		
#Weir, Sluices &Bridges	-0.28	**			-0.32	***	-0.52	***	-0.53	***	-0.40	***	-0.47	***
% Artificial Straightening					-0.29	**	-0.24	**	-0.22	*	-0.25	**	-0.25	**
% Stagnation			-0.18	*	-0.20	*			-0.29	**			-0.25	**
Tree extent	+0.43	***			+0.30	***	+0.25	**	+0.41	***			+0.41	***
% Tree Related bank	+0.31	***			+0.26	**	+0.29	**	+0.35	***	+0.18	*	+0.36	***
% Tree Related channel	+0.42	***			+0.22	*	+0.28	**	+0.41	***			+0.37	***
% Bank Resectioning	**-0.64**	***	-0.19	*	-0.62	***	**-0.60**	***	-0.71	***	-0.40	***	-0.71	***
% Bank Reinforcement	-0.21	*	-0.24	**	-0.27	**	-0.26	**	-0.30	***	-0.31	***	-0.40	***
% Channel Resectioning	-0.47	***	-0.18	*	-0.35	***	-0.23	*	-0.47	***			-0.42	***
% Channel Reinforcement					-0.30	***	-0.19	*	-0.21	*	-0.23	**	-0.29	**
Component 1	**-0.64**	***	-0.22	*	-0.63	***	-0.57	***	**-0.72**	***	-0.40	***	-0.70	***
Component 2							-0.26				-0.22	*		
HMS	-0.58	***	**-0.33**	***	**-0.66**	***	-0.56	***	-0.71	***	**-0.42**	***	**-0.76**	***
HQA	+0.59	***			+0.54	***	+0.53	***	+0.62	***	+0.41	***	+0.60	***
LUIr	+0.54	***			+0.64	***	+0.52	***	+0.52	***	+0.40	***	+0.64	***
LRD	-0.39	***			-0.37	***	-0.20	*	-0.39	***	-0.20	*	-0.28	**
LIMeco	+0.47	***			+0.43	***	+0.30	***	+0.46	***	+0.32	***	+0.43	***
O_2	+0.41	***			+0.24	**			+0.36	***	+0.19	*	+0.25	**
$N\text{-}NH_4$	-0.41	***	-0.26	**	-0.38	***	-0.27	**	-0.36	***	-0.22	*	-0.38	***
$N\text{-}NO_3$	-0.34	***			-0.26	**	-0.20	*	-0.28	**	-0.19	*	-0.23	**
TP[1]	-0.34	***							-0.29	**			-0.18	*

[1] For Temporary streams, $P\text{-}PO_4$ values were available instead of TP.

Table 6.6 Summary of LMM results for the STAR_ICM index. The model includes river type as random effect factor. Pseudo-R-squared: marginal R^2 represents the variance explained by HMS, while conditional R^2 represents the variance explained for the entire model (i.e. by both fixed and random factors).

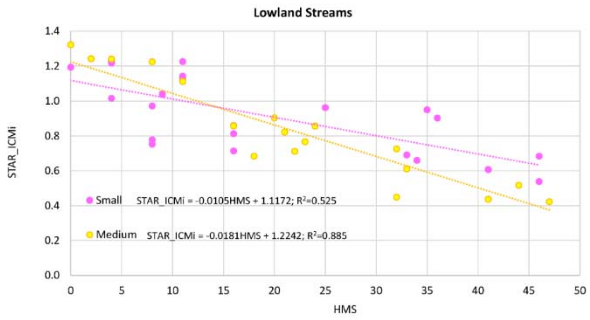
	n	Random effect variable	Pseudo-R-squared		Fixed effect						Correlation of fixed effect
			Marginal	Conditional		Estimate	Std. Error	Df	t value	Pr(>|t|)	
Linear mixed-effects model	132	area	0.601	0.779	HMS	-0.192	0.044	4.47	-4.40	0.00907 **	-
					(Intercept)	0.905	0.024	5.08	38.42	1.83e-07 ***	0.668

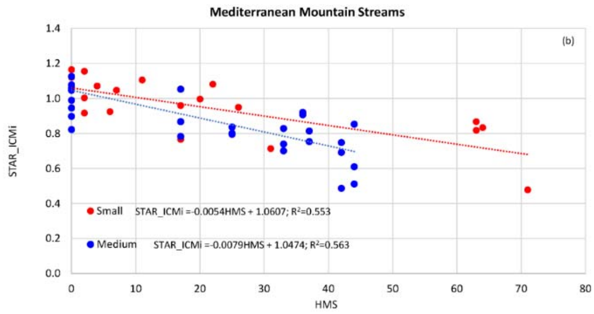
The second axis is accounting for a percentage of explanation ≈ 18% and seems linked to channel modification and presence of artificial straightening (Table 4a). Other derived axes were not considered because having a global low percentage of explanation (≤ 10%). Results of the multivariate analysis showed similar trends in morphological alteration in the different river types (Fig. 2) with similar length of gradient along Component 1.

Biological metrics response to morphological alteration

Spearman results evidence strong correlations between biological metrics and morphological impairment, excluding relevant association of water quality variables with biological metrics in the investigated dataset (Table 5). Correlation coefficients of biological metrics with PCA Component 1 and HMS are significant and are among the highest observed. Shannon and sel_EPTD have the highest correlation with HMS, even if differences in ρ values between HMS and PCA Component 1 are not pronounced. 1-GOLD metric presents generally low ρ values with all the considered variables, but its correlation coefficient resulted higher and significant for HMS. The number of families of EPT and the total number of families have higher correlation with the multivariate axis representing morphological impairment, but very similar to HMS. For total number of families the highest correlation value is obtained with bank resectioning. The best performance in terms of correlation coefficient is between HMS and the combined multimetric index STAR_ICMi (ρ= 0.76). Considering the correlations within the single types (Appendix 2) the general trend is confirmed, apart from small lowland rivers for which the land use (i.e. the LUIr index) seems more relevant for biological metrics. In Mediterranean mountain streams the occurrence of lentic and lotic habitats (LRD) also has some effect on STAR_ICMi.

Results of the mixed-effect linear regression model (LMM) between STAR_ICMi (i.e. representing the Ecological Status) and HMS (representing morphological alteration) are presented in Table 6. Although models including N-NO3 as predictor variable for fixed effects were significant, the model displaying the lower AIC includes HMS only. Models with stream type as random effect factor were significant. Both the fixed (HMS) and random (stream type) effects are highly significant (Table 6) in the final model eventually selected, which shows a marginal pseudo R^2 (i.e related to morphological alteration) > 0.6. The final model accounts for varying intercepts and slopes across the different environmental contexts as showed in Figure 3, where the scatterplots of stressor variable (HMS) versus STAR_ICMi are reported. In all stream types the relationship between HMS and STAR_ICMi is similarly good. Compared to other stream types, a difference in regression slopes is noticeable for lowland streams that can be linked with residual effect of water quality degradation. Additional statistics on the final model and the results of models comparison are reported in Appendix 3.





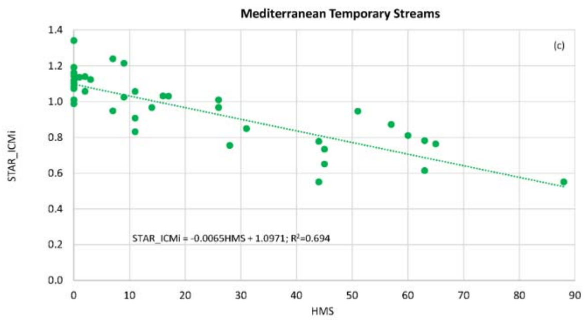


Fig. 6.3 Linear mixed-effects models results between HMS and STAR_ICMi for the different considered river types grouped by environmental contexts. (a) Lowland streams; (b) Mediterranean mountain streams; (c) Temporary streams. R^2 estimated by Ordinary Least Square (OLS) regression.

DISCUSSION

Can benthic metrics read river morphological impairment?

Our research was addressed at testing the response of benthic invertebrate metrics in use for quality classification – i.e. the STAR_ICMi and its component metrics – to morphological alteration. This point is particularly relevant in Italy since the development and /or refinement of metrics is not foreseen in the near future. The significance for such metrics of the outcomes of the two different approaches (HMS values and PCA Component 1 scores) used to quantify morphological impairment was verified.

The strong correlation found between morphological impairment descriptors and biological metrics adds evidence to validate benthic metric sensitivity to river channel and bank modification, as attained by Marzin et al. (2012). These authors, studying 290 French river sites, found strong response of macroinvertebrates metrics to morphological degradation (e.g. riparian vegetation, instream habitat and channel modifications; artificial embankment) even for metrics not specifically developed to detect such alteration. Our study is also supported by the results of Gieswein et al. (2017) who found, in rivers of a mountain catchment, that physical habitat quality (e.g. substrate diversity, bed fixations, bank features like woody debris and bank protection) is able to explain the largest portion of the deviance in aggregate invertebrate metrics.

We here demonstrated that river invertebrates respond to morphological impairment when the main impact is mostly linked to the presence of artificial structures and to the consequent alteration of riverine habitats. The key role of habitat as 'mediator' between benthic community assemblages and physical impairment has been ascertained and discussed for lowland heavily modified water bodies in Buffagni et al. (2019). In our study it is worth noting the performance of the abundance-related metrics 1-GOLD, Sel_EPTD and Shannon that show the highest correlation value with HMS among all the variables indicating anthropogenic impact. These results agree with those of Buffagni et al. (2016) who found in Mediterranean rivers the same three metrics attesting significant separation between morphologically-modified sites and sites at reference conditions. Also, high correlations were found between descriptors of morphological alteration (HMS and PCA Component 1) and number of EPT families, in agreement with other authors (Cooper et al., 2013; Buffagni et al., 2016). Such responsiveness of sensitive organisms to the effects of habitat impairment was evidenced by e.g. Burdon et al. (2013) in agricultural streams and by Buffagni et al. (2019) in lowland highly modified rivers.

In our study, we were able to reduce the potential influence of water quality excluding heavily polluted river sites from the dataset, and the obtained correlation of water quality parameters with biological metrics was weak. Notwithstanding the selection of sites to exclude other possible causes of alteration, some correlation was observed between morphological impairment, water quality parameters, land use (LUIr) and other habitat descriptors (e.g. HQA, LRD). In particular, such correlations are foreseeable (e.g. Erba et al., 2015) since morphological alteration of rivers is usually associated to the protection of specific land uses (e.g. agriculture, urban development) and it is expected to affect habitat structure both in terms of quality and in terms of proportion of lentic and lotic habitats. For lowland rivers it was problematic to locate sites showing low concentrations in water quality parameters. The difficulty to find areas without alteration linked to water quality is acknowledged for lowland rivers and ecological thresholds should be defined on a regional basis (Smith et al., 2013). This circumstance is evident in our lowland sites for nitrate, whose concentration can reach high values in some cases. This is partly due to the spring-fed origin of some of the sites and partly to the agricultural land use. Nonetheless, average N-NO3 values are in line with reference conditions as defined in Pardo et al. (2012) and Bennet et al. (2011). Moreover, in our studied sites only one of four parameters shows high values and comparatively high concentration in nitrates are accompanied by low ammonium and phosphate and high oxygen saturation, thus limiting the effect of overall water quality alteration and impact on the benthic community.

Our results showed that the STAR_ICM multimetric index is the better performing biological metric in relation to morphological impairment, among those tested here. This supports a greater performance of metrics that combine a variety of information on observed and expected community, not treating all individuals equally nor focusing only on specific taxon groups (Leps et al., 2015). In addition, the LMM regression model assigned most of STAR_ICMi variation to the HMS gradient. In Mediterranean rivers the association between STAR_ICMi and morphological alteration has been ascertained in the presence of degraded habitat conditions when both channel and banks modifications are observed (Buffagni et al., 2016). Also, Buffagni et al. (2019) have demonstrated how ecological potential in heavily modified water bodies as expressed by STAR_ICMi is strictly related to microhabitat mosaic and diversity, being these dependent from the level of mitigation measures implementation within a gradient of morphological impairment. Such result is consistent with outcomes from other authors who developed multimetric indices to detect hydromorphological degradation (e.g. Lorenz et al., 2004; Ofenböck et al., 2004; Urbanič, 2014; Theodoropoulos et al., 2020).

Our results seemingly contrast with Golfieri et al. (2018), especially with their general conclusion on the lack of sensitivity of BQE-based indices for hydromorphological alteration. Those authors did not find a significant relationship between MQI and the STAR_ICMi. However, they based their conclusions on only eleven samples located up to 5 km apart from the limits of the respective hydromorphological reach. Such distance can be considered quite far, from a benthic community point of view, e.g. Feld & Hering (2007) demonstrated that hydromorphological alteration at the meso-scale have the strongest influence on benthic invertebrates. Also, Golfieri et al. (2018) did not differentiate among river types and geographical areas. The kind of morphological alteration these authors were studying, as expressed by MQI and according to their experimental design, seemed to be potentially dependent from differences in river types. It is recognized that ecological and geomorphic responses to anthropogenic pressures should be calibrated to the regional context, to define at what degree the responses of stream invertebrate assemblages are region-specific or region-independent and broadly transferable (Villeneuve et al., 2018). We here accounted for potential differences due to stream types by using a mixed-effects model. Such differences might be due to the dominant morphological alterations, which might vary across geographical areas and landscapes. Indeed, the selected regression model demonstrates strong biological responses (i.e., STAR_ICM index) to morphological alteration (i.e., HMS) across stream types.

As a general conclusion, our results support the basic concept that benthic metrics used for ecological status classification in lowland and Mediterraenan rivers, including temporary rivers, are adequate to reveal the major impacts of morphological impairment. Undoubtedly, the link between morphological impairment and biological response can be improved (e.g. Bruno et al., 2014; Chapman et al., 2016), especially when considering specific geographical contexts and hydromorphological modifications not directly linked with bank, channel and riparian condition modification. In such cases (e.g. impact by large hydropower dam) functional approaches and metacommunity studies (Ruhi et al., 2018; Belmar et al., 2019) might increase our capacity of understanding the complex relationships between hydromorphology and biota, including a better estimation of climate change impacts, here not considered.

How can we quantify morphological impairment in relation to ecological status evaluation for the WFD?

We estimated morphological impairment on river sites performing a PCA on a set of variables known to possibly affect biological communities, expressing bank and channel modification and tree-related habitat conditions (e.g. Feld et al., 2014; Buffagni et al., 2016; González del Tánago et al., 2016). Multivariate techniques are widely applied to reduce complex data variability to principal components and defining interpretable gradients of variation (e.g. Verdonschot, 2009; Buffagni et al., 2004; Olsen et al., 2012; Feld et al., 2014). Expectedly, the PCA-derived morphological gradient on the first axis is strictly correlated to HMS index calculated from CARAVAGGIO application. HMS is in fact based on the same information used to run the PCA, but founds on a different approach to condense morphological impairment in a single signal i.e., it is a global score representing the cumulative impact of all specific alterations.

In the intention of the WFD, there are several reasons why hydromorphology has to be included in the assessment (Boon et al., 2010), among these is the need to identify hydromorphological pressures that may be causing a water body to fail to reach its environmental objectives (WFD Annex II, 1.4). This requirement in particular makes crucial to identify methods with clear relevance for the biotic communities.

In Europe, there is no scientific consensus on which methods are the best suited to assess hydromorphological impairment and on which river features should be monitored. This is because many fundamental questions relating hydrological, geomorphological and biological characteristics remain unanswered and because river habitats are monitored to cover a wide variety of objectives (Barquín & Martínez-Capel, 2011). At present, many standard methods are available for river habitat survey (e.g. LAWA, Langhans et al., 2013), some of which - although focused on local habitat features - provide useful information on morphological impairment like River Habitat Survey (Raven et al., 1997) or CARAVAGGIO (Buffagni et al., 2013). Moreover, most restoration measures deal with direct habitat implementation, rather than addressing hydromorphological processes and sediment management (Morandi et al., 2017). Restoration measures aiming at improving in-stream morphology, e.g. large wood placement, bed and bank fixation removal, are expected to have positive influence on macroinvertebrate richness (Kail et al., 2015). By contrary, the link between e.g. restoration of sediment quantity and biological community is less evident (Kail et al., 2015).

In the outlined context HMS seems an appropriate indicator that helps to simplify, quantify, analyze and therefore communicate complex information (Singh et al., 2012). HMS is a simple tool if compared to multivariate approach, this last one strongly dependent from the studied dataset (Sergeant et al., 2016) and not easily replicable. River management principles need tools that are applicable across different geographical contexts (Dunbar et al., 2010). For this reason, and to move away from the concept that any management actions must emphasize the uniqueness of individual rivers - hence requiring extensive site-specific data - we focused on data collected with CARAVAGGIO method and its calculated indices. We demonstrated that this inclusive descriptor (i.e., HMS) is well related to biology both in terms of Spearman results and as expressed through regression modeling, which is able to explain a large part of macroinvertebrates response to morphological alteration across the considered stream - types, in the absence of water pollution. The fact that the response between STAR_ICMi and HMS is apparent among different river types covering a wide geographical context makes our results particularly relevant, being representativeness fundamental in developing monitoring plans to assess river condition (Brierley et al., 2010). The use of a EQR scale to quantify the biological response is suitable to support outcomes independently of differences in benthic community variation along e.g. zoo-geographical and climatic gradients. We anyhow evidenced that biological responses to morphological alteration may slightly differ in different ecological settings and/or geographical areas, even when a context-independent EQR scale is used. This consideration supports the importance to avoid mixing different contexts or at least to account for possible confounding effects when quantifying biological responses to habitat degradation.

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CHAPTER 7

Defining Maximum Ecological Potential for heavily modified lowland streams of Northern Italy

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Defining Maximum Ecological Potential for heavily modified lowland streams of Northern Italy

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ABSTRACT

In relation to hydromorphological alteration the Water Framework Directive (WFD), a major piece of European legislation, has introduced the concept of Heavily Modified Water Bodies (HMWB). In water bodies falling in this category, hydromorphological modifications are permanent, significantly alter the character of the river and cannot be removed without compromising the use of the water body. In HMWBs a dedicated approach to the evaluation of their status is set, and their Ecological Potential must be assessed. Crucial to the process is the definition of Maximum Ecological Potential (MEP) as the reference conditions for HMWB. In the present paper we aim to define MEP conditions for Italian heavily modified lowland rivers, affected by strong bank protection (i.e. levees or bank reinforcement) in reason of flood protection and land drainage uses. The approach applied to identify MEP conditions follows the one considered for natural (not heavily modified) rivers in Italy and large part of Europe and bases on the identification of 'reference sites' representative for the river category and alteration. For the selection of MEP sites environmental features representing mitigation measures and/or expected natural features were considered. The ability of such features in discriminating MEP and disturbed sites was verified by multivariate analyses run on abiotic features (Principal Component Analysis) and biological communities (non-metric multidimensional scaling). We demostrated differences both in terms of invertebrate community and biological metrics used to assess ecological status (and potential) between MEP and impaired river stretches. Finally, we recognised relevant habitat features able to clearly separate MEP reaches from nonMEP reaches with indication on the type and quantity of measures significant for benthic invertebrates and applicable in lowland heavily modified water bodies.

1. INTRODUCTION

The Water Framework Directive (WFD, EC 2000/60) is a major piece of European environmental legislation aimed at protecting and enhancing surface waters and groundwaters. The implementation of the WFD has been a long and complex process, centred on the key objective of achieving good ecological status for all water bodies by 2015 (Carré et al., 2017). Although four years have passed from this ambitious deadline, the WFD implementation remains far from being concluded. Critical reviews on achievements and open challenges related to WFD topics were made available from many authors (e.g. Carvalho et al., 2019; Hering et al., 2010; 2018; Reyjol et al., 2014).

Major improvements in the quality of European rivers have been achieved like e.g. marked efforts to enhance water quality and to implement water monitoring plans and ecological status of some biological quality elements (EEA, 2018). Nevertheless, hydromorphological modification still remains one of the major problems affecting river health (EEA, 2018; Vaughan et al., 2009). In relation to hydromorphological alteration the WFD has introduced the concept of Heavily Modified Water Body (HMWB). Such concept, which exists in reason of the WFD, is strictly linked to the presence of major physical alterations, introduced to allow for a range of water uses. In HMWB hydromorphological modifications are permanent and significantly alter the morphological and hydrological characteristics of the water body (Halleraker et al., 2016). By definition, the removal of major physical modifications from a HMWB compromises the use of the water body. According to Article 4(3) (a) of the WFD, water regulation, flood protection and land drainage are considered among the activities likely to result in a water body being designated as HMWB. When a water body is designated as heavily modified its environmental objective changes from Good Ecological Status (GES) to Good Ecological Potential (GEP). The GEP is conceptually very similar to the GES, although it takes into consideration the limitations imposed by the water body use. As for natural water bodies, and regardless of the adopted classification approach, ecological potential in HMWBs has to be based on the assessment of biological conditions.

The definition of Maximum Ecological Potential (MEP), i.e. the reference condition for HMWB, can be crucial in the process of assessing ecological potential. Such condition is described as the best approximation to a natural aquatic ecosystem that could be achieved given the hydromorphological alterations that cannot be removed without significant adverse effects on the specified use or the wider environment (CIS, 2003). MEP selection criteria are central to the assessment of ecological potential in the same way as reference conditions and reference site selection principles are for ecological status definition (Feio et al., 2014; Pardo et al, 2012). Because in HMWB entirely unaltered conditions cannot be attained, the MEP can be obtained by applying all the mitigation measures compatible with the specified use (CIS, 2003). MEP conditions can be defined and assessed on the basis of actual characteristics found in a specific river reach where the environmental conditions are the best available, provided that all possible mitigation measures are implemented.

Within the program of “Common Implementation Strategy (CIS)” for WFD implementation numerous documents in form of practical guidance on a series of technical issues related to the Directive, including the HMWB topic (CIS, 2003; 2005) were produced. Anyhow, the evaluation of ecological quality of HMWBs is considered as needing in-depth investigation. In the authors knowledge, only few scientific papers are available on this subject (e.g. Fernández et al., 2012; Ibrekk and Pedersen, 2005; Kail and Walter, 2013; Buffagni et al., 2019), some of which not specifically focussing on rivers (Borja and Elliot, 2007; Molozzi et al., 2013). Also, available papers are mostly not updated with the progress done at European level in the different CIS working groups. Likewise, in several countries the classification criteria for HMWBs and Artificial Water Bodies (AWBs), in particular the definition of the ecological potential, are still under development (EEA, 2018), notwithstanding the economic interest linked with HMWB management. Practical experience of defining MEP and GEP seems currently limited and complex. In this context Buffagni et al. (2019) provided useful elements for ecological status/potential evaluation recognizing the positive effect of mitigation measures on in-stream habitat conditions and, subsequently, on benthic communities.

Accordingly to what above, the aim of the present paper is to describe MEP conditions as defined for Italian heavily modified lowland rivers in relation to flood protection and land drainage. The studied context is comparable to what outlined in Buffagni et al. (2019) and consists of river reaches bearing levees or bank reinforcement both constructed to preserve the use of designation. Starting from some of the mitigation measures there defined, which implied gentle riparian vegetation management with a passive restoration approach for in-stream habitats, we will discuss in detail which measures and/or expected natural features are the most important in determining macroinvertebrate and sites ordinations and how these measures are related to MEP definition. We hypothesized that patterns in macroinvertebrate assemblages reflect differences between MEP and impaired sites and are supported by specific habitat features. We kept separately leveed and reinforced reaches. These two kinds of modification are predominantly associated respectively to agricultural (levees) or urban (bank reinforcement) patches. Such contexts have a great influence on riparian vegetation development, presence of organic debris, presence and extension of berms and in-channel substrate. Consequently, leveed and reinforced reaches can be managed according to different options and distinctive mitigation measures can be applied. Moreover, their supposed different habitat composition, related to such contexts, may influence the biological community assemblages.

In detail, our objectives were: (i) to select appropriate MEP sites for the studied context; (ii) to explore the variation of a range of morphological and habitat characteristics to verify if they can discriminate between MEP and nonMEP sites; (iii) to examine if such distinction is supported by differences in the macroinvertebrate community.

2. METHODS

2.1 Study area

We studied 30 river stretches (Figure 1) belonging to Heavily Modified Water Bodies (HMWBs) because of flood protection and land drainage. Sites are located in the Drainage Basin of Venice Lagoon (Italy, Veneto) and are characterised by the presence of levees or bank reinforcement. River sites were selected within a gradient of hydromorphological degradation (Figure 2). All studied rivers belong to water bodies classified from High to Moderate status according to LIMeco descriptor formally used in National Italian legislation (MATTM, 2010) for a water chemistry-based classification. This condition excluded strong water quality degradation as driver for biological communities. Main water quality parameters are reported in Table 1. Major anions were determined by suppressed ion chromatography (Dionex system), whilst NH_4 and PO_4 were detected by molecular absorption spectrometry (Perkin Elmer, UV–VIS Lambda25). Conductivity, pH and oxygen concentration were measured in field.

The selected stretches were unambiguously assigned to the leveed/embanked (*Emb*) or to the reinforced (*Rin*) group. Although embankments and reinforcements are not mutually exclusive, leveed reaches were clearly identifiable by distinctive levees made of earth, without outer visible reinforcement or reinforcement not in direct contact with water and limited to a portion of the bank. Reinforced reaches presented reinforced banks of concrete or other artificial material (including embankment walls) for most of their extension.

2.2 MEP reaches selection

Selection of potential MEP reaches (also referred to as MEPp) was initially based on large scale information. Data relating to the shape of the banks, banktop lines, buffer strips extension, channel conformation and bedforms were digitised and characterised as shapefiles in a QGIS environment. The land use in the catchment was also quantified by means of standard CORINE land cover (CLC) map (1:50.000) and more detailed land use cover map of Veneto region (CLC based, scale 1:10.000, De Gennaro et al., 2007). Aggregated land use data are provided in Figure A.1. In the studied area the land use in the wider environment (catchment) is comparable among different sites, and does not allow a distinction between MEP and nonMEP sites. At the reach scale, land use is implicitly considered in the two categories ‘reinforced’ and ‘leveed’, being the first characterised by a more urban context for both MEP and nonMEP and the latter characterised by agricultural context.

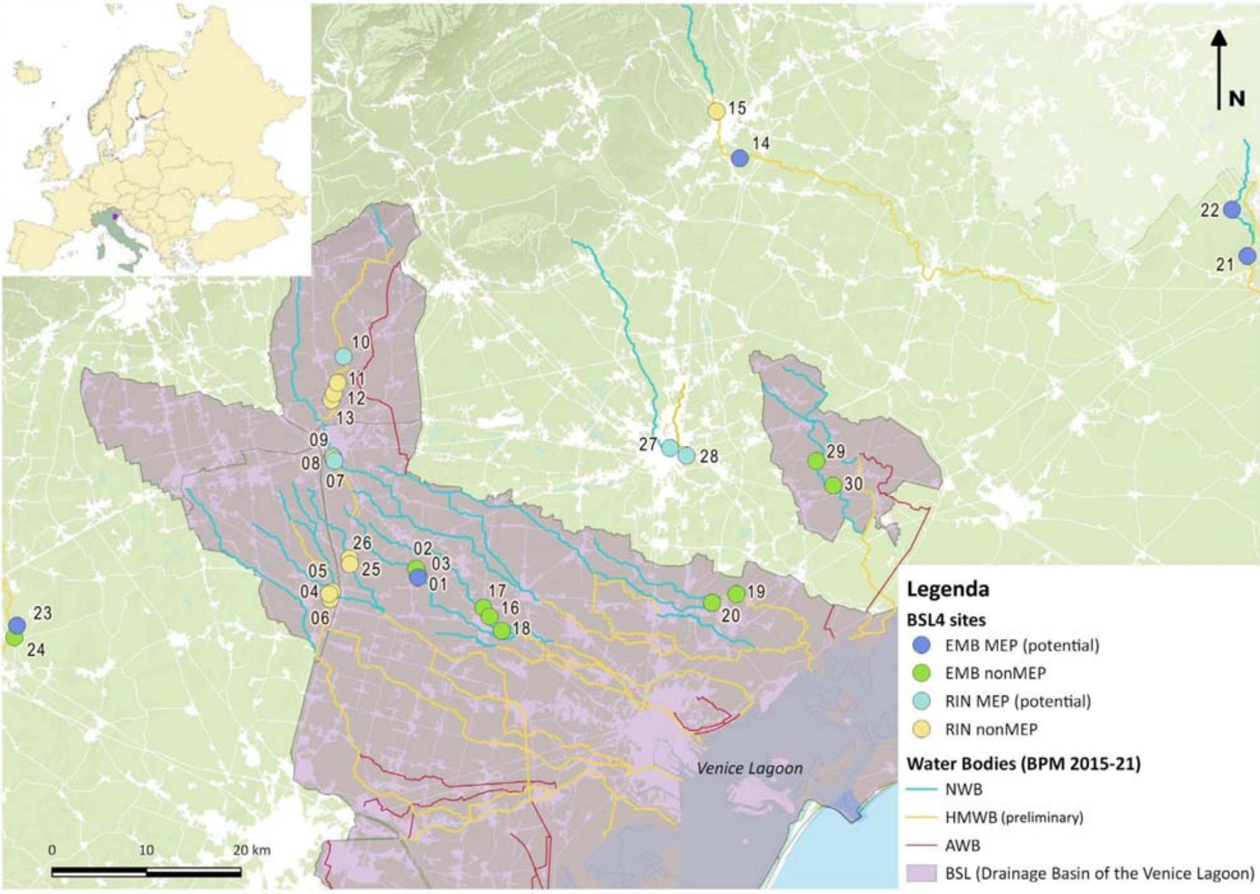

Fig. 7.1. River stretches location. NWB: Non-heavily Modified (i.e. natural) Water Bodies; HMWB: Heavily Modified Water Bodies; AWB: Artificial Water Bodies. 'Preliminary': designation to be confirmed by competent water authorities. MEP (potential) indicated in the map includes reaches excluded following the selection procedure. In legend: EMB leveed/embanked; RIN reinforced.

Large scale analysis for MEPp selection was supported by sites inspection on the field; altogether large scale analysis and field inspection considered the following four components: i) riparian vegetation complexity, ii) in-channel habitat diversification, iii) embankment characteristics, and iv) reinforcement characteristics. For each of the four components we assessed the presence of natural habitat features, each representing possible mitigation measures, considered as guiding elements for MEPp stretches selection, as hereafter described. i) Herbaceous / shrub / arboreal components of riparian vegetation were evaluated in terms of longitudinal continuity and lateral extension (up to and beyond the levee when present). The presence of allochthonous riparian species and/or species not belonging to a typical riparian vegetation association was considered a downgrading factor. ii) The diversification of in-channel habitats, the lack of channel incision and of in-channel alterations, possibly accompanied by the presence of bed forms (e.g. submerged bars, longitudinal bars), were considered positive elements for the riverbed. iii) Levees were characterised as comparatively 'positive' if placed at a distance proportionate to the width of the riverbed and if associated with the presence of internal floodplains and/or berms (interposed between embankment and river channel). iv) The reinforcements, likewise, to be considered positive must consist of materials / techniques of soft engineering and / or permitting the establishment of natural elements on the river bank. Discontinuity in the occurrence of reinforcement was also considered. In general terms, when considering the above mentioned components, we mainly focused on the presence of tree-related bank and channel habitats on the river reach. In fact, where mitigation measures are well implemented (e.g. at MEP reaches), we expect active or passive restoration may resolve in an increase of trees and associated natural features and in an improvement of in-channel diversification as also defined in Buffagni et al. (2019).

Fig. 7.2. Gradient of hydromorphological alteration in the investigated reaches and examples of sites at Maximum Ecological Potential (MEP). (a) MEP (21 and 10); (b) Slightly modified (16 and 28); (c) Obviously modified (17 and 11), respectively for leveed (Emb) and reinforced (Rin) categories.

Table 7.1. Main water quality parameters measured at sampling sites, separately considering MEP, Slightly modified and Obviously modified reaches.

Environmental variable		**MEP**			**Slightly modified**			**Obviously modified**		
		min	**mean**	**max**	**min**	**mean**	**max**	**min**	**mean**	**max**
$P\text{-}PO_4$	µg/l	6.6	22.3	47.9	1.2	25.2	42.9	1.7	22.9	74.1
$N\text{-}NH_4$	mg/l	0.003	0.03	0.09	0.01	0.05	0.18	0.004	0.04	0.18
$N\text{-}NO_3$	mg/l	0.51	1.04	1.70	0.55	2.21	5.90	0.40	1.86	5.89
Oxygen Saturation	%	89.5	104.7	118.0	58.0	88.5	116.0	60.0	92.6	123.2
pH		8.12	8.37	8.51	7.88	8.07	8.43	7.90	8.19	8.56
Conductivity	µS/cm	341	415	493	367	459	581	355	445	604

As a result of this selection 15 reaches were identified as potential MEP, 8 in the leveed and 7 in the reinforced category. A second screening was subsequently performed on MEPp sites to quantify their natural habitat features / mitigation measures and preliminarily confirm or exclude their MEP status. To each potential MEP a 1 to 5 score was attributed to the features composing the four components considered in the first selection. As detailed in Table A.1a, the highest score was given when different natural features are present for each component. For example, dynamic and active channels with typical forms (e.g. bars) scored 5, over-deepened channels with a constant section received a score of 1 and intermediate scores were assigned along a gradient of natural variability in cross sections. Riparian vegetation got 5 if mainly constituted by trees with a good lateral development; if constituted mainly by shrubs only fairly extended, it got 4. Herbaceous or bare structures got respectively 2 and 1. The embankment and reinforcement received a score of 5 if always distant, decreasing to 4 if they got in contact with the water in some parts of the reach; the score was 1 if always in contact with the water (almost without active berms). Obtained scores were then averaged and converted in a 0-1 scale. MEPp reaches were accepted and selected as MEP if having a score equal or higher than the 75th percentile of all potential MEP sites. MEPp sites below the 75th percentile were assigned to the 'Slightly modified' category. Separate percentile values were calculated for the reinforced and leveed sites as a different combination of features was considered for scoring in the two categories (see Table A.1a). This procedure allowed us to objectively define a threshold of acceptability in the application of mitigation measures. This second screening led to the selection of 7 MEP reaches: 4 in the leveed category and 3 in the reinforced category. Table A.1b is provided with the scoring system for MEP selection, including the final score assigned. In addition, we verified for the 7 selected MEP sites that the applied management practices were sustainable (i.e. not invasive and not involving the whole bank and channel extension when applied) and that water quality as per LIMeco descriptor was never lower than good.

Since the 75th percentile value considered in the MEP selection can depend on the observed gradient we further investigated consistency in the selected MEP in comparison to the whole pool of sites by means of multivariate analysis on abiotic data and invertebrate communities.

2.3 Environmental data

To characterise river stretches and to select habitat variables able to discriminate between MEP and other reaches, we applied the CARAVAGGIO method (Buffagni et al., 2013) developed on the basis of the River Habitat Survey protocol (RHS, Raven et al., 1997). The CARAVAGGIO method consists of a comprehensive survey of habitat features and alteration along a 500 m channel length and was applied to a section representative of the wider scale. Large spatial scale QGIS analysis performed for potential MEP selection was beneficial to confirm the representativeness of the stretch investigated with the CARAVAGGIO method with respect to the whole water body.

CARAVAGGIO surveys were undertaken between September and November 2016, simultaneously to biological and water sampling. The variables collected with CARAVAGGIO and used in the present paper to confirm MEP attribution are reported in Tables 2 and 3. In particular, we focused on features representing the alteration of designation (Table 2) and possible mitigation measures (Table 3) of such alteration according to the available literature and current work done in European working groups (e.g. Brookes et al., 2009; Bussettini et al., 2018; Vartia et al., 2018).

The variables used to represent mitigation measures were derived from Buffagni et al. (2016; 2019).

Table 7.2. Environmental variables representing morphological alteration linked to flood protection and land drainage designation uses. Expected morphological alteration and related impact were derived from Bussettini et al., 2018 and Vartia et al., 2018.

River section	Morphological alteration	Impact	Considered environmental variable	Code	Description
Bank	Loss of continuity, alteration of morphodynamic characteristics	Loss of riparian and marginal habitats	Levee/embankment	b_EM	% embankment
			Bank reinforcement	b_RI	% bank reinforced
			Bank resectioning	b_RS	% bank resectioning (excluding embankment, and reinforcement)
Channel	Morphological changes in cross section, loss of bed forms and structures, channel incision	Altered in-stream habitat, loss of morphological diversity and habitat	Channel resectioning	c_RS	% of channel resectioning

Table 7.3. Environmental variables representing mitigation measures and/or expected natural features (modified from Buffagni et al., 2019)
(*): from Brookes et al., 2009; Bussettini et al., 2018; Vartia et al., 2018

River section	Mitigation measure(*)	Environmental variable	Acronym	Description
Bank / channel	Develop riparian forest; rehabilitation of banks and riparian zone with autochthonous trees and shrubs; enhance aquatic and riparian habitats	Presence of Shrubs	Shrub	Presence of shrubs in the river area (channel and banks, %)
		Herbaceous bank	b_gras	% of bank covered by tall herb, grassland, rank vegetation
		Tree on the bank	b_tree	% of bank covered by trees
		Continuity of tree vegetation along the banks	TreeCov	Continuity of woody vegetation along banks (mainly trees, ≥ 2 m high): Arithmetic mean of extent on left and right bank, after numeric conversion (None: 0, Isolated/scattered: 1, Regularly spaced-single: 2, Occasional clumps: 3, Semi-continuous: 4, Continuous: 5)
	Creation of natural-like irregularities; provide space for natural bank adjustment to occur; set-back embankments	Back embankments (or back reinforcement)	Back_EM	% of back embankment
		Bank erosion	b_Ero	% of bank erosion
Channel	Enhance aquatic and marginal habitats	Submerged macrophytes	SO	% of submerged macrophytes
		Emergent macrophytes	EM	% of emergent macrophytes
		Large woody debris (Xylal)	XY	% of large woody debris
		Coarse Particulate Organic Matter	CPOM	% of Coarse Particulate Organic Matter
	Increase bed complexity and river bed variation; reduce channel narrowing and/or resectioning	Channel form	TWW / TCW	Total Water Width / Total Channel Width

2.4 Biological data

In each river stretch macroinvertebrate data were collected with a multihabitat sampling procedure (Buffagni et al., 2001; Buffagni et al., 2004). Each sampling unit consists of a Surber net sample (mesh size 500 µm, area 0.05 m^2). For each site two samples made of 10 sampling units are available (G1 and G2). This corresponds to the required sampling effort for surveillance monitoring by Italian National legislation (MATTM, 2010).

For the two taxalists per river stretch we calculated the following biological metrics: ASPT (Average Score per Taxon; Armitage et al., 1983); N_families (Total Number of Families; e.g. Thorne and Williams, 1997); EPT (number of Ephemeroptera, Plecoptera and Trichoptera taxa; Barbour et al., 1999); Sel_EPTD (log abundance of selected taxa of Ephemeroptera, Plecoptera, Trichoptera and Diptera; Buffagni et al., 2004); Shannon (Shannon-Wiener Diversity index; Shannon and Weaver, 1949); 1-GOLD (1 - relative abundance of Gastropoda, Oligochaeta and Diptera; Pinto et al., 2004). These six metrics are the ones composing the STAR_ICM index (STAR Intercalibration Common Metric index, Buffagni et al., 2007), formally in use in Italy to assess river ecological status. For the calculation of the above-mentioned metrics taxa were identified at family level and the MacrOper.ICM software (ver. 1.0.5, Buffagni and Belfiore, 2013) used.

2.5 Statistical analysis

A principal components analysis (PCA) was applied to environmental variables described in Tables 2 and 3 to determine if the potential MEP sites can be distinguished from disturbed reaches. The same analysis was used to provide information on the observed gradient and on the relative importance of the considered abiotic variables in defining such gradients. According to PCA results it was also possible to confirm/refuse site attribution to the MEP category. Prior to analysis, variables were log transformed or arc-sin square root transformed according to be continuous or proportional. The PCA resulting diagram was represented as a distance bi-plot (Legendre and Legendre, 1998). A non-metric multidimensional scaling (nMDS) analysis was performed to recognise distribution patterns among benthic invertebrate abundance (logarithmic transformation; Bray–Curtis similarity) of the different sampled stretches. Analysis of similarities (ANOSIM) using Bray–Curtis distance (Clarke, 1993) was carried out to test whether there was a significant difference between the proposed groups (MEP, Slightly modified and Obviously modified) in terms of macroinvertebrate communities. Multivariate analyses and ANOSIM were run by means of the PAST software (Hammer et al., 2001).

Lastly, to test differences in the metrics values of the MEP and disturbed reaches, we used the non parametric Mann-Whitney U-test (one-sided). The effect size (i.e. difference in location) was also calculated, based on the Hodges-Lehmann estimator (Hodges and Lehmann, 1963), that is an efficient nonparametric estimator resistant to unusual values (i.e. outliers). It was calculated as the median of all possible pairwise differences between samples from MEP sites and samples from disturbed sites, and can be used to determine how much the two groups differ. The estimator maintains the original unit of the metric under investigation and can thus be interpreted straightforwardly. Calculations were performed with the package 'stats' in the R v.3.4.2 software environment (R Core Team, 2017).

3. RESULTS

3.1 Environmental gradient description

PCA results attributed an overall percentage of explanation of 67.7% to the first two axes (PCA 1: 43.5; PCA 2: 24.2). The first axis is guided by the presence of xylal, set-back embankments, shrubs and trees on one hand of the axis and by channel resectioning and bank reinforcement on the other hand. A clear separation between MEP and disturbed reaches can be observed along this axis. The second axis places in opposition embankment and bank resectioning, distinguishing reinforced from leveed reaches.

PCA results support the initial MEP screening evidencing that there are affinities between MEP and Slightly modified sites (i.e. sites initially selected as potential MEP). These two groups (blue and green colors in Figure 3) are in close contact. In particular, the sites of the Slightly modified groups closer to MEP have good habitat conditions (e.g. are fluvial SCIs - Sites of Community Importance) but exhibit a comparatively low water quality (with respect to MEP group) or present allochthonous riparian species.

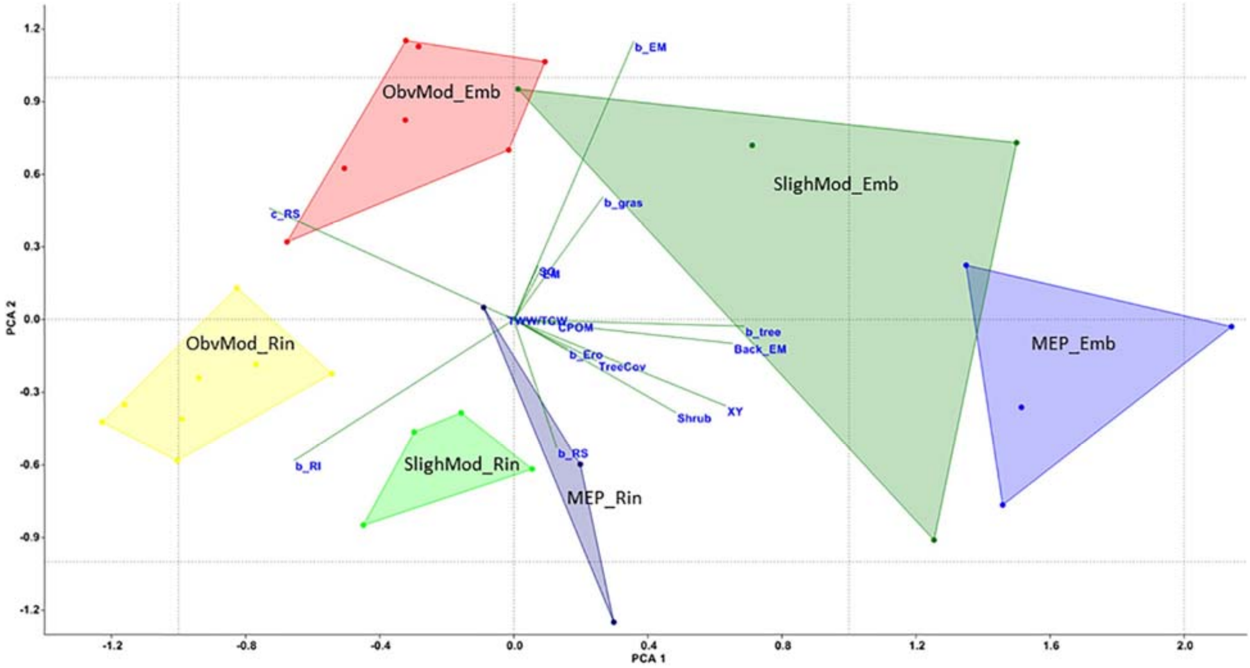

Fig. 7.3. PCA output of environmental variables (segments) in the 30 investigates river reaches (dots). Blue: MEP leveed; dark green: slightly modified leveed; red: obviously modified leveed; dark blue: MEP reinforced; pale green: slightly modified reinforced; yellow: obviously modified reinforced. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Range values characterising the different groups are reported in Table 4 for all the variables used to run PCA analysis. MEP stretches were distinguished by higher presence of natural features improving channel and bank diversity and heterogeneity than the modified reaches of the same group (i.e. embanked *vs* reinforced). In particular, higher percentage of Xylal, CPOM, marginal aquatic habitats (e.g. presence of emergent reeds and sedges), shrubs (e.g. willows) on banks and inside channel were observed in MEP reaches compared to disturbed reaches. The continuity of tree vegetation on the bank was on average semi-continuous in MEP reaches while disturbed reaches had isolated or occasional clumps of trees. As far as aquatic macrophytes are concerned, a higher percentage of such habitat was observed at disturbed embanked reaches.

Table 7.4. Range and mean values of each habitat variable. Numbers are separately reported for leveed (Emb) and reinforced (Rin) reaches, also considering MEP, Slightly and Obviously modified reaches. (number of sites: MEP_Emb: 4; SlighMod_Emb: 4; ObvMod_Emb: 7; MEP_Rin: 3; SlighMod_Rin: 4; ObvMod_Rin: 8).

	MEP_Emb			SlighMod_Emb			ObvMod_Emb		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
c_RS	0.00	0.08	0.30	0.00	0.50	1.00	0.20	0.89	1.00
b_RS	0.00	0.13	0.50	0.00	0.22	0.88	0.00	0.03	0.10
b_EM	0.10	0.62	1.00	0.00	0.74	1.00	0.48	0.88	1.00
b_RI	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.55
Shrub	0.45	0.71	1.00	0.05	0.27	0.45	0.00	0.01	0.05
SO	0.03	0.19	0.36	0.29	0.38	0.48	0.14	0.31	0.50
EM	0.04	0.09	0.13	0.08	0.16	0.23	0.06	0.15	0.22
XY	1.00	1.00	1.00	0.00	0.32	0.71	0.00	0.04	0.29
Back_EM	0.05	0.71	1.00	0.00	0.41	1.00	0.00	0.00	0.00
b_Ero	0.07	0.14	0.20	0.00	0.09	0.23	0.00	0.07	0.33
b_gras	0.05	0.31	0.70	0.40	0.60	1.00	0.05	0.42	1.00
b_tree	0.15	0.64	1.00	0.00	0.61	1.00	0.00	0.00	0.00
CPOM	0.09	0.14	0.19	0.03	0.09	0.15	0.02	0.06	0.10
TreeCov	2.00	3.38	4.00	1.50	2.63	4.00	0.50	1.00	2.00
TWW/TCW	0.30	0.78	1.00	0.99	1.00	1.00	1.00	1.00	1.00
	MEP_Rin			SlighMod _Rin			ObvMod_Rin		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
c_RS	0.00	0.67	1.00	0.10	0.68	1.00	0.00	0.70	1.00
b_RS	0.00	0.48	0.95	0.10	0.34	0.55	0.00	0.00	0.00
b_EM	0.00	0.28	0.83	0.00	0.03	0.10	0.00	0.11	0.38
b_RI	0.20	0.32	0.50	0.05	0.35	0.60	0.70	0.91	1.00
Shrub	0.40	0.63	0.80	0.10	0.21	0.40	0.00	0.06	0.30
SO	0.03	0.17	0.41	0.00	0.25	0.51	0.00	0.16	0.34
EM	0.05	0.10	0.19	0.00	0.06	0.19	0.00	0.06	0.20
XY	0.28	0.28	0.28	0.28	0.28	0.28	0.00	0.04	0.28
Back_EM	0.00	0.17	0.50	0.00	0.01	0.05	0.00	0.00	0.00
b_Ero	0.00	0.17	0.40	0.03	0.16	0.37	0.00	0.03	0.20
b_gras	0.00	0.10	0.30	0.00	0.18	0.30	0.00	0.09	0.30
b_tree	0.00	0.17	0.50	0.00	0.01	0.05	0.00	0.00	0.00
CPOM	0.10	0.11	0.13	0.00	0.09	0.18	0.00	0.04	0.10
TreeCov	3.00	3.67	4.00	2.00	2.25	2.50	0.50	1.00	2.00
TWW/TCW	1.00	1.00	1.00	0.90	0.97	1.00	0.99	1.00	1.00

3.2 Biological assemblages characterising MEP and impaired reaches

93379 individuals belonging to 71 different families were collected in the studied reaches. Figure 4 reports in percentage the relative abundance of the different taxa found at investigated sites, separately for MEP and nonMEP stretches (these last ones including Slightly and Obviously modified reaches) in the leveed and reinforced categories. Highest percentages of Ephemeroptera and Trichoptera were found in MEP reaches, with smaller differences when looking at reinforced category.

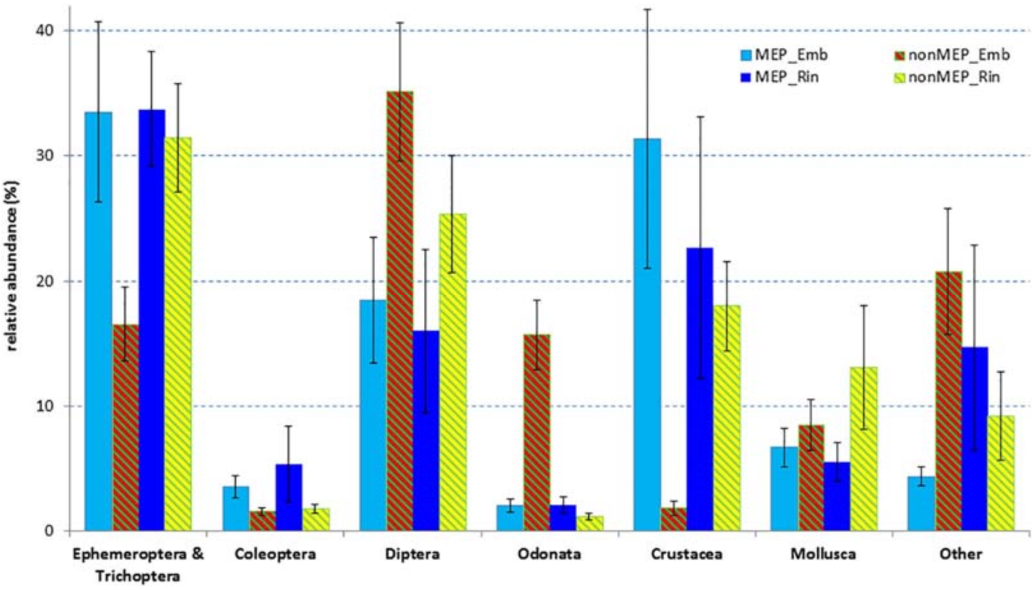

Fig. 7.4. Percentage of relative taxa abundance found at investigated sites, separately for MEP and nonMEP stretches (these including slightly and obviously modified stretches) in the leveed (Emb) and reinforced (Rin) categories. Mean standard errors bars are also reported.

Also Coleoptera, even if representing a small proportion of benthic community abundance, were mostly found in MEP. Diptera were principally present at impaired reaches. Crustacea, mainly Gammaridae and Asellidae, were greatly contributing to the overall invertebrate abundance.

Results of non metric multidimensional scaling (Stress 0.206, Figure 5) supported by ANOSIM test (Table 5) evidenced significant differences for biological communities in all but two sites groups. Biological communities of MEP reaches are mostly different from nonMEP and reinforced reaches are different from leveed ones. The sole non-significant differences were observed in the reinforced category between MEP and Slightly modified and between these latter and Obviously modified. In the reinforced group the biological communities are spread along a more continuous gradient and it is more difficult to find sharp differences. However, the strongly significant difference between MEP and disturbed sites in terms of biological communities provides a validation of MEP sites selection both for leveed and reinforced reaches.

nMDS results are consistent with PCA findings. The most important environmental variables in determining differences in biological communities were: shrubs, continuity in tree cover vegetation and presence of woody debris on one hand of the axis opposite to the presence of aquatic vegetation, embankment, grassland on the banks and channel resectioning.

Biological benthic metrics were tested comparing MEP stretches *vs* all other stretches (Table 6). Leveed MEP and impaired reaches showed strong significant differences for all biological metrics in use for ecological status evaluation, excluding the Shannon index. For example, significant differences between MEP and impaired sites in the EPT and total number of families for leveed reaches correspond to respectively around 3 and 8 families (effect size, in Table 6). This emphasizes that important differences exist between MEP and disturbed sites. Differences were less pronounced when comparing MEP and disturbed reinforced reaches. Remarkably, the STAR_ICMi has the same effect size for both leveed and reinforced stretches significantly distinguishes MEP and nonMEP sites in both categories.

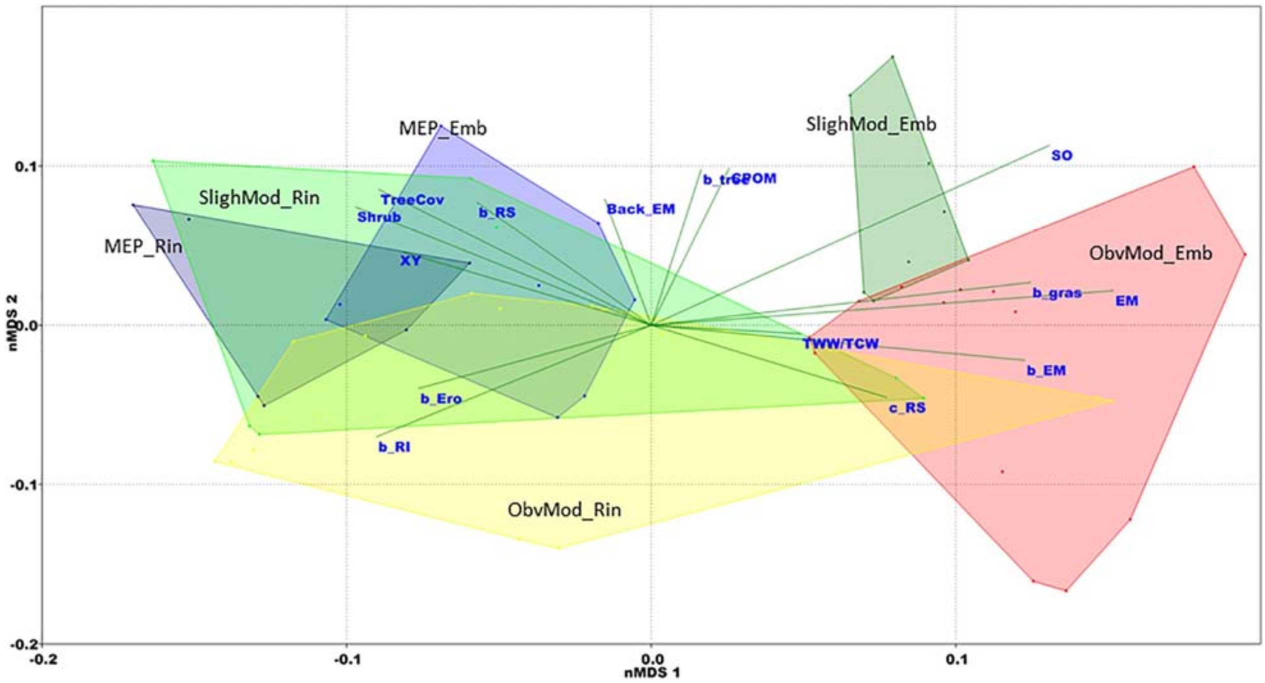

Fig. 7.5. Non-metric multidimensional scaling (nMDS) of 60 benthic invertebrate assemblages. MEP Leveed (blue); MEP reinforced (dark blue), slightly modified leveed (dark green); slightly modified reinforced (pale green), obviously modified Leveed (red); obviously modified reinforced (yellow). Solid vectors represent habitat/mitigation measures, with direction and relative length related to the correlation to the community matrix. Habitat vectors are labelled by variable. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 7.5. One-way ANOSIM results (R=0.443, p=0.0001) between groups. (*)<0.1; *<0.05; **<0.01; ***<0.005; 'ns' not significant.

		Leveed						Reinforced			
		MEP		SlighMod		ObvMod		MEP		SlighMod	
Leveed	SlighMod	0.0002	***								
	ObvMod	0.0001	***	0.0202	*						
Rin	MEP	0.0008	***	0.0004	***	0.0001	***				
	SlighMod	0.0004	***	0.0005	***	0.0002	***	0.1352	ns		
	ObvMod	0.0004	***	0.0001	***	0.0001	***	0.0445	*	0.0775	ns

Table 7.6. Results of the Mann-Whitney U-test between MEP and nonMEP reaches, for the 'leveed' and 'reinforced' groups separately. (*)<0.1; *<0.05; **<0.01; ***<0.005; 'ns' not significant. Effect size is also reported for significant relations.

		ASPT		n_FAM		n_EPT		1-GOLD		Shannon		Log (Sel EPTD+1)		STAR_ICMi	
Leveed	p-value	0.0015	***	0.0078	**	0.0149	*	0.0278	*	0.6234	ns	0.0098	**	0.0029	***
	effect_size	0.55		7.8		2.8		0.25		-		0.59		0.35	
Reinforced	p-value	0.1165	ns	0.0739	(*)	0.2124	ns	0.1824	ns	0.1473	ns	0.0123	*	0.0022	***
	effect_size	-		4		-		-				1.02		0.36	

4. DISCUSSION

4.1 Which habitat features and mitigation measures support MEP definition?

The present paper focused on features recognised as important mitigation measures for heavily modified rivers (Brookes et al., 2009; Bussettini et al., 2018; Vartia et al., 2018). We intended to evidence the effect of mitigation measures more than the measures themselves and for this reason we largely referred to habitat characteristics. By means of habitat, we established a direct link between measures and their effectiveness on biological attributes. This approach was advised by Buffagni et al. (2019) who evidenced that macroinvertebrates response to mitigation measures implementation in HMWBs is mediated by habitat mosaic and heterogeneity.

We investigated a typical lowland context where rivers are characterised by important physical modifications linked to flood protection and land drainage. In such context the effect of agricultural and urban land use at the catchment scale can be not relevant, while the reach scale can be central to determine ecological status as per river invertebrates (Kail & Wolter, 2013). We thus evaluated a set of features representing mitigation measures and natural characteristics at the reach scale, to assess if such features are important in discerning Maximum Ecological Potential (MEP).

The concept of MEP is strictly related to the implementation of all possible mitigation measures relevant to the hydromorphological alterations, not having a significant adverse effect on water use and proving to be ecologically effective in the physical context of the water body (CIS, 2003; 2006). To demonstrate that a defined proportion of mitigation measures has been applied is central for the two available approaches agreed at European level for ecological potential definition, namely 'CIS' and 'Prague' (Vartia et al., 2018). The CIS approach emphasizes the application of all possible measures to identify MEP conditions, while the Prague approach allows for the implementation of fewer measures excluding those providing only a slight ecological benefit, directly assessing GEP. Such basic concept in HMWB management is not easy to standardise, since there are no mitigation measures that are never possible and no measures that are always necessary (Kampa and Kranz, 2005). To overcome this we based on an observed situation where sites present different assemblages of natural features representing mitigation measures and we defined a statistical threshold for MEP acceptability. Such approach combined to multivariate analysis allowed for the definition of habitat features clearly separating MEP from nonMEP reaches. The identified multivariate space provides indication on type and quantity of measures ecologically significant and applicable in lowland heavily modified water bodies providing useful elements for the definition of MEP according to CIS approach. Meanwhile, in the investigated gradient of habitat features and mitigation measures the sites here defined as Slightly modified can represent the Good Ecological Potential (GEP), as far as the abiotic conditions are concerned (i.e. sites where only measures able to determine an ecological improvement are applied, without impacting on the use of designation). This can represent a starting point for a possible application of the Prague approach.

As far as which are the important features to consider, we verified that local restoration linked with the presence of adequate riparian vegetation and associated features, can effectively support the definition of Maximum Ecological Potential. Tree cover, large woody debris, set back embankment, as opposed to channel resectioning resulted the most important variables determining differences between MEP and impacted sites. Features like tree continuity, presence of trees and shrubs on the banks, channel shadowing are widely recognized as important measures of river functioning and naturalness (e.g. Balestrini et al., 2018; Buffagni et al., 2016; Hausner et al., 2018).

It is widely acknowledged that naturalness of channel planform (Kail & Wolter, 2013), unaffected channel morphology, diversity and heterogeneity of substrates are necessary for preserving biological integrity (Bączyk et al., 2018; Shi et al., 2018) and can control ecological status as per benthic invertebrates (Buffagni et al., 2016; 2019). In particular, and remarkably, we ascertained that a gradient in tree-related habitats and channel forms can be observed also when morphology is strongly compromised, resulting as a key element for MEP distinction. In our studied sites distance of levees from river channel is in opposition to channel resectioning, supporting the statement that when enough space is provided to rivers, natural processes of bank and channel adjustment are favoured (Martínez-Fernández et al., 2017). Our results also confirm that levees and reinforcement are often the main impediment to the settlement of appropriate riparian vegetation (e.g. *Salix spp*.) sustaining the need for suitable levees manipulation (i.e. move levees backwards) accompanied by abandonment of human activities on the banks (González et al., 2018; Gumiero et al., 2013). In particular, we demonstrated that even in a highly anthropized territory it is possible to observe leveed rivers profiting from acceptable side space and this results in the presence of natural features linked to an adequate presence of tree-related vegetation along banks.

As well, our results support the hypothesis that in-channel vegetation must be present for a river reach to be classified as MEP. On this regard, emergent and submerged macrophytes are known to contribute to specific macroinvertebrate taxa abundance and diversity (e.g. Ambrožič et al., 2018, Demars et al., 2012). In the studied context, such habitats can become dominant in disturbed reaches in absence of appropriate riparian vegetation cover, causing absence of shadow and favouring massive aquatic macrophytes growth. Correct management options of aquatic vegetation must be planned (e.g. Balestrini et al., 2018) in order to maintain such habitats in the expected MEP ranges, possibly reducing dominance of single macrophyte species and increasing plant diversity.

4.2 Do river invertebrates reflect differences between MEP and impaired stretches?

In our study reach-scale mitigation measures linked with the improvement of in-stream and riparian habitat had strong impacts on macroinvertebrate assemblages.

Our analyses evidenced that sites ordination along the two nMDS axes supported a clear separation between MEP and impaired sites. We did not focus on investigating which taxa dominate the different groups but we identified the most important drivers of biological community among the factors representing MEP conditions, directly correlating such factors to nMDS axes. The shared large scale context and water quality conditions among sampling locations put into evidence the role of habitat features in determining differences between macroinvertebrate communities, as demonstrated in Buffagni et al. (2019).

Channel resectioning was an important feature discriminating MEP and disturbed sites. This is also supported by differences in biological communities. Likewise, the presence of aquatic macrophytes as dominant in-channel habitat (> 40%) has a negative effect on invertebrate communities. It can be argued that when macrophytes become dominant they are constituted of only few taxa, no more supporting invertebrate richness and diversity as different macrophyte taxa do (Humphries, 1996). A dominance of aquatic macrophytes could also deplete in-stream habitat mosaic and heterogeneity with negative effects on biological communities.

The fact that large woody debris and presence of shrubs along the banks are important features for river benthic community is in strong agreement with many authors (e.g. Buffagni et al., 2016; Gerhard and Reich, 2000; Hasselquist et al., 2018). In our study, the presence of Xylal is one biological community driver along nMDS axis 1, in accordance with Nakano et al. (2018) who demonstrated that large wood improves macroinvertebrate richness having a significant effect in creating stable macroinvertebrates' habitats. It is also recognized that bank stabilization and management can deplete benthic community richness, especially if such practices do not ensure adequate plant coverage and associated roots habitats (Cavaillé et al., 2018). In our study, in terms of biological communities CPOM is not strictly discerning MEP and impacted stretches, perhaps because, even if this habitat is generally more abundant at MEP sites, it is also highly present in some impaired sites.

The variables highly correlated to nMDS axis 1 are those discriminating between MEP and impacted stretches along axis 1 of PCA, demonstrating a concordance between abiotic and biotic data. At Slightly modified leveed sites, where habitat features are similar to MEP conditions, the biological communities are very efficient in evidencing effects of water quality (even if water quality is not heavily compromised). In such situation ANOSIM analysis detected differences between Slightly modified and MEP rivers. Differences are weaker when considering reinforced reaches. Study insights would be necessary to emphasize these differences, focusing on the possibility to widen the studied gradient.

Even if the focus of the paper is not on proving the ability of biological metrics currently in use in biomonitoring in representing ecological potential, we have explored the performance of such metrics for MEP status characterisation. Our results demonstrated for invertebrates a shift towards sensitive taxa e.g. number of families of Ephemeroptera and Trichoptera in relation to habitat quality improvement, agreeing with the work of Favata et al. (2018) who studied and evidenced the same sensitivity shift on fish communities. Our results are also in agreement with the findings of other authors (e.g. Shell and Collier, 2018) stressing the importance of maintaining diversified bank features and in-channel habitats to optimize macroinvertebrate metrics, especially in leveed river reaches. Our results fit with Buffagni et al. (2019) stating that a full implementation of measures enhances in-stream habitat mosaic and diversity and benthic ecological potential.

Decline in richness of sensitive EPT and shifts towards more tolerant and atypical communities were also evidenced by Leps et al. (2015) in relation to an increase in stress. Again, also in terms of biological metrics, differences between MEP and nonMEP for the reinforced reaches are scarcely significant, apart for differences in the combined multimetric index used for ecological status evaluation (i.e. STAR_ICMi). It can be inferred that in our study reinforced reaches exhibit a relatively good in-stream habitat quality, as attested by the shorter gradient along PCA axis 1, and thus support similar results in benthic metrics. Specific metrics should be developed for such group of sites in combination to a possible extension in the studied gradient, even if the fact that STAR_ICMi is detecting differences between MEP and nonMEP is quite encouraging. In fact, a greater performance in metrics might be achieved if information on the community as a whole is considered in ecological index calculation (Leps et al., 2015), as is the case for STAR_ICMi.

We can anyhow conclude that differences in habitat features are mirrored by differences in invertebrate communities and these can be read in terms of metrics related to the assessment of ecological status.

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CHAPTER 8

In-stream microhabitat mosaic depicts the success of mitigation measures and controls the Ecological Potential of benthic communities in heavily modified rivers

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In-stream microhabitat mosaic depicts the success of mitigation measures and controls the Ecological Potential of benthic communities in heavily modified rivers

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ABSTRACT

The positive effect of mitigation measures on in-stream habitat conditions and the benthic community is recognised. In heavily modified rivers, though, the response of aquatic invertebrates to mitigation measures and habitat mosaic changes is scarcely documented.

We used non-metric multidimensional scaling to explore the benthic community of leveed rivers in the agricultural lowlands of Northern Italy. The relevance of in-stream substrate microhabitat for the benthic community was assessed, together with the impact of mitigation measures. We proposed a straightforward approach to quantify similarity of microhabitat mosaic between sites testing its statistical significance based on Bayesian statistics. We hypothesised that changes of microhabitat mosaic would reflect the level of implementation of mitigation measures and benthic invertebrates would respond accordingly. Alpha, beta diversity and benthic metrics used to classify ecological status/potential were considered and their variation tested against different levels of measure implementation. Lastly, ecological potential classification was paralleled to both the level of measure implementation and habitat attributes.

The microhabitats found at sites where measures were fully implemented differed from those observed elsewhere and they clearly mirrored morphological alteration and mitigation measures. Moreover, alongside morphological alteration, microhabitat diversity and mosaic were the main factors for benthic community structure. While benthic beta diversity strictly reflected microhabitat diversity, alpha diversity and ecological status metrics copied the mosaic gradient. Microhabitat attributes and most benthic metrics showed significant changes following measure implementation and they were accompanied by a gradual shift in ecological potential classes.

We demonstrated the importance of in-stream substrate microhabitats as a bridge between mitigation measures and the benthic community. Particularly when ecological classification is under focus, microhabitat mosaic should be evaluated for achieving a better understanding of biological responses. The huge amount of data available worldwide could support a straightforward use of river mosaic information for river management.

3 INTRODUCTION

Rivers worldwide are imperilled by hydromorphological pressures that affect habitat quality, impact biological communities and cause the need for mitigation measures or river restoration (e.g. Lepori et al., 2005). In lowland areas, levees and embankments - erected to protect adjacent territories from flooding and facilitate optimal water drainage - confine rivers and require regular management practices (Viero et al., 2019). Such rivers, where habitats are permanently impacted by morphological alteration, are often categorised in Europe as 'heavily modified water bodies' (HMWB; see Kampa and Hansen, 2004), according to the Water Framework Directive (WFD, European Commission, 2000). For these ecosystems, a comprehensive restoration usually cannot be designed without significantly impairing an existing, socio-economic important water use. Hence, a reachable aim is executing a range of mitigation measures, useful to bring the water body to its good or maximum ecological potential (European Commission, 2000). Usually, aquatic invertebrates are the most prevalent biological group used to monitor river health (Birk et al., 2012) and classify ecological status – or potential.

In deeply altered environments, in-stream substrate microhabitats may play a crucial role in controlling the invertebrate community (e.g. Jähnig and Lorenz, 2008;

Lorenz et al., 2009) and ecological status. In fact, substrate type is one of the most important factors in determining benthic invertebrate distribution, abundance and richness in rivers and streams (e.g. Beisel et al., 2000; Graça et al., 2004; Jähnig and Lorenz, 2008). Thus, when the aim is benthic community enhancement, rehabilitation strategies and consequent practical restoration or mitigation measures often aim at creating more heterogeneous substrate conditions based on the assumption that re-establishing physical habitat heterogeneity will increase biodiversity (Miller et al., 2010). However, after an extensive review Palmer et al. (2010) reported only a small proportion of studies found a positive link between biological diversity and in-stream heterogeneity. For highly degraded rivers, these authors argued that habitat heterogeneity as the main element controlling invertebrate diversity could not be supported. In contrast, in a degraded agricultural landscape, Greenwood et al. (2012) found that the strongest determinant of benthic community structure was in-stream habitat. Still, establishing the link between mitigation measures, habitat heterogeneity and the response of biota of deteriorated streams seems to be no trivial task (Spänhoff and Arle, 2007). According to the WFD and other water Directives, quality evaluations (e.g. for classifying Ecological Status) are mostly achieved by comparing 'test' and 'reference' sites, where reference conditions exemplify a reasonably undisturbed (benchmark) situation. Hence, a direct assessment of habitat conditions e.g. by contrasting the number and proportion of in-stream benthic habitats between sites in conjunction with invertebrate assessments, may provide useful information for both managing rivers and better understanding biological responses to pressures. This is likely to be particularly relevant in HMWBs, where disturbance of physical habitat can shape homogeneous channel units (Rabeni et al., 2002) and substrate microhabitats can be of extreme importance for the local distribution and abundance of benthic invertebrates (Wohl et al., 1995).

Among the final goals of river rehabilitation and mitigation measure implementation, the achievement of a given ecological status or potential (i.e. good or better) has an influential position, because it will affect forthcoming management plans and resources allocation (Villeneuve et al., 2018). For non-heavily modified water bodies, few examples of the effect of hydromorphological measures on river ecological status are available, based on WFD-compliant assessment systems (Haase et al., 2013). However, there is a lack of evidence for invertebrate response in riverine HMWBs.

For the purpose of this study, we focused on heavily modified, leveed rivers of the Italian lowlands, where habitat conditions are inevitably linked to the degree of morphological alteration. In a general habitat shortage, we expect in-stream substrate microhabitats to play a central role in controlling aquatic invertebrates. A series of river reaches were selected, covering an extensive range of completion of mitigation measures. Those measures were mainly centred on gentle management practices and improvement of riparian vegetation. The goal of this study is to explore the response of benthic invertebrates to different levels of mitigation measures in leveed rivers, while focusing on the role of the in-stream microhabitat mosaic. Aspects of alpha and beta diversity will also be analysed, together with benthic metrics used in Europe to classify ecological status and potential. To achieve our goal, we set the following objectives: i) describing a simple approach to quantify similarity between in-stream microhabitat mosaics; ii) assessing if and how the level of implementation of mitigation measures is reflected by benthic metrics and river microhabitats; iii) investigating the relationships between in-stream microhabitats, mitigation measures, the benthic community and ecological classification.

The potential of microhabitat mosaic to support the interpretation of benthic data will also be briefly discussed.

4 MATERIAL AND METHODS

The methodological approach employed in the present paper is illustrated in a flow chart (Fig. 1) summarising the connections between the selected variables and the procedures for the data analysis. To facilitate reading comprehension, a list of the main terms, abbreviations and definitions connected with the key concepts of the present paper is reported in Table 1.

2.1 Data collection

2.1.1 Study area and mitigation measures

The studied rivers are located in North-Eastern Italy, mostly in the drainage basin of the Venice Lagoon in an agriculturally-used landscape; they belong to the 'Heavily Modified Water Body' category, *sensu* WFD. Sampling sites coordinates and main typological features are reported in Table A.1. For all sites, the case for designation as HMWB is flood protection and land drainage. The majority of investigated river stretches belong to small lowland rivers (i.e. distance from source < 25 km), while a few sites are mid-sized (distance from source < 75 km; Buffagni et al., 2006). Thus, even if they would belong to different types if not classified as HMWB, according to the Italian official regulation (MATTM, 2016), their biological conditions for benthic invertebrates can/should be assessed referring to the most frequent river type in the area (i.e. small lowland). This type is clustered into a loosely-defined macro-type, together with similar types observed in the area. This is because the overwhelming (hydro)morphological modification is expected to affect biological communities more than river typological features. Sampling sites were selected to cover an increasing level of mitigation measure implementation, aimed at reducing impacts of morphological alteration.

Due to their management over decades for land drainage and/or flood control, all the investigated river reaches bear levees. When they were built, river banks and channel were reshaped and thoroughly modified. Embankment and drainage network functionality are regularly maintained, and the presence of nearly-natural features is always due to the implementation of mitigation measures. Because of the strict constraints imposed by the need to preserve the use of designation, such measures rarely encompass all-embracing restoration measures. The latter are often considered in the case of natural rivers and may include enhancement or structural modification of hard structures for flood risk and land drainage management, re-meandering and restoration of backwater habitats, etc. At best-managed sites in the study area, applied mitigation measures implied gentle riparian vegetation management, with a passive restoration approach for in-stream habitats. Sporadically, active restoration was realised, i.e. enlargement and reshaping of artificial berms, re-establishment of riparian buffer strips. Jointly with the decline of intrusive channel maintenance e.g. dredging and macrophyte removal, low-impact practices facilitated stable in-stream habitat improvements. The list of mitigation measures considered here is based on those measures actually adopted (e.g. Regione Veneto, 2015) even if only locally or potentially appropriate in the study area. For instance, the mitigation measures implemented did not include direct sediment management, while the presence of obsolete flood defence infrastructures was not observed in the studied rivers. Therefore, structures removal and sediment management were not contemplated in this research. The list of the main mitigation measures here considered is reported in Table 2 (measure 1-8) and their percentage of application for each sampling site is shown in Table A.2. The mitigation measures used here were framed for this research also considering available literature (e.g. Brookes et al., 2009). Some of the mitigation measures require a relatively long time to be successful and to show positive effects on habitats and biota, e.g. when they follow river resectioning, artificial berm profiling and levees building. Other mitigation measures need harmonisation with periodic maintenance interventions, e.g. when removing riparian or in-stream vegetation to

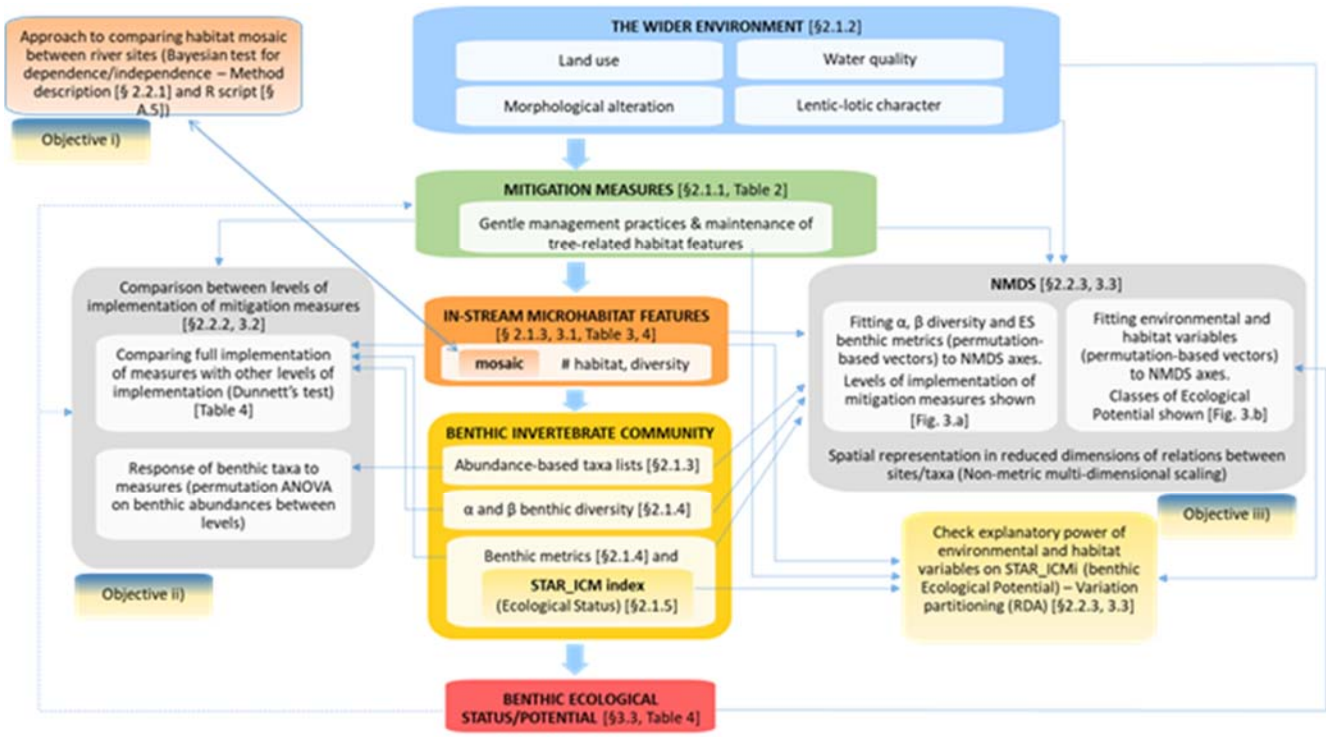


Fig. 8.1. Flowchart summarizing how environmental, habitat and benthic information was used, together with main analytical approaches. The top-to-bottomflowin the central part of the diagram represents an ideal cascade from causes to effects and exemplifies the increasing detail in data usage. Left and right parts refer to statistical approaches, with thin arrows linking data source and analysis. Dashed line: hypothetical use of Ecological Potential results to guide future mitigation measures; top-left box: methodological results.

support water drainage. Thus, while some mitigation measures need a constant and frequent (on a short time scale) implementation, others must be in place since years to show their potential, positive effects.

In order to provide an overall estimate of the degree of mitigation attained at each site, these mitigation measures were averaged and converted in a 0-1 scale using the observed maximum mean value ('manag_meas^{mm}', in Table A.2; please, refer to the bottom of the table for examples of calculation). All the mitigation measures were considered as having the same weight; in accordance with WFD CIS approach (European Commission, 2018), interactions effect was not considered. Information gained with the application of the CARAVAGGIO protocol or extracted from Geographic Information System (see next paragraph) supported the derivation of values for each mitigation measure at river sites.

Where the above-mentioned mitigation measures are well implemented (e.g. at sites representing Maximum Ecological Potential), restoration frequently resolved in an increase of trees and associated natural features, even if habitat improvement was not a specific target for rehabilitation. The presence of tree-related features, representing a natural component in the heavily modified context under study, was considered as an additional river attribute to complement the implementation of conventional mitigation measures. In fact, the presence of tree-related features is believed to be an important factor with a high intrinsic value in relation to structuring and influencing benthic invertebrate communities and local biodiversity (e.g. Buffagni et al., 2016). For this research, such tree-related features were quantified considering a series of bank and channel habitat attributes (Table 2, row 9).

Subsequently, for statistical analyses, sites were grouped in reason of their degree of mitigation measures' application and presence of tree-related habitats. To build groups, the observed ranges of overall mitigation measures (manag_meas^{mm}) and tree-related features (KOTH) were divided into three equal parts. Next, the resulting levels were combined into

Table 8.1 Definition of concepts and terms dealing with heavily modified water bodies, the benthic community and microhabitats considered in the text.

	Concept	Definition
1.	Heavily Modified Water Body (HMWB)	A water body bearing heavy (hydro)morphological modifications that cannot be removed without significantly impairing an existing, socio-economic important water use (*sensu* Water Framework Directive). For these ecosystems, a comprehensive restoration usually cannot be designed. In this study, leveed rivers in an agricultural landscape were studied, which were designated as HMWB for flood protection and land drainage.
2.	Mitigation measures	The range of operable measures that can be implemented to bring the heavily modified water body to its good or maximum ecological potential. They include active and passive measures, whose application depends on water body characteristics, existing constraints and local strategies. Passive mitigation measures often require longer time than active measures to show their positive effects on the ecosystem. Mitigation measures, pertinent to HMWB, are opposed to e.g. restoration measures, which can be fully applied to rivers where all morphological modifications can be removed.
3.	Reference site	For non-heavily modified water bodies, a site where human pressures and impacts are minimal or none.
4.	MEP site	A river site/water body where all mitigation measures that do not have a significant adverse effect on the use of designation and/or the wider environment were taken. Here, the best obtainable conditions for biological elements can be observed.
5.	MEP condition	A situation where the Maximum Ecological Potential for biological quality elements is found, when all mitigation measures were taken. Here, biological conditions reflect, as much as possible, those associated with the closest comparable natural water body type at reference conditions, once considered the morphological alteration due to the use of designation. Even in such circumstance, different HMWBs can host different biological communities. Therefore, MEP sites encompass a range of biological conditions. For ecological classification purposes, MEP conditions can be exemplified by the median value of each metric/index calculated from samples collected at MEP sites.
6.	Benthic invertebrates	Aquatic fauna (excluding fish and other vertebrate organisms) inhabiting a water body that live in strict contact with the bottom substrate. Here we refer to macroinvertebrates, which are usually visible to the eye without the aid of a microscope i.e. those animals that can be captured by a 500 µm net. A biological quality element largely used to monitor river health.
7.	Benthic metrics	The response of macroinvertebrate communities to pressures is often estimated using "benthic metrics", which express structure, function or other measurable characteristic that changes in a predictable way. A metric is calculated from a taxalist obtained for a benthic sample and bases on presence/absence or abundance data.
8.	STAR_ICMi	The STAR_ICMi (STAR Intercalibration Common Metrics index) is a multimetric index derived for the first WFD Intercalibration phase of European rivers, during which it was largely applied. It is now the standard method in Italy for the classification of ecological status and potential based on benthic invertebrates. It is calculated by averaging six normalised and weighted benthic metrics.
9.	Biological classification	The attribution of the investigated site/sample to a quality class based on a biological element (here benthic invertebrates). Initially, the observed value is related to a reference value by calculating an EQR (Ecological Quality Ratio) value. For HMWB, the EQR is derived by contrasting the "test site" value to the value representing MEP conditions. The class is then obtained after comparing the obtained EQR value to the EQR values representing the threshold between quality classes. It is worth noting that a site categorised as MEP on the basis of mitigation measures or a sample collected there can be classified in "good" or lower ecological potential based on biological information. This is analogous to what may happen for reference sites along natural rivers, sometimes biologically classified below the "high" status.
10.	Substrate microhabitat	An in-stream physical habitat where benthic invertebrates are collected. It is a homogeneous portion of the river bottom identifiable by the dominant substrate attribute of its upper part i.e. mineral substrate, macrophyte/algae cover or presence and kind of organic detritus. A list of in-stream substrate microhabitats present at a site is usually compiled prior to sampling invertebrates and benthic sample units are collected proportionally to the share of microhabitats in the river channel. In deeply altered environments, the presence and share of microhabitats may play a crucial role in controlling the invertebrate community and ecological status.
11.	In-stream microhabitat mosaic	For the aims of this paper, we define the in-stream microhabitat mosaic (or simply habitat mosaic) for a site as the combination of substrate microhabitats, which results from their presence/absence and relative share. More in particular, we will quantify the similarity between habitat mosaics by referring to the situation observed at MEP sites. The characterisation of habitat mosaic relies on the information gained to define where individual benthic sample units have to be collected.

four groups that represent decreasing degrees of mitigation: 1, full mitigation; 2, fair; 3, moderate; 4, poor mitigation (please see Table A.2, column 'Final_Group'). At sites belonging to group 1, all relevant mitigation measures that do not have a significant adverse effect on the use of designation or the wider environment have been implemented and the reaches correspond to 'reference sites' of natural rivers. There, the biological conditions are theoretically expected to be at their Maximum Ecological Potential (MEP). In the studied area, four river reaches had been previously classified as in such conditions (Buffagni et al., 2018) and this classification fits with the group attribution performed here based on mitigation measures and tree-related habitats.

2.1.2 Reach-scale survey and environmental variables

Reach-scale habitat survey was based on the CARAVAGGIO method (Buffagni et al., 2013), derived from the River Habitat Survey protocol (Raven et al., 1997). The CARAVAGGIO method consists of a comprehensive survey of habitat features and alteration along a ≈ 500 metres channel length representative of the wider scale. Survey is undertaken on channel, banks and peri-fluvial areas. From the application of the CARAVAGGIO method a selection of indicators – representative of relevant environmental variables categories – were derived and considered in the analyses. These indicators are the degree of morphological alteration/habitat modification (Habitat Modification Score, 'HMS': Raven et al., 1998), land use degradation (Land Use Index, 'LUI': Erba et al., 2015) and the lentic-lotic character (Lentic-lotic River Descriptor, 'LRD': Buffagni et al., 2010)(Table A.3). Particularly, the Habitat Modification Score quantifies the presence and quantity of artificial features along river channel and banks and is suited to assess overall morphological impairment in relation to aquatic invertebrate response in Italian rivers. Further information was extracted from GIS support (in QGIS environment; QGIS Development Team, 2009) and used to support the calculation of a range of mitigation measures. Such information refers to the presence and extent of bars/islands in the channel, size of active channel, banks and levees, berms, banktop profile, peri-fluvial areas, flood defence structures, channel reinforcement.

Additionally, water quality as required by the Italian legislation for WFD monitoring in relation to ecological status evaluations (LIM_{eco}, MATTM, 2010) was assessed. LIM_{eco} is a comprehensive descriptor covering some aspects of water quality mainly related to nutrients enrichment and incorporates information on N-NO_3, N-NH_4, Total Phosphorous and Oxygen saturation (MATTM, 2010). Whenever possible, habitat survey, biological collection and water sampling were carried out at the same time.

2.1.3 Invertebrate sampling and substrate microhabitats

Benthic invertebrates were collected following a WFD-compliant sampling protocol based on a multi-habitat approach, which requires sampling substrate microhabitats in proportion to their coverage at the river site (Buffagni et al., 2001; Furse et al., 2006). Benthic samples are collected from a list of substrate microhabitats *sensu* Hering et al. (2004) present at the site (Table 3), which are quantified at steps of 5% proportional presence and documented prior to sampling with a Surber net (0.05 m^2 each, mesh size 0.5 mm). The overall number of sampling units collected in each microhabitat, separately for MEP and test sites, is reported in Table 3. The taxalists obtained from 10 sample units collected following this protocol are then merged to build the complete site sample, used for describing the benthic community (family level) and calculating benthic metrics. For most sites, at least two site samples were collected (Table A.1). Only very abundant taxa (i.e. ≈ > 1000 individuals per square meter) were sub-sampled.

Table 8.2 List of mitigation measures and tree-related habitat features considered to group river sites according to the observed level of implementation of measures (% of application in the reach, referred to the maximum observed in the area) and to the observed state of the habitat. Codes are provided for an easier reading of Supplementary material. Combination of measures 1-8 is used later on to derive an overall estimation (manag_meas^{mm}) of implementation of mitigation measures for each river site.

	Mitigation measures	Code
1	Reduction of exposed bank reinforcement. Replacement of hard bank protection by soft engineering solutions. Favouring settlement of natural bank profiles.	no_bRI
2	Favouring the presence of berms and/or bankside areas with fringing reeds. Provide space for natural bank adjustment to occur.	berm_reed
3	Reducing channel narrowing and/or resectioning. Creation of natural-like in-channel irregularities.	no_cRS
4	Rehabilitation of banks and riparian zone with autochthonous trees and shrubs (and removal of allochthonous species). During management operations, maintain or improve the structure and diversity of the riparian zone (i.e. sensitive management).	b_arb_shr
5	Rehabilitation of vegetation on landside of the embankment.	lands_veg
6	Application of sensitive techniques to manage in-channel habitats, including submerged vegetation. Reducing dominance of single macrophyte species and increasing plant diversity.	mng_cHAB
7	Ensuring gentle management of marginal (in-channel) habitats, including emergent vegetation, to be retained along river banks.	mng_mHAB
8	Preventing removal and allow large wood to be retained in the channel where possible. Mainly entire trees that fall into the channel.	mng_HAB
	Habitat features	
9	Presence of natural features related to the occurrence of trees i.e. tree-related bank and channel habitats, also as a consequence of mitigation measures above. Overhanging boughs, exposed bankside roots and fallen/leaning trees on the lower bank, together with fallen trees/shrubs and large woody debris within the channel are comprehensively considered.	KOTH

2.1.4 Benthic metrics

Benthic alpha and beta diversity metrics were derived from the invertebrate community data: overall taxonomic richness (NFAM), richness of Ephemeroptera-Plecoptera-Trichoptera (NEPT), Shannon-Wiener (SHAN) and Sørensen indices, and local site-based contributions to beta diversity (LCBD) (Legendre & De Cáceres, 2013). Even if based on presence-absence data, the Sørensen coefficient can still be considered a valid index when exploring beta diversity (e.g. Anderson et al., 2011; Baselga, 2012; Legendre and De Cáceres, 2013). For instance, it can be particularly useful when evaluating temporal trends based on unsatisfactory abundance estimations, like for most historical benthic datasets. Metrics explicitly related to the assessment of Ecological Status/Potential were also calculated: Average Score Per Taxon (ASPT), 1- (% of Gastropoda-Oligochaeta-Diptera taxa) (1-GOLD), Selected Ephemeroptera-Plecoptera-Trichoptera-Diptera taxa (Selected_EPTD) and the STAR_ICM index (Buffagni et al., 2007). The metric 1-GOLD was arcsine-square-root-transformed before analysis to meet assumptions of normality (Barca et al., 2016). ASPT, the number of total and EPT taxa, and Sørensen index are based on presence/absence data, while the remaining metrics use abundance information.

2.1.5 Classification of Ecological Potential

The classification of ecological potential was evaluated (MATTM, 2010) by means of the STAR_ICMi (STAR Intercalibration Common Metrics index). The STAR_ICMi is a multimetric index (Buffagni et al., 2007), calculated from six normalized and weighted metrics (NFAM, NEPT, ASPT, 1-GOLD, Selected_EPTD, SHAN, please see above for acronyms explanation). The normalisation of component metrics and index itself requires the use of reference values (please see Table 1) explicitly derived for the heavily modified rivers under investigation (spatial approach) or provided by present regulation for each HMWB designation category (MATTM, 2016). The median value of samples collected where all (relevant) mitigation measures are implemented, which determines the possibility to obtain the so-called 'Maximum Ecological Potential', is used to calculate the Ecological Quality Ratio. This makes it possible to obtain STAR_ICMi values > 1. Boundaries used to derive Ecological Potential (EP) classes, based on STAR_ICMi values, are those formally intercalibrated for natural rivers by Italy (MATTM, 2016). To classify biological quality based on invertebrates we use here five Ecological Potential classes: High, Good, Moderate, Poor and Bad, using the same approach adopted for non-heavily modified rivers.

2.2 Data analysis

2.2.1 Designing a descriptor for similarity of microhabitat mosaic

The list of substrate microhabitats (see Table 3) and their respective occurrence, for both organic and inorganic (mineral) substrates, previously assessed to define where to collect benthic invertebrates, were employed to define and compare in-stream microhabitat mosaics.

To meet objective i), we designed a descriptor aimed at assessing the similarity of microhabitat mosaic between river reaches. The comparison of mosaics among rivers was performed by calculating a Bayes Factor between the microhabitat proportions observed at two contrasting sites. The analysis of such index provides a Bayesian alternative to classical hypothesis testing, since it offers a way of quantifying the evidence in favour of each of the considered hypotheses. In practice, the analysis is performed by testing for H1 Dependence over H0 Independence and for H0 Independence over H1 Dependence, such as namely if (log(P(Data| indep)/P(Data| dep))) >0 independent, otherwise dependent (Kass and Raftery, 1995). Comparisons were performed between each site and the four MEP sites (see 2.1.1 and Table A.1). A Bayesian test of proportion was performed (R package BayesianFirstAid: Bååth, 2014) to compare overall microhabitat distribution between MEP and test sites. The R script prepared for the analysis is reported in Appendix A.5. These and further analyses were performed in the R v.3.4.2 software environment (R Core Development Team, 2016).

We assume that the microhabitat mosaic observed at each MEP site effectively represents the 'expected' condition for the studied HMWB river type/category. Based on the Bayes Factor analysis performed against the reference mosaics, four Bayes Factor values were obtained for each test site, and three for MEP sites. Therefore, to derive a single value useful to describe the similarity of in-stream microhabitats with those observed at MEP sites, the average was calculated. Finally, Bayes Factor (BF) values were coded as follows to represent microhabitat mosaic: HF (Habitat Factor) = 1-(lnBF)/10, with HF = 0 when lnBF ≥10 and HF = 1 when lnBF <0. For Habitat Factor values > 0.9, we assume no apparent differences exist between the test site and MEP microhabitat conditions. The descriptor states the similarity of the observed habitat mosaic between a test site and a range of MEP sites. The Habitat Factor varies between 0 (no or minimal similarity) and 1 (maximum similarity). High-similarity values indicate good quality of in-stream microhabitats, while values approaching zero denote altered habitat. The index is a probability-derived coefficient. Hence, the consequential measure of similarity (Habitat Factor) is itself probabilistic, i.e. a standardised complement of the probability that the

Table 8.3 Substrate type and microhabitat description with number of samples collected at sites expected at their maximum ecological potential (MEP) and test sites.

Substrate type	Acronym	Microhabitat description	Number of sample units	
			MEP	test
Pelal /Argillal	ARG	Silt, Clay. Substrate characterized by very fine particle size (≤ 6 µm), which compact and adhere and sometimes comes to form a solid surface	10	205
Psammal	SAB	Fine and coarse sand (> 6 µm - 2 mm)	2	20
Akal	GHI	Fine to medium-sized gravel (> 2 mm - 2 cm)	6	0
Microlithal	MIC	Coarse gravel, small cobbles (> 2cm - 6 cm)	22	0
Mesolithal	MES	Fist to hand-sized cobbles (> 6 cm – 20 cm)	6	14
Artificial	ART	Concrete and all solid non-granular substrates artificially placed in the river	0	6
Submerged macrophytes	SO	Submerged macrophytes, including moss and Characeae	10	125
Emergent macrophytes	EM	Emergent macrophytes (e.g. *Typha*, *Carex*, *Phragmites*)	2	39
Living parts of terrestrial plants	TP	Fine roots, floating riparian vegetation (e.g. Alders' roots)	13	6
Fine particulate organic matter	FP	Deposits of fine particulate organic matter	0	6
Coarse particulate organic matter	CP	Deposits of coarse particulate organic matter (e.g. fallen leaves)	7	27
Xylal	XY	Coarse woody material (diameter at least 10 cm) e.g. tree trunks, dead wood, branches, roots	2	2

closeness between habitat structures of two sites is due to chance.

To further support the definition of in-stream microhabitat structure, in addition to HF and using the same information gathered with the protocol for the collection of benthic taxa, the number of individual substrate microhabitats (n_mh) present at each sampling site and their diversity (SWI: Jähnig et al., 2010), based on the Shannon-Wiener Index, were calculated (Table A.3).

2.2.2 Comparing metrics and microhabitats between levels of implementation of mitigation measures

For comparing benthic metrics and microhabitat features between mitigation measure groups (objective ii), rank based multiple comparison and compatible simultaneous confidence intervals (SCI) for transitive relative effects in unbalanced one-way designs were performed (Konietschke et al., 2012, 2015). A many-to-one comparison approach (Dunnett's test) was applied ('mctp' function in R package 'nparcomp'), which does not assume homogeneous variances (homoscedasticity). The three levels of partial implementation of mitigation measures were compared against the pool of MEP samples. In addition to comparing benthic metrics, we tested taxonomic differences and community shifts between different levels of implementation of mitigation measures by using permutational multivariate ANOVA (function adonis2, in vegan).

2.2.3 Connecting the benthic community to environmental factors and exploring what controls Ecological Potential

To explore the relevance of microhabitat mosaic and structure for the benthic community and to examine the overall scenario (objective iii), we investigated the benthic community with Non-Metric Multidimensional Scaling (NMDS; metaMDS in the vegan package, Oksanen et al., 2018). This is a visualisation technique used to highlight the degree of dissimilarity among a set of investigated sites. The dissimilarity is computed starting from a multivariate input matrix by means of a selected index (here Bray-Curtis dissimilarity), used to quantify the compositional dissimilarity between two different sites, based on invertebrate counts at each site. NMDS output represents the set of objects (sites) along a small and specified number of axes (here 2) and maintains the ordering relationships among them (Borcard et al., 2011). Macroinvertebrate abundance data was subjected to square root transformation and Wisconsin double standardisation. After running the NMDS analysis, we used the vector fitting technique (in vegan R library) based on permutation of environmental variables to see how they related to ordination. These environmental descriptors (Habitat Factor, Shannon-Wiener Index for substrates, Habitat Modification Score, Land Use Index, LIM_{eco}, Lentic-lotic River Descriptor, mitigation measures and tree-related features) summarize the main gradients acting and degradation factors in the study area. Benthic metrics were related to NMDS axes as well, with the same technique. Prior to fitting vectors, the linearity assumption was tested with ordisurf function that fits a 2-d surface to an NMDS solution using a generalised additive model approach in vegan . For all the tested variables, a linear vector fit seemed realistic i.e. the gradient of fitted surface was parallel to the variable vector and the contours between levels were roughly perpendicular to the same vector.

The four levels of implementation of mitigation measures and the classes of ecological potential were used to aid the interpretation of NMDS results and vectors.

The Redundancy analysis (RDA) was used for variation partitioning to determine the explanatory power of different environmental variables in relation to the benthic Ecological Potential (STAR_ICM index) and to check significance of individual fractions (varpart and rda functions, in vegan), following the procedure outlined by Borcard et al. (2011) based on partial RDA. All data were standardised (mean = 0, SD = 1) prior to variation partitioning. Linearity assumption was tested before analysis. Additionally, Spearman rank order correlation between variables was calculated.

3 RESULTS

3.1 In-stream microhabitats and their mosaic

The list of substrate microhabitats observed in the study area and sampled is shown in Table 3. In total, 80 and 450 sampling units were collected from MEP and test sites, respectively.

Microhabitat for MEP and test site proportions are shown in Fig. 2. Overall, 12 microhabitats were found, with silt (ARG), submerged macrophytes (SO) and microlithal (MIC) being the most represented. This latter microhabitat, together with gravel (GHI), was only present at MEP sites. On the other side, artificial substrates (i.e. rip-rap) were only present at test sites. The distribution of substrate microhabitats at MEP sites was different from that found at test sites (BF > 580 ≈ Extreme evidence for differences in relative frequencies). Substrates mainly contributing to difference are MIC ($p < 0.001$) and TP ($p = 0.01$), more frequent at MEP sites, plus ARG ($p < 0.001$) and SO ($p = 0.05$), with a higher presence at test sites.

Microhabitat mosaic (HF) ranged between 0.14 and 0.91, with an average value at MEP sites of 0.82. Number of substrate microhabitats (n_mh) and their diversity (SWI) varied between 1-8 (MEP 6.3) and 0-0.834 (MEP 0.74), respectively (Table A.3b).

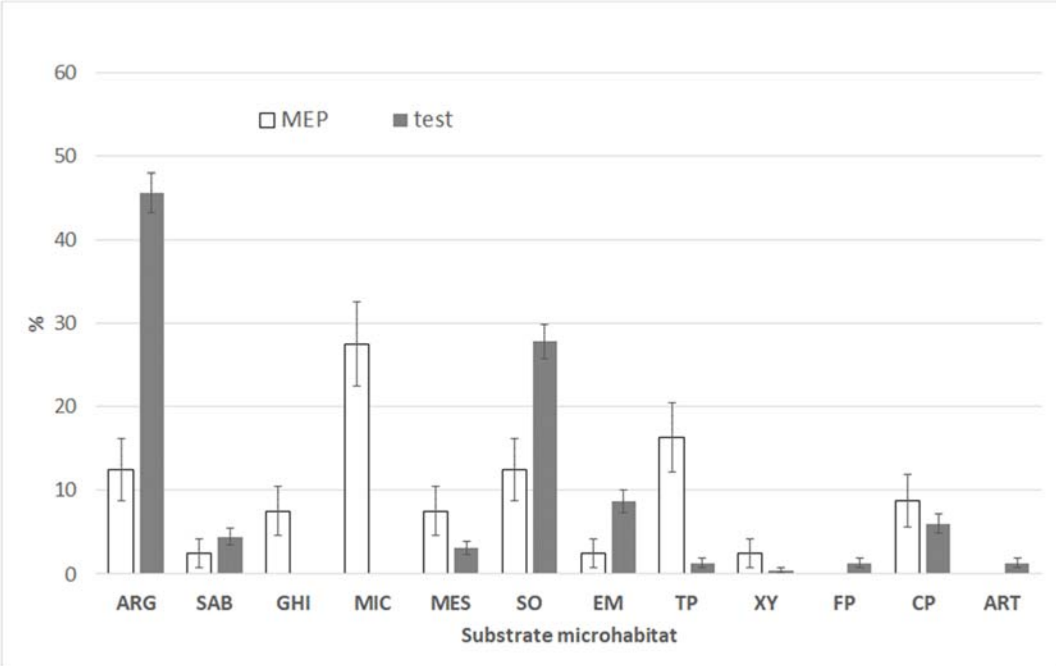


Fig. 8.2 Percentage distribution of substrate microhabitats at MEP and test sites. Bars indicate standard error. Mineral substrates (ARG-MES) on the left; organic substrates (SO-CP) on theright; Artificial substrate (ART) at the right end. Please refer to Table 3 for acronyms.

3.2 Response to the implementation of mitigation measures

The degree of implementation of mitigation measures led to the following sample attribution: Full: 8, Fair: 7, Moderate: 28, Poor: 16 (Table 4). For sites belonging to group with full implementation, high or good water quality based on LIM_{eco} was verified.

The incomplete implementation of mitigation measures (i.e. fair to poor) was noticeably detected by benthic metric values (Table 4a, b). Shannon index (SHAN) apart, all metrics clearly distinguished between full and moderate/poor implementation. A Fair level of implementation was revealed by Sørensen, ASPT, 1-GOLD and, to a lesser extent, Selected_EPTD taxa and STAR_ICMi. Selected_EPTD, NEPT, NFAM and STAR_ICMi show a gradual response to the implementation of mitigation measures, according to the order poor (maximum response) > moderate > fair. The number of Ephemeroptera-Plecoptera-Trichoptera families (NEPT) only reached a maximum value of 7, showing a fairly short range of variation. However, their response to the implementation of mitigation measures was apparent.

Permutation ANOVA on benthic invertebrate abundances performed between the four levels of overall mitigation discovered highly significant differences among groups ($p = 0.001$). More in details, the group with fully implemented mitigation measures was obviously distinct ($p = 0.001$). Sites with moderate and poor implementation showed a clear distinction as well ($p = 0.001$ and 0.006, respectively), while a fair level of implementation was not discriminated from other groups by the benthic community ($p = 0.124$).

The connection among microhabitat attributes and the level of implementation of mitigation measure is illustrated in Table 4b (right). Microhabitat mosaic, the diversity and number (to a lesser extent) of microhabitats all significantly discriminated between full implementation of mitigation measures and other levels of restoration.

Table 8.4 Response of benthic metrics and habitat features to the level of implementation of mitigation measures. Mean values and results of comparison (Dunnett's test) between sites with different levels of measure implementation are shown. The group with the highest level of implementation (Full ≈ reference) was used as benchmark in the test. a) Ecological status/potential metrics and resulting classification (percentages for each class are reported in the last columns); b) The remaing three biological diversity metrics (see text) and habitat metrics (Microhabitat mosaic - HF, number of substrate microhabitats and Shannon-Wiener Index for microhabitats).

a		NFAM			NEPT			ASPT			Selected_EPTD			1-GOLD			STAR_ICMi			Ecological potential (%)				
Measures implementation	n	mean	p-value		mean	p-value		mean	p-value		mean	p-value		mean	p-value		mean	p-value		High	Good	Mod.	Poor	Bad
Full	8	24.6	-		5.4	-		5.2	-		0.5	-		0.72	-		0.91	-		25	75	0	0	0
Fair	7	22.1	0.5840	ns	3.4	0.1100	ns	4.6	0.0000	***	0.1	0.0893	(*)	0.42	0.0040	**	0.65	0.0516	(*)	0	28.6	57.1	14.3	0
Moderate	28	18.6	0.0092	**	3.4	0.0465	*	4.9	0.0328	***	0.1	0.0071	**	0.48	0.0023	**	0.63	0.0003	***	0	25	60.7	10.7	0
Poor	16	12.5	0.0001	***	2.1	0.0007	***	4.3	0.0000	***	0.0	0.0020	***	0.35	0.0031	**	0.46	0.0000	***	0	0	43.8	50	6.3

b		Sørensen			SHAN			LCBD			HF			n_mh			SWI		
Measures implementation	n	mean	p-value		mean	p-value		mean	p-value		mean	p-value		mean	p-value		mean	p-value	
Full	8	0.69	-		1.9	-		0.023	-		0.82	-		6.3	-		0.74	-	
Fair	7	0.52	0.0000	***	2.1	0.5300	ns	0.017	0.2500	ns	0.64	0.0000	***	3.9	0.0133	*	0.50	0.0005	***
Moderate	28	0.42	0.0000	***	1.9	0.9980	ns	0.016	0.0081	**	0.57	0.0000	***	3.2	0.0000	***	0.42	0.0000	***
Poor	16	0.37	0.0000	***	1.6	0.5690	ns	0.016	0.0029	**	0.33	0.0000	***	3.3	0.0002	***	0.42	0.0000	***

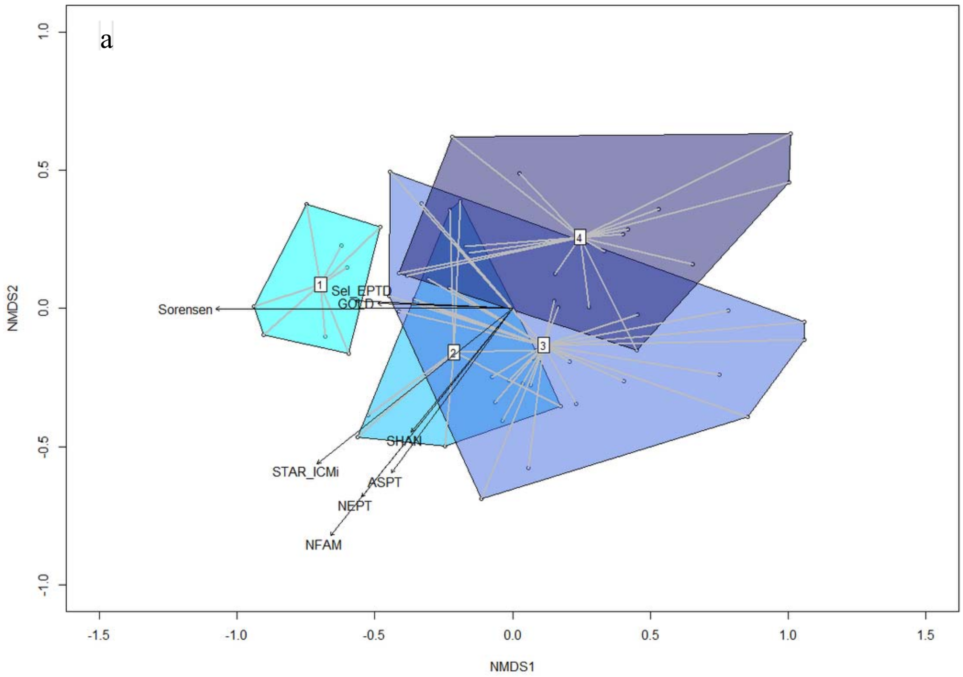
3.3 From mitigation measures to ecological classification

In excess of 36,400 invertebrates were collected and identified, which represented 76 families. NMDS ordination (2D stress 0.179, non-metric fit R^2 0.968) on taxa abundances was used to aid interpretation of benthic response to levels of mitigation measures (Fig. 3a). In general, such levels are well discriminated, with a fair measures implementation (i.e. level 2 in Fig. 3a) that shows an apparent overlap with a moderate implementation of measures (level 3). As far as benthic metrics are concerned, two main alignments can be seen on the NMDS ordination diagram. The first one is the expression of Ecological Status metrics, with NFAM, NEPT, ASPT, SHAN and the consequential STAR_ICMi located within the ordination space aligned to the shift between mitigation measure levels poor (4) to fair (2). The second focal direction is revealed by Sørensen index, together with Selected_EPTD taxa and 1-GOLD, which are well associated with the first NMDS axis. The metric expressing the Local Contribution to β Diversity (LCBD,not shown) is not significantly related to NMDS axes (Table A.4). NMDS diagram showing benthic families location can be found in Fig. A.1.

Another representation of NMDS results useful to investigate further non-trivial relationships, which involved also environmental variables, is shown in Fig. 3b. Here, these variables are displayed as vectors, with the exclusion of land use degradation, not significantly related to NMDS axes (see Table A.4 for coefficients). Due to equivalence and overlap with the diversity of microhabitats (SWI), their number (n_mh) is not shown and not discussed in further detail. In the same figure, polygons encompass sites with the same level of Ecological Potential, based on the STAR_ICM index, ranging from High (1) to Bad (5). This representation supports a comparative interpretation of habitat features, mitigation measures and main pressures in relation to the biological classification based on benthic invertebrates. On the ordination diagram morphological alteration is roughly opposed to mitigation measures and tree-related features and they are approximately parallel with the first axis. Water quality and the presence of lotic conditions, unexpectedly divergent, are somewhat transversal to such axis. Microhabitat diversity (and n_mh, not shown), the variable with the strongest correlation to benthic abundance ($r^2 = 0.69$ with NMDS axes), is clearly opposed to morphological alteration. Microhabitat mosaic was also found to be significantly related to NMDS axes ($r^2 = 0.38$) and its vector lies between those of mitigation measures and the presence of comparatively lotic conditions in the river reach. The classes of ecological potential gradually shift right to left, along the first axis. Minor variations are seen on axis 2, with class 2 (good ecological potential) showing slightly lower values than other classes. The connection between the degree of implementation of mitigation measures and ecological potential is also supported by results presented in Table 4a, right. All samples collected in conditions of full implementation of mitigation measures are biologically classified as good or above. Going to fair and moderate levels of implementation, the corresponding biological classification shifts towards worse classes. Finally, with a poor implementation of mitigation measures, no samples are classified as good or above ecological potential, with most samples attributed to poor/bad classes.

When comparing Fig. 3 a and b, it is apparent how the similarity between microhabitat mosaics (HF) is well associated to the variation of the STAR_ICM index (and other metrics of the same cluster). Furthermore, Sørensen index is aligned with axis 1, dominated by morphological alteration and microhabitat diversity/mitigation measures.

Spearman rank order correlations among microhabitat attributes, selected benthic metrics and environmental variables are reported in Table A.4. Microhabitat mosaic is clearly connected with the implementation of mitigation measures and with the STAR_ICMi.



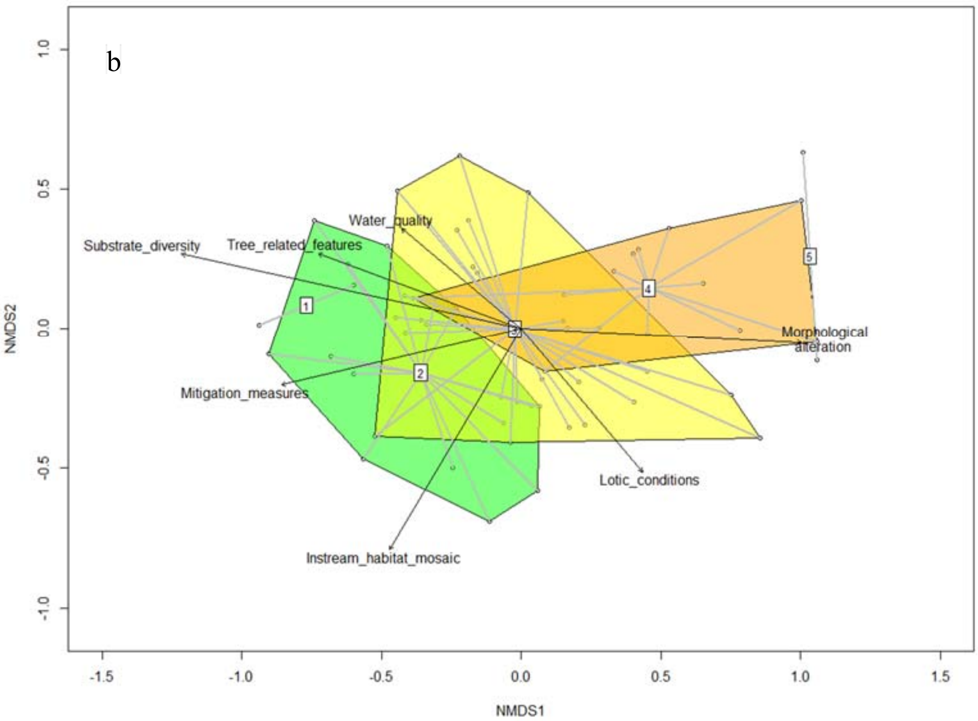


Fig. 8.3 NMDS diagrams based on benthic taxa abundances. (a) Polygons encompass the sites with the same level of implementation of mitigation measures (1 full; 2 fair; 3 moderate; 4 poor) and vectors of benthic metrics. For acronym explanation (benthic metrics) please see the text. (b) Polygons encompassing sites with the same Ecological Potential (EP: 1 High; 2 Good; 3 Moderate; 4 Poor; 5 Bad) and environmental and in-stream habitat vectors. The longer the arrows, the better the concordance between NMDS axes and variable projection (i.e. vectors are scaled by their correlation with the ordination results).

Diversity and number of microhabitats, strongly correlated to each other, are mainly associated with morphological alteration and Sørensen index. The Local Contribution to β Diversity is not significantly related to other biological metrics. However, it is worth noting its negative relation with land use degradation.

Variation partitioning for the STAR_ICM index, which is based on the best tested model, is reported in Fig. 4. Here, fractions with two or more variables overlap correspond to the their joint effects contribution to the explanation of STAR_ICMi variation. Total explained variation (1-residuals) is the overall explained variation (Adj. $R^2 = 0.54$, Pr(>F) = 0.001) of STAR_ICMi, which is the benthic index used to classify ecological status and to define the expected Ecological Potential in heavily modified rivers. The variable showing the highest explanatory power for the STAR_ICMi is Habitat Factor i.e. the similarity of in-stream microhabitat mosaic with MEP sites (Fig. 4), with an individual variation fraction (i.e. after controlling for other variables) of 0.29 (Pr(>F)= 0.001). Individual fractions of the remaining variables (when the effect of other variables is held constant in turn) are smaller but always significant. For morphological alteration this fraction amounts to 0.06 (Pr(>F) = 0.006), for mitigation measures it is 0.03 (Pr(>F) = 0.047) and for tree-related features it is 0.06 (Pr(>F) = 0.01). As expected, some multicollinearity/redundancy between variables emerged (e.g. see overlap regions).

4 DISCUSSION

We studied the benthic community in a range of heavily modified river reaches (i.e. leveed) showing a different level of implementation of mitigation measures, in the lowlands of Northern Italy. We observed a grade of in-stream substrate variables i.e. number, diversity and mosaic of riverbed microhabitats, which mirrored morphological alteration and a gradient in the implementation of mitigation measures. The structure of the benthic community was deeply associated to such features. Also, benthic metrics and biological quality i.e. ecological potential depicted the mitigation measure and habitat breadth.

4.1 Do river microhabitats reflect the implementation of mitigation measures?

At the study sites, a series of mitigation measures to reduce impacts of morphological alteration on the aquatic habitat and biota were increasingly applied. They mainly implied the adoption of sensitive management methods of river maintenance, a few actions on riparian vegetation and a passive restoration approach for in-stream habitats, which exploit flood events as the main drivers for their dynamic development. Microhabitat mosaic, structure and diversity evidently followed the gradual implementation of such mitigation measures. Groll (2017) demonstrated the effectiveness of passive restoration for increasing habitat diversity and the spatial heterogeneity of the riverbed substrates. Most of the sites studied in the present research are free-flowing and their river sections are expected to develop rapidly (Groll, 2017). Additionally, in the coastal lowlands of North-Eastern Italy, the effects of human interventions are even leading to an intensification of extreme events and changes of the flooding dynamics (Viero et al., 2019). With time, such conditions presumably supported a progressive improvement of hydromorphological restructuring of river sections, where substrate habitats could rearrange themselves accordingly.

At river reaches where the MEP sites are located, the presence of high levees paradoxically helped a full implementation of mitigation measures i.e. by promoting a relative isolation from surrounding agricultural areas and limiting bank accessibility. Banks likely acted as natural fences, which are often included among passive restoration measures (e.g. Feld et al., 2011). Such high levees, together with the presence of artificial berms and relatively large riverbeds, supported an effectual passive restoration of

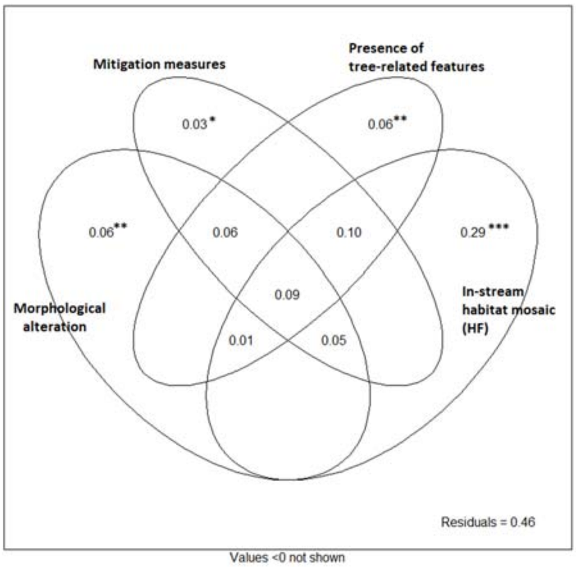


Fig. 8.4 Variation partitioning in RDA(Venn diagram) for the STAR_ICMbenthic index. Total variation (SS): 2.32; Variance: 0.040; No. of observations: 59. In the diagram, the adjusted r-squared values are reported (negative values not shown). Variables included in the model: Morphological alteration; Mitigation measures; Presence of tree-related habitat features; In-stream microhabitat mosaic HF. The level of significance is shown (*p b0.05; **p b 0.01; ***p b 0.001) for individual fractions after controlling for other variables(variation fractions overlapping cannot be tested). The sizes of the ellipses in thediagram are not to scale.

both channel and banks. Hough-Snee et al. (2013) found that, even if improvement of woody species may delay without active plant manipulation, riparian vegetation recovered quickly (i.e. ≤ 4 years) following passive restoration and elimination of the disturbance vector. In the study area, at sites with fully implemented mitigation measures, no removal of riparian plants has been performed for years (≥ 10; see Table A.2). This has supported the presence of tree-related habitat features, which correlated with in-channel substrate diversity.

The proportion of some microhabitats was higher at MEP sites, such as gravel, pebble and living parts of terrestrial plants. In contrast, silt, submerged and emergent macrophytes were more represented at altered sites. Our study was conducted in an agriculturally-dominated landscape. Hence, nonpoint sources arising from agricultural land uses may have caused an increase of stream sedimentation (e.g. Walling, 1990) and input of fine inorganic sediments (Allan, 2004). Indeed, at test sites silt microhabitat accounted for more than 40 percent of all in-stream substrate habitats. Such a high percentage seems to confirm the statement that, in general terms, effective restoration measures should produce adequate changes in the cover of specific substrate types to influence macroinvertebrate diversity (Verdonschot et al., 2016).

Similarly to our study, Lorenz et al. (2009) found that restored reaches were generally wider and had a higher number of in-stream microhabitats, with the appearance of substrates with larger size and an increase of living parts of terrestrial plants. Inversely, these authors noticed an increase of macrophytes after re-meandering, whereas we found lesser macrophytes in fully restored sites compared to altered sites, where macrophytes are often dominant (together with silt). Such smaller occurrence of macrophytes was presumably due to the incidence of extensively shaded stream sections, connected with the recovery of trees and shrubs. Encouraged by gentle management measures, this led to a greater presence of the ‘living parts of terrestrial plants’ microhabitat along the channel banks.

4.2 Microhabitats, mitigation measures and benthic invertebrates

Any valuable appraisal of habitat structure should be applicable to a range of habitats and have relevance to their associated fauna (Warfe et al., 2008). This makes it difficult to select comprehensive habitat indicators suitable for wide-ranging studies and comparisons. On these premises, we directly quantified habitat structure by counting the number of the main in-stream microhabitats and assessing their diversity. Additionally, we proposed a simple approach to quantify similarity of microhabitat mosaic between sites (i.e. the HF). All the three habitat attributes were clearly related to mitigation measures and benthic invertebrate response. HF accounts for complexity of in-stream microhabitat structure (the abundance of substrate and cover features) and, partly, heterogeneity (the composition of different substrate and cover features) (see Barnes et al., 2013). This factor straightforwardly compares the observed microhabitat composition with that expected in restored (MEP) conditions.

The relevance of microhabitat mosaic has been recently affirmed by Villeneuve et al. (2018) who demonstrated its connection to hydromorphological alteration. They also disclosed the crucial direct role of mosaic for invertebrates at the site scale, in relation to ecological status interpretation. Accordingly to such conclusions, we found that both mosaic similarity and diversity of in-stream microhabitats well reflect the level of implementation of mitigation measures and correlate with the benthic community. Habitat diversity evidently mirrors morphological alteration whereas similarity among mosaics reflects the concurrence of mitigation measures and an increasing presence of lotic habitats (Buffagni et al., 2010).

In general terms, the detection of positive effect of habitat improvement on biology can be hindered by a few limiting factors. Among these we mention (Feld et al., 2011): restoration applied at a wrong scale; inappropriate (or insufficient) timing of monitoring; unfitting indicators or target biological groups; confounding effects of natural variability and/or multiple pressures. Such factors apparently did not affect our research, which demonstrated a clear biological response to substrate habitat improvement. In a similar way, Lorenz et al., (2009) found that the number of substrate types was significantly lower in straightened river segments compared to restored sections. Following hydromorphological improvements, invertebrate richness and diversity increased after restoration, with additional taxa collected especially in the newly attained substrates (Lorenz et al., 2009). This fits with our results, which unveil how benthic diversity metrics depend upon microhabitat number, diversity and mosaic. Particularly, beta diversity (Sørensen coefficient) positively correlated with in-stream microhabitat number and diversity. Similarly, Jähnig and Lorenz (2008) showed how the increased substrate diversity might result in higher beta diversity. On the other hand, in our study difference in microhabitat mosaic best explained differences in alpha diversity (i.e. richness, diversity) and benthic quality metrics (i.e. directly related to ecological status/potential). This trend confirms the general conclusions of Miller et al. (2010) who reported that increasing habitat heterogeneity had significant, positive effects on macroinvertebrate richness, i.e. more substrate types at a river site promote higher numbers of taxa (see Jähnig and Lorenz, 2008).

As far as the EPT taxa are concerned, their number increased with increasing microhabitat mosaic similarity; the abundance of selected families (see the Selected_EPTD metric) rose with substrate diversity. These metrics reveal information on sensitive organisms (i.e. Ephemeroptera, Plecoptera and Trichoptera), which are known to be responsive to the combined effects of habitat loss (Burdon et al., 2013). Burdon et al. (2013) demonstrated that in agricultural streams habitat availability (i.e., presence of coarse substrate and interstices) was the most important factor for the relative abundance of EPT taxa. As well, these authors found that the benthic community varied accordingly with the gradient of deposited fine sediment, which caused an obvious regression of EPT taxa when present above the ≈ 20 percent. These findings are in line with our results, which show that the number of EPT taxa varied according with differences in substrate coarseness.

As far as riparian buffers are concerned, even if their role in controlling in-stream habitat structure seems clear, the next step would be demonstrating evidence for any relationship with aquatic organisms, and it is still largely missing (Feld et al., 2011). For instance, Giling et al. (2016) found that, in heavily modified (agriculturally-used) landscapes, replanting at the reach scale may not be sufficient to recover biodiversity, even after decades. This data contrasts with our results that attest a significant relationship between the presence of tree-related features - an effect of mitigation measures - and the benthic community. In a highly degraded agricultural system, Greenwood et al. (2012) similarly observed enhancements in stream communities associated with riparian management. Such authors worked in a context similar to Northern Italy lowlands, where active management was limited and the aquatic habitats were relatively degraded, specifically the waterways of Canterbury Plains, in New Zealand. Similarly, and in the same geographical area, Burdon et al. (2013) found that riparian conditions controlled benthic habitat resources through direct sediment input and flow-mediated sedimentation of silt in lowland agricultural streams impacted by fine sediments.

4.3 From the implementation of mitigation measures to ecological classification

In the lowlands of Northern Italy, morphological alteration of rivers demands a massive implementation of restoration or mitigation measures to fulfil the requirements of water legislation and fit environmental goals. In this study, we demonstrated that mitigation measures increasingly led to in-stream habitat improvement and emphasised how this affected benthic community. Benthic invertebrates are the major biological element used as an indicator to classify ecological status/potential (Birk et al., 2012) and their evaluation is crucial to assess mitigation measure success and/or need for refinement. Therefore, we need to consider the resultant effects on the ecological quality of benthic invertebrates. This seems quite an innovative task. A few papers directly focused on assessing ecological status for benthic invertebrates in fresh or transitional waters, e.g. in small lakes in Flanders (northern Belgium) (Denys et al., 2014) or in estuarine water bodies in the Basque Country (northern Spain) (Borja et al., 2013). Essentially, we did not find scientific references relating mitigation measures, habitat implementation and benthic ecological status in heavily modified rivers. For instance, Shuker et al. (2015) focused on the potential application of a new method, i.e. the Urban River Survey (URS), for assessment of the hydromorphological condition in heavily modified rivers, after the implementation of mitigation measures. However, no biological appraisal was performed. In a similar context, Weber et al. (2017) demonstrated that rehabilitated sites exhibited an improved benthic diversity compared to nearby non-restored sites. Nonetheless, their biological evaluation did not contemplate the core metrics of the legally required national assessment method, thus missing the final step of ecological classification.

We demonstrated that benthic ecological potential, together with alpha and beta diversities, were significantly related to the level of implementation of mitigation measures. As well, we found that microhabitat mosaic and diversity relate to those measures, with ecological potential (STAR_ICMi) chiefly encapsulating microhabitat mosaic and measure information. Among all environmental variables considered, the similarity of microhabitat mosaic between MEP and test sites explained the largest proportion of STAR_ICMi variation.

4.4 Perspectives on using microhabitat mosaic information

Even if more detailed habitat survey approaches would be appropriate, the direct visual assessment of habitat features, as used here, is believed to be a cost-effective and scientifically sound approach for interpreting the benthic invertebrate response (e.g. Silva et al., 2014; Wilson et al., 2007). Highly significant relations were found between habitat characteristics, benthic response and environmental variables, the latter collected at a larger scale. This self-demonstrates that a visual assessment of habitat features is appropriate in the studied context. In any case, the described method for quantifying microhabitat mosaic can be applied to a wide range of data sources, not necessarily based on a visual survey, and can be used to compare different situations and environments. The script syntax is such that it can be applied to any kind of data, if proportionally collected and structured in percentage classes. For river habitats, not only can it be used to compare in-stream substrate microhabitats, but any habitat attribute. For instance, it seems well suited to promptly fit data acquired with the River Habitat Survey method, for which large amount of data is available (e.g. Naura et al., 2016).

Information on the consequences of mitigation measures implementation on habitat is central to appreciate success of river rehabilitation. With mitigation measures driving the process in the background, the link between in-stream substrate mosaic and ecological potential encourages a direct use of habitat information. Firstly, the similarity of microhabitat mosaic together with substrate diversity can be used directly to assess the overall quality of microhabitat structure and heterogeneity. In this way, relevance for the benthic community at the proper scale will be guaranteed (see Lepori et al., 2005; Jähnig et al., 2010). Secondly, when biological sampling is performed concurrently, these habitat indicators make it possible to assess if in-stream microhabitat influences the benthic community structure (e.g. Jähnig et al., 2009). Last but not least, when the aim is to infer on benthic invertebrate potential/status, especially in a multi-pressure context, the evaluation of in-stream substrate mosaic (i.e. HF) will provide a relevant habitat representation for benthic indices used for classification (Villeneuve et al., 2018). Potential for applying these microhabitat metrics to invertebrate monitoring is huge. In fact, in Europe and outside, thousands of benthic samples have been and are constantly being collected with a multi-habitat, proportional approach (e.g. Bennett et al., 2011). Such a field approach for collecting benthic taxa often requires that in-stream microhabitats are listed and their proportion assessed, prior to proceeding with invertebrate sampling (e.g. Buffagni et al., 2001; Hering et al., 2004). Thus, in the last 15-20 years, an enormous amount of substrate microhabitat data for rivers and streams has been collected and stored in environment agencies databases, summing up to at least 89.000 water bodies in Europe (EEA, 2018). Among them, about 12,000 are classified as heavily modified (EEA, 2018) and many are sampled more than once per monitoring cycle. Real numbers are presumably even larger as they include sites not subjected to EU reporting. For example, there are more than 13.000 sites in Germany alone, of which ≈ 35 % are HMWB (Arle et al., 2016). Surprisingly, at least in Italy, the use of such microhabitat information, which is always collected concomitantly with invertebrates, has been marginal or definitively null. The computation on that data of in-stream microhabitat mosaic and diversity as defined in the present study would bring a massive piece of information. This would be precious data towards a better understanding of benthic community behaviour and response to pressures.

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I directly covered all main aspects of the manuscript. Co-authors mainly provided support for field sampling, taxonomic identification, data curation, statistical setting and review & editing

CHAPTER 9 – CONCLUSIONS

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9.1. Conclusions of Chapter 3 to 5 – Macroinvertebrate response to changes of flow-related habitat features

Conclusions of the first paper (chapter 3), "**The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers**":

Response of benthic taxa to flow-related habitat factors

In this research, we studied habitat attributes observed at the time of benthic invertebrate sampling by collecting information on representative river transects and deriving reach-scale prospects. Next, we related the mean reach habitat conditions to the abundance of benthic taxa at the microhabitat scale. Among our goals, we aimed to identify the flow-related habitat factors that influenced the benthic community. More particularly, we focused on those features shaping the lentic-lotic character of rivers, i.e., the overall reach condition defined by the lentic and lotic habitat characteristics. The taxon distribution was strongly related to the relative presence of distinctive flow types (sensu Padmore, 1998), with unbroken waves and non-perceptible flow representing the flow types most associated with benthic differences.

The method we used to quantify the ratio of lentic to lotic habitats i.e., the LRD descriptor, is based on a range of individual pieces of information, picked up at different scales and summed up at the reach level (≈ 500 m). The results of the paper sustain the relevance of different habitat scales, e.g., the micro, meso and reach scales, in defining invertebrate assemblages and species distributions. Accordingly, we found that flow types, as well as other habitat features studied at the reach scale, related well with benthic invertebrates collected at the microhabitat scale.

Association of taxa to specific lentic-lotic conditions

In the studied rivers, most benthic taxa exhibited a significant quantitative relationship with the gradual shift in lentic-lotic conditions. This attests that the existing lentic and lotic habitat characteristics are relevant not only when droughts are approaching but also across the range of lentic-lotic conditions and aquatic states. In fact, more than 85% of taxa showed a significant response to LRD variation.

Taxonomic groups and the lentic-lotic gradient

We found that most taxonomic groups exhibited a clustered positioning of optima along the lentic-lotic gradient and that the highest optimum densities were often located in different ranges of lentic-lotic conditions for different groups. A relatively high number of taxa exhibited an LRD optimum in balanced lentic-lotic conditions, where the number of dissimilar flow-related local environments increases as an outcome of the coexistence and summation of distinctive lotic, transitional and lentic features and habitats. This habitat diversification can generate suitable or advantageous conditions for a range of taxa, thus increasing the prospect that the optima of many taxa' will be detected.

However, the majority of taxon optima (≈ 60%) fell in the lentic range. In particular, many Odonata, Coleoptera, Hemiptera (i.e., OCH) and Mollusca taxa were associated with extremely lentic conditions. In such situations, extreme habitat variability regulates the presence and distribution of taxa. This helps clarify why extremely lentic conditions show the highest number of associated taxa.

General conclusion

The interpretation of the results in this paper supports a wide understanding of the following points:

i) The main factor governing the distribution and optima of taxa along the lentic-lotic gradient is the range of flow types at the site. This determines the main taxonomic variations from the very lotic end of the gradient to the end where the water velocity and turbulence are null, resulting in flow cessation. In this range, near-bed hydraulics and the resulting benthic forces act intensively on benthic communities.

ii) Along the continuum of flow-type variation (i), where diverse lotic, lentic and transitional features coexist in the river stretch i.e., under intermediate lentic-lotic conditions, high habitat diversification is observed, and many taxa can find suitable conditions to colonize the river and survive. We expect the highest alpha diversity (e.g., taxonomic richness) under these conditions.

iii) In the low flow season and/or when a drought is approaching, if/when the flow becomes predominantly or totally imperceptible, the degree of connectivity among aquatic habitats, the physio-chemical properties of the water and biological interactions strongly influence the presence of taxa. In these extremely lentic conditions, a large variety of different, alternative habitat conditions can be observed. Correspondingly, taxa associated with extremely lentic habitats are selectively present, depending on a range of environmental features that are no longer dependent on surface flow, e.g., temperature, water physio-chemical characteristics, the presence of organic detritus, and vertical connectivity. River reaches under these conditions (oligorheic or arheic aquatic state) are expected to show high levels of beta diversity.

As far as the applied issues are concerned, most invertebrate-based assessment systems involve the use of metrics and indices that assign progressive indicator values (and scores) to the presence of taxa belonging to different taxonomic groups. Most benthic metrics, when used to support an evaluation of the ecological status, likely incorporate a relevant reflection of the variation related to the lentic-lotic character of rivers that is not necessarily linked to the degree of habitat alteration and water quality variation. Additionally, the definition of biological reference conditions, both for taxonomy- and trait-based metrics, will presumably need adaptation to the actual lentic-lotic conditions found at different river types and sites. Especially under a changing climate scenario, the lentic and lotic characteristics of habitats should be regularly assessed to strengthen the biological understanding of river ecosystems.

Conclusions of the second paper (chapter 4), "**The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification**":

Do macroinvertebrate metrics respond to the lentic-lotic habitat conditions?

The relationship between biological metrics and the lentic-lotic conditions assessed at the reach scale (i.e., LRD) was evident both in pool and riffle areas and explained a high percentage of variance for a range of metrics e.g. up to 28% for multi-metric indices. However, for some metrics the response in pools generally explained a higher proportion of biological variation. The observed differences can be explained by the physical features of the two mesohabitats. The harsher physio-chemical conditions found in pools when drought is approaching, together with a limited habitat availability and stronger biological interactions, also support the higher association to lentic-lotic conditions observed in pools for the proportion of ovoviviparous taxa, richness and diversity metrics. However, both metrics based on averaged scores and multi-metric indices i.e., those most frequently used to assessing ecological status, showed a comparable average level of explained variance in pools and riffles. Therefore, the natural variability of the ratio of lentic to lotic habitats should be contemplated when using such indices, independently from what mesohabitat is chosen for the collection of macroinvertebrate samples.

What kind of response to lentic-lotic conditions?

A parabolic response to the ratio of lentic to lotic habitats was found for most metrics. However, for EPT_families, which includes rheophilous invertebrates, and for other metrics a linear response was observed, with decreasing values from lotic to lentic ranges.

The linear trend observed here in response to lentic-lotic conditions, as opposed to a parabolic trend, can be explained by the relatively short gradient covered by this study on the lotic side, with truly lotic environments marginally covered. Hence, the opportunity to depict a likely decrease of these metrics on the lotic side was limited. To describe exhaustively the shape of the response curve for EPT_families and for the other metrics that showed a decreasing linear response, a gradient including more extreme lotic conditions should be explored.

Does the ratio of lentic to lotic habitats influence ecological status classification?

Sampling variability i.e., the variation that would occur among replicate samples collected at a site at the same time, is usually confounded with other types of variation in ecological assessments. In addition to differences due to life-history traits of invertebrate taxa, floods, short- and mid-term temporal variation related to climate and drought periods can lead to biased predictions of reference conditions and incorrect inferences. Because of the resulting variation in invertebrate assemblages, evaluations based on comparisons of samples taken with specific lentic-lotic conditions with reference expectations derived from other lentic-lotic conditions will introduce a systematic bias in assessing ecological status. The observed bias between values of the STAR_ICM index found at optimal conditions i.e., where lentic and lotic features are rather balanced, and values expected at the extreme LRD ranges is up to > 20% (lentic side).

Based on the response curve of the STAR_ICMi, we modelled this index to estimate the habitat-adjusted expectation for the index along the lentic-lotic gradient and to calculate EQR values corrected for environmental variability. In terms of classification, the effect of the lentic-lotic conditions was more apparent in pools, where ≈ 28% and 30% samples, for non-reference and reference sites respectively, improved their ecological status class after refining the expectation based on LRD values observed at the time of sampling. In riffles, where the association of the STAR_ICMi with lentic-lotic conditions is weaker, the analogous shift in classification is ≈ 13% and 25%. This exemplifies the potential impact of ignoring lentic-lotic conditions – as largely done in Mediterranean regions – when assessing ecological status. Because the relationship with lentic-lotic conditions was significant for most of the invertebrate metrics used in the EU and outside to assess ecological status, we presume the impact on classification is widespread. We expect more important effects in the Mediterranean area that is characterized by high flow variability and heterogeneity, with many rivers naturally exhibiting a non-perennial flow regime and frequently experiencing limiting lentic conditions.

Most Mediterranean countries use formally a multi-metric method that relies on a typological approach and usually does not depend on site-specific, abiotic data for its implementation. The infrequent availability of widely applicable, predictive models is due to a range of factors, which include data scarcity, large environmental variability, partly unknown taxonomy and complex biogeography. Likely, the same reasons will slow the development of predictive systems in the near future, at least in many south European countries. Therefore, approaches oriented at developing habitat-adjusted reference conditions for existing methods, like that proposed in this paper, can represent a ready-to-use solution for improving ecological assessment. In addition, Europe has recently completed the harmonization of official methods – largely multi-metric - used for classifying ecological status. The comparison of invertebrate methods across the EU has been largely based on the STAR_ICMi. Due to STAR_ICMi response to the lentic-lotic conditions, the results of such evaluations might need a revision focused on habitat discrepancies among rivers and site-specific tuning.

Needs, opportunities, and challenges using lentic-lotic condition approach in ecological assessment

A relevant outcome of this study concerns the description of biological reference conditions for ecological classification. Ignoring lentic-lotic information might result in scarce representativeness, (i.e. relevant bias) or large and seemingly unexplained biological variability (i.e. scarce classification precision). For such situations, the use of biological reference conditions modelled on the basis of the ratio of lentic to lotic features would support a site-specific tuning. This would increase classification performance and interpretability of results.

Additionally, an alteration of the ratio of lentic to lotic features per se can be useful to assess and quantify the effect of discharge reduction at river sites, because the lentic lotic conditions are affected by altered hydrology. Long-term hydrological and ecological data are often required to apply existing e-flows methods. Unfortunately, such data are not promptly available for many south European river catchments and field information on the ratio of lentic to lotic habitat conditions, joined with macroinvertebrate data, may provide support in developing environmental flow standards.

In conclusion, the responses of benthic invertebrate metrics to the ratio of lentic to lotic habitat features observed at the time of sampling should not be ignored. Consequences would imply the difficulty of understanding biological responses to pressures (best scenario) and/or a largely biased classification of ecological status in many circumstances (worst scenario). When amendments to assessment systems are to be founded on reach scale habitat information, the ratio of lentic to lotic habitats should be considered in what Hawkins, Cao & Roper (2010) called 'the challenge of adjusting for natural variability' in the Mediterranean area.

Conclusions of the third paper (chapter 5), "**Hydrology under climate change in a temporary river system: Potential impact on water balance and flow regime**":

The main purpose of the present study was to assess the climate change impact on flow regime in a Mediterranean basin characterised by a river system with an intermittent flow. Our assessment is based on the availability of recent climate change simulations on a global and regional scale. However, predictions of the hydrological response of a river basin under climate change conditions are affected by several sources of uncertainties that depend both on the used hydrological models and on the climate change scenarios. In addition, some of the assumptions made (i.e. that land use does not change in the future) could be incorrect as climate change could also result in a significant alteration of land cover. Hence, we have to consider projections not as a predictive method, but as a tool that may be used to assess changes in process dynamics.

Although we did not analyse the simulated water quality, we can say that decreasing precipitation and the consequent predicted exacerbation of the extreme low flow conditions may increase the risk of water source contamination from sewage and nonpoint source pollutants to water courses.

The results of our study show that climate change will bring a reduction of water resource availability and some alterations in the hydrological regime. Hydrological alterations due to climate change predicted in the near-natural reaches were evaluated using a number of hydrological indicators, based on the range of variability approach (RVA) that takes into account the natural inter-annual variability present in the indicator values in the baseline period and in a future period. Such indicators can be used to infer potential implications for river ecosystems. Assumed that the impact on river ecosystems is relevant when the indicator difference is outside a range of ±30%, we found that the majority of indicators showed a relevant alteration.

Such alterations can be relevant for different biological elements and ecosystem functions. In a WFD context, the assessment of river ecosystem's health implies a comparison to type- or site-specific reference conditions. Reference conditions will have to be redefined if a water body changes its type e.g. shifting from perennial to intermittent. This was not the case for the study reaches. Nonetheless, in general terms, it is worth noting that reference conditions cannot be considered as static and will change, echoing the effect of climate change on the physical and chemical conditions of water bodies. If, in the future, a river reach moves toward more temporary conditions, the biological reference conditions are realistically expected to be different, at least in the low-flow period. In fact, biological assemblages observed in phases close to the dry season can show quite different attributes to those of the other time periods. This reflects the overall balance between lentic and lotic habitats present in a river reach, which should be taken into account when assessing the ecological quality of rivers, because they can greatly affect the assignment of ecological status and the interpretation of biological data.

9.2. Conclusions of Chapter 6 to 8 – Macroinvertebrate response to morphological alteration and to the implementation of mitigation measures

Conclusions of the fourth paper (chapter 6), "**Macroinvertebrate metrics responses to morphological alteration in Italian rivers**":

Can benthic metrics read river morphological impairment?

Our research was addressed at testing the response of benthic invertebrate metrics in use for quality classification – i.e. the STAR_ICMi and its component metrics – to morphological alteration. This point is particularly relevant in Italy since the development and /or refinement of metrics is not foreseen in the near future. The strong correlation found between morphological impairment descriptors and biological metrics adds evidence to validate benthic metric sensitivity to river channel and bank modification. We here demonstrated that river invertebrates respond to morphological impairment when the main impact is mostly linked to the presence of artificial structures and to the consequent alteration of riverine habitats. Our results showed that the STAR_ICM multimetric index is the better performing biological metric in relation to morphological impairment, among those tested here. This supports a greater performance of metrics that combine a variety of information on observed and expected community, not treating all individuals equally nor focusing only on specific taxon groups.

As a general conclusion, our results support the basic concept that benthic metrics used for ecological status classification in lowland and Mediterraenan rivers, including temporary rivers, are adequate to reveal the major impacts of morphological impairment. However, the link between morphological impairment and biological response can be improved, especially when considering specific geographical contexts and hydromorphological alteration not directly linked with bank, channel and riparian condition modification.

How can we quantify morphological impairment in relation to ecological status evaluation for the WFD?

We estimated morphological impairment on river sites performing a PCA on a set of variables known to possibly affect biological communities, expressing bank and channel modification and tree-related habitat conditions. Expectedly, the PCA-derived morphological gradient on the first axis is strictly correlated to HMS (Habitat ModificationScore) index calculated from CARAVAGGIO application. HMS is in fact based on the same information used to run the PCA, but founds on a different approach to condense morphological impairment in a single signal i.e., it is a global score representing the cumulative impact of all specific alterations. In the outlined context, HMS seems an appropriate indicator that helps to simplify, quantify, analyze and communicate complex information. The fact that the response of the STAR_ICMi to HMS is apparent among different river types covering a wide geographical context makes our results particularly relevant, being representativeness fundamental in developing monitoring plans to assess river condition. The use of an EQR scale to quantify the biological response is suitable to support outcomes independently of differences in benthic community variation along e.g. zoo-geographical and climatic gradients. We anyhow evidenced that biological responses to morphological alteration may slightly differ in different ecological settings and/or geographical areas, even when a context-independent EQR scale is used. This consideration supports the importance to avoid mixing different contexts or at least to account for possible confounding effects when quantifying biological responses to habitat degradation.

Conclusions of the fifth paper (chapter 7), "**Defining Maximum Ecological Potential for heavily modified lowland streams of Northern Italy**":

Which habitat features and mitigation measures support MEP definition?

We investigated a typical lowland context where rivers are characterised by important physical modifications linked to flood protection and land drainage. We then evaluated a set of features representing mitigation measures and natural characteristics at the reach scale, to assess if such features are important in discerning Maximum Ecological Potential (MEP). The present paper focused on features recognised as important mitigation measures for heavily modified rivers. We intended to evidence the effect of mitigation measures more than the measures themselves and for this reason we largely referred to habitat characteristics. By means of habitat, we established a direct link between measures and their effectiveness on biological attributes.

The CIS approach emphasizes the application of all possible measures to identify MEP conditions, while the Prague approach allows for the implementation of fewer measures excluding those providing only a slight ecological benefit, directly assessing GEP. Such basic concept in HMWB management is not easy to standardise, since there are no mitigation measures that are never possible and no measures that are always necessary. To overcome this we based on an observed situation where sites present different assemblages of natural features representing mitigation measures and we defined a statistical threshold for MEP acceptability. Such approach combined to multivariate analysis allowed for the definition of habitat features clearly separating MEP from non-MEP reaches. We verified that local restoration linked with the presence of adequate riparian vegetation and associated features, can effectively support the definition of Maximum Ecological Potential. Tree cover, large woody debris, set back embankment, as opposed to channel resectioning resulted the most important variables determining differences between MEP and impacted sites.

In particular, and remarkably, we ascertained that a gradient in tree-related habitats and channel forms can be observed also when morphology is strongly compromised, resulting as a key element for MEP distinction. In our studied sites distance of levees from river channel is in opposition to channel resectioning, supporting the statement that when enough space is provided to rivers, natural processes of bank and channel adjustment are favoured.

Our results also confirm that levees and reinforcement are often the main impediment to the settlement of appropriate riparian vegetation sustaining the need for suitable levees manipulation (i.e. move levees backwards) accompanied by abandonment of human activities on the banks. In particular, we demonstrated that even in a highly anthropized territory it is possible to observe leveed rivers profiting from acceptable side space and this results in the presence of natural features linked to an adequate presence of tree-related vegetation along banks.

As well, our results support the hypothesis that in-channel vegetation must be present for a river reach to be classified as MEP. Correct management options of aquatic vegetation must be planned in order to maintain such habitats in the expected MEP ranges, possibly reducing dominance of single macrophyte species and increasing plant diversity.

Do river invertebrates reflect differences between MEP and impaired stretches?

In our study reach-scale mitigation measures linked with the improvement of in-stream and riparian habitat had strong impacts on macroinvertebrate assemblages. Based on macroinvertebrates, MEP and impaired sites clearly separated, in relation to channel resectioning and to the presence of aquatic macrophytes as dominant in-channel habitat (> 40%), which has a negative effect on invertebrate communities. Large woody debris and presence of shrubs along the banks were important as well for the benthic community.

The variables highly correlated to macroinvertebrates are those discriminating between MEP and impacted stretches, demonstrating a concordance between abiotic and biotic information. In general, differences among sites are weaker at reinforced reaches compared to leveed reaches. Study insights would be necessary to emphasize these differences, focusing on the possibility to widen the studied gradient.

Even if the focus of the paper is not on proving the ability of biological metrics currently in use in biomonitoring in representing ecological potential, we have explored the performance of such metrics for MEP status characterisation. Our results demonstrated for invertebrates a shift towards sensitive taxa e.g. number of families of Ephemeroptera and Trichoptera in relation to habitat quality improvement.

In leveed streams, macroinvertebrate metrics well discriminated among sites with different level of alteration. On the contrary, differences between MEP and non-MEP sites for the reinforced reaches were scarcely significant, apart for differences in the combined multimetric index used for ecological status evaluation (i.e. STAR_ICMi). It can be inferred that in our study reinforced reaches exhibit a relatively good in-stream habitat quality, and thus support similar results in benthic metrics. However, specific metrics should be developed for such group of sites in combination to a possible extension in the studied gradient, even if the fact that STAR_ICMi is detecting differences between MEP and non-MEP sites is encouraging.

In general, we can anyhow conclude that differences in habitat features are mirrored by differences in invertebrate communities and these can be read in terms of metrics related to the assessment of ecological status.

Conclusions of the sixth paper (chapter 8), "**In-stream microhabitat mosaic depicts the success of mitigation measures and controls the Ecological Potential of benthic communities in heavily modified rivers**":

We studied the benthic community in a range of heavily modified river reaches (i.e. leveed) showing a different level of implementation of mitigation measures, in the lowlands of Northern Italy. We observed a grade of in-stream substrate variables i.e. number, diversity and mosaic of riverbed microhabitats, which mirrored morphological alteration and a gradient in the implementation of mitigation measures. The structure of the benthic community was deeply associated to such features. Also, benthic metrics and biological quality i.e., ecological potential, depicted the mitigation measure and habitat breadth.

Do river microhabitats reflect the implementation of mitigation measures?

At the study sites, a series of mitigation measures to reduce impacts of morphological alteration on the aquatic habitat and biota were increasingly applied. They mainly implied the adoption of sensitive management methods of river maintenance, a few actions on riparian vegetation and a passive restoration approach for in-stream habitats, which exploit flood events as the main drivers for their dynamic development. Microhabitat mosaic, structure and diversity evidently followed the gradual implementation of such mitigation measures.

At river reaches where the MEP sites are located, the presence of high levees paradoxically helped a full implementation of mitigation measures i.e. by promoting a relative isolation from surrounding agricultural areas and limiting bank accessibility. Banks likely acted as natural fences, which are often included among passive restoration measures. Such high levees, together with the presence of artificial berms and relatively large riverbeds, supported an effectual passive restoration of both channel and banks.

Microhabitats, mitigation measures and benthic invertebrates

Any valuable appraisal of habitat structure should be applicable to a range of habitats and have relevance to their associated fauna. This makes it difficult to select comprehensive habitat indicators suitable for wide-ranging studies and comparisons. On these premises, we directly quantified habitat structure by counting the number of the main in-stream microhabitats and assessing their diversity. Additionally, we proposed a simple approach to quantify similarity of microhabitat mosaic between sites (i.e. the HF – Habitat Factor). All the three habitat attributes were clearly related to mitigation measures and benthic invertebrate response. HF accounts for complexity of in-stream microhabitat structure (the abundance of substrate and cover features) and, partly, heterogeneity (the composition of different substrate and cover features). This factor straightforwardly compares the observed microhabitat composition with that expected in restored (MEP) conditions.

We found that both mosaic similarity and diversity of in-stream microhabitats well reflect the level of implementation of mitigation measures and correlate with the benthic community. Habitat diversity evidently mirrors morphological alteration whereas similarity among mosaics reflects the concurrence of mitigation measures and an increasing presence of lotic habitats.

Beta diversity (Sørensen coefficient) positively correlated with in-stream microhabitat number and diversity. On the other hand, differences in microhabitat mosaic best explained differences in alpha diversity and benthic quality metrics (i.e. directly related to ecological status/potential).

In addition, a significant relationship between the presence of tree-related features - an effect of mitigation measures - and the benthic community was found.

From the implementation of mitigation measures to ecological classification

In the lowlands of Northern Italy, morphological alteration of rivers demands a massive implementation of restoration or mitigation measures to fulfil the requirements of water legislation and fit environmental goals. In this study, we demonstrated that mitigation measures increasingly led to in-stream habitat improvement and emphasised how this affected benthic community.

Essentially, we did not find scientific references relating mitigation measures, habitat implementation and benthic ecological status in heavily modified rivers. We demonstrated that benthic ecological potential, together with alpha and beta diversities, were significantly related to the level of implementation of mitigation measures. As well, we found that microhabitat mosaic and diversity relate to those measures, with ecological potential (STAR_ICMi) chiefly encapsulating microhabitat mosaic and measure information.

Among all environmental variables considered, the similarity of microhabitat mosaic between MEP and test sites explained the largest proportion of STAR_ICMi variation.

Perspectives on using microhabitat mosaic information

Even if more detailed habitat survey approaches would be appropriate, the direct visual assessment of habitat features, as used in this study, is believed to be a cost-effective and scientifically sound approach for interpreting the benthic invertebrate response. Highly significant relations were found between habitat characteristics, benthic response and environmental variables, the latter collected at a larger scale.

In any case, the described method for quantifying microhabitat mosaic can be applied to a wide range of data sources, not necessarily based on a visual survey, and can be used to compare different situations and environments. The R script syntax provided is such that it can be applied to any kind of data, if proportionally collected and structured in percentage classes. For river habitats, not only can it be used to compare in-stream substrate microhabitats, but any habitat attribute. For instance, it seems well suited to promptly fit data acquired with the River Habitat Survey method, for which large amount of data is available worldwide.

Information on the consequences of mitigation measures implementation on habitat is central to appreciate success of river rehabilitation. With mitigation measures driving the process in the background, the link between in-stream substrate mosaic and ecological potential encourages a direct use of habitat information. Firstly, the similarity of microhabitat mosaic together with substrate diversity can be used directly to assess the overall quality of microhabitat structure and heterogeneity. In this way, relevance for the benthic community at the proper scale will be guaranteed. Secondly, when biological sampling is performed concurrently, these habitat indicators make it possible to assess if in-stream microhabitat influences the benthic community structure. Last but not least, when the aim is to infer on benthic invertebrate potential/status, especially in a multi-pressure context, the evaluation of in-stream substrate mosaic (i.e. HF) will provide a relevant habitat representation for benthic indices used for classification. Potential for applying these microhabitat metrics to invertebrate monitoring is huge. In fact, in Europe and outside, thousands of benthic samples have been and are constantly being collected with a multi-habitat, proportional approach. Such a field approach for collecting benthic taxa often requires that in-stream microhabitats are listed and their proportion assessed, prior to proceeding with invertebrate sampling. Thus, in the last 15-20 years, an enormous amount of substrate microhabitat data for rivers and streams has been collected and stored in environment agencies databases, summing up to at least 89.000 water bodies in Europe. Surprisingly, at least in Italy, the use of such microhabitat information, which is always collected concomitantly with invertebrates, has been marginal or definitively null. The computation on that data of in-stream microhabitat mosaic and diversity as defined in the present study would bring a massive piece of information. This would be precious data towards a better understanding of benthic community behaviour and response to pressures.

9.3. Macroinvertebrate research and the third mission: Dealing with the normative context

Invertebrate-based methods to assess ecological status in Italian rivers are established since years (e.g. Buffagni et al., 2006; 2007; CNR-IRSA, 2007; MATTM, 2010; Buffagni & Erba, 2014), and their use for WFD aims is regulated by a series of decrees (see Table 1.1).

Some of these decrees are still in a validation phase, others are under revision and/or updates are expected soon. Likewise, many aspects connected with e.g. the responses of macroinvertebrate assemblages and quality indices to individual stressors and to changing habitat conditions, still need detailed investigation. Freshwater research continuously brings new results and present innovative theories or approaches, some of which may find a relatively prompt application and strengthen a rationale of why the regulations need to be implemented.

Universities and the National Research Council (CNR) are involved in the so-called 'third mission'. In general, the third mission has at least two key priorities. The first is to help resolve societal challenges by targeted use and transfer of academic knowledge. The second is to transfer technologies and innovations in the form of cooperation with public and private enterprises. In practice, research activities should be described, valorised, interconnected and, finally, transferred to institutions able to use them in applied terms. This thesis fits in the CNR context and, according to CNR Statute (Consiglio Nazionale delle Ricerche, 2018: Article 3 - Aims), CNR "[…] b) promotes the enhancement and use of research results; c) provides advice and certification assistance, as well as technical and scientific support, to the Government and public administrations […]".

The papers presented in this dissertation provide experimental evidence of responses of macroinvertebrates (i.e. one of WFD 'Biological Quality Elements') to changes of both flow-related variables and morphological alteration. Papers 4 and 5, which dealt with heavily modified rivers, brought results and drawn conclusions that may directly be incorporated in the actual regulation for the classification of ecological potential, as soon as the relevant decree (DD 341/STA) and related guidance documents will be updated after their test phase. For instance, both papers provide approaches to quantify the level of implementation of mitigation measures and to relate it to the quality of biological elements. These are crucial aspects to standardise the environmental analysis, beyond a simple quantification of morphological degradation, and make the ecological classification comparable across sites, designation cases and areas. As well, the two papers assessed the attitude of the metrics and index (i.e. the STAR_ICMi) in use for the classification of ecological potential in Italy to discriminate between MEP and non-MEP sites (paper 5) and across levels of implementation of mitigation measures (paper 6), largely confirming their appropriateness in the study context.

Paper 6 presented a new approach to compare river habitats, tested for in-stream substrate microhabitats, which may find a very large application for most water bodies included in the WFD river monitoring network. The comparison may be based on habitat estimations always performed when macroinvertebrates are collected for WFD classification purposes. This approach may find routine application over wide areas, at the cost of zero new data collection. The results and conclusions of two papers offer other connections to Ministerial decrees, some of which are summarized in Figure 9.1.

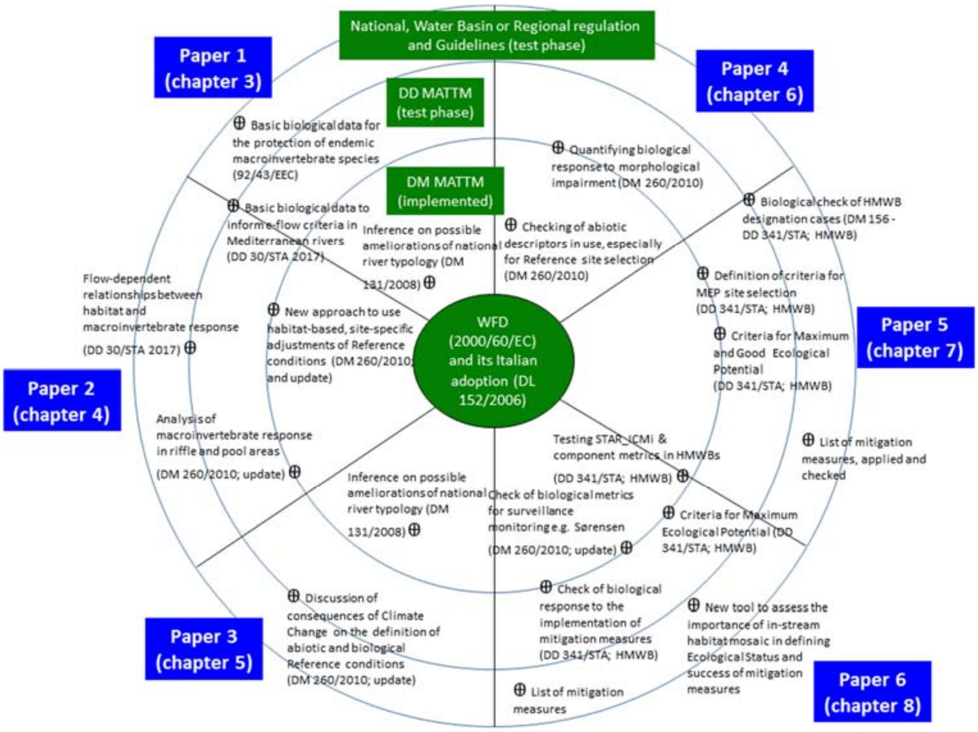

Figure 9.1. Potential relevance of the results of this dissertation for existing Ministerial decrees and other environmental regulation acts (mainly connected to the Water Framework Directive). Closer to the centre of the diagram, more consolidated the regulation acts. Mostly, moving from centre to external parts of the diagram, acts become 'under validation' or 'under development'. For decree codes, please refer to the text.

On the flow-related side, paper 1 provided basic research evidence of what is behind the relationships defined in paper 2 between habitat factors and macroinvertebrate classification metrics. Paper 2 results should be considered in the direction of future site-specific adjustments to reference conditions, thus being relevant for a series of existing regulations e.g. DM 131/2008, DM 260/2010 and updates. As well, its results may be relevant to directly link ecological-flow recommendations to ecological status based on benthic invertebrates (DD 30STA/2017). Moreover, the comparison of invertebrate metrics responses in riffle (‘fast water’) and pool (‘slow water’) areas (*sensu* Hawkins et al., 1993) adds substance to improve the use of invertebrate data in surveillance and investigative monitoring e.g. when applying DM 260/2010 and updates.

Most activities for this thesis have been completed at CNR-IRSA, which had a relevant contributing role or provided assistance to the Ministry of Environment during the last 40 years, in developing the Italian environmental legislation on freshwaters. Apart from DM 156 (MATTM, 2014), all the Ministerial decrees listed in Table 1.1 based on, or gained from national papers/reports prepared by CNR-IRSA for rivers, such as for macroinvertebrates (e.g. Buffagni & Erba. 2007; 2007b; 2014; Buffagni & Belfiore, 2013; Buffagni et al., 2014; CNR-IRSA, 2007), typology (Buffagni et al., 2006), reference conditions (Buffagni et al., 2008; 2013a), habitats (Buffagni et al., 2005; 2013) and e-flows (e.g. Buffagni et al., 2013a). Additionally, part of the technical sections of the abovementioned decrees were drafted by the author of this dissertation. The majority of approaches and methods incorporated into the normative reference have been proposed and developed over time with a strong inter-connection, to favour an organic implementation of the WFD, as far as these aspects are concerned. Hence, it is reasonable to presume that the main results and conclusions of the papers contained in this thesis will be considered in forthcoming updates and refinements of Ministerial decrees dealing with river ecosystems.

In the next future, the outcomes of this thesis may therefore tacitly support the third mission of the National Research Council.

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RIASSUNTO

Lo studio degli habitat fluviali può risultare cruciale per comprendere le risposte degli organismi alla variabilità naturale e alle alterazioni dovute alle attività antropiche. In questa tesi, alcune caratteristiche idromorfologiche che concorrono a definire gli habitat acquatici e ripari sono poste in relazione alle comunità macrobentoniche.

In particolare, sono state approfondite le risposte degli organismi bentonici ad alcune caratteristiche di habitat legate al flusso idrico, con enfasi sui fiumi temporanei in area mediterranea e sui periodi di bassa portata. In un primo lavoro svolto nei fiumi sardi, è stata studiata la risposta dei taxa bentonici al carattere lentico-lotico, i.e., alle condizioni ambientali osservate al momento del rilievo in campo come risultano dall'insieme delle principali caratteristiche di habitat legate al flusso idrico. Per oltre 60 taxa, per i quali tali risposte sono significative, è stato identificato l'optimum per specifici ambiti di carattere lentico-lotico. Le conseguenze della distribuzione disomogenea dei vari taxa e dei principali gruppi tassonomici lungo il gradiente lentico-lotico sono state studiate nel secondo lavoro, focalizzato su metriche e indici usati per la classificazione dello stato ecologico nell'Europa mediterranea. Enfasi è stata posta sulla necessità di promuovere la modulazione delle condizioni di riferimento su base sito-specifica, in funzione degli habitat attesi al momento del campionamento biologico. Infine, in un terzo lavoro relativo al bacino del fiume Candelaro, in Puglia, sono stati stimati gli effetti delle variazioni climatiche attese su una serie di indicatori di alterazione idrologica. Ne sono quindi state discusse le possibili conseguenze sulle biocenosi acquatiche, soprattutto in relazione al carattere lentico-lotico e all'opportunità di modulare condizioni di riferimento corrispondenti alle mutevoli condizioni climatiche.

Parallelamente, in un secondo filone d'indagine sempre legato al ruolo degli habitat fluviali nell'interpretazione dei dati biologici, è stata studiata la relazione tra le comunità bentoniche e il livello di alterazione morfologica dei fiumi. Un primo lavoro ha indagato la risposta delle metriche in uso per la classificazione dello stato ecologico in Italia e altrove all'alterazione dell'habitat a livello di alveo e sponde, soprattutto in termini di presenza di strutture artificiali, in cinque diversi tipi fluviali italiani. In un secondo lavoro, focalizzato su corpi idrici fluviali fortemente modificati in ambito planiziale, dove l'alterazione dell'habitat può raggiungere livelli molto elevati, è stato affrontato il problema della definizione del massimo potenziale ecologico, concetto affine a quello di condizioni di riferimento, valido per questa particolare categoria di corpi idrici. Infine, nello stesso contesto, nell'ultimo lavoro è stata investigata l'importanza del mosaico di microhabitat rinvenuti in alveo come chiave di interpretazione della risposta biologica all'alterazione morfologica di alveo e sponde e al livello di implementazione di misure di mitigazione.

I risultati dei lavori qui presentati consentono di concludere che la caratterizzazione degli habitat fluviali, a vari livelli e varie scale in funzione degli obiettivi, è cruciale per l'interpretazione dei dai biologici. Le risposte biologiche al livello di alterazione dovuto alle attività antropiche e/o alla variabilità naturale legata alla stagionalità, al clima o a differenze tipologiche possono essere efficacemente comprese solo integrando informazioni sull'habitat, rivelatosi un elemento chiave anche in funzione della classificazione dello stato ecologico ai sensi delle attuali normative ambientali.

Scientific publications (included in this thesis)

- Buffagni A. The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers (Sardinia, SW Italy). (submitted)
- Buffagni A., S. Erba, M. Cazzola, E. Barca, C. Belfiore. The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification (submitted)
- De Girolamo A. M., Bouraoui F., Buffagni A., Pappagallo G. & Lo Porto A. 2017. Hydrology under climate change in a temporary river system: Potential impact on water balance and flow regime. River Res. Applic., 33: 1219–1232.

- Erba S., Cazzola M., Belfiore C., A. Buffagni. Macroinvertebrate metrics response to morphological alteration in Italian rivers. (submitted)
- Erba S., L. Terranova, M. Cazzola, M. Cason, A. Buffagni. 2019. Defining Maximum Ecological Potential for heavily modified lowland streams of Northern Italy. Sci. Total Environ., 684: 196–206.
- Buffagni A., Barca, E., Erba, S. & Balestrini, R. 2019. In-stream microhabitat mosaic depicts the success of mitigation measures and controls the Ecological Potential of benthic communities in heavily modified rivers. Sci. Total Environ., 673: 489–501.

APPENDICES

Supplementary material of papers

Supplementary material to:

Chapter 3 (PAPER 1)

Buffagni, A. (submitted) The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers

Buffagni A. The lentic and lotic characteristics of habitats determine the distribution of benthic macroinvertebrates in Mediterranean rivers.

Table S1 Investigated sites: code, sampling date and geographic coordinates. Variables useful for overall reach characterization (Table 1) are also reported, together with those significantly related to benthic NMDS axes (Fig. 1).

Site coordinates				Overall reach characterization											Variables * to benthic NMDS axes								
Site code	Sampling date	Site latitude	Site longitude	Altitude a.s.l. (m)	Distance to source (km)	Slope of the Thalweg (%)	Discharge (field measure)(m^3/s)	Mean channel width (m)	Mean water width of the main channel (m)	Habitat Modification Score (Raven et al., 1998)	Habitat Quality Assessment score (Raven et al., 1998)	Land Use Index (Erba et al., 2016)	Lentic-lotic River Descriptor (Buffagni et al., 2010)	LRD class	Sections with over-depeened channel (count)	'Broken waves' flow type (%)	'Unbroken waves' flow type (%)	'Rippled' flow type (%)	'Not perceptible' flow type (%)	Number of dry sections (flow interruption) in the river channel	'CPOM/FPOM/XY' habitat (%)	Conductivity (µS/cm)	Water temperature (°C)
		WGS84	WGS84	alt	Dist_fin	slope_th	Q_ist	wi_mean	w_chl	HMS	HQA	LUI	LRD	cl_LRD	OD	FP_BW	FP_UW	FP_RP	FP_NP	nDRY_SU	OP_CFXY	Cond	wat_T_is
S1	13/05/2011	41°4'53.11"N	9°13'19.92"E	98	7	0.8	0.066	5.04	4.23	9	57	1.03	11.25	4	3	0.000	0.043	0.493	0.061	0	0.434	574	16.0
S2	09/03/2013	41°05'25.98"N	09°14'07.13"E	88	8.5	1	0.172	6.75	4.55	26	46	1.34	-0.35	3	5	0.041	0.450	0.396	0.000	0	0.454	460	11.3
S3	10/05/2011	41°6'37.30"N	9°13'44.22"E	105	5.5	0.8	0.02	3.23	3.1	2	50	0.5	0	3	2	0.000	0.059	0.274	0.054	0	0.080	467	16.0
S4	13/05/2011	41°4'53.80"N	9°13'21.61"E	98	7	0.8	0.062	5.34	3.97	17	54	3.01	-1.7	3	2	0.000	0.141	0.286	0.000	0	0.352	574	16.0
S5	09/03/2013	41°05'34.15"N	09°14'45.44"E	82	9.5	0.4	0.155	5.35	4.7	16	61	2.36	-6.5	3	2	0.064	0.461	0.348	0.000	0	0.320	470	13.5
S6	08/03/2013	41°07'15.91"N	09°13'30.60"E	118	4	1.2	0.037	3.25	2.75	5	60	3.56	0.1	3	1	0.035	0.433	0.145	0.000	0	0.479	499	11.7
S7	07/03/2013	41°06'36.64"N	09°13'43.83"E	105	5.5	0.8	0.176	5.45	3.4	14	60	1.33	3.49	3	5	0.114	0.378	0.244	0.000	0	0.662	401	14.5
S8	18/08/2004	40°50'42"N	09°33'07"E	51	21.6	0.71	0.005	7.03	6.53	5	49	0.58	53.14	5+	0	0.000	0.000	0.000	0.301	1	0.883	481	21.0
S9	22/05/2011	39°49'22.80"N	9°39'7.02"E	16	5.4	1.1	0.012	9.5	3.48	88	34	11.7	80.34	5+	0	0.000	0.068	0.240	0.064	0	0.618	423	23.0
S10	18/05/2011	40°24'18.79"N	9°38'21.06"E	9	14.7	0.5	0.078	52.1	4.4	42	44	9.83	21.44	4	0	0.000	0.000	0.374	0.115	0	0.177	439	17.3
S11	16/03/2013	40°02'45.12"N	09°31'00.71"E	303	2.5	9.9	0.013	9.8	2.22	9	63	0.9	-9.5	3	0	0.015	0.122	0.715	0.000	0	0.418	258	10.2
S12	11/05/2011	41°7'2.17"N	9°13'3.90"E	118	3.4	1.2	0.004	2.33	1.94	7	57	2.09	59.5	5+	2	0.000	0.019	0.180	0.232	0	0.638	568	15.0
S13	19/05/2011	39°17'27.53"N	9°31'37.63"E	44	11.3	0.7	0.123	16.1	4.7	51	34	11	10.25	4	1	0.000	0.085	0.369	0.163	0	0.000	496	18.0
S14	20/05/2011	39°17'33.97"N	9°31'38.42"E	42	11.5	0.7	0.098	5.75	0.47	79	26	13.1	14.25	4	0	0.000	0.070	0.313	0.058	0	0.102	496	18.0
S15	23/05/2011	40°2'13.99"N	9°31'44.94"E	210	9.8	0.9	0.252	28.4	6.68	21	56	1.03	-38.82	1	3	0.148	0.471	0.295	0.000	0	0.285	340	23.0
S16	18/05/2011	40°13'0.41"N	9°31'6.89"E	231	27.2	2	0.243	30.6	12.25	0	61	0	-11.98	2	2	0.000	0.078	0.388	0.031	0	0.235	292	19.4
S17	21/05/2011	39°55'21.25"N	9°39'41.22"E	14	28	0.6	0.097	21.1	7.1	60	30	10.3	-9.79	3	1	0.045	0.457	0.408	0.032	0	0.000	312	23.0
S18	21/02/2004	39°57'42.02"N	09°32'40.40"E	168	6.1	5.5	0.026	4.24	2.8	0	57	0	-23.5	2	0	0.012	0.173	0.238	0.048	0	1.000	188	11.0
S19	15/03/2013	39°57'39.69"N	09°32'37.94"E	181	6.6	5.5	0.069	5.91	3.18	0	48	0	-20.38	2	3	0.090	0.094	0.432	0.000	0	0.301	135	9.3
S20	07/06/2004	39°57'42.02"N	09°32'40.40"E	168	6.1	5.5	0.015	4.7	3.4	0	57	0	-27.39	2	0	0.094	0.136	0.225	0.127	0	0.953	200	19.3
S21	19/08/2004	39°57'42.02"N	09°32'40.40"E	168	6.1	5.5	3E-04	5.6	2.38	0	56	0	49.75	5	0	0.000	0.000	0.021	0.326	4	0.533	309	24.4
S22	10/05/2011	41°4'52.64"N	9°17'12.73"E	23	36.5	0.2	0.387	34	10.05	2	55	0.89	45.83	5	1	0.000	0.113	0.120	0.128	0	0.514	347	16.1
S23	16/05/2011	40°22'58.26"N	9°23'49.96"E	152	6.1	2.3	0.031	14.5	4.35	0	52	0	8.51	3	2	0.000	0.087	0.446	0.000	0	0.330	421	17.3
S24	17/05/2011	40°22'27.98"N	9°23'57.52"E	118	7.3	2.3	0.029	5.4	4.6	43	46	1.45	4.5	3	4	0.036	0.062	0.413	0.058	0	0.346	421	17.3
S25	22/02/2004	39°57'26.11"N	09°37'3.70"E	32	17.6	0.83	0.421	80.5	7.2	45	45	2.63	-32.05	1	0	0.142	0.481	0.353	0.000	0	0.000	160	11.0
S26	06/06/2004	39°57'25.59"N	09°36'46.32"E	32	17.1	0.83	0.143	15.8	10.9	44	62	0.73	-21.43	2	0	0.025	0.139	0.309	0.000	0	0.644	245	17.0
S27	20/08/2004	39°57'24.69"N	09°36'57.29"E	32	17.6	0.83	0.077	56.7	8.5	46	48	1.9	8.45	3	0	0.048	0.048	0.125	0.229	0	0.629	140	24.6
S28	13/03/2013	39°56'28.60"N	09°34'47.10"E	92	6.2	4.1	0.045	5.65	4.12	0	57	0	-0.87	3	0	0.071	0.143	0.296	0.000	0	0.402	295	11.0
S29	14/03/2013	40°01'12.37"N	09°31'24.87"E	182	5.7	3.4	0.125	27.9	3.95	0	51	0.61	-27.96	2	0	0.125	0.712	0.120	0.000	0	0.084	190	10.5
S30	14/03/2013	40°01'13.70"N	09°31'26.60"E	156	6.5	2.4	0.168	16.9	7.1	44	58	3.57	-23.49	2	0	0.249	0.548	0.156	0.000	0	0.238	167	9.7
S31	09/06/2004	39°41'18.13"N	09°11'41.12"E	432	8.1	0.42	0.049	5	2.45	23	47	3.33	3.75	3	0	0.000	0.109	0.163	0.238	0	0.723	2008	17.0
S32	23/08/2004	39°41'18.13"N	09°11'41.12"E	432	8.1	0.42	0.006	10.9	1.94	45	33	11.6	38.5	5	0	0.000	0.000	0.034	0.254	0	0.663	2142	21.8
S33	23/08/2004	39°39'10.00"N	09°10'27.27"E	325	17.1	0.55	3E-04	7	4	13	55	2.32	35.5	5	0	0.000	0.000	0.000	0.283	0	0.694	2069	21.0
S34	10/06/2004	39°38'40.04"N	09°11'19.32"E	336	19.9	1.66	0.042	10.1	7.3	8	42	0.38	-6.57	3	0	0.000	0.158	0.180	0.123	0	0.322	981	21.3
S35	22/08/2004	39°38'34.78"N	09°11'27.98"E	336	20.6	1.66	4E-04	12.4	2.92	9	53	0.25	64.5	5+	0	0.000	0.000	0.000	0.333	2	0.595	1179	21.0
S36	11/06/2004	39°38'56.83"N	09°10'7.90"E	408	3.1	5	0.007	7.6	2.4	0	48	0	18.39	4	0	0.000	0.000	0.347	0.213	1	0.871	674	17.0
S37	24/08/2004	39°39'1.21"N	09°10'6.59"E	408	3.1	5	0	2	1.65	0	29	0	85.5	5+	0	0.000	0.000	0.000	0.091	6	0.864	806	21.0
S38	22/05/2011	39°46'34.68"N	09°38'52.13"E	21	4.1	1.3	0.097	9.8	9.8	57	32	4.48	69	5+	3	0.000	0.000	0.136	0.160	0	0.447	419	23.0
S39	10/03/2013	41°02'54.00"N	09°19'45.36"E	98	9.5	0.5	0.134	22.7	8.15	26	77	3.84	46.56	5	1	0.000	0.104	0.207	0.157	0	0.693	353	11.5
S40	20/05/2011	39°21'8.75"N	9°29'21.26"E	40	23.5	1.4	0.035	33.8	9.9	7	60	0.14	11.48	4	1	0.070	0.024	0.140	0.172	0	0.493	167	19.3
S41	16/05/2011	40°38'40.15"N	9°35'34.95"E	50	12.4	3.2	0.039	5.5	3.52	0	50	0	8.37	3	0	0.025	0.027	0.095	0.091	0	0.214	143	18.0
S42	15/05/2011	40°39'11.12"N	9°30'9.65"E	111	12.9	0.5	0.607	17.1	11.9	0	62	0	0.71	3	2	0.118	0.184	0.095	0.038	0	0.447	244	18.0
S43	09/06/2004	39°25'57"	08°40'40"	355	11.1	2.5	0.024	9.4	4.02	1	69	0.15	-23.92	2	0	0.000	0.055	0.400	0.050	0	0.449	333	19.4
S44	12/05/2011	41°9'31.61"N	9°10'16.64"E	33	5	2.9	0.004	4.52	3.65	0	70	0	25.34	4	2	0.000	0.053	0.344	0.208	0	0.639	488	20.0
S45	15/03/2013	39°57'37.48"N	09°33'40.80"E	104	12.6	4	0.123	18	7.9	0	72	10	0.18 -15.94	2	0	0.106	0.114	0.380	0.093	0	0.266	137	8.8
S46	17/05/2011	40°23'47.08"N	9°28'52.50"E	65	15.6	1.6	0.024	6.3	4.65	51	29	4.37	23.25	4	0	0.000	0.000	0.197	0.240	0	0.000	689	17.3
S47	20/05/2011	39°9'42.66"N	9°26'53.05"E	50	9.9	2	0.098	40.2	4.03	11	47	3.36	12.5	4	0	0.000	0.116	0.504	0.070	0	0.465	565	18.2
S48	12/05/2011	41°8'51.25"N	9°8'9.31"E	16	17.6	2.6	0.011	7.39	5.429	3	49	0	2.33	3	3	0.000	0.049	0.247	0.212	0	0.000	547	20.0
S49	05/06/2004	40°45'13.31"N	09°31'46.05"E	144	14.1	0.83	0.23	7.3	6.38	20	65	3.9	-32.25	1	0	0.162	0.203	0.358	0.034	0	0.716	218	17.0
S50	20/02/2004	40°43'11"N	09°30'35"E	235	6.6	1.61	0.076	6.8	4.32	0	67	0	-12.54	2	0	0.000	0.095	0.172	0.179	0	0.558	178	8.4
S51	05/06/2004	40°43'7.05"N	09°30'52.23"E	235	7.6	1.61	0.172	9.11	5.95	0	59	0	-26.83	2	0	0.044	0.199	0.146	0.168	0	0.565	166	14.5
S52	18/08/2004	40°43'11"N	09°30'35"E	235	7.6	1.61	0.002	3.56	2.04	0	56	0	37	5	0	0.000	0.000	0.000	0.301	2	0.569	270	21.0
S53	19/08/2004	39°55'06"N	09°37'09"E	31	24.6	0.92	0.056	7	4.4	12	60	3.99	25.99	4	0	0.000	0.047	0.347	0.193	0	0.675	392	25.0
S54	20/02/2004	40°45'29"N	09°31'48"E	100	16.6	0.62	0.105	18	7.85	31	60	3.94	-12.25	2	0	0.000	0.278	0.280	0.110	0	0.848	269	10.4
S55	23/05/2011	40°33'35.03"N	9°20'14.95"E	770	8.3	2	0.008	4.55	2.53	0	52	0.15	-7.84	3	1	0.000	0.061	0.164	0.121	0	0.348	193	18.8
S56	14/05/2011	40°48'57.96"N	9°14'28.79"E	330	9.3	4	0.026	8.35	5.69	11	58	2.85	0.04	3	2	0.026	0.046	0.385	0.098	0	0.271	143	18.0
S57	14/05/2011	40°48'59.18"N	9°14'23.82"E	334	9.2	4	0.026	8.11	6.35	1	56	0.14	13.56	4	1	0.022	0.000	0.235	0.102	0	0.340	143	18.0
S58	06/06/2004	39°57'9.52"N	09°36'12.46"E	48	14.1	0.83	0.222	9	5	14	62	1.61	-10.21	2	0	0.059	0.140	0.243	0.131	0	0.803	286	17.0
S59	12/03/2013	39°57'05.84"N	09°36'16.91"E	54	13.9	1.7	0.05	6.25	3.75	2	84	1	8.75	3	0	0.100	0.258	0.171	0.071	0	0.658	314	12.3
S60	22/02/2004	39°57'9.52"N	09°36'12.46"E	48	14.1	0.83	0.056	5.73	3.678	14	57	3.36	-8.94	3	0	0.000	0.118	0.525	0.175	0	0.736	284	11.0
S61	20/08/2204	39°57'9.52"N	09°36'12.46"E	38	14.6	0.83	0.015	14.6	7.143	26	58	0.88	27.85	4	0	0.000	0.000	0.203	0.154	0	0.638	308	22.3
S62	12/03/2013	39°57'10.27"N	09°36'12.79"E	44	14.1	1.6	0.05	7.4	4.95	17	61	5.38	29.29	4	0	0.000	0.268	0.378	0.000	0	0.727	313	12.8

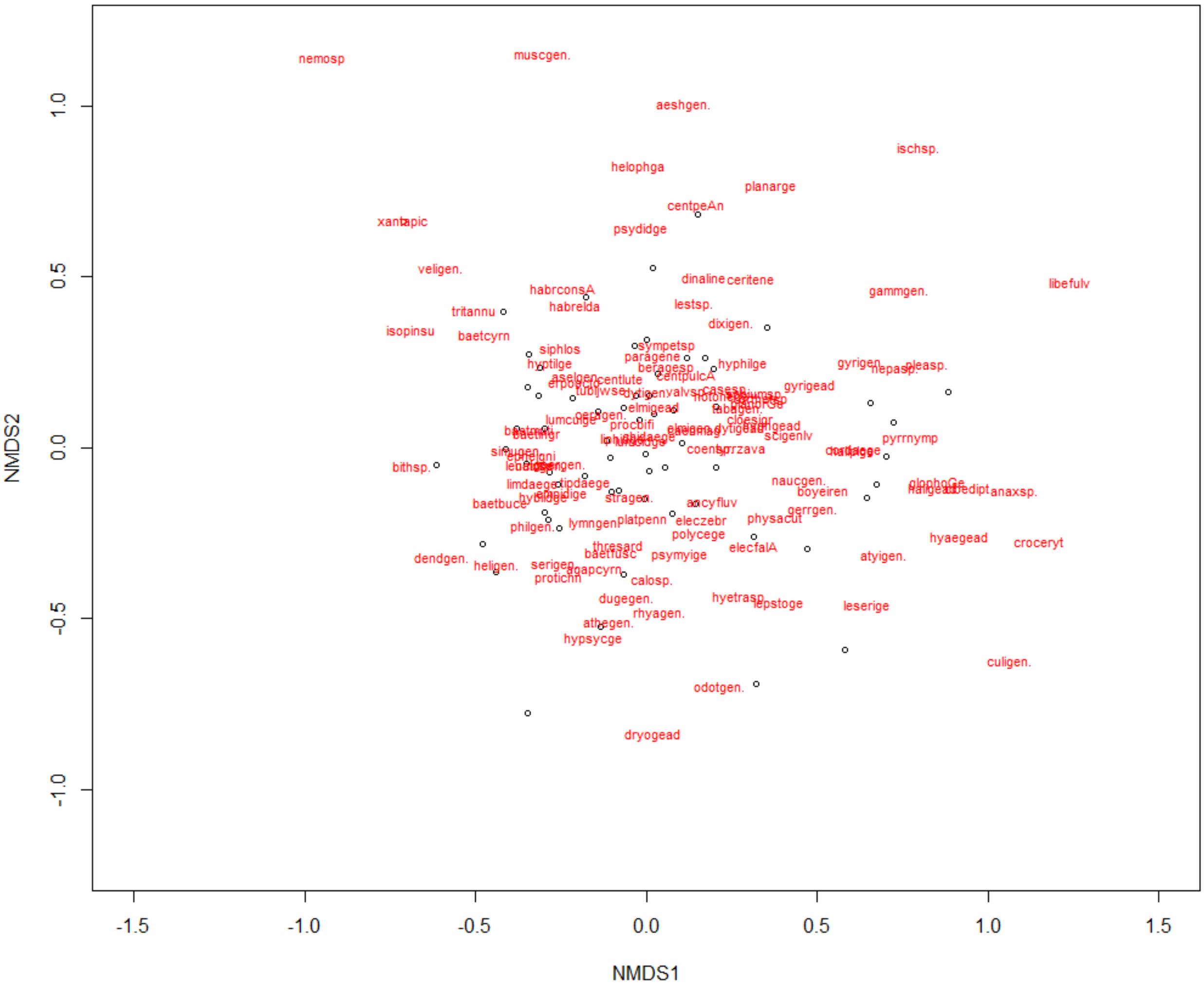

Fig. S1 Relative positioning of benthic taxa in the NMDS space.

Table S2 Results of model selection after 49 random split of the database into training (2/3) and testing (1/3) sub-datasets, with percentage of models selection for each taxon after 100 re-sampling with bootstrap approach. Column mod_49 summarizes the final outcome of such model selection, including the possibility of multiple models (after visual inspection, last column). 'MOD ALL data' indicates the model selected based on the whole dataset. Last column: model finally proposed for selection (same as Fig. 2).

taxon	mod 49	I	II	III	IV	V	MOD ALL data	model check (graph inspection)	Final model
LUMBRICIDAE	V	0	27	6	0	67	V	n	I
LUMBRICULIDAE	V	0	2	22	0	76	V	y	V
NAIDIDAE	V	0	14	6	0	80	V	y	V
TUBIFICIDAE	V	0	0	0	0	100	V	y	V
Dryopidae Gen. sp. Ad.	V	0	0	0	0	100	V	y	V
Dryopidae Gen. sp. Lv.	II-V	0	39	10	0	51	V	y	II-III-V
Dytiscidae Gen. sp. Ad.	II	0	53	16	0	31	II	n	I
Dytiscidae Gen. sp. Lv.	V	0	12	0	0	88	V	y	V
Elmidae Gen. sp. Ad.	V	0	0	0	0	100	IV	y	V-IV
Elmidae Gen. sp. Lv.	V	0	0	6	0	94	V	y	V
Gyrinidae Gen. sp. Ad.	II	0	88	2	0	10	II	y	II
Gyrinidae Gen. sp. Lv.	V	0	2	2	4	92	V	y	V
Haliplidae Gen. sp. Ad.	II-III-V	0	24	37	2	37	III	y	II-III-V
Haliplidae Gen. sp. Lv.	V	0	8	4	0	88	V	y	V
Helophoridae Gen. sp. Ad.	III	0	10	63	2	24	V	n	no
Hydraenidae Gen. sp. Ad.	-	2	39	6	43	10	V	y	V
Hydrophilidae Gen. sp. Ad.	II-III-V	0	51	22	0	27	II	y	II
Hydrophilidae Gen. sp. Lv.	II	0	98	0	0	2	II	y	II-III
ASELLIDAE	V	0	2	6	0	92	V	y	V
ATYIDAE	-	2	20	39	10	29	V	n	no
GAMMARIDAE	II	0	69	2	0	29	II	n	no
ATHERICIDAE	II-V	0	53	8	0	39	II	y	II
CERATOPOGONIDAE	V	0	0	0	0	100	V	y	V
CHIRONOMIDAE	II	0	98	0	0	2	II	y	II
DIXIDAE	II	0	69	4	0	27	II	n	I
EMPIDIDAE	II-V	0	33	18	4	45	IV	n	no
LIMONIIDAE	II-V	0	53	12	0	35	II	y	II-V
PSYCHOMYIIDAE	V	0	0	0	0	100	V	y	V
SIMULIIDAE	II	0	61	8	0	31	II	y	II
STRATIOMYIIDAE	V	0	0	20	4	76	V	y	V
TABANIDAE	II-V	0	47	16	0	37	III	y	III
TIPULIDAE	V	0	27	0	0	73	IV	y	V-IV
Baetis cyrneus	V	0	0	18	0	82	V	y	V
Baetis fuscatus	V	0	0	0	0	100	V	y	V
Baetis ingridae	V	0	8	2	0	90	V	y	V
Baetis cfr. muticus	V	0	20	6	0	73	V	n	I
Caenis macrura-Gr.	III	0	14	67	0	18	III	y	III
Centroptilum luteolum	II-V	0	43	4	0	53	I	y	I
Cloeon dipterum	II-III-V	0	24	22	0	53	V	y	II-III-V
Cloeon simile	II-V	0	53	12	0	35	II	y	II
Electrogena fallax	V	0	12	8	0	80	V	y	V
Electrogena zebrata	V	0	18	10	2	69	III	y	III
Serratella ignita	V	0	0	0	0	100	V	y	V
Habrophlebia consiglioi	V	0	0	0	0	100	V	y	V
Habrophlebia eldae	V	0	8	4	0	88	V	y	V
Procloeon bifidum	V	0	2	8	0	90	V	y	V-III
Siphlonurus lacustris	V	0	0	0	0	100	V	y	V
CORIXIDAE	V	0	8	18	2	71	V	y	V
NAUCORIDAE	II-III-V	0	20	47	0	33	III	y	III
Nepa sp.	II-V	0	49	0	0	51	V	n	I
NOTONECTIDAE	V	0	20	4	0	76	V	y	V
Plea sp.	II-V	0	51	16	0	33	II	y	II
Dina lineata	V	0	0	4	0	96	V	y	V
Helobdella stagnalis	V	0	8	0	0	92	V	y	V
Ancylus fluviatilis	V	0	22	12	0	65	III	n	I
PISIDIIDAE	V	0	4	10	0	86	V	y	V
HYDROBIIDAE	V	0	6	10	0	84	V	y	V
LYMNAEIDAE	V	0	0	0	0	100	V	y	V
Physella acuta	II-V	0	41	10	4	45	II	y	V-II
PLANORBIDAE	II	0	84	0	0	16	II	y	II
Anax sp.	II	0	63	10	8	18	II	y	II
Boyeria irene	V	0	22	2	0	76	V	n	I
Calopteryx sp.	V	0	0	4	0	96	V	y	V
Ceriagrion tenellum	II-V	0	41	2	0	57	V	y	V
Coenagrion sp.	V	0	0	2	0	98	V	y	V
Lestes sp.	V	0	0	10	0	90	V	y	V
Orthetrum sp.	V	0	27	0	0	73	V	y	V-II
Pyrrhosoma nymphula	V	0	8	0	0	92	V	y	V-II
Sympetrum sp.	II	0	84	10	0	6	II	y	II
Isoperla insularis	V	0	0	4	0	96	V	y	V
Leuctra sp.	V	0	12	10	0	78	V	n	III
Tyrrhenoleuctra zavattarii	II-V	0	37	0	0	63	V	y	V-II
Agapetus cyrnensis	V	0	0	2	0	98	V	y	V
BERAEIDAE	II-III-V	0	10	35	0	55	V	y	V
GOERIDAE	V	0	18	8	2	71	V	y	V
HYDROPSYCHIDAE	V	0	0	4	0	96	V	y	V
HYDROPTILIDAE	V	0	4	0	0	96	V	y	V
LEPTOCERIDAE	V	0	27	0	0	73	V	y	V-II
LIMNEPHILIDAE	V	0	18	0	0	82	V	y	V
PHILOPOTAMIDAE	V	0	24	4	0	71	V	y	V
POLYCENTROPODIDAE	V	0	20	8	0	71	V	y	V-III
RHYACOPHILIDAE	II	0	84	0	0	16	II	y	II
SERICOSTOMATIDAE	V	0	0	2	0	98	V	y	V
DUGESIIDAE	V	0	0	0	0	100	V	y	V

Table S3 Overall attribution of models to major benthic groups and main level of identification.

Taxonomic group	Identification level		n taxa	I	II	III	IV	V	mixed
Tricladida	Genus		1	0	0	0	0	1	0
Anellida	Family		4	1	0	0	0	3	0
Hirudinea	Species		2	0	0	0	0	2	0
Mollusca	Genus/Species		6	1	1	0	0	3	1
Crustacea	Genus		3	2	0	0	0	1	0
Ephemeroptera	Species		15	2	1	2	0	8	2
Odonata	Genus/Species		9	1	2	0	0	4	2
Plecoptera	Species		3	0	0	1	0	1	1
Hemiptera	Genus		5	1	1	1	0	2	0
Coleoptera	Family		14	2	2	0	0	6	4
Diptera	Family		11	2	3	1	0	3	2
Trichoptera	Family		11	0	1	0	0	8	2
				I	II	III	IV	V	mixed
Whole community			84	12	11	5	0	42	14
		%		13.1	13.1	6.0	0.0	50.0	16.7

An overall representation of model attribution for benthic taxonomic groups is shown in Figure S2.

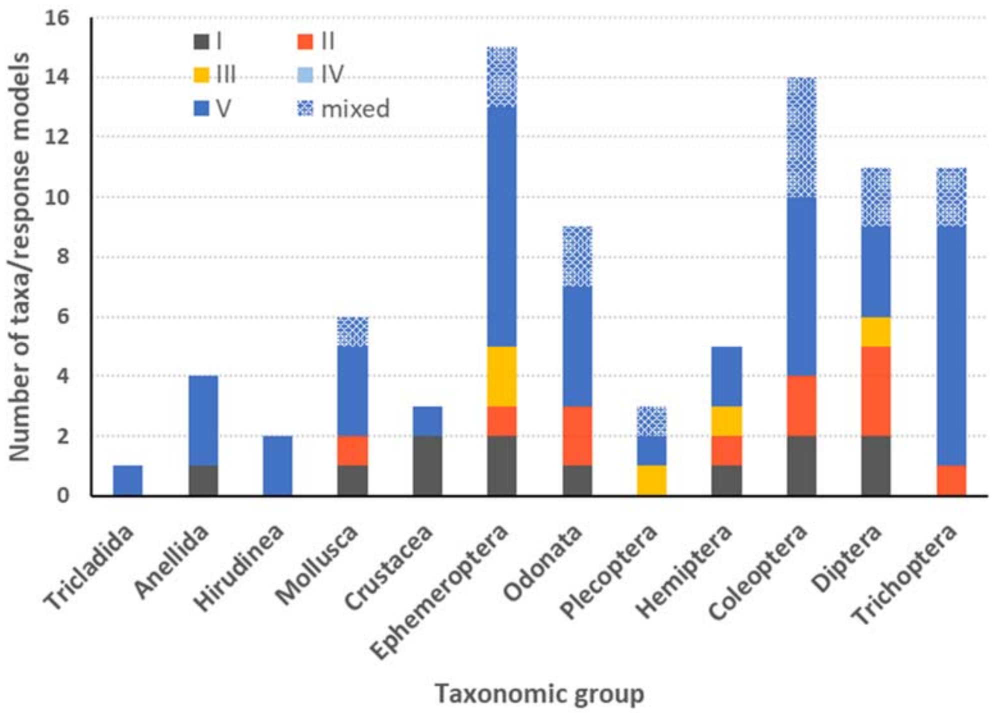

Fig. S2 Response of taxa to the lentic-lotic character (LRD). The type of response model shown by individual taxa for each taxonomic group is presented.

Supplementary material to:

Chapter 4 (PAPER 2)

Buffagni, A., S. Erba, M. Cazzola, E. Barca, C. Belfiore (submitted) The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification.

Table S1. Variability (minimum, median and maximum values) of water quality and geographical variables within sites pressure groups.

Pressure group	Notes		O_2 [mg/l]	Cl [mg/l]	$N\text{-}NH_4$ [mg/l]	$N\text{-}NO_3$ [mg/l]	$P\text{-}PO_4$ [µg/l]	Cond. [µS/cm]	pH	Alt. [m asl]	Dist. to source [km]
Reference	Substantial absence of bank and channel modification and transversal structures. Unmodified channel habitats. Semi-continuous trees and shrubs (including Mediterranean macchia)	min	9	14.5	0.001	<0.01	1	135	7.04	16	3.1
		median	10.7	33.2	0.022	0.20	21	244	7.7	168	7.6
		max	12.5	249	0.050	3.00	70	807	8.6	408	27.2
Least impaired	Some bank reprofiling may be present, substantial absence of bank reinforcement and channel habitat modification. Semi-Continuos trees and shrubs (including Mediterranean macchia)	min	7.2	26.5	0.002	<0.01	3	143	6.9	23	2.5
		median	9.6	75	0.024	0.82	28	421	7.8	105	9.8
		max	11.5	312	0.056	2.00	281	2070	8.41	336	36.5
Slightly altered	Bank reprofiling and reinforcement may be present. Sporadic channel modification, allowing a reference comparable habitat composition. At least discontinuous trees and shrubs (including Mediterranean macchia). In a few cases only herbaceous riparian vegetation present	min	7.2	18	0.007	0.18	2	160	7.18	9	4.1
		median	9.3	61.6	0.036	0.50	41	421	7.69	42	11.5
		max	11.1	321	0.116	2.27	260	2140	8.1	432	28

Table S2. Sites sampling dates, coordinates, Lentic-lotic River Descriptor values (LRD) and pressure group. Sites are sorted by increasing LRD values within the pressure group.

Site code	Pressure group	Sampling date	Site_Lat	Site_Lon	LRD
S66	Reference	14/03/2013	40°01'12.37"N	09°31'24.87"E	-27.96
S31		07/06/2004	39°57'48.37"N	09°32'38.24"E	-27.39
S47		06/06/2004	40°43'07.05"N	09°30'52.23"E	-26.83
S07		08/06/2004	39°25'57.00''N	08°40'40.00''E	-23.92
S29		21/02/2004	39°57'42.02"N	09°32'40.40"E	-23.5
S65		15/03/2013	39°57'39.69"N	09°32'37.94"E	-20.38
S73		15/03/2013	39°57'37.48"N	09°33'40.80"E	-15.94
S48		20/02/2004	40°43'11.00''N	09°30'35.00''E	-12.54
S37		18/05/2011	40°13'00.41"N	09°31'06.89"E	-11.98
S19		13/03/2013	39°56'29.95"N	09°34'49.25"E	-0.87
S58		10/05/2011	41°06'37.30"N	09°13'44.22"E	0
S60		08/03/2013	41°07'15.91"N	09°13'30.60"E	0.1
S44		15/05/2011	40°39'11.12"N	09°30'09.65"E	0.71
S61		12/05/2011	41°08'51.25"N	09°08'09.31"E	2.33
S43		16/05/2011	40°38'40.15"N	09°35'34.95"E	8.37
S39		16/05/2011	40°22'58.26"N	09°23'49.96"E	8.51
S20		12/03/2013	39°57'05.84"N	09°36'16.91"E	8.75
S52		14/05/2011	40°48'59.18"N	09°14'23.82"E	13.56
S08		11/06/2004	39°38'56.83"N	09°10'07.90"E	18.39
S62		12/05/2011	41°09'31.61"N	09°10'16.64"E	25.34
S46		18/08/2004	40°43'00.14"N	09°31'05.11''E	37
S54		10/05/2011	41°04'52.64"N	09°17'12.73"E	45.83
S34		19/08/2004	39°57'56.57"N	09°32'43.03"E	49.75
S53		18/08/2004	40°50'42.00''N	09°33'07.00''E	53.14
S09		24/08/2004	39°39'00.78"N	09°09'59.47"E	85.5
S22	Least impaired	06/06/2004	39°57'09.52"N	09°36'12.46"E	-10.21
S36		16/03/2013	40°02'45.12"N	09°31'00.71"E	-9.5
S21		22/02/2004	39°57'07.90"N	09°36'15.47"E	-8.94
S70		10/06/2004	39°38'40.04"N	09°11'19.32"E	-6.57
S64		09/03/2013	41°05'34.15"N	09°14'45.44"E	-6.5
S56		13/05/2011	41°04'53.80"N	09°13'21.61"E	-1.7
S51		14/05/2011	40°48'57.96"N	09°14'28.79"E	0.04
S57		07/03/2013	41°06'36.64"N	09°13'43.83"E	3.49
S55		13/05/2011	41°04'53.11"N	09°13'19.92"E	11.25
S06		20/05/2011	39°21'08.75"N	09°29'21.26"E	11.48
S01		20/05/2011	39°09'42.66"N	09°26'53.05"E	12.5
S16		19/08/2004	39°55'06.00''N	09°37'09.00''E	25.99
S23		12/03/2013	39°57'10.27"N	09°36'12.79"E	29.29
S69		23/08/2004	39°39'10.00"N	09°10'27.27"E	35.5
S59		11/05/2011	41°07'02.17"N	09°13'03.90"E	59.5
S71		22/08/2004	39°38'34.78"N	09°11'27.98"E	64.5
S49	Slightly altered	05/06/2004	40°45'13.31"N	09°31'46.05"E	-32.25
S35		23/06/2011	40°02'13.99"N	09°31'44.94"E	-38.82
S27		22/02/2004	39°57'26.11"N	09°37'03.70"E	-32.05
S67		14/03/2013	40°01'14.20"N	09°31'55.30"E	-23.49
S26		06/06/2004	39°57'25.59"N	09°36'46.32"E	-21.43
S50		20/02/2004	40°45'29.00''N	09°31'48.00''E	-12.25
S18		21/05/2011	39°55'21.25"N	09°39'41.22"E	-9.79
S63		09/03/2013	41°05'25.98"N	09°14'07.13"E	-0.35
S38		17/05/2011	40°22'27.98"N	09°23'57.52"E	4.5
S02		19/05/2011	39°17'27.53"N	09°31'37.63"E	10.25
S03		19/05/2011	39°17'33.97"N	09°31'38.42"E	14.25
S41		18/05/2011	40°24'18.79"N	09°38'21.06"E	21.44
S40		17/05/2011	40°23'47.08"N	09°28'52.50"E	23.25
S24		20/08/2004	39°57'09.52"N	09°36'12.46"E	27.85
S68		23/08/2004	39°41'18.13"N	09°11'41.12"E	38.5
S72		10/03/2013	41°02'54.00"N	09°19'45.36"E	46.56
S11		22/05/2011	39°46'34.68"N	09°38'52.13"E	69
S13		22/05/2011	39°49'22.80"N	09°39'07.02"E	80.34

Table S3a. Multi-metric indices composition, metrics weight and calculation. Multimetric indices were calculated with MS excel apart STAR_ICMi for which MacrOper.ICM was used

Multi-metric index	Included metrics	Metric weight	Calculation
STAR_ICMi	ASPT	0.333	MacrOper.ICM (Buffagni & Belfiore, 2013)
	N_families	0.167	
	EPT_families	0.083	
	Shannon	0.083	
	1-GOLD	0.067	
	Sel_EPTD	0.266	
$IPtI_N$	N_families	0.25	MacrOper.ICM (Buffagni & Belfiore, 2013)
	EPT_families	0.15	
	Evenness	0.10	Asterics v. 4.04 (AQEM consortium, 2004)
	IASPT	0.30	
	Sel_ETD	0.20	Template MS Excel
$IPtI_S$	N_families	0.40	MacrOper.ICM (Buffagni & Belfiore, 2013)
	EPT_families	0.20	
	IASPT	0.20	Asterics v. 4.04 (AQEM consortium, 2004)
	Sel_EPTCD	0.20	Template MS Excel
IMMi-T	N_families	0.20	MacrOper.ICM (Buffagni & Belfiore, 2013)
	EPT_families	0.20	
	IASPT	0.40	Asterics v. 4.04 (AQEM consortium, 2004)
	Sel_EPTCD	0.20	Template MS Excel

Table S3b. Additional metric calculation (not included in multimetric index)

	Metric	Calculation
	Polyvoltine Ovoviviparous	Template MS Excel (scores from Corneil, 2016)
	HES AHES	Template MS Excel (scores from Lazaridou et al., 2018)
	IBMWP	Asterics v. 4.04 (AQEM consortium, 2004)

Reference

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Lazaridou, M., Ntislidou, C., Karaouzas, I., Skoulikidis, N., 2018. Harmonisation of a new assessment method for estimating the ecological quality status of Greek running waters. Ecol. Indicat. 84, 683–694.

Table S4. Shift in location (Hodges-Lehmann estimator) for the STAR_ICM index after adjusting for the share of lentic and lotic habitats present at the river site and dispersion statistics for the two options of index calculation. The STAR_ICMi original EQRs (STAR) are computed based on a single reference value (standard), while for the STAR_LRD the reference conditions change following the index response to the lentic-lotic gradient. Results are shown for reference, non-reference and pooled samples, for both pools and riffles.

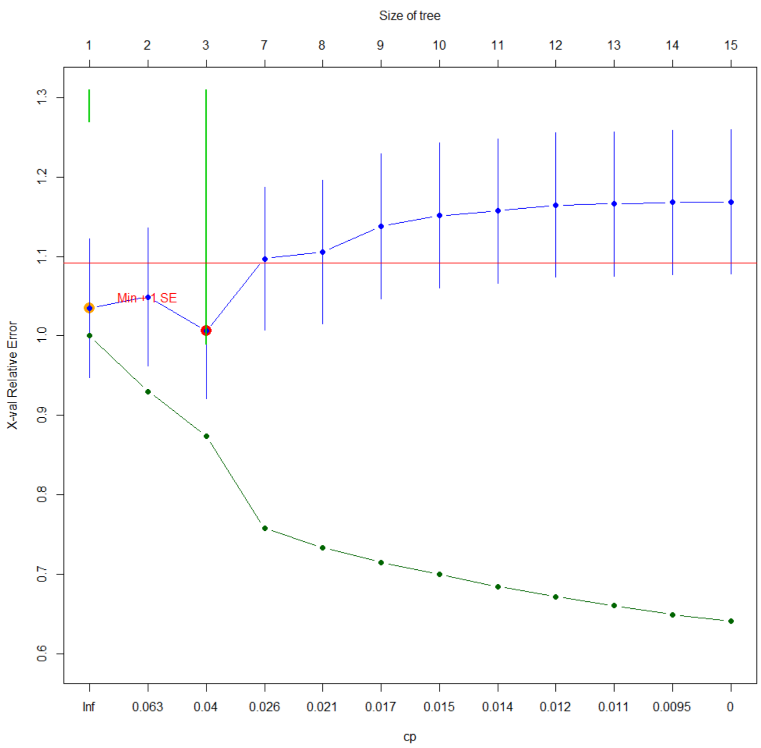
			Estimate	lwr.ci	upr.ci	Median	25%ile	Range	SD	CV
Pool	Reference	STAR				1.000	0.918	0.500	0.109	11.0
		STAR_LRD	0.054	0.053	0.055	1.041	1.005	0.432	0.105	9.9
	non-Reference	STAR				0.904	0.854	0.606	0.138	15.3
		STAR_LRD	0.048	0.047	0.050	0.967	0.886	0.603	0.132	13.8
	all	STAR				0.942	0.882	0.722	0.135	14.5
		STAR_LRD	0.054	0.053	0.055	0.997	0.904	0.603	0.131	13.2
Riffle	Reference	STAR				1.000	0.959	0.598	0.117	11.7
		STAR_LRD	0.054	0.052	0.057	1.061	1.004	0.423	0.109	10.2
	non-Reference	STAR				0.958	0.805	0.734	0.186	19.9
		STAR_LRD	0.050	0.046	0.053	0.994	0.832	0.701	0.183	18.5
	all	STAR				0.971	0.849	0.745	0.168	17.6
		STAR_LRD	0.054	0.052	0.057	1.027	0.915	0.701	0.165	16.2

The ratio of lentic to lotic habitat features strongly affects macroinvertebrate metrics in use in southern Europe for ecological status classification

Andrea Buffagni, Stefania Erba, Marcello Cazzola, Emanuele Barca, Carlo Belfiore

Supplementary Material (continued)

Figure S1. Sequential clustering (MRT) of benthic samples along the lentic-lotic gradient for the identification of potential discontinuities in invertebrate assemblages. Graph of the relative error RE (dark green) and the cross-validated relative error CVRE (blue, with error bars). The red point indicates the solution with the smallest CVRE (3 groups). The green vertical bars reveal the number of times that the solution was selected as the best one during the cross-validation process.



		Pool mesohabitat samples			Riffle mesohabitat samples		
LRD	**site**	**STAR_ICMi**	**ns.p.1**	**ns.p.2**	**STAR_ICMi**	**ns.r.1**	**ns.r.2**
-38.82	15	0.69	0.000	0.000	0.595	0.000	0.000
-32.25	47	1.199	0.117	-0.060	1.12	0.117	-0.060
-32.05	25	0.651	0.120	-0.062	0.578	0.120	-0.062
-27.96	28	0.928	0.188	-0.095	0.973	0.188	-0.095
-27.39	20	1.01	0.197	-0.099	0.976	0.197	-0.099
-26.83	49	0.988	0.206	-0.102	1.056	0.206	-0.102
-23.92	41	1.034	0.249	-0.120	0.95	0.249	-0.120
-23.5	18	1.147	0.255	-0.123	1.031	0.255	-0.123
-23.49	29	0.989	0.255	-0.123	0.801	0.255	-0.123
-21.43	26	0.778	0.283	-0.133	0.685	0.283	-0.133
-20.38	19	1.154	0.297	-0.138	1.117	0.297	-0.138
-15.94	43	1.191	0.351	-0.153	0.93	0.351	-0.153
-12.54	48	1.103	0.387	-0.159	0.989	0.387	-0.159
-12.25	52	1.19	0.390	-0.159	1.026	0.390	-0.159
-11.98	16	1.085	0.393	-0.160	1.005	0.393	-0.160
-10.21	55	0.967	0.409	-0.161	0.983	0.409	-0.161
-9.79	17	0.811	0.413	-0.161	0.714	0.413	-0.161
-9.5	11	1.026	0.416	-0.161	0.722	0.416	-0.161
-8.94	57	1.059	0.421	-0.161	1.169	0.421	-0.161
-6.57	32	0.922	0.441	-0.160	0.955	0.441	-0.160
-6.5	5	1.032	0.441	-0.160	0.954	0.441	-0.160
-1.7	4	1.031	0.476	-0.152	0.942	0.476	-0.152
-0.87	27	1.341	0.481	-0.150	1.246	0.481	-0.150
-0.35	2	0.968	0.485	-0.149	0.942	0.485	-0.149
0	3	1.139	0.487	-0.148	1.087	0.487	-0.148
0.04	53	1.057	0.487	-0.148	0.799	0.487	-0.148
0.1	6	1.108	0.487	-0.147	0.897	0.487	-0.147
0.71	40	1.161	0.491	-0.146	0.947	0.491	-0.146
2.33	46	1.123	0.500	-0.140	1.018	0.500	-0.140
3.49	7	0.967	0.506	-0.136	0.941	0.506	-0.136
4.5	24	1.029	0.511	-0.131	0.827	0.511	-0.131
8.37	39	1.073	0.526	-0.112	1.009	0.526	-0.112
8.51	23	1.119	0.527	-0.112	1.048	0.527	-0.112
8.75	56	1.058	0.528	-0.110	1.06	0.528	-0.110
10.25	13	0.981	0.532	-0.102	0.797	0.532	-0.102
11.25	1	1.214	0.535	-0.096	1.151	0.535	-0.096
11.48	38	1.239	0.536	-0.094	0.978	0.536	-0.094
12.5	45	0.908	0.538	-0.088	0.979	0.538	-0.088
13.56	54	1.135	0.541	-0.080	0.842	0.541	-0.080
14.25	14	0.964	0.542	-0.076	0.659	0.542	-0.076
18.39	34	0.979	0.548	-0.044	0.901	0.548	-0.044
21.44	10	1.068	0.550	-0.018	0.886	0.550	-0.018
23.25	44	0.947	0.550	-0.002	0.927	0.550	-0.002
25.34	42	1.114	0.549	0.018	1.048	0.549	0.018
25.99	51	1.166	0.549	0.024	1.235	0.549	0.024
27.85	58	1.152	0.547	0.043	1.196	0.547	0.043
29.29	59	0.982	0.545	0.058	1.047	0.545	0.058
35.5	31	1.048	0.532	0.127	1.108	0.532	0.127
37	50	0.905	0.528	0.145	1.182	0.528	0.145
38.5	30	0.76	0.524	0.163	0.738	0.524	0.163
45.83	22	1.129	0.496	0.258	0.986	0.496	0.258
46.56	37	1.01	0.493	0.268	0.952	0.493	0.268
49.75	21	0.794	0.478	0.312	0.914	0.478	0.312
53.14	8	1.089	0.461	0.360	0.996	0.461	0.360
59.5	12	0.949	0.426	0.454	0.749	0.426	0.454
64.5	33	0.989	0.395	0.531	1.023	0.395	0.531
69	36	0.873	0.366	0.601	0.786	0.366	0.601
80.34	9	0.551	0.288	0.784	0.513	0.288	0.784
85.5	35	0.976	0.252	0.868	0.658	0.252	0.868
Function coefficients		(Intercept)	K)1	K)2	(Intercept)	K)1	K)2
		0.872	0.344	-0.217	0.777	0.380	-0.191
	Pr(>|t|)	< 2e-16	0.011	0.004	<2e-16	0.015	0.026
	overall:	knot: -27.68			knot: -27.68		
		F-statistic: 8.712 on 2 and 56 DF			F-statistic: 6.293 on 2 and 56 DF		
		p-value: 0.0005081			p-value: 0.003424		

Table S4. LRD values for the studied sites and natural splines values used in piecewise regressions, for pools and riffles. STAR_ICM index values and statistics of the final models selected are also reported.

Figure S2. Summary statistics of the selected models and plots

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

POOL mesohabitat

ITALY

STAR_ICMi

knots = -27.68

```
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                0.87187    0.05775  15.098  < 2e-16 ***
ns(LRD, knot = K.2)1       0.34393    0.13011   2.643  0.01062 *
ns(LRD, knot = K.2)2      -0.21697    0.07200  -3.013  0.00388 **
Residual standard error: 0.1308 on 56 degrees of freedom
Multiple R-squared:  0.2373,  Adjusted R-squared:  0.2101
F-statistic: 8.712 on 2 and 56 DF,  p-value: 0.0005081
```

nTAXAt

knots = 24.3

```
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                 4.7749     0.1606  29.732   <2e-16 ***
ns(LRD, knot = K.1)1        1.1141     0.3711   3.003   0.0040 **
ns(LRD, knot = K.1)2       -0.4627     0.2640  -1.752   0.0852 .
Residual standard error: 0.4322 on 56 degrees of freedom
Multiple R-squared:  0.1879,  Adjusted R-squared:  0.1589
F-statistic: 6.478 on 2 and 56 DF,  p-value: 0.002947
```

EPTt

```
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)        3.3661     0.1073  31.373  < 2e-16 ***
ns(LRD, df = 1)   -1.3500     0.2964  -4.555 2.81e-05 ***
Residual standard error: 0.4271 on 57 degrees of freedom
Multiple R-squared:  0.2668,  Adjusted R-squared:  0.254
F-statistic: 20.74 on 1 and 57 DF,  p-value: 2.813e-05
```

selEPTD [ns]

1-GOLDt

knot= 24.3

```
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                0.75948    0.09096   8.349 2.06e-11 ***
ns(LRD, knot = K.1)1       0.40539    0.21017   1.929   0.0588 .  [Pr(>F) 0.05882 .]
ns(LRD, knot = K.1)2      -0.32513    0.14955  -2.174   0.0339 *
Residual standard error: 0.2448 on 56 degrees of freedom
Multiple R-squared:  0.1402,  Adjusted R-squared:  0.1095
F-statistic: 4.566 on 2 and 56 DF,  p-value: 0.01455
```

ASPT

knot= 24.3

```
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                 6.1014     0.1397  43.671  < 2e-16 ***
ns(LRD, knot = K.1)1       -0.6229     0.3228  -1.930  0.05874 .  [Pr(>F) 0.05874 .]
ns(LRD, knot = K.1)2       -0.7834     0.2297  -3.411  0.00121 **
Residual standard error: 0.376 on 56 degrees of freedom
Multiple R-squared:  0.205,   Adjusted R-squared:  0.1766
F-statistic: 7.222 on 2 and 56 DF,  p-value: 0.001621
```

SHANNON

```
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)        2.1983     0.1112   19.77  < 2e-16 ***
ns(LRD, df = 1)   -0.8757     0.3072   -2.85  0.00607 **
Residual standard error: 0.4427 on 57 degrees of freedom
```

Multiple R-squared: 0.1248, Adjusted R-squared: 0.1094
F-statistic: 8.125 on 1 and 57 DF, p-value: 0.006067

GREECE

HES

knots = c(`50%` = LRD 3.49)

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	33.986	1.656	20.528	<2e-16	***
ns(LRD, df = 2)1	7.763	3.712	2.091	0.0411	*
ns(LRD, df = 2)2	-4.799	2.390	-2.008	0.0494	*

Residual standard error: 4.186 on 56 degrees of freedom
Multiple R-squared: 0.143, Adjusted R-squared: 0.1124
F-statistic: 4.671 on 2 and 56 DF, p-value: 0.0133

AHES

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	55.396	1.096	50.539	< 2e-16	***
ns(LRD, df = 1)	-10.273	3.028	-3.392	0.00127	**

Residual standard error: 4.364 on 57 degrees of freedom
Multiple R-squared: 0.168, Adjusted R-squared: 0.1534
F-statistic: 11.51 on 1 and 57 DF, p-value: 0.001265

SPAIN

IBMWP

knots = -27.68

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	10.1464	0.5857	17.324	<2e-16	***
ns(LRD, knot = K.2)1	3.3160	1.3196	2.513	0.0149	*
ns(LRD, knot = K.2)2	-1.5075	0.7303	-2.064	0.0436	*

Residual standard error: 1.327 on 56 degrees of freedom
Multiple R-squared: 0.1699, Adjusted R-squared: 0.1402
F-statistic: 5.73 on 2 and 56 DF, p-value: 0.005445

IASPT

knots = 24.3

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	5.4399	0.1689	32.213	< 2e-16	***
ns(LRD, knot = K.1)1	-0.8565	0.3902	-2.195	0.032312	*
ns(LRD, knot = K.1)2	-1.0192	0.2776	-3.671	0.000541	***

Residual standard error: 0.4545 on 56 degrees of freedom
Multiple R-squared: 0.2347, Adjusted R-squared: 0.2074
F-statistic: 8.587 on 2 and 56 DF, p-value: 0.0005589

E_sel_EPTCD

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	2.2082	0.1807	12.221	<2e-16	***
ns(LRD, df = 1)	-1.0577	0.4992	-2.119	0.0385	*

Residual standard error: 0.7193 on 57 degrees of freedom
Multiple R-squared: 0.07302, Adjusted R-squared: 0.05675
F-statistic: 4.49 on 1 and 57 DF, p-value: 0.03847

E_IMMiT

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.04803	0.04015	26.104	< 2e-16	***
ns(LRD, df = 1)	-0.37470	0.11091	-3.378	0.00132	**

Residual standard error: 0.1598 on 57 degrees of freedom
Multiple R-squared: 0.1668, Adjusted R-squared: 0.1522
F-statistic: 11.41 on 1 and 57 DF, p-value: 0.001321

PORTUGAL

P_IPtIn
With knots = -27.68

```
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                0.64501    0.05041  12.796  < 2e-16 ***
ns(LRD, knot = K.2)1       0.18455    0.11357   1.625  0.10978
ns(LRD, knot = K.2)2      -0.19552    0.06285  -3.111  0.00293 **
```

Residual standard error: 0.1142 on 56 degrees of freedom
Multiple R-squared: 0.1907, Adjusted R-squared: 0.1617
F-statistic: 6.596 on 2 and 56 DF, p-value: 0.002678

P_IPtIs
knots = -27.68

```
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                0.81882    0.06657  12.300  < 2e-16 ***
ns(LRD, knot = K.2)1       0.31110    0.14999   2.074 0.042669 *
ns(LRD, knot = K.2)2      -0.33646    0.08300  -4.054 0.000157 ***
```

Residual standard error: 0.1508 on 56 degrees of freedom
Multiple R-squared: 0.2838, Adjusted R-squared: 0.2582
F-statistic: 11.09 on 2 and 56 DF, p-value: 8.739e-05

P_sel_ETD [ns]

P_Even

```
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)        0.67680    0.03317  20.404  < 2e-16 ***
ns(LRD, df = 1)   -0.30025    0.09163  -3.277  0.00179 **
```

Residual standard error: 0.1321 on 57 degrees of freedom
Multiple R-squared: 0.1585, Adjusted R-squared: 0.1437
F-statistic: 10.74 on 1 and 57 DF, p-value: 0.001791

FRANCE

POLYV [ns]

```
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)      0.82056    0.03871  21.200   <2e-16 ***
ns(LRD, df = 1)  0.18062    0.10693   1.689   0.0966 .
```

Residual standard error: 0.1541 on 57 degrees of freedom
Multiple R-squared: 0.04767, Adjusted R-squared: 0.03097
F-statistic: 2.853 on 1 and 57 DF, p-value: 0.09664

OVOVI

```
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)      0.10518    0.02186   4.812 1.14e-05 ***
ns(LRD, df = 1)  0.26177    0.06039   4.335 6.00e-05 ***
```

Residual standard error: 0.08702 on 57 degrees of freedom
Multiple R-squared: 0.2479, Adjusted R-squared: 0.2347
F-statistic: 18.79 on 1 and 57 DF, p-value: 6e-05

RIFFLE mesohabitat

ITALY

STAR
knots = -27.68

```
                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                0.77697    0.06689  11.615   <2e-16 ***
ns(LRD, knot = K.2)1       0.37959    0.15071   2.519   0.0147 *
ns(LRD, knot = K.2)2      -0.19129    0.08340  -2.294   0.0256 *
```

Residual standard error: 0.1516 on 56 degrees of freedom
Multiple R-squared: 0.1835, Adjusted R-squared: 0.1544
F-statistic: 6.293 on 2 and 56 DF, p-value: 0.003424

NTAX
knots = 24.3

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	4.7165	0.1997	23.620	<2e-16	***
ns(LRD, knot = K.1)1	0.9649	0.4614	2.091	0.041	*
ns(LRD, knot = K.1)2	-0.4589	0.3283	-1.398	0.168	

Residual standard error: 0.5374 on 56 degrees of freedom
Multiple R-squared: 0.1084, Adjusted R-squared: 0.07656
F-statistic: 3.404 on 2 and 56 DF, p-value: 0.04024

NEPT

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	3.4240	0.1284	26.67	<2e-16	***
ns(LRD, df = 1)	-0.8938	0.3547	-2.52	0.0146	*

Residual standard error: 0.5112 on 57 degrees of freedom
Multiple R-squared: 0.1002, Adjusted R-squared: 0.08443
F-statistic: 6.348 on 1 and 57 DF, p-value: 0.01458

ASPT

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	6.3220	0.1102	57.35	< 2e-16	***
ns(LRD, df = 1)	-1.0171	0.3045	-3.34	0.00148	**

Residual standard error: 0.4389 on 57 degrees of freedom
Multiple R-squared: 0.1637, Adjusted R-squared: 0.149
F-statistic: 11.15 on 1 and 57 DF, p-value: 0.001483

1-GOLD [ns]

SelEPTD
knots = 24.3

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.2342	0.2145	5.754	3.81e-07	***
ns(LRD, knot = K.1)1	1.1108	0.4956	2.241	0.0290	*
ns(LRD, knot = K.1)2	-0.6087	0.3527	-1.726	0.0899	.

Residual standard error: 0.5773 on 56 degrees of freedom
Multiple R-squared: 0.1336, Adjusted R-squared: 0.1027
F-statistic: 4.318 on 2 and 56 DF, p-value: 0.01803

SHAN

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.9407	0.1325	14.651	<2e-16	***
ns(LRD, df = 1)	-0.6268	0.3659	-1.713	0.0922	.

Residual standard error: 0.5274 on 57 degrees of freedom
Multiple R-squared: 0.04895, Adjusted R-squared: 0.03226
F-statistic: 2.934 on 1 and 57 DF, p-value: 0.09219

GREECE

HES
knots = -27.68

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	33.422	2.172	15.389	<2e-16	***
ns(LRD, knot = K.2)1	9.969	4.893	2.037	0.0464	*
ns(LRD, knot = K.2)2	-4.525	2.708	-1.671	0.1000	.

Residual standard error: 4.921 on 56 degrees of freedom
Multiple R-squared: 0.1184, Adjusted R-squared: 0.08694
F-statistic: 3.761 on 2 and 56 DF, p-value: 0.02933

AHES

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	58.447	1.294	45.154	< 2e-16	***
ns(LRD, df = 1)	-12.832	3.576	-3.589	0.000692	***

Residual standard error: 5.153 on 57 degrees of freedom
Multiple R-squared: 0.1843, Adjusted R-squared: 0.17
F-statistic: 12.88 on 1 and 57 DF, p-value: 0.0006919

SPAIN

E_IMMiT

knots = -27.68

Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.79471 0.06236 12.743 < 2e-16 ***
ns(LRD, knot = K.2)1 0.33538 0.14051 2.387 0.020391 *
ns(LRD, knot = K.2)2 -0.32411 0.07776 -4.168 0.000107 ***
Residual standard error: 0.1413 on 56 degrees of freedom
Multiple R-squared: 0.3068, Adjusted R-squared: 0.282
F-statistic: 12.39 on 2 and 56 DF, p-value: 3.503e-05

E_IBMWP

knots = -27.68

Estimate Std. Error t value Pr(>|t|)
(Intercept) 10.1812 0.6733 15.122 <2e-16 ***
ns(LRD, knot = K.2)1 3.1856 1.5168 2.100 0.0402 *
ns(LRD, knot = K.2)2 -1.5309 0.8394 -1.824 0.0735 .
Residual standard error: 1.525 on 56 degrees of freedom
Multiple R-squared: 0.1303, Adjusted R-squared: 0.09921
F-statistic: 4.194 on 2 and 56 DF, p-value: 0.02008

E_IASPT

Estimate Std. Error t value Pr(>|t|)
(Intercept) 5.7876 0.1232 46.98 < 2e-16 ***
ns(LRD, df = 1) -1.1945 0.3403 -3.51 0.000883 ***
Residual standard error: 0.4904 on 57 degrees of freedom
Multiple R-squared: 0.1777, Adjusted R-squared: 0.1633
F-statistic: 12.32 on 1 and 57 DF, p-value: 0.0008835

E_sel_EPTCD

knots = -27.68

Estimate Std. Error t value Pr(>|t|)
(Intercept) 1.1461 0.2506 4.573 2.71e-05 ***
ns(LRD, knot = K.2)1 1.8291 0.5647 3.239 0.00202 **
ns(LRD, knot = K.2)2 -0.8855 0.3125 -2.833 0.00639 **
Residual standard error: 0.5679 on 56 degrees of freedom
Multiple R-squared: 0.264, Adjusted R-squared: 0.2377
F-statistic: 10.04 on 2 and 56 DF, p-value: 0.0001876

PORTUGAL

P_IPtIn

Knot -27.68

Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.63804 0.06155 10.365 1.23e-14 ***
ns(LRD, knot = K.2)1 0.22633 0.13868 1.632 0.1083
ns(LRD, knot = K.2)2 -0.19484 0.07675 -2.539 0.0139 *
Residual standard error: 0.1395 on 56 degrees of freedom
Multiple R-squared: 0.1492, Adjusted R-squared: 0.1188
F-statistic: 4.909 on 2 and 56 DF, p-value: 0.01086

P_sel_ETD [ns]

P_Even

Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.59193 0.03857 15.347 <2e-16 ***
ns(LRD, df = 1) -0.21600 0.10655 -2.027 0.0473 *
Residual standard error: 0.1536 on 57 degrees of freedom
Multiple R-squared: 0.06725, Adjusted R-squared: 0.05088
F-statistic: 4.109 on 1 and 57 DF, p-value: 0.04733

P_IPtIs

knots = -27.68

Estimate Std. Error t value Pr(>|t|)

(Intercept) 0.80501 0.07299 11.030 1.16e-15 ***
ns(LRD, knot = K.2)1 0.40065 0.16443 2.437 0.01803 *
ns(LRD, knot = K.2)2 -0.29376 0.09100 -3.228 0.00208 **
Residual standard error: 0.1654 on 56 degrees of freedom
Multiple R-squared: 0.2401, Adjusted R-squared: 0.213
F-statistic: 8.849 on 2 and 56 DF, p-value: 0.0004575

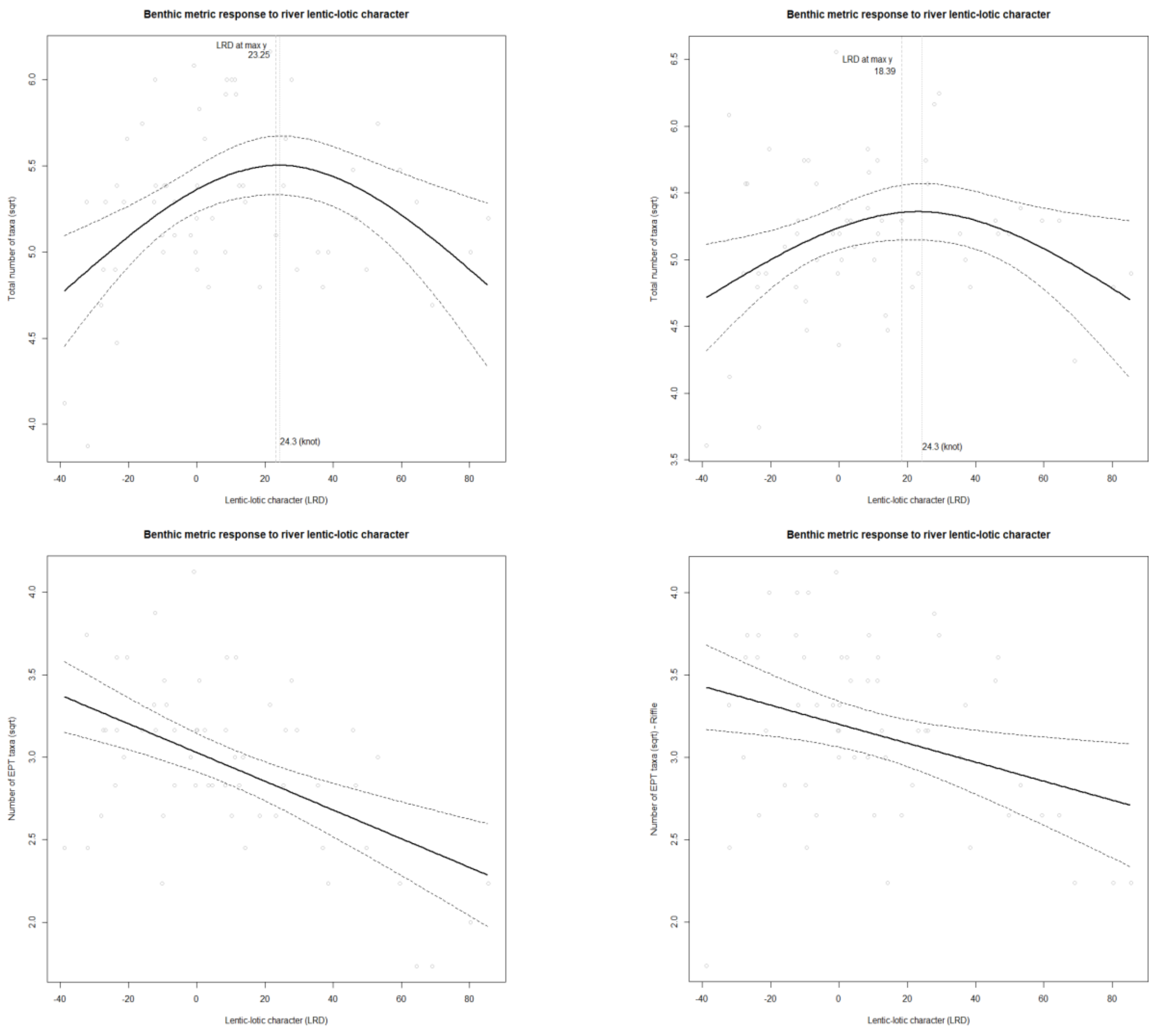
FRANCE

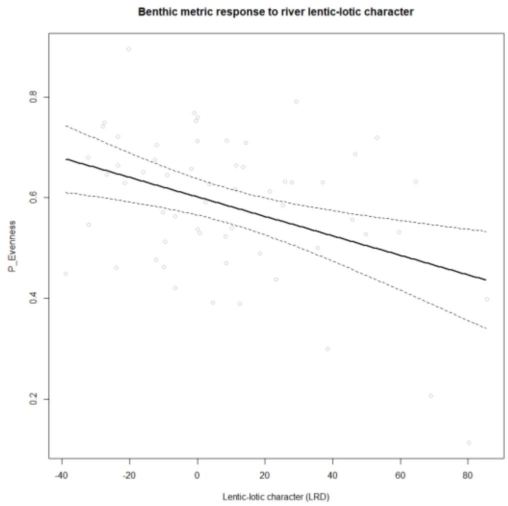
POLYV [ns]
OVOVI

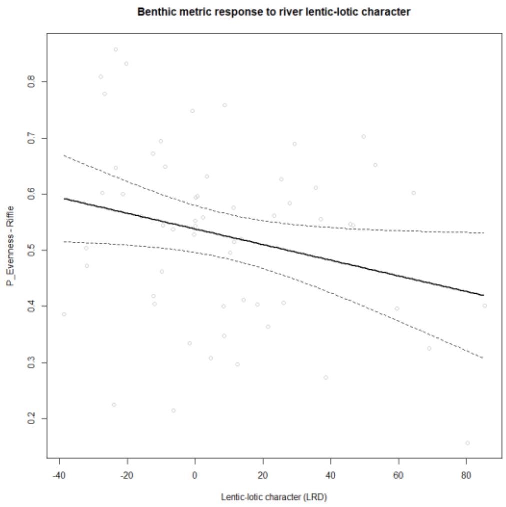
	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.07301	0.02379	3.069	0.00328	**
ns(LRD, df = 1)	0.19739	0.06572	3.004	0.00396	**

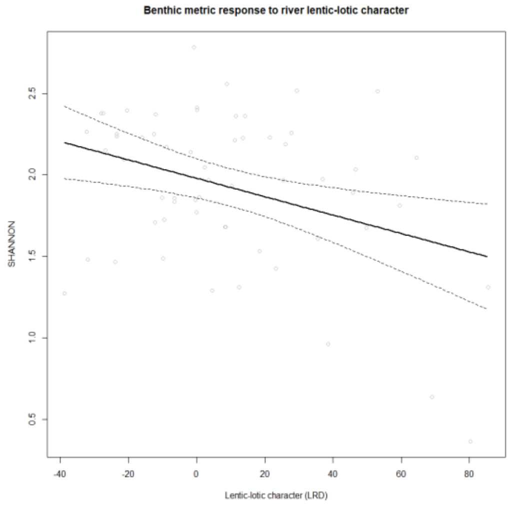
Residual standard error: 0.0947 on 57 degrees of freedom
Multiple R-squared: 0.1367, Adjusted R-squared: 0.1215
F-statistic: 9.022 on 1 and 57 DF, p-value: 0.003957

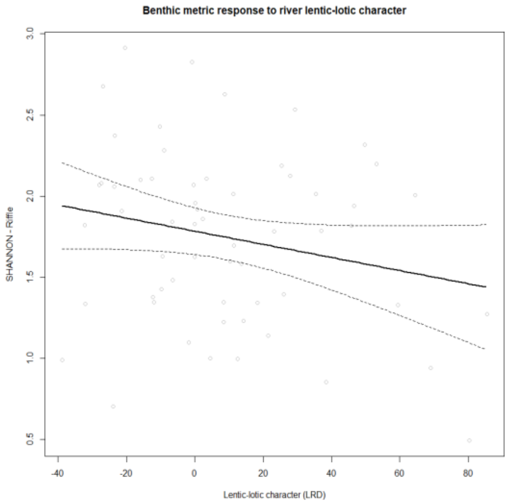
Pool Riffle

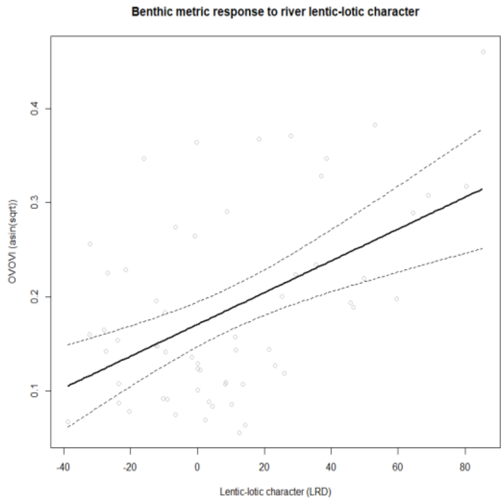


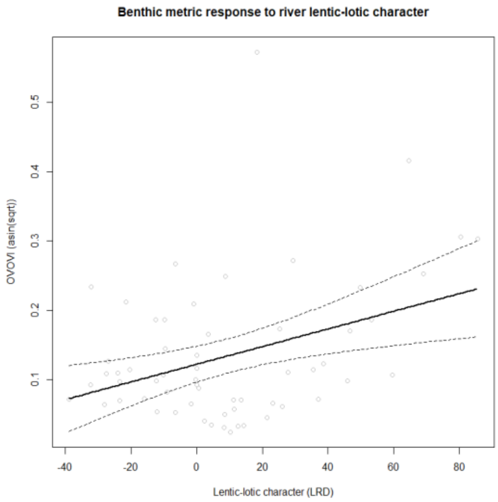

Benthic metric response to river lentic-lotic character
P_Evenness
Lentic-lotic character (LRD)

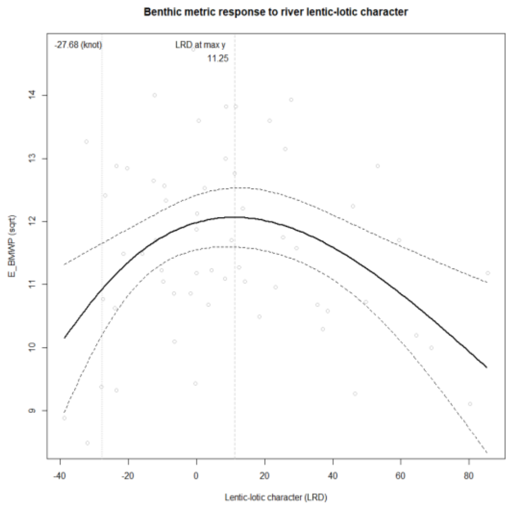

Benthic metric response to river lentic-lotic character
P_Evenness - Riffle
Lentic-lotic character (LRD)

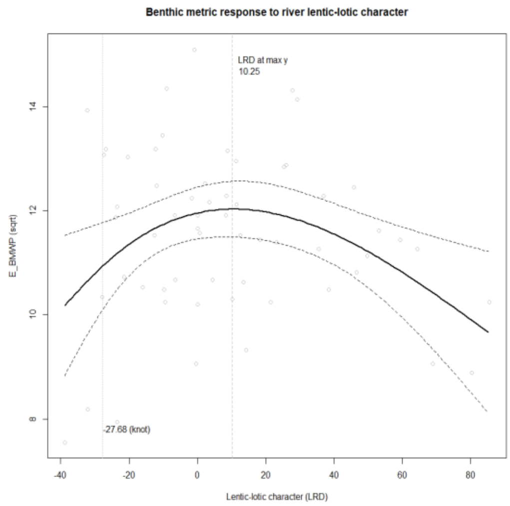

Benthic metric response to river lentic-lotic character
SHANNON
Lentic-lotic character (LRD)

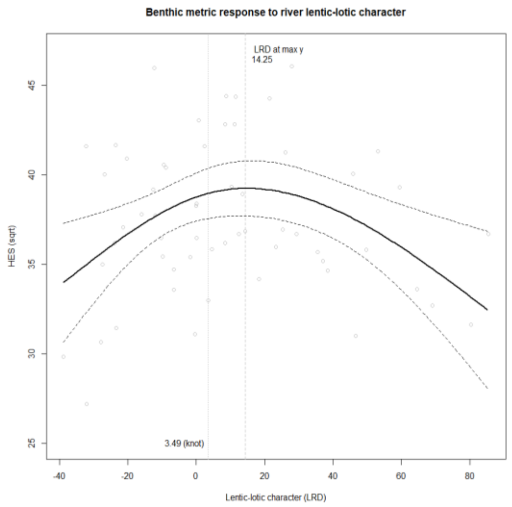

Benthic metric response to river lentic-lotic character
SHANNON - Riffle
Lentic-lotic character (LRD)

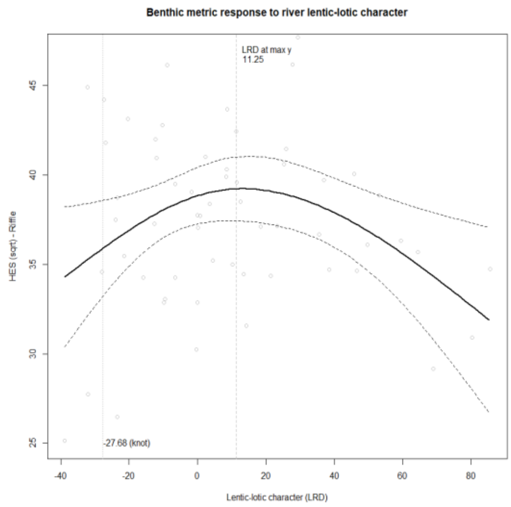

Benthic metric response to river lentic-lotic character
OVOVI (asin(sqrt))
Lentic-lotic character (LRD)

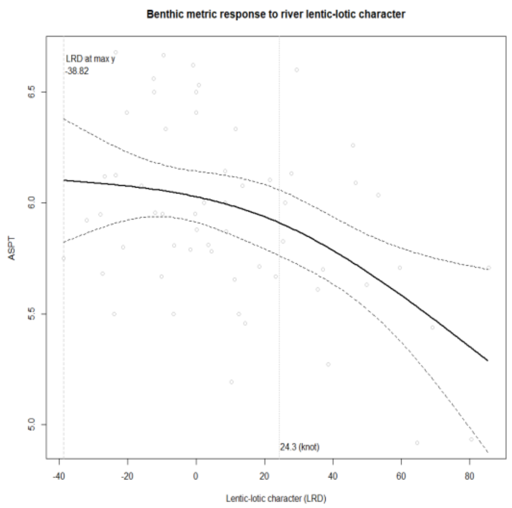

Benthic metric response to river lentic-lotic character
OVOVI (asin(sqrt))
Lentic-lotic character (LRD)

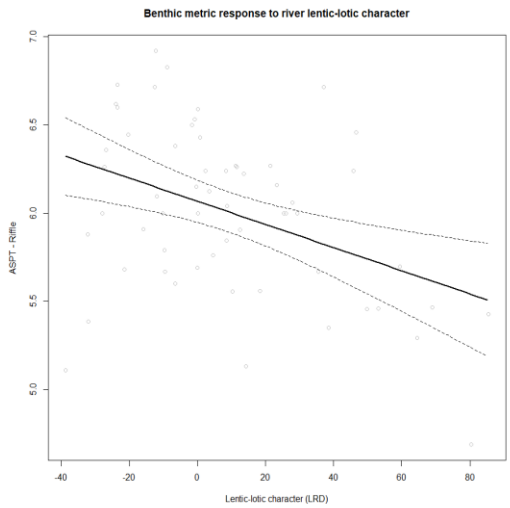

Benthic metric response to river lentic-lotic character
-27.68 (knot)
LRD at max y
11.25
E_BMWP (sqrt)
Lentic-lotic character (LRD)

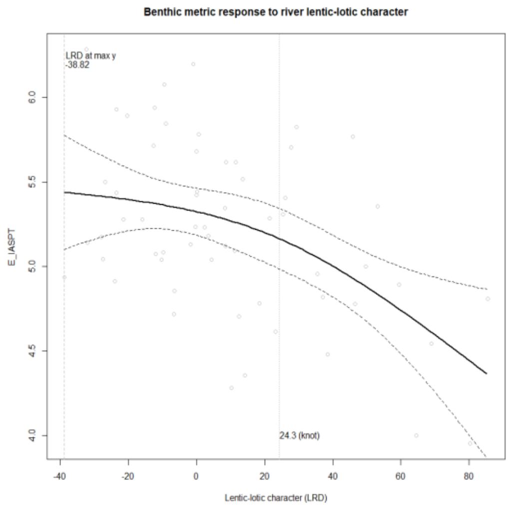

Benthic metric response to river lentic-lotic character
LRD at max y
10.25
-27.68 (knot)
E_BMWP (sqrt)
Lentic-lotic character (LRD)

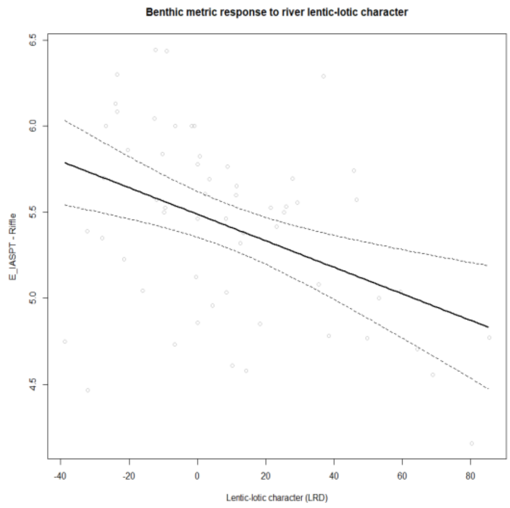

Benthic metric response to river lentic-lotic character
LRD at max y
14.25
3.49 (knot)
HES (sqrt)
Lentic-lotic character (LRD)

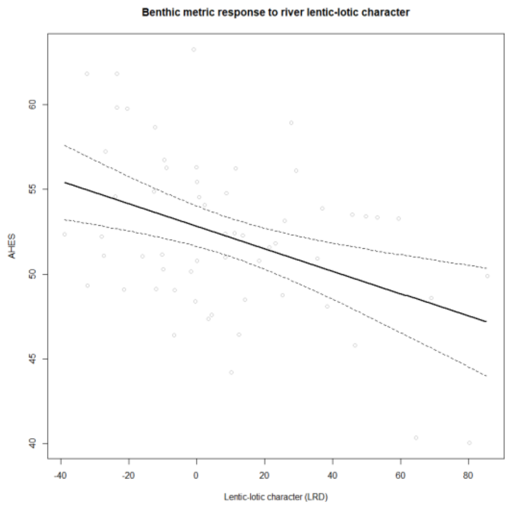

Benthic metric response to river lentic-lotic character
LRD at max y
11.25
-27.68 (knot)
HES (sqrt) - Riffle
Lentic-lotic character (LRD)

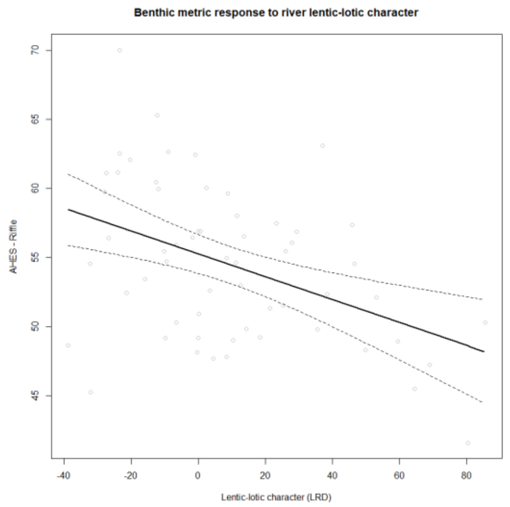

Benthic metric response to river lentic-lotic character
LRD at max y
-38.82
24.3 (knot)
ASPT
Lentic-lotic character (LRD)

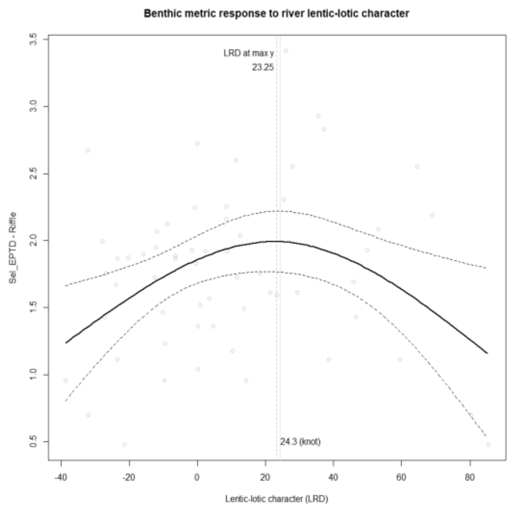

Benthic metric response to river lentic-lotic character
ASPT - Riffle
Lentic-lotic character (LRD)

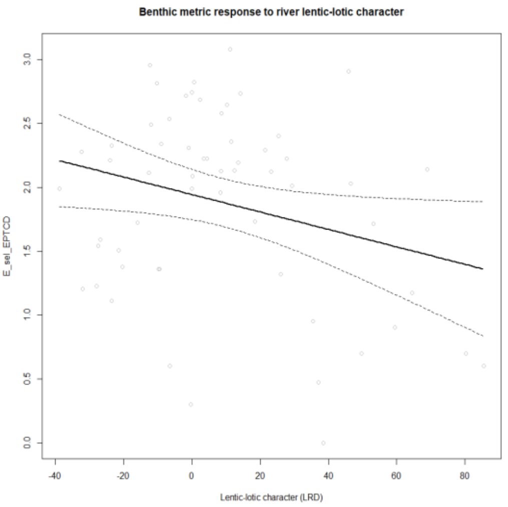

Benthic metric response to river lentic-lotic character
LRD at max y
-38.82
24.3 (knot)
E_IASPT
Lentic-lotic character (LRD)

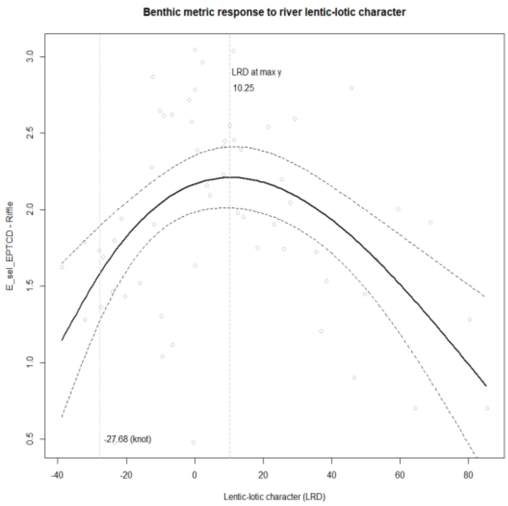

Benthic metric response to river lentic-lotic character
E_IASPT - Riffle
Lentic-lotic character (LRD)

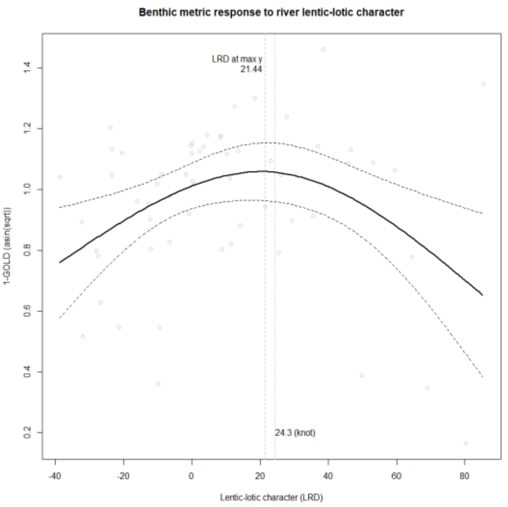

Benthic metric response to river lentic-lotic character
AHES
Lentic-lotic character (LRD)

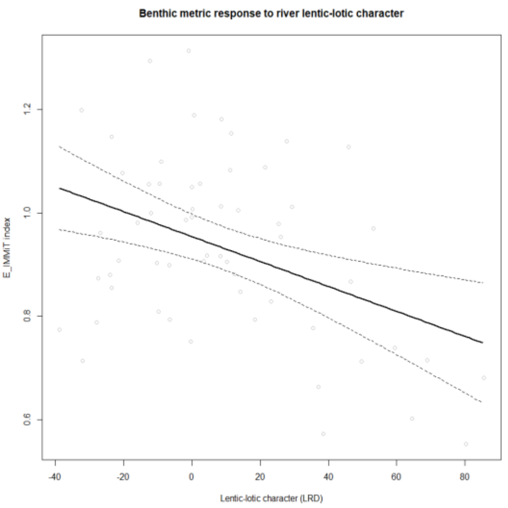

Benthic metric response to river lentic-lotic character
AHES - Riffle
Lentic-lotic character (LRD)

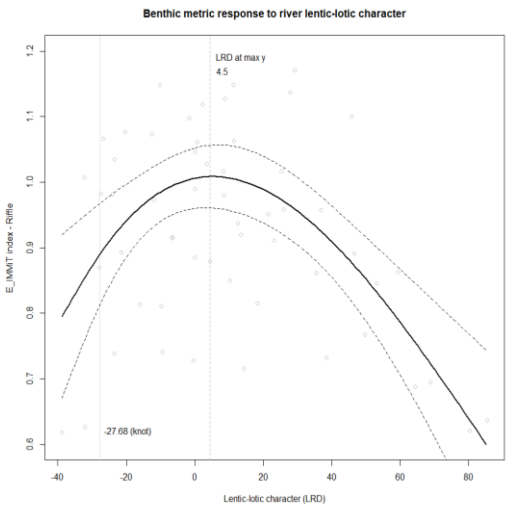

Benthic metric response to river lentic-lotic character
LRD at max y
23.25
24.3 (knot)
Sel_EPTD - Riffle
Lentic-lotic character (LRD)

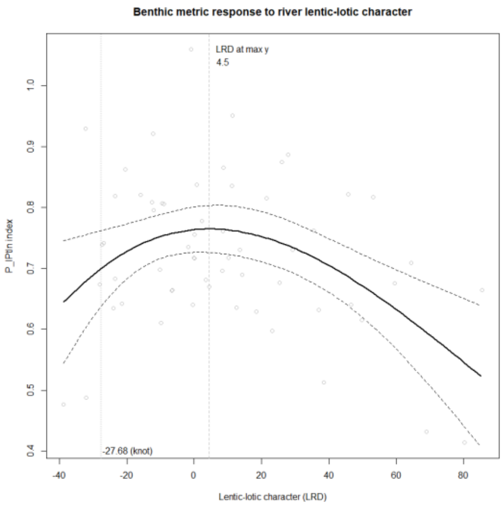

Benthic metric response to river lentic-lotic character
E_sel_EPTCD
Lentic-lotic character (LRD)

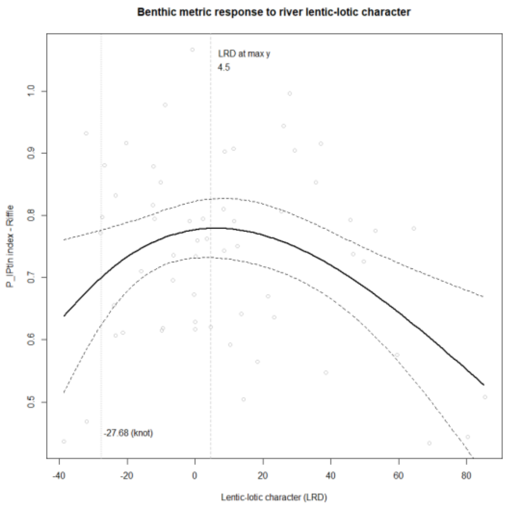

Benthic metric response to river lentic-lotic character
E_sel_EPTCD - Riffle
LRD at max y
10.25
-27.68 (knot)
Lentic-lotic character (LRD)

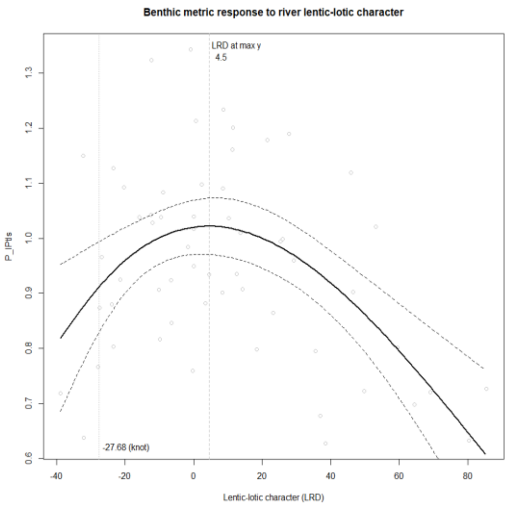

Benthic metric response to river lentic-lotic character
1-GOLD (asin(sqrt))
LRD at max y
21.44
24.3 (knot)
Lentic-lotic character (LRD)

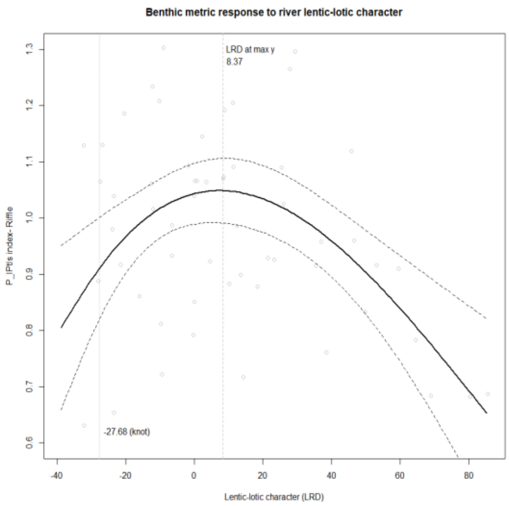

Benthic metric response to river lentic-lotic character
E_IMMIT index
Lentic-lotic character (LRD)

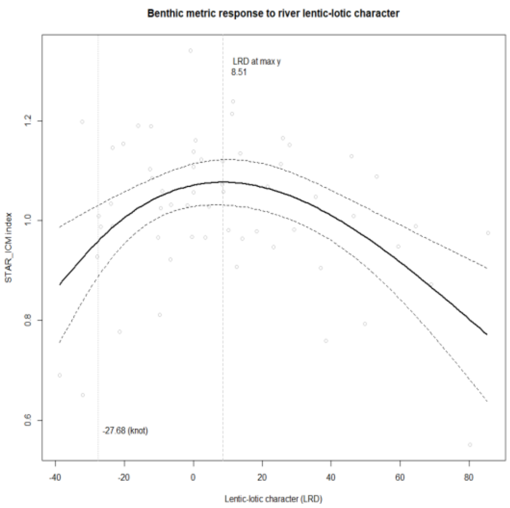

Benthic metric response to river lentic-lotic character
E_IMMIT index - Riffle
LRD at max y
4.5
-27.68 (knot)
Lentic-lotic character (LRD)

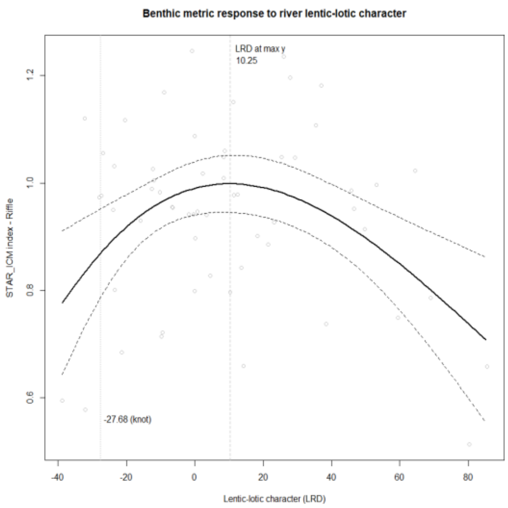

Benthic metric response to river lentic-lotic character
LRD at max y
4.5
-27.68 (knot)
P_IPtIn index
Lentic-lotic character (LRD)


Benthic metric response to river lentic-lotic character
LRD at max y
4.5
-27.68 (knot)
P_IPtIn index - Riffle
Lentic-lotic character (LRD)


Benthic metric response to river lentic-lotic character
LRD at max y
4.5
-27.68 (knot)
P_IPtIs
Lentic-lotic character (LRD)


Benthic metric response to river lentic-lotic character
LRD at max y
8.37
-27.68 (knot)
P_IPtIs index- Riffle
Lentic-lotic character (LRD)


Benthic metric response to river lentic-lotic character
LRD at max y
8.51
-27.68 (knot)
STAR_ICM index
Lentic-lotic character (LRD)


Benthic metric response to river lentic-lotic character
LRD at max y
10.25
-27.68 (knot)
STAR_ICM index - Riffle
Lentic-lotic character (LRD)

Supplementary material to:

Chapter 6 (PAPER 4)

Erba S., M. Cazzola, C. Belfiore & A. Buffagni (submitted) Macroinvertebrate metrics response to morphological alteration in Italian rivers.

Macroinvertebrate metrics response to morphological alteration in Italian rivers - Hydrobiologia

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Appendix 1

Appendix 1a. Investigated rivers, sites code, geographic coordinates and indication of reference sites. Areas: Lowland - Small and Medium sized; Mediterranean Mountain - Small and Medium sized

Area		River	Site code	Site Latitude N	Site Longitude E	Ref site
Lowland	Small sized	Molinetta	pmoli	45°19'27"	08°41'29"	0
	Small sized	Barbavara	pbarb	45°20'55"	08°45'01"	0
	Small sized	Plezza	pplez	45°21'32"	08°43'36"	0
	Small sized	Gottardino	pgott	45°23'16"	08°44'11"	0
	Small sized	Unnamed (Cerano)	psdce	45°24'10"	07°49'07"	0
	Small sized	Tormo	otorm	45°24'15"	09°34'07"	0
	Small sized	Bontempa	obont	45°24'50"	09°30'02"	0
	Small sized	Bardena	obard	45°26'31"	09°48'35"	0
	Small sized	Unnamed (Fontanella)	osdfo	45°26'48"	09°48'44"	0
	Small sized	Cornice	ocorc	45°27'44"	08°48'20"	0
	Small sized	Vera	PVERA	45°28'34"	08°47'07"	1
	Small sized	Lampreda	PLAMP	45°31'27"	08°42'10"	1
	Small sized	Fontanin	PFONT	45°32'34"	08°42'50"	1
	Small sized	Della Rovere	PDROV	45°33'40"	08°41'17"	1
	Small sized	Oriale	poria	45°33'48"	08°41'49"	0
	Medium sized	Radicosa	GRACN	41°20'05"	15°12'19"	1
	Medium sized	Radicosa	GRACF	41°20'09"	15°12'00"	1
	Medium sized	Celone	GCECA	41°20'22"	15°11'28"	1
	Medium sized	Celone	gcetr	41°22'23"	15°16'25"	0
	Medium sized	Celone	gcecf	41°24'13"	15°21'53"	0
	Medium sized	Celone	gcecn	41°24'16"	15°22'07"	0
	Medium sized	San Lorenzo	gloca	41°24'22"	15°19'35"	0
	Medium sized	San Lorenzo	glore	41°24'44"	15°21'42"	0
	Medium sized	Vulgano	gvupu	41°28'58"	15°27'04"	0
	Medium sized	Casanova	gcacf	41°29'48"	15°15'21"	0
	Medium sized	Casanova	gcacn	41°29'56"	15°15'26"	0
	Medium sized	Vulgano tributary	gvuvi	41°31'41"	15°27'36"	0
	Medium sized	Candelaro	gcaca	41°33'07"	15°47'34"	0
	Medium sized	Candelaro	gcama	41°40'00"	15°33'09"	0
	Medium sized	Candelaro	gcasa	41°40'33"	15°32'19"	0
	Medium sized	Triolo	gtrco	41°35'58"	15°25'20"	0
	Medium sized	Salsola	gsade	41°36'19"	15°36'34"	0
	Medium sized	Santa Maria	gsali	41°38'25"	15°20'13"	0
Mediterranean Mountain	Small sized	Argentino	CAROR	39°47'41"	15°55'20"	1
	Small sized	Tanagro	NTARE	40°11'55"	15°43'29"	1
	Small sized	Tanagro	ntaas	40°20'00"	15°36'54"	0
	Small sized	Torno	NTORE	40°17'32"	15°18'58"	1
	Small sized	Torno	ntova	40°17'52"	15°18'23"	0
	Small sized	Calore	ncala	40°20'16"	15°19'49"	0
	Small sized	Zi' Francesca	nziup	40°20'41"	15°33'56"	0
	Small sized	Sammaro	NSARE	40°23'06"	15°21'30"	1
	Medium sized	Taro	ETARE	44°28'03"	09°34'49"	1
	Medium sized	Taro	etadi	44°28'41"	09°44'23"	0
	Medium sized	Taro	etabo	44°29'16"	09°46'18"	0
	Medium sized	Taro	etafs	44°29'40"	09°47'10"	0
	Medium sized	Ceno	ececa	44°38'46"	09°47'46"	0
	Medium sized	Ceno	ECER2	44°67'03"	09°43'23"	1
	Medium sized	Nure	enufa	44°42'48"	09°34'14"	0
	Medium sized	Trebbia	ETRRE	44°44'19"	09°23'11"	1
	Medium sized	Trebbia	etrbo	44°45'24"	09°23'04"	0
	Medium sized	Trebbia	etrpe	44°49'20"	09°30'02"	0

Appendix 2 - Spearman rank correlation results among biological metrics and abiotic impairment descriptors including the first two PCA components (AX1 and AX2). Correlations values are separately reported for the different river types. Only significant correlation values (p<0.05) are reported

CV: Culverts; WSB: Weir, Sluices and Bridges; AS: Artificial Straightening; ST: Artificial Stagnation; ET: Tree extent; TB: Tree related bank habitats; TC: Tree related channel habitats; bRS: Bank resectioning; bRI: Bank reinforcement; cRS: Channel resectioning; cRI: Channel reinforcement.

		ASPT	1-GOLD	Sel_EPTD	N_Families	EPT	Shannon	STAR_ICMi
Small sized lowland streams	CV							
	WSB				-0.56			
	AS				-0.53			
	ST	-0.42						
	ET	0.75	0.48	0.73		0.60		0.70
	TB	0.50		0.63		0.46		0.59
	TC	0.80		0.80	0.63	0.86		0.91
	bRS				-0.60	-0.53		-0.49
	bRI							
	cRS	-0.55		-0.49		-0.45		-0.48
	cRI							
	AX1	-0.72		-0.78	-0.48	-0.69		-0.79
	AX2							
	HMS	-0.55		-0.67	-0.60	-0.68		-0.74
	HQA	0.64		0.63	0.62	0.79		0.77
	LUIr	0.85		0.85	0.72	0.89		0.89
	LRD				0.59		0.62	
	LIMeco	0.30						
Medium sized lowland streams	CV							
	WSB			-0.50	-0.54	-0.47	-0.47	-0.54
	AS	-0.66		-0.63	-0.43	-0.67		-0.70
	ST							
	ET							
	TB	0.52		0.49		0.53		0.53
	TC	0.49	-0.47	0.57		0.56		0.50
	bRS	-0.72		-0.77	-0.71	-0.77		-0.76
	bRI							
	cRS	-0.71		-0.57	-0.64	-0.72		-0.71
	cRI							
	AX1	-0.85		-0.85	-0.69	-0.89		-0.86
	AX2							
	HMS	-0.83		-0.89	-0.79	-0.85		-0.91
	HQA	0.75	-0.50	0.78	0.56	0.75		0.74
	LUIr	0.65		0.50	0.58	0.66		0.59
	LRD	-0.68		-0.81		-0.67		-0.69
	LIMeco	0.79		0.85	0.64	0.81		0.85

		ASPT	1-GOLD	Sel_EPTD	N_Families	EPT	Shannon	STAR_ICMi
Small sized Mediterranean mountain streams	CV							
	WSB				-0.75	-0.62	-0.52	
	AS							
	ST							
	ET							
	TB				-0.57			
	TC				-0.52			
	bRS	-0.77				-0.66	-0.47	-0.63
	bRI	-0.56				-0.59	-0.58	-0.48
	cRS	-0.76				-0.60		-0.54
	cRI						-0.59	
	AX1	-0.74				-0.70	-0.51	-0.64
	AX2			0.60				
	HMS	-0.86				-0.79	-0.48	-0.73
	HQA	0.72				0.67	0.51	0.62
	LUIr	0.47		0.53		0.45		0.48
	LRD	-0.84	-0.59	-0.47		-0.74		-0.67
	LIMeco	0.68				0.69	0.54	0.61
Medium sized Mediterranean mountain streams	CV							
	WSB				-0.66	-0.53		-0.48
	AS							
	ST			-0.54		-0.45		-0.51
	ET				0.55	0.61		0.46
	TB		0.51	0.56	0.75	0.73		0.68
	TC	0.51	0.47		0.51	0.59		0.45
	bRS	-0.48	-0.65	-0.59	-0.62	-0.69		-0.72
	bRI		-0.48	-0.51		-0.47		-0.52
	cRS		-0.57	-0.52	-0.58	-0.58		-0.61
	cRI			-0.51				-0.50
	AX1	-0.46	-0.52	-0.62	-0.69	-0.72	-0.43	-0.73
	AX2							
	HMS	-0.47	-0.60	-0.59	-0.66	-0.73	-0.48	-0.74
	HQA	0.41		0.40	0.40	0.50		0.47
	LUIr	0.43						
	LRD	-0.41		-0.80	-0.62	-0.73	-0.40	-0.79
	LIMeco	0.39			0.51	0.58		0.48

		ASPT	1-GOLD	Sel_EPTD	N_Families	EPT	Shannon	STAR_ICMi
Temporary	CV							
	WSB				-0.42	-0.47	-0.37	-0.45
	AS			-0.38				-0.42
	ST		-0.31			-0.34	-0.36	-0.30
	ET	0.48		0.49	0.65	0.64	0.55	0.67
	TB						0.42	
	TC	0.43			0.35	0.47	0.33	
	bRS	-0.53		-0.70	-0.62	-0.71	-0.56	-0.83
	bRI	-0.57		-0.64	-0.55	-0.68	-0.52	-0.76
	cRS	-0.33		-0.32		-0.40	-0.37	-0.40
	cRI			-0.53	-0.43	-0.45	-0.31	-0.55
	AX1	-0.49		-0.53	-0.59	-0.68	-0.59	-0.70
	AX2			-0.38	-0.47	-0.42	-0.35	-0.48
	HMS	-0.58		-0.70	-0.59	-0.69	-0.58	-0.83
	HQA	0.46		0.35	0.46	0.55	0.58	0.49
	LUIr	0.51		0.58	0.49	0.60	0.62	0.70
	LRD	-0.32				-0.32		
	LIMeco			0.47		0.36	0.45	0.50

Appendix 1b. Investigated rivers, sites code, geographic coordinates and indication of reference sites. Area: Mediterranean Temporary

Area		River	Site code	Site Latitude N	Site Longitude E	Ref site
Mediterranean	Temporary	Solanas	rsoso	39°09'43"	09°26'53"	0
	Temporary	Corr e'Pruna	rcorv	39°17'45"	09°31'41"	0
	Temporary	Porceddu	rporc	39°19'38"	09°31'24"	0
	Temporary	Picocca	RPICO	39°21'09"	09°29'21"	1
	Temporary	Mulargia	rmudd	39°38'33"	09°11'19"	0
	Temporary	Museddu	rmuse	39°46'35"	09°38'52"	0
	Temporary	Bau Samuccu	rcamd	39°49'23"	09°39'07"	0
	Temporary	Fodeddu	rfodd	39°55'21"	09°39'41"	0
	Temporary	Su corongiu	rsuva	39°55'26"	09°38'27"	0
	Temporary	Fodeddu	rfodt	39°55'26"	09°39'28"	0
	Temporary	Foddeddu	rcofo	39°55'35"	09°38'15"	0
	Temporary	Tricarai	RMONP	39°56'29"	09°34'47"	1
	Temporary	Tricarai	RTRRE	39°57'06"	09°36'17"	1
	Temporary	Santa Lucia	rtrpo	39°57'10"	09°36'12"	0
	Temporary	Mirenu	rmicu	39°57'24"	09°36'42"	0
	Temporary	Mirenu	rmicp	39°57'25"	09°36'56"	0
	Temporary	Sa Teula	RSATE	39°57'37"	09°33'41"	1
	Temporary	Gorbini	RGORF	39°57'40"	09°32'38"	1
	Temporary	Gorbini	RGORM	39°57'43"	09°32'40"	1
	Temporary	Gorbini	RGORN	39°57'48"	09°32'38"	1
	Temporary	Girasole	rgiff	39°57'47"	09°39'16"	0
	Temporary	Girasole	rgifn	39°57'49"	09°39'06"	0
	Temporary	Campu e'Spina	rcesp	40°02'45"	09°31'01"	0
	Temporary	Flumineddu	RFLGO	40°13'00"	09°31'07"	1
	Temporary	Lorana	rlomo	40°22'58"	09°23'50"	0
	Temporary	San Giuseppe	rsgss	40°23'47"	09°28'52"	0
	Temporary	Tirso	RTIRS	40°33'35"	09°20'15"	1
	Temporary	Posada	RPOAF	40°38'40"	09°35'35"	1
	Temporary	Posada	RPOVA	40°39'11"	09°30'09"	1
	Temporary	Su Lernu	RSURU	40°43'03"	09°30'58"	1
	Temporary	Su Lernu	RSURE	40°43'11"	09°30'35"	1
	Temporary	Sud Limbara	rtmav	40°48'58"	09°14'29"	0
	Temporary	Sud Limbara	RTMRE	40°48'59"	09°14'24"	1
	Temporary	Oddastru	rodvp	41°02'54"	09°19'45"	0
	Temporary	Malchittu	rmalu	41°03'49"	09°24'18"	0
	Temporary	Baldu	rbava	41°05'34"	09°14'45"	0
	Temporary	Baldu	rbamo	41°04'53"	09°13'20"	0
	Temporary	Baldu	rbavc	41°04'54"	09°13'22"	0
	Temporary	Baldu	rbamp	41°05'26"	09°14'07"	0
	Temporary	Barrastoni	rbrvp	41°06'37"	09°13'44"	0
	Temporary	Barrastoni	rbarr	41°06'37"	09°13'44"	0
	Temporary	Cialdeniddu	rcial	41°07'02"	09°13'04"	0
	Temporary	Sperandeu	RSPER	41°08'51"	09°08'09"	1
	Temporary	Safaa	RSAAL	41°09'32"	09°10'17"	1

Supplementary material to:

Chapter 7 (PAPER 5)

Erba S., L. Terranova, M. Cazzola M. Cason, A. Buffagni (2019) Defining Maximum Ecological Potential for heavily modified lowland streams of Northern Italy. *Science of the Total Environment,* 684, 196-206.

Figure A.1 - Average percentage of different land use categories observed in the catchment respectively for reinforced MEP reaches (a), Reinforced nonMEP reaches (b) Leveed MEP reaches (c), and Leeved nonMEP reaches (d).

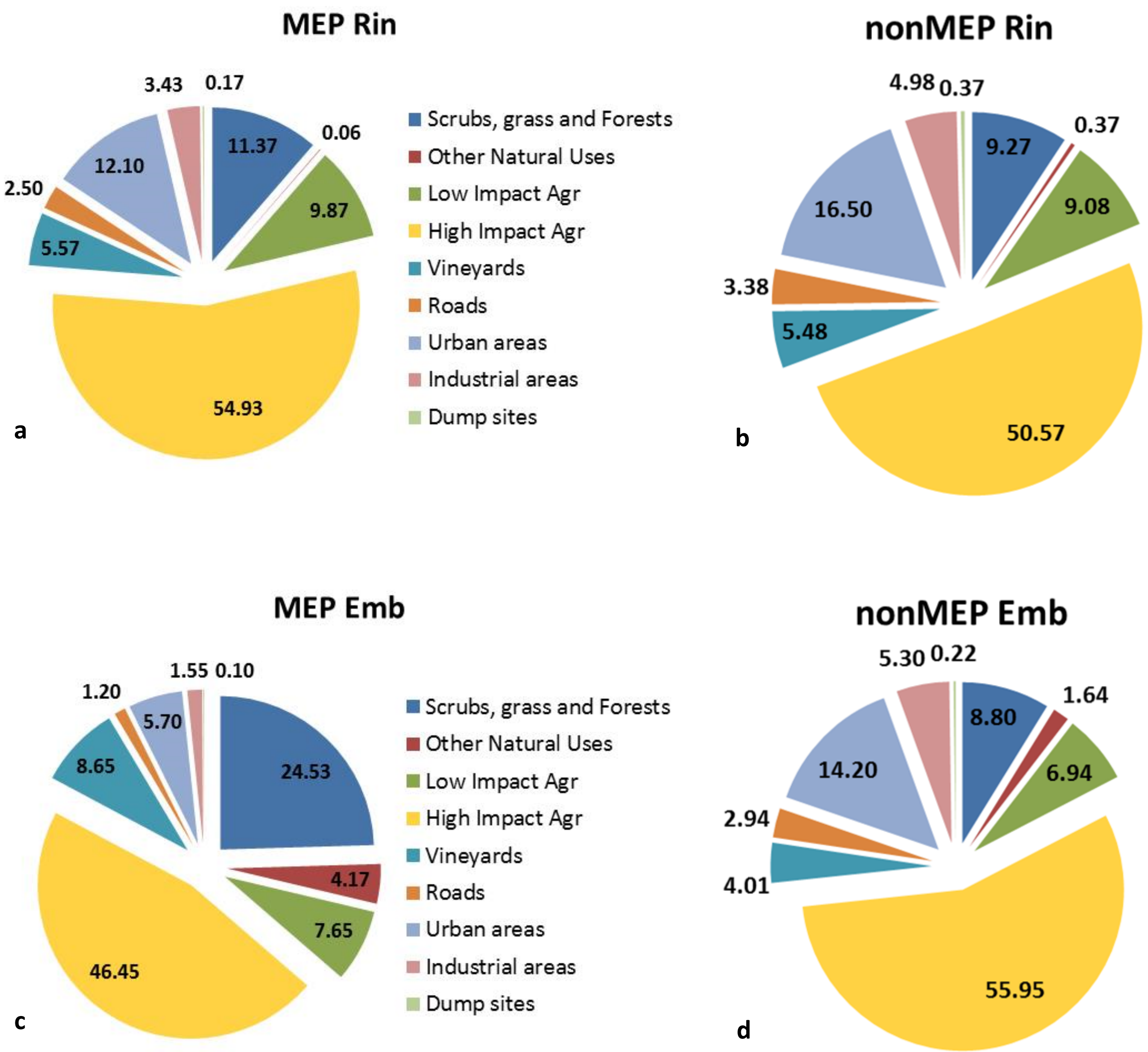

Table A.1a - Features considered for MEP sites selection and used scoring system for MEP attribution.

	Channel Description	Score	Riparian vegetation Description	Score	Levee Description	Score	Reinforcement Description	Score
Leveed reaches	Dynamic, active, typical forms (e.g. bars) present	**5**	Semi-continuous trees and shrubs, good lateral development (Average ≈ 6 m)	**5**	Always distant, wide internal floodplain, presence of active berm	**5**	Absence of visible reinforcement	**5**
	Variable cross section, not overdeepended	**4**	Semi-Continuos trees and shrubs, fair lateral development (Average ≈ 4 m)	**4**	Often distant, internal floodplain, presence of active berm. If in contact only in part of the reach.	**4**	Few reinforcement in the lowest part of the bank, made of natural material	**4**
	Variable cross section, partly overdeepended	**3**	Discontinuous trees and shrubs, fair lateral development (Average ≈ 4 m)	**3**	Internal floodplain and presence of active berm in part of the reach	**3**	Few reinforcement along all the bank	**3**
	Sinuos, overdepeened, constant section of a regular trapezoidal form affected by the presence of artificial elements	**2**	Only herbaceous, woodland only present landside of embankment	**2**	In conctact, small active berms	**2**		
	Realigned, overdepeened, constant section of a regular trapezoidal form	**1**	Bare	**1**	In conctact, no berms	**1**		
Reinforced reaches	Dynamic, active, typical forms (e.g. bars) present	**5**	Semi-continuos trees and shrubs, lateral development < 4 m	**5**	Absence of levees	**5**	Discontinuos reinforcement in the lowest part of the bank, made of natural material	**5**
	Variable cross section, not overdeepended	**4**	Discontinuous trees and shrubs, lateral development < 4 m	**4**	In conctact, but discontinuos	**4**	Reinformcement accompanied by natural elements e.g. active berm	**4**
	Regular cross section, overdeepened	**3**	Discontinuous trees and shrubs, lateral development < 4 m, only on one bank	**3**	Internal floodplain and presence of active berm in part of the reach	**3**	Discontinuos reinforcement made up with rip-rap and or artificial material	**3**
	Regular cross section, with few natural elements	**2**	Variable presence of trees and shrubs, many gardens and allochthonous species replace natural riparian vegetation	**2**	In conctact, small active berms	**2**	Semi-continuos reinforcement made up with rip-rap and or artificial material	**2**
	Constant and regular cross section, with un-natural elements, often trivial	**1**	Bare	**1**	In conctact, no berms	**1**		

Table A.1b - Scores and group definition for selection of MEP sites. Two different groups are identified. Proposed MEP: score ≥ 0.85 (Emb) or ≥ 0.80 (Rin); slightly modified stretches: getting a score lower than 75th percentile of the overall potential MEP sites. For a more complete characterization of reaches we indicated the scale of occurring scoring habitat features (≥ m) and years elapsed since main changes, linked with active and/or passive restoration.

	Channel			**Riparian vegetation**			**Levee**			**Reinforcement**				
Reach	Score	Considered scale (m)	Duration (years)	Score	Considered scale (m)	Duration (years)	Score	Considered scale (m)	Duration (years)	Score	Considered scale (m)	Duration (years)	Final score	Group/Notes
21_Emb	4	2500	>15	5	2500	>30	5	5000	>30	5	2500	>30	**0.95**	**MEP**
23_Emb	5	1000	10	4	1000	10	4	5000	>30	4	2000	>15	**0.85**	**MEP**
14_Emb	5	2000	10	4	2000	>15	4	3000	>30	4	2000	>15	**0.85**	**MEP**
22_Emb	4	2500	>15	4	2500	>30	4	2500	>15	5	2500	>15	**0.85**	**MEP**
01_Emb	3	600	5	2	600	10	2	2000	10	5	2000	10	0.60	Slightly modified
16_Emb	3	600	5	4	600	10	3	2000	10	5	2000	10	0.75	Slightly modified / LIMeco Moderate
19_Emb	3	2000	10	4	2000	15	3	2000	15	5	2000	15	0.75	Slightly modified / LIMeco Moderate
24_Emb	2	1000	10	3	2500	10	3	5000	>30	4	2500	>30	0.60	Slightly modified / LIMeco Moderate
10_Rin	4	1500	5	4	1500	>15				4	1500	10	**0.80**	**MEP**
07_Rin	4	1200	5	5	1200	>15				5	1200	10	**0.93**	**MEP**
09_Rin	4	250	5	4	250	10				4	250	10	**0.80**	**MEP**
27_Rin	2	1200	5	2	1200	10		not recorded		4	1200	10	0.53	Slightly modified
13_Rin	2	1000	5	3	1000	10				3	1000	10	0.53	Slightly modified
25_Rin	3	1000	5	2	1000	10				3	1000	10	0.53	Slightly modified
28_Rin	4	1000	10	2	1000	10				4	1000	10	0.67	Slightly modified / LIMeco Moderate

Supplementary material to:

Chapter 8 (PAPER 6)

Buffagni, A., Barca, E., Erba, S., Balestrini, R. (2019) In-stream microhabitat mosaic depicts the success of mitigation measures and controls the Ecological Potential of benthic communities in heavily modified rivers. *Science of the Total Environment,* 673, 489-501.

Table A.1 - Main typological features and geographical coordinates of sampling sites

Site code	Distance from Source (km)	Altitude (m a.s.l.)	Mean Banface extension (m)	Mean Water width (m)	Max water depth (m)	Latitude	Longitude	N_samples
IM200013	9.7	20	1.7	9.7	1.25	45°34.926'N	12°0.885'E	2
IM200023	8.8	20	1.7	5.3	1.30	45°35.319'N	12°00.716'E	2
IM200033	9.1	20	2.1	6.4	1.44	45°35.178'N	12°00.846'E	2
IM200143	17.2	45	5.8	10.5	0.81	45°51.694'N	12°19.201'E	2
IM200163	16.3	14	4.3	6.2	0.89	45°33.209'N	12°04.983'E	2
IM200173	15.0	16	3.6	8.8	0.80	45°33.712'N	12°04.488'E	2
IM200183	18.3	14	3.6	4.4	0.92	45°32.738'N	12°05.930'E	2
IM200193	34.1	3	6.9	18.3	1.45	45°34.305'N	12°18.697'E	2
IM200203	32.0	4	2.8	8.5	1.40	45°33.873'N	12°17.610'E	2
IM200213	27.1	3	6	16.1	0.91	45°47.685'N	12°48.197'E	2
IC00223	14.9	7	4.4	10	0.75	45°49.537'N	12°47.398'E	2
IM200233	39.2	28	5.7	14.6	1.23	45°32.918'N	11°38.060'E	2
IM200243	40.2	33	6.5	20.5	1.58	45°32.441'N	11°37.863'E	2
IC000293	10.2	7	3.7	8.4	0.85	45°39.583'N	12°23.614'E	2
IC000303	13.4	5	2.8	7.8	1.01	45°38.544'N	12°24.482'E	2
IC01191	15.3	17	4.1	7.6	0.97	45°35.645'N	12°4.692'E	6
IC01231	18.6	14	3.9	7.6	0.60	45°32.755'N	12°4.763'E	3
IC01281	73.6	4	4.3	9.3	na	45°26.329'N	12°6.884'E	1
I0201351	73.6	4	4.3	9.3	na	45°26.329'N	12°6.884'E	2
I-901431	37.2	2	6	14.6	na	45°33.762'N	12°21.005'E	3
I-904811	35.8	0	6.9	13.9	na	45°31.776'N	12°16.247'E	3
I-904831	32.4	5	5	9.35	na	45°30.794'N	12°12.363'E	3
IC04851	51.3	14	2.6	11.2	na	45°29.818'N	11°56.26'E	3
IC05051	7.5	25	3.2	6.2	0.80	45°36.931'N	12°0.783'E	1
IC28101	25.3	32	2.4	2.8	0.30	45°38.007'N	11°59.746'E	2
IC01051	38.3	25	2.5	6.7	na	45°34.771'N	11°52.52'E	1
IC04181	3.7	26	1.5	3.6	0.64	45°35.571'N	11°54.956'E	1

Table A.2 - Values of the different mitigation measures (% of application in the river reach) for the study sites. 'Final_Group' represents decreasing levels of overall application of mitigation measures: 1 full implementation; 2 fair; 3 moderate; 4 poor implementation. The group was derived from the combination of 'group_meas' and 'group_KOTH' (see text for further details). The last column indicates years elapsed from the starting date of implementation of measures related to the improvement of riparian and in-channel vegetation, after hard maintenance works on levees morphology. NM: no mItigation measures applied; WT: adjacent reacreated wetland not directly connected to the river. Additional measures (*), scale of measure application and 'application time' are reported for further characterizing the study area, even if not directly used in the present research. Part of the data were collected by the environmental protection agency of the Veneto Region (ARPAV). (Please refer to Tab. 1 for acronyms)

Site code	no_bRI	berm_reed	no_cRS	b_arb_shr	lands_veg	mng_cHAB	mng_mHAB	mng_HAB	manag_meas^{mm}	group_meas	KOTH	group_KOTH	Final_Group	composite *	sinuos *	adj_bank *	Scale of application (≥ m)	Years elapsed since first rehabilitation of bank vegetation
IM200013	100	20	0	5	30	42.6	22.8	28.6	0.45	2	0.18	3	3	0	6	0	600	10
IM200023	100	30	0	0	0	41.5	22	0	0.35	2	0.00	3	3	0	6	0	800	10
IM200033	100	15	0	0	0	49.7	14.9	0	0.33	3	0.00	3	4	0	3	0	300	10
IM200143	100	60	70	50	0	5.9	12.5	100	0.73	1	0.48	2	1	100	51	10	2000	10
IM200163	100	20	0	35	50	28.5	13.5	28.6	0.50	2	0.26	3	3	20	8	23	2000	15
IM200173	100	0	0	0	0	23.2	12.8	0	0.25	3	0.18	3	4	0	0	0	--	NM
IM200183	45	0	0	5	0	28.2	20	0	0.18	3	0.18	3	4	0	6	0	--	NM
IM200193	100	70	100	23	50	48.3	18	0	0.75	1	0.18	3	2	15	6	0	2300	15
IM200203	100	0	80	0	0	14	5.9	0	0.36	2	0.00	3	3	0	0	0	1000	10
[e]IM200213	100	100	100	100	2	36.2	10.1	100	1.00	1	1.00	1	1	100	34	20	2500	> 15
IC00223	100	5	100	90	80	32.2	3.9	100	0.93	1	0.74	1	1	30	28	7	2500	> 15
IM200233	100	10	100	45	0	3.2	9.1	100	0.67	1	0.37	2	1	60	54	20	1000	> 15
IM200243	100	7	100	45	0	32.8	8.4	71.4	0.67	2	0.57	2	2	60	29	13	3000	10
IC000293	100	5	0	1	5	24.9	11.1	28.6	0.32	3	0.18	3	4	0	6	33	--	NM
IC000303	100	0	0	0	1	34.9	17.9	0	0.28	3	0.00	3	4	0	3	17	--	NM
IC01191	100	0	100	0	0	29.5	4.3	0	0.43	2	0.18	3	3	0	15	0	1000	10
IC01231	100	10	100	0	5	4.6	7.1	0	0.41	2	0.00	3	3	0	20	40	500	10
IC01281	90	0	0	20	0	5.1	5.6	0	0.22	3	0.18	3	4	0	0	20	--	WT
I0201351	90	0	0	20	0	5.1	5.6	0	0.22	3	0.18	3	4	0	0	20	--	WT
I-901431	70	45	100	20	5	0	10	0	0.46	2	0.18	3	3	0	15	0	3000	10
I-904811	100	10	100	0	0	0	9	0	0.40	2	0.00	3	3	0	10	45	120	10
I-904831	100	0	0	0	0	8.2	8.7	0	0.21	3	0.00	3	4	0	0	40	--	NM
IC04851	95	5	70	20	5	35.4	13.6	0	0.45	2	0.00	3	3	5	15	0	800	10
IC05051	90	5	100	0	10	15.7	6	0	0.41	2	0.00	3	3	0	0	0	500	10
IC28101	100	0	100	20	0	8.6	0.9	0	0.42	2	0.48	2	2	0	0	40	1000	10
IC01051	100	0	100	20	5	44.3	23.4	0	0.53	2	0.00	3	3	0	0	0	100	10
IC04181	100	0	100	0	0	41.2	39.2	0	0.51	2	0.32	2	2	0	0	5	1500	10

manag_meas^{mm} : Overall value of measure implementation. Score 1 is obtained for a real case-study (i.e. [e]IM200213, with the largest observed implementation of measures) hereafter exemplified: 5 measures applied in the 100% of the reach, 1 in 36% of the reach, 1 in 10% and 1 measure applied in 2% of the reach (not all the measures are equally implemented). Accordingly, score 1 would have been obtained also from different % of application of measures e.g. 3 measures applied in the 100% of the reach, 1 measure applied in 82%, 2 in 60% of the reach, 1 in 36% of the reach, 1 in 10% and of the reach. Values for other sites are re-scaled accordingly.

* Additional measures	composite	Increasing bank morphological diversity (e.g. creation of composite bank profiles)
	sinuos	Increasing channel morphological diversity (e.g. guaranteeing water sinuosity)
	adj_bank	Re-establishment of bank adjustment processes

Table A.3a - Summary description of the main environmental indices used to characterize river reaches and of the Ecological Status/Potential benthic metrics.

Information category	Acronym	Description	Reference	Notes
Water Quality	LIMeco	Calculated on the basis of the concentration of selected physico-chemical parameters (i.e. TP, N-NH_4, N-NO_3 and Oxygen saturation). Decreasing scores are assigned in relation to increasing pollutant concentrations.	DM 260/2010	Laboratory measures carried on water samples.
Land Use	LUI	Information on land use degradation, obtained assigning scores to impacting land uses (urban, industrial, agricultural land uses) recorded at reach scale	Erba et al., 2015	Derived from CARAVAGGIO (Buffagni and Kemp, 2002; Buffagni et al., 2013) application. CARAVAGGIO was developed on the basis of the River Habitat Survey protocol (RHS, Raven et al., 1997). The method consists of a comprehensive survey of habitat features and modification along 500 m channel length. Based on the collected information a series of habitat descriptors can be calculated.
Lotic conditions	LRD	Furnishes information about the lentic-lotic character of the river stretch. Positive values of the index represent rivers with a lentic character (dominated from slow flowing or still water) while negative values represent lotic rivers (dominated from features linked with high turbulence and fast flowing water).	Buffagni et al., 2010	
Morphological degradation	HMS	Presence and quantity of artificial features along river channel and banks. Consists in the sum of the scores assigned to selected features each representing a type of morphological alteration (e.g. bank modifications, presence of weirs). The index increases with the increasing of morphological impact.	Raven et al., 1998	
Ecological status	STAR_ICMi	Index formally in use in Italy for river classification based on macrobenthic fauna. STAR_ICMi is a multimetric index formed by 6 normalized and weighted metrics.The following biological metrics (see also Buffagni et al., 2016) were calculated to family level for each sample: ASPT (Average Score per Taxon; Armitage et al., 1983); N_families (Total Number of Families; e.g. Thorne and Williams, 1997); EPT (number of Ephemeroptera, Plecoptera and Trichoptera taxa; Barbour et al., 1999); Sel_EPTD (log abundance of selected taxa of Ephemeroptera, Plecoptera, Trichoptera and Diptera; Buffagni et al., 2004); Shannon (Shannon-Wiener Diversity index; Shannon and Weaver, 1949); 1-GOLD (1 - relative abundance of Gastropoda, Oligochaeta and Diptera; Pinto et al., 2004).	Buffagni et al., 2007; DM 260/2010	Calculated from taxalist derived from multihabitat proportional sampling. The software MacrOper.ICM was used (Buffagni & Belfiore, 2013).

Reference

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Table A.3b - Values of habitat variables and envirionmental indices characterizing the studied river reaches. Samples from 1 to 30 collected in September/October 2016. Other samples collected between 2011 and 2013 in different sampling seasons. Part of the data collected by the environmental protection agency of the Veneto Region (ARPAV).

sample_ord	Site code	HF	SWI	n_mh	LIMeco	LUI	LRD	HMS	STAR_ICMi	EP class
1	IM200013a	0.48	0.66	5	0.53	13.89	54	35	0.586	Moderate
2	IM200013b	0.48	0.66	5	0.53	13.89	54	35	0.634	Moderate
3	IM200023a	0.46	0.56	4	0.56	12.80	51	40	0.587	Moderate
4	IM200023b	0.46	0.56	4	0.56	12.80	51	40	0.523	Moderate
5	IM200033a	0.26	0.56	4	0.56	14.62	53	40	0.460	Poor
6	IM200033b	0.26	0.56	4	0.56	14.62	53	40	0.494	Moderate
7	IM200143a	0.77	0.70	6	0.58	11.38	11	40	0.765	Good
8	IM200143b	0.77	0.70	6	0.58	11.38	11	40	0.782	Good
9	IM200163a	0.37	0.56	4	0.49	10.59	45	32	0.596	Moderate
10	IM200163b	0.37	0.56	4	0.49	10.59	45	32	0.567	Moderate
11	IM200173a	0.25	0.56	4	0.39	17.65	47	44	0.537	Moderate
12	IM200173b	0.25	0.56	4	0.39	17.65	47	44	0.622	Moderate
13	IM200183a	0.54	0.68	5	0.38	12.01	58	71	0.641	Moderate
14	IM200183b	0.54	0.68	5	0.38	12.01	58	71	0.622	Moderate
15	IM200193a	0.73	0.68	5	0.49	9.11	47	21	0.559	Moderate
16	IM200193b	0.73	0.68	5	0.49	9.11	47	21	0.529	Moderate
17	IM200203a	0.38	0.56	4	0.50	15.49	18	41	0.857	Good
18	IM200203b	0.38	0.56	4	0.50	15.49	18	41	0.886	Good
19	IM200213a	0.82	0.69	5	0.84	8.38	39	26	0.822	Good
20	IM200213b	0.82	0.69	5	0.84	8.38	39	26	0.955	Good
21	IC00223a	0.79	0.74	6	0.57	5.12	24	23	0.874	Good
22	IC00223b	0.79	0.74	6	0.57	5.12	24	23	1.148	High
23	IM200233a	0.91	0.83	8	0.61	12.97	29	22	1.031	High
24	IM200233b	0.91	0.83	8	0.61	12.97	29	22	0.914	Good
25	IM200243a	0.68	0.65	5	0.37	9.90	56	36	0.886	Good
26	IM200243b	0.68	0.65	5	0.37	9.90	56	36	0.672	Moderate
27	IC000293a	0.14	0.44	3	0.50	15.81	53	30	0.396	Poor
28	IC000293b	0.14	0.44	3	0.50	15.81	53	30	0.338	Poor
29	IC000303a	0.23	0.56	4	0.46	15.38	55	41	0.346	Poor
30	IC000303b	0.23	0.56	4	0.46	15.38	55	41	0.307	Poor
31	IC01191a	0.72	0.45	3	0.38	12.21	23	46	0.689	Moderate
32	IC01191b	0.71	0.46	3	0.50	12.21	23	46	0.928	Good
33	IC01191c	0.71	0.45	3	0.50	12.21	23	46	0.615	Moderate
34	IC01191d	0.59	0.35	3	0.34	12.21	23	46	0.703	Moderate
35	IC01191e	0.80	0.51	4	0.31	12.21	23	46	0.724	Good
36	IC01191f	0.82	0.53	4	0.56	12.21	23	46	0.760	Good
37	IC01231a	0.62	0	1	0.44	12.21	27	50	0.538	Moderate
38	IC01231b	0.89	0.29	2	0.44	12.21	27	50	0.567	Moderate
39	IC01231c	0.65	0.39	3	0.34	12.21	27	50	0.566	Moderate
40	IC01281a	0.17	0	1	0.53	9.77	17	64	0.235	Bad
41	I0201351b	0.15	0	1	0.50	9.77	17	64	0.262	Poor
42	I0201351c	0.19	0	1	0.50	9.77	17	64	0.405	Poor
43	I-901431a	0.54	0.28	3	0.66	6.84	10	64	0.605	Moderate
44	I-901431b	0.63	0.41	3	0.38	6.84	10	64	0.475	Poor
45	I-901431c	0.56	0.28	3	0.25	6.84	10	64	0.424	Poor
46	I-904811a	0.36	0.14	2	0.33	9.55	23	52	0.596	Moderate
47	I-904811b	0.48	0.27	2	0.50	9.55	23	52	0.231	Bad
48	I-904811c	0.34	0.14	2	0.56	9.55	23	52	0.361	Poor
49	I-904831a	0.36	0.22	2	0.41	14.25	33	54	0.641	Moderate
50	I-904831b	0.85	0.56	4	0.50	14.25	33	54	0.584	Moderate
51	I-904831c	0.75	0.41	4	0.50	14.25	33	54	0.466	Poor
52	IC04851a	0.72	0.45	3	0.67	6.29	34	44	0.730	Good
53	IC04851b	0.58	0.35	3	0.46	6.29	34	44	0.703	Moderate
54	IC04851c	0.55	0.27	2	0.66	6.29	34	44	0.714	Moderate
55	IC05051	0.52	0.47	3	0.25	10.31	25	56	0.613	Moderate
56	IC28101a	0.46	0.22	2	0.50	10.34	27	30	0.469	Poor
57	IC28101b	0.47	0.22	2	0.25	10.34	27	30	0.532	Moderate
58	IC01051	0.84	0.58	4	0.31	12.17	16	35	0.761	Good
59	IC04181	0.70	0.41	3	0.53	10.85	50	30	0.919	Good

TABLE A.4a Scores, r^2 and p-value for environmental variables as vectors on NMDS ordination axes.

Environmental variable	NMDS1	NMDS2	r^2	Pr(>r)	
Diversity of substrate micro-habitats (SWI)	-0.976	0.215	0.695	0.0001	***
Morphological alteration (HMS)	0.998	0.049	0.453	0.0001	***
In-stream habitat mosaic (HF)	-0.512	-0.858	0.380	0.0001	***
Implementation of mitigation measures ($manag\_meas^{mm}$)	-0.973	-0.229	0.347	0.0001	***
Presence of tree-related features (KOTH)	-0.937	0.347	0.269	0.0004	***
Presence of lotic habitats (based on LRD)	0.645	-0.764	0.202	0.0015	**
Water quality (LIM_{eco})	-0.766	0.642	0.015	0.015	*
Land_use_degradation (LUI)	0.066	-0.998	0.086	0.1982	ns

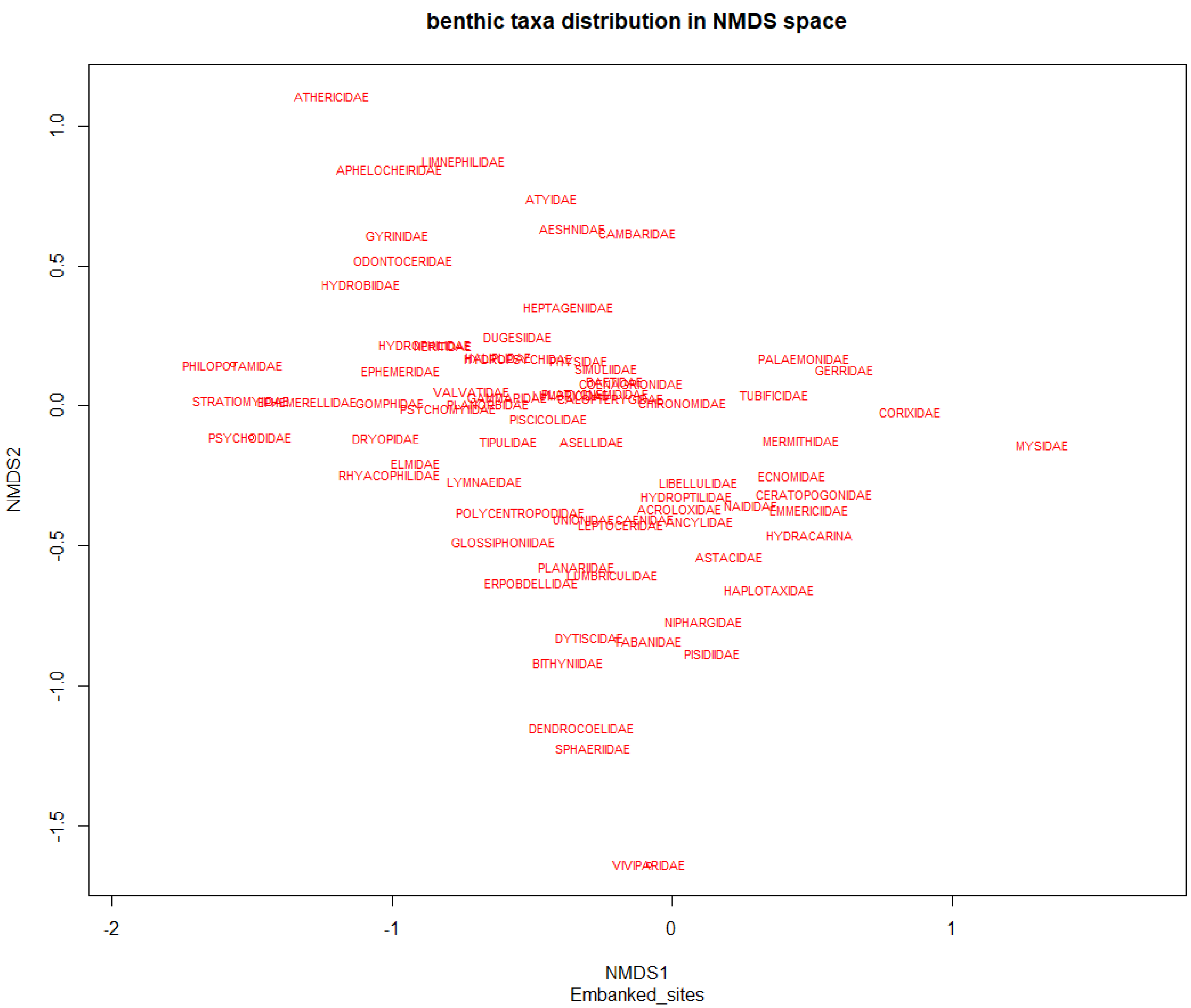
TABLE A.4b Scores, r^2 and p-value for benthic metrics as vectors on NMDS ordination axes.

Metric category	Benthic metric	NMDS1	NMDS2	r^2	Pr(>r)	
Beta diversity	Sørensen	-1	0.00195	0.877	0.001	***
	LCBD site	-0.179	0.983	0.008	0.814	ns
Alpha diversity and	NFAM	-0.627	-0.778	0.839	0.001	***
Ecological Status/Potential	NEPT	-0.628	-0.778	0.579	0.001	***
	SHAN	-0.638	-0.769	0.254	0.002	**
Ecological Status/Potential	ASPT	-0.595	-0.803	0.414	0.001	***
	1-GOLD	-0.998	-0.047	0.180	0.004	**
	LGSE	-0.997	-0.066	0.248	0.001	***
	STAR_ICM index	-0.787	-0.616	0.617	0.001	***

TABLE A.4c Spearman Rank Order Correlations between variables representing habitat mosaic, benthic attributes, impact and mitigation measures. Correlations in italic/red are significant at p <0.05.

	Habitat mosaic			Diversity			Ecological Potential	Impact and mitigation measures					
	HF	n_mh	SWI	NFAM	Sørensen	LCBD	STAR_ICMi	Morphological alteration	Mitigation measures	Lentic-lotic character	Tree-related features	Land use degradation	Water quality
HF	-	*0.44*	*0.43*	*0.54*	*0.37*	0.07	*0.64*	-0.22	*0.64*	-0.19	*0.30*	*-0.36*	0.14
n_mh	*0.44*	*-*	*0.97*	*0.47*	*0.72*	0.13	*0.50*	*-0.55*	*0.45*	*0.37*	*0.44*	0.05	*0.29*
SWI	*0.43*	*0.97*	-	*0.50*	*0.74*	0.14	*0.52*	*-0.59*	*0.45*	*0.39*	*0.42*	*0.06*	*0.30*
NFAM	*0.54*	*0.47*	*0.50*	-	*0.58*	-0.07	*0.75*	*-0.54*	*0.55*	0.12	*0.37*	-0.02	0.21
Sørensen	*0.37*	*0.72*	*0.74*	*0.58*	-	0.10	*0.62*	*-0.70*	*0.56*	*0.27*	*0.54*	-0.06	*0.37*
LCBD	0.07	0.13	0.14	-0.07	0.10	-	0.02	-0.15	*0.31*	0.01	*0.27*	*-0.45*	0.17
STAR_ICMi	*0.64*	*0.50*	*0.52*	*0.75*	*0.62*	0.02	-	*-0.32*	*0.57*	-0.07	*0.39*	-0.17	0.24
Morphological alteration	-0.22	*-0.55*	*-0.59*	*-0.54*	*-0.70*	-0.15	*-0.32*	-	*-0.60*	*-0.37*	*-0.49*	0.03	*-0.37*
Mitigation measures	*0.64*	*0.45*	*0.45*	*0.55*	*0.56*	*0.31*	*0.57*	*-0.60*	-	-0.15	*0.59*	*-0.61*	0.25
Lentic-lotic character	-0.19	*0.37*	*0.39*	0.12	*0.27*	0.01	-0.07	*-0.37*	-0.15	-	0.01	*0.34*	0.01
Tree-related features	*0.30*	*0.44*	*0.42*	*0.37*	*0.54*	*0.27*	*0.39*	*-0.49*	*0.59*	0.01	-	*-0.35*	0.17
Land use degradation	*-0.36*	0.05	0.06	-0.02	-0.06	*-0.45*	-0.17	0.03	*-0.61*	*0.34*	*-0.35*	-	-0.14
Water quality	0.14	*0.29*	*0.30*	0.21	*0.37*	0.17	0.24	*-0.37*	0.25	0.01	0.17	-0.14	-

FIGURE A.1 Relative positioning of benthic taxa in the NMDS space.



Appendix A.5

HF_Bayes_simplified.R

General Info

This R script has been compiled by Emanuele Barca and Andrea Buffagni and is annexed to the paper:
"In-stream habitat mosaic controls the benthic community and depicts the success of mitigation measures in heavily modified rivers"
prepared by Andrea Buffagni, Emanuele Barca, Stefania Erba & Raffaella Balestrini,
submitted to Science of the Total Environment on January 2019.
Please, refer to this paper for explanation of how/when to use the script.

The script supports the calculation of the Bayes Factor (BF) used to derive a Habitat Factor (HF) [HF = 1-(lnBF)/10, with HF = 0 when lnBF >=10]
HF allows the comparison between habitat mosaics observed in different conditions/sites.
We assume that the habitat mosaic observed at each reference site (user-defined) effectively represents the ‘expected’ condition. By calculating the average distance from reference conditions, you get the site HF.

```
#  ____SCRIPT____   #
### DATA PREPARATION

#  input file
#  first column:
#       row1 (micro-habitat): MH
#       then: list the micro-habitats proportionally found at yor site e.g. SO, SO, SO, ARG, TP, ... for each site
#  second column:
#       row1 (site/sample name): site
#       then: write the name of your site/sample
#__________


require(openxlsx)
require(rjags)
require(BayesianFirstAid)
require(BayesFactor)
require(LearnBayes)

##setwd("D:/C_DOCUMENTS/script_R/bayes01") # you can set your working directory here
Data_ini <- read.xlsx("mydata.xlsx", sheet = 1, colNames = TRUE)#startRow = 2,

fix(Data_ini)

sites<-unique(Data_ini[ , 2])

##### Bayes #####
lsites<-length(sites)
lsitesm1<-lsites-1
```

```
#### ANALYSIS ####

sink("results.txt", append=FALSE, split=FALSE) # specify the name for your
output file here
cat("__add your db name here___, site pairs comparison") # add the name of your
dataset/area here
cat("\r\n \r\n")

for (i in 1:lsitesm1){
  site1<-sites[i]
  ini<-i+1
 for (j in ini:lsites) {
  site2<-sites[j]
  string_sites<-paste(site1, site2, sep = " vs ", collapse = NULL)
  Data<-Data_ini[I(Data_ini$site == site1) | I(Data_ini$site == site2), ]

cat("****** NEW PAIR  ", string_sites, "   ******")
cat("\r\n \r\n")
cat("\r\n-----------MH in the two sites/site groups - Contingency
table--------------\r\n") #ab
cont_tab<-with(Data, table(site, MH))
print(cont_tab)

#create a matrix of 1s to test independence vs dependence
M <- cont_tab
l_M<-length(M)#conta gli elementi della matrice
col_M<-nrow(M)
row_M<-ncol(M)

a=matrix(rep(1,l_M),c(col_M,row_M))

log.BF_ct<-log(ctable(M, a)) #dip vs. indep - log(P(Data| indip)/P(Data| dip))
se >0 indip altrimenti dip

BF10<-"H1/H0"
BF01 =  "H0/H1"

cat("\r\n-----------Presentation of Bayes Factor analysis
results--------------\r\n")
cat("Bayes Factor(BF10) for H1 Dependence over H0 Independence: ",BF10,"\r\n")
cat("Bayes Factor(BF01) for H0 Independence over H1 Dependence: ",BF01,"\r\n")
log.K=seq(2,7)
compute.log.BF=function(log.K)
  log(bfindep(M,exp(log.K),100000)$bf)

log.BF=sapply(log.K,compute.log.BF)
BF=exp(log.BF)

#BF in support of the alternative model close to independence
#Bayes factor against independence assuming alternatives close to independence
```

```
cat("\r\n-----------Bayes Factor - overall results for the sites
pair--------------\r\n")
cat("Bayes Factor(BFa20) for Ha2 Close to Independence over H0 Independence:
",round(max(BF),4),"\r\n")
cat("Bayes Factor(BF0a2) for H0 Independence over Ha2 Close to Independence:
",round(1/max(BF),4),"\r\n")

n<-cbind(c(sum(M[1, ]), sum(M[2, ])))

cat("\r\n-----------Additional BF Model Results--------------\r\n")
cat("Bayes factor in support of the model close to independence versus the
model of independence:\r\n")
print(round(data.frame(log.K,log.BF,BF),2))

cat("\r\n-------Bayesian proportion test - Detailed results for each MH in the
sites pair---------\r\n")

for (i in 1:row_M){
###
  v1<-as.numeric(M[, i])
  v2<-as.numeric(n[, 1])
  fit <- bayes.prop.test(v1, v2)
  print(fit) }

cat("END OF COMPARISON FOR: ", string_sites)
cat("\r\n \r\n") #ab
}
}
sink()

### it will take some time... (many comparisons, just wait ;-)

# an example input file (e.g. "mydata") follows
```

MH	site
GHI	REF_site1
SO	REF_site1
GHI	REF_site1
TP	REF_site1
CP	REF_site1
ARG	REF_site1
TP	REF_site1
CP	REF_site1
GHI	REF_site1
GHI	REF_site1
SO	REF_site1
SO	REF_site1
GHI	REF_site1
MIC	REF_site1
MIC	REF_site1
TP	REF_site1
ARG	REF_site1
CP	REF_site1
SO	REF_site1
GHI	REF_site1
SAB	REF_site1
MIC	REF_site2
MIC	REF_site2
MIC	REF_site2
MIC	REF_site2
MIC	REF_site2
MIC	REF_site2
SO	REF_site2
SO	REF_site2
EM	REF_site2
EM	REF_site2
SAB	REF_site2
SAB	REF_site2
MIC	REF_site2
MES	REF_site2
MIC	REF_site2
MES	REF_site2
TP	REF_site2
MES	REF_site2
MES	REF_site2
TP	REF_site2
ARG	REF_site3
ARG	REF_site3
ARG	REF_site3
ARG	REF_site3
ARG	REF_site3
ARG	REF_site3
ARG	REF_site3
ARG	REF_site3
MIC	REF_site3

MIC	REF_site3
MIC	REF_site3
MIC	REF_site3
CP	REF_site3
CP	REF_site3
TP	REF_site3
TP	REF_site3
TP	REF_site3
TP	REF_site3
SO	REF_site3
SO	REF_site3
MIC	REF_site4
MIC	REF_site4
MIC	REF_site4
MIC	REF_site4
MIC	REF_site4
MIC	REF_site4
MIC	REF_site4
MIC	REF_site4
MES	REF_site4
MES	REF_site4
CP	REF_site4
CP	REF_site4
XY	REF_site4
XY	REF_site4
TP	REF_site4
TP	REF_site4
TP	REF_site4
TP	REF_site4
SO	REF_site4
SO	REF_site4
SAB	test_site1
SO	test_site1
SAB	test_site1
SAB	test_site1
SO	test_site1
CP	test_site1
EM	test_site1
CP	test_site1
ARG	test_site1
ARG	test_site2
SAB	test_site2
EM	test_site2
SO	test_site2
SO	test_site2
CP	test_site2
SO	test_site2
EM	test_site2
SAB	test_site2
SO	test_site2
SO	test_site3

ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
ARG	test_site3
MES	test_site3
MES	test_site3
SO	test_site3
SO	test_site3
MES	test_site3
CP	test_site3
SO	test_site3
SO	test_site4
MES	test_site4
SAB	test_site4
SO	test_site4
MES	test_site4
MES	test_site4
MES	test_site4
EM	test_site4
SO	test_site4
ARG	test_site4
ARG	test_site4
MES	test_site4
SAB	test_site4
SAB	test_site4
SAB	test_site4
EM	test_site4
SO	test_site4
SO	test_site4
CP	test_site4
CP	test_site4