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Flow-T index validation for the meso-scale habitat modelling of macroinvertebrate communities in three Italian braided rivers

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ABSTRACT

In the current context of climate change, water is becoming an increasingly contested resource [2, 8, 15, 26, 31], requiring tools that can balance human uses—domestic, agricultural, and hydroelectric—with the maintenance of good ecological status, as required by the European Water Framework Directive 2000/60/EC. One such tool is the MesoHABSIM methodology [20], which can help determine Environmental Flow conditions for river reaches based on habitat availability for aquatic species [10].

In Italy, the methodology has been officially adopted since 2017, and published in the ISPRA Technical-Operational Manual 154/2017 [29]. Furthermore, in the Aosta Valley region, it has been incorporated into the strategic water protection plan and is employed within a multicriteria analysis framework to support decision-making processes concerning riverine habitat management and ecological flow assessment [27].

Originally developed for fish, the method has recently been extended to include benthic macroinvertebrate communities [7, 21, 22], widely recognized as reliable indicators of ecological quality [5, 6, 18].

This thesis focuses on the validation of the Flow-T index, a new metric designed within the BentHab project to describe macroinvertebrate preferences for flow velocity [17], based on an European free acces database describing the flow current preferencies of more than 500 taxa [25].

The study combines field surveys—topographic, bathymetric, and biological—with hydrodynamic modelling on three tributaries of the Po River (Taro, Trebbia, and Enza). Results demonstrate a consistent relationship between measured and modelled Flow-T values, confirming the suitability of the index as a sensitive descriptor of hydromorphological alterations. The findings highlight the potential of Flow-T to strengthen environmental flow assessments and support sustainable water management in regulated river systems.

C'era una volta un giovane ruscello
color di perla, che alla vecchia valle
tra molli giunchi e pratoline gialle,
correva snello;

e c'era un bimbo che gli tendea le mani
dicendo: "A che tutto cotesto foco?
Posa un po' qui: si gioca un caro gioco
se tu rimani.

Se tu rimani, o movi adagio i passi,
un lago nasce e nell'argento fresco
della bell'acqua io, con le mani, pesco
gemme di sassi.

Fermati dunque, non fuggir così!
L'uccello che cinguetta ora sul ramo
ancor cinguetterà, se noi giochiamo
taciti qui".

Rise il ruscello e tremolò commosso
al cenno delle amiche mani tese;
e con un tono di voce cortese
disse: "Non posso!

Vorrei: non posso! il cuor mi vola: ho fretta.
A mezzo il piano, a leghe di cammino,
la sollecita ruota del mulino
c'è che mi aspetta;

e c'è la vispa e provvida massaia
che risciacquar la nuova tela deve
e sciorinarla sì che al sole neve
candida paia;

e v'è il gregge, che a sera porge il muso
avido a bere di quest'onda chiara,
e gode s'io lo sazio, poi ripara
contento al chiuso.

Lasciami dunque" terminò il ruscello
"correre dove il mio dover mi vuole".
E giù pel piano, luccicando al sole,
disparve snello.

- Angiolo Silvio Novaro, Il Ruscello

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1. Introduction

In 2008 the technical paper on Climate Change and water of the IPCC stated that "*observational records and climate projections provide abundant evidence that freshwater resources are vulnerable and have the potential to be strongly impacted by climate change, with wide-ranging consequences for human societies and ecosystems.*" [2]
The following year, the United Nations *World Water Assessment Programme* (WWAP, 2009) further stressed that water scarcity is one of the main global challenges of the 21st century, driven by both climate change and increasing human demand [31].

Among the most relevant hydrological impacts of climate change is the alteration of global precipitation patterns, snow storage, and consequently of local hydrographs, leading to an increase in river intermittency and to a decline in the predictability and resilience of freshwater availability [19]. This phenomenon is expected to aggravate the already widespread scarcity of water resources, potentially exacerbating social conflicts [15, 26], and therefore making it essential to develop tools for managing the resource among competing sectors such as agriculture, hydropower, industry, household uses, and ecosystem services.

In Europe, water management is primarily regulated by the *Water Framework Directive* (WFD, 2000/60/EC) [11], which establishes a framework for the protection of inland surface waters, transitional waters, coastal waters, and groundwater. A key objective of the directive is to achieve "good ecological status" of water bodies, defined as the condition in which the biological, hydromorphological, and physicochemical quality elements deviate only slightly from undisturbed natural conditions.

In Italy, this regulatory framework has been transposed through the Testo Unico Ambientale (TUA, D.lgs. 3/04/2006 n.152). In the TUA the Italian territoty is divided into jurisdiction districts responsible for the basin water managment. River Basin Authorities are required to develop Water Protection Plans, within which the values of the Minimum Vital Flow (DMV) must be established. The DMV is defined as "the quantity of water necessary to ensure the preservation of the physical characteristics of the water body, the balance of the related ecosystems, and the legitimate existing uses" (Legislative Decree 152/2006, Art. 95).

The MesoHABSIM methodology (Mesohabitat Simulation Model) was first proposed in the scientific literature by Parasiewicz [20] as a tool to analyze the spatiotemporal variability of aquatic habitats based on discharge flow and river morphology. It can be used for diverse purposes: quantification and modulation of DMV (Deflusso Minimo Vitale), definition of E-flow, monitoring releases from dam operations, river restoration projects design and monitoring, fish suitability estimation for a watercourse, conservation of specific protected aquatic species and calculation of the River Integrity Index (IH). The method was adapted for the Italian context by Vezza et al. [28], and formally recognized by ISPRA in the technical-operative guidelines MLG 131/2016 [29]. At the European level, the 2019 Directive on Environmental Flows explicitly acknowledges MesoHABSIM among the reference models for defining and assessing E-flow. [8, 10].

The evaluation of river ecological status in Italy has been largely based on benthic macroinvertebrates, through the STAR_ICMi index, which remains the institutional tool for biological monitoring under the WFD and TUA [5, 6].
Benthic macroinvertebrates are invertebrate animals living on or within the substrate of freshwater ecosystems, including aquatic insects, mollusks, crustaceans, and worms. They are widely recognized as reliable bioindicators of river ecological status due to their relatively long life cycles, habitat specificity, and sensitivity to chemical, physical, and hydromorphological alterations [18].
While the STAR_ICMi index is effective in reflecting chemical quality and general biological conditions, it shows limited sensitivity to hydromorphological alterations, such as modifications in flow regime or channel structure [5, 6].

To overcome this limitation, recent developments have extended the MesoHABSIM approach to include habitat modelling for benthic macroinvertebrates [7, 21, 22]. This innovation is currently being tested in Italy through the BentHab project, coordinated by Politecnico di Torino, Università di Parma, Autorità di Bacino del Fiume Po (AdBPo), and Associazione Nazionale Bonifiche Italiane (ANBI). Within this framework, a new metric, Flow-T, has been developed [17]. It is based on a European open-access database describing the velocity preferences of about 500 taxa of macroinvertebrates [25]. Flow-T has proven to be more sensitive to hydromorphological variations, making it particularly promising for studying the ecological response of benthic communities to flow alterations [17].

This thesis builds on the studies conducted by Vezza, Laini, and Pinna in 2019 and 2020, when biological and hydromorphological surveys were carried out in the Trebbia, Taro, and Enza Rivers to calibrate and cross-validate the Flow-T index [21, 22]. The summer period was chosen as it is characterized by reduced precipitation and intensified irrigation withdrawals, making it particularly critical for testing flow-related pressures.

In 2023, additional biological, hydrological, and photogrammetric surveys were undertaken in the same hydromorphologically homogeneous and representative reaches, with the purpose of performing an independent validation of the index. The objective of this thesis is therefore to test the performance of Flow-T by comparing observed and modelled data, and to assess its potential as a reliable tool for incorporating hydro-morphological variability into ecological status evaluations.

In this thesis work, the author took part in the field survey campaign conducted from 25 to 28 June 2023, supporting the activities coordinated by Prof. Alex Laini from the *Department of Life Sciences and Systems Biology* (DBIOS), University of Turin, and Dr. Beatrice Pinna from the DIATI, Politecnico di Torino. The author measured water depth and flow velocity at the biological sampling sites along the Trebbia River, and determined their relative positions using a current meter and a laser rangefinder. She also participated in the hydraulic and bathymetric surveys of the Enza and Taro rivers, as described in the **2.4 Field Surveys** section.

During the data processing and analysis phase, the author developed the bathymetric model of the Taro River and carried out the correction of the DTM's bottom. The corresponding procedures for the Trebbia and Enza rivers were performed by Dr. Pinna (see **Section 3.1.2** and **3.2.1**). The author also conducted sixteen out of the eighteen hydrodynamic simulations (excluding those at measured discharge for the Trebbia and Enza rivers; see **Sections 2.5.2** and **3.2.2**) and produced all eighteen HMU mosaics presented in **3.2.3 - HMU count and mosaic** and **4.2 - HMU physical evolution** following the methodology described in **2.5.3 - HMU definition**. Furthermore, she prepared and formatted the files related to the eighteen HMU mosaics for implementation in the macroinvertebrate habitat model developed by Pinna et al. [21, 22] and integrated into the *SimStream* web platform. Using this tool, the author derived the three Flow-T rating curves discussed in **Section 4.3** and obtained the Flow-T values of each HMU, which were then compared with DBIOS' data to perform the validation analysis described in **3.3 - Flow-T validation**.

2. Materials and Methods

2.1 Study Area

The rivers considered in the analysis are in Emilia Romagna and are three of the main right bank tributaries of the Po River: Taro, Trebbia and Enza.

Taro basin has an extension of about 2030 km^2, 77% of which is in the mountain area. It represents the 2,9% of the Italian Po basin. The Taro River is born on Monte Penna (1735 masl) and, after about 150 km, enters the Po north to Parma, at Gramignazzo. Its main development direction is SW-NE, it creates a fan at the outlet of the plain, with its apex between Fornovo and Collecchio.

The hydrographic network is sufficiently developed, as evidenced by a low hierarchization index, but it maintains very variable bifurcation ratio values, indicative of a very unstable situation. The main branch in the plain section has a branched riverbed, characterised by a high width (500-600 m) and modest incision of the banks (2.0-2.5 m), with consistent formations of banks lithoid material and branches of the low bed with a tortuous and unstable path.

The Trebbia basin has an overall area of about 1070 km^2 (1.5% of the total area of the Po basin), much of which is in hilly-mountainous areas (86%). Administratively, the Trebbia basin falls almost entirely within Emilia-Romagna (18 municipalities in the province of Piacenza) and Liguria (16 municipalities in the province of Genoa), and only one municipality in Lombardy.

The Trebbia River originates from Mount S. Lazzaro in the Ligurian Apennines and flows into the Po, just west of Piacenza, after about 116 km. The main course of the Trebbia can be divided into two distinct sections based on morphological, morphometric, and hydraulic behaviour characteristics: the mountainous section, which extends from the source to Rivergaro, for a length of about 95 km, and the plain section, with a typically braided riverbed, up to its confluence with the Po.

The first section is consistently entrenched, deeply incised into the bedrock, with a morphology characterised by irregular rock meanders, generally with high curvature, evolving slowly. In the final Apennine section, the riverbed tends to straighten and assumes a braided typology. The plain section maintains the braided character, with wide floodplain areas and significant alluvial deposits.

The Trebbia is characterised by a remarkable solid transport capacity, which has been reduced in recent years due to the arrangement of its tributaries. The river has a deeply entrenched bed with rocky banks and steep slopes in the upper part. In the intermediate section, the riverbed consists of materials of low consistency, and in the final section, it flows in a wide cone that extends to its mouth in the Po. There are no significant regulation reservoirs for flood control purposes.

The Enza basin has an overall area of about 890 km^2 (1.3% of the total area of the Po basin), with 64% of it falling within mountainous terrain. The

Fig.1 Geographical context of the Trebbia, Taro, and Enza rivers.

Enza stream originates between the Giogo Pass (1262 m above sea level) and Mount Palerà (1425 m above sea level), near the Tuscan-Emilian watershed. From its source to Canossa, the watercourse flows north-eastward, then predominantly northward until it reaches the plains, where it forms a vast cone with its apex at S. Polo; it then continues embankment until it meets the Po River at Brescello. From its source to its confluence with the Po, the riverbed spans about 100 km in length.
The hydrographic network of the basin is fairly hierarchical compared to other Apennine watercourses, as indicated by low values of the bifurcation index and slight variations in the bifurcation ratio. However, there are various sectors within the basin with erosion-prone channel segments, indicating an evolving situation. An obvious anomaly is the asymmetry between the right-bank sector with a more developed hydrographic network and the left-bank sector. The plain section of the main channel is of a braided type, formed in a paleo-conoid extending for over 20 km, composed of gravel, sand, silt, and clay deposits, conducive to channel meandering; the riverbed is typically wide and shallow with frequent lithoid material banks formations; overall, it exhibits unstable behaviour with frequent meandering phenomena. In the lower section until its mouth at the Po, the riverbed forms frequent meanders, with gentle bottom slopes, in formations consisting of clayey and silty soils.
The three hydrographic basins and their respective river reaches are indicated in Fig.1, with red points marking the locations where surveys were conducted.

The traits interested by the surveys cover an area of about 0,5 km^2 per every stream: the length is about 1 km and the width of the active streambed is about 500 m. These traits have an extension and heterogeneity of habitats that allow them to be considered representative sub-traits of the homogeneous trait of the stream, according to the SUM classification, that is better described in the following paragraph and in the paragraph **2.5.3 - HMU definition.**

2.2 MesoHABSIM Methodology

The MesoHABSIM (MesoHabitat Simulation Model) methodology is designed to assess the hydromorphological status of river reaches with reference to one or more target species, operating at the meso-scale spatial dimension (10^0-10^2 meters). Unlike traditional micro-scale approaches (10^{-1}-10^1 meters) historically used for habitat assessment in fluvial environments [3, 14], the meso-scale framework offers several advantages in terms of both data acquisition and ecological relevance.

First, data collection follows the robust hierarchical structure of morphological classification provided by the IDRAIM system [24], and can be carried out efficiently using lightweight field instruments (see Section **2.4, Field Surveys**). Second, meso-scale resolution enables the inclusion of a broader set of environmental variables to describe river habitats. This facilitates a more comprehensive analysis at both the species and community levels, with the added potential of accounting for biotic interactions among target organisms. Third, MesoHABSIM can be implemented with or without hydraulic simulation models, making it a highly flexible tool suitable for application across both complex and simple morphologies.
At the European level, MesoHABSIM is recognized among the recommended methodologies for morphological assessment [10], and in Italy it has been formally endorsed by ISPRA, the national environmental institute [29].

In the present case study, the methodology was applied through a combination of field mapping and remote analysis. During the field campaign, hydro-morphological units (HMUs) were preliminarily classified in situ at the measured discharge. HMUs represent the physical submerged spatial units at the meso-scale. In the studied rivers, five main HMU types were identified—glide, pool, riffle, backwater, and isolated pond—classified according to flow direction and velocity, channel morphology, and water slope. They are better described in the paragraph **2.5.3 HMU definition**.
Each HMU was further characterized by descriptors essential for habitat suitability assessment, including connectivity with adjacent units, substrate composition, and the presence of specific cover elements.

The HMU raw map was subsequently refined and digitized using Digital Terrain Models (DTMs), orthophotos, and hydrodynamic simulations.
The HMU mosaic and associated descriptors formed the basis for habitat modelling. Analyses were conducted using the Sim-Stream-Web application, which hosts several biological models. In this study, the recently developed macroinvertebrate community model was applied. This model, which assigns a Flow-T value to each HMU, is based on the Random Forest machine learning algorithm [21, 22].
The Flow-T values obtained through this procedure are referred to as *modelled values*. These must be compared against the *observed values* of Flow-T,

which are derived from the laboratory analysis of collected samples in order to be validated.

Moreover, the Sim-Stream platform allows for the generation of habitat availability curves. To construct habitat availability curves, six discharge scenarios were considered for each river reach: one measured in the field and five simulated with the *HEC-RAS 5.0.7* software (see Section **2.5.2, Hydrodynamic Simulation**). For each discharge level, an HMU map was produced and, by calculating an area-weighted average of Flow-T across all HMUs, a mean value was obtained for each discharge scenario.
These values were then used to generate a continuous curve linking discharge rates to predicted Flow-T values. Such curves are further examined in the **4.Discussion** section.

2.3 Flow-T Index

The Flow-T index for macroinvertebrates is a biological index used in freshwater ecology to assess the impact of flow regime changes on aquatic macroinvertebrate communities. This index is particularly useful for evaluating the ecological health of rivers and streams, especially in the context of hydrological alterations such as damming, water abstraction, or flow regulation.
Flow-T stands for Flow Traits, it is a functional index developed by Laini et al. 2022 [17], to evaluate the benthic macroinvertebrate community according to the organisms' flow preference. In ecology, in fact, a "trait" refers to a characteristic or attribute of an organism that can be measured or observed and that influences its fitness, survival, reproduction, or ecological role. For macroinvertebrates the functional trait "current velocity" has been proposed by Tachet et. al, 2010 [25], and a dataset containing more than 500 macroinvertebrate taxa flow preferences has been made available.
Flow-T showed remarkable transferability and applicability with respect to historically used approaches such as Star-ICMI [5] and LIFE index [12]. The latter is an index developed using data from UK rivers and a locally calibrated scoring system. LIFE index is widely used also outside the UK [12], but in every country or site a new calibration and local defined scores have to be developed for each macroinvertebrate taxon, recorded at family or species level. Flow-T on the other hand, proved its efficiency in describing benthic macroinvertebrate communities precisely in relation to the performance of the LIFE index despite the absence of on-site specific scoring assignment,

this allows the use of Flow-T without the geographical area limitation [17].

Flow-T is calculated for each sample using the current velocity preference categories, defined by Tachet et. al (2010), according to the following formula:

$$Flow\!-\!T = \frac{\sum_i^n \log\left(A_i + 1\right) \times \left(m_i + f_i\right)}{\sum_i^n \log\ \left(A_i + 1\right)} \times 100$$

In the above n represents the number of taxa in a sample, A_i the abundance of the i^{th} taxon, m_i and f_i the “moderate” and “fast” velocity preference classes of the i^{th} taxon.
The Flow-T index can be applied at different taxonomic resolutions and at various spatial scales.

A mesoscale habitat model for benthic macroinvertebrates has recently been proposed by Pinna, 2024 [22] and a validation of the model is performed in this thesis work.
The physical habitat model has been developed using *Random Forest* RF, a robust machine learning technique [30], that creates combinations of a high number of decision trees (thus creating a “forest”) and is then able to assign a rank to the different variables in input with the aim to select the minimum number of non correlated variables. The model has been built compatible with the MesoHABSIM methodology, meaning that the same variables and HMU descriptors have been used to train the model. In the end the variables with the higher rank were twelve, and are reported in Tab.1, and the relative partial dependance plots are reported in Fig.2 These variables are the minimum independent ones, thus they have been used to define the Flow-T parsimonious model.

Tab.1 Indipendent variables used to define the parsimoniuos Flow-T model [22].

INDEPENDENT VARIABLE	VARIABLE TYPE AND RANGE
PELAL	Substrate (<0.06 cm, clay)
MICROLITHAL	Substrate (2-6 cm, coarse gravel)
CV75_90	Class of current velocity (75-90 cm/s)
CV45_60	Class of current velocity (45-60 cm/s)
CV60_75	Class of current velocity (60-75 cm/s)
CV90_105	Class of current velocity (90-105 cm/s)
CV120	Class of current velocity (>120 cm/s)
CV15_30	Class of current velocity (15-30 cm/s)
CV15	Class of current velocity (< 15 cm/s)
CONNECTI-VITY	Categorical (True-False)
D60_75	Class of water depth (60-75 cm/s)
D15_30	Class of water depth (15-30 cm)

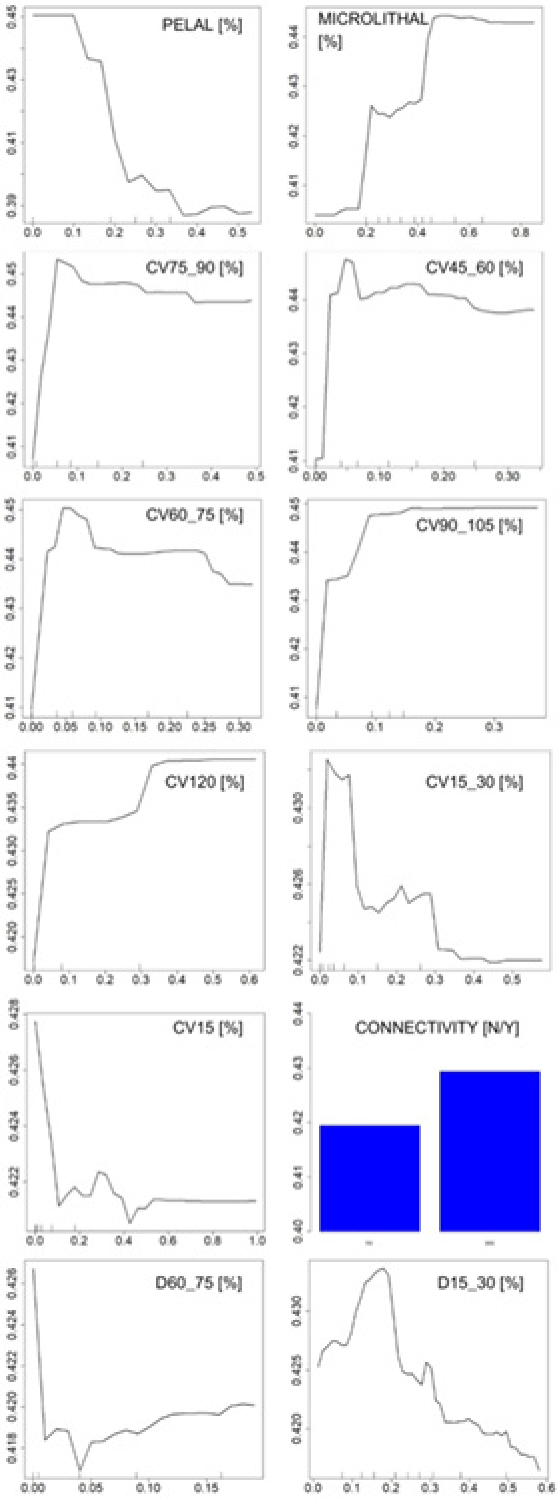

Fig.2 RF Parsimonious Model's Partial Dependence Plot, taken by Pinna et. al 2024 [22].

2.4 Field Surveys

Field surveys have been carried out between the 25 and the 28 of June 2023. The discharge measured in the three traits were about 2,50 m^3/s in the Trebbia, 2,00 m^3/s in Taro and 0,5 m^3/s in Enza.

The aims of the surveys were to obtain topographic, bathymetric and biological descriptions of the sites. Eight operators were involved in the survey activities. The author participated both in the Biological Surveys in the Trebbia River and in the Bathymetric Surveys in the Taro and Enza rivers.

For the topographic description it has been used UAV technology and photogrammetry elaboration. Two operators realised the Ground Control Points GCP with spray washable markers and GNSS antenna (**Fig.3**) while an other operator designed and performed the drone flight.

The bathymetric survey has been carried out using a floating plobe able to register water depth and velocity in every vertical column: three operators were involved in this activity.

Fig.3 Ground Control Points realization during field survey using GNSS antenna and colored washable spray.

Finally, two operators realised the biological survey consisting in the collection of macroinvertebrates samples using a surber net with fine grid and a plastic jar collector. These samples were taken in a representative number of HMU in every trait, they were filtered and collected in alcohol to then be examined in the laboratory.

2.4.1 Topographic Survey

The photogrammetric surveys have been realised by the Ing. Luca Astegiano using UAV device *DJI Phantom 4 Rtk* (**Fig.4**) flying at a height of about 50 m to gain a GSD between 2-3 cm.

To obtain the three traits' ortho-mosaics and Digital Terrain Models DTM the software *Pix4D Mapper Pro* (Pix4D SA, Losanna, Switzerland) was used. It is based on structure from motion techniques that utilise 2D images in series to reconstruct a 3D scene. Implementing also the georeferenced GCP, very high performances, in terms of resolution and accuracy, can be reached.

Fig.4 DJI Phantom 4 RTK Uncrewed Aerial Vehicle UAV used in the field surveys to obtain the topographic description

2.4.2 Bathymetric Survey

Photogrammetry alone is not sufficient to appropriately describe the entire surface area: when water has depth greater than 20 cm an optical effect generates a distortion that cannot be automatically corrected with photogrammetric techniques. So, the bathymetry shape in critical sections was captured together with the velocity field using the sensor *RiverSurveyor M9* (Sontek, San Diego, CA, USA) set on a buoyant platform (**Fig.5**). The data collected using this method were both used to define the flowing discharge in

the defined section, to assess the bathymetric model correction and, in the end, to validate the hydraulic model.
The technology is based on an ADCP device that uses sound waves to measure the speed and direction of currents throughout the water column. The system operates based on the Doppler effect, caused by the interaction between emitted waves and water waves, with a temporal acquisition frequency of 1 second. This setup allows for tracking relative displacements from the initial measurement point. To achieve a georeferenced morphological description, the acquisition system is supplemented with a dual-frequency GNSS (Global Navigation Satellite System) antenna. The temporal acquisition frequency of the GNSS is 4 seconds, ensuring that every three depth/velocity points measured by the boat include one georeferenced point. Coordinates for all measured points are interpolated using the recorded track of the boat. The sensor, combined with the software *RiverSurveyor Live (RSL) - V 4.3* enabled a real-time acquisition (**Fig.5**) of high-precision depth data, specifically measuring two types of depth: Bottom Track and Vertical Beam depth. The Bottom Track method records the vessel's speed over the ground, providing approximate depth values, especially when GPS is unavailable. Alternatively, the system offers an RTK GPS option for ultra-precise positioning. The Vertical Beam method is used to obtain more accurate measurements of water depth. At the same time, the system computes velocity profiles along the water column at each sampling point, generating a grid of mean velocity across the entire section.

In addition, the *RiverSurveyor Live* software allows for the extraction and visualization of the acquired data, including the georeferenced track of the vessel, the morphology of the stream bed, water depth, and velocity distribution. Importantly, by analysing an appropriate cross-section of the river, the system also enables the determination of flow rate.

The bathymetric surveys were carried on working within a three-member field team. These operations required two operators to maneuver the floating platform and one to manage, in real time, the LiveStream software connected to the instrumentation. These operations were conducted under the coordination of Dr. Beatrice Pinna, with the support of Eng. Gianmarco Manfreda and of the author of the present work. All three operators alternated between tasks for both didactic and logistic purposes. The author only participated in the bathymetric surveys of the Taro and Enza rivers. The elaboration of the data were, mostly, developed by Dr. Pinna, being that the RTK sincronization, the discharge evaluation and the data cleaning.

Fig.5 Up-left: River Surveyor M9 Acoustic Doppler senson. Up-right: Bouyant platfonrm containing the ADCP sensor and the GNSS antenna in use during field surveys. Down: River Surveyor Live software in use during field surveys.

2.4.3 Biological Survey

The collection of macroinvertebrates samples has been carried on thanks to the collaboration with the DBIOS, University of Turin, under the co-ordination of the Professor Alex Laini. The author assisted in the biological surveys conducted on the Trebbia River, supporting the collection of water current velocity data using a current meter and recording the survey positions with a laser rangefinder. When not simultaneously employed for other survey operations (e.g., GCP acquisition), the GPS antenna was occasionally used to define the position of the biological sampling point in the place of the rangefinder, as shown in Fig.6.

The operators used a surber net with a mesh of 500 μm and frame 0.05 m^2, the aim was the description of macroinvertebrates community and then the evaluation of the Flow-T index. For each river a number of ten HMU has been sampled, two for each HMU type (glide, riffle pool, backwater and isolated pond). In every HMU, ten samples distributed among the HMU area according to a random stratification procedure were taken. The random stratification procedure involves selecting samples that accurately reflect the variations in substrate composition, water depth, and flow velocity within a habitat unit (HMU). The procedure allocates the number of samples proportionally to the prevalence and characteristics of each distinct area within the HMU. This is done to ensure that all significant variations in the physical environment are adequately represented in the sampling process.
Formally, the procedure can be described as follows:

- Identify Strata: Divide the HMU into distinct strata based on variations in substrate composition, water depth, and flow velocity.

- Determine Proportions: Assess the proportional area of each stratum within the HMU.

- Allocate Samples: Assign the number of samples to each stratum proportionally to its area. This ensures that strata occupying a larger area within the HMU are sampled more extensively, while smaller strata are sampled less frequently.

- Random Sampling: Within each stratum, select sampling points randomly to capture the variability within the stratum.

This procedure ensures a comprehensive and representative sampling of the physical characteristics within the HMU, facilitating accurate ecological assessments and analyses.
The samples were taken from down-stream to upstream in order to not cause disturbances and local alteration in the macroinvertebrates community. Each survey is conducted by positioning the mouth of the net upstream so that the current flows into it. The substrate in front of it is then moved using hands or feet for a period of 3-5 minutes. The samples collected this way were filtered using a sieve, and then conserved in plastic jars in denatured alcohol 90º.
Every sample spot has been georeferenced using a fixed point, visible from the orthophoto, and a rangefinder or GNSS antenna (see Fig.6). Simultaneously the mean vertical flow velocity $v_{m,v}$ and the bottom one, $v_{b'}$ have been registered using a current meter, according to the formula $v_{m,v} = v_{0.6d'}$ in which $v_{0.6d}$ is the velocity value measured at the 60% of the punctual depth.
The samples have then been analysed in the laboratory. The macroinvertebrates were identified under a stereomicroscope at a mixed taxonomic level. For Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Heteroptera, and Mollusca, identification was carried out at the genus level, while for other taxonomic groups, it was done at the family or subfamily level. The organisms were separated from the detritus to determine their dry weight by drying in oven at 105ºC. The coarse organic component of the detritus (size >1 mm) was separated from the mineral fraction through elutriation and weighed after drying in an oven at 105ºC.

2.5 Hydromorphological Data Analysis

Using photogrammetric and bathymetric survey data, a comprehensive description of the topography of the Trebbia, Taro, and Enza sites was obtained, these outputs were provided thanks to the work of Eng. Luca Astegiano.

The DTMs riverbeds were then reconstructed, within a GIS environment, by integrating boat-acquired bathymetric data with the 3D models derived from photogrammetric surveys. The resulting DTMs served as the basis for two-dimensional hydrodynamic simulations conducted in the HEC-RAS environment. These simulations were validated against field measurements collected during the surveys. Once satisfactory agreement was achieved, the DTMs were used to perform two-dimensional flow simulations at the same measured and simulated discharges recorded during the 2020 field

Fig.6 Up-left: Biological sampling with surber net and GNSS antenna. Up-right: rangefinder used for positioning when GNSS available not available. Down-left: Detail of a biological sample. Down-right: local depth and flow velocity description using current meter and paper notes. Also the alcoohol and plastic jars for samples conservation and trasport are visible.

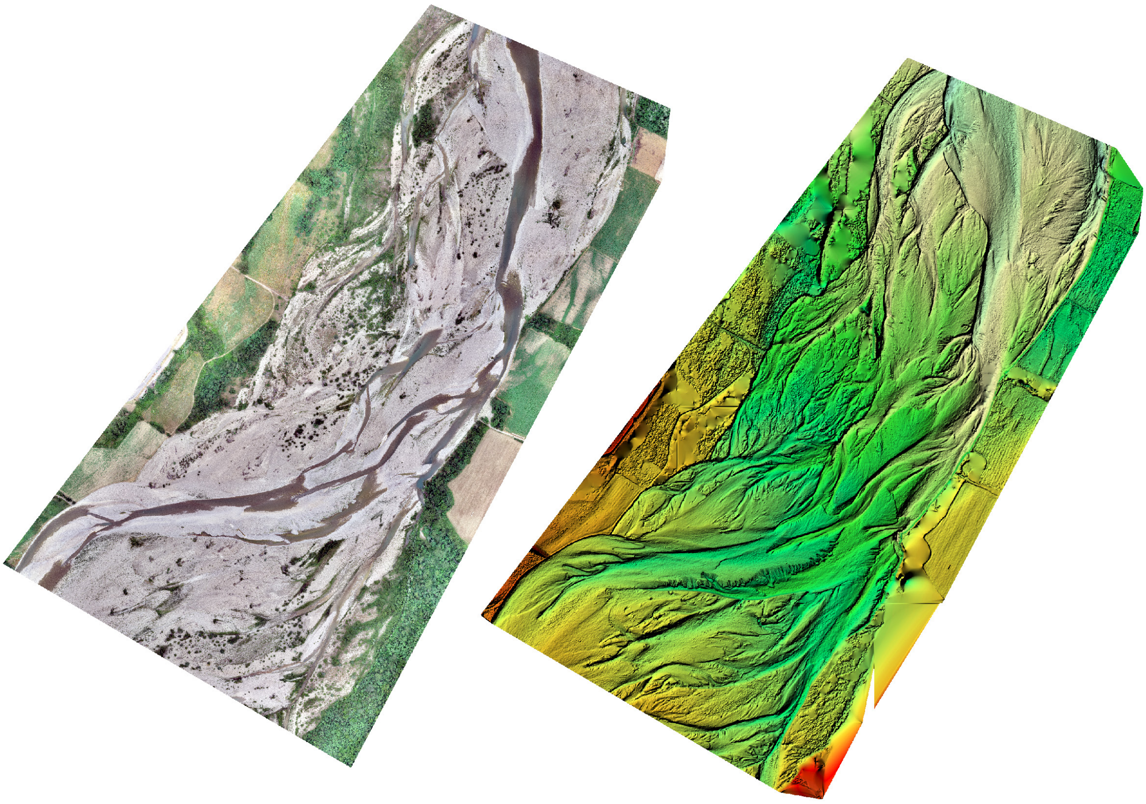

Fig.7 Representative output of the photogrammetric process. Left: Taro river orthomosaic (2 cm resolution). Right: Taro RIver Digital Terrain Model DTM (10 cm resolution).

campaign, thereby enabling a direct comparison between the two years. Beyond the assessment of wetted areas under varying flow conditions, the hydrodynamic simulations and DTMs were further employed to delineate hydro-morphological units (HMUs), following the guidelines of the SUM manual. These HMUs, corresponding to the mesohabitat spatial scale, are suitable for the application of the MesoHABSIM methodology, and were enriched with information on cover and substrate composition. Properly formatted shapefiles describing HMUs at different discharges were subsequently uploaded to the online Sim-Stream platform. By selecting benthic macroinvertebrates as the target group, Flow-T rating curves were generated for the three study reaches.

The author realized the entire procedure for the Taro River, while the two simulations at the measured discharge for Trebbia and Enza were carried on by Doc. Pinna.

2.5.1 DTMs Corrections

The photogrammetric survey produced orthomosaics in RGB and DTMs of the three sections with a spatial resolution of 2 cm and 10 cm, respectively. As an example, the Taro photogrammetric products are shown in **Fig.7**.
Since the DTM is to be used as a basis for hydraulic modelling, the refractive effect of water must be taken into account. The refraction of water in the construction of the DTM has the effect of underestimating depths. In fact, the optical ray, when passing from a less dense medium, such as air, to a denser medium like water, is distorted as described by Snell's law and illustrated in **Fig.8**.
The ratio between the real depths and the fictious depths corresponds to the ratio between the refractive index of water and that of air. Since these are 1.33 and 1.0003 respectively, the following relationship is obtained:
$D_r = 1.33\ D_f$
Where D_r are the real depths that we want to evaluate and D_f are the fictious ones contained in the DTMs. The abovementioned relation is the one valid in the ideal case and, in the field of riverine analysis, is called bathymetric model.
The coefficient in the linear relationship can vary due to different factors, among which the illumination conditions of the day, the clarity of the water, the drone flight angle and height. To evaluate the distortion coefficient relative to the survey days and settings, the depth points measured within the scope of the bathymetric survey were considered as *real depths* and used to

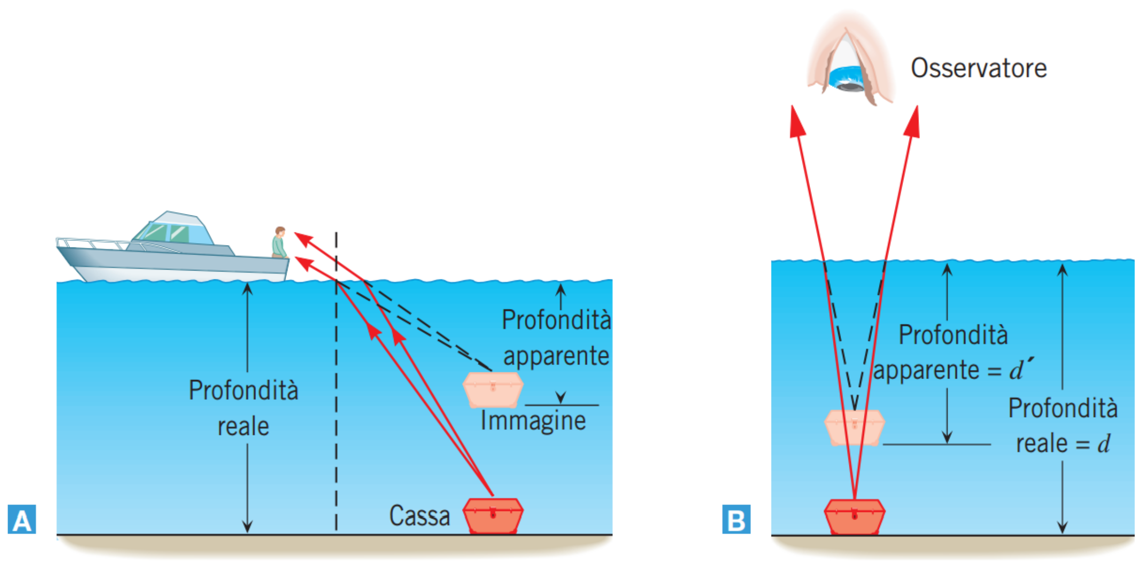

Fig.8 Snell's Law optical distortion principle. Figure modified from John D. Cutnell 2012 [9].

calibrate the bathymetric model. The real values measured by the *River Surveyor* sensor and the fictious ones contained in the DTM in the corresponding positions are plotted, and a linear relation among the two can be derived by linear regression as shown in Fig.9.
The coefficient obtained in this way, by using a little number of real values, can be used to derive all the remaining. For each river and stream a coefficient was found.
It has been proven that for depths lower than 20 cm the distortion effect is negligible; the correction equation is then used only for the pixel values of the DTM which fall within a wet area deeper than 20 cm. Using the proper bathymetric model in each of the three traits of Taro, Trebbia ed Enza, the corrected DTMs provided with real depth values are obtained.

The author developed the bathymetric model of the Taro River, while Dr. Pinna produced those of the Trebbia and Enza rivers.

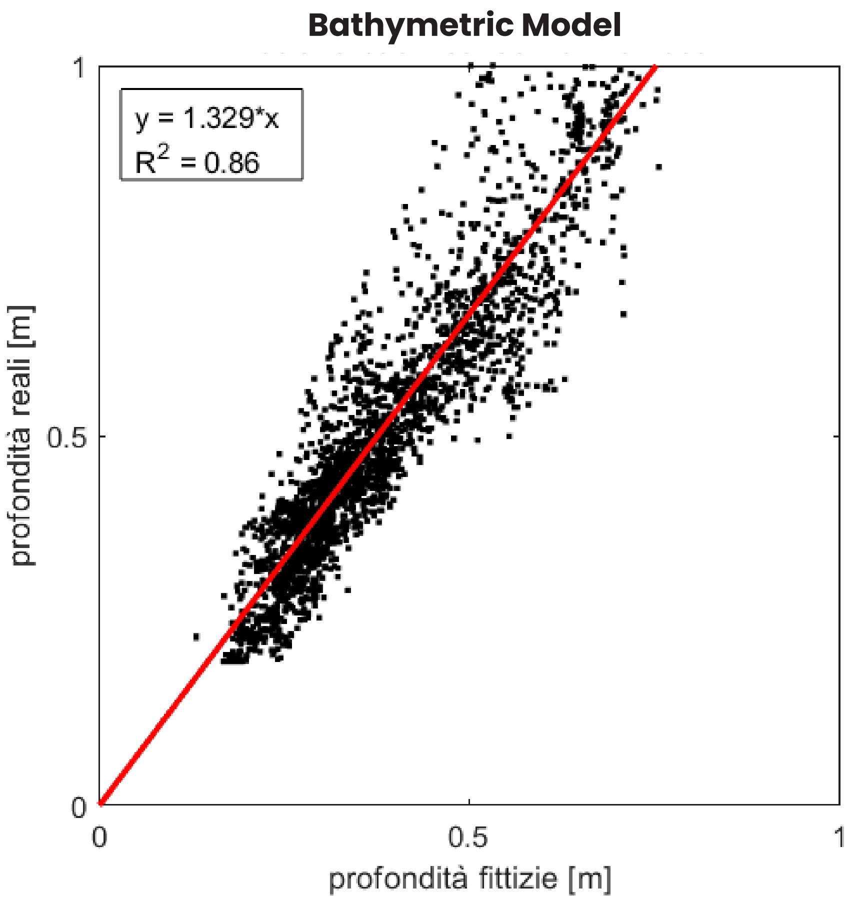

Fig.9 Bathymetric model obtained by the linear interpolation between *real depth* (*River Surveyor* measured values) and *fictious depths* (DTM derived values).

2.5.2 Hydrodynamic Simulations

The 2-D hydrodynamic simulations on the Trebbia, Taro ed Enza traits were performed using the software *HEC-RAS* (US Army Corps of Engineers, Hydrologic Engineering Center, Davis, California) version 5.0.7.
The software is widely used to perform hydraulic simulations, it has several modes, spatial scale options, and can be used with different focuses. In the case of braided river flow simulation, a 2D unsteady flow analysis was chosen. The 2D unsteady flow analysis in HEC-RAS is based on the definition of a 2D flow area that is the domain in which the continuity and momentum conservation equations are solved.

The continuity equation is the following:

$$\frac{\delta H}{\delta t} + \frac{\delta(hu)}{\delta x} + \frac{\delta(hv)}{\delta y} + q = 0$$

In which H is the hydraulic tie rod, while u and v are the horizontal components of the velocity vector (the vertical component is neglected in 2D analysis). The term q considers possible source/sink contributions.

The momentum equation is:

$$\frac{\delta V}{\delta t} + V\nabla V = -g\nabla H + v_t\nabla^2 V - c_f V + fk \times V$$

The above, exploded in the two horizontal components of the velocity $V(u,v)$ becomes:

$$\frac{\delta u}{\delta t} + u\frac{\delta u}{\delta x} + v\frac{\delta u}{\delta y} = -g\frac{\delta H}{\delta x} + v_t\left(\frac{\delta^2 u}{\delta x^2} + \frac{\delta^2 u}{\delta y^2}\right) - c_f u + fv$$

$$\frac{\delta v}{\delta t} + u\frac{\delta v}{\delta x} + v\frac{\delta v}{\delta y} = -g\frac{\delta H}{\delta y} + v_t\left(\frac{\delta^2 v}{\delta x^2} + \frac{\delta^2 v}{\delta y^2}\right) - c_f v + fu$$

In which g is the gravitational acceleration, v_t is the horizontal eddy viscosity coefficient, c_f is the bottom friction coefficient and f is the Coriolis parameter. On the left-hand side of the equations there are the acceleration terms, while on the right-hand side the internal or external forces acting on the fluid can be found.

The outputs obtained by HEC-RAS hydraulic simulations can be various. A shapefile and three rasters for every simulated discharge are extracted:

The shapefile that contours the wet area
The depths raster, that in every pixel has a value of depth
The velocities raster, that in every pixel has a value of velocity
The water surface elevation raster (WSE), that in every pixel has a value of WSE

In the RAS-Mapper interface, the first input to insert is the terrain surface, that is a raster file containing, in every pixel, a value of surface elevation. In the cases under examination the terrain surfaces are the DTMs obtained by photogrammetric survey and then corrected in GIS environment according to the specific bathymetric model (see previous paragraph).
The DTM is imported with the default rounding precision of 1/128 and original resolution: in the studied cases all three DTMs have a spatial resolution of 10 cm.
Secondly the orthophoto in transparency can be useful to properly assess where the riverbed and the wet areas are located and correctly sketch the 2D floodable area.
In fact, the DTM is read by the software like an impermeable surface, some of

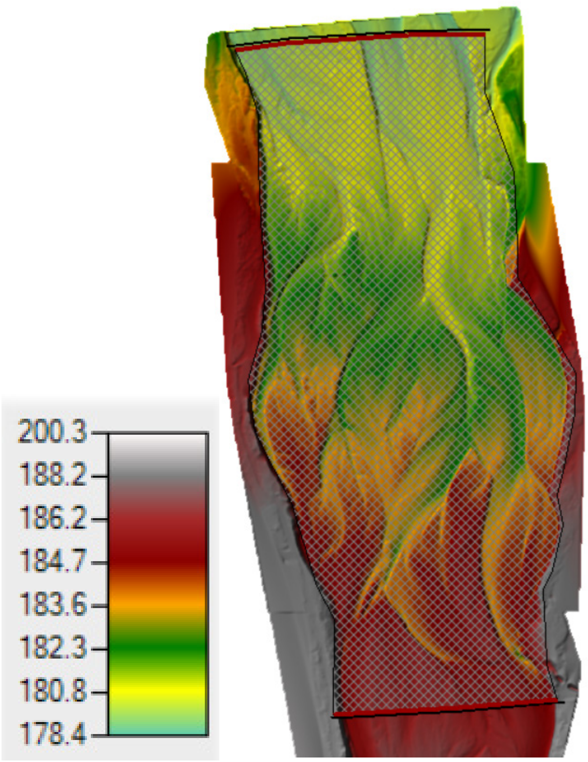

Fig.10 HEC-RAS environment: terrain, floodable area and boundary conditions.

which can be flooded through defined permeable boundary conditions lines. For example, in **Fig.10** there is the Enza 2D flow area, in red the boundary condition lines (south: upstream inflow, north: downstream outflow).
Once defined the 2D flow area perimeter, the software creates a mesh with a given edge size and generates several computation points. The given edge size for all three Trebbia Taro and Enza traits is 1 m. By **Fig.11** the mesh detail can be appreciated. It is made by squares of 1m x 1m, is generated inside the domain perimeter, over the DTM and at the centre of every square there is a point in which the software solves the water flow equations.

In this phase is given as input also the Manning's coefficient, that is a measure of the surface roughness.
The determination of the Manning's coefficient for the considered streams are the result of previous analyses. To be specific the Taro's Manning is 0.036, while Trebbia's and Enza's Manning are both 0.035. The first simulation in every trait is done using the flow rate measured during the field campaign with *RiverSurveyor* system:

Q= 2,00 m^3/s for Taro
Q= 2,50 m^3/s for Trebbia
Q= 0,50 m^3/s for Enza

The first evaluation of the correctness of the results is a visual comparison between the wet area obtained by the simulation and the one visible from the orthophoto.
If the two do not correspond adjustments in floodable area, boundary conditions extension and location can be made. Since the systems we aim to describe are very complex it is possible that this phase proceeds through trial and error and the optimal input conditions are not trivial.
Moreover it has to be taken into account that riverbeds are not impermeable surfaces and, especially in complex systems as braided rivers, some water volume can be exchanged with sub-channels. Yet these interactions are difficult to model, especially at the wide scale for which these analyses are being carried out. We hypothesise these contributions are negligible given that discharge measurements upstream and downstream are consistent.
Once an acceptable configuration of the wet area is reached in the first simulation, a numerical validation is performed for every trait in terms of depths accuracy reached.
To do this the depth values obtained by the simulations are compared with

the ones measured during the field campaign. It means that the depth values contained in the pixels that spatially correspond to the points measured using River Surveyor are extracted and plotted in a scatter plot and their correlation coefficient R^2 and root mean square error value $RMSE$ are calculated.
Also in this case, if R^2 and $RMSE$ are unacceptable, the procedure must be repeated changing parameters or their combinations in the previous steps.
Once performed the validations it means that an accurate correction of the DTM has been reached, therefore it can be used as the basis for hydrodynamic simulations at different values of discharge.
The chosen values of discharge correspond to the ones measured/simulated in 2020. The values of discharge used are summarised in **Tab. 2**, in bold the discharge values measured during the 2023 field survey campaign.

The author carried out sixteen of the eighteen total simulations, while the two simulations at measured discharge for the Trebbia and Enza rivers were performed by Dr. Pinna during the DTM correction phase.

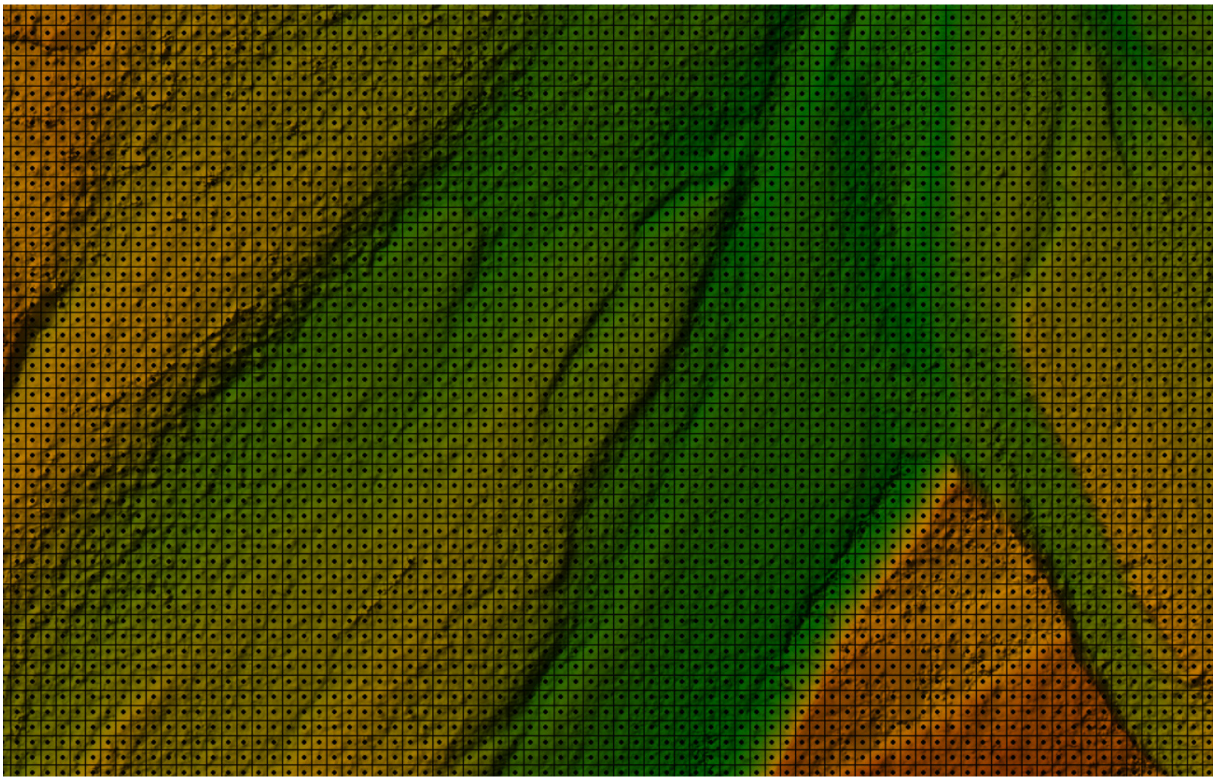

Fig.11 HEC-RAS environment: mesh detail. The squares are 1mx1m, at the center of every square there is the point in which the flow equation are solved.

Tab.2 Discharge values in m^3/s simulated per River.

Q (m^3/s)	TARO	TREBBIA	ENZA
Q_1	0.8	0.5	0.33
Q_2	**2**	1.65	**0.5**
Q_3	3	**2.5**	1
Q_4	6.4	5	1.5
Q_5	13	10	3
Q_6	20	20	5

2.5.3 HMU Definitions

The HMU definition phase is again performed in GIS environment. As for the preliminary classification performed on the field, the remote classification of HMU is based on the SUM methodology [23]. This methodology is robust and versatile, it provides a comprehensive definition of the morphological units of the riverbed and the floodplain, but in the case of the application of the MesoHABSIM methodology only the classification of submerged units is required.

The author developed the HMU mosaics for all the eighteen hydrodynamic simulations, and corredated each HMU with the covers, connectivity and substrate data.
In the study cases the HMU expected are mostly glide, riffle and pools together with backwaters and isolated ponds. The reaches, in fact, are unconfined and slightly inclined, they are in the alluvial plain downstream of the mountain range from which they originate, and the abovementioned meso-scale morphologies are typical of these traits.

The definitions taken from the SUM manual follows:

GLIDE
"These units are characterised by a rather regular longitudinal profile of the riverbed and the free surface. The free surface may appear slightly rippled but is nearly parallel to the riverbed (conditions very close to those of uniform flow) and visually shows little turbulence and aeration. [...] Nevertheless, glides are also common in low-gradient gravel-bed streams as well as in sandy-bottomed streams, where they are typically located downstream of pools or upstream of riffles."

RIFFLE

"These units are characterised by shallower and faster flow compared to adjacent units, composed of relatively uniform sediment (gravel and small pebbles) that rarely emerge from the water. The differences in depth and velocity between riffles and adjacent units (typically pools and glides) decrease as discharge increases. Riffles form at points of inflection between the banks in meandering alluvial streams, where the channel is dominated by a sequence of alternating bars."

POOL

"These units are primarily erosional in origin and appear as topographic depressions on the riverbed with a downstream sloping portion, occupying the entire cross-sectional area. They are characterised by relatively high depths and reduced velocities, but also by complex hydrodynamic features (turbulent fluctuations). The sediment often appears finer compared to adjacent units, in cases where sedimentation has occurred, otherwise it can also be very coarse. Pools often alternate with steps or riffles, respectively in high-gradient streams with boulders and pebbles and in gravel-bed channels, but they can also be found in sandy-bottomed streams associated with meanders. There are indeed numerous processes underlying their formation, thus giving rise to different subtypes."

BACKWATER

"These are generally areas located at the margins of the low-flow channel, corresponding to indentations in the shoreline (e.g., between two successive trees along the bank, following erosion) or individual woody elements that cause backflow and consequent slowing of the current near stagnant water. They can also form upstream of woody debris accumulations (dam jams). These areas are often used as refuge zones from the current for numerous aquatic organisms and promote the development of aquatic vegetation in river stretches where this would be limited by the high turbulence of the current."

ISOLATED POND

"An isolated pool is a morphological unit that is most commonly found in streams with multiple channels; it consists of a portion of a secondary channel in the riverbed completely disconnected from the rest of the low-flow channel network."

The velocity, depth and WSE rasters obtained by the hydrodynamic simulations in HEC-RAS for every discharge value are imported in ArcGIS Pro. Then an appropriate symbology representation is chosen. Depth and velocity rasters' values are divided in classes, according to the ones on which the MesoHABSIM is based, then a color scale is assigned to each class. The classes intervals are the following (*m* for depths or *m/s* for velocities):

1st class:	0-0.15
2nd class:	0.15-0.30
3rd class:	0.30-0.45
4th class:	0.45-0.60
5th class:	0.60-0.75
6th class:	0.75-0.90
7th class:	0.90-1.05
8th class:	1.05-1.20
9th class:	> 1.20

At the WSE rasters an automatic visualisation is assigned, it allows to easily distinguish more inclined surfaces from flat ones.
Combining the layers is possible to evaluate the subdivision among different HMU, an example is shown in **Fig.12**.

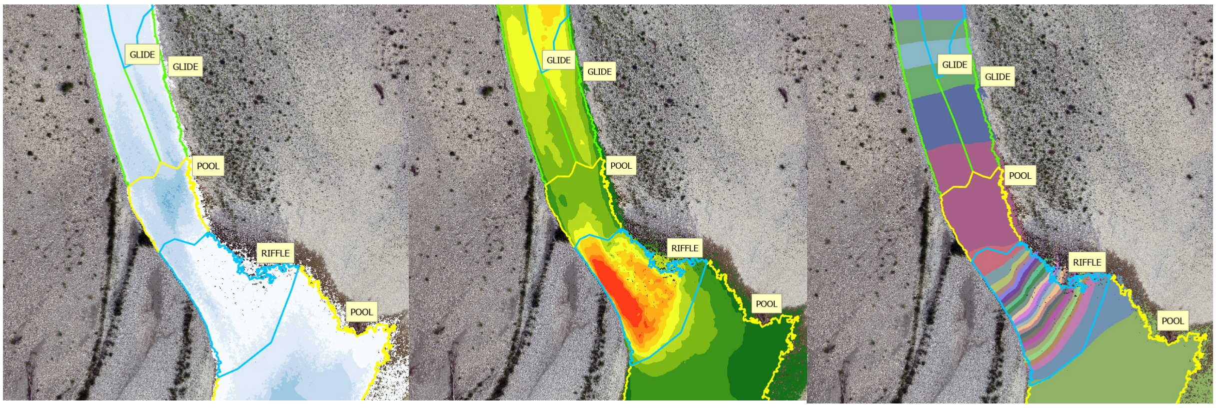

Fig.12 Example of HMU definition according to water depth, velocity and surface elevation rasters classified visualization.

3. Results

3.1 Field Surveys

3.1.1 Topographic Results

The topographic surveys resulted in the production of three orthomosaics and corresponding DTMs, which are presented in Fig. 13. A total of 8 010 images were calibrated, and 171 georeferenced GCPs were employed. The subdivision of these data by river reach is provided in Tab. 3, which also reports the average number of keypoints per image and the associated RMSE values. The average ground sampling distance (GSD) was 2.61 cm for the Trebbia River, 2.91 cm for the Taro River, and 2.64 cm for the Enza River. All DTMs were produced at a resolution of 10 cm/pixel and subsequently upscaled using the Inverse Distance Weighting (IDW) method.

Tab.3 Photogrammetric process details per reach.

	TREBBIA	TARO	ENZA
Mean Keypoints per image	12471	14122	14224
N° of calibrated images	3294	3572	1144
GCPs	71	47	53
$RMSE_x$ [m]	0.044	0.049	0.037
$RMSE_y$ [m]	0.034	0.043	0.04
$RMSE_z$ [m]	0.022	0.034	0.019
$RMSE_{mean}$ [m]	0.033	0.042	0.032

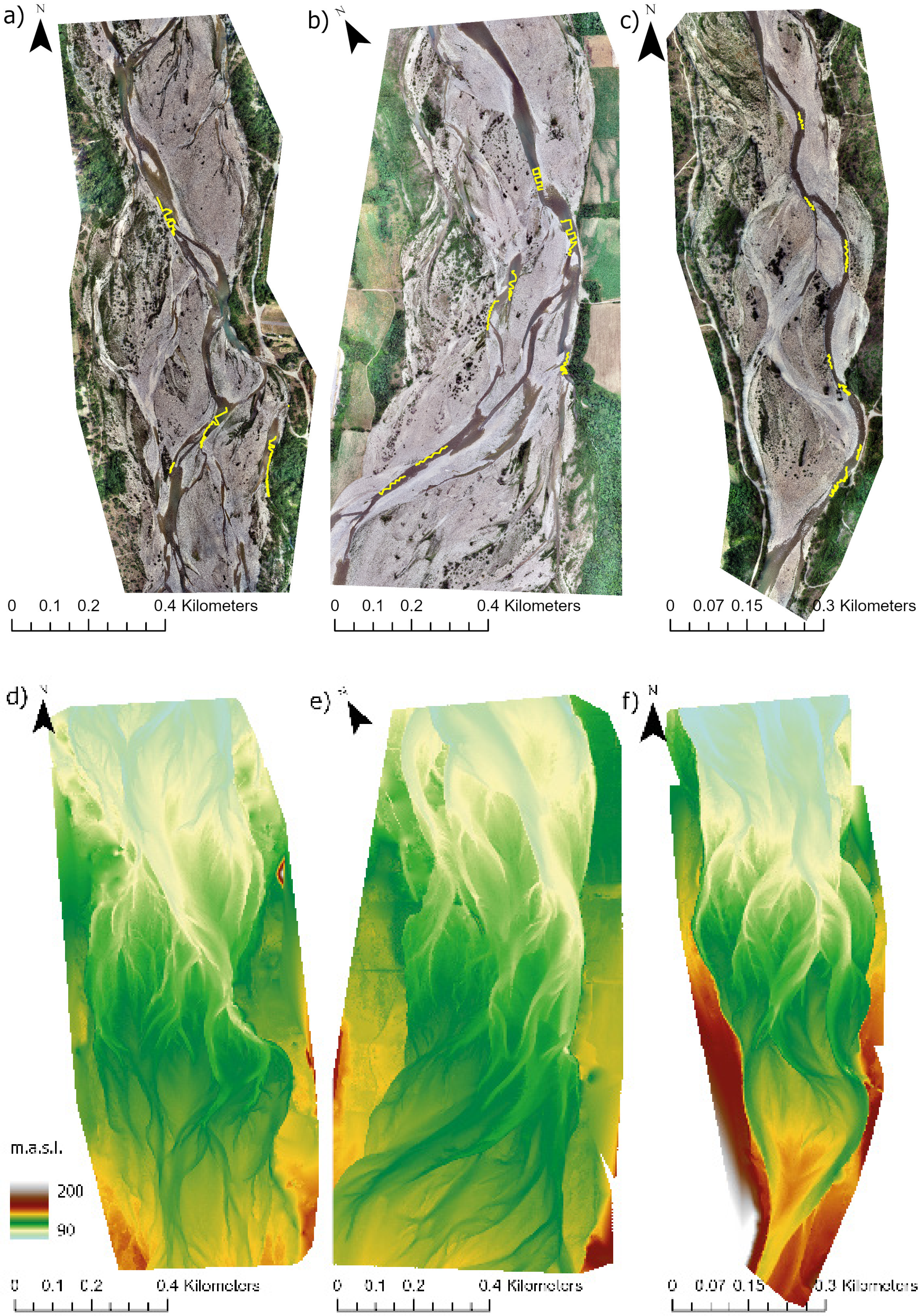

Fig.13 Thopographic results for Trebbia a), d) Taro b), e) and Enza c), f). For each stream an orthophoto with 2 cm resolution and a DTM with 10 cm resolution were obtained. In yellow the track of the floating platform used for the bathymetric model.

3.1.2 Bathymetric Results

Bathymetric results also are shown in **Fig. 13 a) b)** and **c)**. The yellow lines represent the tracks of the floating platform, in Trebbia **(a)** there were 4 takens for a total of 3011 points. In Taro **(b)** seven different segments were taken for a total of 3388 points. Finally in the Enza **(c)** stream there are 2551 points in seven traits. In each of these points values of water depth and velocity were taken. In straight section also the values of discharge have been measured. The discharges measured upstream in the three rivers are consistent with the ones measured downstream, meaning that the submerged flow can be disregarded. The discharged measured in Trebbia, Taro and Enza were respectively of 2.5, 2.0 and 0.5 m^3/s.

Using this data, and the ones modelled in the hydrodynamic simulations, it was possible to define the three bathymetric models, with bathymetric coefficient of 1.351, 1.329 and 1.386 respectively for Trebbia, Taro and Enza (**Fig. 13**).

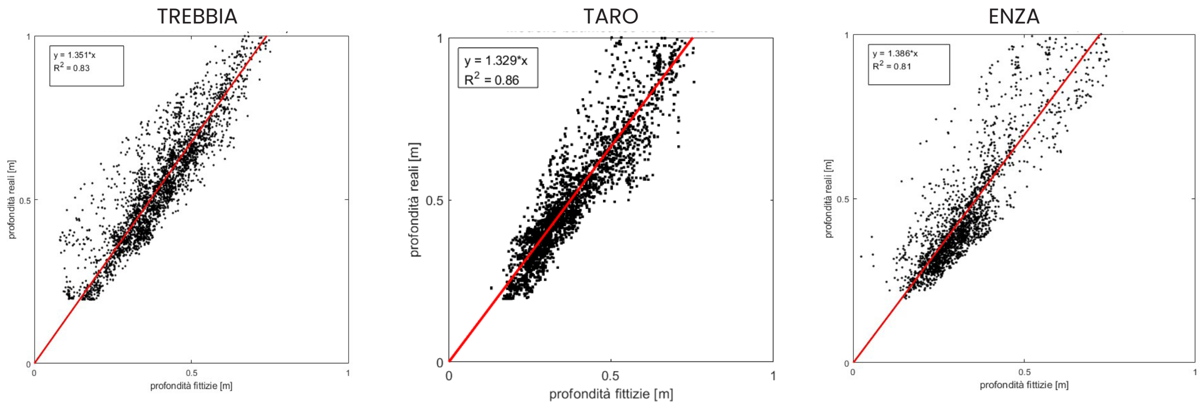

Fig.14 Bathymetric model and bathymetric coefficient for Trebbia, Taro and Enza.

3.1.3 Biological Results

During the biological sampling, a total of 30 samples were collected, 10 for each river, 6 for each HMU type. In total, 40 200 organisms were classified, with Chironomidae (Diptera), Simulidae (Diptera), and Baetis (Ephemeroptera) being the most abundant taxa.

These 40 200 individuals belong to 97 taxa (95 + Cladocera + Copepoda). Of these 97 taxa, 53 were classified at the genus level, the other 44 at the family level or higher.

Flow-T values derived from field samples ranged between 0.233 and 0.573 (**Tab. 4**) in which it can be appreciated that riffles, as expected, have the higher values of Flow-T, while isolated ponds the lower ones.

Tab.4 Flow-T values calculated per every sample.

	HMU	Flow-T
TREBBIA	BACKWATER 31	0.266
	BACKWATER 96	0.326
	GLIDE 20	0.421
	GLIDE 90	0.357
	POOL 30	0.298
	POOL 95	0.273
	RIFFLE 25	0.487
	RIFFLE 89	0.498
	ISOLATED POND 102	0.278
	ISOLATED POND 103	0.251
TARO	BACKWATER 17	0.356
	BACKWATER 66	0.446
	GLIDE 19	0.541
	GLIDE 63	0.317
	POOL 45	0.329
	POOL 59	0.257
	RIFFLE 18	0.573
	RIFFLE 64	0.429
	ISOLATED POND 77	0.295
	ISOLATED POND 79	0.233
ENZA	BACKWATER 16	0.315
	BACKWATER 22	0.356
	GLIDE 5	0.465
	GLIDE 13	0.431
	POOL 11	0.403
	POOL 23	0.383
	RIFFLE 6	0.512
	RIFFLE 9	0.504
	ISOLATED POND 30	0.337
	ISOLATED POND 31	0.394

3.2 Hydromorphological Results

3.2.1 DTM Corrections

Starting from the DTMs obtained by the potogrammetric processing a bathimetric model for each stream was realized (Fig. 14) and used to correct the DTM.
In the virgen DTM the points with depths greater than 20 cm were extracted, in these zones the depth value of every pixel was increased by a factor around 1.33, according to the proper trait's bathymetric model. The areas corrected this way were then mosaicked back in the original DTM (Fig.15).
The corrected DTMs have then been used as base for the hydrodynamic simulations.

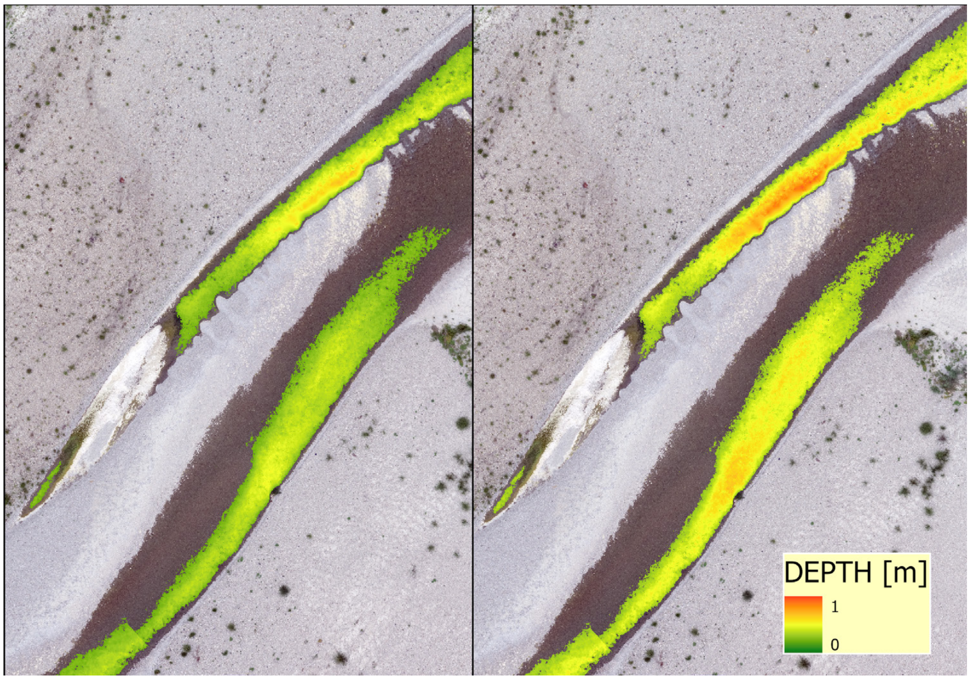

Fig.15 DTM correction: Taro detail. On the left the areas with depths greater than 20 cm before the correction. On the right the same areas after the correction. It can be appreciated that depths are greater after the correction.

3.2.2 Hydrodynamic Simulations

The hydrodynamic simulations were performed as descibed in the paragraph 2.5.2. At first the simulations were performed for the three river traits imposing an inpunt of discharge rate equal to the one measured during the field campaign. The outputs obtained were, per each trait, a raster of depths, one of velocities and one of water surface elevation. These were uploaded in GIS environment

and can be appreciated in **Fig.17**. It can be observed that the wet area obtained by the simulations perfectly ovelaps the one visible from the ortophotos. It can also be noticed that zones with greater depth correspond to lower velocities, while zones with greater velocities also have higher slopes of water surface. These results are physically consitents.
To objectively verify the goodness of the hydrodynamic simulation results a validation of depth values has been perfomed.
For every yellow point in **Fig. 13 - a) b) c)** the measured depth value was compared with the simulated depth value in the corresponding rater's pixel in **Fig. 17 - a) b) c)**. The scatter plots of the comparison and their linear regressions are reported in **Fig. 16**.
The depth validation performances were high considering R^2 values of 0.79, 0.98 and 0.93 respectively for Trebbia, Taro and Enza, therefore the corrected DTMs were considered valid and have been used to perform the hydrodynamic simulations at the lower and higher discharges choosen.
In the end 18 simulations have been performed. The wet areas' shapefiles at different discharge rates are shown in **Fig.18**.

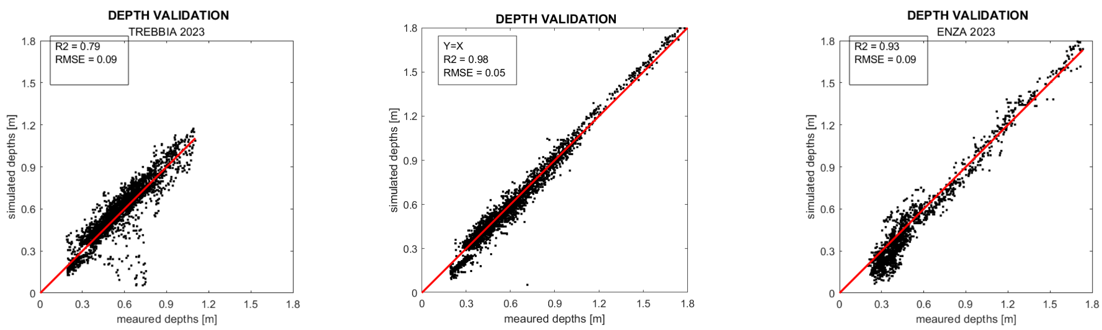

Fig.16 Bathymetric model validation for Trebbia Taro and Enza.

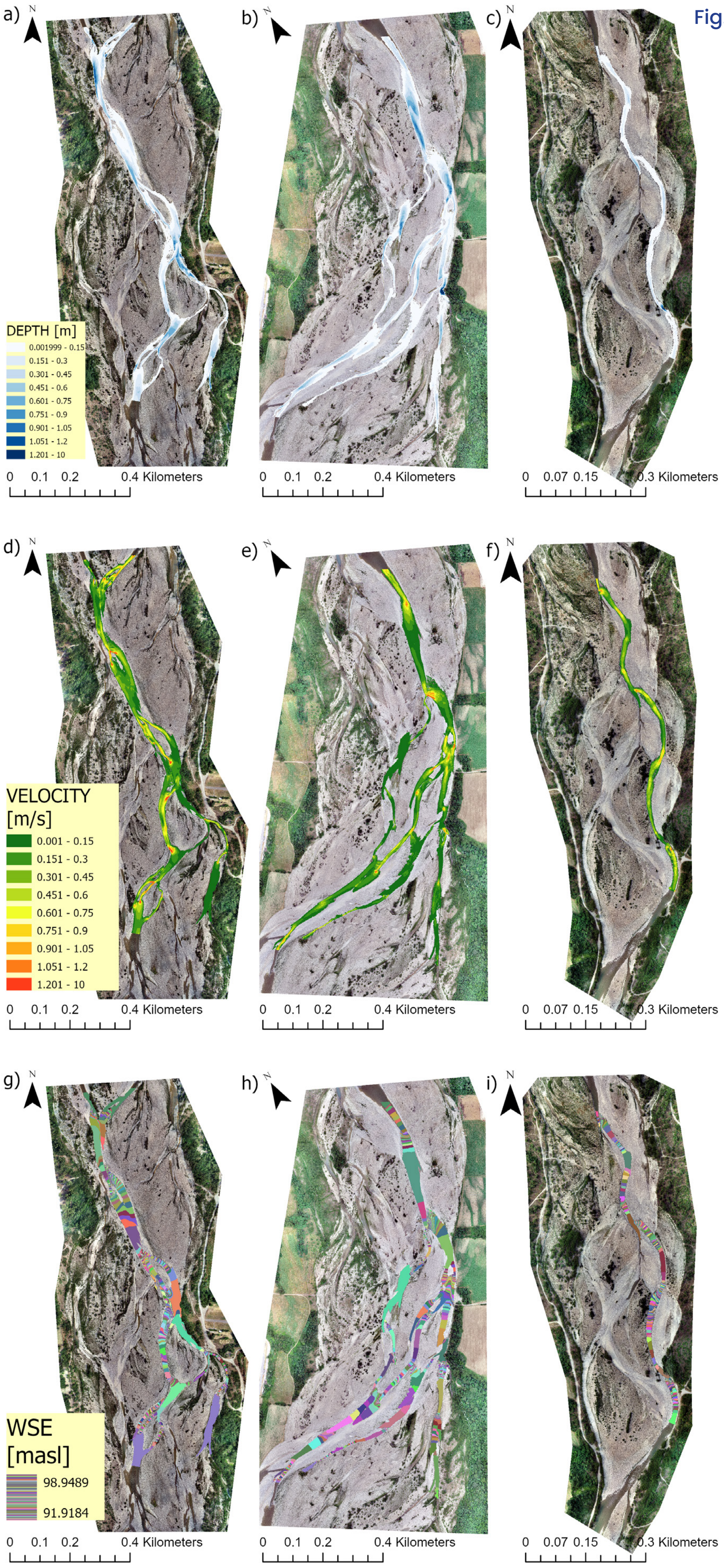

Fig.17 Hydrodynamic simuluation results at the discharge measured in field.

Q=2,50 m3/s for Trebbia in a), d) and g).

Q=2,00 m3/s for Taro in b), e) and h).

Q=0.50 m3/s for Enza in c), f) and i).

It can be appreciated that the wet areas of the simulation correspond to the ones of the ortophotos.

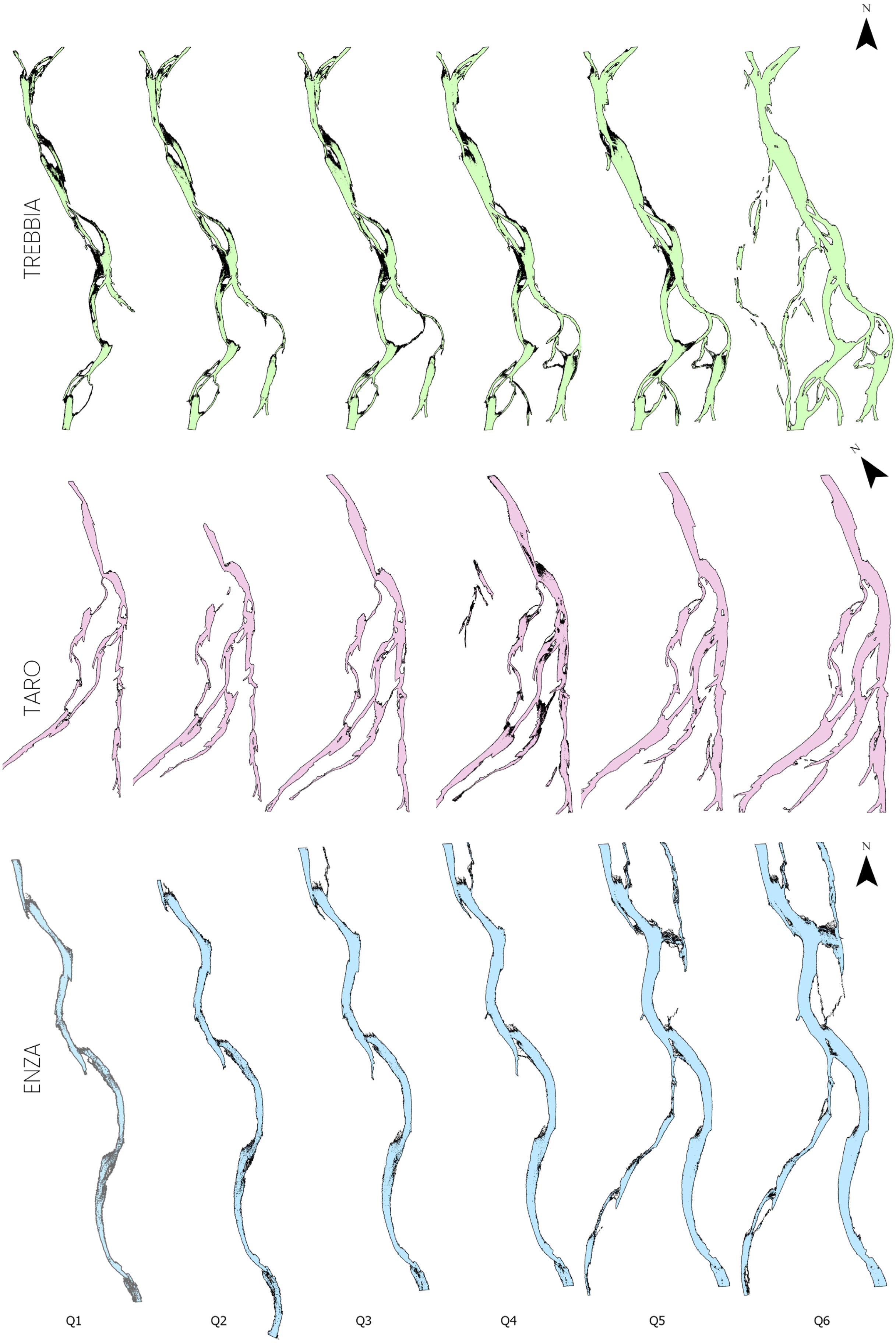

Fig.18 Wet areas obtained by hydrodynamic simulations for the three rivers' traits. The corrispondent value of Q1-6 can be found in the Table 2.

3.2.3 HMU Count and Mosaic

From the eighteen hydraulic simulations, an equal number of HMU mosaics were obtained, six for each river reach. At the measured discharge of Q_3 = 2.5 m³/s in the Trebbia River, 14 backwaters, 35 glides, 20 pools, 28 riffles, and 15 isolated ponds were identified. In the Taro River, at Q_2 = 2.0 m³/s, 9 backwaters, 25 glides, 18 pools, 20 riffles, and 6 isolated ponds were observed. In the Enza River, at Q_2 = 0.5 m³/s, 4 backwaters, 8 glides, 6 pools, 9 riffles, and 7 isolated ponds were mapped.

The complete HMU counts for all eighteen scenarios are reported in **Tab. 5**, while the corresponding HMU mosaics are illustrated in **Fig. 19–21**.

Tab.5 HMU count per every measured (in bold) and simulated scenario. B=backwater, G=glide, P=pool, R=riffle, IP=isolated pond.

	TREBBIA						TARO						ENZA					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**
B	9	11	**14**	10	11	17	11	**9**	8	5	13	14	4	**4**	3	2	4	6
G	45	46	**35**	39	42	48	32	**25**	29	25	22	26	9	**8**	8	6	21	23
P	26	33	**20**	28	24	22	19	**18**	21	20	18	15	3	**6**	4	4	9	10
R	12	24	**28**	34	38	42	18	**20**	22	29	26	33	9	**9**	8	8	13	17
IP	8	17	**15**	16	15	27	6	**6**	5	5	5	4	7	**7**	7	7	1	2

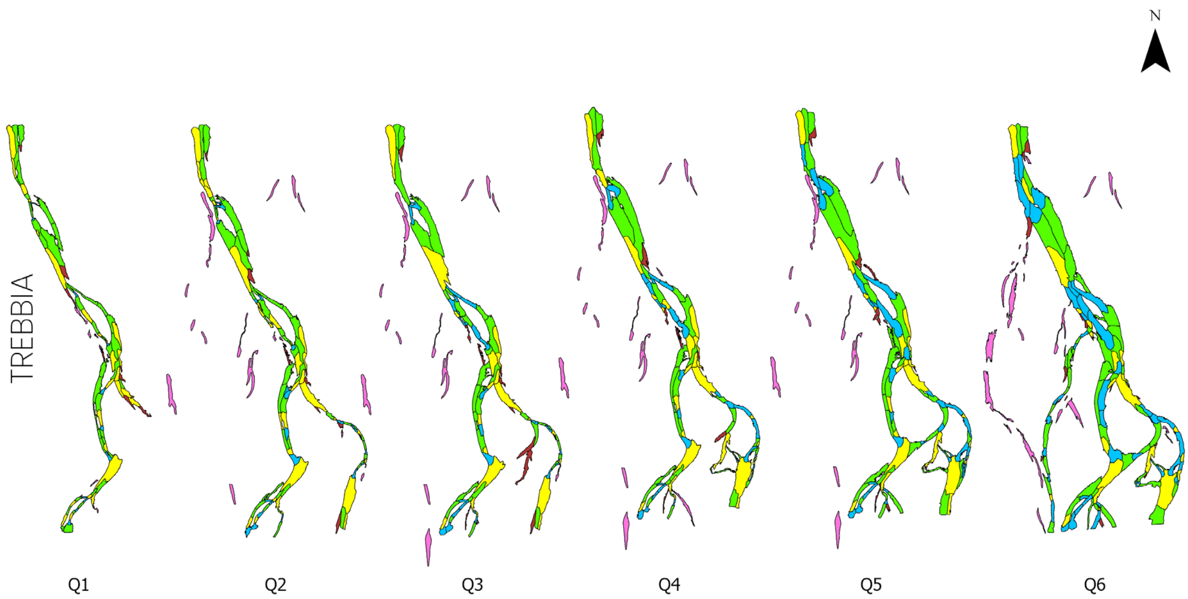

Fig.19 HMU mosaic of the Trebbia River at the six different discharges in scale 1:15000. The corrispondent value of Q1-6 can be found in the Table 2.

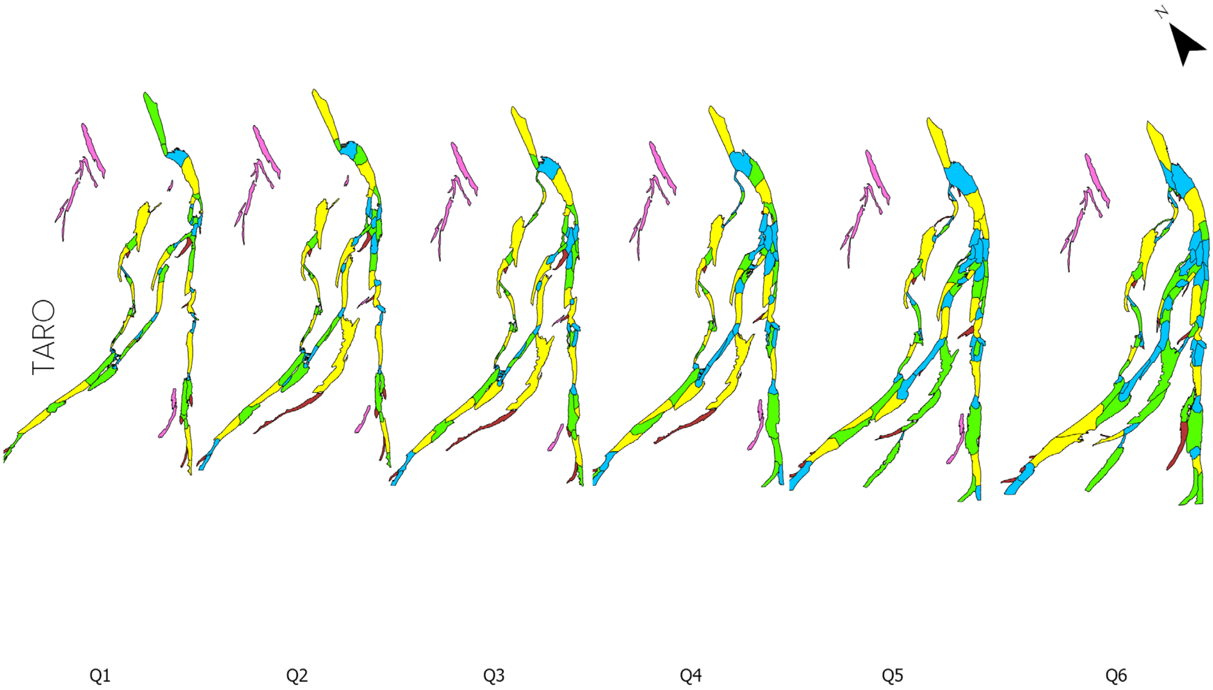

Fig.20 HMU mosaic of the Taro River at the six different discharges in scale 1:15000. The corrispondent value of Q1-6 can be found in the Table 2.

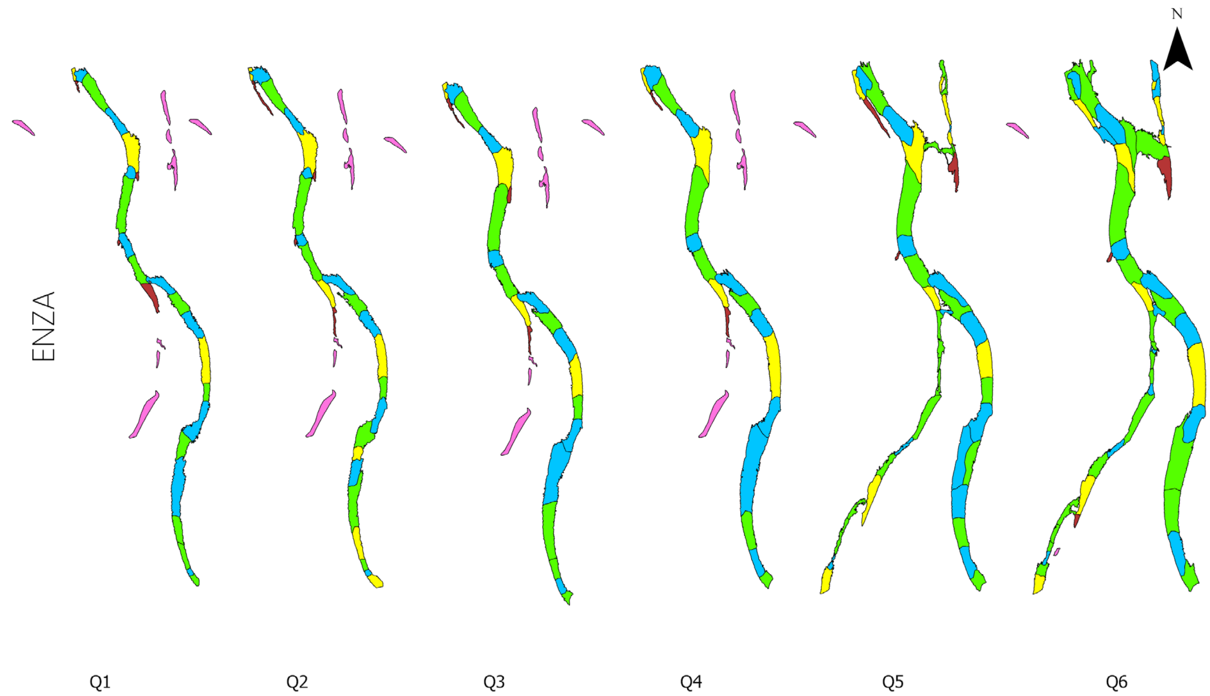

Fig.21 HMU mosaic of the Enza River at the six different discharges in scale 1:15000. The corrispondent value of Q1-6 can be found in the Table 2.

3.3 Flow-T Validation

The shapefiles containing the HMU configurations, together with the text files describing velocity, depth, and substrate for each HMU, were uploaded to the SimStream Web platform. As described in Section 2.3, the web application computes a Flow-T value for each HMU. The Flow-T values obtained from the model ranged between 0.24 and 0.62 (Fig. 23), a variation range consistent with the field samples' one. The variation ranges from the two approaches are therefore comparable. In this study, Flow-T values obtained from field surveys are considered as reference, while the values derived from the model required validation. Validation was carried out by comparing paired field and modelled values through scatter plots and by calculating the R^2 and RMSE. For each HMU listed in Tab. 4, the corresponding simulated Flow-T value was extracted. The R^2 correlation coefficient was 0.632 for the Trebbia River (Fig. 24), 0.643 for the Taro River (Fig. 25), and 0.610 for the Enza Stream (Fig. 26). The RMSE values were 0.053, 0.066, and 0.040, respectively. When considering all three rivers together, the validation yielded an overall R^2 of 0.663 and an RMSE of 0.054 (Fig. 22).

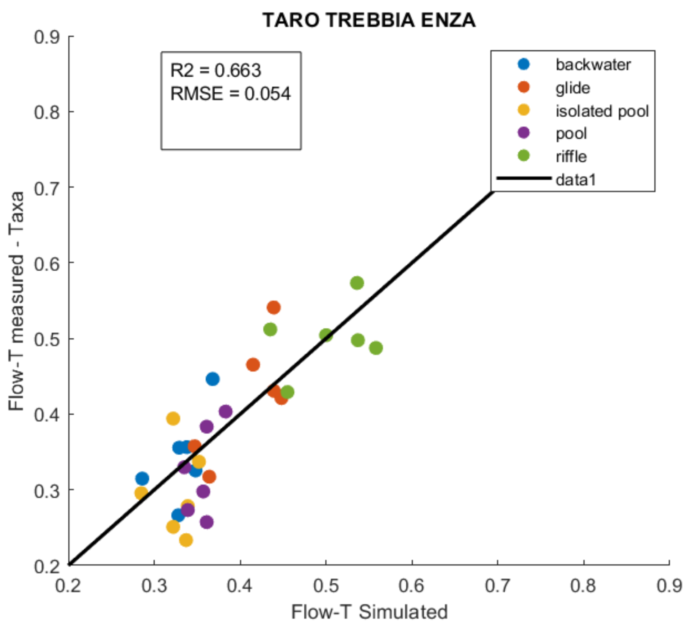

Fig.22 Flow-T overall validation.

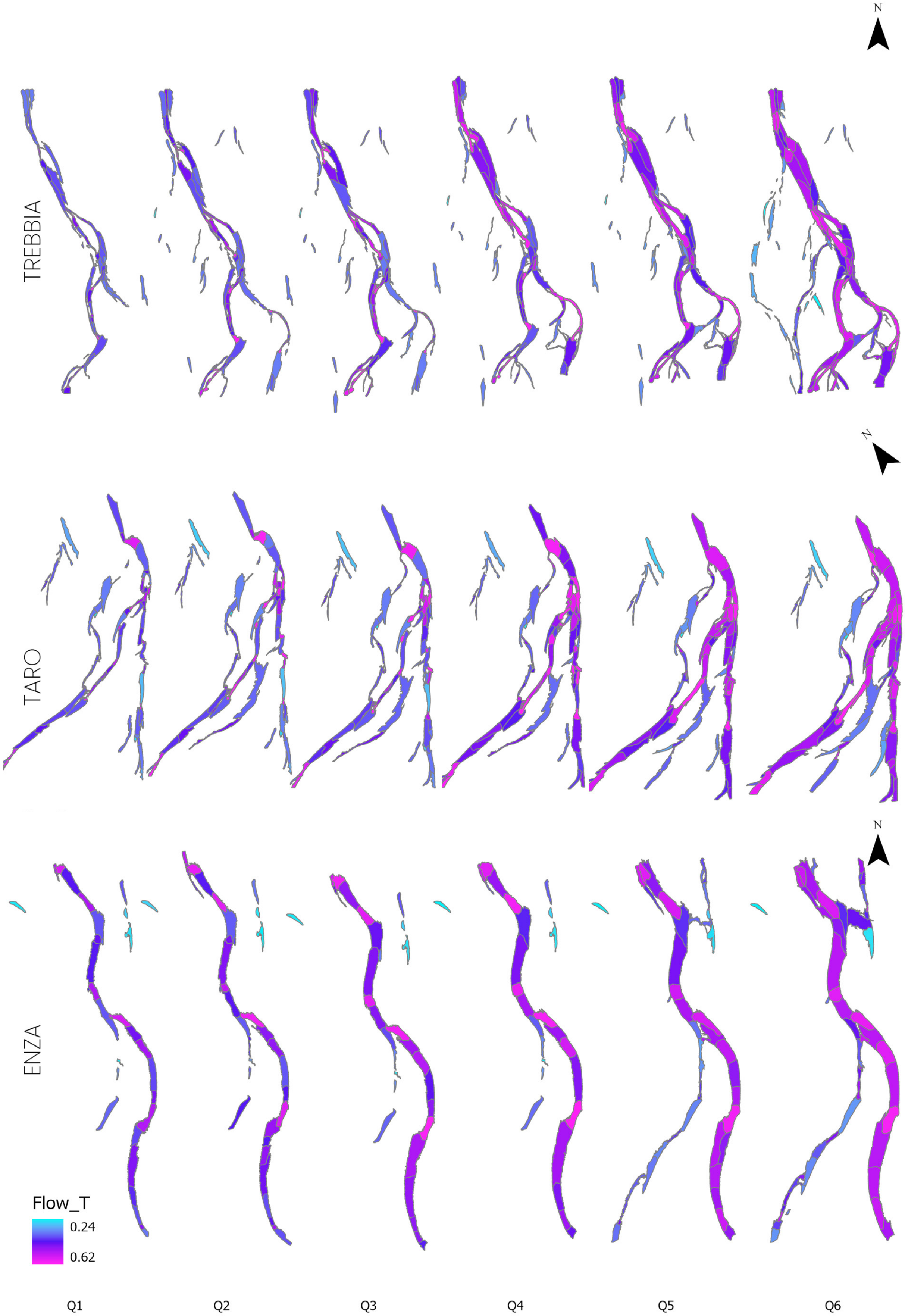

Fig.23 Flow-T values derived from the Macroinvertebrate Random Forest model implemented in SimStream Web.

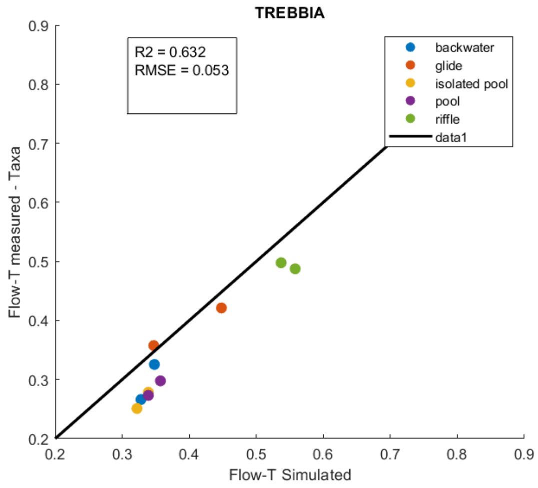

Fig.24 TREBBIA Flow-T validation.

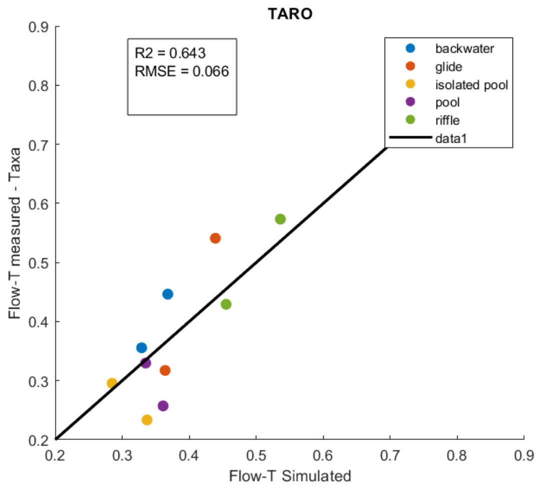

Fig.25 TARO Flow-T validation.

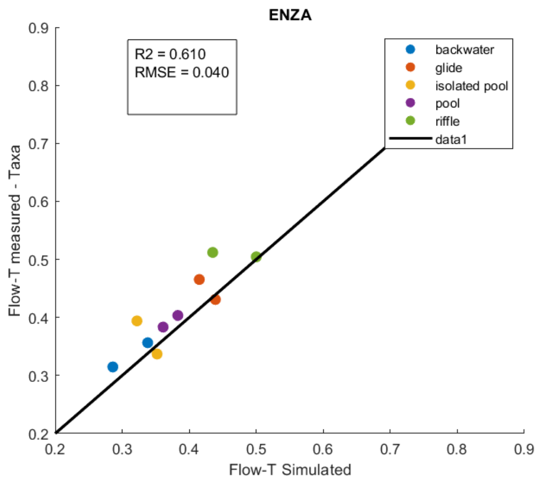

Fig.26 ENZA Flow-T validation.

4. Discussion and Conclusion

4.1 Flow-T Distribution

The analysis of field data revealed clear variability in Flow-T values among different mesohabitat types. This heterogeneity reflects the influence of hydro-morphological conditions on benthic macroinvertebrate communities, with certain mesohabitats providing more favorable flow regimes than others.In Fig. 27 it can be appreciated that riffles, as expected, have the higher values of Flow-T, while isolated ponds the lower ones. Glides on the other hand, have intermediate values and show the largest variance. These results are consistents with the findings of Brugazzi et al. 2021 [7].

The same resut is observed in the simulated Flow-T values, in wich Flow-T has been modelled for every HMU. As expected, HMUs characterized by higher flow velocities and a greater presence of microlithal substrate exhibited higher Flow-T values, whereas HMUs with lower velocities, such as backwaters, or lacking connectivity, such as isolated ponds, showed lower values.

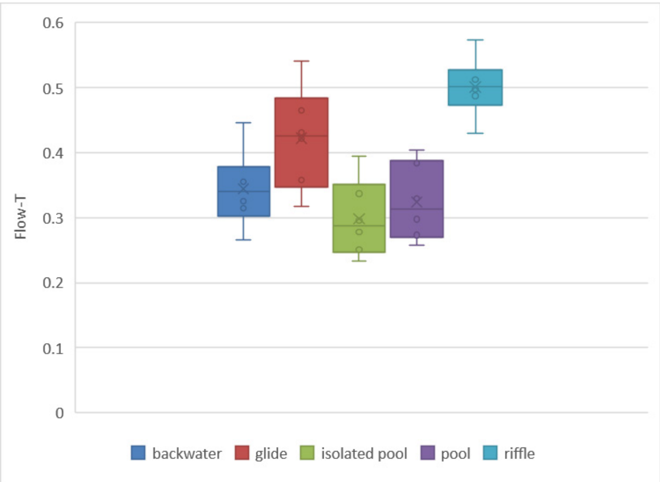

Fig.27 Flow-T values calculated per HMU type.

4.2 HMU Physical Evolution

The HMU mosaics are consistent with the physical evolution of flow under increasing discharge values, in fact, it can be observed that with increasing flow rate, not only the wet area increases, but also depths and velocities. Across the three reaches, beyond the overall expansion of wetted area, Fig. 28-30 show that the rate of increase in riffle area is generally higher than that of glides. By contrast, the relative proportion of pools, isolated ponds, and backwaters remains largely unchanged with respect to the total wetted area. In the Enza River (**Fig. 30**), at discharges Q5 and Q6, the percentage of isolated ponds decreases sharply, as they become submerged by two new branches of the river that, according to the simulations, are activated at higher flows. With increasing discharge, some glides evolve into riffles as flow velocity rises significantly. In other cases, higher discharge results in increased depth and reduced velocity, leading instead to a more distinct identification of HMUs of the pool type.

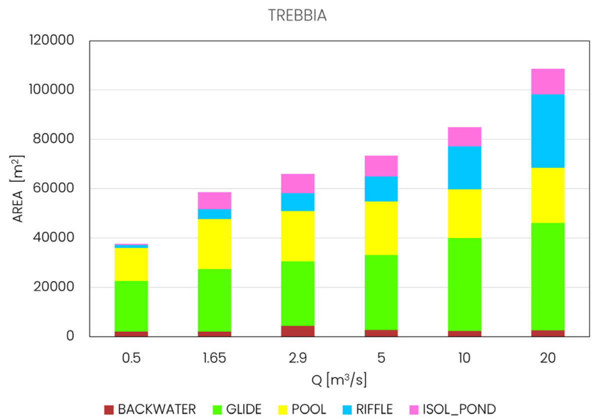

Fig.28 Trebbia's HMU wet area in the six scenarios

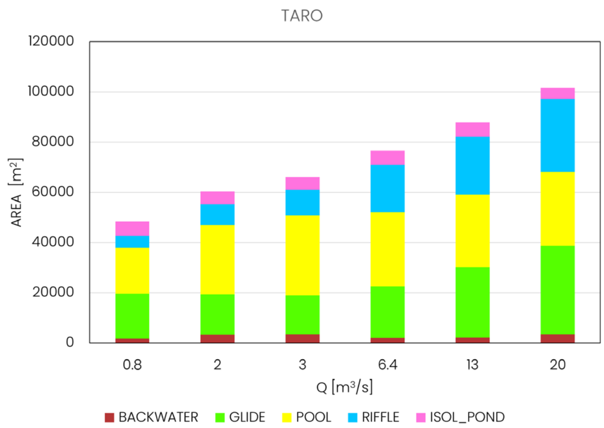

Fig.29 Taro's HMU wet area in the six scenarios

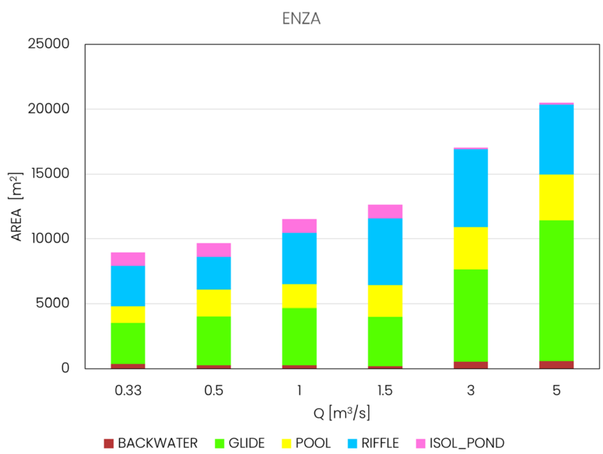

Fig.30 Enza's HMU wet area in the six scenarios

4.3 Flow-T Rating Curves

n addition to individual Flow-T values for each HMU, SimStream generates Flow-T rating curves, shown for each river in Fig. 31–33. For each simulated discharge, a mean Flow-T value is computed as the area-weighted average across HMUs, and the resulting curve is obtained by interpolating these averaged values. This approach enables the prediction of mean Flow-T values at discharges other than the simulated ones.

The Flow-T–discharge curves closely mirror the shape of the wetted area–discharge relationships. The discharge range considered spans from 0.5 to 20 m^3/s for both the Trebbia and Taro Rivers. Although Trebbia exhibits generally higher values of wetted area and Flow-T, the overall trends remain comparable between the two rivers. In contrast, the Enza Stream was analyzed over a narrower range (0.33–5 m^3/s), resulting in consistently lower values of both wetted area and Flow-T. Furthermore, the Enza displays a more monotonic pattern, while the Trebbia and Taro curves both show a distinct inflection around $Q \approx 2.0$ m^3/s.
The Flow-T rating curves can be considered the macroinvertebrate analogue of the habitat–discharge curves that SimStream generates for each fish species model in the catalogue. By capturing the response of macroinvertebrates to varying flow conditions and habitat types, these curves provide a quantitative tool to evaluate ecological functionality at the mesohabitat scale. Importantly, this approach opens the possibility of incorporating macroinvertebrate-based assessments into the River Habitat Integrity Index (IH), thus complementing fish-based metrics and offering a more holistic evaluation of riverine ecological conditions. Such integration could enhance the capacity of water managers to account for multiple trophic levels and community types when defining environmental flows and habitat conservation strategies.

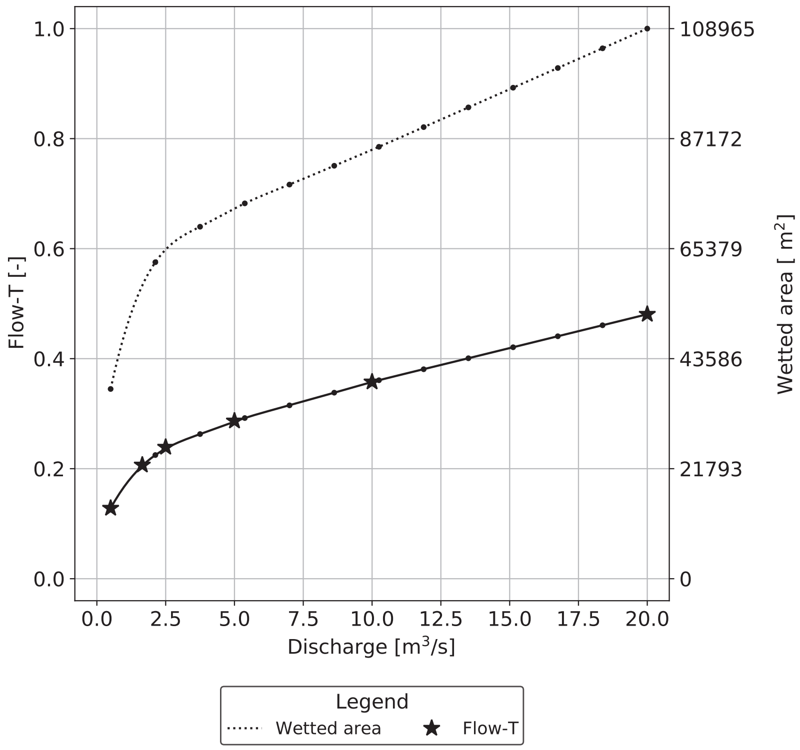

Fig.31 TREBBIA Flow-T rating curve.

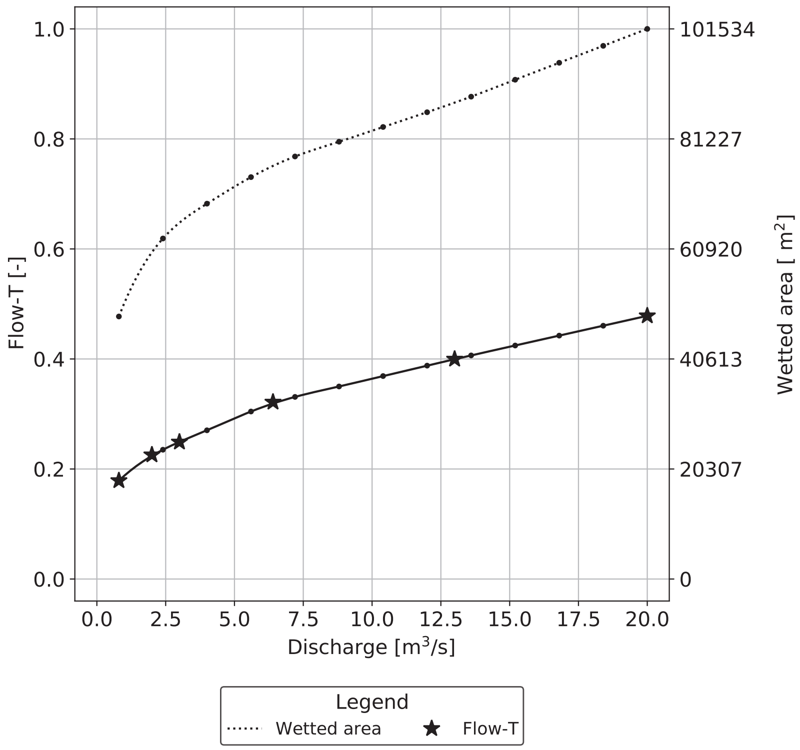

Fig.32 TARO Flow-T rating curve.

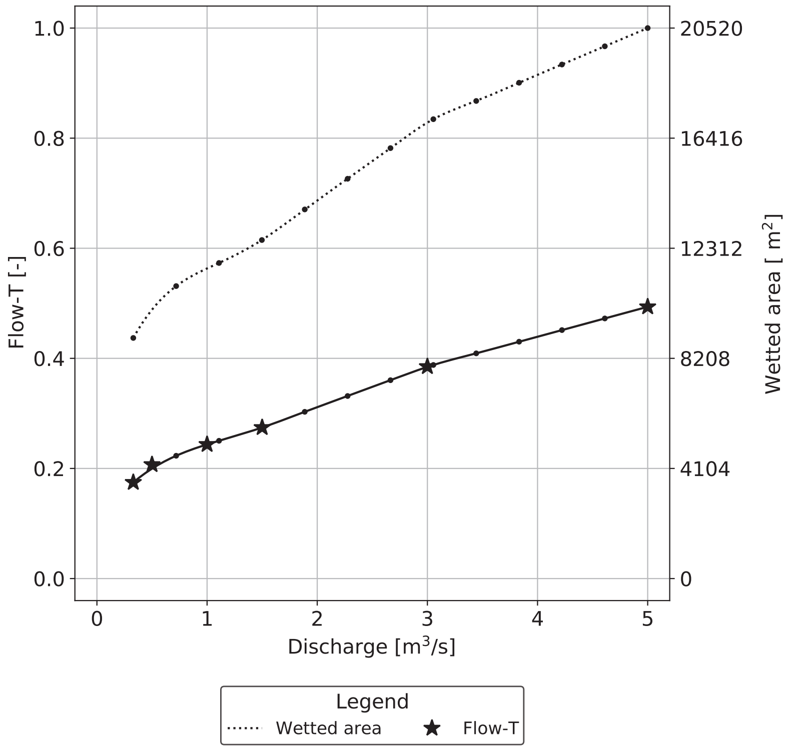

Fig.33 ENZA Flow-T rating curve.

5. Conclusions

In continuity with the objectives of the BentHab project, this thesis work focused on the validation of the Flow-T index in order to extend the application of the mesoHABSIM methodology to benthic macroinvertebrate communities. As already is done for fish, the methodology allows for the assessment of habitat availability for benthic macroinvertebrates based on the physical characteristics of the river at the mesoscale — including depth, velocity, substrate, and the presence of cover or refuges — and on the biological model developed by Pinna et al. [22].

The model, developed using the Random Forest decision tree statistical technique, identified the main variables influencing the composition of macroinvertebrate communities within specific mesohabitats, including certain velocity and depth classes as well as different substrate types. Accurate field data collection and subsequent correct data modeling were therefore essential for the success of the study.

Field surveys conducted in 2023 were based on previous surveys carried out in 2019 and 2020 in the Trebbia, Taro, and Enza rivers during the summer low-flow period. In each river, 100 samples were collected during the biological survey, 10 samples per unit, yielding a Flow-T value for each specific unit, which was considered the "true" value.
Additionally, bathymetric surveys were conducted in critical sections, and topographic surveys using UAVs and ground control points (GCPs) were carried out along the full extent of the study reaches. This allowed the generation of Digital Terrain Models (DTMs), which, after correction and removal of water refraction effects, were used as a basis for hydrodynamic modeling in HEC-RAS.

Sixteen flow scenarios were simulated, three of which corresponded to field-measured discharges. For each scenario, HMU mosaics were created along with frequency distributions of depth, velocity, substrate, and presence/absence of cover and refuges. These datasets were then loaded into SimStream to apply the macroinvertebrate biological model.

Specifically, simulated Flow-T values from the three scenarios corresponding to field-measured discharges were compared with the "real" biological survey values. The validation was successfully achieved, with a determination coefficient of R^2 = 0.66. The remaining scenarios were used to generate, for each river, the Flow-T rating curves, one of the most versatile and useful tools within the MesoHABSIM methodology.

The validation carried out in the Trebbia, Taro, and Enza Rivers confirmed that the index reflects ecological expectations across habitat types and can be reliably reproduced through hydrodynamic modelling. These findings strengthen the role of Flow-T as a complementary metric within the MesoHABSIM framework, offering an innovative approach for defining ecologically sustainable flows and supporting the objectives of the Water Framework Directive.

Nevertheless, some limitations must be acknowledged. The present study was conducted during low-flow summer conditions, which are especially critical for Mediterranean rivers but may not capture the full hydrological variability characterizing annual cycles. Extending validation to different geographic and ecological contexts within Europe would also be crucial to verify the transferability of the metric and to refine its calibration.

Future applications of the Flow-T index have significant potential in the study of intermittent rivers, which are becoming more and more critical under climate change and for which fish-based biological models cannot be applied. The procedure can also be effectively combined with remote sensing tecniques, particularly in non-wadeable rivers, where traditional field-based procedures would be extremely time-consuming if not unfeasible.

AUTHOR CONTRIBUTION

This work was carried out within the BentHab project, in collaboration with the Po River Basin Authority (AdBPo). Prof. Alex Laini (Department of Life Sciences and Systems Biology — DBios, University of Turin) together with Prof. Paolo Vezza (Department of Land and Infrastructure - DIATI, Politecnico di Torino) acted as scientific lead and was responsible for the design and coordination of the field surveys. Dr. Simone Guareschi (DBios, University of Turin) performed the taxonomic analysis of macroinvertebrate samples and calculated the observed Flow-T values for the 30 sampled HMUs.

Dr. Beatrice Pinna (DIATI, Politecnico di Torino) coordinated the field campaign and produced the bathymetric models for the Trebbia and Enza rivers.

Bathymetric surveys were carried out jointly by Dr. Pinna, Eng. Gianmarco Manfreda, and the author. Eng. Luca Astegiano (Gallery AS Rilievi Srl, Cuneo) planned and executed the UAV flights and his enterprise processed the resulting data (point-cloud cleaning, orthophoto and DTM generation). Ground control points (GCPs) were collected by Dr. Alessandro Guglielmetto (DIATI, Politecnico di Torino) and Dr. Arianna Paschetto (Department of Earth Sciences, University of Turin).

Overall methodological supervision and integration within the MesoHABSIM framework were ensured by Prof. Paolo Vezza (DIATI, Politecnico di Torino).

GLOSSARY

ADCP Acoustic Doppler Current Profile
DTM Digital Terrain Model
DSM Digital Surface Model
GCP Ground Control Points
GIS Geographic Information System
GPS Global Positioning System
GSD Ground Sample Distance
HMU Hydro Morphological Unit
IDW Inverse Distance Weightening
IH Habitat Integrity Index
RF Random Forest
RMSE Root Mean Square Error
SUM Sistema di rilevamento e classificazione delle Unità Morfologiche dei corsi d'acqua
UAV Uncrewed Aerial Vehicle
WFD Water Framework Directive 2000/60/EC
WSE Water Surface Elevation

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