


Evaluating ecological flow river-fed by carbonate aquifers: results from an integrated multidisciplinary approach on the Nera River (Central Italy)

Valutazione del deflusso ecologico in fiumi alimentati da acquiferi carbonatici: risultati di un approccio integrato multidisciplinare sul Fiume Nera (Italia centrale)

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Abstract

Estimating Ecological Flows is essential in rivers to achieve the objectives defined by the EU Water Framework Directive 2000/60/EC (WFD). In the framework of an agreement with the Autorità di Bacino Distrettuale dell'Appennino Centrale, the work aims to present the results of an integrated approach developed to consider various factors affecting the river's ecological flow (hydrological, hydrobiological, hydrogeological, hydro-morphological, and hydrochemical). The present study focuses on the Nera River, a main tributary of the Tiber River, which is primarily fed by groundwater from limestone aquifers. The river catchment hosts strategic water resources that are crucial for providing drinking water and sustaining aquatic ecosystems. Moreover, river water is utilized for hydropower generation, fish farming, and various recreational activities along the river. The Ecological Status was rated as good based on the New Index of the Ecological Status of Fish Communities (NISECI), along with physicochemical characterization of river water and hydromorphological characteristics, as criteria for its definition. Based on a regional model specifically developed for brown trout of rivers in the Tiber River basin (thus applicable to the Nera River), the minimum ecological flow was set at approximately 2.92 m³/s. Based on flow-duration curves, the average and maximum ecological flow values were also identified as 3.71 and 5.07 m³/s, respectively. The analyses carried out by the Water Exploitation Index Plus (WEI⁺) revealed medium-to-high stress in the catchment; therefore, withdrawals should be carefully re-planned to minimize further impacts on water-dependent ecosystems. The approach proposed for the Nera River highlights the importance of conducting focused, multidisciplinary studies in areas where groundwater withdrawals interact with groundwater-dependent ecosystems, thereby supporting regional management plans that address the needs of both humans and aquatic ecosystems.

Riassunto

La stima del deflusso ecologico dei fiumi è essenziale per raggiungere gli obiettivi definiti dalla Direttiva Quadro sulle Acque (2000/60/CE). Nell'ambito di un accordo con l'Autorità di Bacino Distrettuale dell'Appennino Centrale, è stato sviluppato un approccio integrato che tiene conto di diversi fattori che coinvolgono il sistema fluviale (idrologici, idrobiologici, idrogeologici, idromorfologici e idrogeochimici). Il presente studio analizza il fiume Nera, alimentato principalmente dalle acque sotterranee provenienti da falde acquifere in rocce calcaree. Il bacino idrografico del fiume ospita risorse idriche strategiche, fondamentali per l'approvvigionamento idropotabile e il sostentamento degli ecosistemi acquatici. Inoltre, l'acqua del fiume viene utilizzata per la produzione di energia idroelettrica e per l'allevamento ittico ed è indispensabile per assicurare varie attività ricreative lungo il corso del fiume stesso. Lo stato ecologico, valutato considerando il Nuovo Indice dello Stato Ecologico delle Comunità Ittiche (NISECI), combinato con la caratterizzazione fisico chimica dell'acqua fluviale e delle caratteristiche idromorfologiche, è risultato buono. Grazie a un modello geostatistico regionale sviluppato per la trota fario, la portata ecologica minima è stata fissata a circa 2.92 m³/s. Sulla base delle curve di durata della portata, sono stati identificati anche i valori medi e massimi del deflusso ecologico, rispettivamente pari a 3.71 e 5.07 m³/s. Le analisi effettuate mediante il Water Exploitation Index Plus (WEI⁺) hanno evidenziato uno stress medio/alto sul bacino idrografico; pertanto, ulteriori prelievi pianificati dovrebbero essere valutati con attenzione per non produrre ulteriori criticità legate agli usi dell'acqua sull'ambiente. L'approccio proposto per il fiume Nera sottolinea l'importanza di condurre studi mirati e multidisciplinari nelle aree in cui i prelievi di acque sotterranee si intersecano con le risorse che dipendono dalle acque sotterranee, sostenendo così piani di gestione regionali che rispondano alle esigenze antropiche e a quelle degli ecosistemi acquatici.

Introduction

Ecological flow (EF) is a crucial concept in water resource management, referring to the quantity, timing, and quality of water flows necessary to sustain freshwater ecosystems (Arthington et al., 2018). The problem of defining EF has been increasingly investigated since widespread hydrological alterations resulting from river damming, water withdrawals, and climate change have caused ecological damage and impacted the health of river system communities on a global scale (Carlisle et al., 2011; Di Matteo et al., 2017; de Jalón et al., 2019). The definition of EF enhances traditional water management by aligning with the requirements of the EU Water Framework Directive 2000/60/EC (WFD), which aims to achieve good ecological status for water bodies by considering biological, hydromorphological, and physical-chemical elements. The lowest-scoring element determines the overall ecological status. According to Lorenzoni et al. (2005) and Sapounidis et al. (2019), fish fauna serve as sensitive indicators of flow alteration in running waters, in terms of population or community changes (Jungwirth et al., 2000; Poff & Zimmerman, 2010; Schiemer, 2000). Groundwater-fed rivers are widely distributed globally, as groundwater sustains $59\% \pm 7\%$ of global river flows (Xie et al., 2024). This provides stable flow regimes that sustain fish populations by maintaining consistent habitat conditions and supporting cold-water species resilient to extreme low flows and temperature changes.

As recently reported by Baaner et al. (2025), groundwater-dependent ecosystems may be defined geographically at the landscape level, based on specific populations and networks of groundwater-dependent habitats. The WFD recognizes that ecologically appropriate hydrological regimes are essential for achieving good ecological status; however, the practical application of EFs remains a major issue, primarily due to the lack of standardized protocols for setting EFs at the local scale (Veza et al., 2012; Leone et al., 2023). As reported by Leone et al. (2023), most EU Member States have adopted EF methodologies that rely on statistical hydrological evaluations at a macro scale, neglecting ecological and morphological implications. For example, hydrological variables were often used to evaluate the ecological consequences of flow regime changes (e.g., Tharme, 2003); in this way the Minimum Vital Flow (MVF) was introduced (e.g., the minimum amount of water in a river or stream needed to maintain the physical characteristics of the water body, the quality of the water itself, and the local natural aquatic ecosystem). Recently, a debate over the balance between ecosystem conservation and human water needs has gained prominence in the literature, especially in areas affected by prolonged drought. A recent analysis by Bianucci et al. (2024) of 620 large basins and 3,839 sub-basins in Europe found that the problem of ecological flow is predominant, considering that more than 70% of basins are affected by a reduction in the mean annual discharge under severe emission scenarios generated by Regional Climate Models (RCM). To address these apparent conflicts, multiple factors should be considered when

defining the EF, including the accurate assessment of water use and its impact on ecosystems. According to Yarnell et al. (2022), the definition of EF requires an integrated approach that encompasses the evaluation of surface water (SW) - groundwater (GW) interactions and their relationships with fluvial communities, river hydro-morphology, and water quality. In this framework, the present study aims to propose and apply an integrated experimental approach to define the EF for the Nera River catchment, which represents the most important river fed by GW hosted in limestone aquifers in Central Italy (Boni et al., 1986). A multidisciplinary, integrated approach is proposed that encompasses hydrological and hydrogeological characterization, bioecological assessment, hydromorphological surveys, and hydrochemical analyses. A study of water use was also conducted to examine the current stress on the fluvial environment. These aspects are necessary to determine the flow regime required for the long-term health and functioning of ecological systems sustained by the complex interactions between GW and SW.

Materials and methods

Study area

The Nera River is included in the protection zone of the Nera River Regional Park (<https://www.parcodelnera.it/wp-content/uploads/120502A19SS1.pdf>), which has a high environmental value due to its connection to the fluvial ecosystem and its associated habitats. The Nera River catchment at Torre Orsina ($A = 1,362 \text{ km}^2$) is characterized by calcareous rocks of the Umbria-Marche-Sabine tectonic unit (Cosentino et al., 2017; Fig. 1a). It hosts Jurassic-Cretaceous carbonate aquifers, with underlying Triassic evaporites acting as an aquiclude. Two hydrogeological complexes characterized by high relative permeability are widely distributed in the river catchment: the Basal Limestones Complex (BLC, composed of the Calcare Massiccio and Corniola formations) and the Maiolica Complex (MAC). Proceeding upward in the stratigraphic succession, another important hydrogeological complex is found, the Scaglia Calcareo Complex (SCC). Overall, the hydrogeological complexes host important aquifers due to intense fracturing (BLC, MAC, and SCC) and karstification (for BLC) and are related to the complex tectonic evolution of the arc-shaped folds and thrusts of the Central Apennines, later followed by extensional structures, NW-SE trending normal faults (e.g., Barchi et al., 1998).

GW resources of limestone aquifers sustain the Nera River discharge through punctual and linear springs from the headwaters to the Torre Orsina river section (e.g., Boni et al., 1986; Boni & Preziosi, 1994; Boni et al., 2010; Preziosi et al., 2022; Azimi et al., 2023; Ortenzi et al., 2025); Figure 1b shows the river stretches mainly fed by GW. According to Boni et al. (1986), the Base Flow Index (BFI) at the Torre Orsina section exceeds 75%, indicating the key role of GW in sustaining the SW (Cencetti et al., 1989). After the last 2016 seismic sequence, whose main shock occurred on October 30th (Mw 6.5), the hydrogeological properties of limestone aquifers in the headwaters of the Nera River have been deeply affected

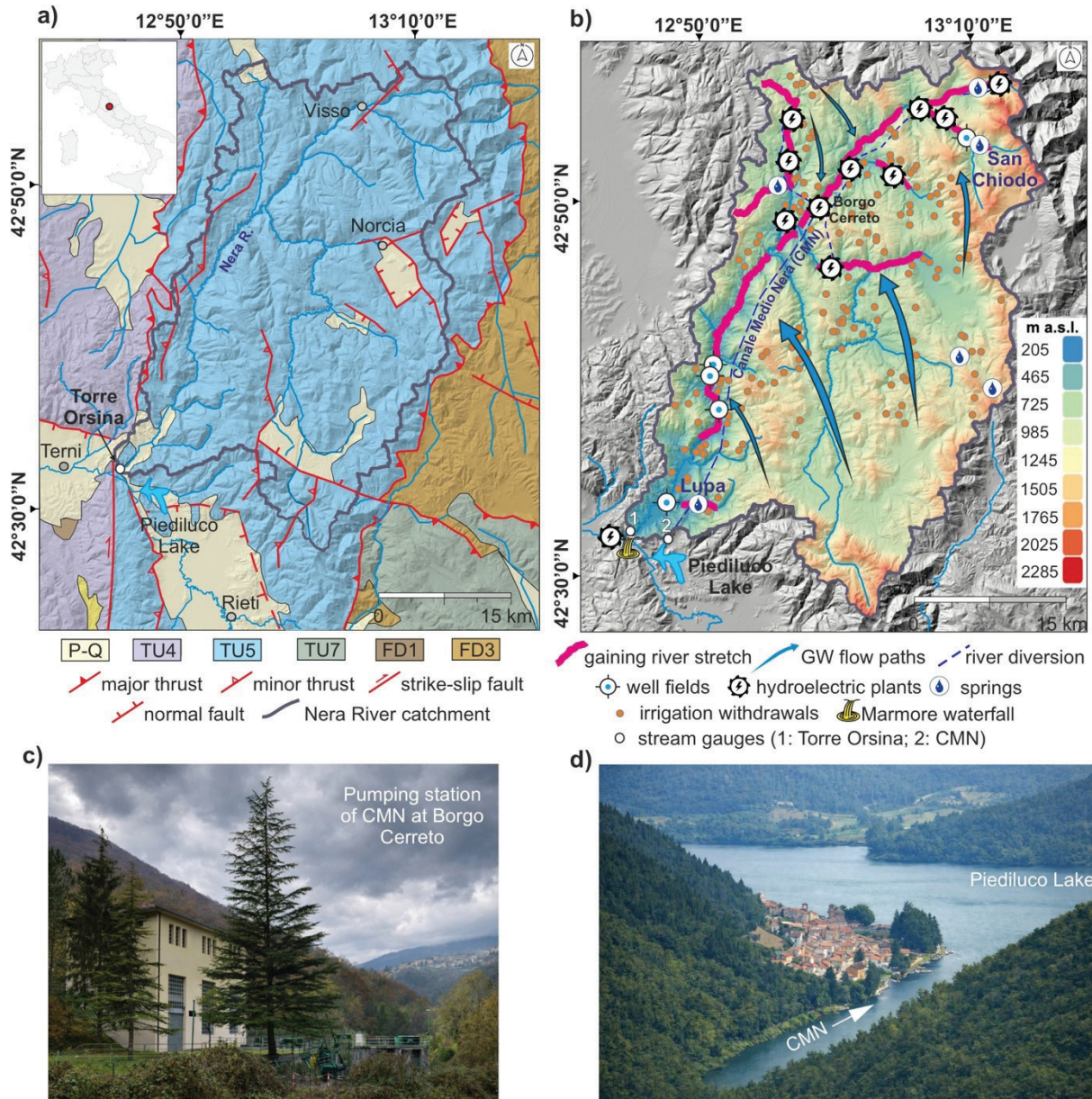


Fig. 1 - a) Structural sketch of the Nera River catchment area (modified from Cosentino et al., 2017). P-Q – Pliocene–Quaternary marine and continental deposits; TU4 – Inner Umbria tectonic unit; TU5 – Umbria-Marchean-Sabine tectonic unit; TU7 – Gran Sasso–Western Marsica tectonic unit; FD1 – Burdigalian foredeep deposits; FD3 – Messinian foredeep deposits. b) Location of the main anthropogenic pressure on the Nera River catchment, with the principal GW flow path of limestone aquifers feeding the river. c) pumping station at Borgo Cerreto, which supplies water into the Canale Medio Nera (CMN) in the middle-high part of the Nera River. d) panoramic view of the CMN outlet in the Piediluco Lake.

Fig. 1 - a) Schema strutturale del bacino idrografico del F. Nera (modificato da Cosentino et al., 2017). P-Q – depositi continentali e marini del Pliocene–Quaternario; TU4 – Unità tettonica dell’Umbria interna; TU5 – Unità tettonica Umbro-Marchigiana-Sabina; TU7 – Unità tettonica Gran Sasso–Ovest Marsica; FD1 – Depositi di avanfossa del Burdigaliano; FD3 – Depositi di avanfossa del Messiniano. b) Localizzazione delle principali opere antropiche nel bacino del F. Nera con le direzioni di flusso principali degli acquiferi carbonatici drenanti verso il fiume. c) stazione di sollevamento dell’acqua verso il Canale Medio Nera (CMN) a Borgo Cerreto (parte medio-alta del bacino del F. Nera). d) foto panoramiche del CNM in ingresso al Lago di Piediluco.

by different transient co-seismic effects, which increased the river discharge (Petitta et al., 2018; Di Matteo et al., 2020; Mastrorillo et al., 2020; Di Matteo et al., 2021; Cambi et al., 2022; Mastrorillo et al., 2023). According to Di Matteo et al. (2021), the effects of the seismic sequence lasted about three years after the main shock; thus, currently, meteo-climatic processes and anthropogenic withdrawals/releases regulate the GW-SW interactions.

GW-SW interactions are vital for native fish communities, especially during periods of limited or absent recharge

from precipitation, or during extended droughts, and are key resources for drinking water, hydroelectric power, and aquaculture. As shown in Figure 1b, hydroelectric plants and river diversions significantly affect the Nera River discharge; the most significant diversion is the Canale Medio Nera (CMN), which draws water from the upper part of the catchment toward Piediluco Lake, with a discharge of about 14 m³/s. Moreover, the withdrawals of drinking water from springs and wells are about 0.95 m³/s, while those for agricultural purposes are about 0.048 m³/s. The land use of

the Nera River catchment is mainly characterized by forest (75%), followed by agricultural land (20%), grassland and transitional woodland shrub (4.5%), and urban areas (0.5%).

Workflow for the definition of the ecological flow

To consider the various aspects involved in defining EF (bioecological, hydrological, hydrogeological, hydromorphological, and hydrochemical), the research followed the steps outlined below. The following is a stepwise workflow chart for defining ecological flow, divided into three main steps, with two additional ones to verify the effects of EF modulation on river water quality and to assess water stress across the catchment (Fig. 2).

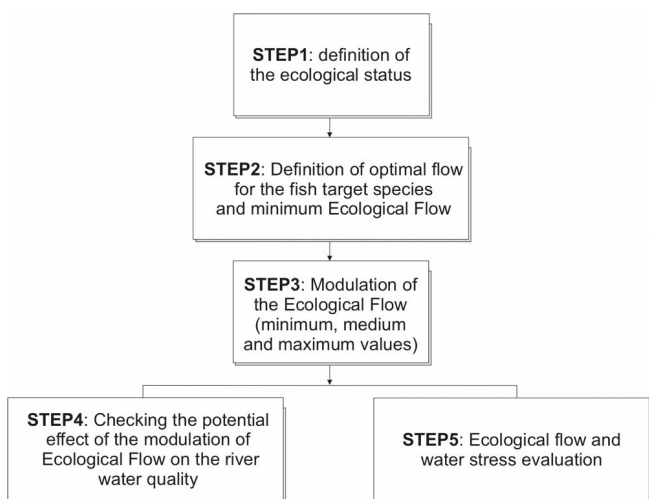


Fig. 2 - Stepwise workflow chart for defining ecological flow values.
 Fig. 2 - Workflow con i vari step per la definizione dei valori di deflusso ecologico.

STEP 1: Definition of the ecological status

The environmental status is assessed according to the WFD, considering the health of aquatic ecosystems by evaluating biological, hydro-morphological, physical-chemical, and chemical characteristics of the river system. Figure 3 illustrates the workflow for determining the ecological status in accordance with Italian environmental regulations that incorporate the WFD into Italian law (D.M. 260/2010; https://www.arpa.umbria.it/au/norme/nazionali/DM%20260_2010%20classificazione%20acque.pdf).

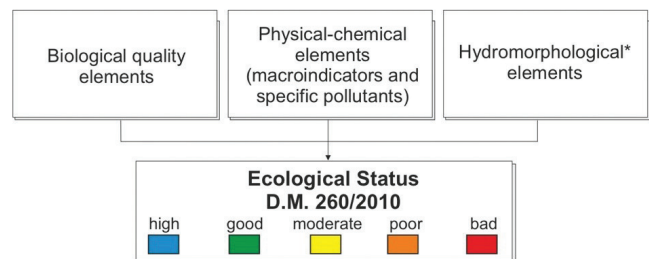


Fig. 3 - Italian procedure to define the ecological status of surface water (D.M. 260/2010). *The hydromorphological quality is necessary to validate the ecological status when the score of biological and physical-chemical elements is high.
 Fig. 3 - Procedura italiana per la definizione dello stato ambientale delle acque superficiali (D.M. 260/2010). *Lo stato elevato (biologico e fisico-chimico) deve essere confermato dalla qualità idromorfologica.

The biological quality has been evaluated by the New Ecological Status of Fish Communities Index (NISECI; Macchio et al., 2017), which is a multimetric index essentially based on the evaluation of the deviation of observed fish community composition from a pristine condition (i.e., in the absence of anthropogenic alterations), in which the reference community is represented by a set of expected native species, identified according to zoogeographical and ecological criteria (Rossi et al., 2021). The NISECI is computed by Eq. 1 (Macchio et al., 2017); the characteristics of the observed fish communities are evaluated with the following three metrics, each ranging from 0 to 1:

- i. Metric x_1 = the presence of expected native species, some of which are endemic and of great conservational value.
- ii. Metric x_2 = the biological condition of their populations, concerning abundance and age structure.
- iii. Metric x_3 = the presence of alien species, which are classified into three categories based on their levels of impact on indigenous populations: high, medium, and moderate harmfulness.

$$NISECI = 0.1 \cdot x_1^{0.5} + 0.1 \cdot x_2^{0.5} + 0.8 \cdot (x_1 \cdot x_2) - 0.1 \cdot (1 - x_3) \cdot \{0.1 \cdot x_1^{0.5} + 0.1 \cdot x_2^{0.5} + 0.8 \cdot (x_1 \cdot x_2)\} \quad 1$$

To meet the WFD requirements, the NISECI values are expressed as the Ecological Quality Ratio (EQR), as shown in Eq. 2 (Macchio et al., 2017).

$$EQR_{NISECI} = (\log_{NISECI} + 1.1283) \cdot 1.0603 \quad 2$$

For further details on the calculation of metrics and the NISECI equation, refer to Macchio et al. (2017) and Rossi et al. (2021). Based on these metrics, NISECI and EQR_{NISECI} enable the assignment of each sampling site to one of the five predefined quality classes, as detailed in Table 1.

Tab. 1 - NISECI classes for the definition of the ecological status (from Macchio et al., 2017).

Tab. 1 - Classi NISECI per la definizione dello stato ecologico (da Macchio et al., 2017).

Classes	Level	NISECI values intervals	EQR _{NISECI} values intervals
1	High	0.525 ≤ NISECI	0.80 ≤ EQR _{NISECI}
2	Good	0.322 ≤ NISECI < 0.525	0.60 ≤ EQR _{NISECI} < 0.80
3	Moderate	0.198 ≤ NISECI < 0.322	0.40 ≤ EQR _{NISECI} < 0.60
4	Poor	0.121 ≤ NISECI < 0.198	0.20 ≤ EQR _{NISECI} < 0.40
5	Bad	NISECI < 0.121	EQR _{NISECI} < 0.20

The physical-chemical and chemical quality elements support the assessment of ecological status by monitoring nutrients (nitrogen and phosphorus), dissolved oxygen, and other specific pollutants that affect aquatic ecosystems (Table 1/B in D.M. 260/2010). Chemical-physical parameter and water samples for geochemical analyses have been collected during the 2023-2024 period (four campaigns during both low and high flow periods), and the results of geochemical analyses have been integrated with those coming from the monitoring campaigns carried out by Agenzia Regionale per l’Ambiente dell’Umbria (ARPA Umbria) during 2008-2023

Tab. 2 - Thresholds for assigning scores to individual parameters to obtain the LIMeco (from D.M. 260/2010).

Tab. 2 - Soglie per l'assegnazione dei punteggi ai singoli parametri per ottenere il valore LIMeco (da D.M. 260/2010).

Parameter	Level 1 (score 1)	Level 2 (score 0.5)	Level 3 (score 0.25)	Level 4 (score 0.125)	Level 5 (score 0)
100-O ₂ %	≤ 10	≤ 20	≤ 40	≤ 80	> 80
N-NH ₄ (mg/L)	< 0.03	≤ 0.06	≤ 0.12	≤ 0.24	> 0.24
N-NO ₃ (mg/L)	< 0.6	≤ 1.2	≤ 2.4	≤ 4.8	> 4.8
P _{tot} (µg/L)	< 50	≤ 100	≤ 200	≤ 400	> 400

in a sampling section located about 700 m upstream of the Torre Orsina section (NER4, Cingolani & Charavgis, 2024; <https://apps.arpa.umbria.it/acqua/qualita-acque-superficiali>). The LIMeco index (*Livello di Inquinamento da Macrodescriptors per lo stato ecologico*, D.M. 260/2010) was used as a descriptor to derive the physical-chemical quality class, supporting biological quality elements. The procedure for calculating LIMeco involves calculating individual scores for dissolved oxygen (100-O₂% saturation) and for nutrients (N-NH₄, N-NO₃, and P_{tot}), according to the limits specified in D.M. 260/2010 (Table 2). The average LIMeco value calculated for the sampling period is used to assign a quality class to the investigated river section.

The Morphological Quality Index (MQI; Rinaldi et al., 2014) is used to assess the physical quality of the river stretch in terms of its compliance with natural processes. The method was designed to comply with WFD requirements and analyze how much human activities have altered its natural morphology and geomorphic processes. The MQI index is determined through successive levels of analysis, using a hierarchical approach at different scales: catchment scale, segment scale (macro stretch), and finally at river stretch scale. Figure 4 presents a general methodological framework divided into two phases, along with a list of the main aspects to be considered when computing the MQI. As reported by

Rinaldi et al. (2014), the index is determined on the basis of a set of 28 indicators, grouped into three macro-categories: functionality, artificiality, and morphological changes (Fig. 4). Each indicator is assessed using a survey form (<https://www.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/guidebook-idraim.pdf>) with a score assigned in proportion to the degree of alteration. The final score is given by the Morphological Alteration Index (MAI, Eq. 3), which compares the total deviation (S_{tot}) from unaltered conditions with the maximum possible deviation for the given watercourse type (S_{max}).

$$MAI = S_{tot} / S_{max} \quad 3)$$

The MQI is then defined by Eq. 4, as complementary to Eq. 3.

$$MQI = 1 - MAI \quad 4)$$

The MQI classifies streams into five morphological quality classes: very poor (MQI = 0÷0.3); poor (MQI = 0.3÷0.5); moderate (MQI = 0.5÷0.7); good (MQI = 0.7÷0.85); and very good (MQI > 0.85).

For the Nera River, a stretch of approximately 8,860 m has been surveyed to define the MQI, including both upstream and downstream portions of the Torre Orsina section. It should be noted that hydromorphological quality is necessary to validate ecological status (Fig. 3), particularly when the scores for both biological and physical-chemical components are high (D.M. 260/2010).

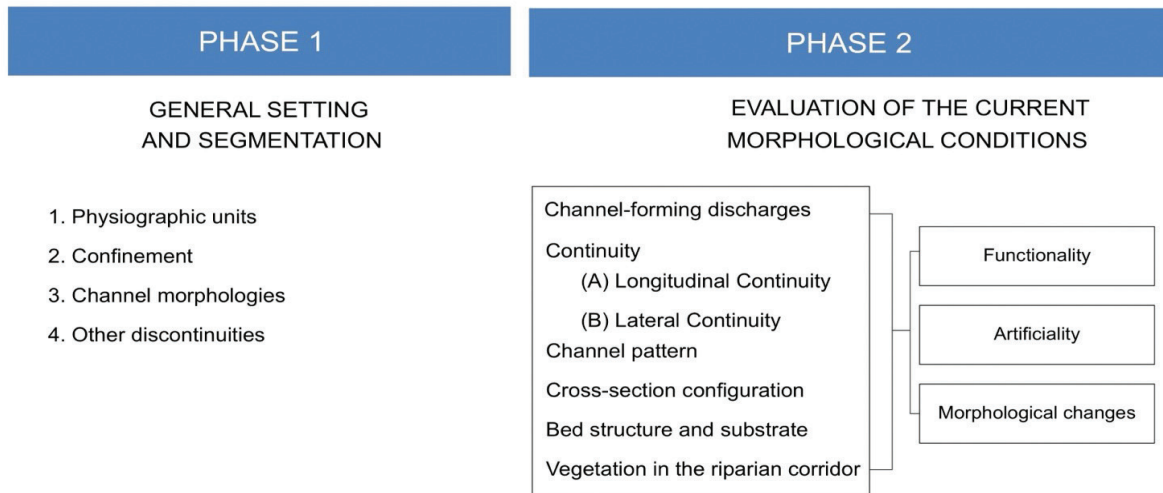


Fig. 4 - General methodological framework illustrating the overall procedure of hydromorphological analysis useful for computing the MQI index (modified from Rinaldi et al., 2014).

Fig. 4 - Schema metodologica che illustra la procedura di analisi idromorfologica, utile al calcolo dell'indice IQM (modificato da Rinaldi et al., 2014).

STEP 2: Definition of optimal flow for the fish target species and minimum EF

The optimal flow (Q_{opt}) indicates the discharge for which fishes have the greatest habitat availability, with hydraulic and structural characteristics suitable for their development. Equation 5 presents an empirical relationship for the brown trout (*Salmo trutta complex*) developed by Carosi et al. (2026); it relates the Q_{opt} (in m^3/s) and the watershed area (S , in km^2) and is applicable to all rivers in the Tiber River basin, including the Nera River, which has a typical hydraulic regime with current speeds ranging between 0.1 and 1.3 m/s (e.g., Bicchi et al., 2006; Carosi et al., 2020; 2025). The coefficient 0.2103 is dimensioned so that Q_{opt} is in m^3/s and S in km^2 .

$$Q_{opt} = 0.2103 \cdot S^{0.3953} \quad (5)$$

The Q_{opt} has been used to define the minimum Ecological Flow (EF_{min}) for summer flow rates, which correspond to the low-flow season. In general, if the in-force Minimum Vital Flow by the previous management plans (e.g., PTA 2016-2021, <https://www.regione.umbria.it/-/piano-di-tutela-delle-acque-aggiornamento-2016-2021>) assured a good ecological status of the river stretch, the EF_{min} was assigned at least equal to the MVF value, with the possibility to increase it considering the need to maintain the river's diluting capacity.

STEP 3: Modulation of the EF

In this work, the modulation of the EF is represented by a set of temporally distributed values, following the natural flow paradigm principle, that is a foundational principle in river conservation and management that states that the ecological integrity of river ecosystems is largely sustained by the natural, dynamic, and variable flow of water (Poff et al., 1997; Faergemann, 2012).

Therefore, the variability of EF is linked to the variability of natural flows through a flow modulation criterion that ensures the needs of aquatic organisms and bank vegetation. To modulate the EF, the Flow Duration Curve (FDC) of the Nera River at Torre Orsina stream gauge has been reconstructed using available daily discharge data for the 1997-2021 period. The FDC provides an estimate of the percentage of time a given flow rate value is equalled or exceeded within an assigned historical period; specifically, each flow rate represented on the y-axis is associated with the curve with its exceedance duration in days represented on the x-axis; consequently, the area under the curve represents the volume of water runoff (Vogel & Fennessey, 1995). To estimate the natural FDC (FDC_{nat} ; Fig. 5), the mean daily natural discharge (Q_{nat}) was first reconstructed using a regionalization procedure studied across the entire Tiber basin, including the Nera River (e.g., Bellezza et al., 2009; Casadei et al., 2018).

In this way, the naturalized discharge that the catchment would supply in the absence of withdrawals and diversions is considered, rather than the discharge measured at the Torre Orsina section. Thereafter, the flow duration curve of EF (FDC_{EF}) was obtained following the procedure described in Casadei et al. (2025), which reconstructs the FDC_{EF} curve

(Fig. 5) using daily ecological flow values (EF_t) computed as 10% of the difference between $Q_{nat(t)}$ and EF_{min} (Eq. 6). EF_{min} is then kept constant for $1 < t < 365$. It should be noted that the FDC_{nat} (mean duration curve) does not capture the interannual variability of flows. However, for the purpose of analyzing ecological flow variability, it appears to be the most feasible approach, since it is unlikely in practice to impose constraints requiring EF_{med} and EF_{max} to vary from year to year.

$$EF_t = EF_{min} + \{(Q_{nat(t)} - EF_{min}) \cdot 0.10\} \quad (6)$$

where:

EF_t = mean daily ecological flow at time t (m^3/s).

EF_{min} = minimum ecological flow (m^3/s).

$Q_{nat(t)}$ = mean daily natural flow at time t (m^3/s).

As described by Casadei et al. (2025), EF_{min} is kept constant for durations $t \geq 274$ days, corresponding to the streamflow equalled or exceeded 75% of the time (Q_{75} ; Demirel et al., 2015). To avoid abrupt changes at this threshold, the curve is then smoothed by logarithmic interpolation, yielding the environmental flow duration curve (FDC_{EF}).

269 Using the FDC_{EF} curve, the EF_{med} and EF_{max} values were identified by applying the flow 269 Using the FDC_{EF} curve, the EF_{med} and EF_{max} values were identified by applying the flow.

Using the FDC_{EF} curve, the EF_{med} and EF_{max} values were identified by applying the flow rates at 182 and 90 days, following the approach described in Casadei et al. (2025).

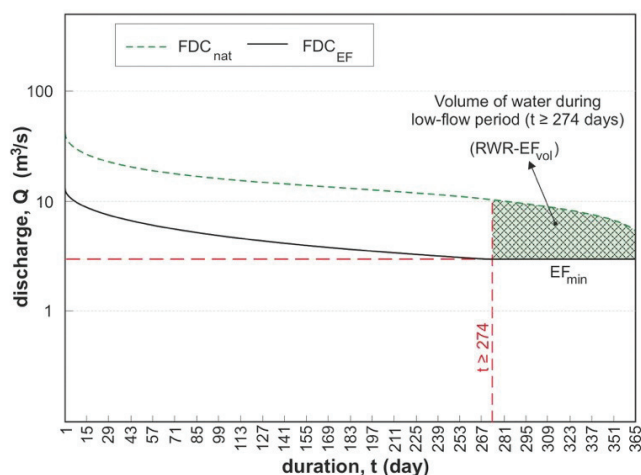


Fig. 5 - Example of natural Flow Duration Curve (FDC_{nat}) with FDC_{EF} curve. The green area represents the difference between the natural volume of water (RWR) and that allocated for the ecological flow (EF_{vol}) for the low-flow period ($t \geq 274$ days).

Fig. 5 - Esempio di curva di durata delle portate naturali (FDC_{nat}) e dell' FDC_{EF} . L'area in verde rappresenta la differenza del volume di acqua naturale (RWR) e quella destinata al deflusso ecologico per $t \geq 274$ giorni.

STEP 4: Checking the potential effect of the modulation of EF on the river water quality

In the literature, correlations between water-solute concentration (C) and flow rate (Q), known as C-Q correlations, are widely used to characterize the sources, mobilization, and transport of solutes at the catchment scale (e.g., Speir et al.,

2023). There are numerous metrics of C-Q correlations (e.g., Fig. 6), each of which documents the variations in solute concentration under different flow conditions. Two of the most applied C-Q metrics are the C-Q slope (denoted as beta, or b) and the ratio of the coefficients of variation for C and Q (CV_C/CV_Q). The beta parameter is the exponent (b) of the power equation $C = aQ^b$, where a and b are constants, and are often estimated from the regression of C on Q, in a log-log space (Godsey et al., 2009; Musolff et al., 2017). As reported in Figure 6b, the correlations between C and Q are generally classified into three groups based on the values of b: enrichment ($b > 0$), dilution ($b < 0$), and constant ($b \approx 0$).

Invariant concentrations with respect to flow rate variations define a chemostatic behavior, while concentration variations in response to flow rate variations define a chemodynamic behavior. A chemostatic or chemodynamic behavior can be defined in more detail by combining the values of b with the ratio of the coefficients of variation of C and Q (CV_C/CV_Q) (Musolff et al., 2015; Fig. 6b).

In the present study, for the analysis of the C-Q correlation, the concentrations of the relevant parameters were taken from the ARPA Umbria database (<https://apps.arpa.umbria.it/acqua/qualita-acque-superficiali>). The Sezione Difesa e Gestione Idraulica of the Umbria Region provided the average river discharge for the sampling day.

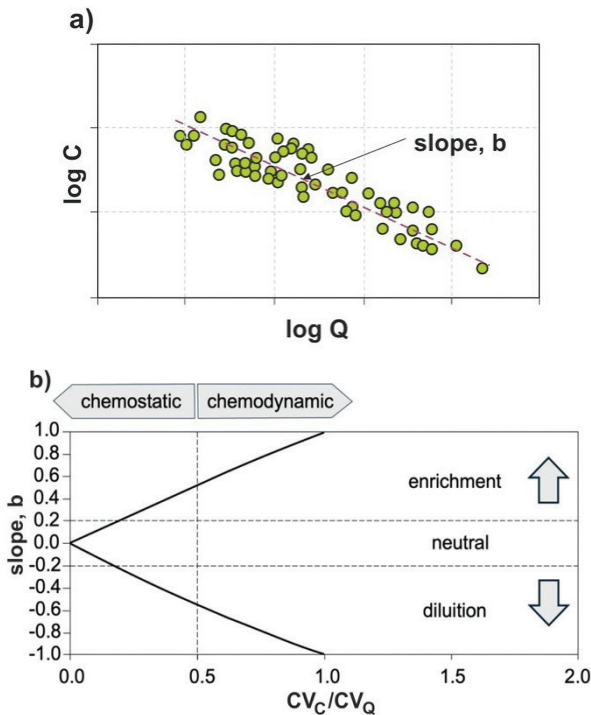


Fig. 6 - a) Esempio di un possibile plot C-Q; b) Rappresentazione grafica della pendenza b vs. CV_C/CV_Q .

STEP 5: Ecological flow and water stress evaluation

Estimating water resources for the catchment depends on the balance between available and accessible water resources, as well as the various uses envisioned in a context of compatibility and sustainability (e.g., Ciaravino & Ciaravino, 2016). The Water Exploitation Index (WEI) is used as an indicator of water stress, updated in its WEI⁺ form by the European Commission’s Committee of Experts (Faergemann, 2012). In this study, we employed a new index, WEI⁺_{EF} (Eq. 7), modified by Casadei et al. (2020, 2025) and incorporating the volume of environmental flow (EF_{vol}).

$$WEI_{EF}^+ = \frac{(abstractions - returns)}{RWR - EF_{vol}} \cdot 100 \tag{7}$$

where:

abstractions-returns = volume of net water withdrawals (m³).

RWR = Renewable Water Resource, m³ (estimated as the area under the FDC_{nat} curve).

EF_{vol} = volume for environmental flow, m³ (estimated as the area under the FDC_{EF} curve).

As reported in Figure 5, the WEI⁺_{EF} can be computed considering all the observation period or only the low-flow period ($t \geq 274$ days); in this case, the denominator of Eq. 7 ($RWR - EF_{vol}$) is computed only for times higher than 274 days (green zone in Fig. 5).

Table 3 presents the WEI⁺_{EF} classes, as classified by Estrela-Segrelles et al. (2024) and Casadei et al. (2025), ranging from no stress (class 1) to critical (class 5)

Tab. 3 - WEI⁺_{EF} classes (from Casadei et al., 2025).

Tab. 3 - Classi di WEI⁺_{EF} (ripresa da Casadei et al., 2025).

Classes	Level	WEI ⁺ _{EF} values intervals (%)
1	No stress	1 < WEI ⁺ _{EF} ≤ 25
2	Low	25 < WEI ⁺ _{EF} ≤ 50
3	Medium	50 < WEI ⁺ _{EF} ≤ 75
4	High	75 < WEI ⁺ _{EF} ≤ 100
5	Critical	WEI ⁺ _{EF} > 100

Results

The approach presented for defining EF has been tested in the Nera River catchment at the Torre Orsina stream gauge (upstream of the confluence with the main tributary, the Velino River). By applying Equations 1 and 2, the NISECI and EQR_{NISECI} values were computed for the brown trout population at the Torre Orsina site, yielding approximately 0.93 and 1.00, respectively, which fall into the high-quality class (see Table 1). For the computation, the following metrics have been used in Equation 1 ($x_1 = 1.00$; $x_2 = 1.00$; $x_3 = 0.25$). The high class found by the EQR_{NISECI} has also been confirmed by the LIMeco index and by the concentrations of the supporting chemical elements (STEP 1). The hydromorphological survey gives a MQI value of 0.57, indicating moderate quality of

the stream segment. Overall, the high ecological status, as defined by biological and physical-chemical elements, is not supported by hydro-morphological elements. Although the moderate hydro-morphological quality can be considered reasonably acceptable, according to D.M. 260/2010, the ecological status is downgraded to good, which still meets the WFD goal.

Using Equation 5, the optimal flow rate for the brown trout (Q_{opt}) was computed (STEP 2), yielding approximately $3.65 \text{ m}^3/\text{s}$. Considering the high environmental value of the Torre Orsina river stretch (i.e., it is included in the conservation zone of the Nera River Regional Park), the EF_{min} was set in 80% of the Q_{opt} ($EF_{min} = 2.92 \text{ m}^3/\text{s}$). After computing the EF_{min} , the ecological flow was modulated according to the procedure in STEP 3, yielding EF_{med} and EF_{max} values from the FDC_{EF} (black line in Fig. 7).

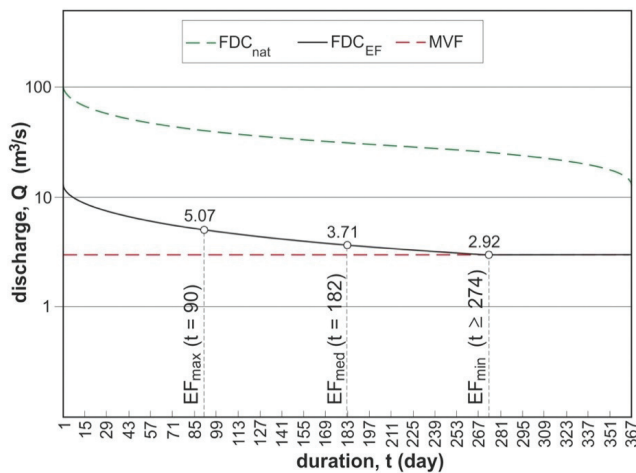


Fig. 7 - Flow Duration Curves (FDC) with EF_{med} and EF_{max} values for the Torre Orsina section computed at the duration (t) proposed by Casadei et al. (2018).

Fig. 7 - Curve di durata (FDC) con i valori di EF_{med} e EF_{max} ricavati per i tempi (t) proposti dall'approccio riportato in Casadei et al. (2018).

To verify the performance of EF modulation on river water quality, the relationships between discharge and nutrient concentrations (Q-C plots) were investigated (see STEP 4). Figure 8a,b shows the Q- P_{tot} and Q- $N-NO_3$ plots based on the historical monitoring carried out by ARPA Umbria

at the NER4 section (Cingolani & Charavgis, 2024). The experimental data for both P_{tot} and $N-NO_3$ do not show a significant trend with increasing flow rate (i.e., slope $b \approx 0$). Moreover, it is worth noting that in most of the monitored periods, the concentrations of P_{tot} and $N-NO_3$ fall below the threshold values for a good quality (level 2, see Table 2), except for a few samples (Fig. 8b).

The WEI+EF index was used to evaluate the effects of current withdrawals and how they can be managed, considering the needs for environmental flow (see STEP 5). Table 4 synthesizes the different components of the WEI+EF index (Eq. 7), considering RWR and EFvol computed for $1 \leq t \leq 365$ days and $t \geq 274$ days (the low-flow period). In both cases, the WEI+EF values fall within the medium water stress class, indicating that the level of water use requires attention to SW-GW-dependent ecosystems.

Discussions

Effect of the modulation of EF on the river water quality

The EF_{min} proposed ($2.92 \text{ m}^3/\text{s}$) confirms the in-force MVF (about $2.90 \text{ m}^3/\text{s}$; PTA 2016-2021), which was already obtained using the microhabitat method, considering fishes as the target for environmental evaluations (Bicchi et al., 2006). The physical-chemical quality of the Nera River water does not show any significant change with increasing discharge, as confirmed by the C-Q plots of nutrients (Fig. 8). However, according to Arora et al. (2020), analysis from C-Q relationships at individual sampling stations can be incomplete for understanding the solute behavior upstream or downstream of the sampling station. In general, the differential C-Q approach (e.g., C-Q analysis along multiple river sections) aims to capture the difference in concentration within a river segment relative to the difference in discharge, thereby accounting for gains, losses, or fractional solute turnover between sampling stations. Although aware of the problem, we have chosen the single point for C-Q analyses in our work since no historic discharge data were available a few kilometers upstream and downstream of the Torre Orsina section. Anyway, despite no dilution processes being observed at the Torre Orsina section in C-Q plots, the nutrient values ($N-NO_3$ and P_{tot}) recorded during more than forty sampling campaigns mainly remained below the concentration limits

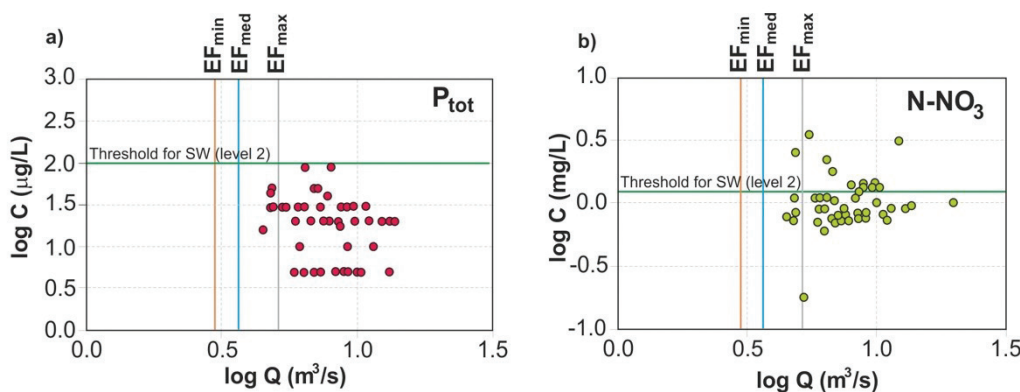


Fig. 8 - Q- P_{tot} plot a) and Q- $N-NO_3$ plot b) for the Nera River at Torre Orsina section based on ARPA Umbria monitoring data (<https://apps.arpa.umbria.it/acqua/qualita-acqua-superficiali>).

Fig. 8 - Grafici Q- P_{tot} a) e Q- $N-NO_3$ b) per il F. Nera alla sezione di Torre Orsina basati sui dati di monitoraggio dell'ARPA Umbria (<https://apps.arpa.umbria.it/acqua/qualita-acqua-superficiali>).

Tab. 4 - WEI⁺_{EF} index at Torre Orsina section estimated over the mean annual period and the mean low-flow period ($t \geq 274$ days). RWR and EF_{vol} values are obtained from FDCs reported in Figure 7, following the approach in Figure 5.

Tab. 4 - Indici WEI⁺_{EF} a Torre Orsina stimati per il periodo medio annuo e per il periodo medio di magra ($t \geq 274$ giorni). I valori di RWR e EF_{vol} sono stati ottenuti dalle FDCs di Figura 7, seguendo l'approccio di Figura 5.

Period of computation ($1 \leq t \leq 365$ days)							
Abstractions (Mm ³)				Returns (Mm ³)	RWR (Mm ³)	EF _{vol} (Mm ³)	WEI ⁺ _{EF}
Canale Medio Nera	Drinking water	Mineral water	Irrigation				
449.9	30.8	0.9	1.5	4.3	992.5	127.7	55% (medium)
Period of computation ($t \geq 274$ days)							
Abstractions (Mm ³)				Returns (Mm ³)	RWR (Mm ³)	EF _{vol} (Mm ³)	WEI ⁺ _{EF}
Canale Medio Nera	Drinking water	Mineral water	Irrigation				
89.1	7.8	0.2	0.9	1.1	163.0	23.2	69% (medium)

that provide a good quality of SW in terms of LIMeco index, indicating that the previous MVF had ensured high-quality water at the Torre Orsina river section.

Ecological flow and water stress evaluation

While from a physical-chemical, biological, and hydromorphological perspectives, the ecological status of the river appears to be in good condition, the quantitative analysis carried out by the WEI⁺_{EF} index highlights some issues that should not be overlooked. The WEI⁺_{EF} (e.g., 69% for low-period, Table 4) has been computed by considering the RWR as depicted by the green area in Figure 5 ($t \geq 274$ days). For the computation of RWR, the FDC_{nat} curve has been used, reconstructed by a regionalization model developed by Bellezza et al. (2009) and Casadei et al. (2018). The regionalization model is widely used in many practical applications and can also be applied to ungauged basins (Castellarin et al., 2004). It should be noted, as discussed by Faergemann (2012) and Casadei et al. (2025), that the FDC_{nat} curve from the regionalization model can underestimate the impacts of withdrawals in the catchment. Further studies, with more accurate monitoring of actual water use, should be carried out to consider the observed discharge data rather than the modelled ones. In this framework, as a precautionary measure, the water stress level over the catchment should be shifted from medium to high. In current water resource management, approximately 94% of withdrawals from the Nera River catchment originate from the Canale Medio Nera, CMN (Table 4). Given the uncertainty in assessing the volume of net water withdrawals in the catchment, the management of withdrawals in current conditions must be supported by accurate hydrogeological and hydrobiological studies. In this framework, a review of existing water diversion concessions would be advisable to ensure that both environmental requirements and drinking water supplies are met, also considering potential effects of prolonged drought periods in the mountain region of Central Italy recorded

by ground-based and remote sensing data (Cambi et al., 2010; Di Matteo et al., 2013; Romano & Preziosi, 2013; Mastrotillo et al., 2023; Ortenzi et al., 2023; Casadei et al., 2025). As reported by Matono et al. (2012) and Burak et al. (2016), prolonged drought periods can still jeopardize the survival of aquatic species; this underscores the urgency of establishing sustainable management of water resources in the Mediterranean Region. This is a key point that overlaps with another critical aspect. Recently, the drinking water supply from springs in the Nera River catchment (e.g., Lupa, San Chiodo; Fig. 1b) has been supplemented by pumping wells, primarily located in the lowest part of the catchment along the river stretches characterized by groundwater inflow (Fig. 1b). Under these conditions, the cone of depression can interact with the river body, affecting the river ecosystem through changes in the availability and temperature of surface water (e.g., Lapedes et al., 2022) and, in some cases, undermining the primary purpose of pumping, which is to extract high-quality water from limestone aquifers for drinking purposes (e.g., Di Matteo & Dragoni, 2005; Barlow & Leake, 2012; Valigi et al., 2021). Overall, even though the cone of depression of pumping wells does not directly interfere with the river, the groundwater extracted from the wells no longer flows towards the river, thereby reducing the water available to GW-dependent ecosystems. Although the withdrawals from these new wells are under definition by means of updated hydrogeological studies, assuming an additional discharge of 100 L/s (about 3.15 Mm³/year) to the drinking water in Table 4, the WEI⁺_{EF} will increase to about 56% and 70%, for $1 \leq t \leq 365$ days and $t \geq 274$ days, respectively. Overall, the WEI⁺_{EF} class remains in the medium category for both evaluation periods ($50 < WEI_{EF}^+ \leq 75\%$, Table 3). As recently reported by Zipper et al. (2024), integrating streamflow depletion into water management decision support is crucial for preventing the possible consequences of groundwater extraction, such as ecosystem degradation or water conflicts.

Conclusions

An integrated approach for defining the modulated EF has been implemented and applied to the Nera River, accounting for biological, hydromorphological, and physical-chemical aspects. Moreover, the current water stress over the catchment due to the use of water resources has been evaluated and discussed. The following conclusions can be drawn:

- The regional model for brown trout allowed the setting of the minimum ecological flow for the Nera River at the Torre Orsina section, which is characterized by a good ecological status, as defined by the NISECI index, physical-chemical analysis of river water, and hydromorphological parameters.
- The modulation of the ecological flow meets the WFD requirements to ensure the needs of GW-dependent ecosystems not only during low-flow periods but also under other hydrological conditions (e.g., during recharge periods). It is an indispensable tool to understand the direct ecological consequences of water management decisions.
- The modulation of ecological flow does not show any evident effects on water quality (e.g., dilution processes), as highlighted by the relationships between nutrient concentration and flow rates derived from historical monitoring data. It is worth noting that the approach remains a valuable tool for assessing the potential impact of managing river discharge.
- The WEI_{EF}^+ has proven reliable for assessing water stress over the Nera catchment, considering the environmental requirements and supporting regional management plans that also address human needs.

The approach proposed for the Nera River may apply to similar basins in the Mediterranean region, underscoring the need for focused, multidisciplinary studies in areas where GW withdrawals and GW-dependent ecosystems coexist.

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Competing interest

The authors declare no competing interest.

AI use declaration statement

The authors declare no AI was used for the preparation of any part of this paper

Author contributions

All authors contributed to the study conception, design, methodology, and wrote the manuscript. They read and approved the final manuscript.

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