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Do people have the power? Citizen Science and Environmental Monitoring: insights from the Ombrone River basin and European experiences

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Abstract

Freshwater ecosystems are widely threatened by multiple anthropogenic pressures, while conventional monitoring programmes remain limited in spatial and temporal coverage. In this context, Citizen Science (CS) has emerged as a promising approach to complement traditional monitoring, although concerns regarding data quality and methodological consistency persist. This PhD thesis provides an evaluation of participatory approaches for assessing the ecological quality of riverine ecosystems, based on a series of targeted field experiments conducted across different biological components.

The research initially focuses on the development and validation of a simplified CS protocol using benthic macroinvertebrates as habitat quality bioindicators. The results showed that CS-based methods can produce ecologically meaningful results comparable to expert-based assessments, provided that robust standardisation of sampling procedures and experimental design is ensured (Chapter 1).

A comparative analysis of European simplified macroinvertebrate protocols further reveals that methodological differences in sampling design influence data comparability, highlighting the need for further investigation to harmonise approaches at a continental scale (Chapter 2).

Expanding beyond aquatic biota, the thesis evaluates a novel protocol for riparian vegetation monitoring, showing that volunteers are capable of generating high-quality data when protocols are carefully designed to minimise observer bias. The findings also emphasise the importance of targeted training and iterative feedback for improving data reliability (Chapter 3).

Finally, the thesis presents the CS4Rivers project, a transdisciplinary framework integrating citizen participation, scientific research, and environmental monitoring, within which the aforementioned CS protocols were developed and/or tested. The results obtained from citizen-generated data highlight the potential of the simplified protocols as effective early warning tools, capable of capturing spatial heterogeneity and ecological gradients within the study area (Chapter 4).

Overall, the thesis demonstrates that Citizen Scientists can generate reliable, high-quality data when supported by robust, standardised protocols and continuous training with iterative feedback. Practical field experience further enhances volunteer

performance over time, underscoring the importance of long-term, well-structured CS programmes. The integration of participatory and traditional monitoring approaches could provide a more comprehensive assessment of ecosystem quality. Collectively, these findings strengthen the scientific basis for the inclusion of CS within official freshwater monitoring frameworks and support its role in long-term environmental assessment and management.

Riassunto

Gli ecosistemi di acqua dolce sono ampiamente minacciati da molteplici pressioni antropiche e i programmi di monitoraggio ufficiali risultano limitati in termini di copertura spaziale e temporale. In questo contesto, la Citizen Science (CS) si sta affermando come un approccio chiave per integrare il monitoraggio ufficiale, sebbene permangano preoccupazioni riguardo alla qualità dei dati e alla validità metodologica. Questa tesi di dottorato fornisce una valutazione di approcci partecipativi per la valutazione della qualità ecologica degli ecosistemi fluviali, basata su una serie di esperimenti in campo condotti su differenti componenti biologiche.

La ricerca si concentra inizialmente sullo sviluppo e sulla validazione di un protocollo CS semplificato basato sui macroinvertebrati bentonici come bioindicatori della qualità dell'habitat. I risultati dimostrano che i metodi semplificati possono produrre risultati comparabili a quelli ottenuti da esperti, a condizione che siano garantite una rigorosa standardizzazione delle procedure di campionamento e del disegno sperimentale (Capitolo 1).

Un'analisi comparativa di protocolli semplificati europei basati sui macroinvertebrati bentonici evidenzia come le differenze nel disegno di campionamento influenzino la comparabilità dei dati raccolti, sottolineando la necessità di ulteriori approfondimenti per armonizzare i protocolli a scala continentale (Capitolo 2).

Estendendo l'analisi, la tesi valuta un nuovo protocollo per il monitoraggio della vegetazione ripariale, mostrando che i volontari sono in grado di produrre dati di elevata qualità quando i protocolli sono progettati in modo da minimizzare il bias dell'osservatore. I risultati evidenziano inoltre l'importanza della formazione mirata e del feedback iterativo nel migliorare l'affidabilità dei dati (Capitolo 3).

Infine, la tesi presenta il progetto CS4Rivers, un framework transdisciplinare che integra partecipazione dei cittadini, ricerca scientifica e monitoraggio ambientale, all'interno del quale i protocolli CS sono stati sviluppati e/o testati. I risultati ottenuti dai dati raccolti dai cittadini evidenziano il potenziale dei protocolli semplificati come efficaci strumenti di allerta precoce, in grado di rilevare eterogeneità spaziali e gradienti ecologici nell'area di studio (Capitolo 4).

Nel complesso, la tesi dimostra che i volontari dei progetti di CS possono generare dati affidabili e di alta qualità quando supportati da protocolli robusti e standardizzati e da una formazione continua con feedback iterativo. L'esperienza pratica sul campo

migliora ulteriormente le prestazioni dei volontari nel tempo, sottolineando l'importanza di programmi di CS strutturati e di lungo periodo. L'integrazione tra approcci partecipativi e tradizionali può consentire una valutazione più completa della qualità degli ecosistemi. Nel loro insieme, questi risultati rafforzano le basi scientifiche per l'inclusione della CS nei programmi ufficiali di monitoraggio delle acque dolci e ne supportano il ruolo nella gestione e valutazione ambientale a lungo termine.

Overview of the PhD thesis

This doctoral thesis addresses the growing need for innovative, participatory, and reliable approaches to assess the ecological quality of freshwater ecosystems. These environments are, in fact, widely threatened by multiple anthropogenic pressures, while conventional monitoring programs remain constrained by limited spatial and temporal coverage. Within this context, the thesis explores how participatory approaches (Citizen Science - CS) can assess the ecological quality of riverine ecosystems. The work is structured around a series of field-based experiments that primarily examine the reliability, comparability, and applicability of CS-based protocols, across different biological components of riverine ecosystems.

A central focus is placed on the use of benthic macroinvertebrates as indicators of ecological status, given their established role within regulatory frameworks such as the Water Framework Directive (EC, 2000). The first part of the thesis introduces and evaluates a newly developed simplified protocol (EasyBenthos), specifically designed for non-expert applications, on the assessment of its performance under controlled experimental conditions and its ability to generate consistent ecological information (Chapter 1).

Building upon this, the second chapter expands the perspective to a broader European scale, comparing multiple simplified macroinvertebrate sampling protocols developed in different national contexts. By testing these approaches under comparable field conditions, this section investigates the degree of methodological interoperability and highlights the factors that influence the consistency of results obtained with different CS protocols (Chapter 2). This comparative approach provides an essential step toward understanding the feasibility of harmonised CS-based monitoring across Europe.

The thesis then focuses on riparian ecosystems, which are a critical, yet often underrepresented, component of ecological assessment frameworks. A novel protocol for riparian vegetation monitoring (RiVe) is introduced and evaluated, with a particular focus on data quality, and the role of training in improving volunteer performance (Chapter 3).

Finally, the thesis puts these individual methodological contributions within the broader operational framework in which the simplified approaches were developed and/or tested: the CS4Rivers project. This chapter presents the key descriptive and

operational components that enabled the design, implementation, and functioning of the project over three years of field-based experimentation (Chapter 4).

Thesis Introduction

1.1 Why a Citizen Science project?

In recent decades, issues concerning the governance of science - particularly its sustainability and accessibility - have increasingly emerged, encompassing not only the acquisition and dissemination of knowledge, but also its generation and co-production. Furthermore, evolving legislation, technology, and culture have raised fundamental questions about scientific excellence, funding, patentability, and the principles of open science. In addition, many authors propose to manage science as a commons. The term 'commons' refers to a form of community management of a shared resource. Good governance of the commons implies that the communities that share access and/or use of a resource manage their behaviour through a self-established set of rules (Ostrom 1992; Madison et al. 2019). The commons results from a collaborative, open, and experimental process, and necessarily involves the community of practice. Each community not only produces the commons but is simultaneously produced in the common acting (Dardot et al., 2019).

In this framework, the role of Citizen Science (CS) - as the active engagement of the general public in scientific research tasks (Bonney et al., 2014) - is crucial, as it involves a research practice in which every citizen, entity, and community can find their place and share responsibility. As a dynamic and integrative approach, CS offers valuable responses by fostering collaboration, sustainability, accessibility, and quality in scientific knowledge production, contributing to improved governance of science (Freschi et al., 2024). CS, in particular, extends beyond dialogue and participation in decision-making processes to enable non-experts to generate knowledge throughout all stages of the scientific process (Leach et al., 2005).

Worldwide and across Europe, the prevalence of CS practices is increasing at both European and national levels. Since 2011, several CS projects have been funded by the European Commission, indicating the institutionalisation of CS (European Commission, 2021).

Since CS is a rapidly expanding field that spans a wide range of disciplines in science, technology, engineering, and mathematics (STEM) (Mason et al., 2025), in this thesis, we will refer to environmental CS and its application in environmental monitoring.

1.2 Benefits and Challenges of CS in Environmental Monitoring

CS has been widely recognised for delivering substantial benefits across scientific and social domains. Documented advantages include the promotion of scientific literacy, public engagement, and ecosystem stewardship (Evans et al., 2005; Pollock et al., 2005). By fostering information exchange between scientists and non-scientists, CS contributes to the democratisation of science and integrates multidisciplinary approaches to inform policy and conservation programs (Robertson et al., 2001; Carr, 2004); furthermore, it also makes scientists more aware of local knowledge and expertise (Carolan, 2006). Moreover, CS offers cost-effective monitoring solutions for government agencies, expanding temporal and spatial coverage beyond what institutional resources alone can provide (Whitelaw et al., 2003; Conrad & Daoust, 2008).

At the societal level, CS contributes to strengthening trust, cooperation, and leadership within communities and between civil society and the scientific community (Whitelaw et al., 2003; Schwartz, 2006). These dynamics encourage community resilience, foster stewardship ethics, and promote more sustainable local governance (Cooper et al., 2007). Participation in CS projects enhances public understanding of scientific processes, reinforces environmental education, and strengthens community awareness of local environmental dynamics (Au et al., 2000; Nali & Lorenzini, 2007).

Despite these benefits and positive attitudes, significant challenges persist. These can be grouped into epistemic, ethical, and political dimensions (Resnik et al., 2015).

Epistemic challenges concern data quality, participant training, and methodological rigour. Common issues include limited experimental design and inconsistent data validation procedures, leading to skepticism about data accuracy (Whitelaw et al., 2003; Gouveia et al., 2004). Concerns about data quality persist, particularly among researchers and natural resource managers who rely on high-precision datasets (Aceves-Bueno et al., 2015; Brown et al., 2019). Addressing these challenges requires clear data governance protocols, improved visibility of validated CS initiatives (Galanos et al., 2022), and good practices that guide quality assurance. In addition, many project managers report recruiting and retaining long-term participants as a key barrier to effective project implementation (L'Astorina et al., 2023). Strategies to address these gaps include adopting engagement frameworks that promote sustained participation through recognition, feedback, and community building (Liñán et al., 2022).

Ethical challenges include preventing misappropriation of research results, exploitation of participants, ensuring transparency, and safeguarding equitable recognition for citizen contributors (Purdam, 2014).

Finally, political challenges relate to strengthening cooperative governance structures and integrating data collected through CS into formal monitoring frameworks and policy processes. Among natural resource practitioners, the emphasis on generating robust datasets often outweighs efforts to ensure their institutional integration, revealing a persistent gap between data production and its uptake within official systems (L'Astorina et al., 2023).

Overall, CS continues to evolve as a crucial instrument linking knowledge generation, democratic participation, and sustainable governance. To fully realise its potential, sustained institutional support, effective coordination, and transparent data management frameworks are essential to ensure both scientific robustness and long-term policy relevance.

1.3 The importance of Data Quality

A practical definition of high-quality data in CS refers to datasets that exhibit levels of accuracy and bias comparable to those generated by professionals and trained experts (Bonney et al., 2014; Cooper et al., 2014; Theobald et al., 2015).

Evidence shows that well-designed CS projects can achieve data accuracy equal to or exceeding that of professional monitoring programs. Kosmala et al. (2016) identify a set of guiding points for evaluating the robustness and suitability of CS datasets.

1. High-quality CS data depend on iterative project design, where protocols and tools are refined through pilot testing to minimise confusion and systematic error. Task complexity is a major determinant of accuracy: simple, well-structured activities typically yield more reliable results, whereas complex tasks require targeted training, pre-tests, expert validation, replication, and statistical correction to control for bias.
2. Ensuring systematic procedures for data capture and entry are essential. Projects should employ standardised digital forms, controlled input fields (e.g., numerical constraints for counts), and categorical selection menus to reduce transcription errors. Similarly, standardised and calibrated equipment should be used across volunteers to maintain measurement consistency.

3. The inclusion of relevant metadata (such as environmental conditions, device settings, and participant characteristics) is critical for quantifying and modelling sources of bias.
4. Sampling effort should be standardised (e.g., by location, time, or duration). In projects where this is not feasible, effort data must be recorded and incorporated into analytical models to account for uneven sampling intensity.
5. Comparisons between volunteer and professional-collected data are necessary. Importantly, accuracy among professionals should also be evaluated to distinguish inter-observer variability from differences between professional and volunteer datasets.
6. Data should be fit for purpose, covering appropriate temporal, spatial, and quantitative dimensions aligned with the research objectives. Good data management practices are also essential (Borer et al. 2009; Michener and Jones 2012; Wiggins et al. 2013). These include secure storage, consistent and interpretable data formatting, and accessibility for end users.

In many cases, the CS datasets are the only available data that have the level of geographical coverage required to investigate large-scale ecological questions (Dickinson et al., 2010). Therefore, it is necessary to understand the level of data quality and address these concerns to strengthen the CS approach within scientific research.

1.4 Freshwater Ecosystems and Citizen Science: the CS4Rivers project

In recent decades, national and international freshwater policies have been implemented to enhance the ecological status of surface waters and counteract their historical degradation. The United Nations Sustainable Development Goal (SDG) 6 on Clean Water and Sanitation (UN, 2018) aims to improve water quality by reducing pollution and hazardous chemical use (target 6.3) and to safeguard water-related ecosystems (target 6.6). In Europe, the Water Framework Directive (WFD, 2000/60/EC) mandates the member states to restore or maintain a “good ecological status” in all surface water bodies. Nevertheless, approximately 60% of European rivers remain below this threshold (EEA, 2018).

Monitoring the condition of freshwater ecosystems - including water quality, biodiversity, hydromorphology and riparian vegetation - poses significant challenges for relevant authorities, in terms of time, labour, and financial resources, given the

extensive length of stream networks and the high number of small standing waters (Kelly-Quinn et al., 2023). In this context, CS offers a cost-effective and participatory approach to address these monitoring gaps by expanding temporal and spatial data collection coverage (Brooks et al., 2019).

All over the world, many CS projects focus on different components of freshwater ecosystems. In the most valuable cases, they actively collaborate with environmental agencies to assess the habitat quality of riverine ecosystems (e.g., Anglers' Riverfly Monitoring Initiative, https://www.riverflies.org/).

In Italy, a new CS project has been developed for this purpose. CS4Rivers ("Citizen Science for Rivers", cs4rivers.unisi.it/) is a CS program led by the University of Siena (Spoke 3 of the National Biodiversity Future Centre) and funded by the National Recovery and Resilience Plan (NRRP). Its primary focus is the establishment of a "Riverine Biodiversity Observatory" on the Ombrone river basin (South of Tuscany, Italy) through active involvement of local communities, stakeholders, and citizen scientists.

The main objectives of the project are: i) to generate new scientific knowledge on river-ecosystem biodiversity to advocate on local, regional and national policy; ii) to enhance community psychophysical well-being by engaging volunteers in outdoor field activities in natural river settings; iii) to facilitate networking among local, regional and national stakeholders focused on riverine ecosystem conservation.

CS4Rivers is developed to monitor multiple components of river ecosystem; in fact, it is active throughout different participatory monitoring activities: chemical water quality, macroinvertebrate community and riparian vegetation. Thanks to the CS approach, the project seeks to address critical information gaps in small-stream and tributary systems, often under-monitored by conventional programmes and emphasises shared responsibility between scientists, practitioners and citizens in the stewardship of the Ombrone river basin.

Volunteers are trained for standardised protocols to ensure data reliability and compatibility with scientific and management uses. Training, tools and resources are provided to support participation in data collection and monitoring activities.

Within this thesis, the CS4Rivers project serves as a case study for assessing the scientific strength of CS approaches in freshwater ecosystem monitoring. In parallel, it also considers the role of well-established biomonitoring methods in providing an expert-based perspective on environmental quality. These official approaches align

with the broader perspective adopted in this work, which recognises the value of combining institutional and CS methodologies to achieve more comprehensive ecological assessments. In this sense, such official methods may also represent a potential avenue for future integration within CS initiatives.

Study Area

The Ombrone River basin (ORB) is a highly heterogeneous fluvial system both in geomorphological and environmental terms. Geographically, it extends across the provinces of Siena and Grosseto, and includes several major tributaries, such as the Arbia, Merse, Orcia, and Trasubbie rivers. (Fig. 1).

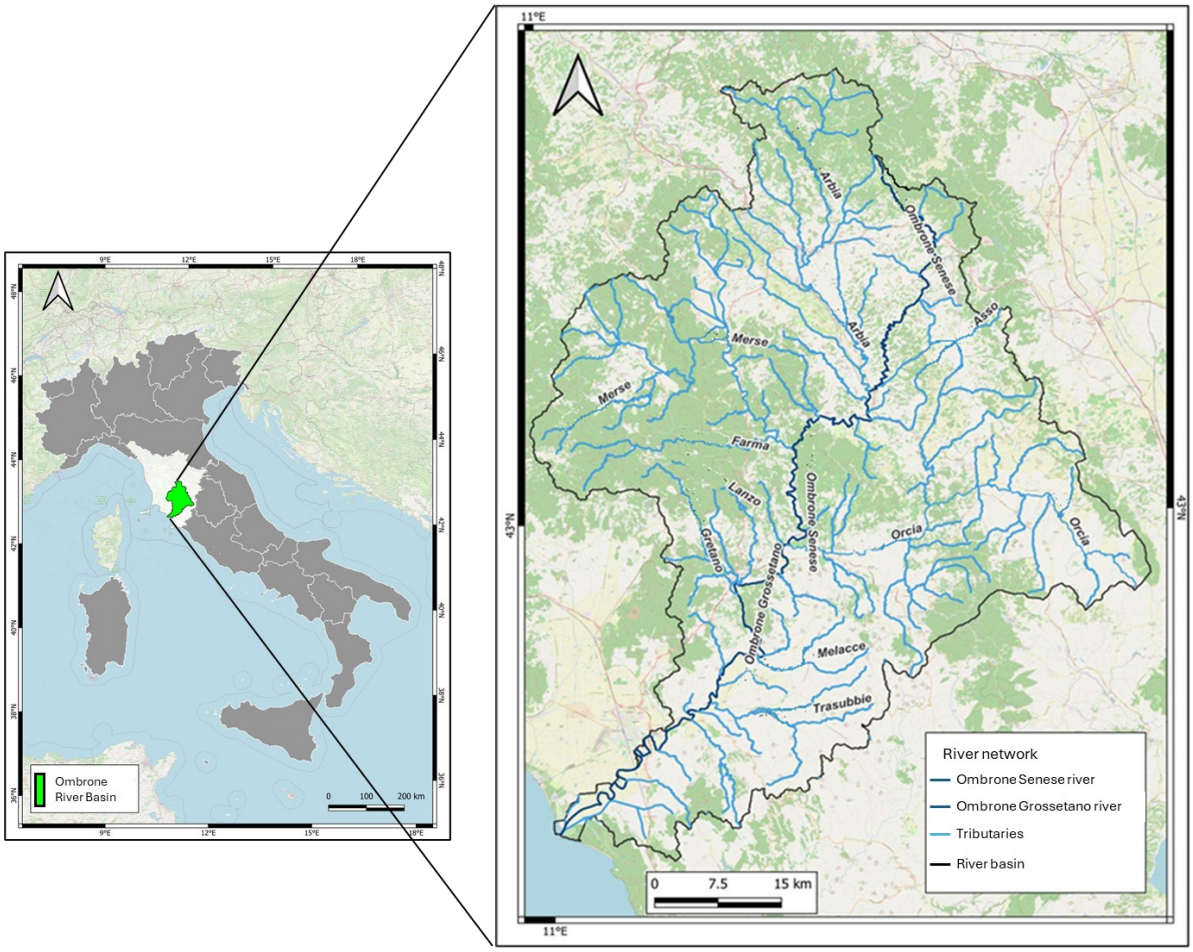


Figure 1. The Ombrone River basin and its tributaries

Climatically, the ORB is strongly influenced by the Tyrrhenian Sea, which produces a moderating effect, as well as by its predominant westward exposure. The Apennine range provides a natural barrier against cold air masses originating from the east and northeast. Atmospheric circulation is dominated by westerly and northwesterly currents, while the wind regime is further shaped by Mediterranean depressions and

orographic effects. During the winter, these conditions favour the advection of humid air masses from the south over Tuscany (Sanna et al., 2024).

ORB territory is characterised by rich natural and cultural resources and historically has represented a key identity element for southeastern Tuscany (Rombai, 2009). In recent decades, these features have supported the development of tourism-related activities promoted by local associations, including rafting, navigation, cycling, and trekking along the river corridor (Fig. 2). However, the basin is also subject to significant anthropogenic pressures. The ORB is characterised by a dispersed settlement pattern, with an average population density of approximately 50 inhabitants per km². Between 2001 and 2011, the population increased by 5.5%, reaching 157,399 inhabitants, followed by a decrease of 4.6% between 2011 and 2021, down to 150,185 inhabitants. Over the same period, the number of buildings increased by 9%, with a particularly marked rise in productive, commercial, and tourism-related structures (+237%), compared to a modest increase in residential buildings (+6%) (istat.it).



Figure 2. Rafting activities alongside the Ombrone river. Ph. Chiara Vitillo

Land use within the basin is dominated by agricultural areas (56%) and forested land (42.5%), while artificial surfaces account for slightly more than 1% (European Environment Agency, 2019). Intensive land use practices and deforestation have contributed to increased soil erosion and delta formation. Subsequently, the delta has undergone significant erosional processes due to reduced sediment supply, aggregate extraction, and land subsidence, resulting in coastline retreat and saltwater intrusion into groundwater systems. Environmental pressures also include contamination

phenomena, particularly arsenic and metal pollution affecting the Merse River, mainly due to mining activities in the Colline Metallifere area, as well as water abstraction for intensive agriculture. For these reasons, part of the experimental work of this thesis focused primarily on the Merse River.

The main hydrological axis of the basin is represented by the Ombrone River. It extends for 161 km and drains a catchment area of 3,494 km². Its upstream reaches are characterised by an extremely torrential regime, whereas the terminal section exhibits features typical of lentic environments, including reduced flow velocity, low dissolved oxygen concentrations, and high loads of suspended inorganic and organic matter. This marked longitudinal variability supports high levels of biodiversity, as evidenced by the presence of numerous protected areas along the river course (ARPAT, 2001). The riverside vegetation varies, with dense forests upstream (*Alnus* sp., *Cornus* sp., *Fraxinus* sp., and *Quercus ilex*). Near the mouth, *Salix* sp., *Populus* sp., *Juniperus* sp., and herbaceous plants predominate (Fig. 3). This riverine environment supports a unique fauna, including the endangered fish Etruscan chub (*Squalius lucumonis*) (Rombai, 2009).



Figure 3. Riparian vegetation alongside the Ombrone river. Ph. Chiara Vitillo

Among the main tributaries, the Merse River is 71.7 km long and drains a basin of 705.8 km². It receives the Feccia River and Rosia Stream on the left, and the Gonna Stream and Farma River on the right and ultimately flows into the Ombrone River. Hydrologically, peak discharges occur in autumn-winter, whereas minimum flows are recorded in summer; however, baseflow remains relatively stable due to numerous perennial springs sustaining discharge even during dry periods (Benvenuti et al., 1971).

From a geological perspective, the area is mainly characterised by Quaternary alluvial deposits, both recent and ancient (Landi et al., 2002).

The Merse basin is largely covered by forested areas interspersed with pasture and agricultural land. Vegetation is dominated by mixed deciduous oak forests (*Erico-Quercetum cerridis* Arrigoni 1990), with thermophilous holm oak stands (*Viburno-Quercetum ilicis*) on warmer slopes and serpentine substrates. Siliceous terrains host forests dominated by *Quercus cerris* and *Castanea sativa* (*Quercetalia robori-petraeae*), while conifer reforestations, primarily *Pinus pinaster,* are widespread (De

Dominicis et al., 1997). The high ecological value of these habitats, together with the rich faunal diversity, has led to the establishment of several protected areas, including regional and national reserves and Sites of Community Importance (SIC). In its upper reaches, river waters often exhibit a reddish colouration caused by the erosion of mineral-rich deposits linked to historical mining activities (Fig. 4) (ARPAT, 2001).

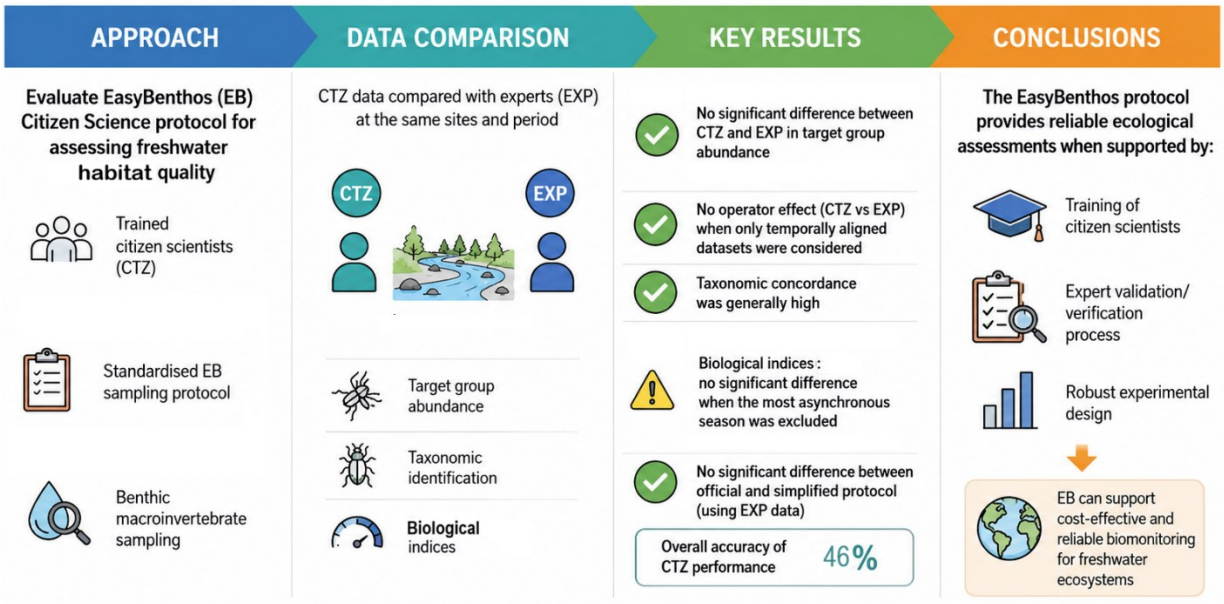


Figure 4. Reddish water colouration of the Merse River near the Colline Metallifere mining area. Ph. Chiara Vitillo

Chapter 1.

Assessing freshwater quality through Citizen Science: evaluation of the EasyBenthos protocol based on benthic macroinvertebrates

Graphical abstract



Abstract

This study evaluates the EasyBenthos (EB) Citizen Science (CS) protocol for assessing the habitat quality of freshwater ecosystems using benthic macroinvertebrates in the Ombrone River basin (Tuscany, Italy). Non-expert volunteers (CTZ), after a training course and following standardised sampling procedures, collected data used to calculate a Simplified Biotic Index (SBI). Volunteer-generated data were compared with data collected by experts (EXP) at the same sites and over the same period, focusing on abundance estimates, taxonomic identification of target groups, and ecological quality indices. Results showed no significant difference between CTZ and EXP in estimating target group abundance, although a significant difference was observed in the overall abundance. The temporal asynchrony between CTZ and EXP sampling events significantly influenced total abundance discrepancies, while no operator effect (EXP vs CTZ) was observed when only temporally aligned datasets were considered. Taxonomic concordance was generally high. Biological indices derived from CTZ and EXP data revealed no significant difference when the sampling season with the greatest temporal

discrepancy was excluded. Quality class results generally differed by one ecological class. When comparing results obtained with the official and simplified protocol using data collected by the same operator (EXP), no statistically significant difference was detected. The overall accuracy of the CTZ performance was 46%. These results indicate that the EB provides reliable ecological assessments when supported by training, EXP validation/verification process, and a robust experimental design.

1. Introduction

Freshwater environments provide numerous ecosystem services (Naselli-Flores & Padisák, 2022), which are considered unique and irreplaceable on Earth (Allan & Castillo, 2007; Hanna et al., 2018). Despite their ecological importance, these environments are among the most impacted and modified globally, facing multiple anthropogenic pressures including nutrient enrichment, pesticide runoff, and morphological alterations (Blake & Rhanor, 2020; Albert et al., 2021; von Gönner et al., 2024).

In response to widespread freshwater quality degradation in Europe, the Water Framework Directive (WFD) sets ambitious objectives to achieve a good ecological status for all surface waters and good ecological potential for heavily modified water bodies (EC, 2000). Furthermore, the Directive establishes common standards and methodological frameworks for all EU Member States, along with shared environmental objectives. A key innovation of the WFD lies in its strong emphasis on biological quality elements (BQEs) - including macroinvertebrates, fish, diatoms, and macrophytes - as fundamental components for evaluating the ecological status (Roccatello et al., 2025).

Benthic macroinvertebrates have become the most used BQEs for assessing freshwater biological quality (Buss et al., 2015). Due to their sedentary nature, long life cycle, and diverse sensitivity to chemical and physical stressors, macroinvertebrate communities are particularly suitable for ecological assessments (Wallace et al., 1996; Barbour et al., 1999). Despite this well-established framework, the practical application of the WFD remains challenging. National and regional authorities face substantial constraints in terms of financial resources, personnel, and logistical capacity, particularly given the vast extent and fragmentation of European river networks. In addition, official monitoring programmes tend to prioritise large rivers, leading to

persistent spatial and temporal data gaps in freshwater quality assessments (Kelly-Quinn et al., 2022; Weisner et al., 2022; von Gönner et al., 2024).

In this context, Citizen Science (CS) has emerged as a transformative tool to overcome these limitations by actively engaging the public in scientific inquiry (Bonney et al., 2014). Participatory programs enable the collection of large-scale datasets across diverse landscapes, providing insights into ecological trends that would be logistically impossible for professional scientists alone (Blake & Rhanor, 2020; Callaghan et al., 2021).

Regardless of its potential, CS faces relevant methodological challenges. Scientists often remain sceptical of volunteer data due to potential spatial biases, reporting preferences for charismatic species and misidentifications (Kosmala et al., 2016; Johnston et al., 2022). Furthermore, taxonomic identification accuracy is highly resolution-dependent; while volunteers excel at order or family levels, accuracy significantly declines at genus or species levels (Moffett & Neale, 2015; von Gönner et al., 2023). To avoid these limitations in using CS, ensuring good data quality and a robust standard protocol for data collection remains a fundamental prerequisite for the effective integration of CS into official water management frameworks, for its acceptance as a credible complementary research approach, and for fully exploiting its scientific potential. In addition, data accuracy is influenced by multiple factors, including task complexity, volunteer expertise, and prior familiarity with the target organisms (Kosmala et al., 2016). Therefore, the selection of study organisms is a critical element in the design of an effective CS program.

Benthic macroinvertebrates have several characteristics that make them particularly well-suited for CS initiatives. They are relatively easy to sample and identify, which allows non-specialists to collect reliable data; they are widely distributed across freshwater habitats, making them accessible in most rivers and streams; and they have limited mobility and long-lasting aquatic life stages, which increase the likelihood of encountering them during sampling and simplify repeated observations (Roccatello et al., 2025).

Within the CS4Rivers project, we developed “EasyBenthos” (EB), the first Italian simplified protocol specifically designed for CS-based assessment of riverine ecological quality through the analysis of macroinvertebrate communities. In this study, we present the protocol and evaluate the quality of the data obtained with its application through an experimental assessment.

2. Materials and Methods

2.1 Study Area

The research was conducted as part of the CS4Rivers project, which is centred on the Ombrone river basin in southern Tuscany, Italy. Field activities took place along the Merse river and its principal tributary, the Farma river, selected for their variety of sites that are particularly suitable for monitoring benthic macroinvertebrate communities and for testing this type of ecological assessment approach (Fig. 1).

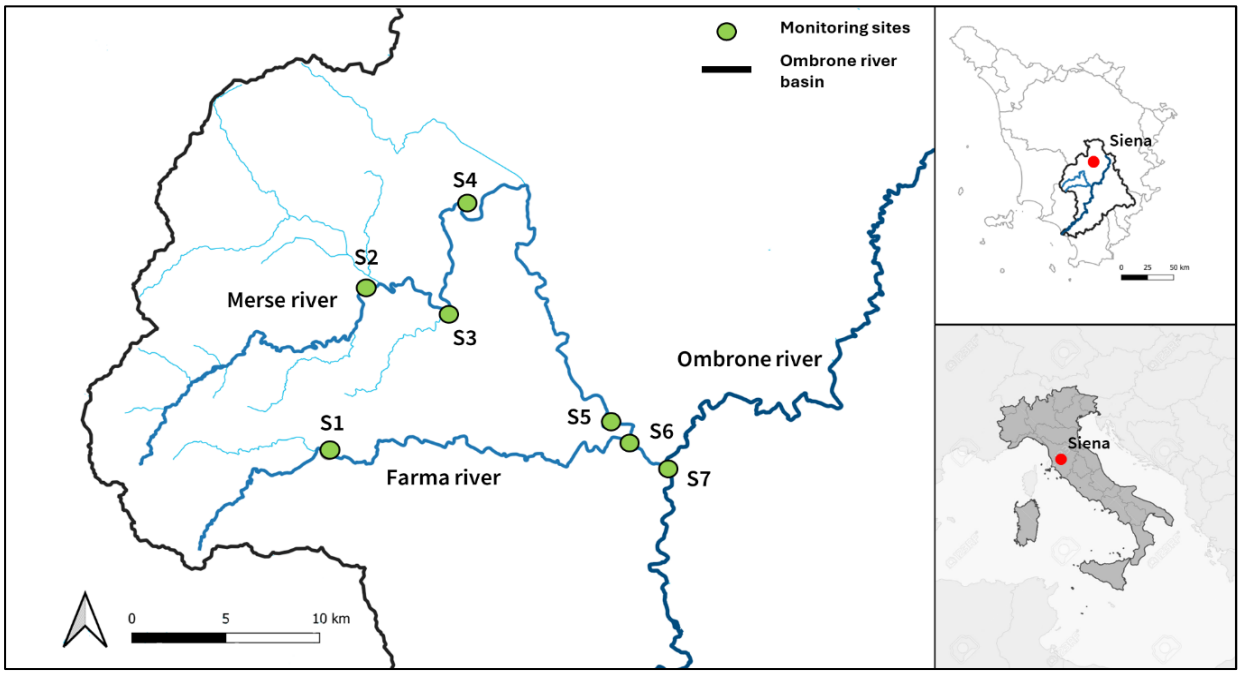


Figure 1. Study area and the monitoring sampling sites

2.2 The EasyBenthos protocol within the CS4Rivers project

EasyBenthos (EB) is the first Italian simplified protocol designed to evaluate the ecological quality of riverine ecosystems through the analysis of macroinvertebrate communities using a CS approach. EB allows for calculating the Simplified Biotic Index (SBI), which was specifically developed as a streamlined version of the official, well-established Extended Biotic Index (EBI) (Ghetti, 1997), a qualitative index that facilitates volunteers' participation while maintaining scientific rigour. The SBI is not a novel biotic index in terms of calculation; rather, it represents a simplified index for deriving EBI values from reduced taxonomic levels collected by trained volunteers.

The EB protocol is organised into two consecutive steps: 1) training, and 2) data collection.

1) Training

Before participating in field activities, all volunteers attended a standardised training session. The training, led by a CS4Rivers researcher, covered the ecological role of macroinvertebrate community, the application of the sampling protocol, and the use of supporting materials, including a monitoring handbook, a simplified dichotomous key, and field data sheets. A practical field exercise was included to ensure familiarity with the protocol, promote consistency in data collection, and reduce observer-related variability.

2) Data collection

During the data collection phase, volunteers/citizen scientists (CTZ) carried out monitoring activities independently. The monitoring procedure was divided into three phases: a) environmental characterisation, b) macroinvertebrate sampling, and c) taxonomic identification.

a) At each sampling site, CTZ first assessed safety conditions (clear water and depth below the knee) and verified the feasibility of sampling based on river flow characteristics (stable flow conditions). Where sampling was deemed safe and applicable, CTZ used a standardised datasheet to systematically record general ecosystem information, including the microhabitat's presence, physical and hydromorphological characteristics of the river reach, georeferenced site coordinates, and photographic documentation (Supplementary Materials, Fig. 1S).

b) Macroinvertebrate sampling was conducted using the kick sampling technique and a standardised sampling net with a 250 mm frame and a 500 mm deep net bag.

The mesh size was 1 mm, which effectively excludes very small organisms and ensures methodological consistency with other CS monitoring programmes. During the sampling, large cobbles were lifted and manually cleaned in front of the net.

The sampling effort was distributed proportionally to the presence of different microhabitats identified within the riverbed to ensure a representative collection of the benthic community. Sampling activities were conducted within a river reach ranging from a minimum of 10 m to a maximum of 50 m in length. CTZ moved from one bank to the opposite bank and then returned to the starting bank, following a systematic transect tracing a V-shaped path across the river reach. Each sampling lasted 3-5 minutes.

c) During the sorting and identification phase, CTZ used a simplified dichotomous key together with sampling materials developed by CS4Rivers researchers to classify

organisms into predefined target groups (Fig. 2). These target groups were established within the CS4Rivers project and correspond to the sensitivity groups employed in the EBI calculation table (Tab.2), where their presence determines the horizontal entry used to derive the final index value. The groups are explicitly selected according to their sensitivity to habitat alteration, forming an ordered gradient from the most to the least sensitive taxa. In addition, they were chosen as the most frequent, representative, and easy-to-identify in the study area. Accordingly, the target groups were recorded in the SBI tabular datasheet: the rows represent target groups organised by decreasing sensitivity to habitat alteration, while the columns indicate the total number of organisms of each group. Instead of counting every individual, abundance was recorded in discrete classes (class 1: 1-2 organisms; class 2: 3-10 organisms; class 3: >10 organisms), which also allows consideration of drift effects (Ghetti, 1997). For Plecoptera, Ephemetoptera, Tricoptera and Chironomidae-Oligochaeta, an additional row was used to record the number of families in terms of 1 or >1 (Supplementary Materials, Fig. 2S). To guarantee data quality and taxonomic rigour, CTZ, following a specifically developed protocol, take and transmit high-resolution photographs of each identified target group to the experts (EXP). These images allowed EXP to verify the taxonomic identification at family level and calculate the SBI (Fig. 3). CTZ also sent by email all the other data collected in the field to EXP for validation. This validation/verification step also provides post-sampling feedback to volunteers, supporting learning and improving the quality and consistency of subsequent data collection. Here we consider validation as the process through which records are checked to ensure the data have been submitted correctly, and verification as the process of checking records for correctness, confirming taxonomic identity (Tweddle et al., 2012).

All organisms collected were released back at the sampling site at the end of the sorting phase.



Figure 2. Citizen Scientists during the sorting phase. Ph Gaia Cappelletti

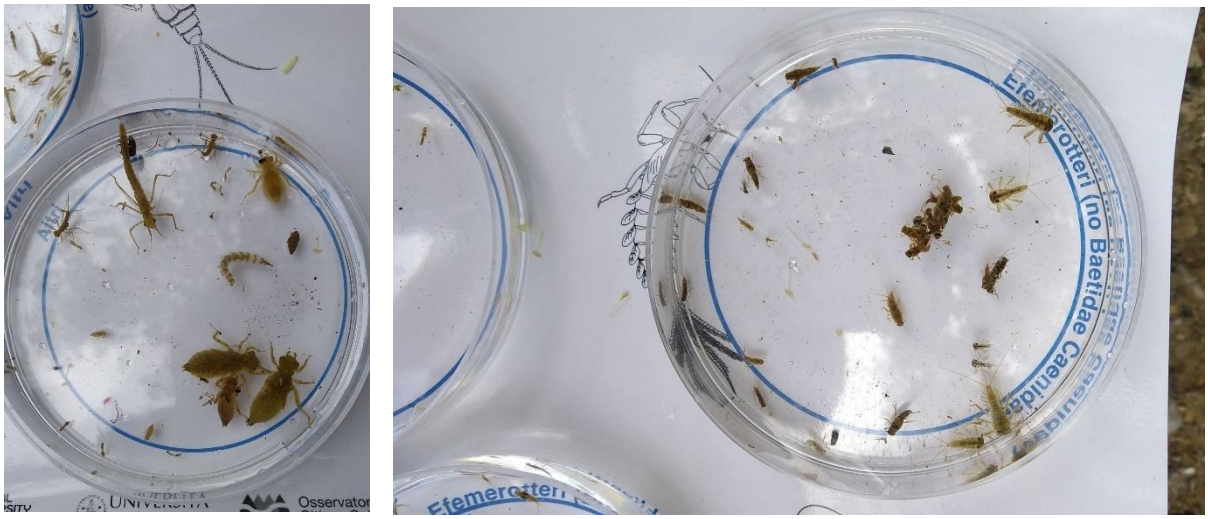


Figure 3. Target groups' pictures sent by Citizen Scientists, examples. Ph. Barbara Anselmi

2.3 Experimental Design

To assess the quality and reliability of CTZ-generated data and the overall robustness of the EB protocol, an experimental field assessment was conducted. EXP collected reference data sampling the same monitoring sites and using the same CTZ protocol. EXP surveys were conducted at the same period as the CTZ's sampling: April-May for the spring sampling and October-November for the autumn one. Monitoring sites were selected during a co-design session involving the CS4Rivers researchers and

volunteers, based on accessibility and safety criteria, and on the selection of sites with similar microhabitat presence to ensure similar macroinvertebrate community composition (Tab. 1).

Table 1. Microhabitat presence (%) per monitoring site as the mean value for the three sampling campaigns

Site	Clay/ sand	Gravel	Medium pebbles Ø max 20cm	Large pebbles Ø max 40cm	Stones >40cm	Rock slabs	Artificial
S1	10	50	20	20	0	0	0
S2	10	20	40	20	5	0	5
S3	20	30	40	10	0	0	0
S4	10	20	50	20	0	0	0
S5	10	20	20	30	10	5	5
S6	30	50	10	10	0	0	0
S7	30	0	40	30	0	0	0

A total of 15 volunteers, organised into groups of at least two people, surveyed 7 monitoring sites during the Spring 2024, Autumn 2024 and Spring 2025. Each monitoring site was surveyed three times by the same group; however, during the autumn campaign, deviations from the planned sampling effort occurred due to inadequate field conditions, resulting in two surveys at two sites. As a pilot test, one site was surveyed four times.

Abundance estimation of the target group was compared between CTZ and EXP. Abundances were estimated as the mean of the class range; the maximum abundance class corresponding to the mean number of organisms observed per group (15 individuals).

Quality classes obtained with CTZ and EXP-generated data using the simplified protocol were compared.

At each monitoring site, EXP also conducted a second sampling using the official method, which employs a standard net with a 250 mm frame, a 500 mm deep net bag,

and a mesh size of 500 μm and preserved organisms in a solution of 70% ethanol and 30% water and transported to the laboratory. Following a more detailed taxonomic analysis, through specific taxonomic manuals (Sansoni, 1988; Camapioli et al., 1994), EXP calculated the official EBI for each monitoring site. The quality classes obtained using the simplified and the official protocol were also compared.

2.4 Simplified and Extended Biotic Index

For each monitoring site, two biotic indices were calculated: 1) a simplified index (SBI) based on data collected by CTZ (SBI-ctz) and EXP (SBI-exp), and 2) the official one based only on data collected by EXP (EBI). In both cases, the ecological quality class was assessed following the official protocol developed by Ghetti (1997).

1) The operator (CTZ and EXP) identified only the target taxonomic groups in the field and CTZ documented the collected organisms through photographic records, which were subsequently sent to EXP for verification. EXP reviewed the CTZ data and identified all collected organisms at the family level as prescribed by the simplified protocol, rather than the official one. The data were then used to calculate EBI values using the official reference table (Tab. 2). In this experimental assessment, EXP also used the photographic verification procedure to calculate the correct version of SBI-ctz (SBI-corr).

Table 2. Official table for EBI values calculation (Ghetti, 1997)

		Total number of Systematic Units (SU) constituting the community (vertical input)								
Faunal groups that determine with their presence the horizontal entry into the table (horizontal entry)		0-1	2-5	6-10	11-15	16-20	21-25	26-30	31-35	36…
Plecoptera (*_Leuctra_)	More than 1 SU	-	-	8	9	10	11	12	13***	14***
	1 SU	-	-	7	8	9	10	11	12	13***
Ephemetoptera ** (excluding Baetidae and Caenidae)	More than 1 SU	-	-	7	8	9	10	11	12	-
	1 SU	-	-	6	7	8	9	10	11	-
Tricoptera (including Baetidae and Caenidae)	More than 1 SU	-	5	6	7	8	9	10	11	-
	1 SU	-	4	5	6	7	8	9	10	-
Gammaridae and/or Atiidae and/or Palemonidae	All the SU above absent	-	4	5	6	7	8	9	10	-
Asellidae and/or Nifargidae	All the SU above absent	-	3	4	5	6	7	8	9	-
Oligocheta or chironomidae	All the SU above absent	1	2	3	4	5	-	-	-	-
Others	All the SU above absent	0	1-	2-	3-	-	-	-	-	-

*: In communities where Leuctra is present as the only Plecoptera taxon, and Ephemeroptera are absent (except possibly for genera of the Baetidae and Caenidae families), Leuctra must be considered at the Trichoptera level to define the horizontal entry in the table;
**: For the definition of horizontal entry in the table, each genus of the Baetidae and Caenidae families must be considered at the Trichoptera level;
-: doubtful judgment, due to sampling error, the presence of "drift" organisms incorrectly considered in the calculation, an inadequately colonised environment, or a typology that cannot be assessed with the EBI (e.g., springs, snowmelt water, still water, deltaic areas, brackish water);
***: These index values are rarely reached in Italian flowing water ecosystems, so care must be taken both to avoid the accumulation of biotypes (artificial increase in the number of taxa) and to evaluate the potential effects of pollution, given that these are environments with a high taxa richness.

2) Only EXP identified in the laboratory all the organisms collected according to the official protocol (Tab. 3), and calculated the EBI values using the official reference table (Tab. 2).

Table 3. Taxonomic identification levels to determine the systematic units (Ghetti, 1997)

Faunistic Groups	**Levels of taxonomic determination to define "Systematic Units"**
Plecoptera	genus
Ephemeroptera	genus
Trichoptera	family
Coleoptera	family
Odonata	genus
Diptera	family
Heteroptera	family
Crustacea	family
Gasteropoda	family
Bivalvia	family
Tricladida	genus
Irudinea	genus
Oligochaeta	family
Other taxa to consider in calculating the EBI	
Sialidae (Megaloptera)	
Osmylidae (Planipennia)	
Prostoma (Nemertea)	
Gordiidae (Nematomorpha)	

The EBI values obtained with the simplified and official protocols were converted into an ecological quality class, as shown in Table 4.

Table 4. Conversion of EBI values into quality classes, with the corresponding ecological assessment and colour code for cartographic representation.

Quality class	EBI values	Quality assessment	Colour and/or screening related to the quality class	
Class I	10-11-12…	Environment not significantly altered	Light blue	
Class II	8-9	Environment with moderate symptoms of alteration	Green	
Class III	6-7	Altered environment	Yellow	
Class IV	4-5	Environment severely altered	Orange	
Class V	0-1-2-3	Environment severely degraded	Red	

2.5 Statistical analysis

As most variables deviated from normality (Shapiro-Wilk test, p>0.05), non-parametric statistics were used. Specifically, the Wilcoxon test was used to assess significant differences between CTZ and EXP-generated data, while the Spearman's rank correlation coefficient was used to evaluate the correlation between datasets.

The time interval between CTZ and EXP sampling events was calculated as the difference between sampling dates, expressed as days elapsed since the beginning of the year. For each sampling campaign, median ± MAD (median absolute deviation) values were calculated. The time interval was included as a fixed effect in a linear mixed model (LMM) with a Gaussian error distribution to test its influence on the difference in the estimated number of organisms detected by the two operators, with site and year fitted as random factors.

Overall abundance was analysed using a Tweedie generalised linear mixed model (GLMM), with operator as a fixed effect and site as a random effect. The significance of fixed effects was assessed using an ANOVA Type III Wald χ^2 test.

To evaluate CTZ taxonomic identification errors, the concordance between the CTZ samples and the verification samples performed by EXP based on target group pictures was calculated using the Concordance Correlation Coefficient (CCC) (Lawrence et al., 1989).

To provide an overall measure of CTZ group performance at the site level, absolute errors (AE) across each target group were calculated and aggregated. Subsequently, they were standardised by the total number of target groups included in the analysis:

$$AE_i = \frac{1}{n}\sum_{j=1}^{n} \left|\frac{cs_{i,j} - ex_{i,j}}{15}\right|$$

where n is the number of target groups detected by EXP for each sampling; cs and ex are the abundance estimated by CTZ and by the EXP, respectively, for target group *j* in site *i*. This approach produces a normalised absolute error for each sampling site, with values ranging from 0 (perfect agreement) to 1 (maximum disagreement).

The overall accuracy of the CTZ dataset was subsequently expressed as percentage agreement, a metric widely adopted in similar assessments (Aceves-Bueno et al., 2017).

$$Accuracy = (1 - AE_{mean}) \times 100$$

where AE_{mean} is the mean site-level normalised absolute error across all monitoring sites.

To investigate the relationship between the number of target groups detected per site and the resulting accuracy, a Spearman's rank correlation analysis was performed.

Regarding biological indices, when a significant difference was detected, the AE between the corresponding quality classes was calculated. AE median ± MAD and mean ± sd were then calculated to quantify the deviation between classes.

All calculations were performed using the free software R (R Core Team, 2025).

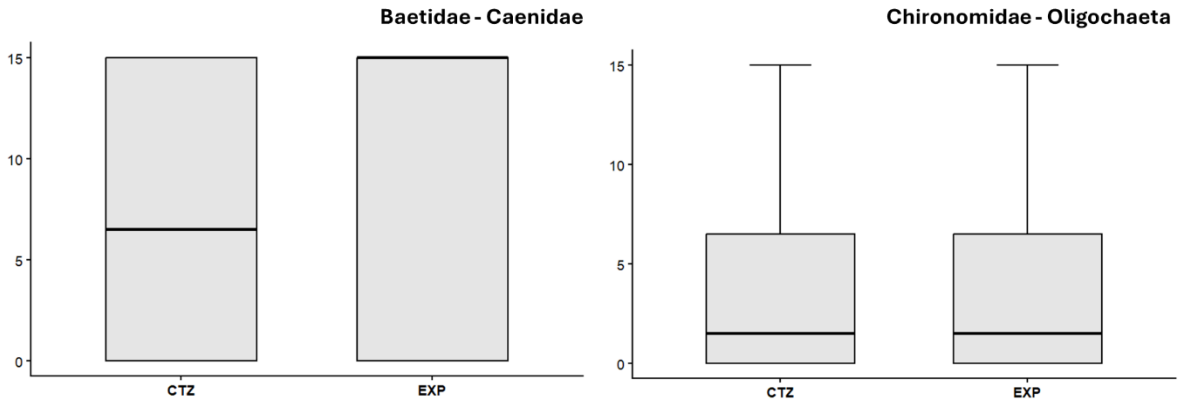
3. Results

The raw abundance data for each target group per site are reported in the Supplementary Materials (Tab. 1S).

To evaluate the quality of the data collected by CTZ, we compared observations across different analytical levels of the EB protocol. Specifically, results are organised into: (i) organism abundance estimation; (ii) taxonomic identification errors; (iii) biological indices; and (iv) performance of CTZ groups.

3.1 Organism abundance estimation

Significant differences were not observed between CTZ and EXP in estimating the abundance of each target group ($p>0.05$) when applying the same simplified protocol. Taxa for which both CTZ and EXP recorded no observations were omitted from the analysis (Fig. 4).



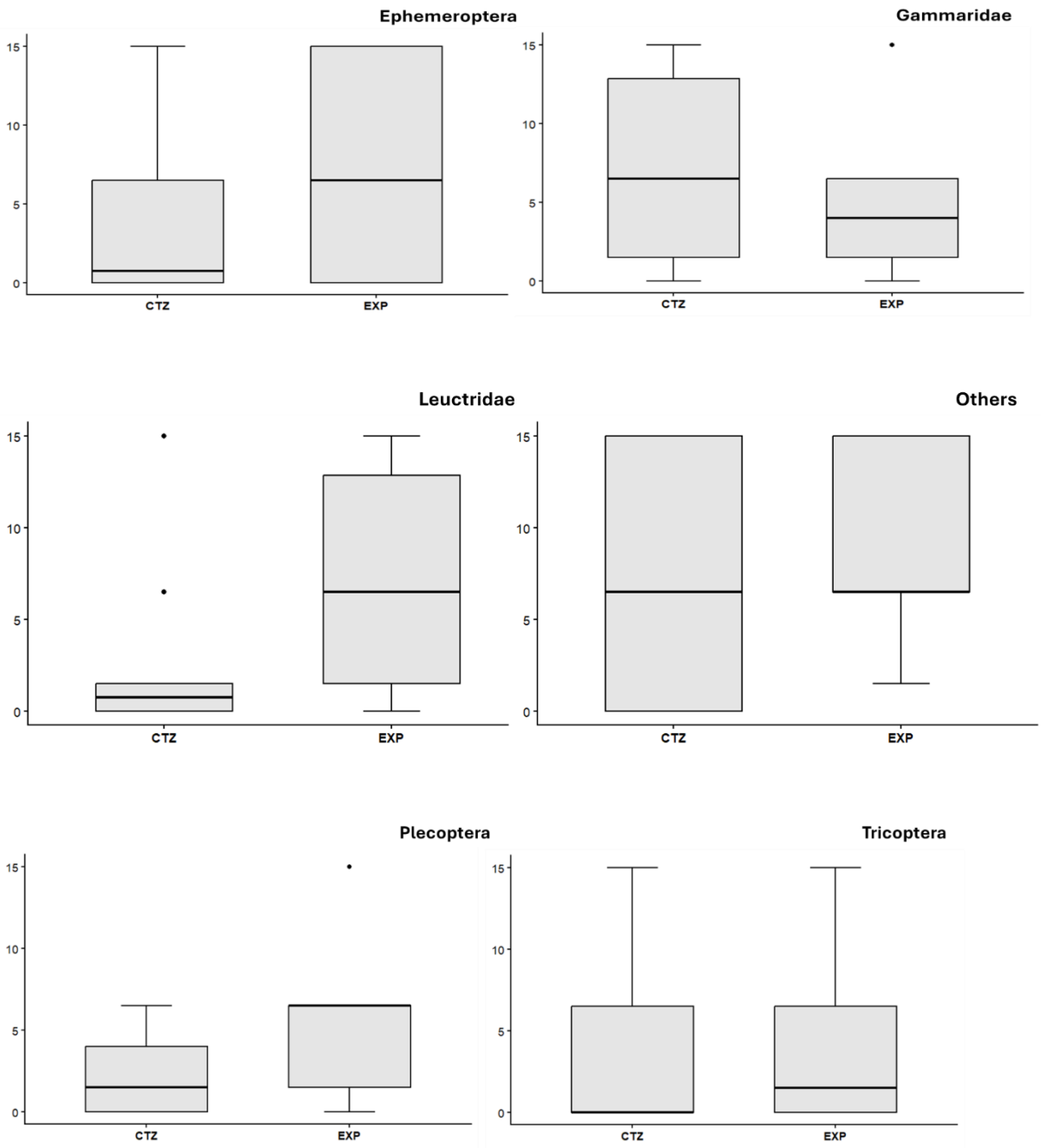


Figure 4. Boxplots showing abundance-estimation comparison by target group. The target groups correspond to the faunistic categories employed in the EBI calculation. The category “Others” encompasses all organisms used for the EBI value calculation and that are not assigned to any of the other defined groups (excluding Niphargidi, Atyidae and Palaemonidae, due to their rarity in the study area).

Considering all the observation pairs (N=161, Fig. 5), a significant difference emerged between CTZ and EXP abundance estimates ($p<0.05$). No significant correlation was observed between the two datasets (Spearman $r = -0.03$, $p>0.05$). Taxa for which CTZ and EXP recorded no observations were omitted from the analysis.

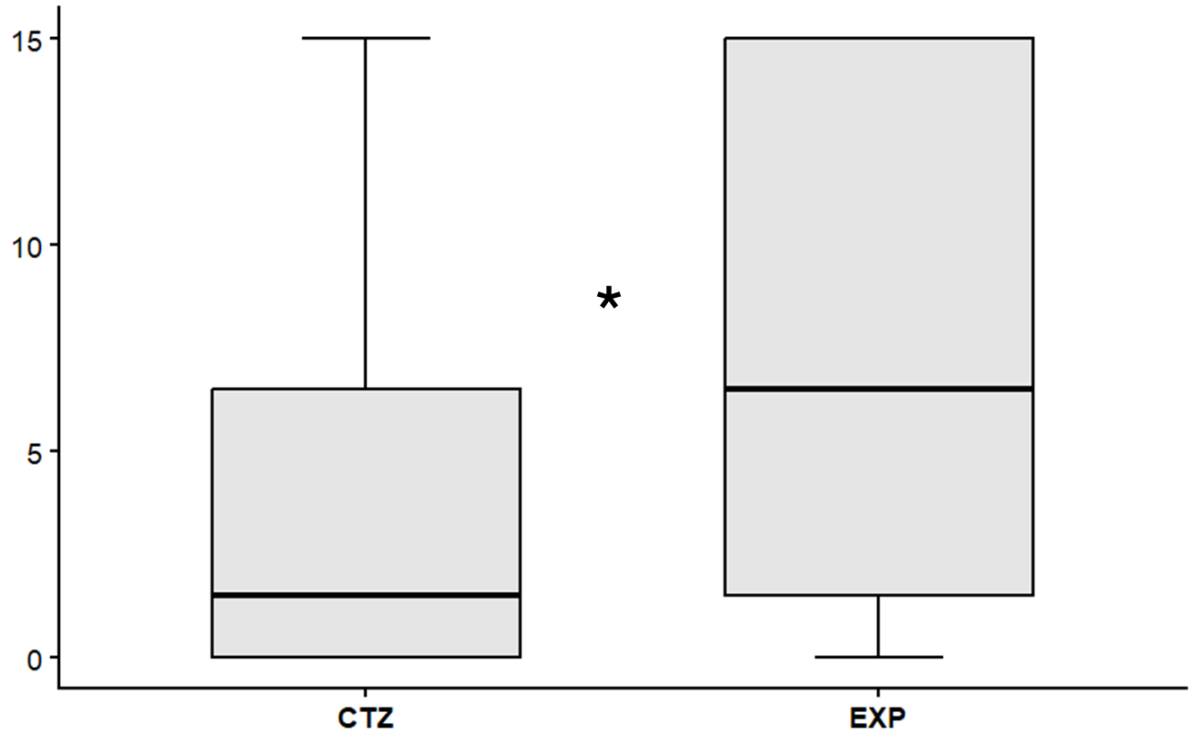


Figure 5. Boxplot showing the total abundance of organisms collected by CTZ and EXP. CTZ generally samples fewer organisms than EXP

To investigate this difference, we examined the effect of the time interval between CTZ and EXP sampling events. Due to logistical constraints, this interval was substantially longer during the Spring 2025 campaign (Tab. 5).

Table 5. Median ± MAD of intervals between operator-day sampling events, calculated as the difference between EXP and CTZ sampling dates and expressed in days since the beginning of the year

Time interval	**median ± MAD**
Spring 2024	38.00 ± 14.00
Autumn 2024	1.00 ± 1.00
Spring 2025	54.00 ± 16.00

The LMM revealed a significant positive effect of time on the difference between EXP and CTZ estimates of organism abundance ($\beta = 0.279 \pm 0.095$ SE, $t = 2.94$, $p<0.05$).

When the Spring 2025 data were excluded, no significant difference was detected between CTZ and EXP abundance estimates (N 117, $p>0.05$), and no significant correlation persisted ($r = -0.01$, $p>0.05$).

Furthermore, the Tweedie GLMM showed no significant effect of operator on target group abundance estimation during Spring 2024 and Autumn 2024 (Wald $\chi^2 = 0.93$, $df = 1$, $p>0.05$; Fig. 6).

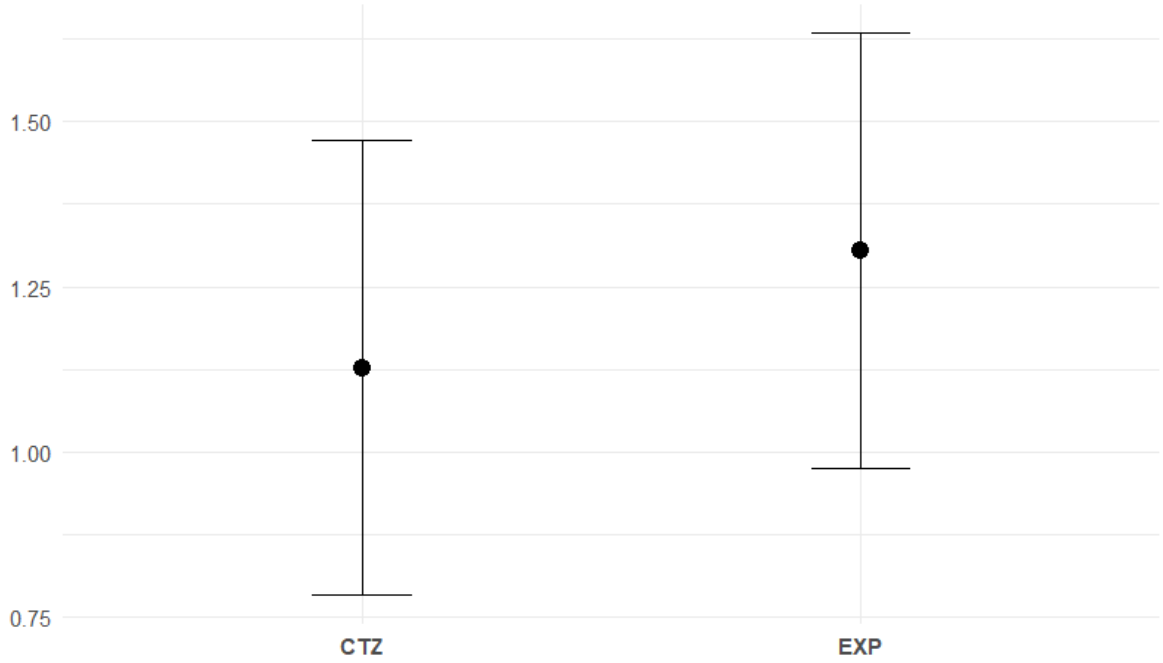


Figure 6. Estimated marginal means of abundance estimation performed by the operators (CTZ vs EXP). Error bars represent 95% confidence intervals

3.2 Taxonomic identification errors

No significant difference was detected between CTZ field identifications and the photographic verification performed by EXP ($p>0.05$).

Thanks to the preliminary photographic verification, target group CCC analysis revealed excellent agreement for Tricoptera (CCC=0.889), Gammaridae (CCC=0.880), Ephemeroptera (CCC=0.852) and “Others” (CCC=0.753). Moderate agreement was observed for Baetidae-Caenidae and Plecoptera (CCC=0.557), while agreement was poor for Chironomidae/Oligochaeta (CCC=0.471). Leuctridae was excluded from the analysis due to frequent zero counts in both assessments.

3.3 Biological Indices

Significant difference was found between quality classes calculated with SBI-ctz and SBI-exp (data not shown). The median ± MAD and the mean±sd difference between quality class calculated with SBI-ctz and SBI-exp across sites were respectively 1±0 and 0.69±0.48 (Fig. 7).

Cases where it was not possible to calculate the ecological quality classes due to poor data quality or inadequate sampling conditions for data collection were omitted from the analysis.

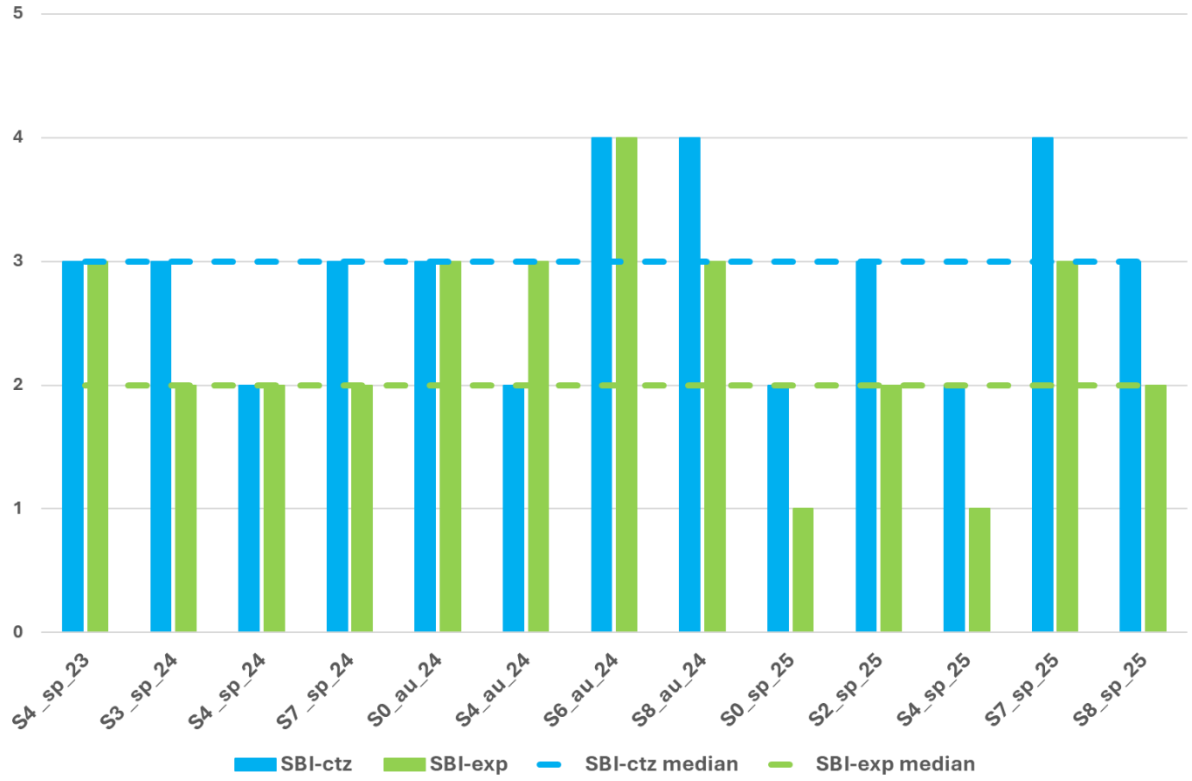


Figure 7. Barplot represents the quality class calculated per site with CTZ and EXP-generated data using the simplified protocol

Excluding the sampling season characterised by the largest temporal gap between CTZ and EXP surveys (Spring 2025), no significant difference was observed (N=8; data not shown).

Still, no significant difference was found between quality classes calculated with SBI-ctz and SBI-corr (data not shown).

Following the more detailed analysis performed by EXP, significant difference was found between quality classes calculated with SBI-ctz and EBI (data not shown).

The median±MAD and mean±sd difference between quality classes calculated with SBI-ctz and EBI across sites were respectively 1±1 and 0.85±0.55 (Fig. 8).
Cases where it was not possible to calculate the ecological quality classes due to poor data quality or inadequate sampling conditions for data collection were omitted from the analysis.

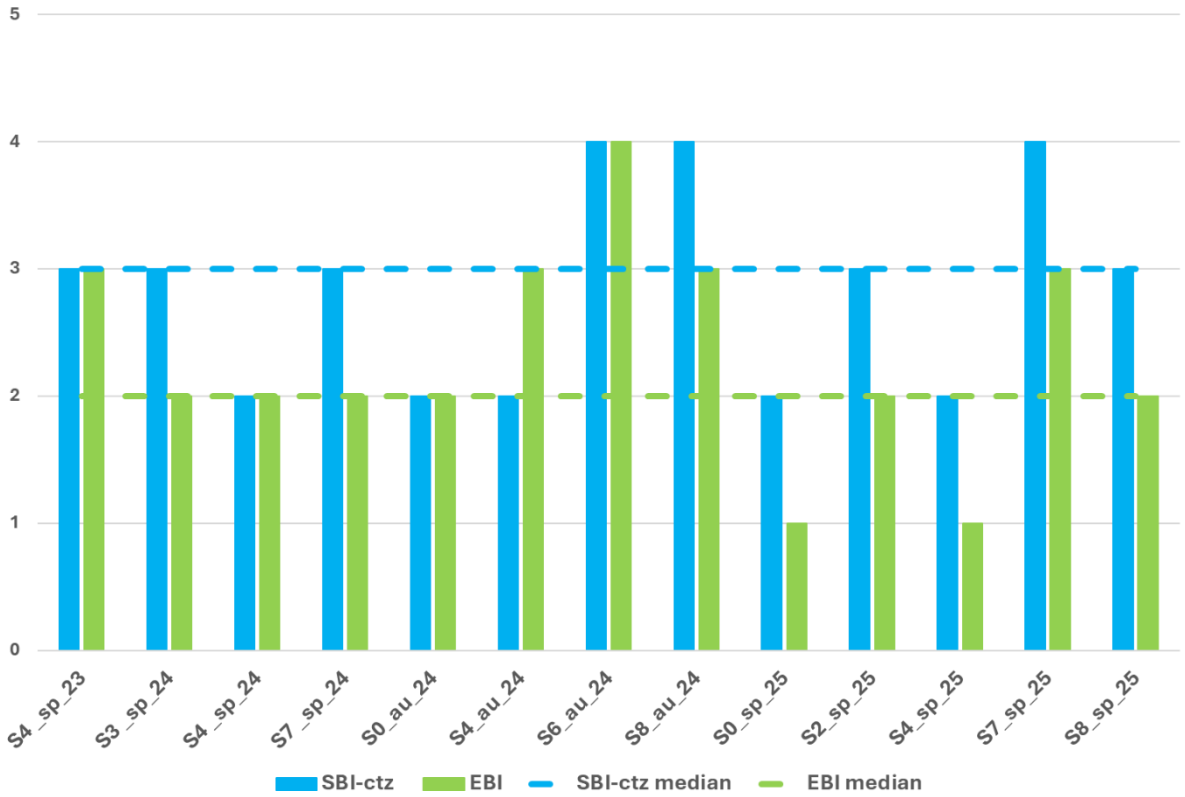


Figure 8. Barplot represents the quality class calculated per site with the two protocols: SBI-ctz and EBI.

Excluding the sampling season characterised by the largest temporal gap between CTZ and EXP surveys (Spring 2025), no significant difference was observed (N=8; data not shown).
No significant difference was detected between the SBI-cor and EBI and between the SBI-exp and EBI (data not shown).

3.4 Citizen Scientists group performance

Since the Spearman correlation between the number of taxa target detected by EXP and the accuracy was not significant ($p>0.05$, $r=0.13$), the CTZ group accuracy was

calculated. The total accuracy was 47±27%Accuracy ranged from 304% to 64%, with the highest value recorded for group G3 (64±25%) and the lowest for group G8 (30±29%) (Tab. 6).

Table 6. Accuracy (%) for each CTZ group

G0	G2	G3	G4	G6	G7	G8
56 ± 18	59 ± 4	64 ± 25	40± 24	46± 39	31± 57	30± 29

4. Discussion

4.1 Organism abundance estimation

The comparison of organism abundance between CTZ and EXP indicates that the EasyBenthos protocol provides reliable data under controlled temporal conditions.

In fact, no significant difference was observed at the level of each target group, suggesting that trained CTZ were able to detect and sample the predefined faunistic categories with performance comparable to EXP when applying the same protocol. However, when all abundance observations were analysed altogether, a significant difference emerged, with CTZ collecting fewer individuals than EXP. Moreover, no significant correlation was observed between datasets, indicating that CTZ observations did not consistently reproduce the abundance gradient recorded by EXP. This difference may be related to differences in sampling efficiency and sorting accuracy between CTZ and EXP. Even though both operators sampled the same monitoring sites and followed a standardised protocol, the selection of macrohabitats is operator-dependent and strongly influences the macroinvertebrate community sampled (Beisel et al., 1998; Robson & Chester, 1999; Callisto et al., 2001; Lamouroux et al., 2004). Less experienced operators may therefore have sampled microhabitats less representatively, encountering the well-known spatial bias typical of CS projects (August et al., 2020) and resulting in lower sampling efficiency compared to EXP.

Moreover, the sorting phase may lead to missed individuals, particularly when operator working in the field with live and mobile organisms. Without the use of a microscope, it is more challenging to detect and collect small or cryptic organisms, which can easily be overlooked by a less experienced operator (Hamilton et al., 2024).

The results highlight another well-known bias in CS projects related to temporal availability (Bird et al., 2014): CTZ often sample at times that are more convenient for them, which may not always align optimally with research requirements. In this study, although operators sampled within the same general time window, the LMM revealed a significant positive effect of the time interval between sampling events on the number of organisms collected. This finding indicates that days gaps (Tab. 4) may confound comparisons between operators. Similar studies provide comparable insights. For example, von Gönner et al. (2023) showed that a limited temporal gap of approximately two weeks (14.4±9.3 days) between sampling events did not produce statistically significant effects on biological indices or hydromorphological parameters. Storey et al. (2016), who compared CTZ and professional macroinvertebrate samples collected at the same sites and dates, reported a relatively strong correlation between macroinvertebrate metrics (r=0.85). In contrast, Moffett and Neale (2015) found a weaker correlation (r=0.54) when CTZ and professional samples were collected at slightly different times.

To further evaluate the influence of temporal asynchrony on the results, the dataset affected by extended temporal gaps (Spring 2025) was excluded from the analysis. When these data were removed, no significant difference between CTZ and EXP abundance estimate was detected. Moreover, the GLMM confirmed no significant operator effect during campaigns conducted within shorter temporal windows.

These results suggest that the simplified protocol does not intrinsically bias data quality and that temporal asynchrony appears to be a contributing factor, alongside potential differences in sampling efficiency. A previous study by Agnello et al. (2022) demonstrated that individual productivity in CS projects is strongly influenced by opportunities for social interaction and learning, with participants more likely to invest time and effort when perceiving direct personal benefits. In addition, fixed sampling efforts per microhabitat can further reduce spatial variability (Brooks et al., 2019), while additional training and field experience may help minimise discrepancies between CTZ and EXP during the sampling phase (Callaghan et al., 2021). This finding underscores the importance of synchronised sampling and more standardised sampling protocols in future validation studies.

4.2 Taxonomic identification errors

Photographic verification showed no significant difference between CTZ field identifications and EXP identifications derived from the photographic review of CTZ samples, suggesting that taxonomic misclassification was not a major source of error. CCC values ranged from excellent to moderate agreement across target groups (with poor agreement only for Chironomidae/Oligochaeta), indicating that identification accuracy is taxon-dependent and influenced primarily by morphological recognisability. In general, volunteers tend to identify organisms with distinctive morphological patterns more accurately (Barbato et al., 2021; Hamilton et al., 2024), as observed for Trichoptera and Gammaridae (CCC= 0.889 and CCC= 0.880, respectively), or when working at higher taxonomic levels (von Gönner et al., 2023), such as Ephemeroptera compared to Baetidae-Caenidae (CCC= 0.852 and CCC= 0.557, respectively). Furthermore, CTZ appears to identify taxa that are morphologically similar to others worse (Swanson et al., 2016). This pattern appears particularly evident for Chironomidae/Oligochaeta (CCC= 0.471), whose vermiform morphology can lead to confusion with other benthic organisms exhibiting similar body shapes.

EXP photographic validation/verification remains a key methodological component in CS frameworks, as it helps maintain and confirm the taxonomic reliability and overall quality of the collected data (Gilfedder et al., 2019). It further allows for the identification of critical issues associated with CTZ related to target groups. This insight has supported the design of ad hoc training tailored to the specific needs of CTZ during the project.

4.3 Biological indices

The comparison of biological indices provides insight into the ecological implications of taxonomic simplification and the quality of CTZ-generated data.

A significant difference was detected between SBI-ctz and SBI-exp, indicating residual variability likely attributable to the sampling phase. As taxonomic misidentification did not emerge as a major source of bias, this discrepancy is more plausibly linked to differences in sampling performance, potentially associated with the time lag between CTZ and EXP sampling events. This interpretation is further supported by the absence of a significant difference when excluding the sampling season characterised by the largest temporal gap; however, this result is based on a limited sample size (N = 8) and should therefore be interpreted with caution.

No significant difference was found between SBI-ctz and SBI-corr, indicating that CTZ were generally able to correctly identify the most sensitive taxa, which determine the horizontal entry in the EBI calculation table and ultimately drive ecological classification.

On the other hand, a significant difference was observed between SBI-ctz and EBI, but not between SBI-exp and EBI, indicating that discrepancies from the official protocol are primarily driven by operator-dependent rather than limitations inherent to taxonomic simplification. The absence of a significant difference between SBI-exp and EBI further confirms that methodological simplification does not compromise ecological quality classification when sampling is conducted by the same operator (EXP). Consistently, the lack of significant differences between SBI-corr and EBI suggests that the verification procedure effectively harmonised taxonomic resolution within the simplified protocol.

These findings are consistent with previous studies showing that reduced taxonomic resolution does not impair the ability to derive reliable ecological quality classifications (Storey et al., 2017; Brooks et al., 2019; Hamilton et al., 2024; von Gönner et al., 2023; Harrison et al., 2025; Roccatello et al., 2025).

Overall, these results support the robustness of the simplified approach while highlighting the importance of standardising sampling procedures. Furthermore, in cases where significant differences were observed, the median deviation corresponded to one quality class, indicating a limited impact on ecological assessment. Within a five-class system such as the EBI, this effect is likely to be particularly constrained within intermediate quality classes.

4.4 Citizen Scientists group performance

The overall accuracy of CTZ groups (46 ± 27%) indicates a sufficient level of reliability in data collection under the EasyBenthos framework. The accuracy values observed are lower than those reported in comparable studies. For instance, CTZ classification accuracy ranges between 80% and 90% compared with EXP (von Gönner et al., 2023; Hamilton et al., 2024). Anyway, the application of standard training, supporting materials (simplified dichotomous key, standardised datasheets) and post-sampling EXP feedback likely contributed to maintaining data quality across campaigns. These elements align with best practices in CS monitoring, where iterative

validation/verification and learning loops are critical for long-term reliability (van der Wal et al. 2016).

5. Conclusions

This study evaluated the reliability of the EasyBenthos simplified protocol for freshwater macroinvertebrate monitoring within a CS framework. Our experimental results indicate that it can generate ecologically meaningful data when applied under controlled conditions and supported by appropriate training, standardised procedures, and EXP validation/verification. Temporal and spatial biases may however affect data comparability. Maintaining participant motivation and engagement is likely critical for minimising temporal gaps.

Taxonomic identification errors did not represent a major source of bias: the use of predefined target groups likely facilitated identification by CTZ, while EXP verification ensured taxonomic consistency and simultaneously promoted sustained participation in monitoring activities.

In terms of biological indices, the EasyBenthos protocol provides a reliable ecological assessment when compared with the official protocol. Verification procedures effectively harmonised taxonomic resolution with the official protocol, and under the same operator, the simplified protocol leads to the same ecological quality classes. Where differences occurred, median deviations were limited to a single quality class, suggesting minimal impact on ecological classification, particularly within intermediate categories of the five-class EBI system.

Overall, these findings suggest that the EasyBenthos protocol can support freshwater biomonitoring by enabling CTZ-generated data under controlled experimental and procedural conditions. Strategies to reduce possible sources of bias and noise should be implemented in future studies.

6. Acknowledgements

The author would like to thank Prof. Loppi and Prof. Gumiero for their scientific support in developing the experimental design and for their feedback on this chapter. The author also wishes to express gratitude to Prof. Monaci for his assistance and support during the field activities.

Supplementary Materials

citizen science FOR RIVERS

NATIONAL BIODIVERSITY FUTURE CENTER

UNIVERSITÀ DI SIENA 1240

Osservatorio Citizen Science

Scheda monitoraggio comunità macrobentonica

Scatta almeno 2 foto georeferenziate della zona presa in esame (1 verso valle, 1 verso monte) da allegare ai dati

Nome osservatore _____

E-mail _____

Data monitoraggio _____ Condizioni meteo _____

Nome della località _____ Nome del fiume _____

Latitudine _____ Longitudine _____

Lunghezza del tratto monitorato (minimo 10 m - massimo 50 m) _____

1 Come si presenta l'acqua nell'alveo?

- ◯ portata minima
- ◯ portata media

2 Qual è la larghezza dell'alveo bagnato, rispetto all'alveo totale?

Larghezza dell'alveo bagnato

metri _____

Alveo totale

metri _____

3 Come si presenta la vegetazione riparia?

- ◯ la fascia riparia è costituita prevalentemente da specie arboree (ampia > 5 m)
- ◯ la fascia riparia è costituita prevalentemente da specie arboree (stretta < 5 m)
- ◯ la fascia riparia è costituita prevalentemente da specie arbustive
- ◯ la fascia riparia è costituita prevalentemente da specie erbacee
- ◯ non è presente nessuna fascia riparia

4 Sono presenti indizi di attività antropiche nei pressi del fiume?

- ◯ sì
- ◯ no

Se sì, specificare quale: _____

5 Qual è la velocità media della corrente?

- ◯ impercettibile o molto lenta
- ◯ lenta
- ◯ veloce
- ◯ turbolenta

6 Stima la presenza in percentuale dei diversi microhabitat minerali

Microhabitat	Presenza in %
Argilla / Sabbia	
Ghiaia	
Ciottoli medi Ø max 20 cm	
Ciottoli grandi Ø max 40 cm	
Pietre di grosse dimensioni > 40 cm	
Lastre di roccia	
Artificiale	

7 Sono presenti altri tipi di substrato?

Substrato	Presenza in %
Alghe filamentose	
Radici degli alberi esposte nell'alveo	
Fogliame	
Muschi	
Detriti legnosi	

Figure 1S. Standardised datasheet used to systematically record general ecosystem information: the microhabitat's presence, physical and hydromorphological characteristics of the river reach, georeferenced site coordinates, and photographic documentation

citizen science FOR RIVERS

NATIONAL BIODIVERSITY FUTURE CENTER · UNIVERSITÀ DI SIENA 1240 · Osservatorio Citizen Science

Indice Biotico Semplificato (IBS)

data _____

Analisi delle comunità di macroinvertebrati che colonizzano gli ecosistemi fluviali

luogo _____

Gruppi faunistici		Stima di presenza		
		1 - 2	3 - 10	> 10
Plecotteri (escludere Leuctridae) 	> 1			
	1			
Plecotteri (Leuctridae) 				
Efemerotteri (escludere Baetidae e Caenidae) 	> 1			
	1			
Efemerotteri Baetidae e Caenidae) 	2			
	1			
Tricotteri 	> 1			
	1			
Gammaridi 				
Asellidi 				
Chironomidi o Oligocheti 	> 1			
	1			
Altri Odonati, Coleotteri adulti e Ditteri				

www.cs4rivers.unisi.it

Figure 2S. SBI tabular datasheet: in the row, the target groups organised by decreasing sensitivity to habitat alteration; in the columns, the total number of organisms per group recorded in discrete classes

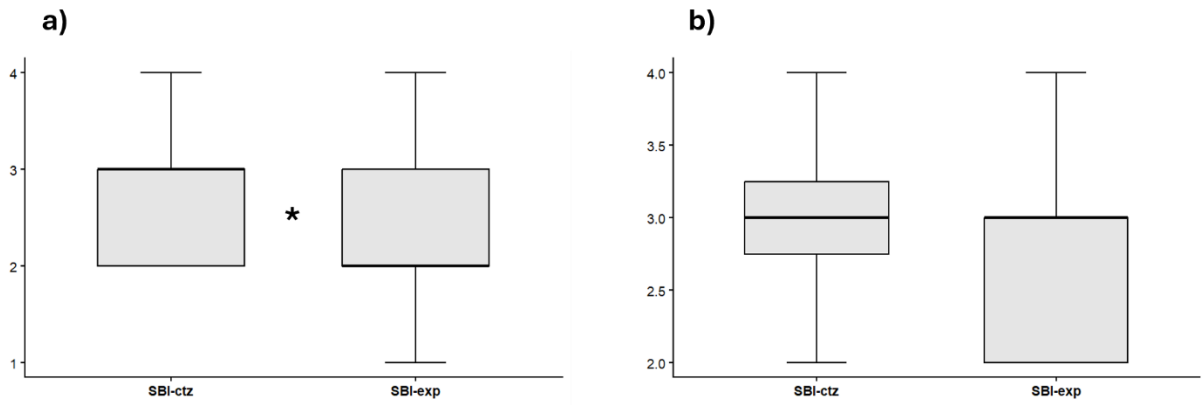


Figure 3S. Boxplots showinfg the significant difference between the simplified index calculated with citizen-generated data (SBI-ctz) and the simplified index calculated with experts-generated data (SBI-ex) (a). When Spring 2025 was excluded from the analysis, no significant difference was observed (b)

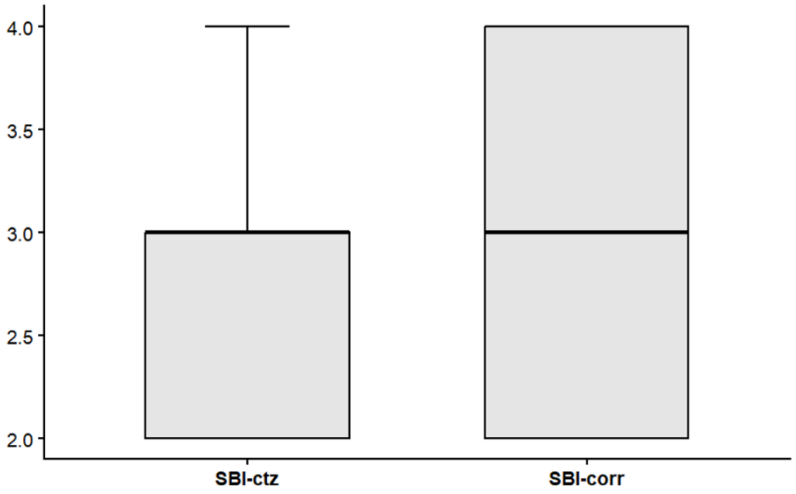


Figure 4S. Boxplots showing the absence of significant differences between the simplified index calculated using citizen-generated data (SBI-ctz) and the simplified index calculated following the photographic verification (SBI-corr)

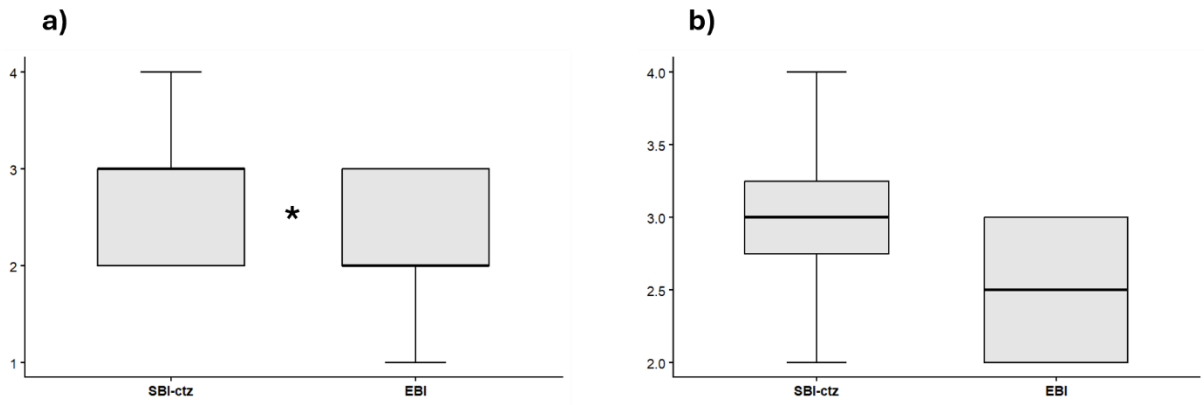


Figure 5S. Boxplots showing the significant difference between the simplified index calculated using citizen-generated data (SBI-ctz) and the official index calculated by experts (EBI) (a). However, when Spring 2025 was excluded from the analysis, no significant difference was detected between the two indices (b)

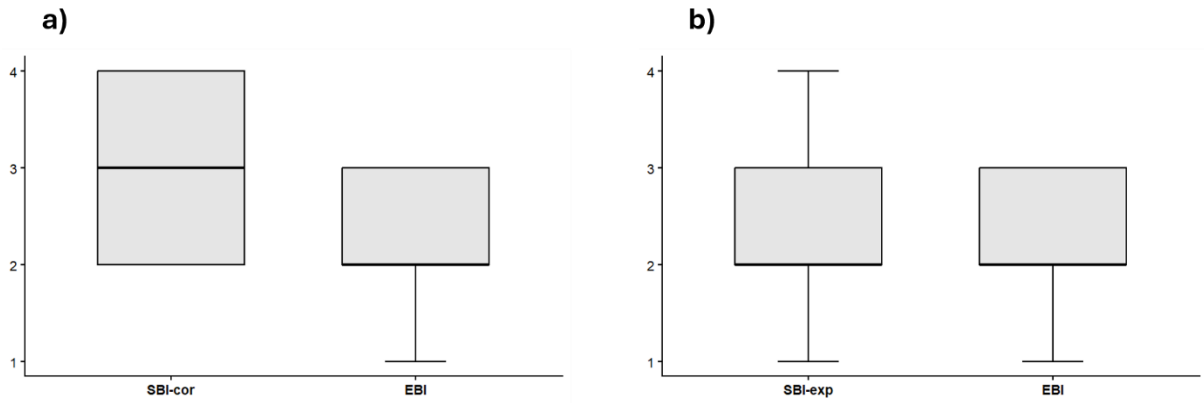


Figure 6S. Boxplots comparing the simplified index calculated following photographic verification (SBI-corr) with the official expert-derived index (EBI), showing a significant difference (a). Panel (b) illustrates the absence of significant difference between the simplified index calculated using expert-generated data (SBI-exp) and the official expert-derived index (EBI)

Table 1S. abundance per target group per site monitoring by CTZ and EXP; sp = Spring; au = Autumn; 23, 24, and 25 refer respectively to 2023, 2024 and 2025

Target groups	S4_sp_23		S0_sp_24		S2_sp_24		S3_sp_24		S4_sp_24		S6_sp_24	
	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP
Plecoptera (no Leuctridae) >1				1.5								
Plecoptera (no Leuctridae) 1	6.5	6.5				1.5						
Plecoptera (Leuctridae)			1.5	15	15	6.5		1.5	6.5	15	1.5	
Ephemeroptera (no Baetidae, Caenidae) >1	15		15	15	15	6.5	15	15	6.5	15	15	
Ephemeroptera (no Baetidae, Caenidae) 1		6.5										
Ephemeroptera (Baetidae, Caenidae) 2		15		15	15	15		15	15	15		
Ephemeroptera (Baetidae, Caenidae) 1	6.5						15				6.5	6.5
Tricoptera >1	1.5		6.5	1.5	6.5				15	6.5		
Tricoptera 1						6.5		1.5				
Gammaridae		1.5			15	15	6.5	6.5				
Asellidae												
Chironomidae o Oligochaeta >1			6.5		6.5		6.5		6.5	6.5	6.5	
Chironomidae o Oligochaeta 1	6.5					6.5		6.5				15

Others	6.5	6.5		15	15	6.5	15	15	15	15		6.5

Target groups	S7_sp_24		S8_sp_24		S0_au_24		S3_au_24		S4_au_24		S6_au_24	
	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP
Plecoptera (no Leuctridae) >1												
Plecoptera (no Leuctridae) 1					6.5	1.5			6.5	15		
Plecoptera (Leuctridae)	1.5					15	1.5	1.5				
Ephemeroptera (no Baetidae, Caenidae) >1	15	6.5		6.5						15		
Ephemeroptera (no Baetidae, Caenidae) 1			6.5		1.5	1.5		1.5	6.5		6.5	6.5
Ephemeroptera (Baetidae, Caenidae) 2		15		15	1.5				16	15		
Ephemeroptera (Baetidae, Caenidae) 1	15		15			1.5						
Tricoptera >1		6.5							6.5			
Tricoptera 1	6.5			1.5						6.5		
Gammaridae			6.5			1.5	1.5	1.5				
Asellidae												
Chironomidae o Oligochaeta >1	15				1.5	1.5						6.5

Chironomidae o Oligochaeta 1		15		15		1.5		6.5	6.5	1.5		
Others	15	15	15	6.5		6.5		1.5	15	6.5		6.5

Target groups	**S8_au_24**		**S0_sp_25**		**S2_sp_25**		**S3_sp_25**		**S4_sp_25**		**S6_sp_25**	
	CTZ	**EXP**	**CTZ**	**EXP**	**CTZ**	**EXP**	**CTZ**	**EXP**	**CTZ**	**EXP**	**CTZ**	**EXP**
Plecoptera (no Leuctridae) >1			1.5	6.5						6.5		
Plecoptera (no Leuctridae) 1						6.5	1.5					
Plecoptera (Leuctridae)				1.5		6.5		15	15	6.5		
Ephemeroptera (no Baetidae, Caenidae) >1			6.5	15				15	6.5	15	6.5	15
Ephemeroptera (no Baetidae, Caenidae) 1		6.5			1.5	1.5						
Ephemeroptera (Baetidae, Caenidae) 2	15	15		15	6.5					15		
Ephemeroptera (Baetidae, Caenidae) 1			15			15	15	15	15			
Tricoptera >1	6.5		6.5	6.5				1.5	15	6.5		
Tricoptera 1		8				1.5	1.5					1.5
Gammaridae			1.5	6.5	15	15	15	6.5	6.5			
Asellidae												

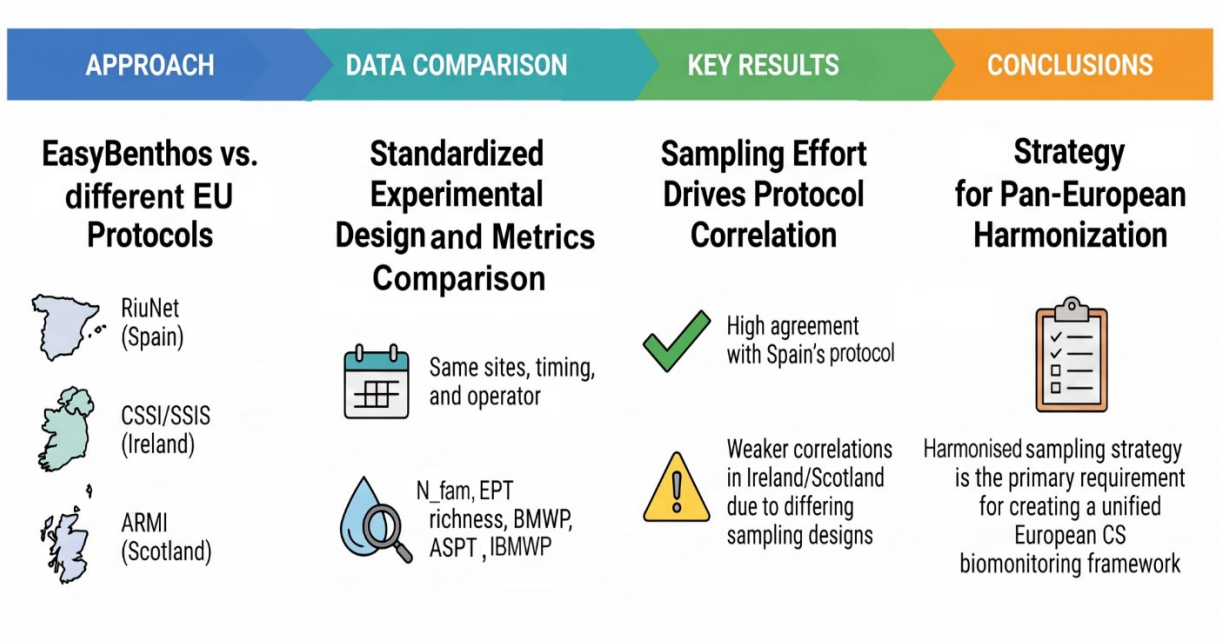
Chironomidae o Oligochaeta >1	15										
Chironomidae o Oligochaeta 1		15	1.5	1.5		1.5		1.5	6.5		1.5
Others	1.5	1.5	15	15	6.5	15	1.5	15	15	15	6.5

Target groups	S7_sp_25		S8_sp_25	
	CTZ	EXP	CTZ	EXP
Plecoptera (no Leuctridae) >1				
Plecoptera (no Leuctridae) 1			1.5	6.5
Plecoptera (Leuctridae)				1.5
Ephemeroptera (no Baetidae, Caenidae) >1				6.5
Ephemeroptera (no Baetidae, Caenidae) 1		1.5		
Ephemeroptera (Baetidae, Caenidae) 2			15	15
Ephemeroptera (Baetidae, Caenidae) 1	15	15		
Tricoptera >1		1.5		
Tricoptera 1	1.5			15
Gammaridae				
Asellidae				
Chironomidae o Oligochaeta >1				
Chironomidae o Oligochaeta 1	6.5	15	1.5	
Others	15	6.5	6.5	15

Chapter 2.

Toward a simplified Pan-European protocol for biomonitoring freshwater quality: a comparative assessment of Citizen Science methods using macroinvertebrates

Graphical abstract



Abstract

This study evaluates the comparability of simplified macroinvertebrate-based Citizen Science (CS) protocols for freshwater biomonitoring across Europe, to develop a harmonised Pan-European approach. A field experiment was conducted in Spain, Ireland, and Scotland, where the Italian EasyBenthos (EB) protocol was applied alongside national CS methods (RiuNet, CSSI, SSIS, and ARMI) under comparable conditions (same sites, same time, same operator, different protocols). Metrics including taxa richness, EPT richness, BMWP, and ASPT were calculated using presence-absence data, and statistical agreement was assessed through non-parametric tests and correlation analyses. Results showed high interoperability between EB and the Spanish RiuNet protocol, with strong correlations across metrics and full agreement in ecological quality classes. In contrast, comparisons with Irish and Scottish protocols revealed weaker correlations and lower agreement, likely driven by differences in sampling effort and design. These findings highlight sampling strategy

as a key factor influencing protocol comparability and underscore the need for further research to support the development of a standardised European CS biomonitoring framework.

1. Introduction

The European Water Framework Directive (WFD; 2000/60/EC; EC, 2000) serves as the reference point for European Union legislation on aquatic protection. It establishes an operative framework based on Biological Quality Elements (BQEs) - including macroinvertebrates, fish, diatoms, and macrophytes - to define the "ecological status" of water bodies (Copetti et al., 2024).

A central mandate of the WFD is to achieve at least "good ecological status" for all surface waters, a goal that requires a common and consistent interpretation across all Member States (Poikane et al., 2014). To ensure this, the Directive instituted the intercalibration process, a scientific and political effort designed to harmonise national assessment systems and resolve inconsistencies in quality class boundaries (Birk et al., 2006). Through this process, experts from different countries compared national biological assessment methods and aligned them against common European benchmarks. The intercalibration exercise demonstrated that, despite methodological differences, national statutory monitoring systems could be successfully harmonised within a shared assessment framework (Buffagni et al., 2006; Bennett et al., 2011). This process has been fundamental in establishing a common scientific basis for ecological assessment across Europe.

In parallel, recent years have seen the rapid expansion of various simplified monitoring methodologies based on macroinvertebrates (Brooks et al., 2019; Kelly-Quinn et al., 2022; Harrison et al., 2025; Fortuño et al., 2025) that have emerged under the umbrella of Citizen Science (CS), defined as active engagement of the general public in scientific research tasks (Vohland et al., 2021). CS has been increasingly recognised as an important tool for environmental research and monitoring (Silvertown, 2009). This growing relevance also extends to riverine ecosystems (DiFiore & Fitch, 2016; Shuker et al., 2017), where CS activities increase sampling frequency and density, reducing the spatial and temporal gaps of conventional monitoring programmes (Brooks et al., 2019; Kelly-Quinn et al., 2022), and address WFD requirements for public participation (Article 14) (Harrison et al., 2025).

However, an important gap remains: while official methods have been rigorously harmonised, no comparable effort has been undertaken for CS protocols. The lack of a standardised Pan-European simplified protocol poses a major challenge for large-scale data integration, harmonisation and interpretation. Currently, the simplified tools are almost exclusively derived from the official statutory protocols of their respective countries of origin. For instance, the Anglers' Riverfly Monitoring Initiative (ARMI) was developed as a simplified version of the UK statutory macroinvertebrate monitoring program (Brooks et al., 2019), whereas the RiuNet project is based on the IBMWP index, which is officially adopted in the Iberian Peninsula (Fortuño et al., 2025). In Ireland, simplified approaches such as the Citizen Science Stream Index (CSSI) and the Small Stream Impact Score (SSIS) have also been developed, relying on the same underlying principle of taxa sensitivity used in the national monitoring framework based on the Q-value system (Kelly-Quinn et al., 2022; Harrison et al., 2025). Similarly, in Italy, the first simplified protocol specifically designed for CS applications, EasyBenthos (EB), derived from the official Extended Biotic Index, is currently under development. In general, each country aims to achieve an appropriate trade-off between applicability by non-experts and accuracy in the development of the simplified methods; consequently, the methodological simplifications involving the sampling protocol, as well as the index and quality class calculation, differ according to national priorities and contexts.

Consequently, these simplified methods remain uncoupled from any standardisation. Without a standardised Pan-European simplified protocol, data collected in different ecoregions remain incomparable (Buffagni et al., 2007), hindering the integration of citizen-generated data into a unified "neighbourhood watch" for European river conservation (Brooks et al., 2019; Kelly-Quinn et al., 2022). The development of a Pan-European simplified protocol is therefore fundamental to bridging the gap between local volunteer engagement and high-level WFD policy requirements.

Testing the comparability of these simplified approaches under controlled field conditions, therefore, represents an important step toward understanding whether different CS protocols can provide consistent ecological assessments.

The objective of this study was therefore to conduct a field experiment comparing the simplified macroinvertebrate sampling protocol developed in Italy (EasyBenthos protocol - EB) with those in Spain, the UK (Scotland) and Ireland. By applying the different sampling protocols at the same sites under comparable conditions, the study

aimed to evaluate the extent to which these methods produce consistent, interoperable results across diverse geographical and environmental contexts, thereby laying the foundation for a simplified Pan-European sampling protocol.

2. Materials and Methods

2.1 Study Areas

The experiment was conducted in Spain, Ireland, and Scotland. These countries were selected for their long histories and experience with macroinvertebrate monitoring and CS, as well as for the presence of well-established, structured CS programs focused on freshwater ecosystems.

In Spain, 6 waterbodies were sampled near Vic and southward: Riera de Vallcarquera, Riera Picamena, Gurri, Torrent de Sant Cugat, Congost, and Meder. Sites were selected to represent a gradient of biological quality: Riera de Vallcarquera and Riera Picamena as good-quality, Gurri and Torrent de Sant Cugat as moderate-quality, and Congost and Meder as poor-quality. All rivers belong to the small mid-altitude river typology and generally have a perennial hydrological regime, except Torrent de Sant Cugat, which is temporary. Riparian vegetation is generally discontinuous, except at Riera de Vallcarquera, which has a continuous, predominantly tree-covered riparian zone extending more than 5 m (Fig. 1).

In Ireland, 9 sites distributed across seven streams were surveyed within and around Dublin city: Owenadoher (two sites), Shanganagh (two sites), Carrickmines Stream, Poddle, Grange Stream, Dodder, and Glencullen. Sites were selected to represent a gradient of biological quality: Owenadoher (upstream), Dodder, and Glencullen as good-quality; Shanganagh (upstream), Owenadoher (downstream), and Carrickmines Stream as moderate-quality; and Poddle, Kill of the Grange Stream, and Shanganagh (downstream) as poor-quality. Riparian vegetation is generally tree-dominated, with a width of less than 5 m at all sites. All streams belong to the small water body category (catchment area <10 km²), with a wetted channel width of 2–5 m (Fig. 1).

In Scotland, 9 sites were surveyed along several streams: North Esk (two sites), South Esk, Allt Ogline, Rusky Burn, Teith, Dreel Burn, Esk, North Calder Water, and one site in the town of Motherwell. Sites were selected to represent a gradient of biological

quality: North Esk, South Esk, and Allt Ogline as good-quality; Rusky Burn, Teith, and North Calder Water as moderate-quality; and Dreel Burn, Esk, and the site in Motherwell as poor-quality. Riparian vegetation is generally tree-dominated, with a width greater than 5 m at North Esk and South Esk, and less than 5 m at all other sites, except at Motherwell, which is dominated by herbaceous vegetation (Fig. 1).

Overall, lower-quality sites across all regions were generally located near urban or predominantly agricultural areas and were primarily affected by wastewater discharges and agricultural impacts, such as eutrophication. Higher-quality sites were situated in more remote areas, away from settlements. The monitoring sites selected in each country are constantly monitored by local citizen scientists: they were therefore deemed suitable in terms of safety and accessibility, as well as appropriate for the study objectives.

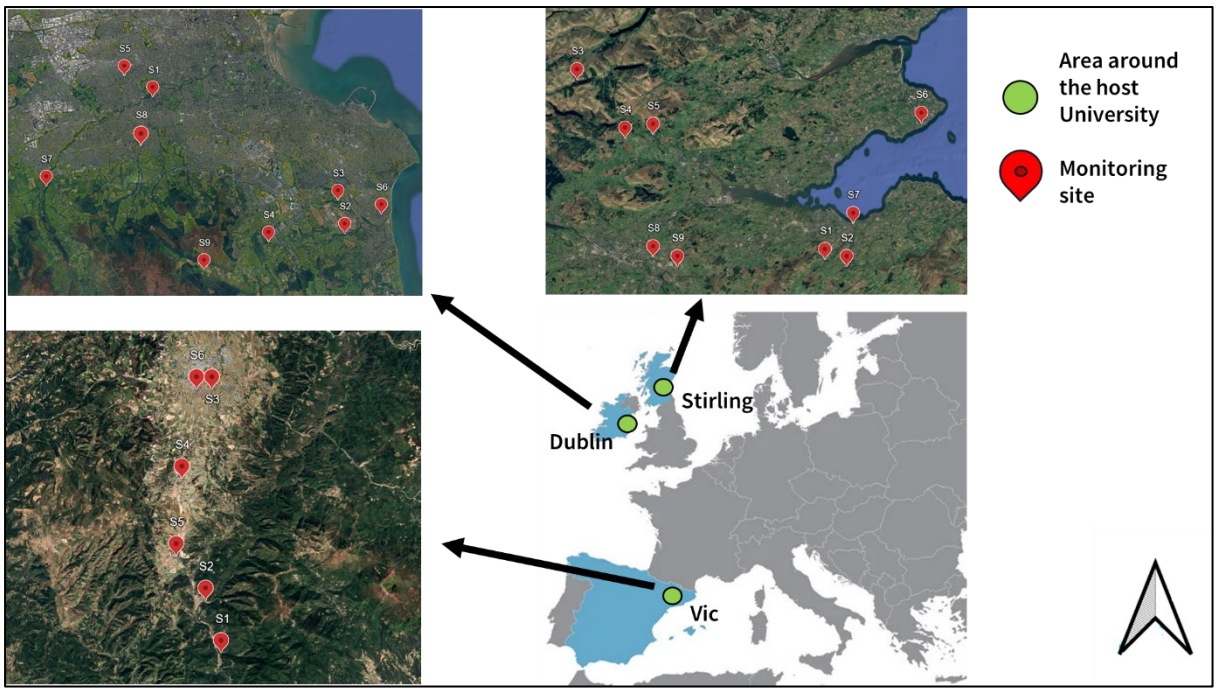


Figure 1. Study areas of each host country and the monitoring sites

2.2 Experimental design

The experimental design was refined in collaboration with the host universities and research centres: Universitat de Vic and BETA Lab (Biodiversity, Ecology, Environmental and Food Technology), Spain; School of Biology and Environmental Science, University College Dublin, Ireland; and Stirling University in partnership with Buglife - The Invertebrate Conservation Trust, Scotland.

24 monitoring sites were selected across the study countries: 9 per country, except in Spain, where only 6 sites were sampled due to adverse meteorological conditions. Site selection was informed by data provided by relevant environmental monitoring authorities, together with the expert knowledge of the host researchers. To test the protocols under different conditions, sites were chosen to represent three levels of ecological quality (poor, intermediate, and good), with 3 monitoring sites per category (2 per category in Spain).

At each monitoring site, two macroinvertebrate sampling protocols were applied by the same operator: the EB protocol and the protocol adopted in the host country. To minimise environmental variability, sampling sites were selected within river stretches characterised by similar ecological and hydromorphological conditions, extending for at least 1 km. The two sampling sites were spatially separated by a minimum distance of 10 m to ensure independence between the samples and did not influence each other. Each sampling protocol was conducted moving from downstream to upstream to avoid disturbance of the sampling area.

During the sorting phase in the field, the host country protocol was followed. Furthermore, organisms were identified at the family level, except for Oligochaeta and Nematomorpha, which were identified respectively at the subclass and phylum level. When field identification was uncertain, organisms were preserved in a 70% ethanol - 30% water solution and transported to the laboratory for subsequent identification through specific taxonomic manuals.

For each monitoring site, quality classes (expressed as an ordinal scale) were calculated according to the different national protocols. For the Anglers' Riverfly Monitoring Initiative (ARMI) protocol, which provides a score rather than discrete quality classes, the ARMI score was calculated.

2.3 The macroinvertebrate simplified protocol per country

For each country, here we present only the project aspects relevant to the field experiment, particularly those related to macroinvertebrate community monitoring and analysis, carried out in this study as described in the experimental design section.

2.3.1 RiuNet project (Spain)

The RiuNet project is a CS initiative designed to engage the public in the assessment and management of rivers in Spain. The RiuNet App, developed by the Freshwater Ecology, Hydrology and Management (FEHM-Lab) research group, enables users to

evaluate both the hydrological and ecological status of a river segment. The ecological status includes the assessment of biological quality, which is determined through macroinvertebrate analysis. For this purpose, macroinvertebrates are used as bioindicators through an adapted version of the IBMWP index, the official biological index for Mediterranean rivers in the Iberian Peninsula (Alba-Tercedor et al., 2002).

The RiuNet App tailors macroinvertebrate family sensitivity levels to river typology, which is preliminarily defined through the assessment of the hydrological status (sensu Gallart et al., 2017). This status is determined by identifying the river's hydrological regime - whether perennial or temporary- and by detecting any potential alteration.

Macroinvertebrate sampling is performed using the kick sampling technique and an aquarium net (1 mm mesh), collecting macroinvertebrates from various microhabitats within the river reach. Large cobbles are also lifted and manually cleaned in front of the net. The operator moves from downstream to upstream along a 100 m transect. The sorting phase is conducted in the field using simplified dichotomous keys from the RiuNet App. All organisms are identified at the family level, except for Oligochaeta and Odonata, which are identified at the subclass or suborder level, respectively.

Data are recorded using the RiuNet app, which employs the IBMWP index - calculated as the sum of predefined scores assigned to each benthic macroinvertebrate taxon - to determine the sensitivity of bioindicators. The app then calculates a simplified biological quality index ranging from 1 to 10. Results are expressed using a five-class colour-coded system: High (blue), Good (green), Moderate (yellow), Poor (orange), and Very Poor (red) (Fortuño et al., 2025) (Tab. 1)

Table 1. Biological quality classes according to the RiuNet protocol and their corresponding index values

High: 9-10	
Good: 7-8	
Moderate: 5-6	
Poor: 3-4	
Very Poor: 1-2	

2.3.2 Citizen Science Stream Index and Small Stream Impact Score (Ireland)

The Citizen Science Stream Index (CSSI) was developed by Simon Harrison and colleagues at University College Cork (Ireland) as a rapid biomonitoring protocol for assessing the ecological status of streams (Harrison et al., 2025). The index is based on six macroinvertebrate indicator taxa selected according to three criteria: ease of identification by non-specialists, sensitivity to organic pollution gradients, and broad geographic distribution.

Sampling is conducted using a standardised 30-second kick-sampling technique with a freshwater net (250 mm frame, 500 mm depth, 1 mm mesh) with three replicate samples collected per site at ~2 m intervals. Organisms are sorted in the field using a white tray. The CSSI applies a presence/absence scoring system: within each replicate, each pollution-sensitive taxa (Plecoptera, Heptageniidae, Rhyacophilidae) contribute +1, while each pollution-tolerant taxa (Hirudinea, Asellidae, Gastropoda) contribute -1. The cumulative score classifies the monitoring site into three quality classes (Heavily polluted, Moderately polluted, Clean) (Harrison et al., 2025) (Tab. 2).

Table 2. Biological quality classes defined by the CSSI protocol and their corresponding values

Clean: +5 to +9	
Moderately polluted: -4 to +4	
Heavily polluted: -9 to -5	

The Small Stream Impact Score (SSIS), developed as a rapid, in-field risk assessment for watercourses (McGarrigle, 2014), was adapted to CS approach by Mary Kelly-Quinn, Jan Robert Baars, Bryan Kennedy and Fran Igoe at University College Dublin (Ireland), represents a simplified derivative of the Q-value index (Toner et al., 2005) used by the Irish Environmental Protection Agency (EPA).

Benthic macroinvertebrates are collected using a standardised kick-sampling technique with a freshwater net (250-mm frame and 500-mm deep net bag with 1-mm mesh). To ensure representative sampling across the study site, the operator systematically moves among different microhabitats. Organisms attached to coarse substrates are collected by manually lifting and washing stones directly into the net.
The operator progressed diagonally across the stream channel upstream while continuously disturbing the substrate for a total of 2 minutes.
Samples are rinsed in the net (~30 s) to remove fine sediments, and large debris is carefully washed and removed while retaining organisms. The samples are transferred to a white tray for sorting, with prior photographic documentation of the whole sample and selected taxa. Identification is conducted at different taxonomic levels depending on the organisms and using standard keys, with a minimum sorting time of 20 minutes. Relative abundance (RA) is assigned to each taxonomic group. The SSIS score is calculated by: (i) recording taxa within target indicator groups and their RA; (ii) deriving group-specific scores based on taxa richness and RA; (iii) additional scoring derived from the abundance of tolerant taxa; and (iv) summing partial scores, normalising (÷5), and scaling (×2). Final scores are expressed as three ecological quality classes (probably unimpacted, evidence of impact, probably impacted), representing a simplified alternative to the five-class EPA system (Kelly-Quinn et al., 2022) (Tab. 3).

Table 3. Biological quality classes established by the SSIS protocol and their corresponding index values

Probably unimpacted: >7.25	
Evidence of impact: 6.5-7.25	
Probably impacted: <6.5	

2.3.3 Anglers' Riverfly Monitoring Initiative (Scotland)

The Anglers' Riverfly Monitoring Initiative (ARMI) is a standardised CS framework designed to complement statutory river quality assessments through high-frequency biological monitoring. It provides a simplified version of the official Biological Monitoring Working Party (BMWP) index. The Riverfly Partnership developed the ARMI protocol in close collaboration with the Environment Agency (EA) of England and has since been exported across the UK.

The sampling methodology utilises a standard freshwater net (250-mm frame and 500-mm deep net bag with 1-mm mesh). The core field procedure consists of a multi-habitat 3-minute kick-sample. This primary collection phase is supplemented by a 1-minute manual stone-washing period.

Following collection, the sample is transferred to a white sorting tray for taxonomic analysis (Fig. 2). The protocol focuses on eight key macroinvertebrate target taxa, identified at order or family level, as follows: cased and caseless Trichoptera, Ephemeridae, Ephemerellidae, Baetidae, Heptageniidae, Plecoptera, and Gammaridae. These groups were selected based on their established sensitivities to pollution, year-round availability, and ease of identification by trained non-professionals. Abundance for each group is estimated using a $\log_{10}$ scale, where observed densities are translated into ordinal scores ranging from 1 to 4. The final ARMI score is the summation of these individual scores, which is then evaluated against a "trigger level", a site-specific threshold established by EA, based on historical baseline data. If the ARMI score is below this level twice in a row at the same site, it indicates a potential pollution incident, which triggers a formal investigation by the authorities (Brooks et al., 2019).



Figure 2. Sorting phase during the Scottish field experiment. Ph Bruna Gumiero

2.4 Metrics

For each monitoring site, the following metrics were calculated:

- N_families (n_fam): calculated as the sum of all macroinvertebrate families recorded at each site (Ofenböck et al., 2004).
- EPT richness: number of families belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera, commonly considered sensitive to environmental disturbance (Ofenböck et al., 2004; Böhmer et al., 2004).
- Biological Monitoring Working Party (BMWP): calculated as the sum of predetermined scores assigned to each benthic macroinvertebrate family (except for Oligochaeta, identified at the class level) (Chesters, 1980).
- Average Score Per Taxon (ASPT): calculated by summing the individual BMWP scores of all macroinvertebrate families present and then dividing that sum by the total number of families (Armitage et al., 1983).
- Iberian Biological Monitoring Working Party (IBMWP): the official biological index for Mediterranean rivers in the Iberian Peninsula, representing an adaptation of the BMWP (Alba-Tercedor et al., 2002). It was calculated solely for comparison with Spanish rivers.

2.5 Statistical analysis

Since the data did not approach a normal distribution for most variables (Shapiro-Wilk test, $p>0.05$), non-parametric tests were used.

For each monitoring site, macroinvertebrate-based metrics were calculated to compare the consistency of the monitoring protocol. Given that the protocols provide qualitative or semi-quantitative measurements, all metrics were calculated based on presence-absence data. To evaluate the agreement among protocols, the Wilcoxon test ($p<0.05$) and Spearman's rank correlation ($p<0.05$) were calculated for each metric pair. To minimise the drift effect, taxa represented by only one or two individuals were excluded from the metric-based analyses.

Quality classes or ARMI scores were compared using the Wilcoxon test ($p<0.05$). Agreement among protocols was assessed as the percentage of sites assigned to the same quality class/ARMI score. For this analysis, the drift effect was controlled in accordance with each protocol's specifications.

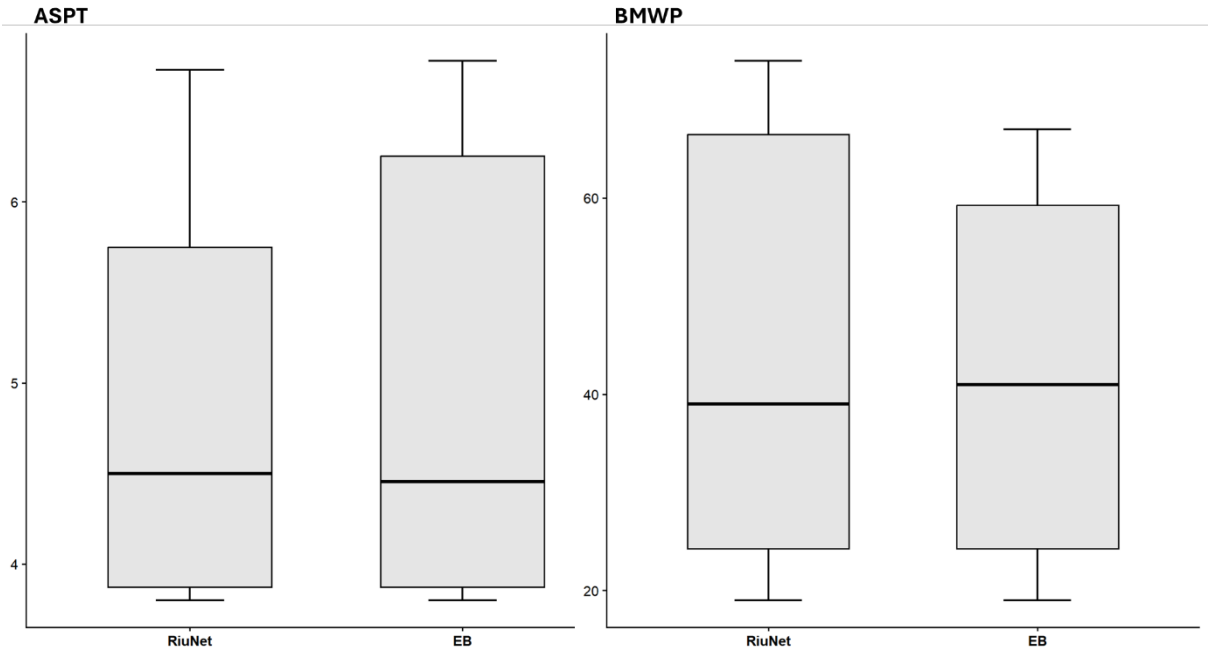
All calculations were run using the R software (R Core Team, 2025).

3. Results

All comparisons were conducted between the EasyBenthos (EB) protocol and the host country's protocol. Raw data are reported in the Supplementary Materials (Tab. 1S).

3.1 EasyBenthos vs RiuNet protocol: metrics and quality classes

Comparisons between the Riunet and EB protocols revealed no significant difference across all considered metrics ($p>0.05$) (Fig. 3).



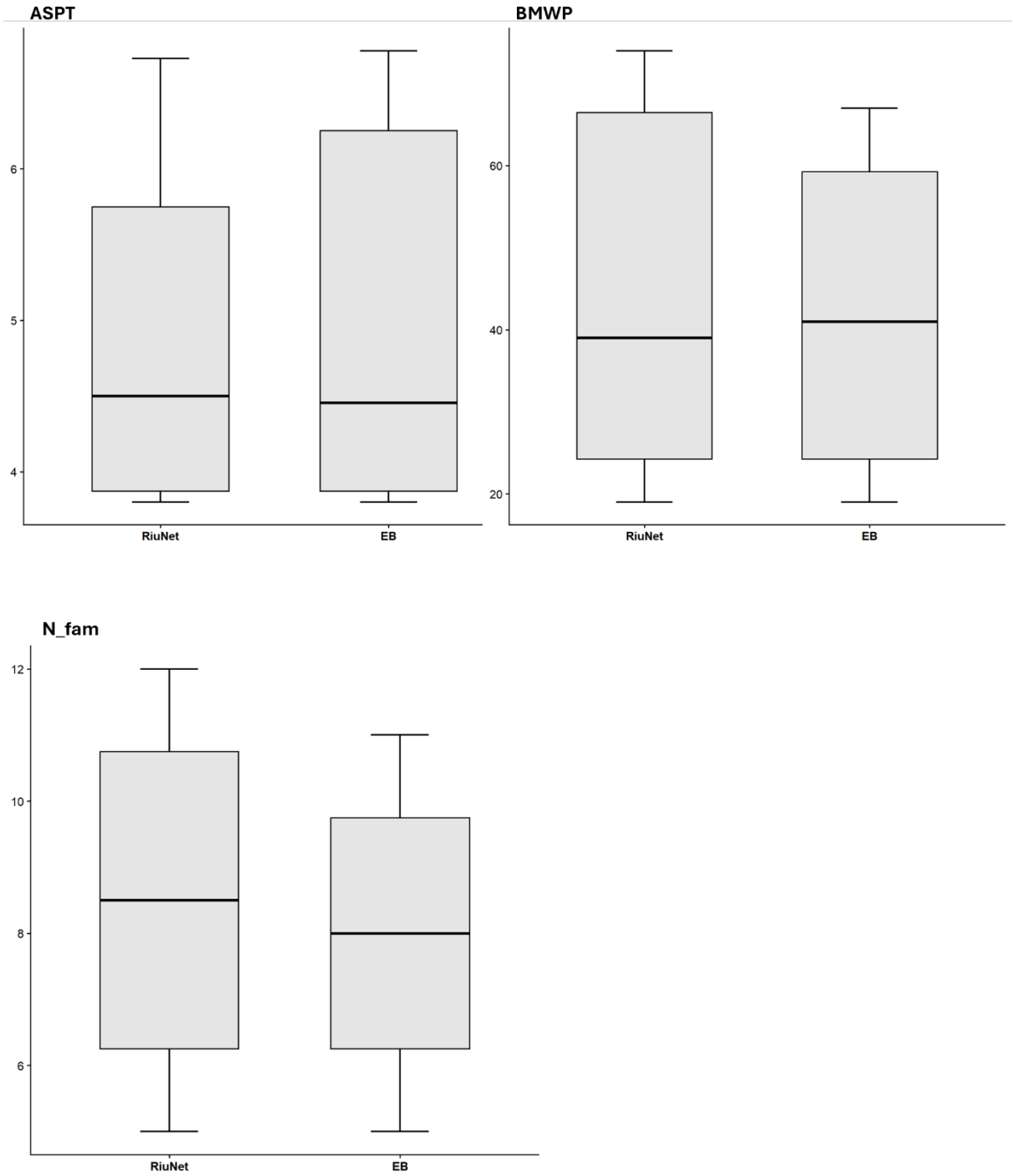


Figure 3. Boxplots comparing metrics calculated with the RiuNet and EB protocols

Spearman's rank correlation analysis indicated strong to very strong positive correlations between the two protocols for most metrics, with significant correlations observed for ASPT ($r=0.94$, $p<0.05$), BMWP ($r=1$, $p<0.05$), IBMWP ($r=0.94$, $p<0.05$), and EPT ($r=1$, $p<0.05$), while n_fam showed a non-significant correlation ($r=0.77$, $p>0.05$).

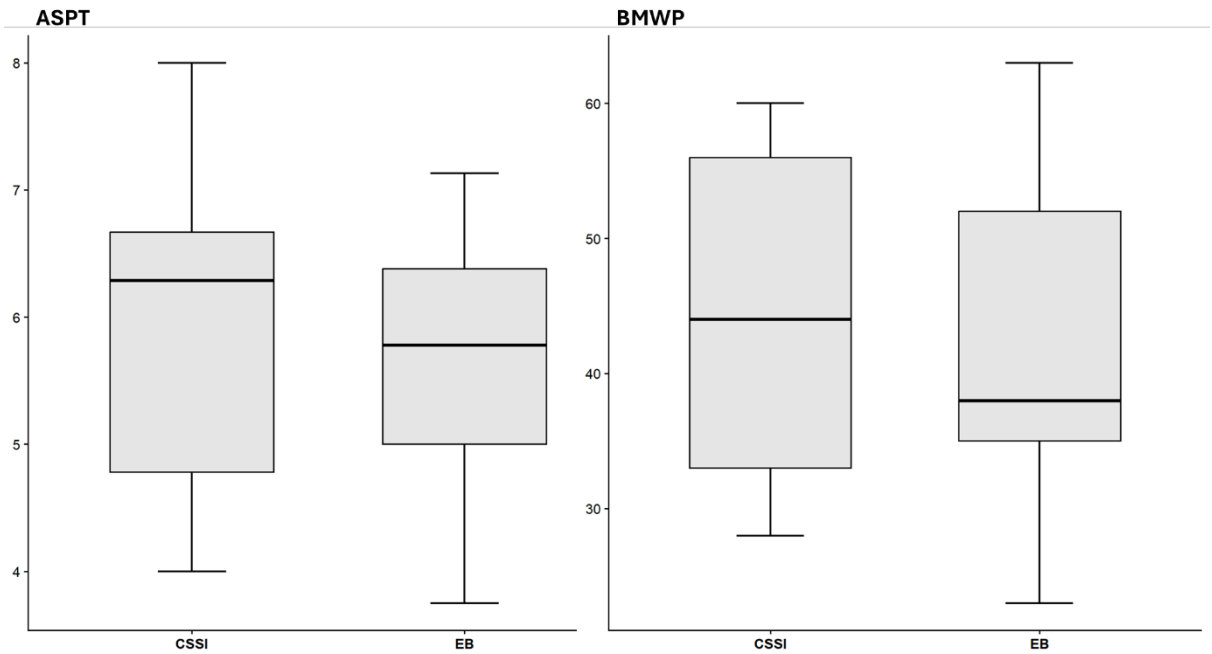
The Wilcoxon test indicated no statistically significant difference (p>0.05) between quality classes obtained with the RiuNet and EB protocols (data not shown). Agreement between protocols was complete, with 100% of sites assigned the same class (Tab. 4).

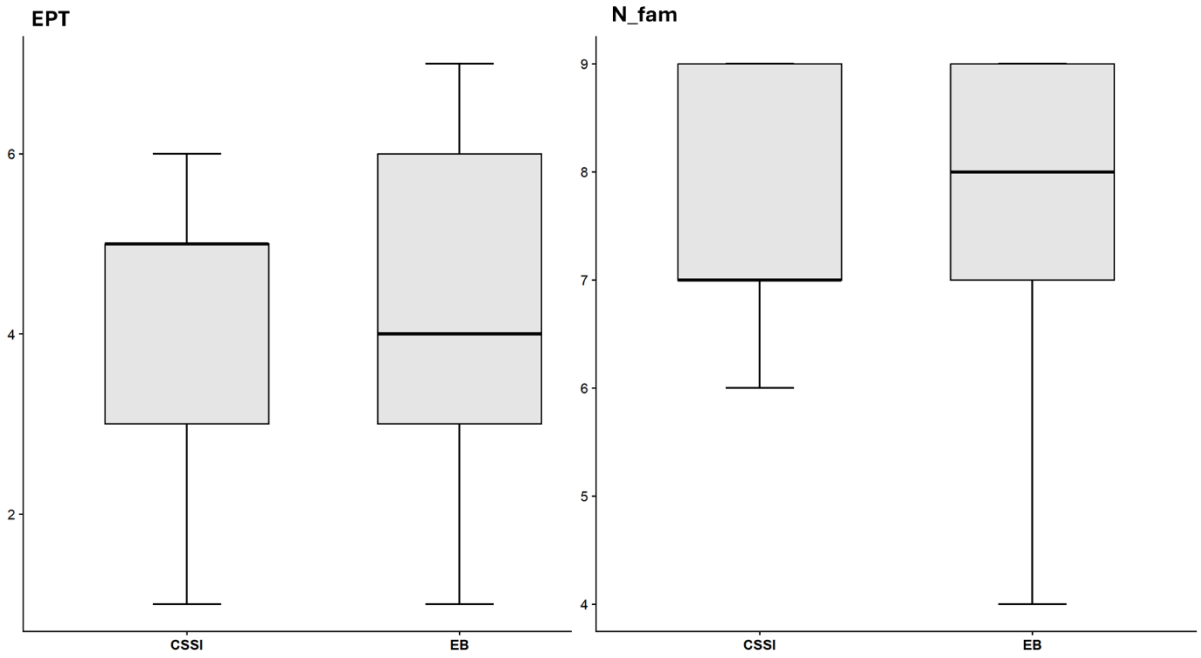
Table 4. Comparison of biological quality classes assigned by the RiuNet and EB protocols across all sampling sites

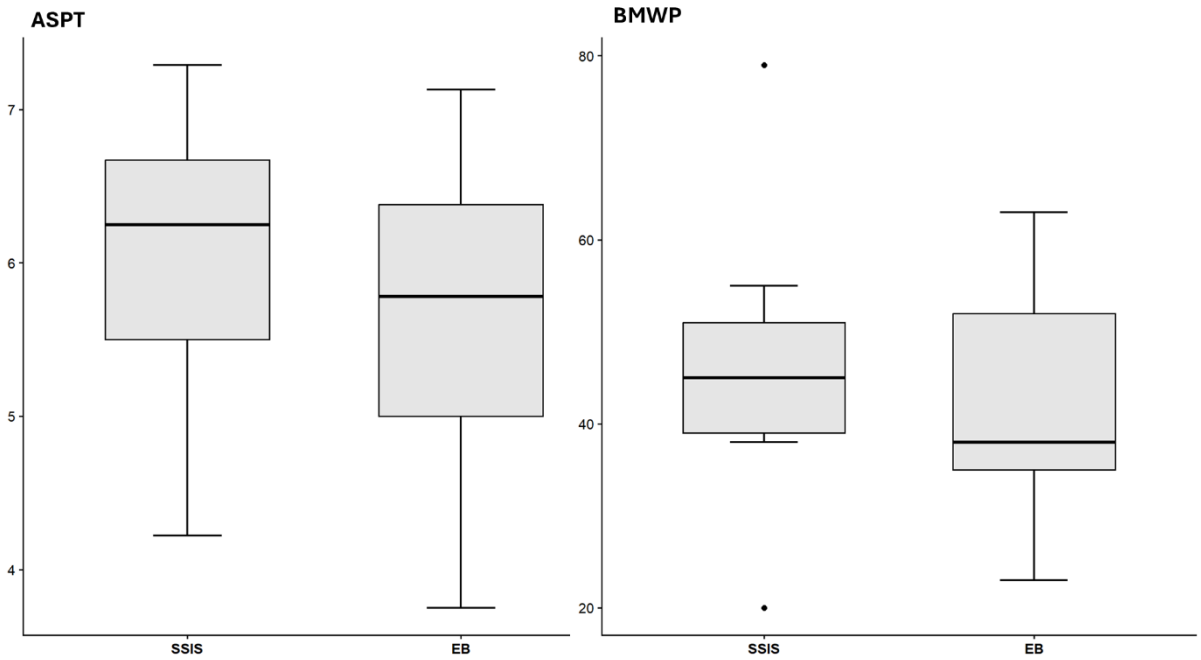
	s1	s2	s3	s4	s5	s6
RiuNet						
EB						

3.2 EasyBenthos vs Citizen Science Stream Index and Small Stream Impact Score protocol: metrics and quality classes

The Wilcoxon tests showed no significant difference between protocols for any of the investigated metrics (p>0.05) (Fig. 4)




EPT
6
4
2
CSSI
EB
N_fam
9
8
7
6
5
4
CSSI
EB


ASPT
7
6
5
4
SSIS
EB
BMWP
80
60
40
20
SSIS
EB

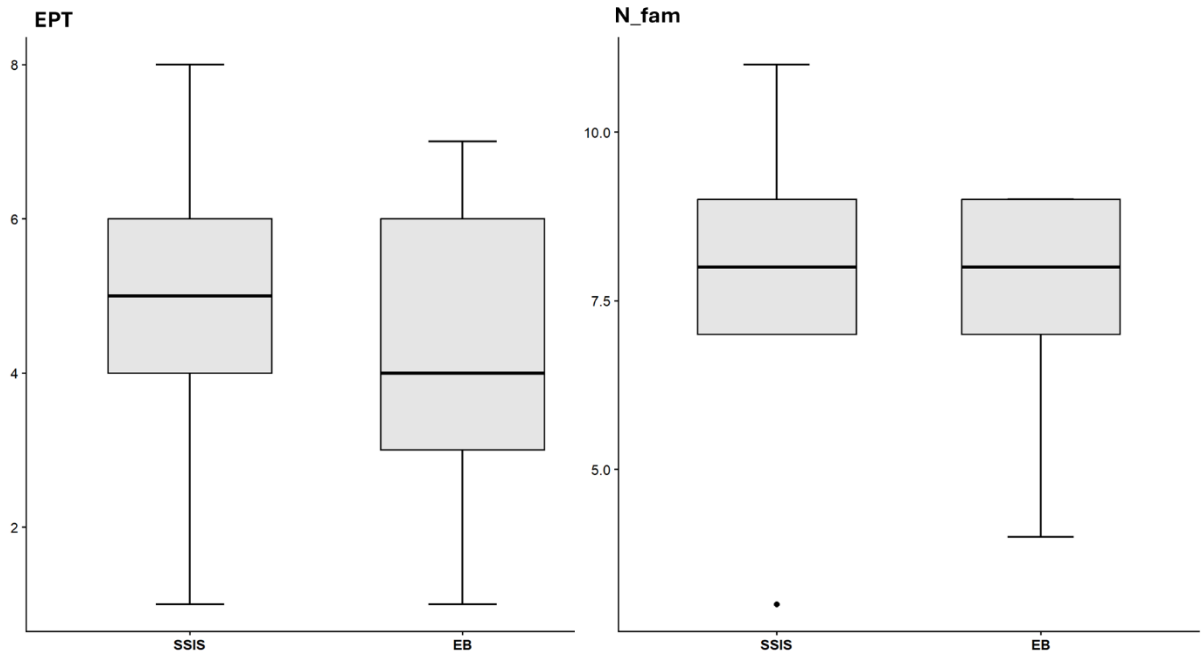


Figure 4. Boxplots comparing metrics calculated with the different protocols: the upper panels show CSSI versus EB, and the lower panels show SSIS versus EB.

Correlation patterns differed between protocols and metrics. The ASPT metric showed significant correlation between CSSI and EB ($r=0.82$, $p<0.05$) but not between SSIS and EB ($r=0.62$, $p>0.05$). BMWP, EPT, and n_fam exhibited no significant correlation between CSSI and EB ($r=0.52$, $r=0.53$, and $r=0.27$, respectively; $p>0.05$), in contrast to significant correlations between SSIS and EB ($r=0.70$, $r=0.68$, and $r=0.80$, respectively; $p<0.05$).

The Wilcoxon test indicated no statistically significant difference ($p>0.05$) between quality classes obtained with the different protocols (data not shown). Agreement between protocols was 44.4% between CSSI and EB, and 55.5% between SSIS and EB. (Tab. 5).

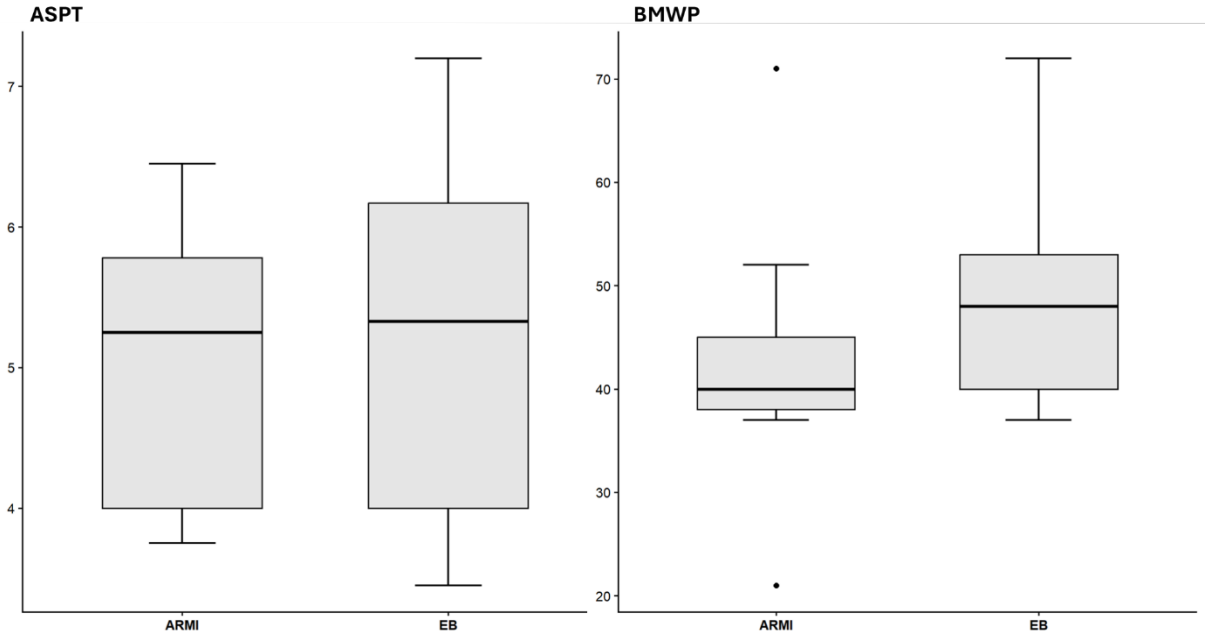
Table 5. Comparison of biological quality classes assigned by the CSSI, SSIS, and EB protocols across sampling sites

	s1	s2	s3	s4	s5	s6	s7	s8	s9
CSSI									
EB									

SSIS									
EB									

3.3 EasyBenthos vs Anglers' Riverfly Monitoring Initiative protocol: metrics and ARMI scores

Comparisons between the ARMI and EB protocols revealed no significant difference for ASPT and BMWP ($p>0.05$), while a significant difference emerged between EPT and n_fam ($p<0.05$) (Fig. 5).



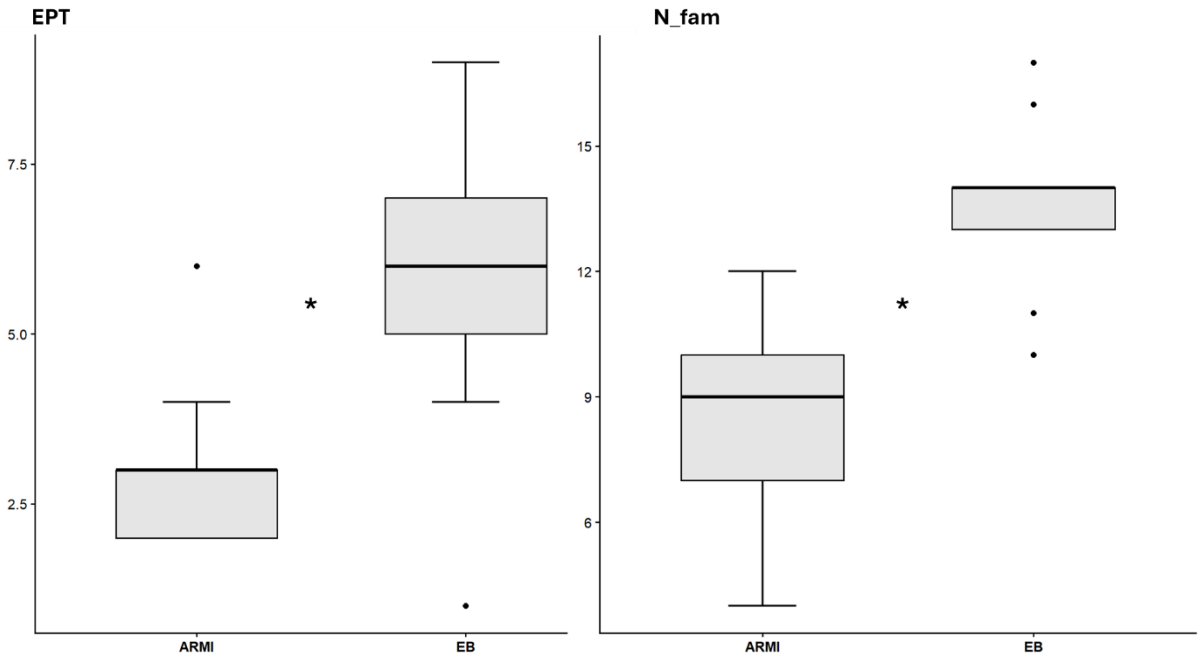


Figure 5. Boxplots comparing ARMI and EB protocols for each pair of analysed metrics

The ASPT, BMPW and n_fam metrics showed no significant correlation between protocols (r=0.59, r=0.46, r =-0.04, respectively; p>0.05). In contrast, the EPT exhibited a positive and statistically significant correlation (r=0.72, p<0.05).

The Wilcoxon test revealed no significant difference (p>0.05) in the ARMI scores calculated using the two protocols (data not shown). Agreement between protocols was 11.1% (Fig. 6).

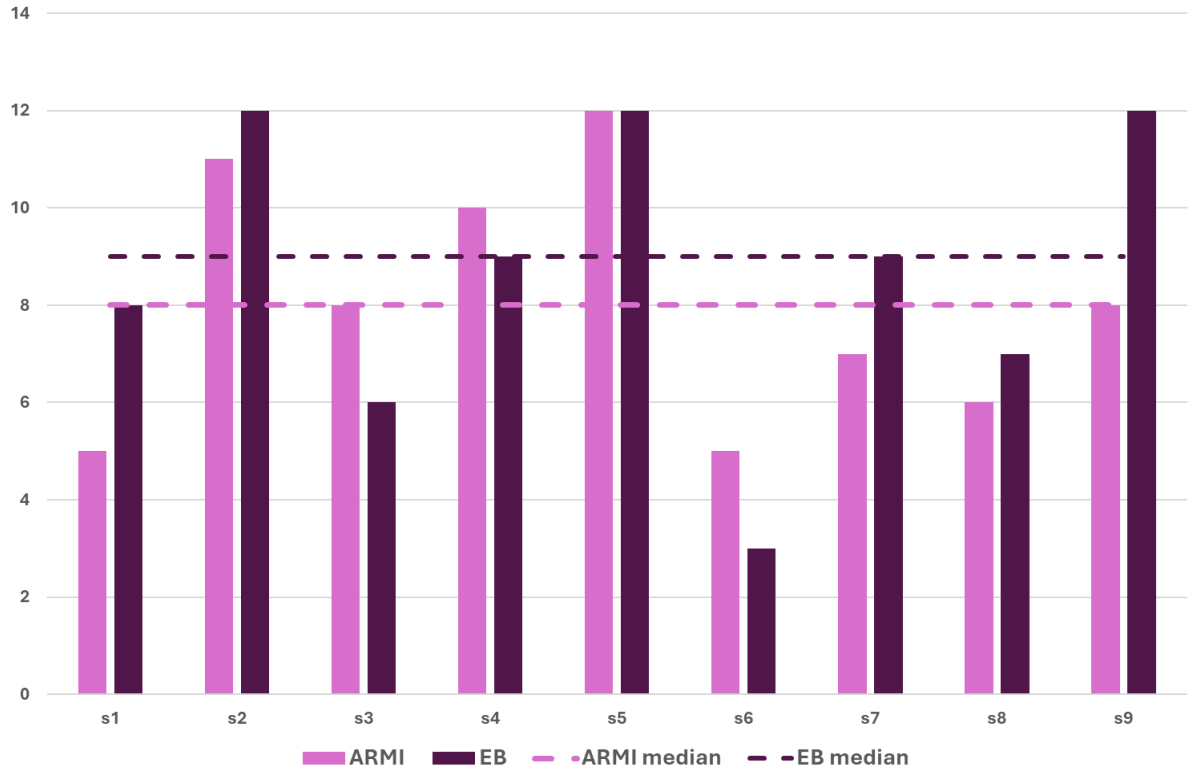


Figure 6. Scatterplot comparing ARMI scores obtained using the two protocols (EB and ARMI)

4. Discussion

This study compared the performance of different CS sampling protocols under comparable field conditions (same monitoring sites and operator). The results are presented through pairwise comparisons between protocols.

4.1 EasyBenthos vs RiuNet protocol: metrics and quality classes

The comparison between the EB and RiuNet protocols demonstrated high consistency, supporting interoperability between the two sampling approaches under comparable field conditions. Despite differences in sampling procedures, no significant difference was observed across all investigated metrics, and correlation analyses revealed strong to very strong positive relationships for ASPT, BMWP, IBMWP, and EPT. This indicates that the EB protocol can capture macroinvertebrate community patterns consistently with the Spanish protocol.

The lack of a significant correlation for n_fam suggests that the EB protocol alternately underestimates or overestimates family richness relative to RiuNet. This pattern is likely attributable to differences in sampling equipment: although both protocols use a 1 mm mesh size, the smaller hand net adopted in RiuNet could more effectively access

interstitial spaces among coarse substrates. This may enhance taxon capture efficiency, particularly in narrow and shallow streams typical of the Spanish CS monitoring program. Consequently, discrepancies in n_fam are likely driven by differences in microhabitat accessibility during sampling rather than by inherent methodological inconsistency. Given that the influence of sampling device characteristics on the estimation of taxonomic richness is well documented (Mackey et al., 1984; Davies, 2001; Hafezul et al., 2016; Pinna et al., 2023), the key challenge is identifying the approach that is more practical for volunteers, while maintaining an appropriate trade-off between sampling efficiency and ease of use.

The complete agreement (100%) in ecological quality class assignment further reinforces the strong interoperability between the EB and the RiuNet protocols. This is particularly relevant in the context of the study objective, as it may suggest that differences in sampling equipment do not necessarily translate into differences in the evaluation of the ecological status, encouraging the interoperability between protocols (Friberg et al., 2006).

4.2 EasyBenthos vs CSSI and SSIS protocols: metrics and quality classes

The comparison with the CSSI and the SSIS protocols revealed a complex pattern. While statistically significant differences were not observed in metric values, correlation analyses showed variable levels of agreement depending on both the metric and the protocol considered.

In general, SSIS exhibited stronger and more consistent correlations with EB than CSSI, particularly for BMWP, EPT, and n_fam. This suggests that the SSIS sampling approach may be more aligned with EB to capture macroinvertebrate community structure. In contrast, the CSSI showed weaker and mostly non-significant correlations between metrics, likely influenced by the difference in sampling effort. While CSSI is based on three static 30-second replicas, EB provides for continuous 5-minute sampling, thereby increasing the likelihood of detecting taxa (Hughes et al., 2012). Furthermore, the fact that one method is stationary whereas the other involves active movement across the site may amplify microhabitat selection effects, which in turn can influence the composition and representativeness of the sampled community (Larras et al., 2020). This partially constrains interoperability, particularly between EB and CSSI. Previous studies (Friberg et al., 2006) also demonstrated that taxon richness is primarily affected by the sorting and identification phases (which were kept constant

across sampling events in this study) rather than by sampling effort. Moreover, field manipulation of live organisms and the removal of material from sorting trays may reduce comparability of the results due to the potential loss of taxa (Haase et al., 2004b). Field handling is strongly dependent on operator skill (although in this case, the same operator conducted all samplings) and can also be affected by external factors such as weather conditions (Dines et al., 2000). Thus, even though conditions were controlled in this field experiment, other sources of variability between samples other than sampling effort cannot be excluded.

The agreement in the assessment of ecological quality classes between EB and the Irish protocols was relatively low (44.4% for CSSI and 55.5% for SSIS), despite the absence of a statistically significant difference.

In particular, greater disagreement between CSSI and EB was observed at the extremes of the classification scale ("Heavily Polluted" and "Clean"), while intermediate classes showed higher concordance. In contrast, no consistent pattern of agreement was evident between SSIS and EB across quality classes. These results indicate that interoperability between protocols should be interpreted with caution, as heterogeneity in sampling strategies can introduce significant variability in community characterisation and, consequently, in the assessment of the ecological quality class (Larras et al., 2020).

4.3 EasyBenthos vs ARMI protocol: metrics and ARMI scores

The comparison of the EB protocol with the ARMI protocol outlines a distinct scenario. While ASPT and BMWP did not differ significantly between protocols, a significant difference was observed for EPT and n_fam, indicating that EB and ARMI differ in their sensitivity to community composition, with EB generally producing higher median values for both metrics.

Generally, weak and non-significant correlations for most metrics were found, except for EPT, which exhibited a moderate and statistically significant correlation. These results are likely linked to the higher level of standardisation in the ARMI protocol, which combines a 3-minute kick-sample with a 1-minute manual stone search, including approximately 10 seconds of kicking at each point before moving on. This structured approach reduces operator-dependent variability in sampling effort, leading to differences with EB in terms of microhabitat selection and overall sampling effort, ultimately affecting the taxa collected.

As discussed in the previous paragraph, sampling effort, microhabitat selection, and operator-induced variability can affect sampling outcomes and, consequently, the composition of the sampled macroinvertebrate community. Another study has shown that, although a standardised sampling effort at the catchment scale may be sufficient to characterise taxonomic richness, at the scale of individual monitoring sites, taxon richness continues to increase with sampling effort without reaching an asymptote (Silva et al., 2016). This aspect may have influenced the results of the present study, which are based on direct site-by-site comparisons, each relying on a single sampling event that may not have fully captured the actual taxonomic richness present at each site.

Despite the absence of significant differences in ARMI scores, the very low agreement between these (11.1%) indicates substantial divergence between outcomes derived from EB and ARMI protocols.

Although the ARMI score is calculated as the sum of abundance estimates of selected target groups, the observed discrepancies could once again reflect differences in sampling protocol between the two methods. As already mentioned, this suggests that variations in sampling design can influence the detection and estimation of taxa, thereby limiting the interoperability between protocols (Larras et al., 2020).

5. Conclusions

The comparison of different CS protocols in use in some Countries showed different patterns of agreement and suggested that the EB protocol has high interoperability with some national protocols, while its comparability with others is more variable. It is also important to note that the relatively small sample size in each country limits the strength of the conclusions that can be drawn.

This study provides, to our knowledge, the first field-based comparison of simplified macroinvertebrate sampling protocols used in CS programmes across Europe. By applying different sampling protocols under similar conditions and comparing the results, it offers preliminary insights into the degree of protocol interoperability. The lack of comparative studies invalidates the demonstration of the effectiveness of CS and the comparability of their outputs, an essential condition for elevating these practices to a robust pillar of Open Science, at least on the European scale. This highlights the critical importance of our findings, which therefore provide preliminary evidence in this context.

Overall, the Italian EB protocol showed high comparability with the Spanish RiuNet protocol, with consistent metric values and full agreement in quality classes results. This indicates that, under comparable conditions, differences in sampling design do not necessarily affect assessment outcomes, supporting potential interoperability between these protocols.

In contrast, comparisons with the Irish (CSSI and SSIS) and Scottish (ARMI) protocols revealed more variable results. Although metric values were often not significantly different, correlations between metrics and agreement in quality classes/ARMI scores indicated lower interoperability. These discrepancies highlight the influence of sampling protocols on the results, especially for protocols based on shorter (CSSI) or more structured (ARMI) sampling efforts that may amplify differences among protocols.

These findings highlight sampling design as one of the key factors influencing the interoperability of monitoring methods, even though other sources of variability cannot be excluded. Further research will be necessary to consolidate these results and to more clearly define the extent of interoperability among CS sampling protocols.

6. Acknowledgements

The author would like to thank the host research teams for their collaboration and assistance throughout the field campaigns carried out in the different countries involved in this study. Particular thanks are extended to Prof. Gumiero for her guidance during the fieldwork conducted in Scotland.

Special thanks are extended to Prof. Loppi and Prof. Gumiero for their input in the development of the experimental design and for their review of this chapter.

Supplementary Materials

Table 1S. Presence-absence data collected with different protocols: RiuNet, Citizen Science Stream Index (CSSI), Small Stream Impact Score (SSIS), Anglers' Riverfly Monitoring Initiative (ARMI), EasyBenthos (EB)

Macroinvertebrate taxa	S1		S2		S3		S4	
	RiuNet	EB	RiuNet	EB	RiuNet	EB	RiuNet	EB
Plecoptera								
Perlodidae				x				
Ephemeroptera								
Baetidae	x	x	x	x	x	x	x	x
Caenidae			x	x	x	x	x	x
Ephemerellidae	x	x	x	x		x		
Ephemeridae	x		x					
Heptageniidae	x		x	x				
Leptophlebiidae	x	x	x	x			x	x
Trichoptera								
Hydropsichidae	x		x	x		x		
Leptoceridae	x	x	x	x				
Limnephillidae	x	x					x	
Ryachophillidae				x				
Sericostomatidae	x	x						
Coleoptera								
Dytiscidae	x		x	x		x	x	x
Elmidae	x							
Odonata								
Aeshnidae	x	x						

Calopterygidae			x		x		x	x
Gomphidae	x	x		x				
Diptera								
Athericidae			x	x				
Ceratopogonidae		x	x	x	x	x	x	x
Chironomidae	x	x	x	x	x	x	x	x
Simulidae	x	x	x	x	x	x	x	x
Tabanidae								x
Tipulidae	x		x	x			x	x
Heteroptera								
Gerridae					x	x		
Hydrometridae							x	x
Notonecnidae			x					x
Veliidae	x	x	x				x	x
Crustacea								
Cambaridae								
Gastropoda								
Ancylidae	x		x					
Hydrobiidae	x		x	x			x	x
Lymneidae				x				
Physidae			x		x		x	
Hirudinea								
Erpobdellidae	x		x			x		
Oligochaeta	x	x	x	x	x	x	x	x
Others								

Hydrachnidiae	x			

Macroinvertebrate taxa	S5		S6	
	RiuNet	EB	RiuNet	EB
Plecoptera				
Perlodidae				
Ephemeroptera				
Baetidae	x	x	x	x
Caenidae	x	x	x	x
Ephemerellidae				
Ephemeridae				
Heptageniidae				
Leptophlebiidae				
Trichoptera				
Hydropsichidae				
Leptoceridae				
Limnephillidae				
Ryachophillidae				
Sericostomatidae				
Coleoptera				
Dytiscidae				
Elmidae				
Odonata				
Aeshnidae				

Calopterygidae				
Gomphidae				
Diptera				
Athericidae				
Ceratopogonidae				
Chironomidae	x	x	x	x
Simulidae	x	x	x	x
Tabanidae		x		
Tipulidae	x	x		
Heteroptera				
Gerridae				
Hydrometridae				
Notonecnidae				
Veliidae				
Crustacea				
Cambaridae			x	x
Gastropoda				
Ancylidae	x	x		
Hydrobiidae				
Lymneidae				
Physidae		x		
Hirudinea				
Erpobdellidae	x	x		
Oligochaeta	x	x	x	x
Others				

Hydrachnidiae

Macroinvertebrate taxa	S1			S2			S3			S4		
	CSSI	SSIS	EB	CSSI	SSIS	EB	CSSI	SSIS	EB	CSSI	SSIS	EB
Plecoptera												
Chloroperlidae												
Leuctridae		x										
Nemouridae												
Perlodidae												
Ephemeroptera												
Baetidae	x	x	x	x	x	x	x	x	x	x	x	x
Caenidae												
Ephemerellidae	x	x	x	x	x	x	x	x	x		x	x
Heptageniidae	x	x	x	x	x	x	x	x	x	x	x	x
Leptophlebiidae												
Trichoptera												
Glossosomatidae	x	x	x	x	x	x	x	x		x	x	x
Goeridae							x	x		x		x
Hydropsichidae	x	x	x		x	x	x	x	x	x		x
Hydroptilidae												
Lepidostomatidae												
Leptoceridae												
Limnephillidae			x								x	x
Philopotamidae								x	x			

Polycentropodidae		x							x			x
Ryachophillidae	x	x	x	x		x	x		x	x	x	x
Sericostomatidae	x		x	x	x	x	x	x	x	x		
Coleoptera												
Dytiscidae	x										x	
Elmintidae	x	x	x	x	x	x	x	x	x	x	x	x
Odonata												
Aeshnidae												
Calopterygidae												
Libellulidae												
Diptera												
Athericidae												
Ceratopogonidae	x		x				x					
Chironomidae	x	x	x	x			x	x				
Pedicidae	x	x	x									
Simulidae	x	x	x	x	x	x	x	x		x	x	x
Tabanidae												
Tipulidae			x									
Heteroptera												
Gerridae												
Hydrometridae												
Notonecnidae												
Veliidae												
Crustacea												
Asellidae							x					
Gammaridae	x	x	x	x	x	x	x	x	x	x	x	x

Gastropoda												
Ancylidae												
Hydrobiidae				x			x					
Lymneidae												
Tateidae					x	x						
Hirudinea												
Erpobdellidae	x								x			
Oligochaeta	x		x	x	x	x	x	x	x	x		
Tricladida												
Planariidae		x	x	x	x			x		x	x	x

Macroinvertebrate taxa	**S5**			**S6**			**S7**			**S8**		
	CSSI	**SSIS**	**EB**	**CSSI**	**SSIS**	**EB**	**CSSI**	**SSIS**	**EB**	**CSSI**	**SSIS**	**EB**
Plecoptera												
Chloroperlidae											x	
Leuctridae							x	x	x	x	x	
Nemouridae												
Perlodidae												
Ephemeroptera												
Baetidae				x	x	x	x	x	x	x	x	x
Caenidae												
Ephemerellidae	x	x	x	x	x	x	x	x	x	x	x	x
Heptageniidae							x	x	x	x	x	x
Leptophlebiidae												

Trichoptera													
Glossosomatidae			x									x	x
Goeridae													
Hydropsichidae				x	x	x	x	x	x	x	x	x	x
Hydroptilidae												x	
Lepidostomatidae							x		x				
Leptoceridae													
Limnephillidae													x
Philopotamidae											x		
Polycentropodidae							x	x	x				
Ryachophillidae								x	x	x	x		
Sericostomatidae					x		x	x	x	x	x	x	x
Coleoptera													
Dytiscidae													
Elmintidae							x	x	x				
Odonata													
Aeshnidae													
Calopterygidae			x										
Libellulidae													
Diptera													
Athericidae													
Ceratopogonidae				x		x							
Chironomidae	x	x	x				x	x	x				
Pedicidae													
Simulidae		x	x				x	x	x	x	x	x	
Tabanidae													

Tipulidae										x		x
Heteroptera												
Gerridae												
Hydrometridae												
Notonecnidae												
Veliidae												
Crustacea												
Asellidae	x	x	x	x	x	x						
Gammaridae				x	x	x	x	x		x	x	x
Gastropoda												
Ancylidae	x	x	x									
Hydrobiidae	x	x	x									
Lymneidae	x	x	x									
Tateidae												
Hirudinea												
Erpobdellidae				x	x	x						
Oligochaeta	x	x	x	x	x	x	x		x	x	x	x
Tricladida												
Planariidae		x	x	x				x		x	x	x

Macroinvertebrate taxa	**S9**		
	CSSI	**SSIS**	**EB**
Plecoptera			
Chloroperlidae			

Leuctridae	x	x	x
Nemouridae		x	x
Perlodidae	x	x	x
Ephemeroptera			
Baetidae	x	x	x
Caenidae			
Ephemerellidae	x	x	x
Heptageniidae	x	x	x
Leptophlebiidae			
Trichoptera			
Glossosomatidae			
Goeridae			
Hydropsichidae	x	x	
Hydroptilidae			
Lepidostomatidae			
Leptoceridae			
Limnephillidae			
Philopotamidae	x		
Polycentropodidae	x	x	x
Ryachophillidae			x
Sericostomatidae	x	x	x
Coleoptera			
Dytiscidae	x		
Elmintidae		x	x
Odonata			
Aeshnidae			

Calopterygidae			
Libellulidae			
Diptera			
Athericidae			
Ceratopogonidae			
Chironomidae		x	x
Pedicidae			
Simulidae	x	x	x
Tabanidae			
Tipulidae			
Heteroptera			
Gerridae			
Hydrometridae			
Notonecnidae			
Veliidae			
Crustacea			
Asellidae			
Gammaridae			
Gastropoda			
Ancylidae			
Hydrobiidae			
Lymneidae			
Tateidae			
Hirudinea			
Erpobdellidae			
Oligochaeta			

Tricladida								
Planariidae						x		

Macroinvertebrate taxa	S1		S2		S3		S4	
	ARMI	EB	ARMI	EB	ARMI	EB	ARMI	EB
Plecoptera								
Chloroperlidae							x	
Leuctridae	x	x	x	x			x	x
Nemouridae		x						
Perlidae					x	x		
Ephemeroptera								
Baetidae	x	x	x	x	x	x	x	x
Caenidae								
Ephemerellidae	x	x	x	x	x	x	x	x
Ephemeridae			x	x			x	
Heptageniidae	x	x	x	x	x	x	x	x
Leptophlebiidae								
Trichoptera								
Hydropsichidae								
Hydroptilidae								
Leptoceridae			x	x				
Lepidostomatidae								
Limnephillidae			x					
Odontoceridae				x				

Philopotamidae					x			x
Ryachophillidae		x		x	x	x	x	x
Sericostomatidae		x		x	x			
Coleoptera								
Dytiscidae						x	x	
Elminthidae		x		x		x	x	x
Odonata								
Aeshnidae								
Calopterygidae								
Gomphidae								
Diptera								
Athericidae								x
Ceratopogonidae								
Chironomidae	x	x	x	x			x	x
Limonidae			x	x	x			
Simulidae	x	x				x	x	x
Tabanidae								
Tipulidae			x					
Heteroptera								
Gerridae								
Hydrometridae								
Notonecnidae								
Veliidae								
Crustacea								
Asellidae	x	x					x	x
Gammaridae			x	x			x	x

Gastropoda								
Ancylidae								
Hydrobiidae								
Lymneidae								
Physidae								
Planorbidae								
Hirudinea								
Erpobdellidae					x	x		
Oligochaeta	x	x	x	x		x	x	x
Tricladida								
Planarie	x	x		x			x	x
Nematomorpha	x	x		x				

Macroinvertebrate taxa	**S5**		**S6**		**S7**		**S8**	
	ARMI	**EB**	**ARMI**	**EB**	**ARMI**	**EB**	**ARMI**	**EB**
Plecoptera								
Chloroperlidae								
Leuctridae	x	x			x	x		
Nemouridae		x						
Perlidae	x							
Ephemeroptera								
Baetidae	x	x	x	x	x	x	x	x
Caenidae								
Ephemerellidae	x	x					x	x

Ephemeridae								
Heptageniidae	x	x			x	x		x
Leptophlebiidae								
Trichoptera								
Hydropsichidae								
Hydroptilidae			x		x	x		
Leptoceridae								
Lepidostomatidae								
Limnephillidae								
Odontoceridae	x	x						
Philopotamidae						x		
Ryachophillidae	x	x	x		x	x	x	x
Sericostomatidae	x	x			x	x		
Coleoptera								
Dytiscidae	x		x	x	x	x	x	x
Elminthidae					x	x		
Odonata								
Aeshnidae								
Calopterygidae								
Gomphidae								
Diptera								
Athericidae								
Ceratopogonidae								
Chironomidae	x	x	x	x	x	x	x	x
Limonidae					x	x		
Simulidae			x					x

Tabanidae								
Tipulidae			x	x			x	
Heteroptera								
Gerridae								
Hydrometridae								
Notonecnidae								
Veliidae								
Crustacea								
Asellidae	x	x	x	x	x	x	x	x
Gammaridae	x	x	x	x			x	x
Gastropoda								
Ancylidae		x						
Hydrobiidae			x	x	x	x	x	x
Lymneidae	x	x	x	x		x	x	x
Physidae								
Planorbidae			x	x				
Hirudinea								
Erpobdellidae	x		x	x			x	x
Oligochaeta	x	x	x	x			x	x
Tricladida								
Planarie								
Nematomorpha								

Macroinvertebrate taxa	S9	
	ARMI	EB
Plecoptera		
Chloroperlidae		
Leuctridae	x	x
Nemouridae		
Perlidae		
Ephemeroptera		
Baetidae	x	x
Caenidae		
Ephemerellidae		
Ephemeridae		
Heptageniidae		
Leptophlebiidae		
Trichoptera		
Hydropsichidae		
Hydroptilidae		
Leptoceridae		
Lepidostomatidae		x
Limnephillidae		
Odontoceridae		
Philopotamidae		x
Ryachophillidae		x
Sericostomatidae		x
Coleoptera		

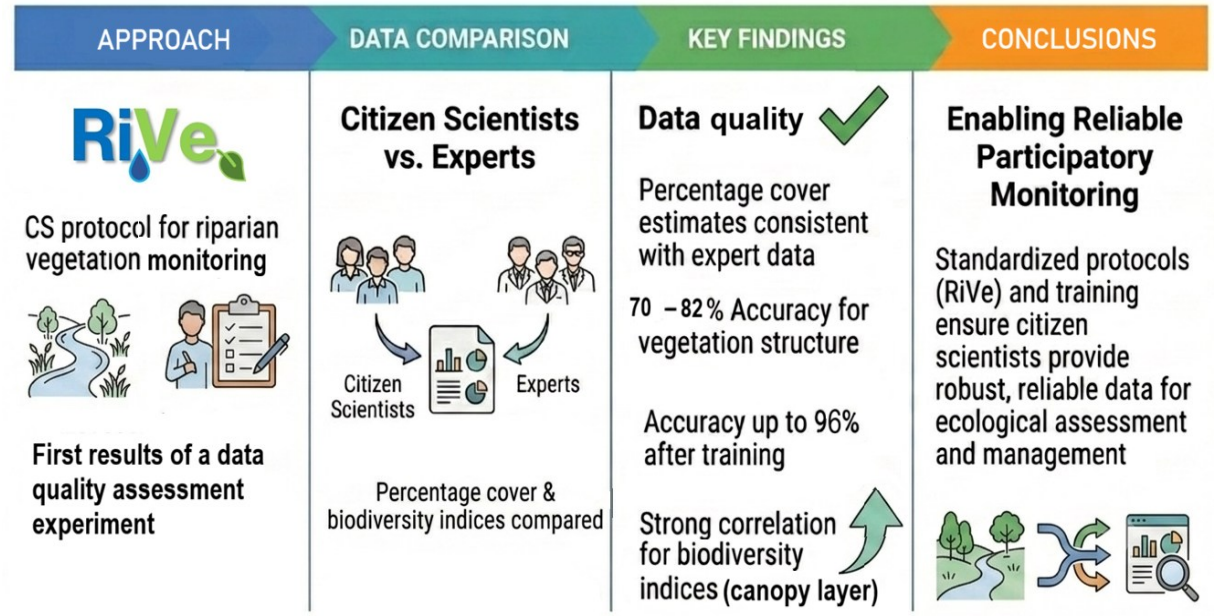
Dytiscidae	x	x
Elminthidae		
Odonata		
Aeshnidae		
Calopterygidae		
Gomphidae		
Diptera		
Athericidae		
Ceratopogonidae		
Chironomidae	x	x
Limonidae	x	x
Simulidae	x	x
Tabanidae		
Tipulidae		
Heteroptera		
Gerridae		
Hydrometridae		
Notonecnidae		x
Veliidae		
Crustacea		
Asellidae	x	x
Gammaridae	x	x
Gastropoda		
Ancylidae		
Hydrobiidae	x	x
Lymneidae	x	x

Physidae		
Planorbidae		
Hirudinea		
Erpobdellidae	x	x
Oligochaeta	x	x
Tricladida		
Planarie		

Chapter 3.

Data Quality in Citizen Science Riparian Vegetation Monitoring: First Results from the RiVe Protocol

Graphical abstract



Abstract

This study evaluates the data quality of a new Citizen Science (CS) protocol (RiVe) designed for riparian vegetation monitoring and tested within the CS4Rivers project (Tuscany, Italy). Data collected by trained citizen scientists (CTZ) were compared with expert assessments (EXP), focusing on percentage cover estimates and biodiversity indices (Shannon and Pielou). Results showed no significant difference between CTZ and EXP in percentage cover at either the taxon level (with the exception of *Robinia pseudacacia*, understory layer) or the overall level, with moderate but significant correlations. No observer bias was detected by the generalised linear mixed model (GLMM). Biodiversity indices showed no significant difference but non-significant correlations. Overall data accuracy was high (ranging from 70% to 82%, sd between ±8% and ±11%). Following targeted training and additional field practice for the lowest-accuracy group, accuracy increased from 70% to 96%. Furthermore, previously non-significant correlations became significant and stronger. These results demonstrate that, with standardised protocol design and appropriate training, CTZ can reliably

assess riparian vegetation structure. Furthermore, the RiVe protocol represents a robust tool for participatory monitoring, supporting the integration of CS data into ecological assessment and management frameworks.

1. Introduction

Riparian zones are highly dynamic, transitional ecosystems that link aquatic and terrestrial environments along rivers, lakes, streams and wetlands (Dinca et al., 2025). The unique ecological position of riparian areas supports a wide range of ecosystem services essential to both freshwater ecosystems and human well-being (Cole et al., 2020; Basak et al., 2021). Among these, riparian vegetation plays a central role in flood regulation by increasing surface roughness, reducing flow velocity, enhancing infiltration, and lowering downstream flood peaks (Keesstra et al., 2018; Riis et al., 2020). Dense root systems stabilise riverbanks and reduce erosion (Feld et al., 2018), while large woody debris influences sediment deposition, channel morphology, and habitat complexity (Swanson et al., 2021; Dunn et al., 2022). Canopy shading regulates microclimate, moderates stream temperature, limits algal blooms, and maintains dissolved oxygen levels, which are critical for aquatic organisms (Garner et al., 2017; Trimmel et al., 2018). Furthermore, riparian corridors enhance habitat heterogeneity, provide refugia and nursery areas for numerous aquatic and terrestrial species, and ensure ecological connectivity, thereby reducing fragmentation and increasing ecosystem resilience (Naiman & Décamps, 1997; Tabacchi et al., 2003; Camporeale et al., 2013). They also support markedly distinct species pools compared to adjacent terrestrial habitats. As a result, these systems increase regional richness (- diversity) globally by approximately 50% on average. Consequently, conservation planners can substantially enhance the number of species included within a regional conservation portfolio simply by incorporating riverine systems into terrestrial biodiversity reserves (Sabo et al., 2005).

Riparian zones also act as effective buffer systems, improving water quality through sediment filtration, pollution retention, erosion control (Cole et al., 2020) and denitrification (Gumiero et al., 2011). They contribute coarse particulate organic matter to aquatic food webs, sustaining detritivores and decomposers (Giri et al., 2025). Furthermore, riparian zones contribute to carbon sequestration: restored riparian sites store greater amounts of carbon than unrestored ones, highlighting their potential to mitigate climate change impacts (Matzek et al., 2022). Finally, recreational and cultural

services are also provided by riparian vegetation, which support activities such as fishing, canoeing, hiking, and birdwatching. Their aesthetic and experiential value contributes to human well-being and reinforces cultural identity (Cameron et al., 2020).
Despite their well-recognised ecological importance, riparian ecosystems remain widely impacted, mismanaged (Zaimes, 2020) and not explicitly considered in the assessment of the ecological status under the EU Water Framework Directive (WFD 2000/60/EC) (González del Tánago et al., 2021). This mismatch between ecological relevance and policy assessment frameworks hampers the effective conservation and restoration of riparian ecosystems. In Europe, a key legal instrument is the Habitats Directive (European Commission, 1992). A central component of the Directive is the requirement for continuous and systematic monitoring, as the European Commission mandates regular assessments of the conservation status of species and habitats at both national and biogeographical levels on a six-year reporting cycle (Evans and Arvela, 2011). More recently, the EU Biodiversity Strategy for 2030, with the EU Nature Restoration Law, explicitly promote the restoration and strengthening of ecological corridors, including riparian and fluvial corridors, as key components of a trans-European ecological network (European Commission, Regulation EU 2024/1991). Achieving these restoration objectives requires not only implementation measures but also a collaboration among policymakers, institutions, scientists, and communities (Urbanič et al., 2022) and a significant investment in resources.
In this context, Citizen Science (CS) - as the active engagement of the general public in scientific research tasks (Bonney et al., 2014) - represents a valuable opportunity to enhance riparian ecosystem monitoring, increase public awareness, and foster stakeholder engagement. Although CS is frequently promoted as a promising approach for environmental monitoring - particularly due to its cost-effectiveness and its ability to generate data with broad spatial, temporal, and taxonomic coverage (Chandler et al., 2017; McKinley et al., 2017) - concerns regarding the quality and reliability of citizen-generated data remain widespread within the scientific community (Lukyanenko et al., 2016; Hochmair et al., 2020).
Accordingly, here we introduce (i) a novel CS protocol (RiVe) specifically designed for monitoring riparian vegetation, which is one of the few protocols fully focused on these ecosystems, and (ii) the first results of a data quality assessment experiment conducted on this protocol, providing an initial evaluation of the reliability of citizen-generated data collected using the RiVe protocol.

2. Materials and Methods

2.1 Study Area

The study was carried out within the CS4Rivers project, which focuses on the Ombrone River basin (southern Tuscany, Italy). This experiment was conducted along the Ombrone, the Orcia and the Merse River, including its main tributary, the Farma stream (Fig. 1), because they feature extensive riparian vegetation and areas subjected to management, making them suitable for this type of investigation.

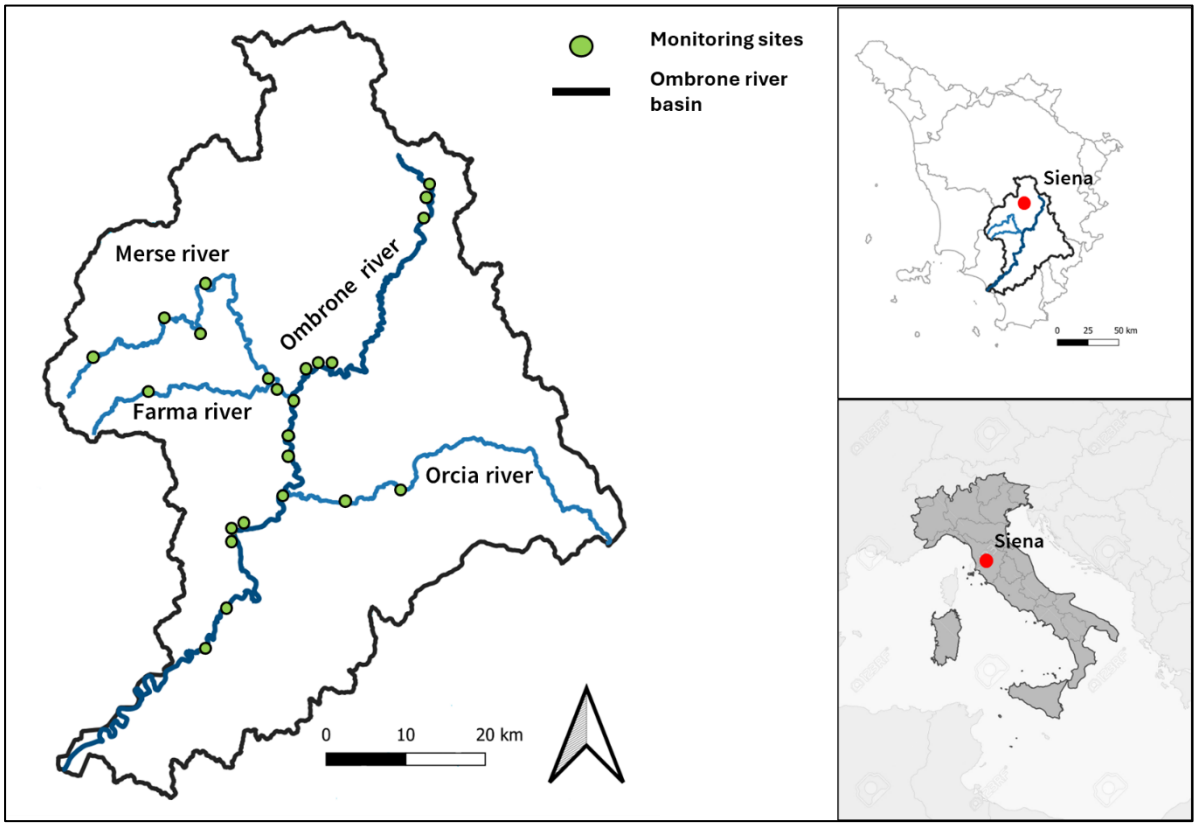


Figure 1. Study area and the monitoring sites

2.2 The RiVe protocol within the CS4Rivers project

The RiVe protocol was developed by the Citizen Science Observatory (osservatoriocitizenscience.org) and the National Biodiversity Network of the Italian Institute for Environmental Protection and Research (nnb.isprambiente.it/it). Within the CS4Rivers project, the protocol was adapted to address the project's objectives and the specific environmental characteristics of the Ombrone River basin: twelve target taxa were selected based on their occurrence within the river basin, and were

complemented by additional herbaceous species and lichen taxa. Below, a description is given of the RiVe protocol as implemented in this study. It is important to note that this is the first assessment of data quality collected by citizens with this protocol.

The RiVe protocol applied in this experiment is organised into two consecutive steps: (i) training, and (ii) data collection.

i) Training:

Before participating in field activities, all volunteers attended a standardised training session. The training, led by a CS4Rivers researcher, covered the ecological role of riparian vegetation, the application of the sampling protocol, and the use of supporting materials, including a monitoring handbook, a simplified dichotomous key, and field data sheets. A practical field exercise was included to ensure familiarity with the protocol and to promote consistency in data collection, as well as to reduce observer-related variability.

ii) Data collection:

During the data collection phase, volunteers/citizen scientists (CTZ) carried out monitoring activities independently. Sampling was conducted within predefined plots of 10 × 15 m, with the longer side parallel to the riverbank. Plot dimensions were measured in the field using a metric wheel. For each plot, data on site characteristics were collected, including georeferenced information and photographic documentation (Supplementary Materials Fig. 1S). CTZ recorded the occurrence of twelve selected woody taxa, six herbaceous, and four lichen taxa (Fig. 2). Target taxa were selected by CS4Rivers researchers based on their ecological relevance and considering their ease of recognition by non-professional operators, and were identified at genus or species level (Tab. 1).

Table 1. Taxa target per layer involved in this study

Taxa target	**Layer**
Ailanthus altissima	Canopy layer (height > 3 m)
Alnus glutinosa	
Carpinus betulus	
Fraxinus sp.	

Populus nigra *Populus x canescens* *Quercus* sp. *Robinia pseudacacia* *Salix alba* *Tamarix* sp. *Ulmus minor*	
Alnus glutinosa *Amorpha fruticosa* *Artemisia verlotiorum* *Artemisia vulgaris* *Carpinus betulus* *Fraxinus* sp. *Helianthus tuberosus* *Populus nigra* *Populus x canescens* *Quercus* sp. *Reynoutria japonica* *Robinia pseudacacia* *Salix alba* *Tamarix* sp. *Ulmus minor*	Understory layer (height between ground and 3 m)

Riparian woody environments, as all forest ecosystems, are usually divided into layers (i.e. canopy, shrub, understory) according to the main height of vegetation. Consequently, plant cover was measured at the canopy and the understory layer (height > 3 m, and between ground and 3 m, respectively). Plant cover was estimated using five abundance or percentage cover classes derived from the Braun-Blanquet (van der Maarel, 1979) scale: 1 (1-5%), 2 (6-25%), 3 (26-50%), 4 (51-75%), and 5 (76-100%). Taxonomic nomenclature used in this work followed the nomenclature of the Portal to the Flora of Italy (2021).

At the end of the sampling activity, CTZs submitted photographic records of the target taxa to the CS4Rivers research team for preliminary data verification. CTZ also sent by email all the other data collected in the field for validation. These steps also provide post-sampling feedback to volunteers, supporting learning and improving the quality and consistency of subsequent data collection. Here we consider validation as the process through which records are checked to ensure the data have been submitted correctly, and verification as the process of checking records for correctness, confirming taxonomic identity (Tweddle et al., 2012).



Figure 2. Citizen Scientists during the sampling phase. Ph. Martino Danielli

2.3 Experimental Design

To assess the data quality of observations collected by CTZ using the RiVe protocol, we compared CTZ-generated data with a reference dataset. Reference data were collected by a group of botanists of the University of Siena, Italy (EXP). Both CTZ and EXP used the RiVe protocol at the same monitoring sites. Specifically, mean

percentage cover estimates (per taxon per layer) and biodiversity indices were compared between CTZs and EXPs to evaluate the quality and reliability of CTZ data. EXP surveys were conducted in parallel with the CTZ's sampling. Monitoring sites were selected during a co-design session involving the CS4Rivers researcher and volunteers, based on accessibility and safety criteria, and on the selection of sites with similar riparian vegetation characteristics in terms of species composition and abundance. A total of 12 volunteers, organised into groups of at least two people, surveyed 15 monitoring sites (at least two sites per group) during the Spring 2023 and 2024. In a separate trial, based on the initial data quality assessment, the group showing the lowest agreement with EXP data received additional training and surveyed nine monitoring sites during Spring 2025 (Fig. 1). Data collected during the 2025 campaign were analysed separately to evaluate the effect of additional training and practice on data quality.

2.4 Biodiversity indices

For each plot, the Shannon (H') and the Pielou (J') indices, as measures of biodiversity and evenness, respectively, were calculated for the canopy and understory layer. In this study, both indices were computed exclusively on the target taxa and were used to assess the quality and reliability of the CTZ-generated data.

The Shannon biodiversity index was calculated as:

$$H' = -\sum_{j=1}^{S} (p_j \times ln\ ln\ (p_j))$$

where S is the total number of taxa in the plot, and p_j is the relative abundance of taxon j. In this study, taxa's relative abundance was estimated using percentage cover, as in many vegetation ecology studies (Dembicz et al., 2025). Therefore, p_j was calculated as the ratio between the cover of taxon j (c_j) and the cumulative cover of all taxa within the plot (C):

$$p_j = \frac{c_j}{C}$$

The Pielou evenness index was calculated as:

$$J' = \frac{H'}{ln(S)}$$

2.5 Statistical analysis

Since the data did not approach a normal distribution for most variables (Shapiro-Wilk test, $p>0.05$), non-parametric tests were run. The Wilcoxon test and Spearman correlation were used to check for significant differences ($p<0.05$) and significant correlations ($p<0.05$) between the data collected by CTZ and EXP, respectively. The analysis was performed at the taxon level and overall. At the taxon level, analyses were limited to taxa with at least three non-zero paired observations per observer.

Taxa percentage coverage records were used as response variables in generalised linear mixed models (GLMMs) to test the effect of observer (CTZ vs EXP) on taxon coverage. GLMMs were fitted using a Tweedie family, which accounts for many values = 0, with a log link function. The model included observer as fixed effect and taxa and site as random effects. The comparison was performed using the likelihood-ratio test to assess the observer's contribution.

To evaluate the performance of CTZ groups, the mean absolute error (AE) between CTZ and EXP estimates of taxon percentage cover was calculated for each plot. To assess overall CTZ group performance at the plot level, absolute errors were aggregated across all monitored taxa within each plot and standardised by the total number of taxa considered:

$$AE_i = \frac{1}{n} \sum_{j=1}^{n} \left| \frac{cs_{i,j} - ex_{i,j}}{100} \right|$$

where n is the number of target taxa detected by EXP in the plot; cs and ex are the percentage cover estimated by CTZ and by the EXP, respectively, for taxon *j* in plot *i*. This procedure provides a normalised absolute error per plot, ranging between 0 (perfect agreement) and 1 (maximum disagreement).

The overall total accuracy of CTZ data were then calculated as a percentage agreement, which is the comparison most commonly used (Aceves-Bueno et al., 2017):

$$Accuracy = (1 - AE_{mean}) \times 100$$

where AE_{mean} is the mean plot-level normalised absolute error across all plots.

To investigate the relationship between the number of target taxa detected per plot and the resulting accuracy, a Spearman's rank correlation analysis was performed.

All calculations were run using the R software (R Core Team, 2025).

3. Results

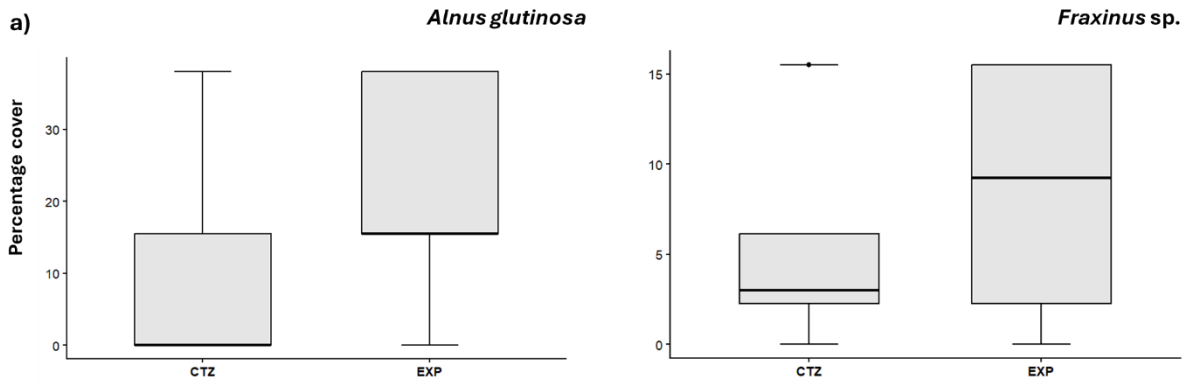
The raw data of percentage coverage per taxon per plot are reported in the Supplementary Materials (Tab. 1S)

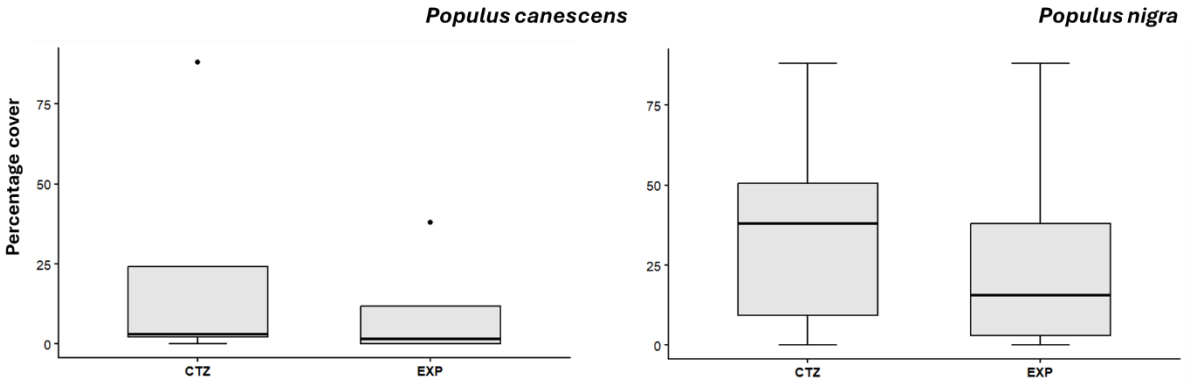
To evaluate the quality of the data collected by CTZ, we compared observations across different analytical levels of the RiVe protocol. Specifically, results are organised into: (i) comparison of percentage cover of target taxa; (ii) comparison of biodiversity indices; and (iii) an evaluation of CTZ groups' performance, including the assessment of additional training and practice on the group showing the preliminary lowest agreement with EXP data.

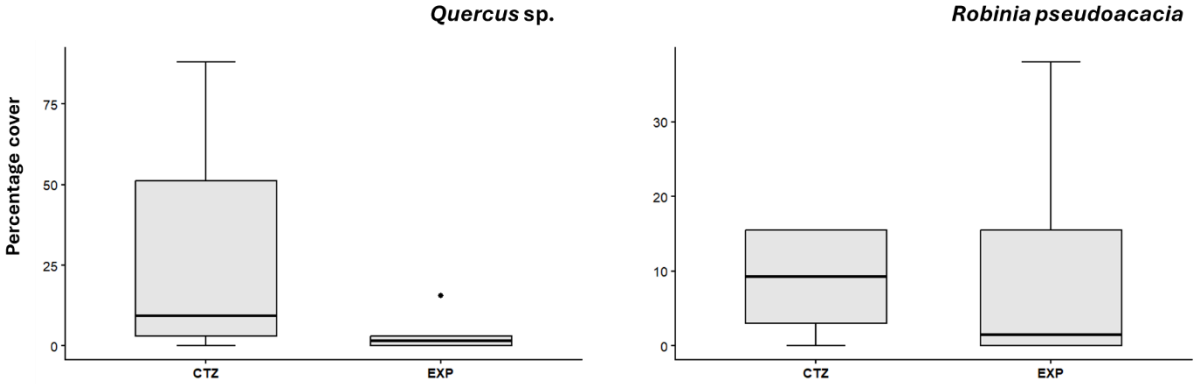
3.1 Percentage cover

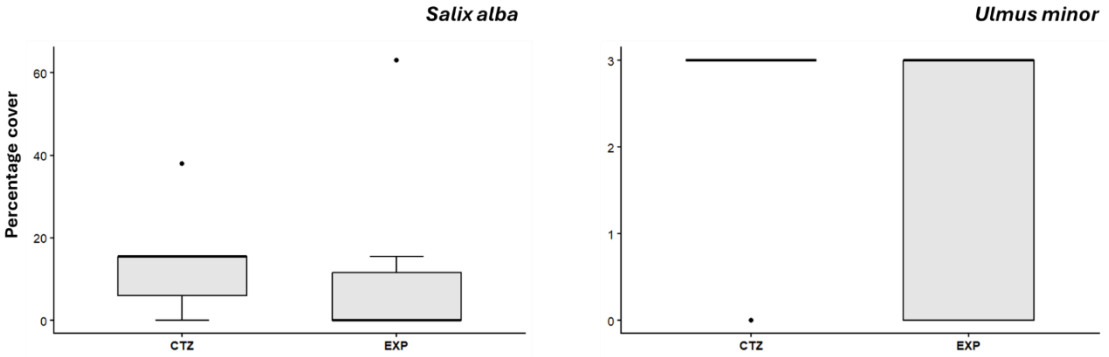
Based on the verification of the taxon photographs submitted by CTZ, it can be concluded that CTZ did not appear to encounter difficulties in the identification of the target taxa.

No significant difference was found between CTZ and EXP percentage cover estimates per taxon per layer ($p > 0.05$), except for *Robinia pseudacacia* layer understory ($p < 0.05$) (Fig. 3).

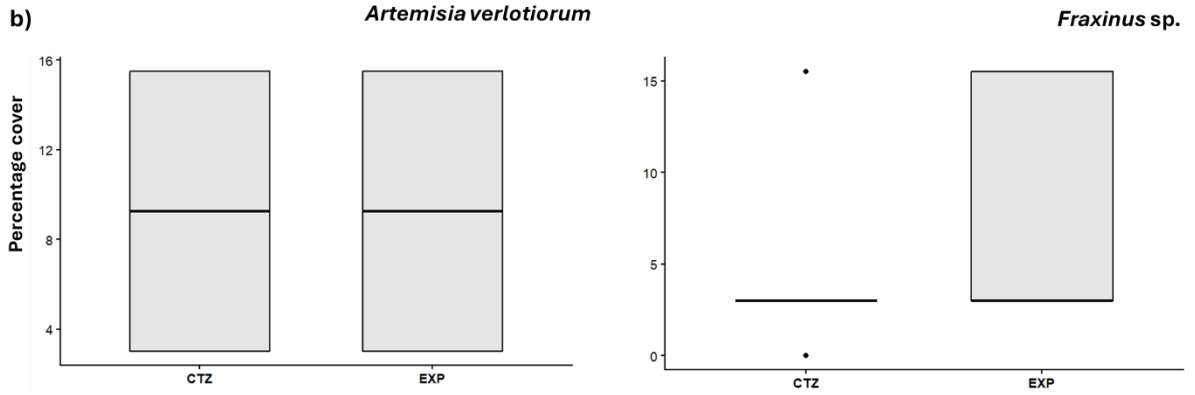



Populus canescens
Populus nigra
Percentage cover
75
50
25
0
CTZ
EXP


Quercus sp.
Robinia pseudoacacia
Percentage cover
75
50
25
0
30
20
10
CTZ
EXP


Salix alba
Ulmus minor
Percentage cover
60
40
20
3
2
1
CTZ
EXP

b)


Artemisia verlotiorum
Fraxinus sp.
Percentage cover
16
12
8
4
15
10
5
CTZ
EXP

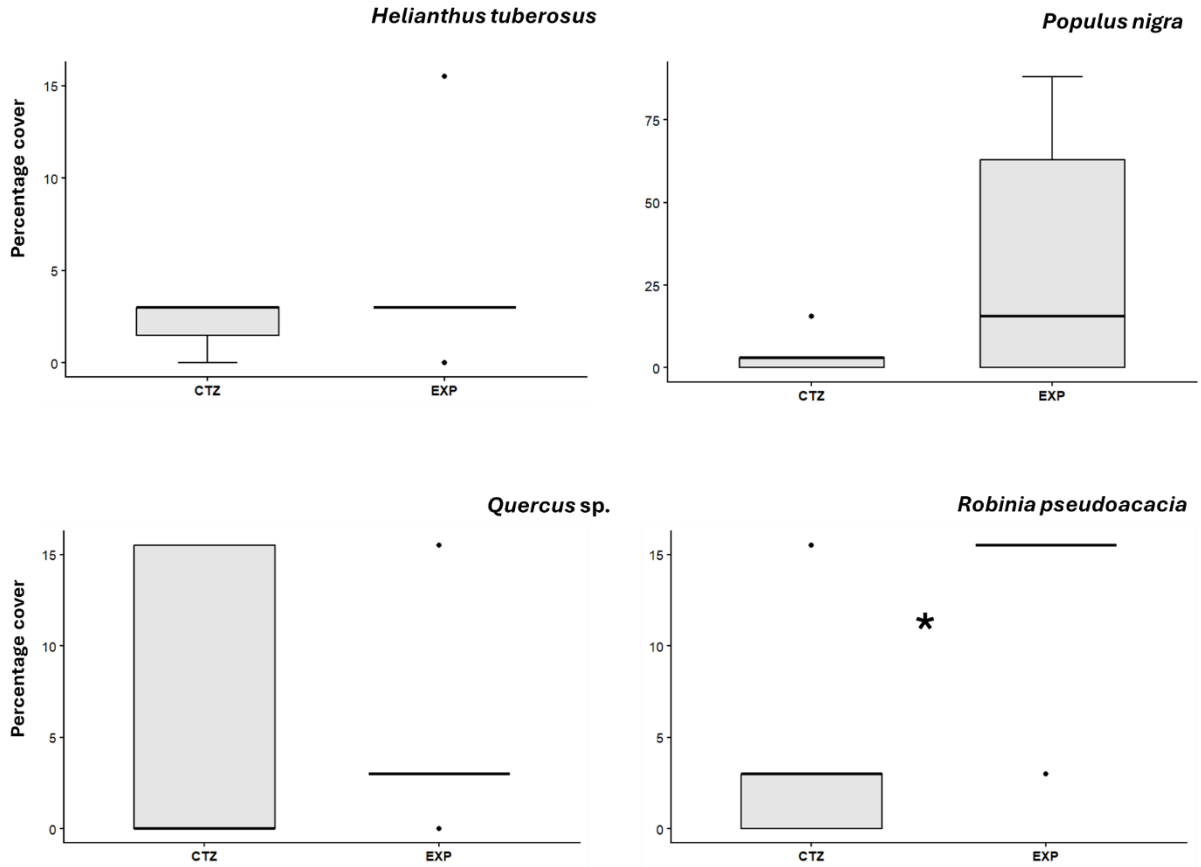


Figure 3. Boxplots showing percentage coverage comparison per taxon per layer: a) canopy layer; b) understory layer

No significant difference was found between CTZ and EXP overall (N=436) percentage cover estimates ($p>0.05$). In addition, a significant positive correlation was observed between CTZ and EXP measurements ($r = 0.45$, $p <0.001$).

GLMMs indicated no evidence of bias in CTZ estimates of taxon coverage ($\chi^2 = 0.042$, $df = 1$, $p > 0.05$). (Fig. 4).

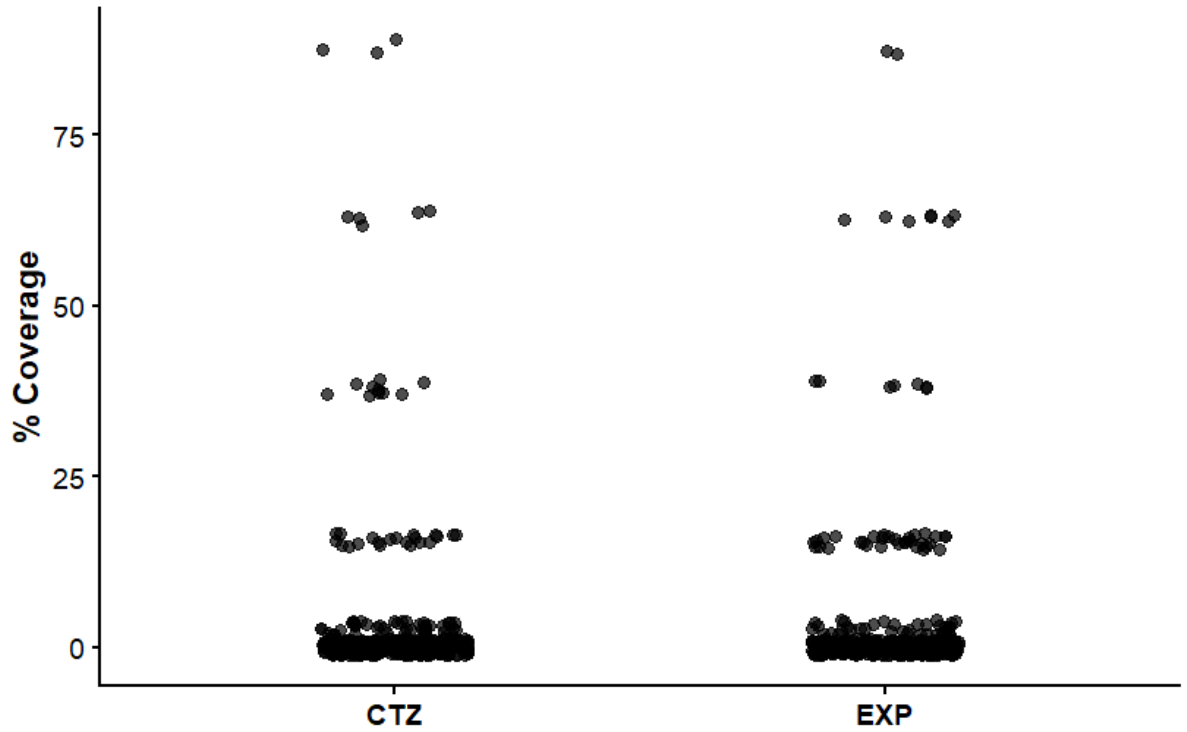
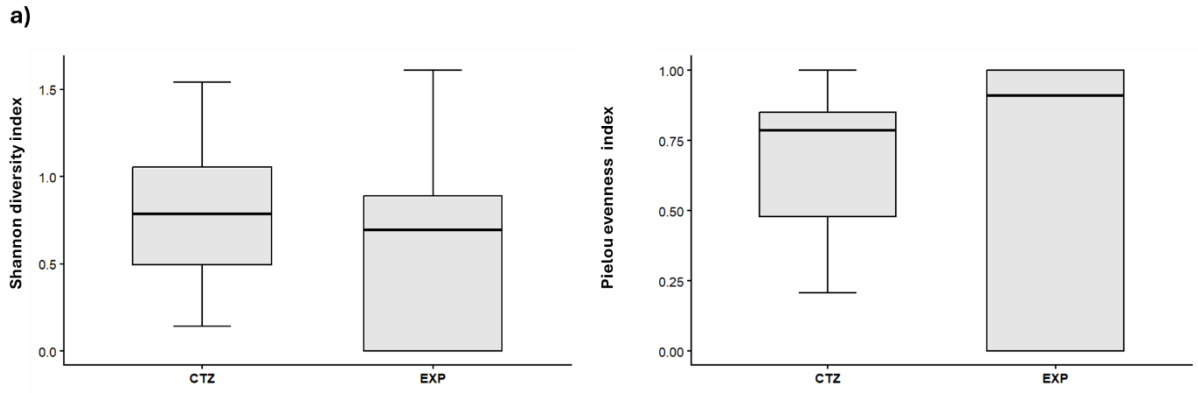


Figure 4. Distribution of percentage cover (% Coverage) for the two observer types (CTZ and EXP). Each point represents an individual observation.

3.2 Biodiversity Indices

The Shannon and Pielou indices per layer were not different between CTZ and EXP ($p>0.05$), but, unlike percentage cover, the Spearman correlation coefficients between CTZ and EXP were not statistically significant for each layer (canopy layer: $r=0.51$, $p>0.05$; $r=0.45$, $p>0.05$ respectively. Understory layer: $r=-0.07$, $p>0.05$; $r=-0.33$, $p>0.05$ respectively) (Fig. 5).



b)

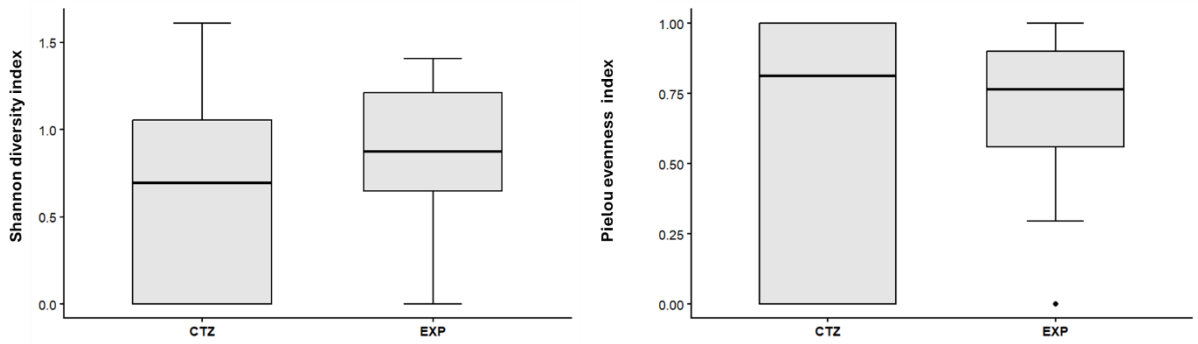


Figure 5. Shannon and Pielou diversity index between CTZ and EXP data for a) the canopy and b) the understory layer.

3.3 Citizen Scientists group performance and the effect of additional training

Since the Spearman correlation between the number of target taxa detected by EXP and the accuracy was not significant ($r=0.23$, $p>0.05$), the CTZ group accuracy was calculated, which was always high (Tab. 2).

Table 2. Accuracy (%) for each CTZ group

G1	G2	G3	G4	G5	G6
70 ± 8	82 ± 11	70 ± 25	80 ± 18	80 ± 8	72 ± 18

Group 1 exhibited the highest deviation from EXP observations. Based on this initial assessment, group 1 received additional targeted training during winter 2025 and subsequently conducted further field surveys in spring 2025.

After the additional training and field practice, the new data collected by group 1 in spring 2025 showed that the percentage cover estimates had no significant difference with EXP ($p>0.05$), and also a significant positive correlation emerged (Spearman's rank correlation, $r = 0.76$, $p<0.001$).

Similar results were obtained for the biodiversity indices regarding canopy layer, but not understory layer (Shannon: canopy layer: $r=0.75$, $p<0.05$; understory layer: $r = 0.06$, $p>0.05$; Pielou: canopy layer: $r=0.78$, $p<0.05$; understory layer: $r = 0.15$, $p>0.05$), and the aggregated absolute error decreased to 0.04 ± 0.04 (accuracy increased from 70% to 94%).

4. Discussion

4.1 Percentage cover

This study provides empirical evidence that CTZ can reliably assess riparian vegetation composition, diversity and structure when supported by a protocol specifically designed for this purpose.

According to the photographs' verification, it can be concluded that CTZ did not appear to encounter difficulties in the identification of the target taxa. This outcome highlights the effectiveness of the protocol design: the target taxa were carefully selected to ensure they were sufficiently distinctive and recognisable in the field. The absence of identification errors suggests that this selection strategy was successful, as CTZ consistently identified the focal species accurately. Thus, the choice of target taxa proved appropriate and robust for reliable implementation within the protocol.

The near absence of estimated differences in percentage cover between CTZ and EXP for each taxon and layer suggests that CTZ reliably captured overall vegetation cover across strata and did not exhibit limitations in the identification of target taxa. This result is particularly relevant for vegetation sampling, where structural heterogeneity and complexity often pose challenges to accurate survey assessments (Palmquist et al., 2019).

Across taxa and plots, estimates of percentage cover collected by CTZ showed no significant differences from EXP assessments and were positively correlated with EXP data. Together, these results indicate the absence of systematic bias in CTZ-based sampling, support the overall accuracy and replicability of the RiVe protocol at the sampling level, and sustain the applicability of the RiVe protocol for this type of monitoring. However, the moderate strength of correlations observed for percentage cover estimates highlights that agreement between CTZ and EXP is not as high as it could be. Nevertheless, rather than indicating poor data quality, this pattern likely reflects the well-known inherent subjectivity of visual cover estimation (Müller et al., 2024; Bergstedt et al., 2009; Hahn & Scheuring, 2003). Several authors have noted that ocular estimates can be particularly challenging in the presence of diverse life forms and complex canopy boundaries (Sykes et al., 1983; Hatton et al., 1986; Floyd and Anderson, 1987). Moreover, ocular approaches require observers to mentally integrate the foliar cover of individual plants, a cognitive process that is inherently more subjective than point-based methods (Godínez-Alvarez et al., 2009).

Similarly, the results from the GLMMs indicated that observer identity (CTZ vs EXP) did not have a significant effect on the estimation of taxon percentage cover, suggesting the absence of systematic differences between CTZ and EXP estimates. This finding provides strong support for the robustness of the RiVe protocol, indicating that variation in cover estimates is primarily driven by ecological heterogeneity rather than by observer-related bias. It suggests that the protocol's design, based on predefined target taxa, stratification, and standardised percentage coverage, effectively minimises subjectivity and supports consistent application across observers with different levels of expertise.

4.2 Biodiversity indices

Comparison of biodiversity indices partially corroborates these findings and highlights the importance of continuous training and practice (see the paragraph below). Shannon and Pielou indices calculated per layer showed no significant differences between CTZ and EXP data, but Spearman correlations were not significant, suggesting that CTZ can only partially capture community diversity and evenness. These patterns suggest that, while overall community structure is reasonably well represented by CTZ data, limitations remain for the indices output, and CTZ does not appear to introduce systematic bias but may occasionally under/overestimate values.

These findings do not undermine the utility of CTZ-generated data; rather, they highlight the need for careful attention during data analysis and the use of robust analytical protocols and indices that account for potential biases, rather than relying solely on classical metrics (Gacutan et al., 2022).

Importantly, it should be noted that these results refer to the initial sampling campaigns, conducted prior to the implementation of additional targeted training and practice.

4.3 Citizen Scientists group performance and the effect of additional training

Training and experience proved to be key factors in improving data quality. The group with the lowest initial accuracy (G1) further increased its following additional practice and targeted training, achieving no significant differences and, importantly, significant correlations for biodiversity indices (canopy layer), which were not observed in the initial sampling campaigns. These findings confirm that structured guidance and iterative feedback enhance the reliability of CTZ-generated data and underscore the importance of continuous fieldwork activities, even if the understory layer appears to be the more difficult to assess.

The observed improvement in total data accuracy further illustrates the role of training and experience in reducing observer-related uncertainty in CS monitoring. For the sampling campaigns 2023 - 2024 the total accuracy was already high (accuracy = 0.76, 76%), and it increases after the additional training (accuracy = 0.96, 96%), indicating a strong agreement between CTZ and EXP estimates. Even if the volunteers' accuracy varies with task difficulty (Kosmala et al., 2016), comparable levels of agreement have been reported in CS studies: Aceves-Bueno et al. (2017) reviewed studies that compared CTZ-collected with EXP-collected datasets and found that over half of these studies had an agreement of 80%. These findings also align with Kosmala et al. (2016), whose assessment of data quality in CS stated that 70-95% accuracy is typical for species identification tasks within CS activities.

These results suggest that observed improvements reflect both volunteer performance and the RiVe protocol suitability. In fact, the improvement in accuracy is demonstrated also in other CS studies in which structured project - e.g., prescribed sampling in space and time by mostly trained and experienced volunteers - (Callaghan et al., 2021; Perry et al., 2021) were necessary to reduce the bias and improve CTZ data quality, reinforcing the importance of careful standardised methodological design.

5. Conclusions

This study aimed to test the reliability of the RiVe method, specifically designed for monitoring riparian vegetation, when applied in a CS framework, with emphasis on the assessment of data quality and reliability of CTZ-generated data using the RiVe protocol.

The results suggest that CTZ can reliably assess riparian vegetation structure when supported by a well-designed project framework, a protocol specifically tailored to the topic, and a carefully structured experimental design. The RiVe protocol effectively minimises observer-related bias at the sampling level, enabling consistent estimation of vegetation cover across taxa and sites.

In our specific case study, the level of agreement observed between CTZ and EXP assessments suggests the possibility of defining an indicative quality threshold for percentage cover estimates of the target taxa. Notably, all CTZ achieved levels of accuracy ≥ 90% relative to EXP evaluations. On this basis, a ≥ 90% accuracy threshold may be considered a plausible benchmark of good data quality within the context of

this study. Although this value was not formally applied as an a priori exclusion criterion, it emerges from our findings as a tentative reference point for data accuracy. Further field-based investigations are necessary to assess its robustness and broader applicability.

While limitations were observed in the biodiversity indices during the initial sampling campaigns, targeted training and continued field practice substantially improved data accuracy and consistency. These results highlight the importance of adaptive training frameworks and standardised protocol in enhancing the quality of citizen-generated data.

From this study, it can be concluded that RiVe could bea suitable method for participatory monitoring of riparian vegetation, particularly for applications focused on structural patterns. When combined with iterative training and validation/verification processes, such approaches could complement expert-led monitoring and contribute to long-term assessment of riparian ecosystems.

6. Acknowledgements

I am grateful to thank Prof. Loppi for his scientific guidance in developing the experimental design and for his feedback on this chapter.

The author also wishes to express gratitude to Dr De Simone, Dr Mascia, and Dr Picchiotti for their assistance during the field activities and for sharing their expertise as "experts" throughout this study.

Thanks are extended to Prof. Gumiero and the National Biodiversity Network (NNB - ISPRA) for developing the original RiVe methodology, which provided the foundation for the work presented in this chapter.

Supplementary Materials

citizen science FOR RIVERS

NATIONAL BIODIVERSITY FUTURE CENTER

UNIVERSITÀ DI SIENA 1240

Osservatorio Citizen Science

Scheda monitoraggio vegetazione riparia

Scegli un tratto di fiume lungo min 500 m, max 1 km e fai il monitoraggio in max 10 plot (aree di campionamento) nella sponda sinistra e max 10 in quella destra, ma solo dove è presente vegetazione arborea o arbustiva.
10 è un numero di plot indicativo dipende dalle dimensioni del bosco ripario e dall'eterogeneità del bosco.

Nome osservatore _____

E-mail _____

Data monitoraggio _____ Condizioni meteo _____

Nome della località _____ Nome del fiume _____

Latitudine vertice nord _____ Longitudine del vertice nord _____

Scatta almeno 2 foto dell'area di campionamento (plot) (una esterna e una ravvicinata) da allegare ai dati.

1 Tratto di fiume in

- ◯ pianura (0-150 metri)
- ◯ collina (150-600 metri)
- ◯ montagna (sopra i 600m)

2 La sezione del fiume è:

- ◯ non visibile
- ◯ molto artificiale: forte presenza di strutture artificiali(es: gabbionate, grossi massi, strutture in cemento ecc.)
- ◯ molto modificata: entrambe le rive sono verticali e alte.
- ◯ parzialmente artificiale (es. massi solo al piede della sponda)
- ◯ senza evidenze di artificializzazione

3 Ci sono evidenze di connessione tra il fiume ed il bosco ripario?

- ◯ non so
- ◯ no
- ◯ sì (es. detriti e sedimenti rilasciati dopo una piena) specificare quali _____

Informazioni che caratterizzano il bosco ripario nel transetto di campionamento

4 Indica la superficie dell'area osservata (consigliato 15x10 = 150 m2): m^2 _____

5 Su quale sponda del fiume si trova (metti le spalle alla sorgente)

- ◯ sinistra
- ◯ destra

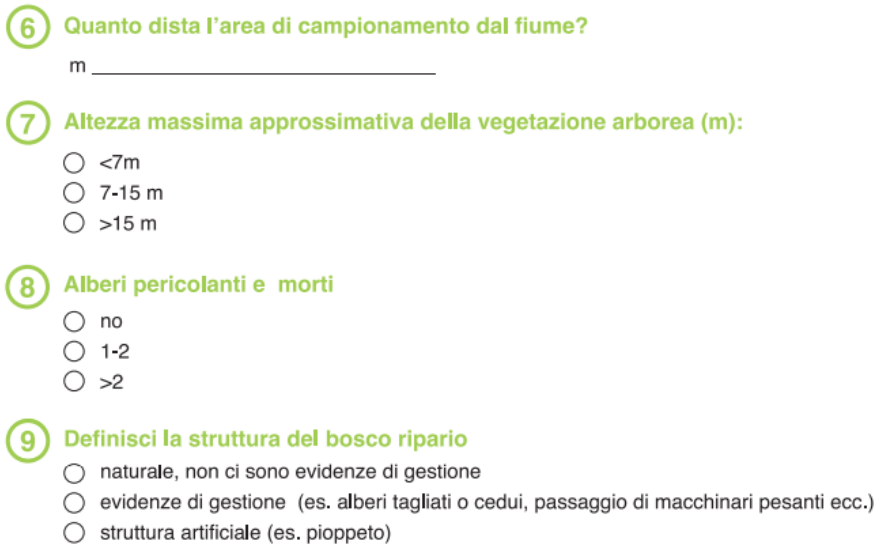


Figure 1S. Standardised field data sheet

Table 1S. percentage coverage per taxa per site monitoring by CTZ and EXP; H = herbaceous, S = shrub, W = woody; mer = Merse and Farma river; omb = Ombrone river; orc = Orcia river

Taxa target	mer0		mer1		mer2		mer3		mer4		mer6	
	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP
Aegopodium podagraria H					3	15.5						
Ailanthus altissima S												
Ailanthus altissima W												
Alnus glutinosa S									3			
Alnus glutinosa W		38		38				15.5	15.5	15.5		
Amorpha fruticosa S												
Artemisia verlotiorum H											3	15.5
Artemisia vulgaris H												15.5
Carpinus betulus S	38											
Carpinus betulus W	63		38						3			
Fraxinus sp. S		15.5	3	15.5			15.5	15.5	3	3		
Fraxinus sp. W	15.5	15.5						15.5	3			
Helianthus tuberosus H									3	3	3	

Populus nigra S	15.5								3			
Populus nigra W	15.5	38	38	38		15.50	38	15.5	15.5	38	38	63
Populus x canescens S												
Populus x canescens W												
Quercus sp. S				15.5			15.5	3		3		3
Quercus sp. W			3				88	15.5	3			
Reynoutria japonica H												
Robinia pseudacacia S								3	3	15.5	3	15.5
Robinia pseudacacia W			15.5	38	3	15.5		15.5	15.5	3	3	
Salix alba S												
Salix alba W							38					
Santolina etrusca H												
Tamarix sp. S												
Tamarix sp. W												
Ulmus minor S												
Ulmus minor W					3							

Taxa target	mer7		mer8		omb0		omb3		omb4		omb5	
	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP
Aegopodium podagraria H												
Ailanthus altissima S				3								
Ailanthus altissima W	3		3									
Alnus glutinosa S												
Alnus glutinosa W												
Amorpha fruticosa S												
Artemisia verlotiorum H	15.5	15.5	3	3				3				
Artemisia vulgaris H												15.5
Carpinus betulus S												
Carpinus betulus W												
Fraxinus sp. S	3	3	3	3					3	3		
Fraxinus sp. W									3	3		
Helianthus tuberosus H	3	15.5					3	3				3
Populus nigra S					3	63	3	3		3	3	
Populus nigra W	63	63	63	88	63	3	3	3		15.5	88	15.5

Populus x canescens S							15.5	63				15.5
Populus x canescens W												38
Quercus sp. S					15.5		3					
Quercus sp. W					15.5	3						
Reynoutria japonica H												
Robinia pseudacacia S		15.5	3	15.5	15.5	15.5						
Robinia pseudacacia W	3				15.5							
Salix alba S							3	3		3		15.5
Salix alba W			15.5						3	63		15.5
Santolina etrusca H												
Tamarix sp. S												
Tamarix sp. W											3	
Ulmus minor S						15.5				3		3
Ulmus minor W									3	3	3	

Taxa target	omb6		omb11		omb12		omb13		omb14		omb16	
	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP
Aegopodium podagraria H												
Ailanthus altissima S												
Ailanthus altissima W												
Alnus glutinosa S			3	3	3			3				
Alnus glutinosa W							38					
Amorpha fruticosa S												
Artemisia verlotiorum H		3										
Artemisia vulgaris H			3	3		3	3	3				
Carpinus betulus S	3	3										
Carpinus betulus W												
Fraxinus sp. S												
Fraxinus sp. W												
Helianthus tuberosus H	3	3	3	3	3	3	3	3				
Populus nigra S			88	88	15.5	63		88		63		
Populus nigra W					38		38		3	3		

Populus x canescens S	63	63			15.5						88	88
Populus x canescens W					3	3			3			
Quercus sp. S												
Quercus sp. W							63			3		
Reynoutria japonica H												
Robinia pseudacacia S										15.5		
Robinia pseudacacia W							15.5					
Salix alba S					38							
Salix alba W					15.5				15.5			
Santolina etrusca H												
Tamarix sp. S												
Tamarix sp. W												
Ulmus minor S										3		
Ulmus minor W				3		3	3	3				

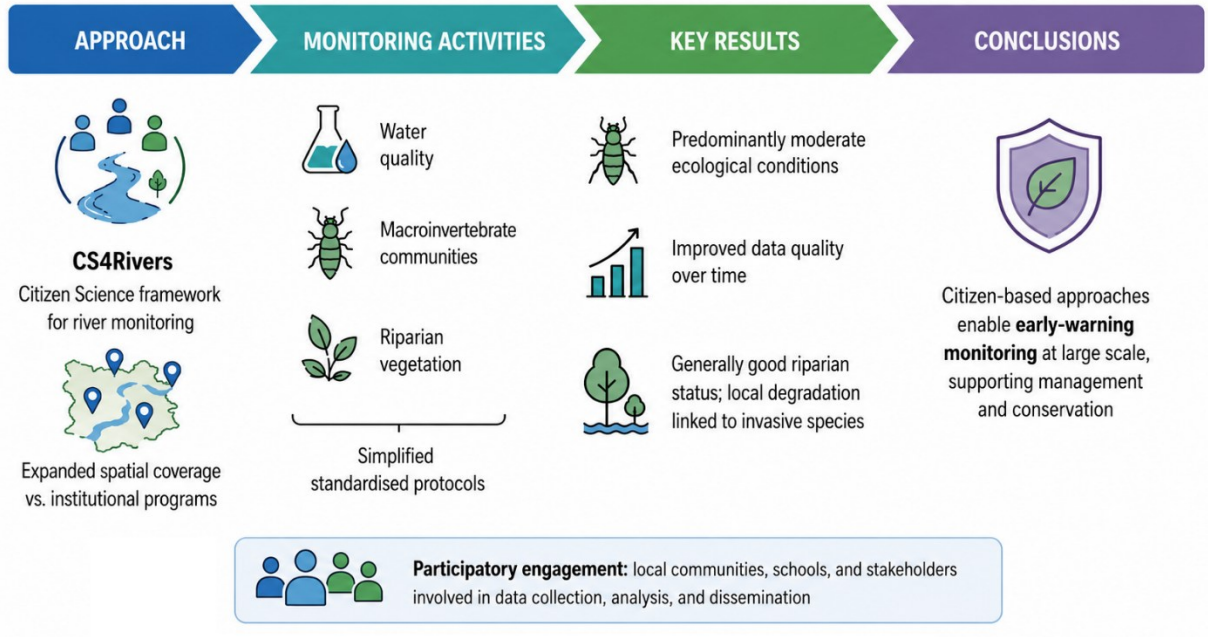
Taxa target	omb17		omb71		omb83		omb94		orc1		orc2	
	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP	CTZ	EXP
Aegopodium podagraria H												
Ailanthus altissima S												
Ailanthus altissima W												
Alnus glutinosa S					3	3					3	3
Alnus glutinosa W						3						
Amorpha fruticosa S												
Artemisia verlotiorum H	15.5	3										
Artemisia vulgaris H								3	3	3		3
Carpinus betulus S												
Carpinus betulus W												
Fraxinus sp. S												
Fraxinus sp. W												
Helianthus tuberosus H		3			3	3	3	3	3	3		
Populus nigra S		15.5	63	63	15.5	3	63	63	3		63	63
Populus nigra W	3	3		3		3						3

Populus x canescens S		63			3	3			3				
Populus x canescens W	88					3	3	3					
Quercus sp. S													
Quercus sp. W			3	3									
Reynoutria japonica H													
Robinia pseudacacia S			15.5	3									
Robinia pseudacacia W													
Salix alba S		3			15.5	3			88	88	3		
Salix alba W										15.5		3	
Santolina etrusca H													
Tamarix sp. S													
Tamarix sp. W													
Ulmus minor S			3	15.5									
Ulmus minor W						3		3					

Chapter 4.

CS4Rivers: a Citizen Science framework for integrated monitoring of riverine ecosystems

Graphical abstract



Abstract

The CS4Rivers project, led by the University of Siena within the National Biodiversity Future Centre (NBFC), has developed a Citizen Science (CS) framework for integrated monitoring of riverine ecosystems and tested it in the Ombrone River basin (Tuscany, Italy). The approach has combined scientific research with participatory engagement, involving local communities, schools, and various stakeholders in data collection, analysis, and dissemination. Monitoring activities focused on water chemical quality, macroinvertebrate communities, and riparian vegetation using simplified but standardised protocols. Results indicated predominantly moderate ecological conditions for macroinvertebrates, with improvements in data quality over time due to iterative training and field practice. Riparian vegetation assessments revealed generally good ecological status, though localised degradation was associated with invasive species, particularly *Robinia pseudoacacia*. The framework enabled an expansion of spatial monitoring coverage compared to institutional programs. Overall, the CS4Rivers project findings suggest that citizen-based approaches can provide

useful tools for early-warning monitoring in large-scale environmental assessment, supporting official management and conservation programs.

1. Introduction

The CS4Rivers project, launched by the University of Siena in autumn 2022 within the framework of the National Biodiversity Future Centre (NBFC), funded by the Italian National Recovery and Resilience Plan (PNRR), aimed to establish a participatory observatory of habitat quality in the Ombrone River basin (South Tuscany, Italy). As part of Spoke 3 of the NBFC, dedicated to terrestrial and freshwater ecosystems, the project adopted a transdisciplinary approach integrating scientific research and Citizen Science (CS). It involves citizens, schoolchildren, and local stakeholders in training, monitoring, data collection, analysis and dissemination activities, contributing to the co-design of conservation strategies.

The project has been structured into a series of steps ranging from the definition of the research question and methodology to data collection and analysis, and finally to the dissemination of results (Fig. 1). The first four phases of the project took place between winter 2023 and spring 2025; the sharing and dissemination results phases began in winter 2025 and are still ongoing (Capineri et al., 2025).

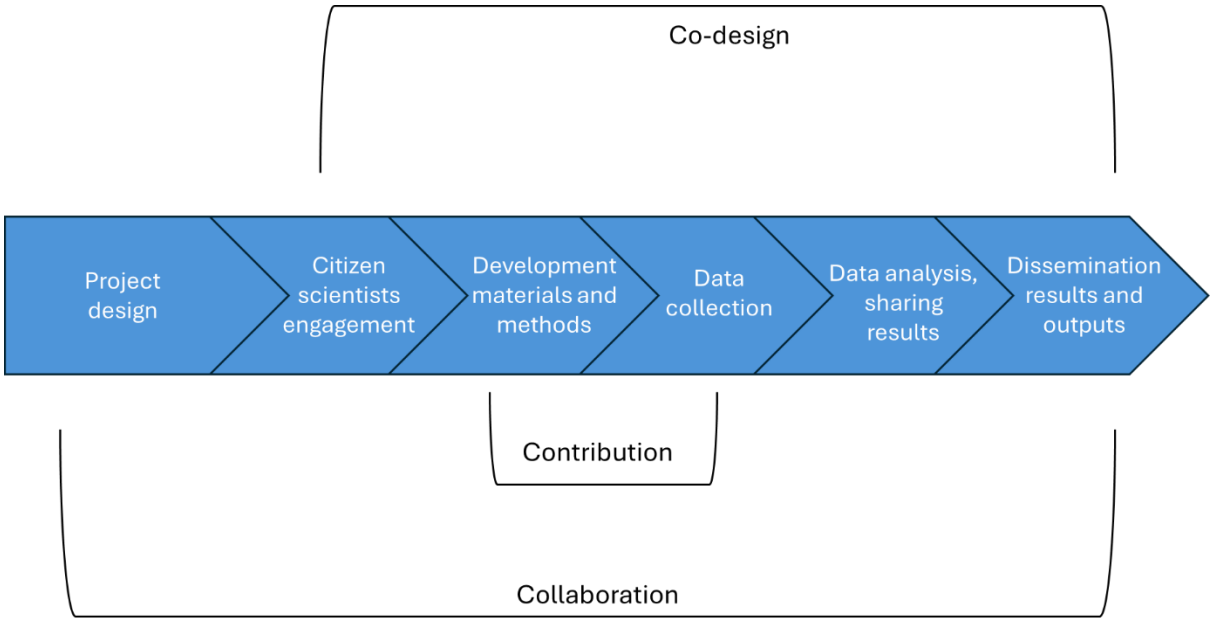


Figure 1. CS4Rivers project workflow (after Capineri et al. 2025).

This chapter presents the main components of the CS4Rivers framework, with particular emphasis on aspects not addressed in the previous chapters devoted to data

quality experiments. In addition, it provides an overview and discussion of the results of the monitoring activities conducted by citizens, focusing specifically on riparian vegetation and the macroinvertebrate community.

2. Materials and Methods

2.1 Study Areas

The project's study area is the Ombrone River basin and its main tributaries. The area was selected by the research team at the University of Siena for both its previous research experience and its proximity to the academic headquarters (Fig. 2).

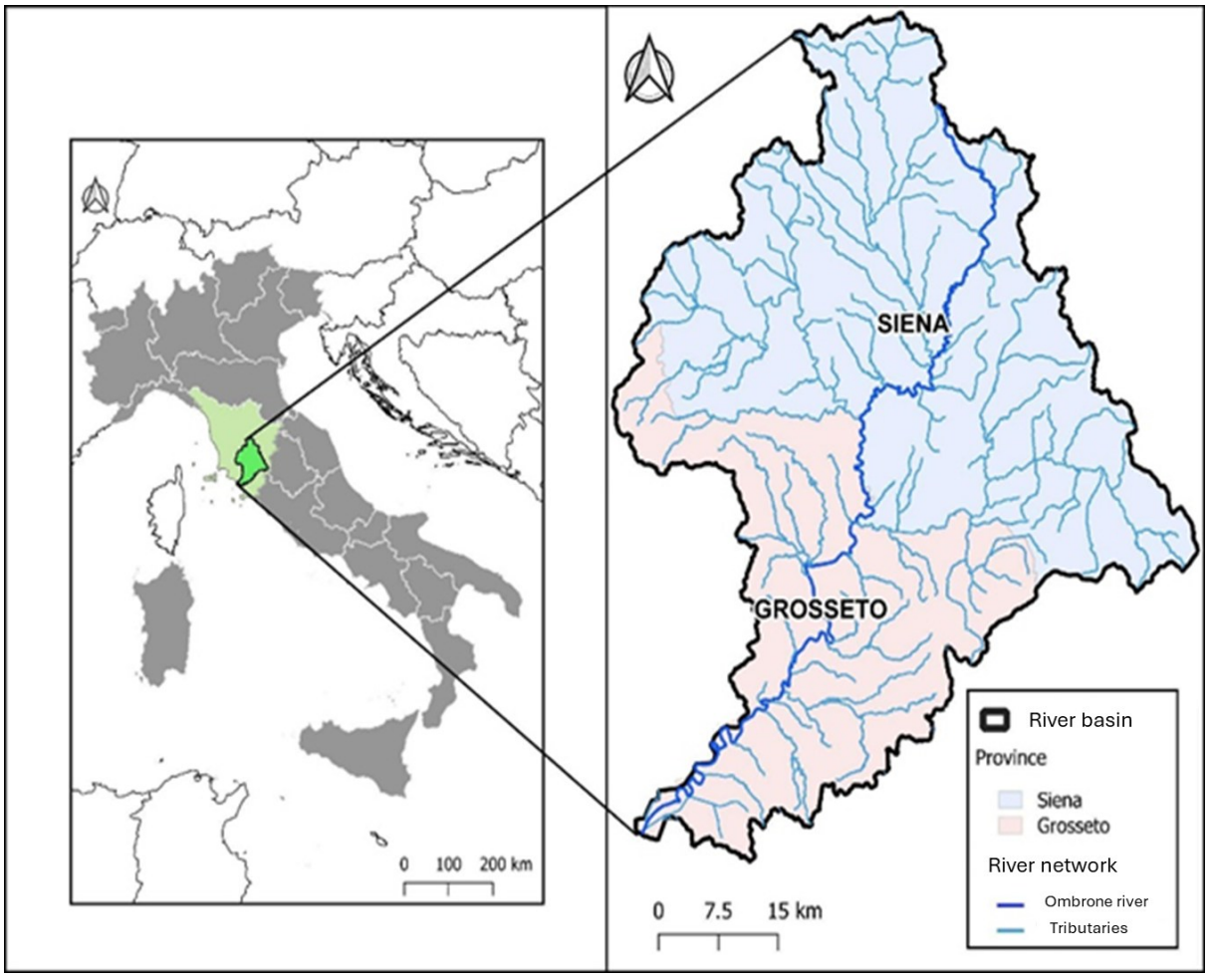


Figure 2. Study Area

Monitoring sites were identified during a co-design session with citizens, taking into account local knowledge, safety, accessibility, and specific interest. A total of 107 monitoring sites were surveyed. It is noteworthy that, within the same study area, the regional environmental authority (Agenzia Regionale per la Protezione Ambientale

della Toscana - ARPAT) is presently monitoring only 32 sites (Sanna et al., 2024) (Fig. 3).

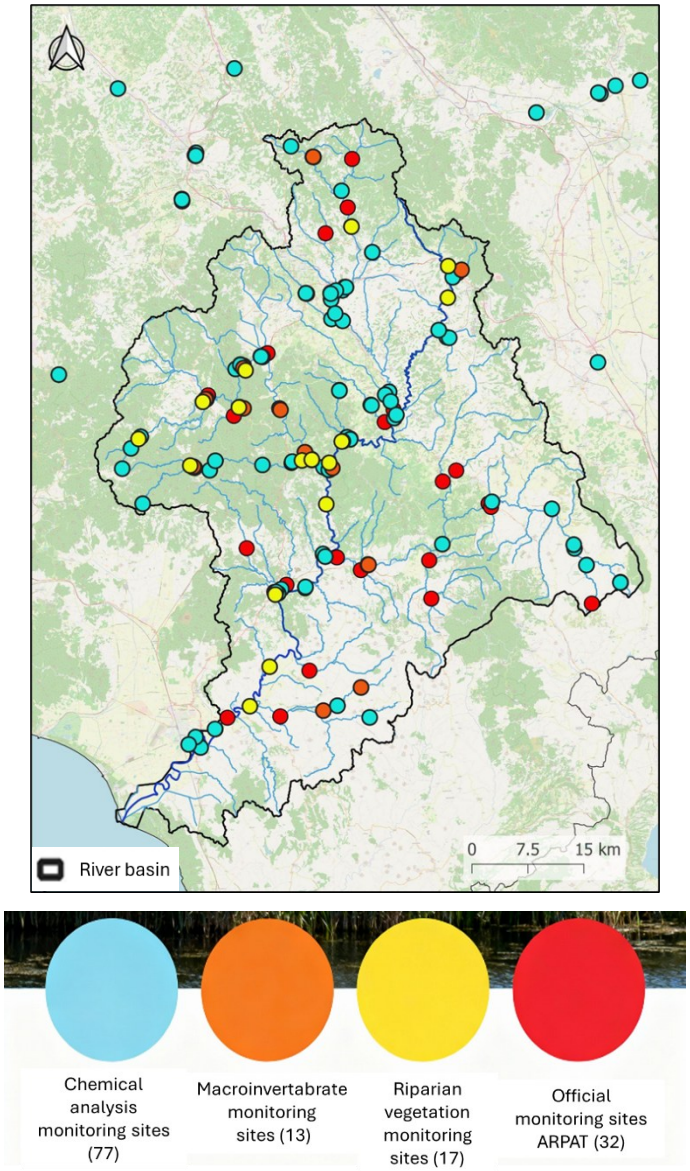


Figure 3. Spatial distribution of monitoring sites by activity (light blue: chemical analyses; orange: macroinvertebrates; yellow: riparian vegetation), alongside ARPAT monitoring sites (red) within the same study area.

2.2 The Citizen Science approach

Stakeholder engagement began with a top-down approach led by researchers and was subsequently expanded through a bottom-up procedure, driven by the "snowball effect" triggered by the diversity of stakeholders involved. Overall, 168 stakeholders from different sectors were involved: academia (universities, schools, research centres), civil society (citizen scientists and cultural, environmental, sports, and

museum organisations), public bodies and institutions (national, regional, and local authorities), and economic activities (farms and agritourism enterprises). According to the quintuple helix model framework (Carayannis et al., 2012), stakeholders were then categorised according to the helix they belong to (academia, government, industry, civil society, environment). The fifth helix, representing the environment, was not explicitly included in the engagement phase, as it serves as the contextual framework for the overall process (Capineri et al., 2025) (Fig.4).

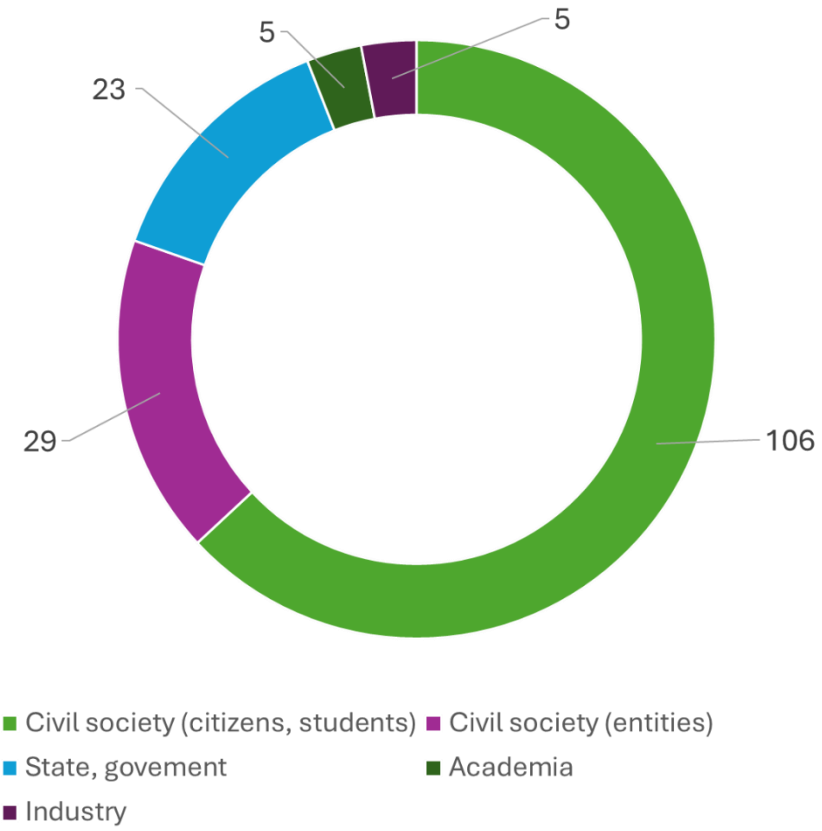


Figure 4. The variety of stakeholders involved in the project (N=168)

Of the 168 stakeholders involved in the project, 46 were citizen scientists who actively and consistently participated in the monitoring activities conducted throughout the project. The remaining participants were either occasional contributors, such as those involved in the RiverBlitz events (see below), or high school students. Socio-demographic data were collected from the 46 regular citizen scientist participants through a structured questionnaire administered for this purpose (Figg. 5,6,7).

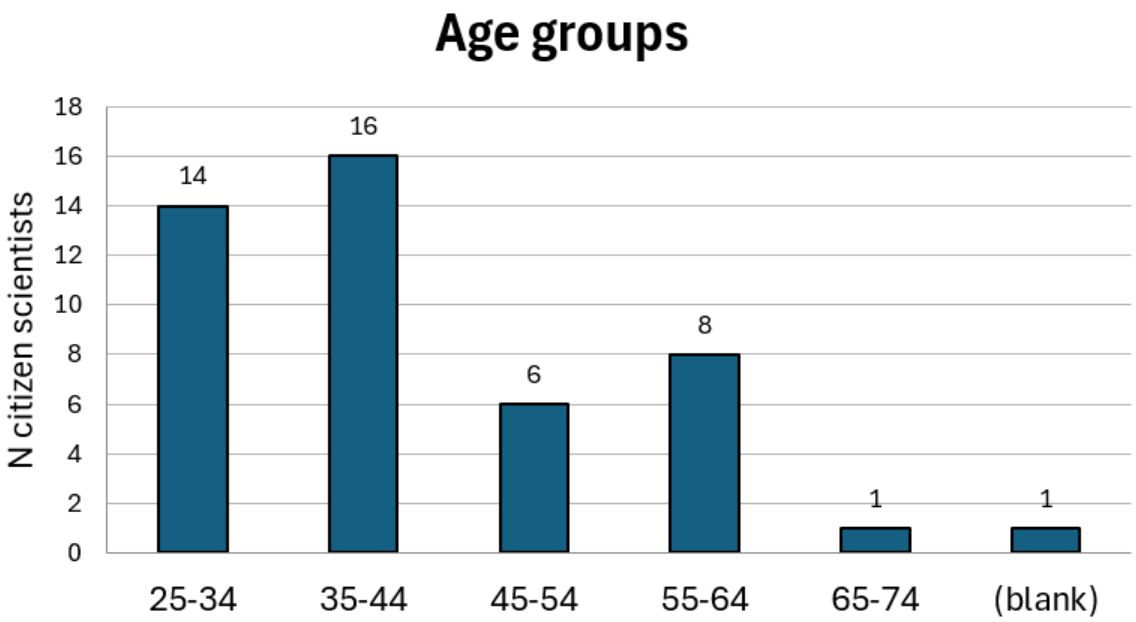


Figure 5. Bar plot showing the distribution of participants across age groups

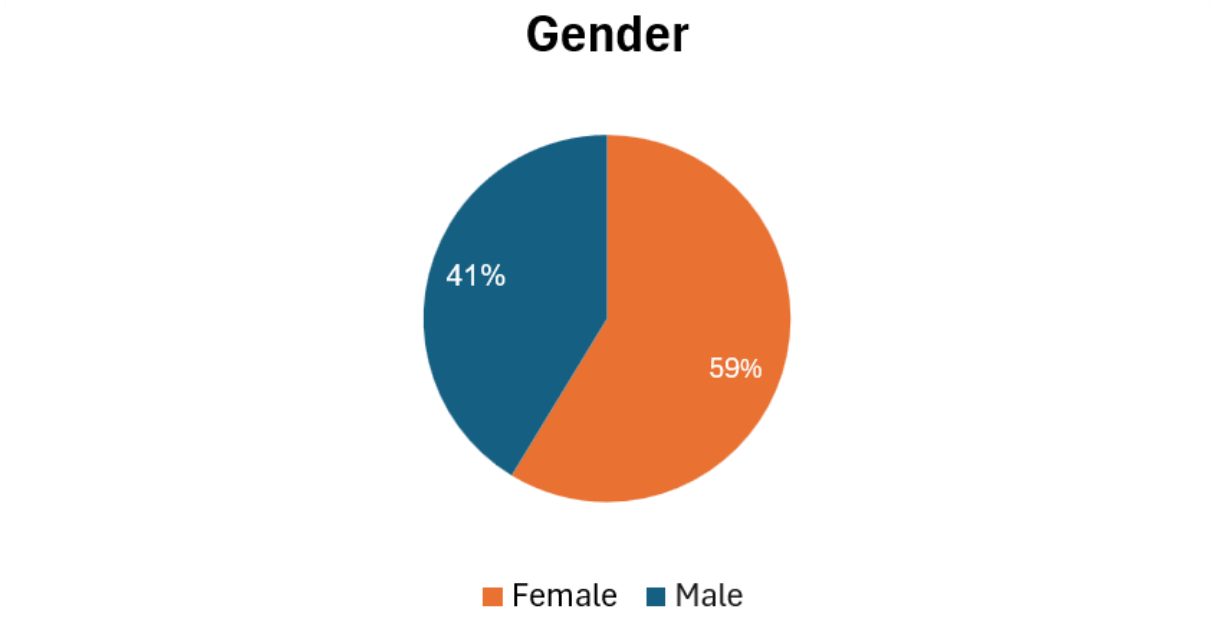


Figure 6. Pie chart illustrating the gender distribution of participants

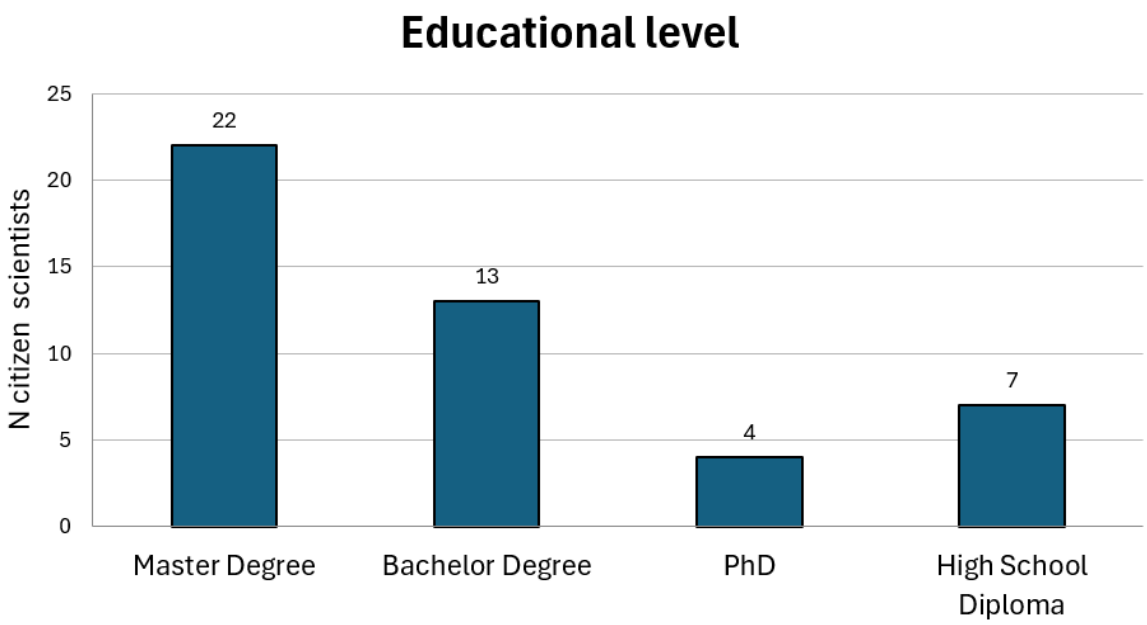


Figure 7. Bar plot representing the distribution of participants by educational attainment level

Within the CS4Rivers framework, all volunteers attended a standardised training session before performing field activities. Two training sessions were organised for each monitoring activity, one in the Province of Grosseto and one in the Province of Siena, to facilitate the participation of local communities (Fig 8). The training, delivered by CS4Rivers researchers, covered the fundamental principles of fluvial ecology, the application of the sampling protocols, and the use of project-specific supporting materials available on the project website (cs4rivers.unisi.it, “Activities” section).



Figure 8. Citizen scientists attending a training session. Ph. Fabrizio Monaci

Throughout the whole duration of the project, volunteers received continuous feedback from experts on the sampling results. In addition, an online feedback event was organised for each monitoring activity at mid-project (autumn 2024), specifically addressed to citizens. A final in-person workshop was held in November 2025 to present and discuss the overall results with both volunteers and other stakeholders interested in the monitoring activities' results.

To test the developed protocols and foster local community engagement, a Riverblitz event was organised in September 2023. The Riverblitz is a public participation event conceived by the Maremma Natural History Museum, aimed at collecting data on biodiversity and habitat quality in river ecosystems. The sampling area included the Ombrone River and its tributaries in the surroundings of Paganico (Grosseto). Participants (N=37), together with researchers (N=9), had the opportunity to directly apply monitoring techniques to assess riverine ecosystem quality, while also enabling CS4Rivers researchers to conduct a preliminary field test of the protocols developed or adapted within the project (Figg. 9, 10).



Figure 9. People applying at the RiverBlitz event. Ph. Andrea Sforzi



Figure 10. Citizen scientists performing sampling techniques. Ph. Chiara Vitillo

Over the three years of the project, the CS4Rivers team also participated in and presented the project at the 2023 and 2024 editions of BRIGHT - the European Researchers' Night, to engage people and disseminate project results (Fig. 11).



Figure 11. CS4River researcher during the Bright Night event. Ph. Michela Teobaldi

In order to further enhance volunteer engagement throughout the various phases of the project, in April 2024, a representative of the citizen scientists involved in macroinvertebrate monitoring collaborated with the CS4Rivers research team in presenting the preliminary results at the 2nd National Conference of Citizen Science Italia (Fig. 12).

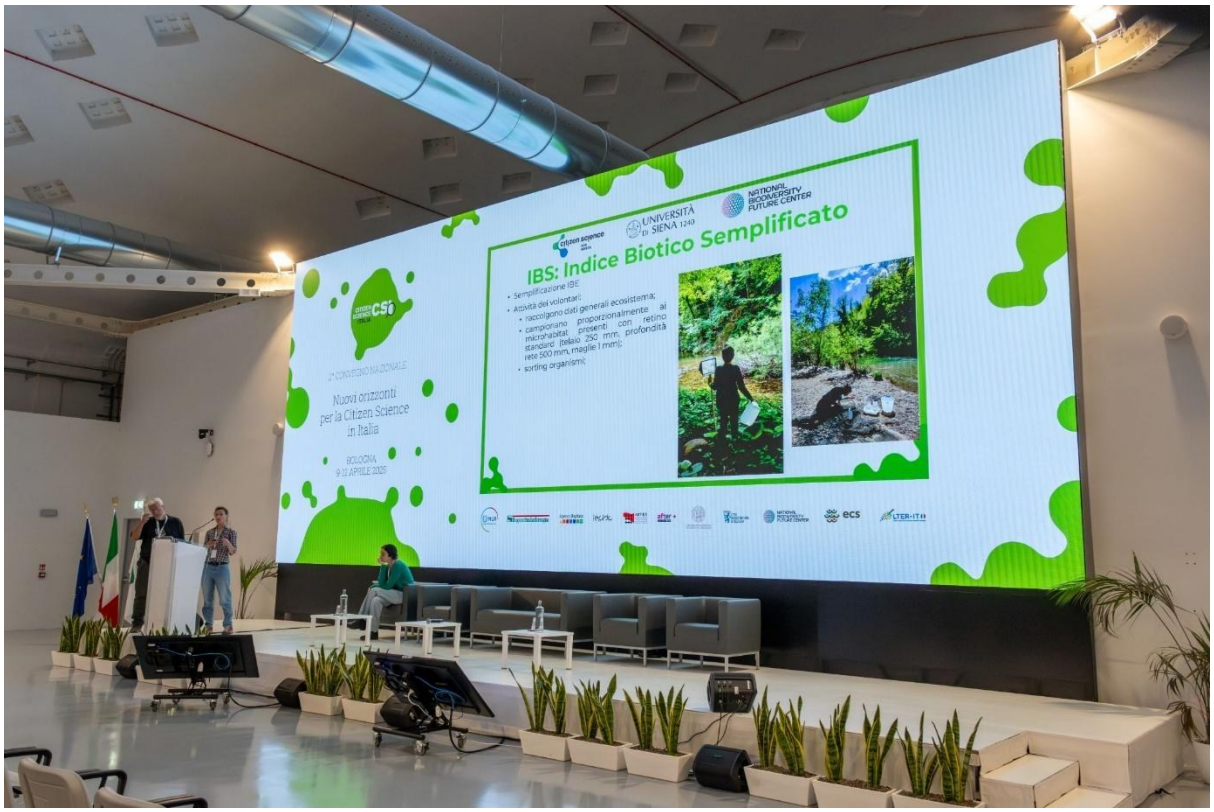


Figure 12. CS4Rivers' representation at the 2nd National Conference of Citizen Science Italia. Ph. Chiara Fedrigotti

2.3 Monitoring activities

Project monitoring activities encompassed: (i) water chemical quality, (ii) macroinvertebrate communities, and (iii) riparian vegetation. The number of citizen scientists per activity is reported in Figure 13.

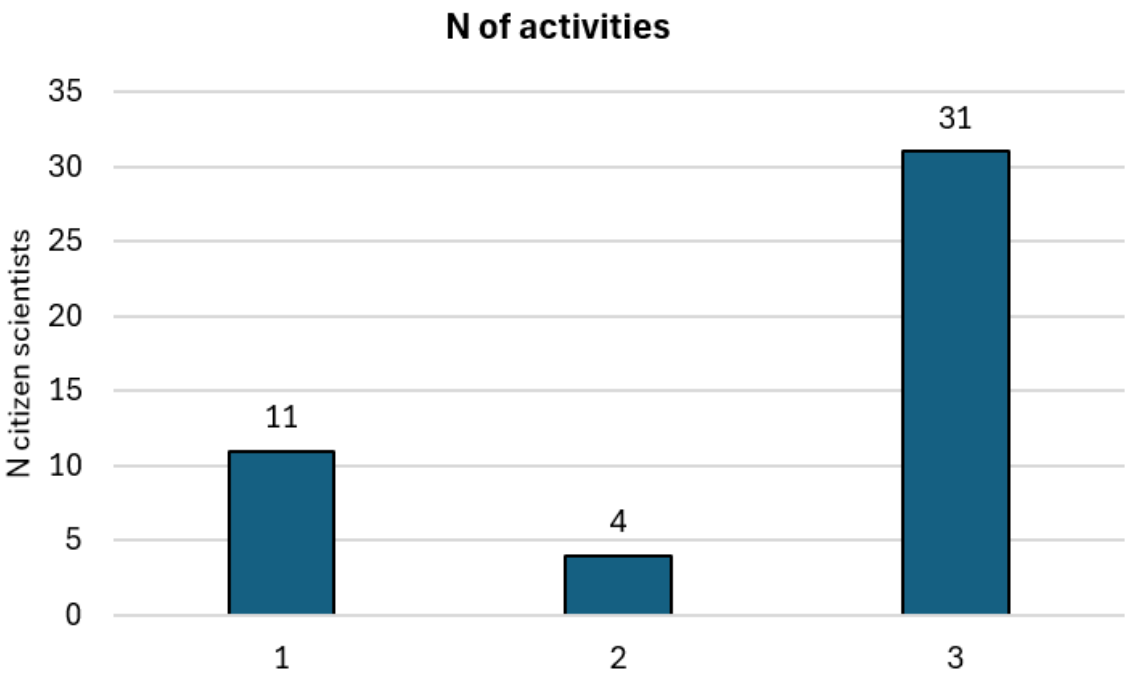


Figure 13. The numbr of citizen scientists per activity

i) Water quality monitoring began in October 2023 and continued until September 2025, encompassing a total of 383 samplings (monthly sampling frequency) across 16 watercourses within the Ombrone River basin. Additional monitored sites included water bodies outside the basin (Fig. 3). Monitoring activities focused on nitrate and phosphate concentrations and water turbidity. The sampling protocol is schematically summarised in Figure 14.

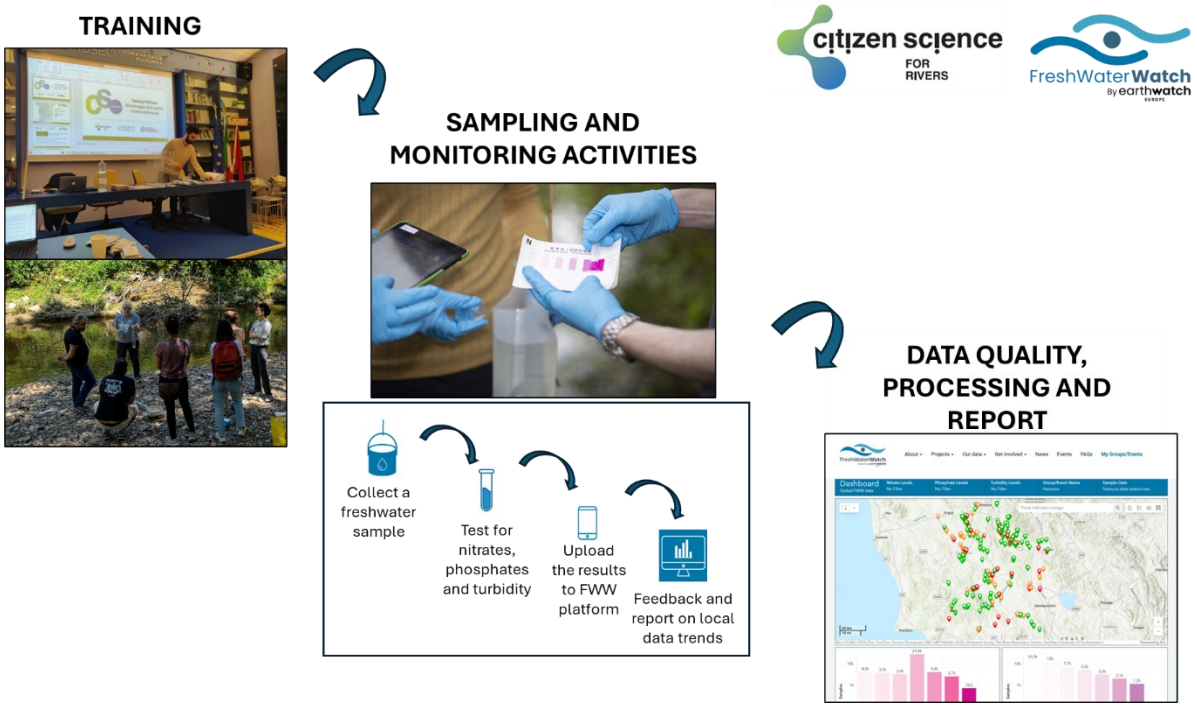


Figure 14. Visual abstract of the water chemical analysis framework adopted in the CS4Rivers project. The protocol is based on FreshWater Watch (FWW), a global CS program developed by the environmental charity Earthwatch Europe (2022).

ii) Macroinvertebrate community monitoring was carried out in spring 2024, autumn 2024, and spring 2025, involving 15 volunteers. Six streams were monitored across 13 monitoring sites (semi-annual sampling frequency). The sampling protocol is summarised in Figure 15.

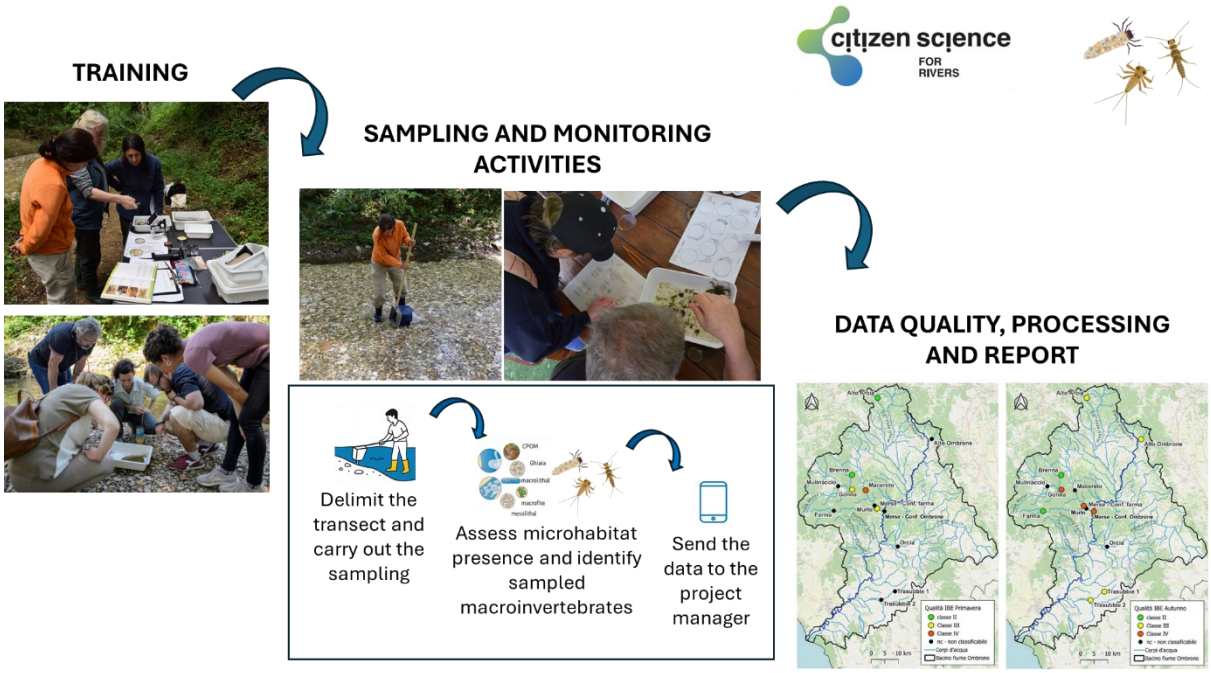


Figure 15. Visual abstract of the macroinvertebrate community monitoring protocol. The protocol is based on the EasyBenthos method, a novel protocol developed by University of Siena researchers within the CS4Rivers project

Using the data collected by citizen scientists and following the EasyBenthos protocol, the CS4Rivers researcher calculated the water quality class of each monitoring site, based on the well-established Extended Biotic Index (EBI) (Ghetti, 1997) (Tab. 1)

Table 1. The five EBI quality classes with their corresponding conventional colours

Quality class	Quality assessment	Colours and related quality class
Class I	Environment not significantly altered	High
Class II	Environment with moderate symptoms of alteration	Good
Class III	Altered environment	Moderate
Class IV	Environment highly altered	Poor
Class V	Environment highly degraded	Bad

iii) Riparian vegetation monitoring was conducted by 12 volunteers from May to October in 2023 and 2024. The activity covered 17 monitoring sites and 99 sampling plots (annual sampling frequency). The sampling protocol is schematically summarised in Figure 16.

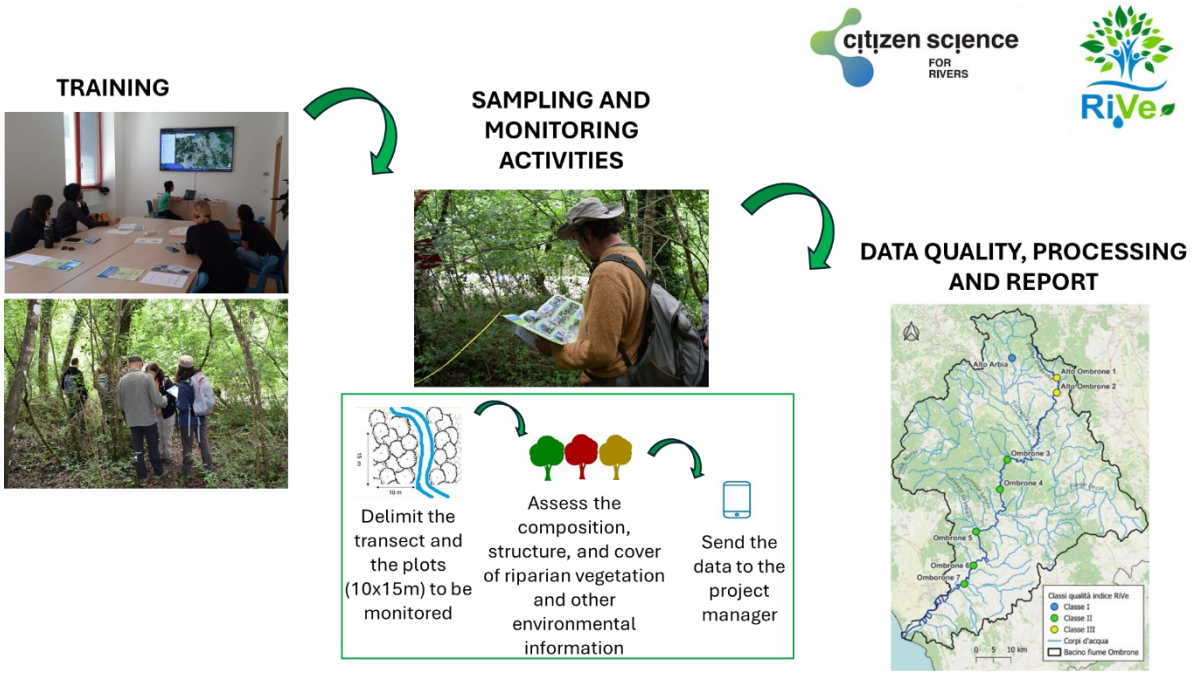


Figure 16. Visual abstract of the riparian vegetation monitoring protocol. The RiVe protocol was developed by the Citizen Science Observatory and the National Biodiversity Network (ISPRA) and was adapted within the CS4Rivers project to address the project's objectives and the specific environmental characteristics of the Ombrone River basin.

Based on the data collected by citizens using the RiVe protocol, the CS4Rivers researcher calculated: a) the sub-index of riparian vegetation invasiveness (IRVI), and b) the RiVe index (IRiVe).

a) The IRVI is a semi-quantitative indicator designed to assess the relative dominance of invasive taxa. The index is calculated as the proportion between the cumulative percentage cover of the invasive taxa and the total percentage cover of all the monitored taxa. IRVI values can range from 0% (absence of invasive species) to 100% (complete dominance by invasive taxa). The IRVI has been calculated separately for the two vegetation layers monitored (canopy layer: >3m; understory layer: 1-3 m. Herbaceous species were excluded from the analysis).

b) The IRiVe is formed by six indicators which describe key structural and compositional attributes of a healthy riparian forest. In this context, a healthy forest is characterised by tall, structurally diverse, and patchy vegetation, the presence of dead trees, and the absence of invasive species in both vertical layers. Each indicator is evaluated using three to five qualitative classes (Tab. 2).

Table 2. The indicators of the RiVe index and their corresponding scores

Indicator	Scoring Criteria	Score
1. River Section	Natural: no or minimal alteration	20
	Partially modified	10
	Significantly modified: riverbed lowering	0
	Significantly modified: artificial	0
2. Vegetation Height	>15 m	20
	7-15 m	10
	<7 m	0
3. Decaying Trees	>2	20
	1-2	10
	0	0
4. Forest Structure	Natural	20
	Managed (e.g., cuts, coppicing)	10
	Artificial (e.g., poplar plantation)	0

	I don’t know	-
5. Invasive Species (>3 m layer)	<5%	20
	5-25%	15
	26-50%	10
	51-75%	5
	76-100%	0
6. Invasive Species (1-3 m layer)	<5%	20
	5-25%	15
	26-50%	10
	51-75%	5
	76-100%	0

The RiVe index is obtained by adding the scores of the six indicators, producing a value between 0 and 120, which is converted to the quality of the riparian forest into five distinct classes (Tab. 3). The index can be calculated at the level of individual plots or the entire transect; in this study, it was computed at the transect level by averaging the summed scores of all plots. Where surveys were conducted on both riverbanks, data from the two sides were aggregated.

Table 3. The five RiVe quality classes and their corresponding standard colours

Quality class	Quality assessment	Colours and related quality class	
Class I	Natural watercourse, intact forest, absence of invasive species	High	
Class II	Largely natural section, limited management, few invasive species	Good	
Class III	Altered forest structure, presence of invasive species	Moderate	
Class IV	Heavily modified watercourse and forest, intensive management	Poor	
Class V	Highly artificial environment, dominance of invasive species	Bad	

3. Results

The results presented in this chapter derive from the data collected by citizen scientists using the simplified protocols. For riparian vegetation, only the data from the 2023 and 2024 sampling campaigns are included, as the 2025 data were collected specifically for the data quality experiment and are limited to plot-level observations rather than transect-level assessments.

3.1 Macroinvertebrate monitoring

The quality classes derived from citizen scientists' data are presented in Figures 17,18 and 19, organised by sampling campaigns.

In spring 2024, Classes II–III predominated, with a critical situation observed at Macereto (Class IV - orange/highly altered environment). Notably, the presence of many unclassifiable stations is due to the data-quality scarcity collected by citizen scientists. It should be emphasised that this was the first independent sampling season for volunteers (Fig. 17).

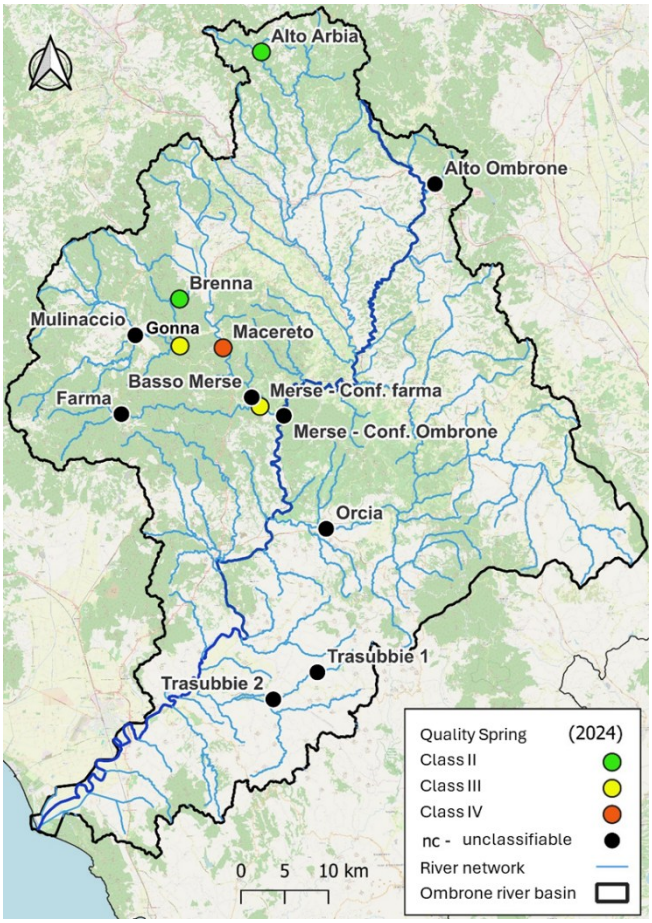


Figure 17. Spatial distribution of monitoring sites with relative quality class, performed in Spring 2024

In autumn 2024, most monitoring sites were classified as Class III (yellow) and Class IV (orange), indicating overall moderate to poor conditions, with some localised deteriorations. The number of monitoring sites without data decreased, reflecting improved data quality collected. It should be noted that during the autumn period, the macrobenthic community naturally declines in both diversity and abundance (Fig. 18).

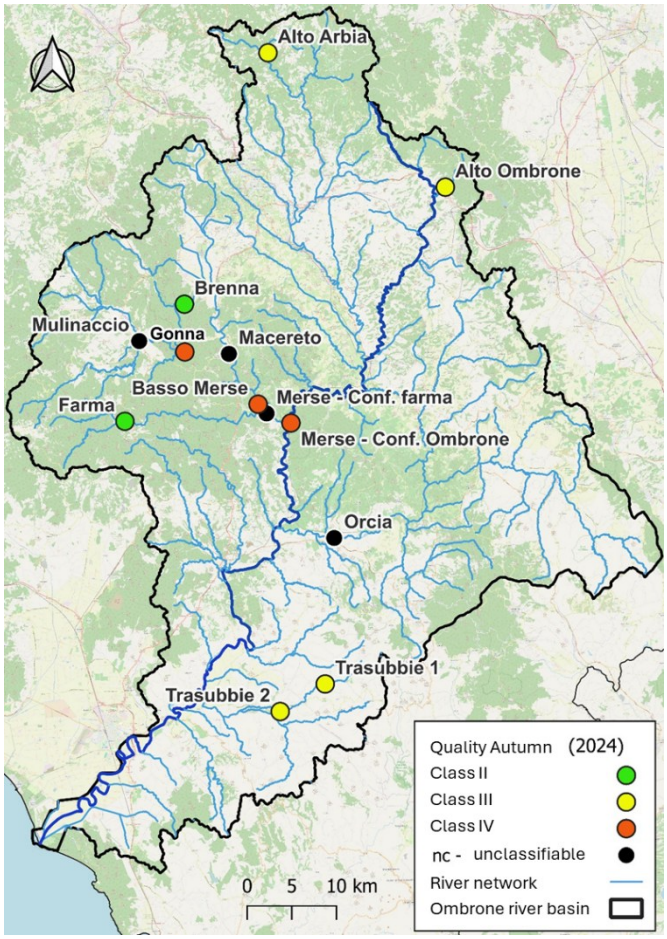


Figure 18. Spatial distribution of monitoring sites with relative quality class, performed in Autumn 2024

During spring 2025, conditions were generally stable, with minor variations and some stations showing improvement. Notably, unclassifiable sites have further decreased (Fig. 19).

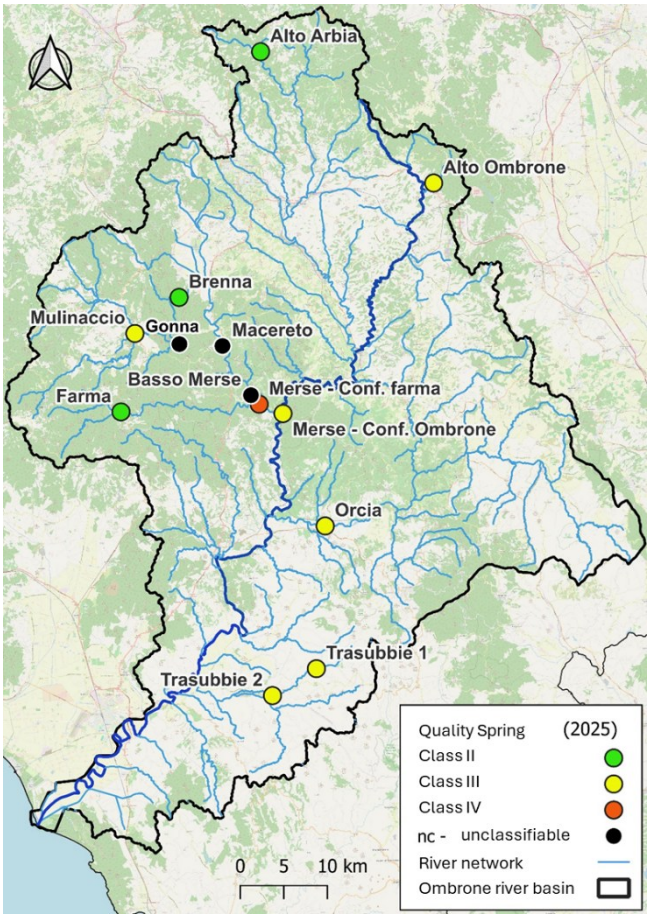


Figure 19. Spatial distribution of monitoring sites with relative quality class, performed in Spring 2025

3.2 Riparian vegetation monitoring

The 2023 sampling campaign surveys took place along the Ombrone and Arbia rivers. The canopy layer was generally dominated by hygrophilous species such as *Populus nigra* and *Populus* × *canescens*, while the understory layer exhibited greater variability, including both native species and, in some stations, a high dominance of the invasive *Robinia pseudoacacia* (Fig. 20).

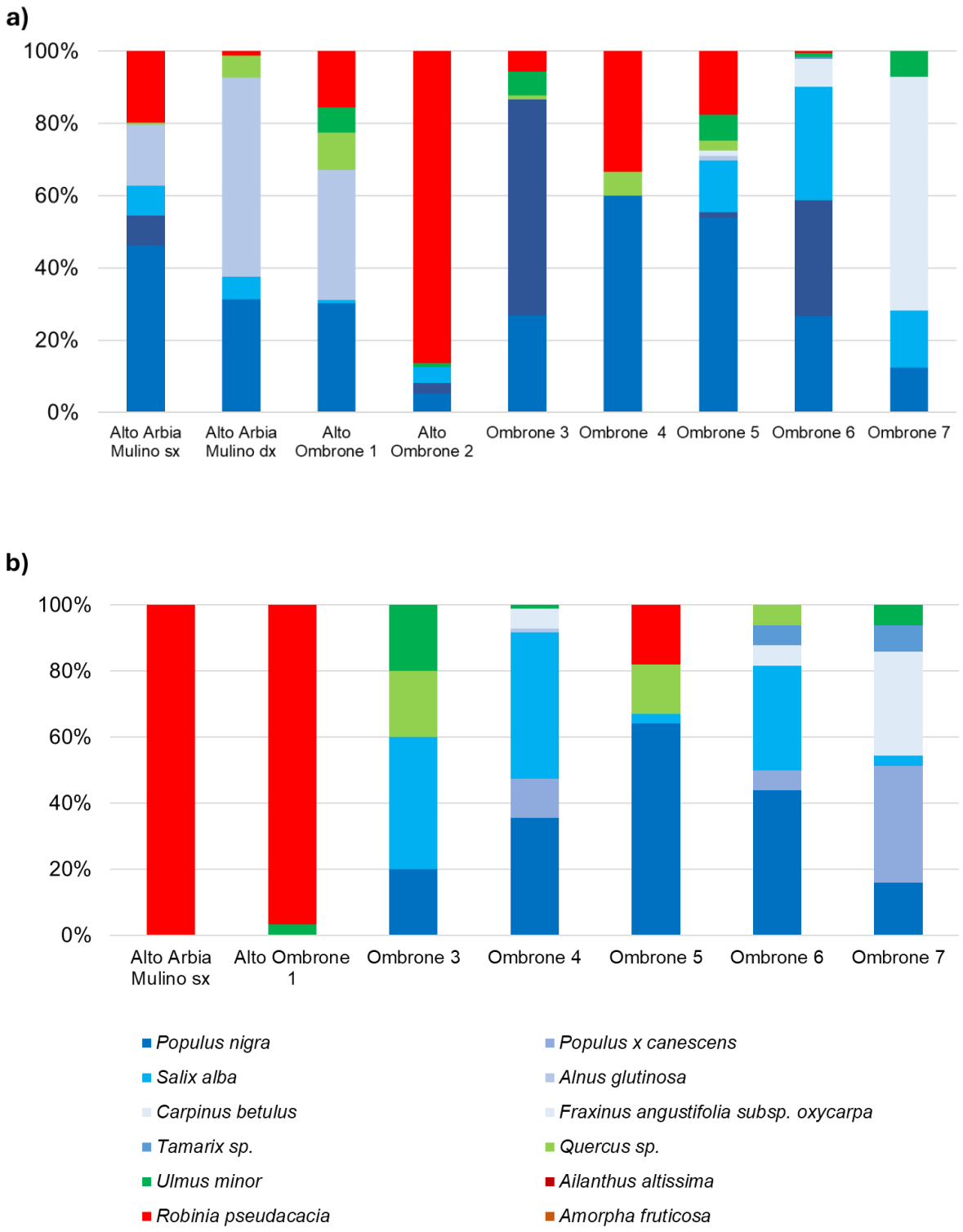


Figure 20. Percentage cover of target species across monitoring sites: a) canopy layer; b) understory layer. In shades of blue: hygrophilous species; in shades of green: mesophilous species; in shades of red: alien species.

The spatial distribution of the RiVe index quality classes across the Ombrone River basin shows a longitudinal pattern characterised by varying ecological conditions. Most

of the basin was characterised by good ecological conditions (Class II). Moderate conditions (Class III) were observed in specific upstream sections, indicating localised pressures. A high-quality site (Class I) was located in the upstream site, referring to the Arbia river. Overall, the pattern depicted a good ecological condition of riparian vegetation quality along the river continuum (Fig. 21).

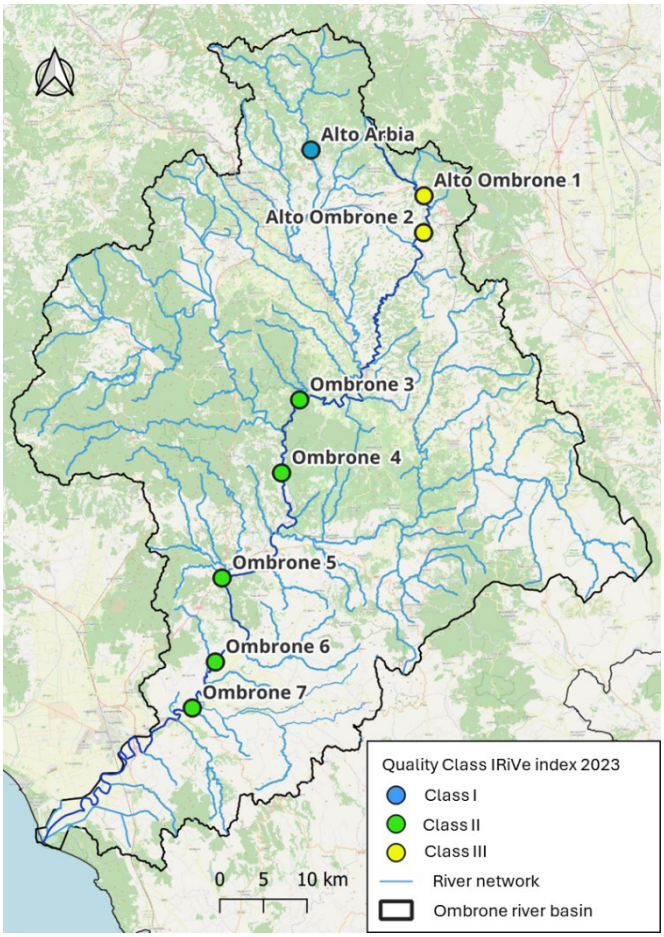


Figure 21. Spatial distribution of monitoring sites with relative quality class, performed in the 2023 sampling campaign

For the sampling campaign in 2024, surveys took place along the Merse river; both the canopy and the understory layer displayed great heterogeneity. For the canopy layer, hygrophilus and mesophylus species were dominant, but in some river ranches (especially for the understory layer), a marked dominance of the invasive *Robinia pseudoacacia* was observed (Fig. 22).

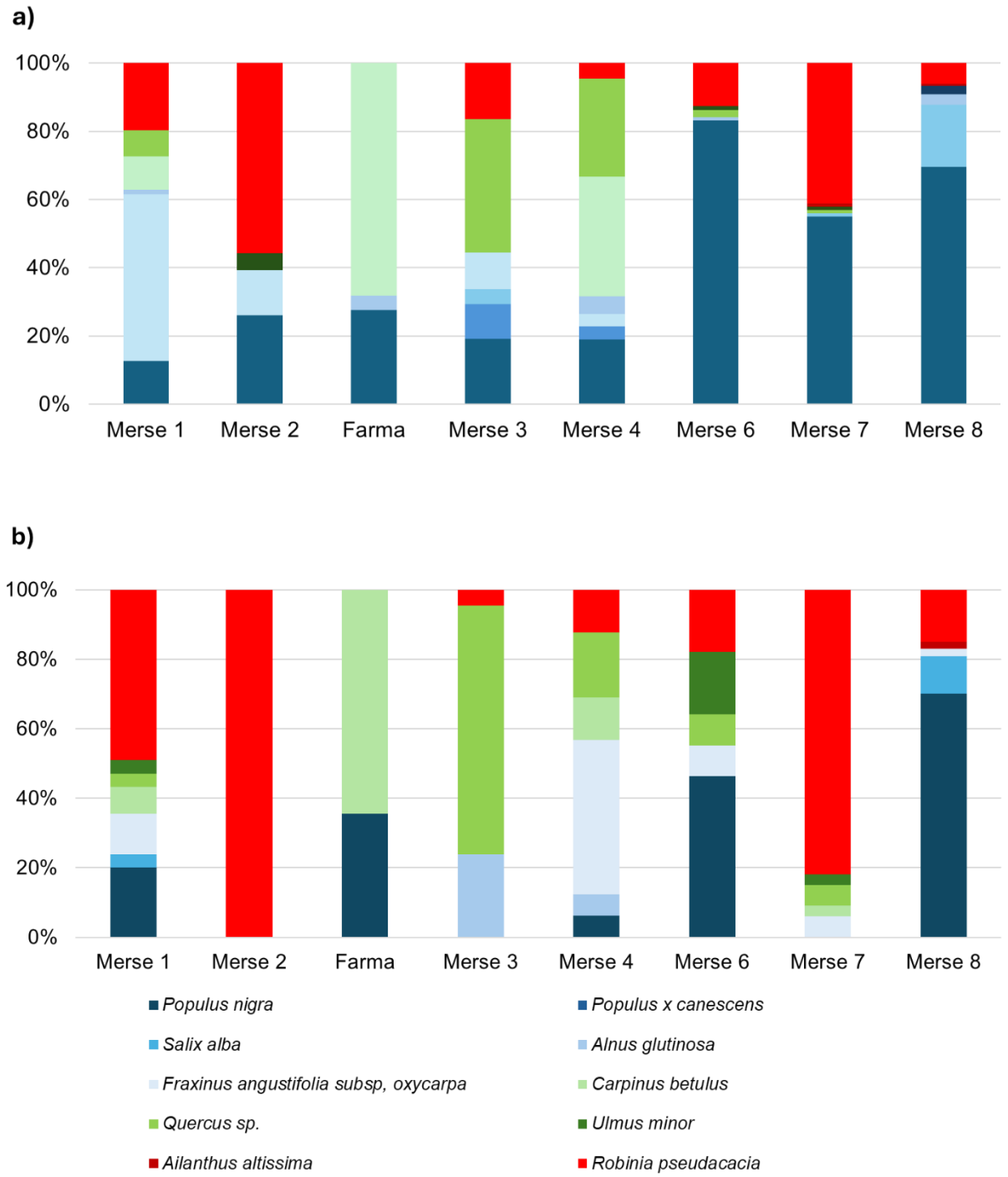


Figure 22. Percentage cover of target species across monitoring sites: a) canopy layer; b) understory layer. In shades of blue: hygrophilous species; in shades of green: mesophilous species; in shades of red: alien species.

The spatial distribution of the RiVe index quality classes revealed a heterogeneous pattern. High-quality conditions (Class I) are observed in two stations, particularly along the Farma river and selected reaches of the Merse River, while other sites fall within Class II (good) and Class III (moderate). This distribution depicted a mosaic of

ecological conditions, with high-quality values interspersed with moderately altered sections, reflecting spatial variability in riparian vegetation structure and ecological integrity (Fig 23).

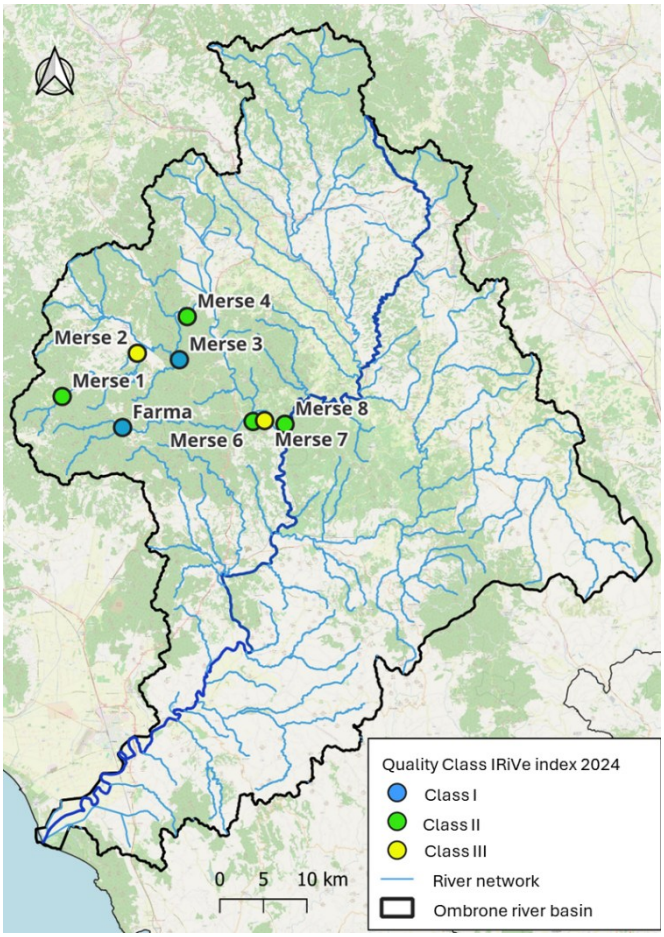


Figure 23. Spatial distribution of monitoring sites with relative quality class, performed in the 2024 sampling campaign

4. Discussion

This section synthesises the main findings of the study, providing a preliminary interpretation of the results. However, additional sampling campaigns repeated over time are needed to further validate both protocols and confirm their reliability in capturing the temporal dynamics of ecosystem conditions.

4.1 Macroinvertebrate monitoring

Regarding the macroinvertebrate results, the data indicate a diffuse condition of environmental alteration (Class III), with moderate conditions observed in some cases (Class II) and a few critical (Class IV). The reduction in “non-classifiable” stations

reflects an improvement in data quality collected by citizen scientists, highlighting the importance of maintaining continuity in training and practical field activities.

4.2 Riparian vegetation monitoring

About the ecological quality of riparian vegetation, the RiVe index indicated generally good ecological quality of riparian vegetation, with the majority of stations falling within Classes I and II, reflecting high naturalness and good structural integrity. However, four reaches were classified as Class III, where the spread of invasive species - particularly *Robinia pseudoacacia* - was the main critical issue, while *Ailanthus altissima* was only sporadically present.

Sections dominated by native hardwood species (e.g., *Carpinus* sp., *Fraxinus* sp., *Quercus* sp.), particularly along the Farma river and in the central reaches of the Merse River, exhibited a near absence of invasive species and balanced regeneration, confirming good ecological functionality. Overall, the system displayed a mosaic pattern, with high-quality areas interspersed with more degraded sections, highlighting the need for regular monitoring and targeted management actions.

5. Conclusions

Despite their simplified nature, the methods adopted in this study demonstrated a great potential as early warning monitoring tools, as they are able to distinguish different levels of ecological quality across sites. Their application in a citizen science context tests and supports their potential feasibility for large-scale, cost-effective environmental assessments.

Long-term monitoring will be essential to consolidate their role in supporting river management and conservation strategies.

6. Acknowledgements

The author would like to thank all members of the CS4Rivers project team for their contributions to the development of the project concept and workflow.

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Thesis Conclusion

This PhD thesis evaluates Citizen Science (CS) approaches for assessing the ecological quality of riverine ecosystems, based on targeted field experiments. Within this framework, the thesis primarily focused on CS protocols and systematically assessed different monitoring techniques, demonstrating their effectiveness as reliable tools for ecological assessment and monitoring. To the best of our knowledge, this work is one of the first comprehensive doctoral-level assessments of CS data quality in Italy.

Within macroinvertebrate monitoring, the findings highlight that CS protocols can provide ecologically meaningful and comparable results to expert-based assessments when supported by robust standardisation of sampling and experimental design. Logistical and operational challenges associated with fieldwork by non-professional operators, especially when handling small and mobile organisms, should also be considered to improve data quality (Chapter 1).

The comparison among European CS macroinvertebrate sampling protocols further contextualises these findings by suggesting that interoperability may be influenced by the sampling design (Chapter 2). Reduced agreement between protocols characterised by heterogeneous sampling effort highlights the sensitivity of macroinvertebrate community characterisation to methodological choices, underscoring the need for greater harmonisation of sampling strategies across national CS frameworks.

Evidence from riparian vegetation monitoring confirms that volunteers can generate high-quality data when protocols are specifically designed to minimise observer bias and aligned with participants' skill levels (Chapter 3). Furthermore, targeted training and iterative feedback remarkably improved performance, highlighting the central role of adaptive learning in enhancing data quality.

Finally, the analysis of citizens-generated data supports the potential of the applied protocols as early warning tools for ecosystem assessment (Chapter 4). Both macroinvertebrate and riparian vegetation results captured spatial heterogeneity in ecological quality, identifying gradients of alteration. However, additional sampling campaigns repeated over time should be performed to further validate the robustness of both protocols.

Overall, this thesis demonstrated that integration across different monitoring techniques could play an important role in achieving a comprehensive picture of the ecological quality of a given study area.

However, the data quality procedure adopted in this research required a substantial investment of time and resources from experts, raising important concerns regarding its long-term sustainability. While data quality assessment is considered essential during the validation phase of a new protocol, ensuring the reliability of the collected data and the robustness of the methodological framework, its continuous application may not be feasible over extended periods. Once a protocol has been adequately validated, data quality controls could be implemented through periodic spot checks rather than through the continuous verification procedures applied throughout the field experiments in this thesis. Such an approach would improve the sustainability of long-term monitoring programmes while maintaining an appropriate level of quality assurance. Examples of this strategy already exist in Europe. Within the Anglers' Riverfly Monitoring Initiative (ARMI) framework (Chapter 2), for instance, each monitoring site is associated with a site-specific trigger level established by experts on the basis of historical baseline data. This threshold-based approach provides a reference baseline against which new observations can be evaluated, allowing potential anomalies and substantial deviations from expected conditions to be rapidly identified. Consequently, expert effort and verification activities can be targeted towards sites or periods showing unusual patterns, while optimising the overall efficiency of monitoring programmes.

Although significant challenges remain, this doctoral thesis represents one of the first doctoral-level investigations into data quality in CS within an Italian case study. By providing insights into the scientific validity of CS data, it contributes to the advancement of CS methodological application in freshwater ecology, as well as supporting its integration into long-term monitoring and management frameworks.

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