UNIVESITÀ DEGLI STUDI DI NAPOLI FEDERICO II

DOCTORAL SCHOOL OF AGRICULTURE AND FOOD SCIENCES
(XXIX Cycle)

TESTING THE PERFORMANCE OF BATS AS INDICATORS OF HABITAT QUALITY IN RIPARIAN ECOSYSTEMS

PhD candidate: Carmelina De Conno

Tutor: Danilo Russo

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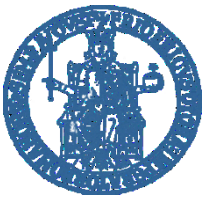
Università degli Studi di Napoli Federico II



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1. INTRODUCTION

1.1. CONCEPT OF BIOINDICATION

Starting from the industrial revolution, human activities have increasingly influenced both the structure and functionality of natural ecosystems, leading to serious consequences, such as environmental pollution, climate change, habitat fragmentation, biodiversity loss, etc. The world must face all of these issues simultaneously. To support the scientific decision-making process, it is fundamental to gather maximal information with minimal resource requirements, in the most efficient way possible. Traditionally, environmental monitoring was done with chemical and physical essays only, but these methods cannot provide information on the response of biota to a pollution/degradation event. They can neither show the indirect effect of different molecules acting together (in synergism or antagonism) in a ecosystem nor test the long term effects of small concentrations of pollutants (such as bioaccumulation).

In the last 30 years, bioindication that both readily reflects and represents the state of the environment has shown to be a powerful tool that allows for efficient monitoring. It has become one of the pillars of modern environmental sciences and an essential part of conservation biology (Markert et al., 2003; McGeoch, 2007).

Generally, bioindicators are described as "biological processes, species, or communities" that "are used to assess the quality of the environment and how it changes over time" (Holt and Miller, 2011). Depending on the specific objective of bioindication, three categories of bioindicators can be identified (McGeoch, 1998):

- Environmental indicators: A species or a group of them that responds predictably, in ways that are readily observed and quantified, to environmental disturbance or to a change in environmental state;

- Ecological indicators: A species or a group of them that demonstrates the effect of environmental changes on biota or biotic systems;

- Biodiversity indicators: A group of taxa or functional group the diversity of which reflects the diversity of other higher taxa in a habitat or set of habitats.

Moreover, according to their provenance, bioindicators can be classified as "active", if they are bred in laboratory and then exposed to a standardized condition in the field for a defined period of time, or "passive", if they naturally occur in ecosystem and their reactions are examined (Markert et al., 2003).

Depending on the specific objective and scale of the study, a good bioindication strategy always requires the selection of suitable bioindicators, which have to fit particular criteria. It is possible to identify two main categories: economic/ logistic suitability and biological efficacy (McGeoch, 1998). A good bioindicator should always be easy and cheap to survey, in terms of cost, time efficiency and personnel requirements. But it is also fundamental that 1) its response to the disturbance or stress is measurable and proportional to the degree of contamination/degradation; 2) it is relatively common, widely distributed geographically and stable, with an adequate population density (rare species are not good for bioindication purpose); and 3) it is taxonomically stable, easy to identify, and with a well known ecology, for a well designed sampling protocol (Holt and Miller, 2011; Jones et al., 2009; McGeoch, 2007, 1998; Russo and Jones, 2015; Sartori, 1998; Syaripuddin et al., 2015). If the taxon studied meets all these criteria and also shows to have a strong, robust and statistically significant relationship with the environmental parameter of interest, then it is a fully functional biological indicator.

1.2. BIOINDICATION IN RIPARIAN ECOSYSTEMS

Freshwater ecosystems cover less than 1% of world surface, but they contain 6-10% of all species and one-third of all vertebrate species worldwide, demonstrating that they are important hotspots of biodiversity (Dudgeon et al., 2006; Balian et al., 2008; Strayer and Dudgeon, 2010). Freshwater ecosystems also provide several services, fundamental for human settlements and productive activities (both agriculture and industrial). On the other hand, human activities generate a high pressure on the natural balance of such ecosystems. Rivers and lakes are losing biodiversity faster than any other terrestrial or marine ecosystem (Jenkins, 2003; Strayer and Dudgeon, 2010). The awareness of ecological and economical importance of these habitats implicated big efforts for conservation and restoration of river environments, especially in the last few decades.

Bioindication is now a necessary supplement to traditional monitoring techniques for riparian ecosystems and is also required by legislation in several countries, like the Water Framework Directive of European Union (European Parliament, 2000). A variety of biological assemblages have been used worldwide in scientific research to assess the ecological status of streams and rivers in the last century, however benthic macroinvertebrates, periphyton and fishes are the most commonly employed at application level in national monitoring programs.

Aquatic macrobenthos organisms have been studied since 1964 for this purpose and are now considered the most suitable as indicator of water quality (Hellawell, 1986; Furse et al., 2006). There are several reasons for this success: macroinvertebrates are widely represented in all water courses and they are relatively easy to sample and classify; they include taxa withdifferent sensitivity tothe various types of pollutants, to which they can react quickly. Their life cycle is long enough (one year or more) to register the effect of occasional event of pollution; they have limited migration patterns, allowing to assess site-specific impacts; and their community is made up of species that cover a broad range of trophic levels and pollution tolerances (Barbour et al., 1998; Sansoni, 1988; Sartori, 1998).

Macrobenthic communities react in predictable ways to environmental changes, mostly showing diversity reduction, the disappearance of sensitive taxa and dominance of opportunistic ones, and the decrease of individual size (Gray, 1989; Li et al., 2010).Typically, bioindication methods study the differences between the composition of an expected community and the current community of a particular site or combine the relative abundance of some taxonomic group with their sensitivity/tolerance to pollution (Armitage et al., 1983; Buffagni and Erba, 2014; Ghetti, 1997; Li et al., 2010; Sansoni, 1988; Sartori, 1998). Several indices have been proposed and used in various countries, including Trent Biotic Index (Woodiwiss, 1964) , then modified in Extended Biotic Index (EBI), Saproby Index (SI), Biological Monitoring Working Party Score System (BMWP), Average Score per Taxon (ASPT), Indice Biologico Esteso (IBE) - the Italian adaptation for EBI- and the multimetric Star_ICMindex.

To the contrary, fish communities are used to assess long term effects and broad habitat conditions because of their long life cycle (of several years) and their mobility along the water course (Karr, 1981). Also in this case, species are distributed along the whole food chain. In addition, fish are eaten by humans, so it is even more important to assess contamination (Barbour et al., 1998; Chandler, 1970; Li et al., 2010; Sartori, 1998; Zerunian et al., 2009). Fish are easy to collect and identify in the field and their environmental requirements are well known, but the presence of numerous alien species in fish community due to the habitual repopulation made especially for sport fishing, can be problematic in the final evaluation of the ecological status (Kennard et al., 2005; Sartori, 1998). A variety of indices based on fish community have been developed starting from the Index of Biotic Integrity (IBI) (Karr, 1981; Karr et al., 1986; Kesminas & Virbickas, 2000; Oberdorff & Hughes, 1992; Zerunian et al., 2009).

Periphyton and its main component diatoms are good environmental indicators and their use is widespread and well developed for standing and flowing waters (Lowe & Pan, 1996; Kelly et al., 2009; King et al., 2000; King et al., 2006; Prygiel et al., 1999; Giorgio et al., 2016). Because of their nutritional needs and their position at the first

level of aquatic food chain, diatoms respond quickly and predictably to a wide range of pollutants, even when the concentration of the latter does not visibly affect other aquatic assemblages (McCormick & Cairns, 1994). Taxa richness and diversity, assemblage similarity, taxonomic composition, Chlorophyll-a and biomass have all been reported as measures to indicate the environmental stress. Most biotic indices, developed on species-specific sensitivities and tolerances, are used for monitoring eutrophication, acidification and organic pollution (Delgado et al., 2012).

Because of continuing new trends in environmental management policies, ecologists require new and effective tools to evaluate the current status of ecosystems and in turn facilitate effective management for conservation and restoration. In recent years, several researchers are trying to introduce molecular techniques to the bioindication field. These methods, that usually focus on DNA-based species identification and evaluation of genetic diversity, are the logical extension of the previously described techniques of measuring the variation of environmental status. (Sharley et al., 2004; Syaripuddin et al., 2015; Szabó et al, 2007). Actually, the identification at genus or species-level for macroinvertebrates and periphytons, for example, can be time consuming and even with high levels of taxonomic skill, misidentifications of species may still result. Thus the introduction of reliable genetic techniques could be an instrumental help in biotic index evaluations.

1.3. BATS AS BIOINDICATORS

Bats meet all the "criteria for a good bioindicator" described above. Their taxonomy is stable and there are already methods for easy sampling and identification. They are on every continent, except Antarctica, so they are geographically widespread and are among the mammal orders with higher diversity, with more than 1300 species (Fenton & Simmons, 2014). Thanks to this worldwide distribution, bats are adapted to different habitats and consequently they have different trophic needs: most of them are almost exclusively insectivorous, some species feed on small vertebrates, three species are hematophagous, while in tropic regions there are also many frugivorous and nectivorous species. There is evidence for a relationship between contamination or environmental alteration and trophic levels (Alleva et al., 2006), so bats could show evidence of pollution earlier than other taxa at lower trophic levels (e.g. invertebrates, that are commonly used as bioindicators) (Jones et al., 2009; McGeoch, 1998). Slow reproductive rates make bats ideal indicators for long term monitoring and for past disturbance, because their populations decline rapidly, but require a healthy environment and a long time to increase again in number (Jones et al., 2009). Bats cover different ecological niches and provide several ecological services. Pteropodids and phyllostomids are important in pollination and seed dispersal of several plants; insectivorous bats are important in agricultural systems as pest controllers, since they can eat a large amount of nocturnal insects even equal to their body mass per night (Boyles et al., 2011; Kurta et al., 1989; Jones et al., 2009; Puig-Montserrat et al., 2015; Williams-Guillén et al., 2008).

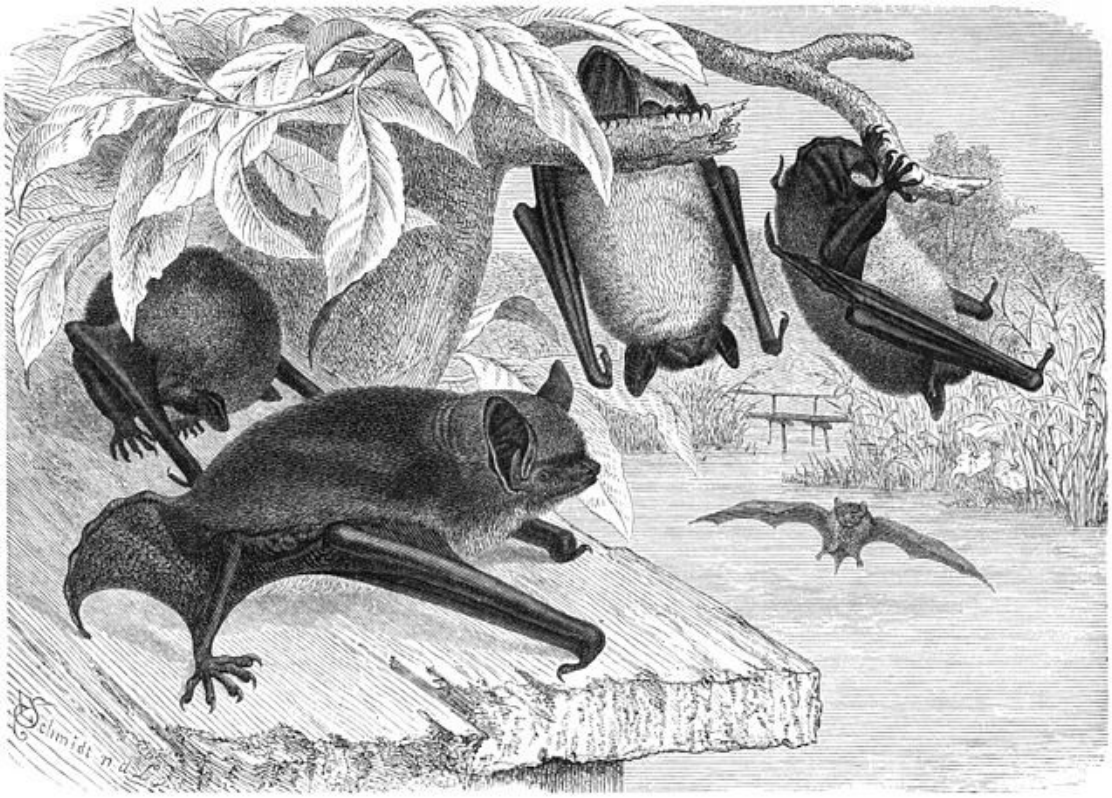


Figure 1. *Myotis daubentonii*, a vespertilionid bat specialized into riparian habitat (drawing of Brehms Tierleben, 1927)

Historically, bat populations were monitored with visual count at roosts. Modern techniques are mostly based on trapping with mist nets or harp traps (that allow to take morphometric measurements and non-invasive genetic measurements), radiotracking (to analyse habitat use and spatial use) and acoustic surveys, in which ultrasonic pulses emitted by bats to orient in the dark and detect prey are recorded (used for studying species presence/absence at a site, activity and foraging/drinking behaviour and habitat use). Acoustic surveys offer a non-invasive way of surveying bat distribution and habitat use, revealing species that often evade capture and would otherwise go unnoticed (Russo and Voigt, 2016). In terms of bioindication, acoustic surveys represent the most promising approach to surveying bat presence and activity as recordings may be carried out for long times using unattended recording systems triggered by bat ultrasonic pulses. The only drawbacks are given by bat species that broadcast faint calls, which may be overlooked, and the difficulty in separating species emitting similar calls (Russo and Voigt, 2016).

1.4. OBJECTIVES

For better understanding food web dynamics in riparian ecosystems, it is important to study and characterize trophic interactions between terrestrial and aquatic systems (Polis et al., 1997). Aquatic-emergent insects are key exporters of contaminants to terrestrial ecosystems (Runck 2007), thus insectivorous bats are a promising link between both of them. Several studies showed that bats depend strongly on water habitat, not only for drinking. As linear landscape elements, rivers are used as preferential pathways for movement and migration (Fenton & Thomas, 1985). Their general activity is higher on rivers and lakes than over other habitats and some species forage exclusively over water or close to riparian vegetation (Adams & Hayes, 2008; Almenar et al., 2009; Biscardi et al., 2007; Hagen and Sabo, 2011; Russo and Jones, 2003; Vaughan et al., 1997).

The main objective of this thesis was to test bats as bioindicators in river ecosystems. We decided to sample bat community over several rivers in Abruzzo and Campania region with passive listening points, to measure species composition, commuting, foraging and drinking activity. We also evaluated the ecological status by analysing the macrobenthic community, using the multimetric STAR_ICM index. Contemporary we calculated the Fluvial Functionality Index (IFF), which considers biotic and abiotic factors for a comprehensive survey of river and riparian ecosystem functionality. The entire set of analyses has been compared with qualitative (species composition) and quantitative information (i.e. species richness, commuting and foraging/drinking activity) obtained by bats monitoring, to verify the relationship between the degree of pollution detected and the bat activity and to determine if their response is proportional to that showed by an index based on a well known and already in use bioindicator, like macrobenthic community.

2. MATERIALS AND METHODS

2.1. FIELD WORK AND STUDY AREA

We selected five rivers in central-south Italy (Figure 2): the Sangro and Sagittario in Abruzzo (Figure 3) and the Calore Irpino, Tammaro and Tusciano in Campania region (Figure 4 and Figure 5). Various sources of environmental disturbance are present in each river basin or directly in the riverbed. These include organic and chemical pollution, intensive agriculture, tourism, channel modification, dams for hydroelectric power production, etc. The twenty-six sampling points are distributed along the whole river courses, from spring to mouth, ranging from 1225 metres to 31 metres above sea level and with an average spacing of approximately 10km. Each sampling point was named with the first three letters of the river name and an ascending number from spring to mouth (i.e. TUS1 is the first sampling point of Tusciano river, the one closest to the spring). Samplings were carried out between May and October; each site was sampled twice for both the macrobenthic (May-June and September/October) and bats communities (June/July and September). During the first session of macrobenthos sampling, we also completed the IFF form for each specific stretch.

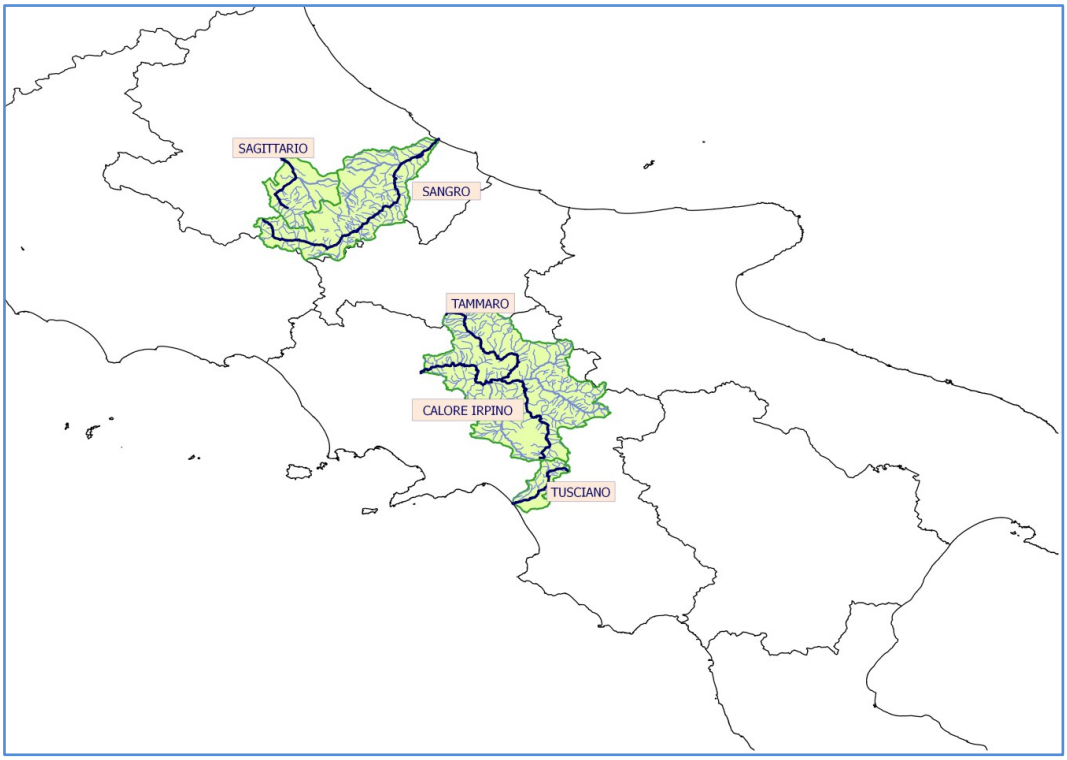


FIGURE 2. In this map are shown our studied rivers. Green areas indicate their basins. Black lines highlight sampled river courses.

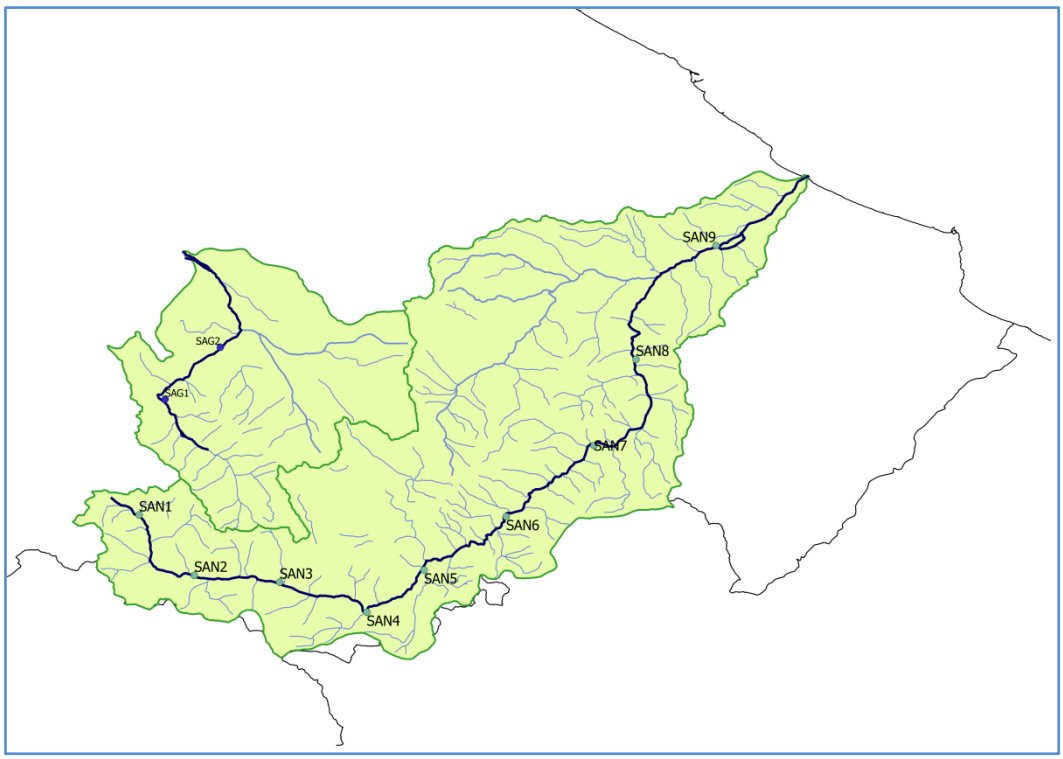


FIGURE 3. Sangro and Sagittario rivers and sampling points.

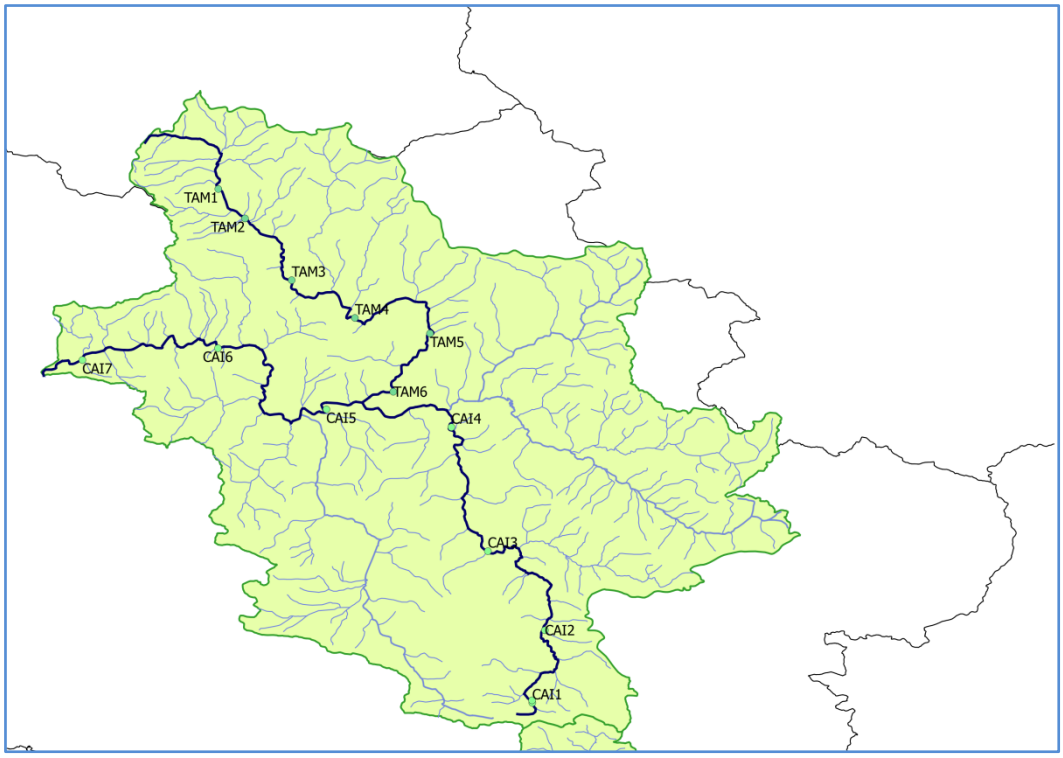


FIGURE 4. Tammaro and Calore Irpino rivers and sampling points.

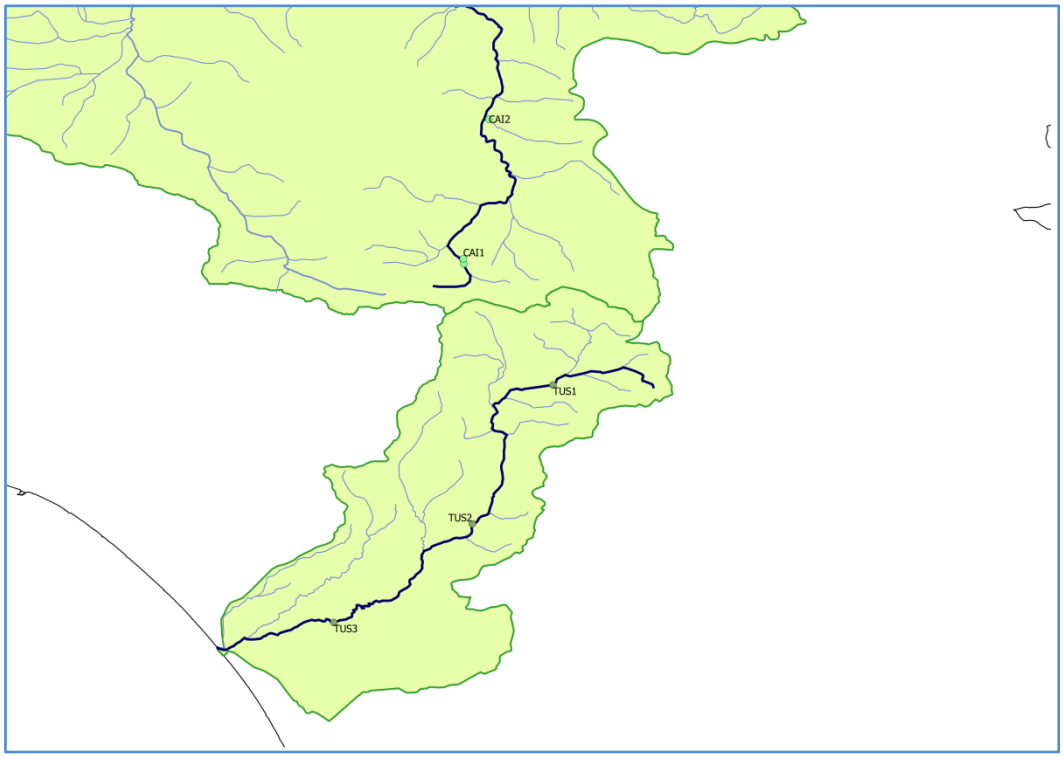


FIGURE 5. Tusciano river and its three sampling points.

2.2. BENTHIC MACROINVERTEBRATES SAMPLING

Macroinvertebrates were sampled using the proportionally distributed multi-habitat method for wadeable rivers, in order to obtain the ecological status classification with the STAR_ICM (Intercalibration Common Metrics) index (Buffagni & Erba, 2007; Buffagni & Erba, 2014; Buffagni et al., 2007). This is a standardised quantitative method, because there is a predetermined sampling area, divided in ten units of 0.05 m^2 or 0.1 m^2 depending on the relevant Hydro-Eco-Region (HER), defined as areas where general characteristics of aquatic ecosystems are highly comparable (Figure6) (Buffagni et al., 2006; Wasson et al., 2006). Our sampling sites belong to the HER 12, 13 and 18, which require a total sampling area of 0.5m^2. We used a Surber net (size 0.23 m × 0.22 m, mesh size 900 µm) to collect macroinvertebrates in the required units. These were allocated according to microhabitat percentage of occurrence, as visually estimated at each site before starting sampling. Two types of microhabitats were considered: abiotic and biotic, but only those with at least 10% of coverage in the sampling station. The first ones were classified according to the size and typology of riverbed rocks: macrolithal, microlithal, gravel, sand, concrete riverbed, etc. The biotic ones could be algae, emergent or submerged macrophytes, wood debris, Coarse Particulate Organic Matter – CPOM - or Fine Particulate Organic Matter – FPOM.

Figure 6. Italians Hydro-Eco-Regions : 01 - Western Alps; 02 - Prealps_Dolomites; 03- Central-Eastern Alps; 04 - Southern Alps; 05 - Monferrato (Piemonte region); 06 – Po valley; 07 – Carso (Karst); 08 – Piemonte Appennines; 09 – Mediterranean Alps; 10 – North Appennines; 11 – Tuscany; 12 – Adriatic coast; 13 – Central Appennines; 14 – Rome_Viterbo_Vesuvius; 15 – Southern Lazio; 16 – Basilicata_Tavoliere; 17 – Puglia_Gargano; 18 – Southern Appennines; 19 – Calabria_Nebrodi; 20 – Sicily; 21 – Sardinia (image taken from MacrOper software, Buffagni&Belfiore, 2013).

We sorted samples in the field, to primarily remove debris and stones and to count and identify macroinvertebrates taxa. Only a small percentage of organisms were preserved in 90% ethanol, if there were uncertain taxa or if we needed a detailed identification (genus or Operational Units for some Ephemeroptera groups). In the laboratory, we completed identification using taxonomical keys (Belfiore, 1983; Consiglio, 1980; Campaioli et al. 1999; Moretti, 1983; Rivosecchi, 1984; Sansoni, 1988;

Tachet et al., 2000). The invertebrate abundances were, then, pooled together to create a unique taxa-list for each site.

FIGURE 7. Sampling In Calore Irpino river. On the right, a Surber net.

For the evaluation of ecological status, we used MacrOper.ICM 1.0.5 software (Buffagni & Belfiore, 2013), which automatically calculates the final STAR_ICMi. This index is based on six different metrics (Table 1): ASPT (Average Score Per Taxon), Log_{10}_Sel_EPDT+1 (where EPDT is the sum of selected Ephemeroptera, Plecoptera, Diptera and Trichoptera taxa), 1-GOLD (where GOLD is the sum of Gastropoda, Oligochaeta, and Diptera), total number of families, total number of EPT (Ephemeroptera, Plecoptera, and Trichoptera) families and the Shannon-Weiner diversity index ($D_{S\text{-}W}$). These metrics are combined together (each metric with a specific weight) into the overall index score. Finally, this score is normalized, dividing it by that of pertinent reference site for each fluvial type (see Annex II of Water Framework Directive).

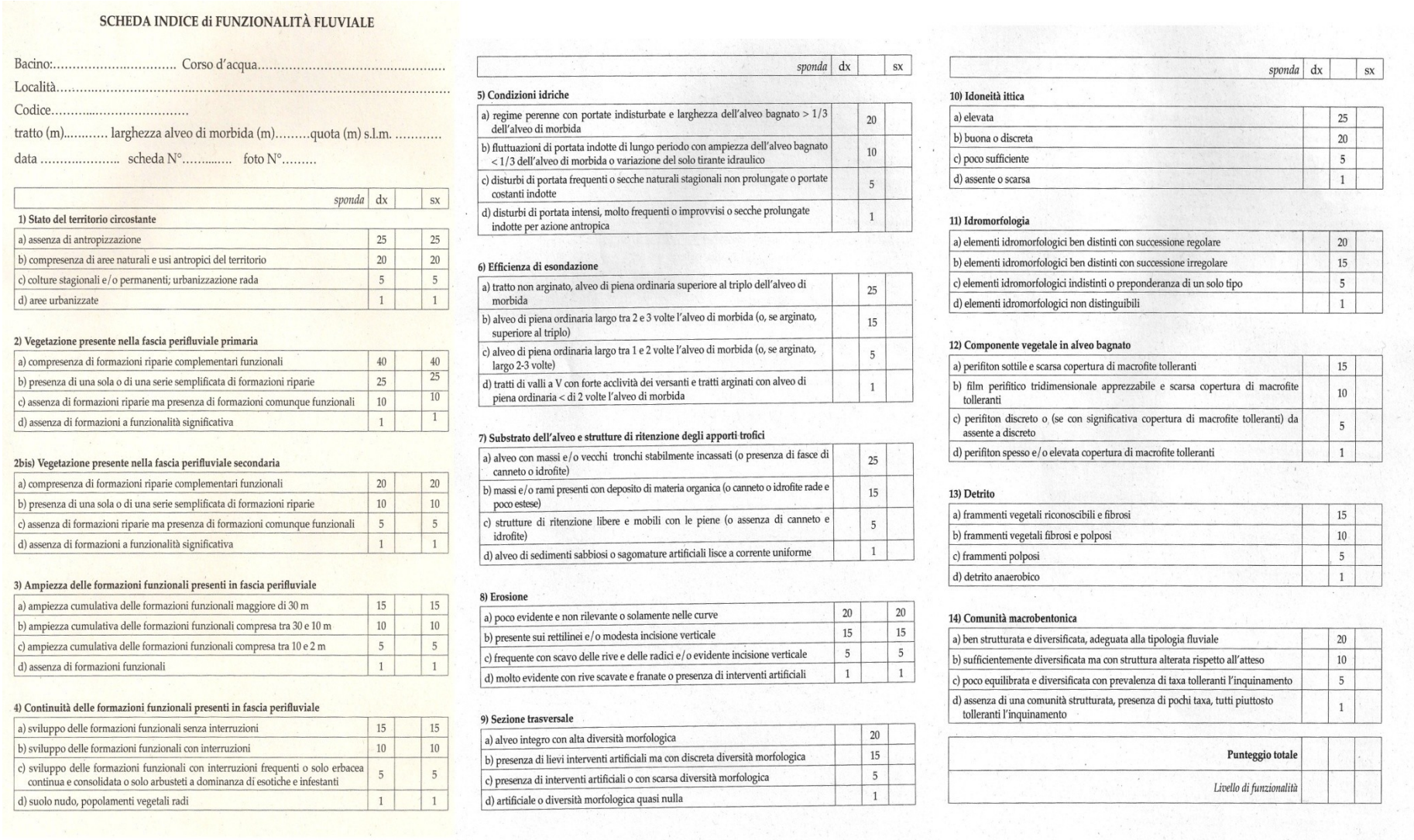
TABLE 1. Metrics Composing STAR_ICM index, and relative weights (Buffagni& Erba, 2007, Modified)

Metric	Considered Taxa	Weight
ASPT	Average Score Per Taxon	0,334
$\mathrm{Log}_{10}(\mathrm{Sel\_EPTD}+1)$	Log_{10}(Sum of abundance of Heptageniidae, Ephemeridae, Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae,Stratyomidae, Dixidae, Empididae, Athericidae and Nemouridae +1)	0,266
1-GOLD	1-(Relative abundance of Gasteropoda, Oligochaeta and Diptera)	0,067
Total number of family	Sum of all families found in a site	0,167
Number of EPT families	Sum of Ephemeroptera, Plecoptera and Trichoptera families	0,083
Shannon-Wiener diversity Index	$D_{S-W}=-\sum_{i=1}^{s}\left(\frac{n_i}{A}\right)*\ln\left(\frac{n_i}{A}\right)$	0,083

For each site, a quality class is attributed according to STAR_ICMi values range, as indicated for each river type in the Ministerial Decree 260/2010.

2.3. FLUVIAL FUNCTIONALITY INDEX

For each sampling point, we applied the Index of Fluvial Functionality (IFF) method, which integrates analyses of macroscopic abiotic aspects of river and surrounding territory with different biotic components (Siligardi et al., 2007). To obtain this index, we considered homogeneous stretches of the rivers, containing the different sampling points; we completed the form shown in figure 7, composed of 14 multiple-choice questions. For every question, there were four possible answers with different weight. The final sum of these weights resulted in the IFF score, which could range from a minimum of 14 (lower functionality) to a maximum of 300 (maximum functionality). Questions 1 to 4 are about the territorial and riparian vegetation characteristics; questions 5 and 6 relate to morphological characteristics of the riverbed; questions 7 to 11 examine structural and hydraulic aspects, going progressively toward a smaller scale; the last three questions consider biological components (Callegari et al., 2010). To answer the macroinvertebrates community question (14), we referred to the results of our samplings.



SCHEDA INDICE di FUNZIONALITÀ FLUVIALE

Bacino:..... Corso d'acqua.....

Località.....

Codice.....

tratto (m)..... larghezza alveo di morbida (m).....quota (m) s.l.m. .....

data ..... scheda N°..... foto N°.....

sponda	dx		sx
1) Stato del territorio circostante			
a) assenza di antropizzazione	25		25
b) compresenza di aree naturali e usi antropici del territorio	20		20
c) colture stagionali e/o permanenti; urbanizzazione rada	5		5
d) aree urbanizzate	1		1
2) Vegetazione presente nella fascia perifluviale primaria			
a) compresenza di formazioni riparie complementari funzionali	40		40
b) presenza di una sola o di una serie semplificata di formazioni riparie	25		25
c) assenza di formazioni riparie ma presenza di formazioni comunque funzionali	10		10
d) assenza di formazioni a funzionalità significativa	1		1
2bis) Vegetazione presente nella fascia perifluviale secondaria			
a) compresenza di formazioni riparie complementari funzionali	20		20
b) presenza di una sola o di una serie semplificata di formazioni riparie	10		10
c) assenza di formazioni riparie ma presenza di formazioni comunque funzionali	5		5
d) assenza di formazioni a funzionalità significativa	1		1
3) Ampiezza delle formazioni funzionali presenti in fascia perifluviale			
a) ampiezza cumulativa delle formazioni funzionali maggiore di 30 m	15		15
b) ampiezza cumulativa delle formazioni funzionali compresa tra 30 e 10 m	10		10
c) ampiezza cumulativa delle formazioni funzionali compresa tra 10 e 2 m	5		5
d) assenza di formazioni funzionali	1		1
4) Continuità delle formazioni funzionali presenti in fascia perifluviale			
a) sviluppo delle formazioni funzionali senza interruzioni	15		15
b) sviluppo delle formazioni funzionali con interruzioni	10		10
c) sviluppo delle formazioni funzionali con interruzioni frequenti o solo erbacea continua e consolidata o solo arbusteti a dominanza di esotiche e infestanti	5		5
d) suolo nudo, popolamenti vegetali radi	1		1

sponda	dx		sx
5) Condizioni idriche			
a) regime perenne con portate indisturbate e larghezza dell'alveo bagnato > 1/3 dell'alveo di morbida		20	
b) fluttuazioni di portata indotte di lungo periodo con ampiezza dell'alveo bagnato < 1/3 dell'alveo di morbida o variazione del solo tirante idraulico		10	
c) disturbi di portata frequenti o secche naturali stagionali non prolungate o portate costanti indotte		5	
d) disturbi di portata intensi, molto frequenti o improvvisi o secche prolungate indotte per azione antropica		1	
6) Efficienza di esondazione			
a) tratto non arginato, alveo di piena ordinaria superiore al triplo dell'alveo di morbida		25	
b) alveo di piena ordinaria largo tra 2 e 3 volte l'alveo di morbida (o, se arginato, superiore al triplo)		15	
c) alveo di piena ordinaria largo tra 1 e 2 volte l'alveo di morbida (o, se arginato, largo 2-3 volte)		5	
d) tratti di valli a V con forte acclività dei versanti e tratti arginati con alveo di piena ordinaria < di 2 volte l'alveo di morbida		1	
7) Substrato dell'alveo e strutture di ritenzione degli apporti trofici			
a) alveo con massi e/o vecchi tronchi stabilmente incassati (o presenza di fasce di canneto o idrofite)		25	
b) massi e/o rami presenti con deposito di materia organica (o canneto o idrofite rade e poco estese)		15	
c) strutture di ritenzione libere e mobili con le piene (o assenza di canneto e idrofite)		5	
d) alveo di sedimenti sabbiosi o sagomature artificiali lisce a corrente uniforme		1	
8) Erosione			
a) poco evidente e non rilevante o solamente nelle curve	20		20
b) presente sui rettilinei e/o modesta incisione verticale	15		15
c) frequente con scavo delle rive e delle radici e/o evidente incisione verticale	5		5
d) molto evidente con rive scavate e franate o presenza di interventi artificiali	1		1
9) Sezione trasversale			
a) alveo integro con alta diversità morfologica		20	
b) presenza di lievi interventi artificiali ma con discreta diversità morfologica		15	
c) presenza di interventi artificiali o con scarsa diversità morfologica		5	
d) artificiale o diversità morfologica quasi nulla		1	

sponda	dx		sx
10) Idoneità ittica			
a) elevata		25	
b) buona o discreta		20	
c) poco sufficiente		5	
d) assente o scarsa		1	
11) Idromorfologia			
a) elementi idromorfologici ben distinti con successione regolare		20	
b) elementi idromorfologici ben distinti con successione irregolare		15	
c) elementi idromorfologici indistinti o preponderanza di un solo tipo		5	
d) elementi idromorfologici non distinguibili		1	
12) Componente vegetale in alveo bagnato			
a) perifiton sottile e scarsa copertura di macrofite tolleranti		15	
b) film perifitico tridimensionale apprezzabile e scarsa copertura di macrofite tolleranti		10	
c) perifiton discreto o (se con significativa copertura di macrofite tolleranti) da assente a discreto		5	
d) perifiton spesso e/o elevata copertura di macrofite tolleranti		1	
13) Detrito			
a) frammenti vegetali riconoscibili e fibrosi		15	
b) frammenti vegetali fibrosi e polposi		10	
c) frammenti polposi		5	
d) detrito anaerobico		1	
14) Comunità macrobentonica			
a) ben strutturata e diversificata, adeguata alla tipologia fluviale		20	
b) sufficientemente diversificata ma con struttura alterata rispetto all'atteso		10	
c) poco equilibrata e diversificata con prevalenza di taxa tolleranti l'inquinamento		5	
d) assenza di una comunità strutturata, presenza di pochi taxa, tutti piuttosto tolleranti l'inquinamento		1	
Punteggio totale			
Livello di funzionalità			

Figure 8. The IFF form (Siligardi et al., 2007). The answers to each question have different weightings according to their different functionality levels.

2.4. ACOUSTIC RECORDINGS

We surveyed bats communities and their activity in each sampling point with stationary and automatically triggered bat detectors D500X (Pettersson Elektronik, Uppsala, Sweden). The D500X remotely records the ultrasonic spectrum up to 190 kHz, in real time without the presence of an operator and it is possible to leave it in the field for a long period of time. This device has a triggering system that allows it to automatically start recording as a sound is detected.

We recorded during warm nights, with air temperature always higher than 10°C, because insects become less active below this temperature (Rydell, Entwistle & Racey, 1996) and no or light wind. We recorded from sunset to dawn, leaving the D500X on the river side, as close as possible to water, oriented at 45° to vertical (Britzke et al., 2010; Wickramasinghe et al., 2003). We placed the recorder where riparian vegetation was not enclosed over the river course and next to smooth water surface, because ultrasonic noise produced by turbulent water could interfere with bat echolocation and prey detection (Greif and Siemers, 2010; Warren et al., 2000).

FIGURE 9. D500X bat detector located at one of the sampling points along the Sangro river shores. In the image A, the bat detectors 45° orientation is shown. In B, the way the detector faced the surface where bats foraged is shown.

We used the following recording settings for the two sampling sessions:

- 500 kHz sampling rate (this value would cover the entire frequency range of all bat species potentially encountered in Italy, up to the ca. 110 kHz of *Rhinolophus hipposideros*);
- 5 seconds of records length from trigger;
- 60 seconds of not recording interval after each record, to prevent oversampling the same bat passing;
- High pass filter enabled at 10kHz;
- Low trigger sensitivity, to avoid recording background noise.

Recordings were saved on Compact Flash cards as WAV files.

2.5. ACOUSTIC IDENTIFICATION

Acoustic identification of bat calls is a complex task, especially in diverse bat communities such as those of central and southern Italy. Although today a range of automated classifiers are available, their performances may be variable and difficult to assess (Russo and Voigt, 2016). For this reason we preferred to adopt a conservative approach to best balance taxonomic resolution vs. reliability in analyses. We used separate criteria to assess species richness and bat activity. All bat recordings were screened visually in BatSound 4.1. Oscillograms, power spectra and spectrograms were generated to measure call variables following Russo and Jones (2002). To generate spectrograms we used a 512-pt FFT Hamming window with a 98% window overlap. A species was recorded as present when at least one bat pass on each recording session showed either "typical echolocation calls" (i.e. echolocation calls whose frequencies, duration and frequency vs. time course allowed safe identification) or diagnostic social calls (Middleton et al., 2014; Nardone et al., in

press; Pfalzer and Kusch, 2003; Russ, 2012; Russo et al., 2009; Russo and Jones, 2002, 1999; Russo and Papadatou, 2014). Generally, "typical " calls are those broadcast respectively in the open by open space or edge specialists and in dense vegetation or near obstacles by clutter specialists. This approach inevitably underestimates the actual richness, but avoid false positives, which is especially desirable for a bioindication exercise such as ours. In this way we managed to recognize the following species or species groups (in brackets it is indicated the diagnostic parameter we used):

- *Rhinolophus ferrumequinum* (peak frequency);
- *Rhinolophus hipposideros*(peak frequency);
- *Rhinolophus Euryale* (peak frequency);
- *Myotis myotis/blythii* (peak frequency, call duration);
- *Myotis daubentonii* (social calls; frequency values in areas where *M. capaccinii* was not known to occur based on previous extensive mistnetting surveys);
- *Myotis emarginatus* (frequency values and bandwidth);
- *Myotis nattereri* (frequency values and bandwidth);
- *Myotis* sp. (steep FM spectrogram shape);
- *Plecotus auritus/austriacus* (spectrogram shape and presence of conspicuous harmonics);
- *Barbastella barbastellus* (presence of type 1, 2 calls; e.g. Denzinger et al., 2001);
- *Eptesicus serotinus* (spectrogram shape and end frequency value);
- *Nyctalus leisleri* (spectrogram shape and end frequency value + alternation of broadband and narrowband calls);
- *Nyctalus noctula* (spectrogram shape and end frequency value + alternation of broadband and narrowband calls);

- *Pipistrellus kuhlii* (end frequency and presence of social calls);
- *Pipistrellus pipistrellus* (end frequency and presence of social calls);
- *Pipistrellus pygmaeus* (end frequency and presence of social calls);
- *Hypsugo savii* (end frequency);
- *Miniopterus schreibersii* (end frequency and presence of social calls);
- *Tadarida teniotis* (end frequency).

To assess bat activity, we grouped together species whose calls show significant overlap and may therefore be misclassified. In this case, bat passes were categorized in bioacustic groups as follows: *Rhinolophus ferrumequinum*; *Rhinolophus hipposideros*; *Rhinolophus euryale*; *Myotis* sp.; *Plecotus auritus/austriacus*; *Barbastella barbastellus*; *Eptesicus/Nyctalus*; *Pipistrellus kuhlii/nathusii*; *Pipistrellus pipistrellus*; *Pipistrellus pygmaeus/Miniopterus schreibersii*; *Hypsugo savii*; *Tadarida teniotis*.

2.6. STATISTICAL ANALYSES

For statistical analyses, we selected as descriptive variables of bats community:

- Species richness: total species number in a site;
- Weighted species richness: at each site, we multiplied present species by the relevant IUCN Conservation Value Index (see Table2) and then summed the obtained values. This variable allowed us to highlight and give more emphasis to the presence of endangered species.
- Total activity: total number of bat passes at a site;
- Species activity: number of passes of each species at a site;
- Relative activity: number of passes of each species or acoustic group divided by the total passes at each site.

TABLE 2. According to IUCN red list category, we gave a conservation value index (Critically Endagered =CR; Endagered=EN; Vulnerable=VU; Near threatened= NT; Least Concern=LC; Data Deficient=DD)

Species	IUCN Red List Category	Conservation Value Index
Rhinolophus ferrumequinum	VU	3
Rhinolophus hipposideros	EN	4
Rhinolophus euryale	VU	3
Myotismyotis/blythii	VU	3
Myotis daubentonii	LC	1
Myotis emarginatus	NT	2
Myotis nattereri	VU	3
Myotis sp.		
Plecotus auritus/austriacus	NT	2
Barbastella barbastellus	EN	4
Eptesicus serotinus	NT	2
Nyctalus leisleri	NT	2
Nyctalus noctula	VU	3
Pipistrellus kuhlii	LC	1
Pipistrellus pipistrellus	LC	1
Pipistrellus pygmaeus	DD	1.5
Hypsugo savii	LC	1
Miniopterus schreibersii	VU	3
Tadarida teniotis	LC	1

We applied Generalized Linear Mixed-effects Models (GLMM) with lme4 package (Bates, 2010) in R software to analyse the influence of several factors on the chosen variables. We fit the model using sampling sites and rivers as "random effect factors", while our "response variables" were: STAR_ICMi, IFF, elevation, river width, session and night duration. Our "fixed effect factors" were: species richness, weighted species richness, total bats activity, species activity, relative activity of species, bioacoustic groups activity, relative activity of bioacoustic groups.

3. RESULTS

3.1. MACROBENTHIC COMMUNITY

In the two sampling sessions, we collected a total of 120,827 macroinvertebrates, belonging to 44 different families.

The mean number of macrobenthic families during spring was 27.3±6.8, while in autumn it was 28.7±6.7, and this difference was not statistically significant(t-test: t=-0.912 , p=0.37). But analysing one the of STAR_ICMi metrics, specifically the EPT families number, we saw that it was significantly lower in autumn (mean EPT I session=11.9±3.6; mean EPT II session=10.1±3.5; t-test: t=2.774, p=0.01) (see FIGURE 10). At the same time, the Shannon-Wiener diversity Index was significantly higher in autumn ($D_{S\text{-}W\ I\ session}$=1.74; $D_{S\text{-}W\ II\ session}$=2.04; t-test: t=-2.84, p=0.008) (see Figure 11).

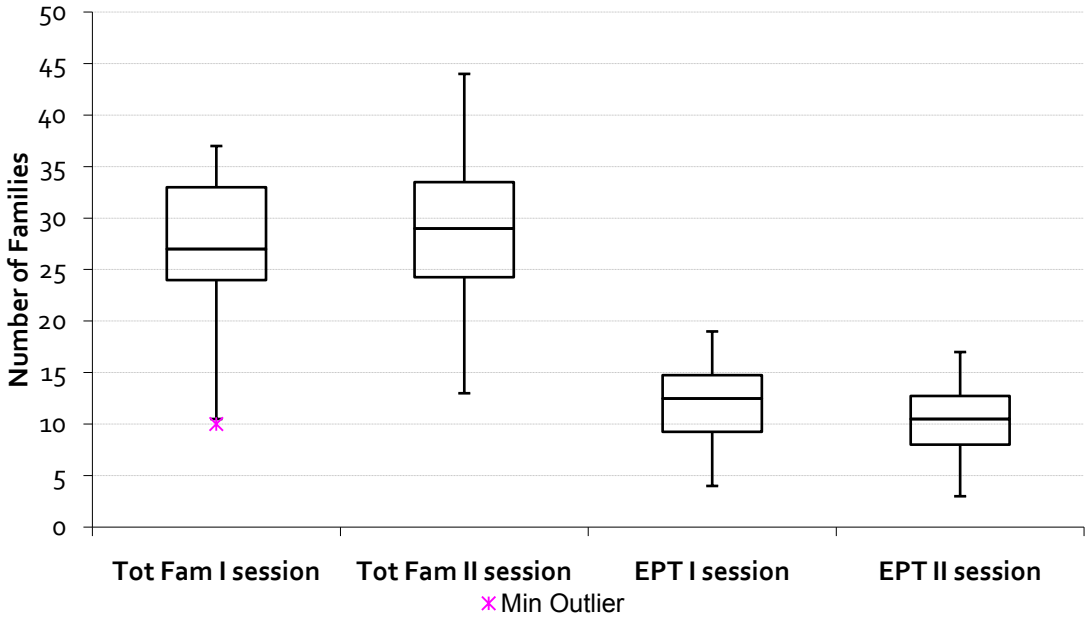


FIGURE 10. Box Plots showing differences in the total number of families and the sum of Ephemeroptera, Plecoptera and Trichoptera (EPT) families found in the two different sessions. The ends of the whiskers are set at 1.5*IQR above the third quartile (Q3) and 1.5*IQR below the first quartile (Q1). Pink asterisks indicate extreme lower values.

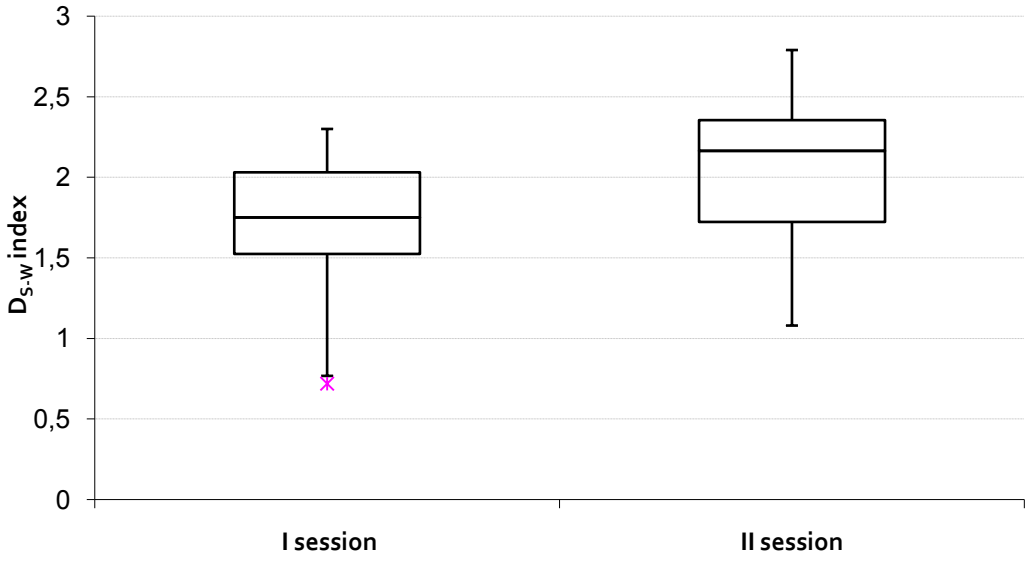


FIGURE 11. Box Plots of Shannon-Wiener Diversity Index in the two sampling session.

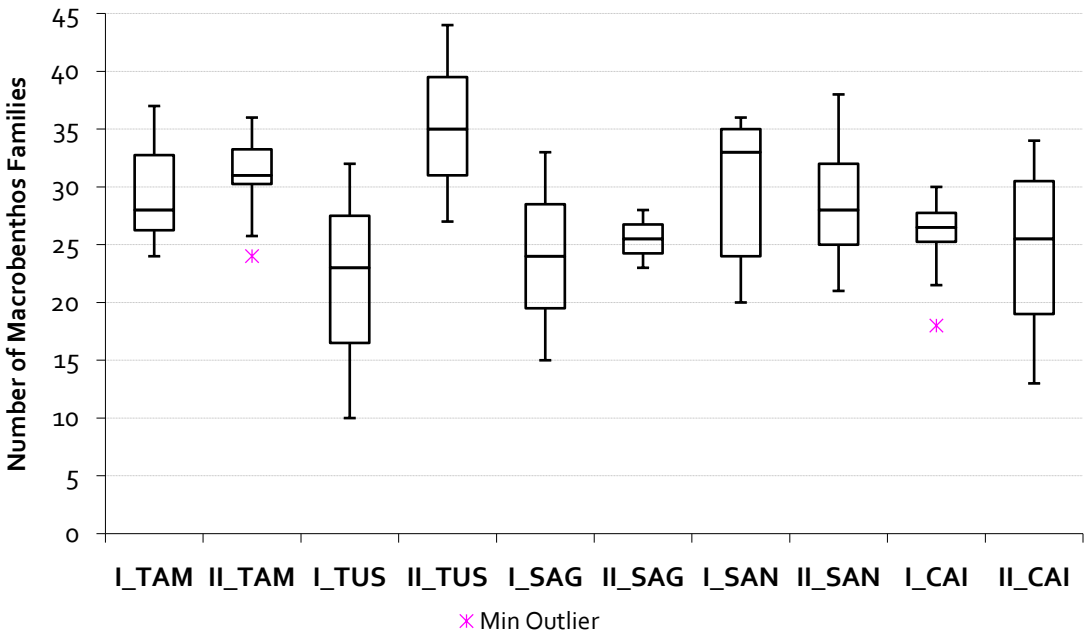


FIGURE 12. Box Plots showing differences in number of families found in the five rivers in the two different sessions. The ends of the whiskers are set at 1.5*IQR above the third quartile (Q3) and 1.5*IQR below the first quartile (Q1). Pink asterisks indicate extreme lower values.

Analysing the five rivers separately, we see different tendencies (figure 12). While the total number of families grows in Tammaro, Tusciano and Sagittario in autumn, it decreases in Sangro and Calore Irpino, which are the biggest rivers.

FIGURE 13. On the left, young of *Austropotamobius pallipes* found in SAN1 and on the right, *Epeorus alpicola* found in CAI1, in ventral and dorsal vision.

As illustrated by Table 3, the ecological status quality, evaluated with the application of STAR_ICMi, varies from 1 (High) to 4 (poor) in the different sampling seasons, but considering the average value, it goes only from 1 to 3 (moderate). In the Tammaro river there are always good values (2), except in TAM3. In the Tusciano and Sagittario rivers we found a gradual deterioration of biological community with decreasing elevation. The Sangro river has an opposite situation, with the lowest values (3=moderate) in SAN2, and high quality class in most part of its course. The Calore Irpino has a trend similar to that of the Tusciano and Sagittario during the first sampling season only. Looking at its average quality class, we cannot see a particular tendency, but only the predominance of the 3rd class.

TABLE 3. The STAR_ICMindex and associated quality class for the two sampling session and the average values considered for the definition of ecological status.

River	Site	STAR_ICMi I session	Quality class I session	STAR_ICMi II session	Quality class II session	Average STAR_ICM index	Average Quality class
Tammaro	TAM1	0.794	2	0.989	1	0.891	2
	TAM2	0.877	2	0.832	2	0.854	2
	TAM3	0.622	3	0.591	3	0.607	3
	TAM4	0.868	2	0.782	2	0.825	2
	TAM5	0.889	2	0.847	2	0.868	2
	TAM6	0.817	2	0.640	3	0.728	2
Tusciano	TUS1	1.047	1	0.974	1	1.011	1
	TUS2	0.739	2	0.992	1	0.866	2
	TUS3	0.480	3	0.638	3	0.559	3
Sagittario	SAG1	1.019	1	0.957	2	0.988	1
	SAG2	0.780	2	0.851	2	0.815	2
Sangro	SAN1	1.199	1	0.994	1	1.096	1
	SAN2	0.815	2	0.441	4	0.628	3
	SAN3	0.853	2	0.736	2	0.795	2
	SAN4	1.027	1	1.001	1	1.014	1
	SAN5	1.043	1	0.927	2	0.985	1
	SAN6	1.004	1	1.040	1	1.022	1
	SAN7	0.902	2	1.030	1	0.966	1
	SAN8	0.943	1	1.017	1	0.980	1
	SAN9	0.779	2	0.833	2	0.806	2
Calore Irpino	CAI1	0.974	1	0.939	2	0.957	2
	CAI2	0.742	2	0.664	3	0.703	3
	CAI3	0.846	2	0.810	2	0.828	2
	CAI4	0.774	2	0.336	4	0.555	3
	CAI5	0.492	3	0.717	2	0.604	3
	CAI6	0.638	3	0.534	3	0.586	3

The average ecological status judgements are summarised in figure 14, 15 and 16.

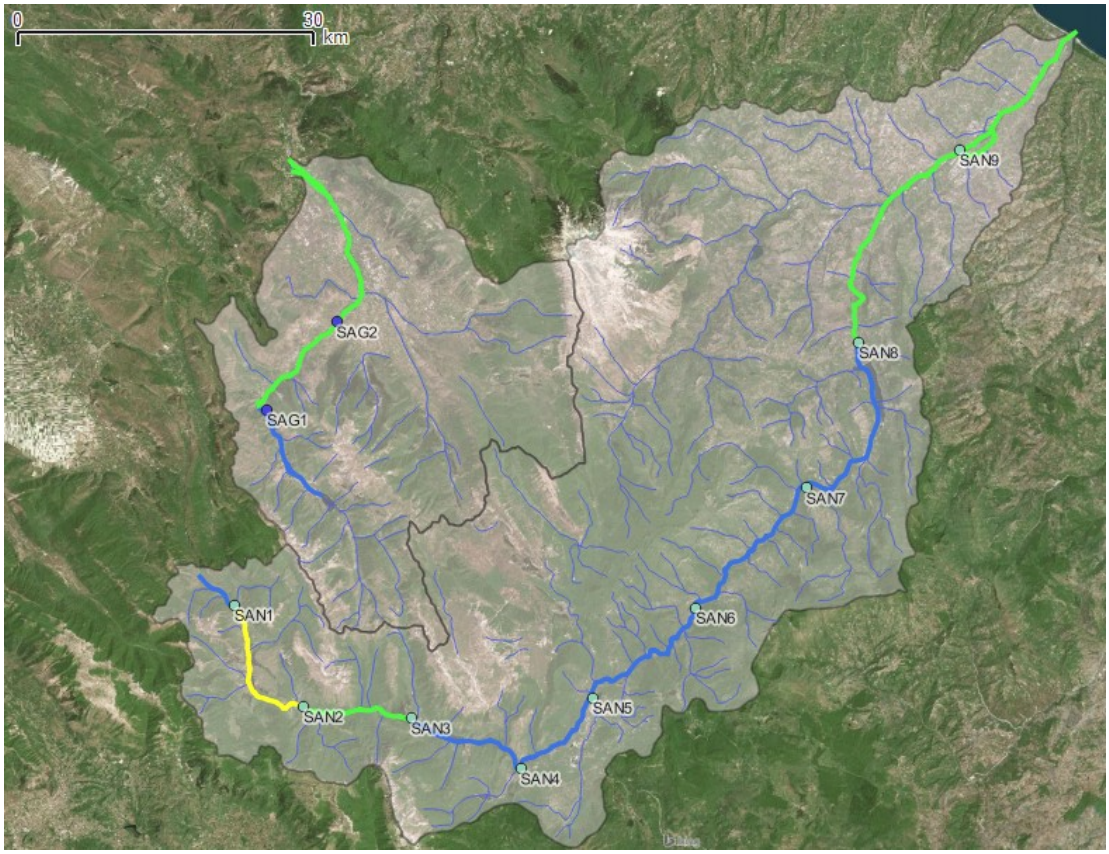


FIGURE 14. Map showing ecological status evaluation of the Sangro and Sagittario rivers. (Blue=excellent; green=good; yellow=moderate)

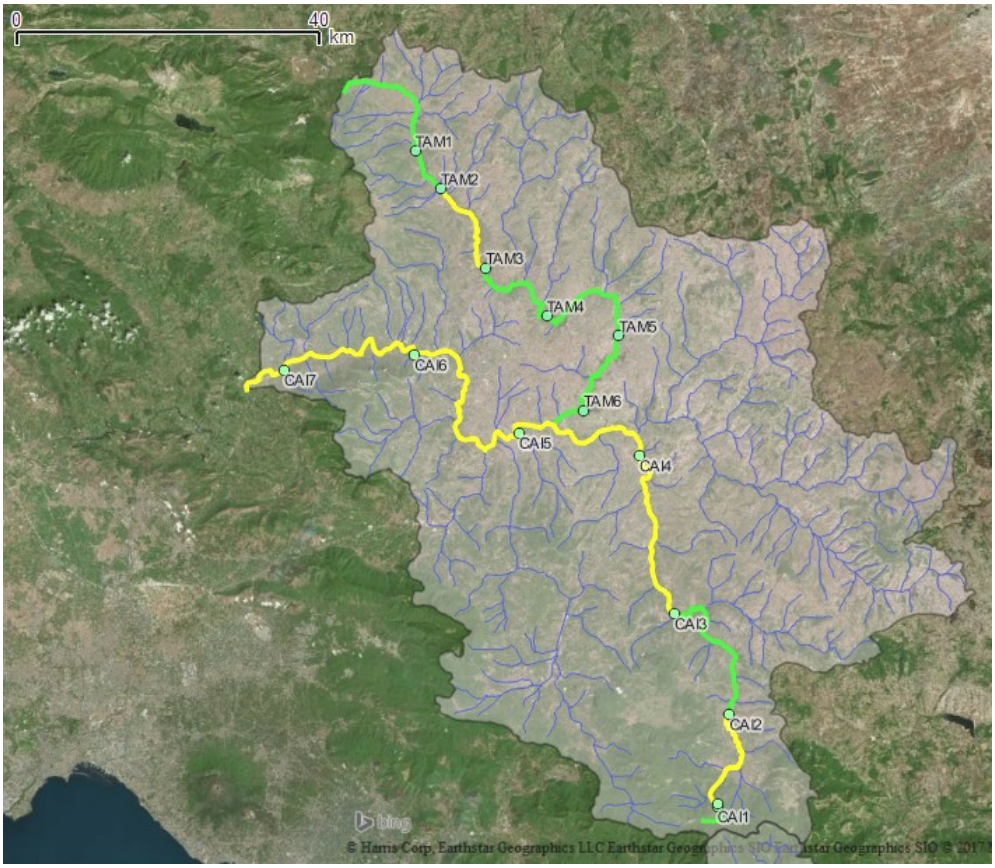


FIGURE 15. Map showing ecological status evaluation of the Tammaro and Calore Irpino rivers. (Blue=excellent; green=good; yellow=moderate)

FIGURE 16. Map showing ecological status evaluation of the Tusciano river. (Blue=excellent; green=good; yellow=moderate)

3.2. FLUVIAL FUNCTIONALITY INDEX (IFF)

In our sampling sites, we did not find excellent functionality levels (see Figure 17 and Table 5). Fifty % of site falls in II or II-III class. The highest values were 246, in CAI1 and 239 in SAG1, the lowest were reached in CAI3, with 111 and TUS3, with 118 respectively. The Tammaro, Tusciano and Sagittario rivers have a decresing functionality trend toward the mouth of the river. The Sangro river showed several ups and downs, while in the Calore Irpino the lowest values were found in the middle, increasing towards the mouth.

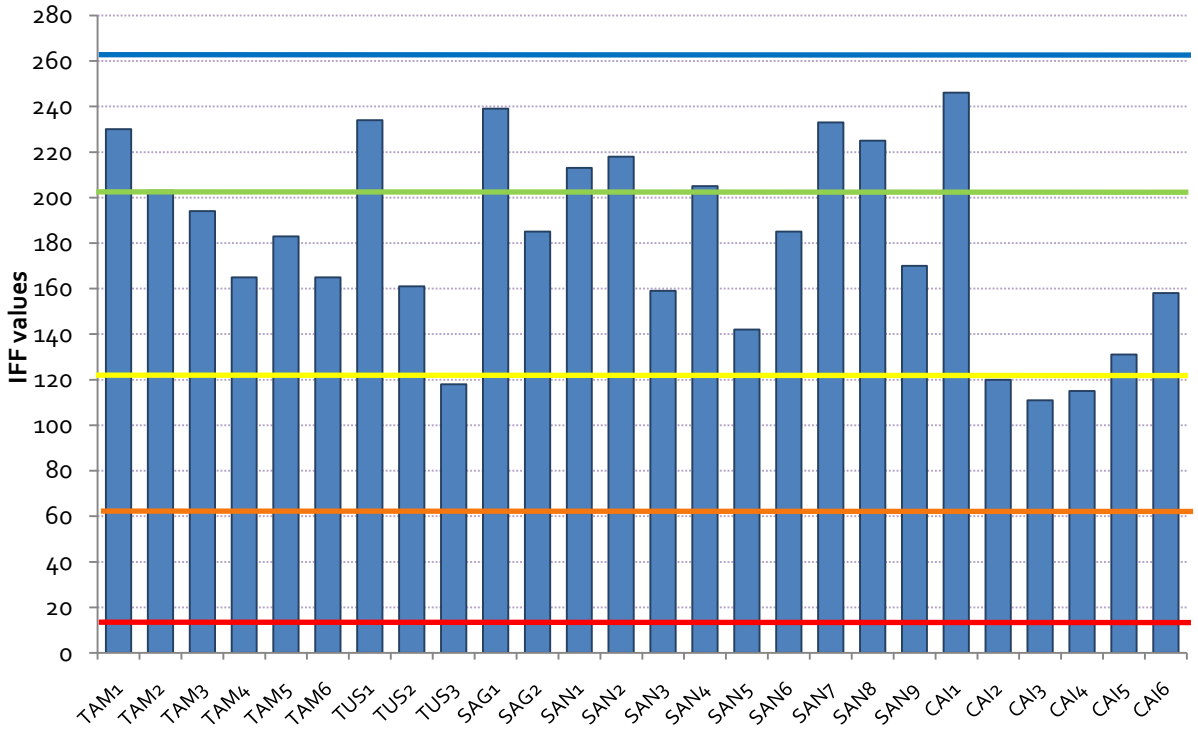


FIGURE 17. Bar diagram comparing IFF values for each site. Coloured lines indicate lower limits of functionality levels. Above the blue line is I level (excellent), values between blue and the green indicate II level (good), between green and yellow is III level (moderate), between yellow and orange is IV level (poor) and between orange and red lines is V level (bad). Intermediate levels between classes are not shown.

3.3. BAT RICHNESS AND ACTIVITY

We sampled bat communities twice in each of the 26 sites for a total of 561 hours of recordings which included 30,475 bats passes.

In our sampling points, we found 20 of the 35 Italian bat species. The most frequent species are *P. pipistrellus, P. kuhlii* and *H. savii*, since they have been found at every site (see Table 4, 5). *Myotis daubentonii*, recorded at every site except SAG1, was the species with the highest number of passes, 11264, followed by *P. pipistrellus* and *P. kuhlii*. The rarest species were *Rhinolophus euryale*, with only 5 passes recorded at CAI1 and TAM1, and *Myotis capaccinii*, with 5 passes recorded at TUS2, TUS3 and SAG1.

We had an average of about 8 bat species at each site in the two sessions. SAN1 has the lowest species richness, with only 3 species recorded during the first sampling night, followed by TUS2 and SAG2 with 4 species also during in the first session. Thirteen species were found at SAN2 during the second session and 12 in CAI1 and CAI3 in the first session.

A visual inspection of data did not reveal a relationship between the STAR_ICMi values or IFF values and bat species richness, which was later confirmed by the GLMM analyses. Indeed, we had an average STAR_ICMi of 1.096 in SAN1 and 1.022 in SAN6, where we had an average of 6.5 and 8 bat species respectively. At the same time, the highest IFF value (246 in CAI1), also had an average of 10 bat species, and at the lowest IFF (111 in CAI3), we had 9.5 species.

TABLE 4. Number of passes for each species in the five rivers and total bat activity (R.eur= *Rhinolophus euryale*; R.fer= *R. ferrumequinum;*R.hip= *R. hipposideros;* M.cap= *Myotis capaccinii*; M.dau= *M. daubentonii*; M.ema= *M. emarginatus*; M.m/b= *Myotis myotis/Myotis blythii*; M. mys= *M. mystacinus*; M.nat= *M. nattereri*; P.kuh= *Pipistrellus kuhlii*; P.pip= *Pipistrellus pipistrellus*;P.pyg= *P. pygmaeus*; N.lei= *Nyctalus leisleri*; N.noc= *N. noctula*;H.sav= *Hypsugo savii*;E.ser= *Eptesicus serotinus*; B.bar= *Barbastella barbastellus*; P.aus/aur= *Plecotus austriacus/Plecotus auritus*; M.sch= *Miniopterus schreibersii*; T.ten= *Tadarida teniotis*; Ept/Nyc= *Eptesicus/Nyctalus*)

River	*R.eur*	*R. fer*	*R. hip*	*M.cap*	*M. dau*	*M.ema*	*M. m/b*	*M.mys*	*M.nat*	*Myotis sp.*	*P.kuh*	*P.pip*	*P.pyg*	*N.lei*	*N.noc*	*H.sav*	*E. ser*	*B.bar*	*P.aus/ aur*	*M.sch*	*T.ten*	*Ept/Nyc*	TOT
Tammaro	1	6	205	0	3418	68	0	0	1	242	1516	2911	10	4	6	237	5	0	1	3	40	5	**8679**
Tusciano	0	1	99	4	721	0	1	0	0	15	1016	200	243	10	0	151	11	5	0	114	4	4	**2599**
Sagittario	0	0	2	1	14	4	10	0	19	3	107	61	0	5	1	33	0	45	5	0	0	1	**311**
Sangro	0	2	158	0	5539	36	3	85	245	139	775	3011	243	144	0	519	3	19	0	4	20	7	**10952**
Calore Irpino	4	1	6	0	1572	1	45	0	19	77	2510	1189	622	32	2	1285	22	7	2	428	76	34	**7934**
Total	**5**	**10**	**470**	**5**	**11264**	**109**	**59**	**85**	**284**	**476**	**5924**	**7372**	**1118**	**195**	**9**	**2225**	**41**	**76**	**8**	**549**	**140**	**51**	**30475**

TABLE 5. Summary table showing bat species found at each site in the two different sampling sessions and corresponding average STAR_ICMi and IFF values.

River	Site	STAR_ICM index	Quality class	IFF	Functionality level	Species richness I session	Species richness II session	Species I session	Species II session
Tammaro	TAM1	0.891	2	230	II	8	8	*R. ferrumequinum, M. daubentonii, M. emarginatus, P. kuhlii, P. pipistrellus, P. pygmaeus, H. savii, E. serotinus*	*R. euryale, R. hipposideros, M. daubentonii, M. emarginatus, P. pipistrellus, H. savii, T. teniotis, Eptesicus / Nyctalus*
	TAM2	0.854	2	203	II	9	8	*M. daubentonii, M. emarginatus, M. nattereri,P.s kuhlii/nathusii, P. pipistrellus, P. pygmaeus, H. savii, E. serotinus, T. teniotis*	*R. ferrumequinum, R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H. savii, M. schreibersii*
	TAM3	0.607	3	194	II-III	6	7	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, H. savii, P.s austriacus, T. teniotis*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, H. savii, M. schreibersii, T. teniotis*
	TAM4	0.825	2	165	III	8	7	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, N. noctula, H. savii, E. serotinus, T. teniotis*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, N. noctula, H. savii, E. serotinus*
	TAM5	0.868	2	183	II-III	5	5	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, H. savii, E. serotinus*	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H. savii*
	TAM6	0.728	2	165	III	7	7	*R. ferrumequinum, R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, H. savii, M. schreibersii*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii*
Tusciano	TUS1	1.011	1	234	II	7	8	*P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, E. serotinus, B. barbastellus*	*R. ferrumequinum, R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. Savii*
	TUS2	0.866	2	161	III	4	5	*M. capaccinii, P. kuhlii/nathusii, P. pipistrellus, H. savii*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. Pygmaeus*
	TUS3	0.559	3	118	III-IV	8	9	*M. daubentonii, M. myotis/blythii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, H. savii, E. serotinus, M.schreibersii*	*M. capaccinii, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, M.schreibersii, T. Teniotis*
Sagittario	SAG1	0.988	1	239	II	9	10	*M. capaccinii, M. emarginatus, M. nattereri, P. kuhlii/nathusii, N. leisleri, N. noctula, H. savii, B. barbastellus, P. austriacus, Eptesicus / Nyctalus*	*R. hipposideros, M. emarginatus, M. myotis/blythii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H.savii, B. barbastellus, P. Austriacus*
	SAG2	0.815	2	185	II-III	4	5	*M.nattereri, P. kuhlii/nathusii, P. pipistrellus, H. savii*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, H. Savii*

Sangro	SAN1	1.096	1	213	II	3	10	*M. daubentonii, P. pipistrellus, N. leisleri*	*M. daubentonii, M. emarginatus, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, B. barbastellus, T. teniotis*
	SAN2	0.628	3	218	II	8	13	*M. daubentonii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H. savii, E. serotinus, B. barbastellus*	*R. ferrumequinum, R. hipposideros, M. daubentonii, M.emarginatus, M. myotis/blythii, M. nattereri, P.kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, M. schreibersii, T. Teniotis*
	SAN3	0.795	2	159	III	7	10	*R. hipposideros, M. daubentonii, M. mystacinus, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, H. savii*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, B. barbastellus, M. schreibersii, T. Teniotis*
	SAN4	1.014	1	205	II	9	10	*R. hipposideros, M. daubentonii, M. mystacinus, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N.leisleri, H. savii*	*R. hipposideros, M. daubentonii, M. emarginatus, M. myotis/blythii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. Savii*
	SAN5	0.985	1	142	III	6	9	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii*	*R. ferrumequinum, M. daubentonii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H. savii, B. barbastellus, T. teniotis*
	SAN6	1.022	1	185	II-III	9	7	*R. hipposideros, M. daubentonii, M. emarginatus, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, M. schreibersii*	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, T. Teniotis*
	SAN7	0.966	1	233	II	11	9	*R. hipposideros, M. daubentonii, M. emarginatus, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, H. savii, E. serotinus, B. barbastellus, T. teniotis, Eptesicus / Nyctalus*	*R. hipposideros, M. daubentonii, M. emarginatus, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H. savii, B. barbastellus, T. Teniotis*
	SAN8	0.980	1	225	II	5	7	*M. daubentonii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, H. savii*	*R. hipposideros, M. daubentonii, M. myotis/blythii, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H. Savii*
	SAN9	0.806	2	170	III	6	6	*M. daubentonii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, H. savii, B. barbastellus*	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, H. savii, B. barbastellus, Eptesicus / Nyctalus*

Calore Irpino	CAI1	0.957	2	246	II	12	8	*R. euryale, M. daubentonii, M. myotis/blythii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, E. serotinus, B. barbastellus, M. schreibersii*	*R. euryale, M. daubentonii, P. pygmaeus, N. leisleri, E. serotinus, B. barbastellus, M. schreibersii, T. Teniotis*
	CAI2	0.703	3	120	III-IV	10	10	*R. hipposideros, M. daubentonii, M. myotis/blythii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, M. schreibersii, T. teniotis*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, B. barbastellus, M. schreibersii, T. Teniotis*
	CAI3	0.828	2	111	III-IV	12	7	*R. hipposideros, M. daubentonii, M. emarginatus, M. myotis/blythii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, N. noctula, H. savii, M. schreibersii*	*R. hipposideros, M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, M. Schreibersii*
	CAI4	0.555	3	115	III-IV	8	8	*R. hipposideros, M. daubentonii, M. myotis/blythii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, H. savii, M. schreibersii*	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, M. schreibersii, T. Teniotis*
	CAI5	0.604	3	131	III	6	8	*M. myotis/blythii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, H. savii, M. schreibersii*	*M. daubentonii, P. kuhlii/nathusii, P. pipistrellus, P. pygmaeus, N. leisleri, H. savii, M. schreibersii, T. Teniotis*
	CAI6	0.586	3	158	III	10	7	*M. daubentonii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, N. leisleri, H. savii, E. serotinus, B. barbastellus, P. austriacus, T. teniotis*	*M. daubentonii, M. myotis/blythii, M. nattereri, P. kuhlii/nathusii, P. pipistrellus, H. savii, T. Teniotis*

GLMM analyses showed (TABLE 6) that species richness values, weighted or not, are not linked to the ecological status expressed by STAR_ICMi or to river functionality level. Simple species richness is positively related only to elevation. Total activity (total number of bat passes) was negatively related to STAR_ICMi, i.e. more bat passes where habitat quality was lower. Activity of two species (*P. kuhlii* and *H. savii*) was negatively associated to elevation. For *P. pygmaeus/M. schreibersii* and *Eptesicus/Nyctalus* acoustic groups, absolute and relative activities (number of passes of the species divided by total number of passes in a site) of the former was negatively related to STAR_ICMi, while those of the latter were positively related to

IFF values. Relative activity of *P. kuhlii/nathusii* indicated a negative response to IFF, relation that not emerged from simple activity. *P. pipistrellus* absolute activity and *Myotis* sp. absolute and relative activities were only positively associated with river channel width.

TABLE 6. GLMM model results. For each fixed effect variable are shown along with F, p and t values. Significative values are shown in red (* if p<0,05; **if p<0,01; ***if p<0,001).

Fixed effect variable	Response variables	F value	p value	t value
Species Richness	STAR_ICMi	0.4790	0.4930	-1.514
	IFF	2.3710	0.1315	0.988
	Elevation	4.6920	0.0363*	1.984
	Width	0.8070	0.3744	1.035
	Duration	0.9990	0.3236	-2.146
	Session	2.5550	0.1178	2.222
Weighted species richness	STAR_ICMi	0.0030	0.9531	-0.557
	IFF	1.5850	0.2154	0.903
	Elevation	1.5670	0.2180	1.438
	Width	0.0080	0.9303	1.086
	Duration	6.0100	0.0187*	-2.332
	Session	3.7820	0.0489*	2.522
Total activity	STAR_ICMi	6.2680	0.0165*	-2.030
	IFF	0.0120	0.9150	1.313
	Elevation	0.0290	0.8657	0.299
	Width	11.6410	0.0015**	2.480
	Duration	0.0470	0.8288	-1.774
	Session	0.6660	0.4194	1.790
Pipistrellus pipistrellus	STAR_ICMi	0.6780	0.4147	-2.026
	IFF	3.3840	0.0726	2.477
	Elevation	0.0010	0.9704	-0.045
	Width	4.7530	0.0346*	1.700
	Duration	0.0030	0.9577	-1.322
	Session	1.9890	0.1655	1.310
Barbastella barbastellus activity	STAR_ICMi	1.4910	0.2286	
	IFF	2.9940	0.0906	
	Elevation	0.0530	0.8193	
	Width	1.1710	0.2851	
	Duration	0.5550	0.4603	
	Session	0.4070	0.5268	
Tadarida teniotis activity	STAR_ICMi	3.6800	0.0616	
	IFF	0.0810	0.7779	
	Elevation	0.1600	0.6911	

	Width	0.0270	0.8696	
	Duration	0.2360	0.6295	
	Session	0.0380	0.8462	
P. kuhlii/nathusii activity	STAR_ICMi	3.4350	0.0705	-0.025
	IFF	4.0160	0.0513	0.211
	Elevation	7.0440	0.0110*	-2.039
	Width	7.5660	0.0086**	2.299
	Duration	0.5480	0.4632	0.433
	Session	0.2150	0.6454	-0.522
H. savii activity	STAR_ICMi	2.0840	0.1550	-1.676
	IFF	0.5860	0.4480	2.042
	Elevation	4.0800	0.0495*	-1.252
	Width	8.4190	0.0058**	3.667
	Duration	3.4580	0.0697	-1.109
	Session	0.7400	0.3945	0.944
Myotis sp. activity	STAR_ICMi	0.248	0.6213	-1.137
	IFF	1.559	0.2185	1.413
	Elevation	3.409	0.0716	1.036
	Width	8.779	0.0049**	2.296
	Duration	0.006	0.9379	-1.668
	Session	3.942	0.0534	-1.680
P. pygmaeus/M. schreibersii activity	STAR_ICMi	20.2900	0.0000486***	-3.143
	IFF	2.9850	0.0910	-1.654
	Elevation	0.8010	0.3760	0.809
	Width	0.0400	0.8420	0.553
	Duration	0.0590	0.8090	-0.226
	Session	0.6320	0.4310	0.212
Eptesicus/Nyctalus activity	STAR_ICMi	1.7610	0.1913	-0.956
	IFF	5.7390	0.0209*	0.431
	Elevation	8.8860	0.0047**	2.586
	Width	2.1890	0.1461	-0.526
	Duration	0.2070	0.6510	-0.936
	Session	0.8510	0.3614	1.004
P. pipistrellus relative activity	STAR_ICMi	0.0000	0.9970	
	IFF	1.0590	0.3100	
	Elevation	0.0830	0.7750	
	Width	0.1250	0.7250	
	Duration	0.4280	0.5170	
	Session	0.0270	0.8700	
B. barballstellus relative activity	STAR_ICMi	1.5200	0.2250	
	IFF	2.8180	0.1010	
	Elevation	0.3250	0.5720	
	Width	0.0470	0.8300	
	Duration	0.0060	0.9390	

	Session	0.0660	0.7990	
Tadarida teniotis relative activity	STAR_ICMi	0.3760	0.5435	
	IFF	0.0620	0.8041	
	Elevation	0.1350	0.7154	
	Width	3.0490	0.0885	
	Duration	0.4440	0.5090	
	Session	1.7850	0.1891	
P. kuhlii/nathusii relative activity	STAR_ICMi	0.8780	0.3543	-0.338
	IFF	3.2310	0.0498*	-0.579
	Elevation	1.2240	0.2752	-2.344
	Width	2.0510	0.1599	-0.214
	Duration	0.0150	0.9034	-0.084
	Session	0.1070	0.7450	0.051
H. savii relative activity	STAR_ICMi	0.5740	0.4533	-0.358
	IFF	2.2990	0.1373	1.008
	Elevation	1.5850	0.2154	-1.196
	Width	1.0930	0.3020	1.329
	Duration	2.2600	0.1406	-1.300
	Session	5.4000	0.0253*	1.185
Myotis sp. relative activity	STAR_ICMi	3.262	0.0784	-1.137
	IFF	0.478	0.4933	1.413
	Elevation	0.042	0.8389	1.036
	Width	5.694	0.0218*	2.296
	Duration	0.000	0.9979	-1.668
	Session	0.043	0.8367	1.680
P. pygmaeus/M. schreibersii relative activity	STAR_ICMi	13.7950	0.0006***	-3.143
	IFF	2.1520	0.1502	-1.654
	Elevation	0.0010	0.9749	0.809
	Width	0.0440	0.8340	0.553
	Duration	0.0670	0.7972	-0.226
	Session	0.5060	0.4810	0.212
Eptesicus/Nyctalus relative activity	STAR_ICMi	0.0420	0.8394	-0.956
	IFF	4.2750	0.0452*	0.431
	Elevation	0.3010	0.5861	2.586
	Width	3.1370	0.0842	-0.526
	Duration	0.1530	0.6978	-0.936
	Session	7.2960	0.0101*	1.004

4. DISCUSSION

The objective of this study was to test if bats could be used as bioindicators for river habitats. Thus, to have a reliable benchmark, we evaluated the ecological status with an index, the STAR_ICMi, based on the analyses of benthic macroinvertebrates, which are accepted worldwide as good bioindicators of river quality (Armitege et al., 1983; Deborde et al., 2016; Sansoni, 1988), and for larger scale comparison, we applied the Fluvial Functionality Index (Siligardi et al., 2007).

Our biological monitoring indicated that the most parts of studied rivers have high or good water quality, but there are still some areas that have not reached "good" status, as targeted by the Water Framework Directive for the end of 2015. Along river courses there are several human-made interventions that limit river connectivity with surrounding territory and negatively compromise the self-depurative capability of water bodies.

While the average STAR_ICMi did not significantly change between the two sampling sessions, the composition of macrobenthic community did. We have a similar total number of families, but pollution sensitive taxa (comprised in Ephemeroptera, Plecoptera and Trichoptera, EPT, orders) generally decreased in the second sampling season and have been replaced by stress tolerant taxa. The number of individuals belonging to EPT families decreased, in favour of the total diversity of the sites, as expressed by the Shannon-Wiener diversity index, resulting in a more equal distribution of organisms in the community.

What we expected from bat community has not been entirely disregarded. Species richness does not seem to be related to river quality, but only to elevation as confirmed by McCain (2007) for temperate regions. This is probably because at higher altitude there is a greater abundance of well-structured riparian forests that can support greater prey diversity (Russo and Jones, 2003).

The variability of total bat activity is, on the contrary, negatively related to water quality. Different results were obtained by Abbott et al. (2009), who assessed the influence of sewage effluents on bat activity and benthic macroinvertebrates. In their case study, biological indices of water quality were lower downstream sewage outputs, while total bat activity did not significantly differ between upstream and downstream. Moreover, López-Baucells et al. (2017), contrary to our results, suggest that, only in the presence of high quality riparian forests in wide rivers, a greater level of bat activity might identify good quality ecosystems.

The same inverse relationship between bat activity and water quality was also found for both absolute and relative activities of the bioacoustic group composed by *Pipistrellus pygmaeus* and *Miniopterus schreibersii,* which are negatively correlated to water quality expressed by STAR_ICMi. These species are largely sympatric in the Mediterranean region (Russo and Papadatou, 2014) and emit similar echolocation calls, with a FM-QCF structure (Frequency Modulated signal followed by a Quasi-Constant Frequency tail) (see Russo and Jones, 2002), suited for open or edge spaces. For both of them, river ecosystems are dominant foraging sites (Russo and Jones, 2003), often followed by urban areas (Vincent et al., 2011).

Our data are consistent with what was found by Abbott et al. (2009) about *P. pygmaeus* activity in Irish rivers, but not with the results of a similar study conducted in South-West England (Vaughan et al., 1996). While the former found that *P. pygmaeus* was more active after sewage effluent, Vaughan found the opposite, arguing that *Pipistrellus pygmaeus* diet may be reliant on pollution-sensitive insects. However, the following year, in a study on bat foraging behaviour in different types of land uses, the same authors came to the hypothesis that feeding activity of *P. pygmaeus* may be linked to pollution tolerant insects (Vaughan et al., 1997). This was proven by subsequent studies, which showed that its diet mainly relies on nematoceran flies, Chironomidae and Ceratopogonidae specifically (Arnold et al, 2002; Barlow, 1997; Bartonicka et al., 2008; Kalko, 1995).

In our sampling sites, the total number of passes and the activity of *P. pipistrellus, P. kuhlii/nathusii, H. savii* and *Myotis* sp. were also positively related to the width of river

channel. This result is confirmed by literature (Biscardi et al., 2007; López-Baucells et al., 2017; Warren et al., 2000). We assume that this happens because river bed is larger at lower altitude and, without considering habitat quality, warmer air and water temperature improve growth and development of larval stage of some flying insects (Grindal et al., 1999; Walker& Cwynar, 2006). Besides, species such as *M. daubentonii* use the water surface as foraging site from which they trawl prey (Nardone et al. 2015), so wider channels simply mean more foraging surface is available.

For pure orthodoxy we decided to keep together *P. kuhlii/nathusii* since their echolocation calls are not distinguishable. However, *P. nathusii* in the study areas are either very rare or not present, so we may safely refer to this group as to *P. kuhlii* alone. Both *P. kuhlii* and *H. savii* are thermophilous species, more common at lower altitudes and are markedly synurbic (Ancillotto et al., 2014), so the negative response to elevation we found was expected. Relative activity of *P. kuhlii/nathusii* is also negatively linked to IFF values. Although rivers are still important habitat for *P. kuhlii* (Serangeli et al., 2012), IFF was likely to decrease in strongly anthropized landscapes, where *P. kuhlii* preferentially roosts. We therefore argue that the habitat effect we found was in fact associated with landscape effects which we did not control in this study.

The *Eptesicus/Nyctalus* bioacoustic group is the only one that is positively linked to fluvial functionality. *Nyctalus leisleri,* the most common species of *Nyctalus* genus, forage at heights up to 150 m and insects at this altitude may not be dependent on the habitat feature on the ground (Russ et al., 2003). Vaughan et al., (1997) found that activity of species belonging to these genera is influenced by different land use, so we can argue that they respond to habitat variation at large scale, just like those expressed by IFF. Alternatively, riparian trees such as *Salix alba* or *Populus* may bear cavities used as roosts by these species, so the link with IFF might reflect roost availability.

As also shown by a recent study (López-Baucells et al., 2017), we found that water quality does not have a significant effect on commuting activity of the *Myotis* group,

mostly represented by *Myotis daubentonii* in river habitats. This species is highly dependent on aquatic insects (Flavin et al., 2001; Vaughan, 1997) and seems to benefit from an increasing availability of pollution-tolerant insects, mostly Diptera, due to eutrophication of inland waters (Kokurewicz, 1995). *Myotis daubentonii* widely vary its feeding habits, both geographically and temporally (during the year), proving to be a highly generalist and opportunistic species that can successfully exploit river stretches with different environmental conditions. With our study, based only on bioacoustic recognition of species, we could not get data on animal's body conditions or sex, that would also provide valuable information on habitat productivity and quality (Nardone et al., 2015; Russo, 2002). This species shows marked intersexual segregation, with females confined to lower elevation, where food resources are more abundant, along with some males, whereas other males only occur at higher altitudes (Nardone et al., 2015; Russo, 2002). Body condition of higher elevation males is generally lower in response to the more oligotrophic habitat they use, but such condition may fluctuate over the year.

5. CONCLUSION AND FUTURE PROSPECTS

Bats are often deemed potentially powerful bioindicators because of their high biological diversity, high taxonomic stability, and sensitivity to human actions (Jones et al., 2009; Russo and Jones, 2015), yet very few studies have so far attempted to test the actual responses of bat communities to differences in habitat quality and only one (López-Baucells et al., 2017) has attempted to use them to bioindicate riparian habitat quality. An important difference between our study and the former, however, is that while Lopez-Baucells et al. (2017) only looked at responses by one species (*M. daubentonii*), we employed a more comprehensive approach and examined the entire bat community. Since bat assemblages feature species known to have different sensitivity towards habitat quality and whose ecomorphology (especially wing profile) and echolocation calls are tailored to pursue different prey size and respond to different habitat structures, a multi-species approach is highly advisable in hope to highlight different reactions.

On the positive side, we detected non-random responses of bats to riverine habitat quality, although mostly inversely related to the latter. This is not particularly surprising since bats have outstanding metabolic requirements (a single bat may consume an insect biomass equivalent of up to its own body mass per night) so only insect-rich habitats may support significant bat activity. That is the case with lower-quality riparian sites, where swarming dipterans abound in response to eutrophication. The other side of the coin, which represents a significant impediment to using bats as bioindicators, is the absence of bat species bio-marking specific habitat conditions, i.e. exclusive to them. Again, this was somewhat expected given the well-known ecological flexibility of many bat species, especially those whose echolocation calls are FM-CF shaped, such as pipistrelloid and nyctaloid bats, which made up for most of our sample.

One limitation of this study is that the area we considered included only rivers with a limited variability of pollution levels, whereas a steeper environmental gradient would have been desirable to reveal currently undetected reactions to extreme situations (highly polluted rivers). My next aim is to collect more data from extreme quality situations in order to increase the pollution gradient and seek potential patterns that have not emerged yet.

The usage of a bioacoustic approach for bioindication has pros and cons. Bats are elusive, difficult to catch, so mistnetting would have probably underestimated richness and activity and overlooked species that are particularly good at evading capture. On the other hand, the taxonomic resolution conferred by acoustic surveys is inevitably coarse, and liberal identification quite often leads to misclassification (Russo and Voigt, 2016) which would be disastrous in any bioindication campaign. Moreover, the big advantage of obtaining large amount of data quickly from recordings is heavily counterbalanced by the time consumed to screen recordings and identify species as well as by the high equipment costs and the need of bat specialists for reliable species recognition. An average time of two years is needed to acquire sufficient practice of bat call identification (D. Russo, pers. comm..), so the approach is definitely out of reach for most staff of public agencies, reserve rangers or volunteers recruited for large-scale campaigns. This said, we are still positive on the fact that according to our results it is possible to lay the foundations for a bioacoustic index based only on the analyses of total activity or bioacoustic group activity, which could be successfully applied by other than bat experts, removing one of major limits of acoustic surveys due to detailed species identification. The ever-growing technology in this field promises that in the near future relatively cheap, automated recorders will be available, so we are confident that also problems related to the costs of this approach will be mitigated.

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