

Original Articles

Testing the performance of bats as indicators of riverine ecosystem quality

Carmelina De Conno^a, Valentina Nardone^a, Leonardo Ancillotto^a, Salvatore De Bonis^b,
Marco Guida^c, Inês Jorge^d, Ugo Scarpa^a, Danilo Russo^{a,e,*}



^a Wildlife Research Unit, Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Università via, 100, 80055 Portici (Napoli), Italy

^b Agenzia Regionale Protezione Ambientale Lazio, Sezione Provinciale di Frosinone, Servizio risorse idriche e naturali – suolo, rifiuti e bonifiche, Unità risorse idriche, via Armando Fabi, 212, 03100 Frosinone, Italy

^c Dipartimento di Biologia Strutturale e Funzionale, via Cinthia, 80126 Napoli, Italy

^d Faculty of Science, University of Porto, Rua Campo Alegre, 4169-007 Porto, Portugal

^e School of Biological Sciences, Life Sciences Building, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ, United Kingdom

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ABSTRACT

Rivers host a wealth of biodiversity and play critical ecological functions, so monitoring their environmental status and tracking its changes has paramount importance for appropriate management. Although some biological groups, and especially benthic macroinvertebrates, are employed routinely to achieve this goal, the quest for bioindicators of river quality is far from over, because finding further suitable organisms may improve indication performances and inform habitat management. Using organisms that are at risk for bioindication also fulfills the goal of providing important information for the conservation of the taxon (or taxa) used for bioindication. Bats are a diverse, threatened mammal group whose characteristics make them potentially suitable bioindicators in many ecosystem types, but research in this field is still limited. In this study, set in Central and Southern Italy, we hypothesize that assemblages of foraging bats will respond to environmental status and quality of riverine ecosystems and that therefore bats may serve as effective bioindicators. We established the environmental status of 50 sampling sites selected along 10 rivers using two indices officially adopted in the country, i.e. the STAR_ICMi (evaluating water quality from macrobenthic invertebrate assemblages) and the fluvial functionality index (*Indice di Funzionalità Fluviale*, IFF), which incorporates several biotic and abiotic components and represents a functional indicator of river ecosystem health. At the sampling sites, we also recorded bat activity with operator-independent real-time bat recorders and classified bat passes to species or phonic groups. We examined 167,371 macroinvertebrates and 55,157 bat passes, corresponding to 15 species or phonic groups. The activity of *Miniopterus schreibersii*/*Pipistrellus pygmaeus* and *Myotis daubentonii/capaccinii* declined with increasing values of STAR_ICMi and IFF, while the activity of *Nyctalus/Eptesicus serotinus* increased with both indices. The activity of *P. kuhlii* also declined as IFF values increased, while we observed the opposite for *Pipistrellus pipistrellus*, *Myotis emarginatus*, *Myotis nattereri* and *Barbastella barbastellus*. Pooling together species whose activity respectively increased or decreased as the values of quality indices increased improved indication performances by strengthening statistical significance. Our work constitutes a significant step towards the use of bats as bioindicators in river ecosystems as we show that differences in bat activity may reveal changes in environmental conditions and may thus demonstrate the effects of habitat alteration on the river biota. We highlight that locally adapted bat populations may show differences in foraging behaviour and food preferences; hence our findings warrant confirmation from other regions. Further constraints are given by the variable degree of taxonomic resolution achieved in bat sound analysis, which may represent an issue especially in species-rich bat assemblages such as those of southern Europe.

1. Introduction

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,

* Corresponding author at: Wildlife Research Unit, Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Università via, 100, 80055 Portici (Napoli), Italy.

E-mail address: danrusso@unina.it (D. Russo).

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2010). Freshwater ecosystems also provide several services, fundamental for human settlements and productive activities (both agriculture and industrial). On the other hand, human activities apply 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 the ecological and economic importance of these habitats implicated large efforts around the conservation and restoration of river environments, especially in the last few decades (e.g. Darby and Sear, 2008).

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 groups with their sensitivity/tolerance to pollution (Armitage et al., 1983; Buffagni and Erba, 2014; Sansoni, 1988). Bioindication is now a necessary supplement to traditional monitoring techniques for riparian ecosystems and is required by legislation such as the Water Framework Directive of the European Union (European Parliament, 2000).

Aquatic macrobenthic organisms have been analyzed for this purpose since the 1960s and are now considered the most suitable as an indicator of water quality (Furse et al., 2006). They are widespread, easy to sample and identify, cover a broad range of trophic levels and pollution tolerances, exhibit fast, taxon-specific reactions to pollutants, and have low mobility: thus, their responses are representative of site-specific conditions (Barbour et al., 1999; Sansoni, 1988). Most biotic indices, developed on species-specific sensitivities and tolerances, are used for monitoring eutrophication, acidification and organic pollution (Delgado et al., 2012). 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). Several bioindication indices have been developed in various countries, e.g.: the Trent Biotic Index (Woodiwiss, 1964), later modified in the Extended Biotic Index (EBI) (Woodiwiss, 1980); the Saprobity Index (SI) (DIN38 410-2, 1990); the Biological Monitoring Working Party Score System (BMWP) (Chesters, 1980); the Average Score per Taxon (ASPT) (Armitage et al., 1983); and the multimetric STAR_ICMi index (Buffagni et al., 2007; Buffagni and Erba, 2014).

The quest for bioindicators of river quality is, however, far from being over, because finding further suitable organisms may speed up or refine monitoring and help improve habitat management. Using organisms whose conservation status also needs to be monitored as a bioindicator is fulfilling two needs with one deed. From this perspective, bats would prove ideal candidates because in many cases they are at risk (e.g. O'Shea et al., 2016; Conenna et al., 2017; Welch and Leppanen, 2017) and thus are in need of systematic monitoring: in Europe, monitoring bat conservation status is an obligation arising from Article 11 of 92/43/EEC "Habitats" Directive. Moreover, bats meet all the criteria for a suitable bioindicator (Jones et al., 2009; Russo and Jones, 2015). Bats are on every continent, except Antarctica, so they are geographically widespread and are among the mammal orders with higher diversity, with > 1300 species (Fenton and Simmons, 2014). Thanks to this worldwide distribution, bats are adapted to different habitats and consequently, they have different trophic needs (Altringham, 2011). Because of their position high in trophic webs, bats could react to pollution faster than other taxa, such as invertebrates (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 suitable environmental conditions and a long time to increase again in number (Jones et al., 2009; Russo et al., 2017). On the other hand, foraging activity is likely to react promptly to fluctuations in insect prey availability driven by habitat changes – in fact, insectivorous bats have been found to track changes in insect availability (Fukui et al., 2006; Hagen and Sabo, 2012).

For better understanding food web dynamics in riverine ecosystems, it is important to 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 these systems. Moreover, bats depend strongly on water habitats. Their foraging activity is typically higher over rivers and lakes than in other habitats and some species forage exclusively over water or close to riparian vegetation (Adams and Hayes, 2008; Almenar et al., 2009; Biscardi et al., 2007; Hagen and Sabo, 2011; Russo and Jones, 2003; Vaughan et al., 1997). Several bat species follow rivers as preferential pathways for movement and migration (e.g. Fenton and Thomas, 1985; Serra-Cobo et al., 2000; Furmankiewicz and Kucharska, 2009) and many use rivers and streams as a source of drinking water (Korine et al., 2016).

Bats are therefore in most cases likely to show clear responses to the quality of riverine habitats, as shown by several studies whose primary aim was to inform bat conservation (Vaughan et al., 1996; Biscardi et al., 2007; Kalcounis-Rueppell et al., 2007; Naidoo et al., 2013; Salvarina, 2016). Fewer studies considered the potential implications of such responses for bioindication in rivers. Two studies (Langton et al., 2010; López-Baucells et al., 2017) adopted a single species approach focused on the vespertilionid bat *Myotis daubentonii* to explore responses to river quality. In England and Wales, this species was more active on larger waterways with more surrounding woodland, with a broad variation possibly caused by site-specific factors (Langton et al., 2010). The study also showed a positive association of *M. daubentonii* activity with good water quality as expressed by macroinvertebrate diversity. However, López-Baucells et al. (2017) concluded that, for the Iberian Peninsula, *M. daubentonii* might complement other bioindicators, but cannot be used alone for evaluating riparian ecosystem conditions. Noticeably, in a study conducted in North Carolina (Li and Kalcounis-Rueppell 2017) the activity of different bat species showed different responses to water quality at a landscape scale, i.e. water quality could be used to predict which bat species occur in a given landscape when local studies are lacking. Assessing community-scale responses might, in fact, provide better performances (Li and Kalcounis-Rueppell, 2017) and open new avenues for practical applications.

In this study, set in Central and Southern Italy, we tested the responses of bat assemblages to the quality of riverine habitat and explored the potential role of bats as bioindicators in river ecosystems. We hypothesized that bat activity will differ according to riverine habitat quality and that given the different degree of ecological flexibility expressed by the various bat species, responses will be species-specific. We also aimed to establish sets of species that best characterize river health, grouping them together in order to increase their indication performances regardless of their taxonomic or ecological relatedness.

Instead of relying on published maps of river quality, we evaluated it at the same sites where we surveyed bat activity. To assess the ecological quality of rivers, we adopted a dual approach. First, we analysed the macrobenthic community, using the STAR_ICMi (Buffagni et al., 2007; Buffagni and Erba, 2014), the multimetric index now in use in Italy, based on a quantitative multi-microhabitat sampling method. We also calculated the Fluvial Functionality Index (*Indice di Funzionalità Fluviale*, hereafter IFF), which considers biotic and abiotic factors for a comprehensive survey of the river and riparian ecosystem functionality (Siligardi et al., 2007; Siligardi and Cappelletti, 2008). We then assessed bat presence and activity through acoustic surveys done at the same sampling points and tested whether species activity of riverine bat assemblages is associated to changes in the values of STAR_ICMi and IFF, whose bioindication performances are well known and reliable.

2. Materials and methods

2.1. Study area and sampling schedule

Fieldwork took place in May to October 2015 and 2016 on ten rivers in Central and Southern Italy: the Aventino, Sangro, and Sagittario Rivers in the Abruzzo Region, the Calore Irpino, Calore Salernitano,

Sabato, Sele, Tammaro and Tusciano Rivers in the Campania Region and the Volturno River between the Molise and Campania Regions. We selected 50 sampling points located along the whole river courses, from spring to mouth, at altitudes between 25 and 1,216 m a.s.l. and on average approximately 10 km apart. We sampled each site twice for both the macrobenthic invertebrates (May–June and September–October) and bats (June–July and September). During the first session of macrobenthos sampling, we also determined IFF for each specific stretch.

2.2. Benthic macroinvertebrates

At each site, we sampled macroinvertebrates along 20 m river stretches using a multi-microhabitat method for wadable rivers as required for the STAR_ICM (Intercalibration Common Metrics) index (Buffagni and Erba, 2007; Buffagni and Erba, 2014; Buffagni et al., 2007), the standardized quantitative sampling method used in Italy in accordance with the European Water Framework Directive (2000/60/EEC). As required by this method, we first visually estimated the percentage of occurrence of the different microhabitats in the selected stretch of riverbed. We considered two microhabitat components: abiotic and biotic. Abiotic components are classified according to the size and typology of riverbed rocks: megalithal, macrolithal, mesolithal, microlithal, gravel, sand, silt/clay, concrete riverbed and hydropetric. The biotic components comprised algae, emergent or submerged macrophytes, roots, wood debris, coarse particulate organic matter or fine particulate organic matter. We then sampled macroinvertebrates with a Surber net (size 0.23 m × 0.22 m, mesh size 900 µm) in 10 replicates that were distributed proportionally among the types of microhabitat present.

We sorted samples in the field, to remove primarily debris and stones and to count and identify macroinvertebrates taxa. We preserved only a small percentage of organisms in 90% ethanol when we needed a more detailed identification. We accomplished 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 taxon-list for each site.

For the evaluation of the ecological status, we used the MacOper.ICM 1.0.5 software (Buffagni & Belfiore, 2013), which automatically calculates the final STAR_ICMi. This index is based on six metrics: ASPT (Average Score Per Taxon), $\text{Log}_{10}\text{Sel}_{\text{EPDT}} + 1$ (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 EPTs (Ephemeroptera, Plecoptera, and Trichoptera) families and the Shannon-Weiner diversity index ($D_{S,W}$). 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 a pertinent reference site for each fluvial type (see Annex II of Water Framework Directive). For each site, we attributed a quality class according to STAR_ICMi values range, as indicated for each river type in the Ministerial Decree 260/2010.

2.3. Fluvial functionality index (IFF)

For each sampling point, we applied the IFF method, which integrates analyses of macroscopic abiotic aspects of the river and its surrounding territory with different biotic components (Siligardi et al., 2007). To obtain this index, we considered 20 m long homogeneous stretches of the rivers comprising the selected bat sampling points. For each sampling point, we completed a form, composed of 14 multiple-choice questions. For every question, there are four possible answers with different weights attached to them. The final sum of these weighted values is the IFF score, which may range from 14 (lowest functionality) to 300 (maximum functionality). Questions 1–4 concern

the territorial and riparian vegetation characteristics; questions 5 and 6 relate to morphological characteristics of the riverbed; questions 7–11 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 used the same data we collected to obtain the STAR_ICM index.

2.4. Bat activity sampling

We surveyed bat activity in each sampling point with stationary, automatically triggered D500X bat detectors (Pettersson Elektronik, Uppsala, Sweden). The D500X remotely records the ultrasonic spectrum up to 190 kHz (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*). We recorded on warm nights, with air temperature > 10 °C, because insects become less active below this temperature (Rydell et al., 1996), and no or light wind. At each site, we recorded bats over an entire night, from sunset to dawn, leaving the D500X on the riverside, as close as possible to water, oriented at 45° to vertical pointing upwards (Britzke et al., 2010; Wickramasinghe et al., 2003). We recorded bats at sites where riparian vegetation did not represent an obstacle to bat flight paths and where water surface was smooth in order to avoid turbulent water, which may interfere with bat activity (Greif and Siemers, 2010; Warren et al., 2000) and decrease signal-to-noise ratio in recordings. We used the following recording settings for the two sampling sessions: 500 kHz sampling rate; 5 s recording length from trigger; 60 s of not recording interval after each record; high pass filter enabled at 10 kHz; low trigger sensitivity, to avoid recording background noise. We saved recordings on Compact Flash cards as WAV files.

2.5. Bat 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 is available, their performances may be variable and difficult to assess (Russo and Voigt, 2016; Rydell et al., 2017). 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 bat activity to the highest possible taxonomic resolution. We screened bat recordings visually in BatSound 4.1. We generated oscillograms, power spectra and spectrograms to measure call variables following Russo and Jones (2002). To obtain spectrograms we used a 1024-pt FFT Hamming window with a 98% window overlap. We identified species using “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., 2017; Pfalzer and Kusch, 2003; Russ, 2012; Russo et al., 2009; Russo and Jones, 2002, 1999; Russo and Papadatou, 2014) when present in recordings. Generally speaking, “typical” calls are those broadcast respectively in the open by aerial hawkers or edge specialists and in dense vegetation or near obstacles by clutter specialists. For comparison, we used reference recordings made from species of known identity and values tabled in Russo and Jones (2002) and Russ (2012).

A few bat species occurring in the study area overlap in most of their spectral and temporal variables, so we established phonic groups made of species broadcasting similar echolocation calls, i.e. pooling *Miniopterus schreibersii* with *Pipistrellus pygmaeus*, *Nyctalus* spp. with *Eptesicus serotinus*, and two *Plecotus* species (*P. auritus* and *P. austriacus*). When calls allowed a reliable identification, we separated *Myotis emarginatus* from *M. nattereri*. In the case of *M. daubentonii* and *M. capaccinii*, we pooled them together in all analyses because they are difficult to tell apart from call recordings, but we highlight that *M. daubentonii* largely predominates in our study sites. Based on capture data,

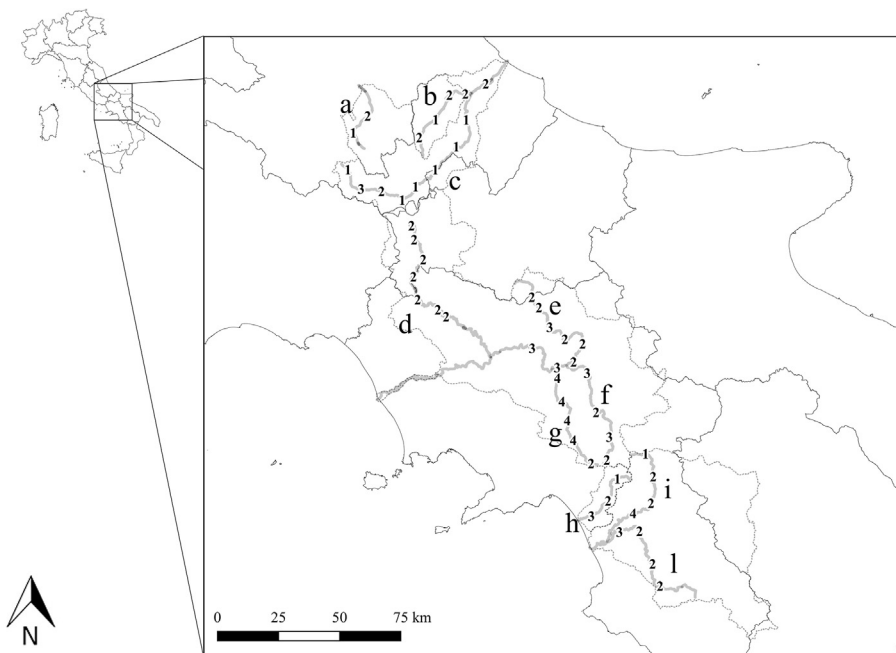


Fig. 1. Average ecological quality established for ten rivers surveyed in Southern and Central Italy based on macrobenthic community sampling. Numbers indicate the different quality classes recorded at sampling sites: 1 = Excellent, 2 = Good, 3 = Moderate, 4 = Poor. Rivers are labelled as follows: a = Sagittario River; b = Aventino River; c = Sangro River; d = Volturno River; e = Tammaro River; f = Calore Irpino River; g = Sabato River; h = Tuscano River; i = Sele River; l = Calore Salernitano River.

we estimate that this species accounted for > 90% of the phonic group. For the same reason, we pooled together *Myotis myotis* and *Myotis blythii*. We also ran analyses with all *Myotis* species pooled as *Myotis* spp. Because we could not identify confidently all species, we refrained from determining species richness and only analysed bat activity.

2.6. Data analysis

We applied Generalized Linear Mixed-effects Models (GLMMs) with a Poisson distribution and a log-link function using the lme4 package (Bates, 2010) in R 3.3.2 (R Core Team 2016) to analyze the influence of several factors on the chosen variables. We fit the model using sampling site and river as random effect factors, while our explaining fixed factors were STAR_ICMi, IFF, and river width. After a preliminary screening, we excluded season (classified as summer or autumn) and night duration as neither had a significant effect on any model. Our response variables were total bat activity as well as the activity of single species or phonic groups. Activity was expressed both as absolute activity (the number of passes recorded for a given species or phonic group at a given site) and relative activity (the number of passes recorded for a given species or phonic group at a given site divided by the total number of passes recorded at that site). When variables did not follow a normal distribution, data were log transformed to meet test assumptions. We tested response variables separately for each bioindication index; in all models, river width was included as fixed factor. We evaluated the direction and magnitude effects on the activity of each variable by checking model estimates and standard errors and set significance at $p < 0.05$. To increase model performance, we also ran a set of models on species grouped ex-post, i.e. after the analyses previously described had been carried out. In such cases, we pooled together, and analysed as a single group, the number of bat passes recorded for the species that had responded consistently to a given index in the previous analyses, i.e. whose activity either increased or declined for increasing values of one of the two indices considered. In this multi-taxon, or “shopping basket” approach (Vane-Wright et al., 1994; Kotze and Samways, 1999; Sauberer et al., 2004), which is useful to strengthen responses and make them easier to measure, the bat species were pooled together only according to consistency in their reactions to river quality, regardless of their taxonomic relatedness or ecological similarity.

3. Results

3.1. Water quality and river functionality

We examined 167,371 macroinvertebrates collected at the 50 sampling points. The ecological status at the sampling points assessed through the STAR_ICM index varied between class 1 (high) and class 4 (poor). On average, over half of our sites showed a good (class 2) water quality (Fig. 1). The Sangro River showed the highest quality, while the Sabato River had the poorest, with four out of five sampling points being class 4. Looking at IFF results, of our sampling sites, only two had an excellent (class I) functionality, while most others showed lower (class II or III) levels (Fig. 2), mostly due to channelization, dredging, alteration and destruction of riparian vegetation.

3.2. Bat activity

We recorded bat activity over 1048 h, which corresponded to 55,157 bat passes, equalling 551.6 ± 445.3 passes/site (Table 1). We identified 15 bat species or phonic groups: those most frequently recorded were *Myotis daubentonii*/*M. capaccinii*, *Pipistrellus pipistrellus*, *P. kuhlii*, *Miniopterus schreibersii*/*P. pygmaeus* and *Hypsugo savii*. Those most rarely recorded were *Rhinolophus euryale* and *Plecotus* spp. (Table 1). The analysis conducted on single species or phonic groups showed a strong association with water quality and especially river functionality, as detailed below. Tables 2 and 3 show only models with significant terms. Tables S1 and S2 show full models, including those not reaching significance. River width influenced a limited number of species or phonic groups: we recorded higher activity levels of *Miniopterus schreibersii*/*Pipistrellus pygmaeus* and *Pipistrellus kuhlii* (absolute and relative activity, all $p < 0.05$) at sites with longer interbank distances. In all cases, relative activity showed a significant positive correlation with absolute activity (Table S3).

3.3. Responses to water quality (STAR_ICMi) and river functionality (IFF)

The absolute and relative activity of *Miniopterus schreibersii*/*Pipistrellus pygmaeus* (respectively, $p < 0.01$ and $p < 0.05$) and *Myotis daubentonii*/*capaccinii* ($p < 0.05$) declined with increasing values of STAR_ICMi (Table 2). Relative activity instead increased along with

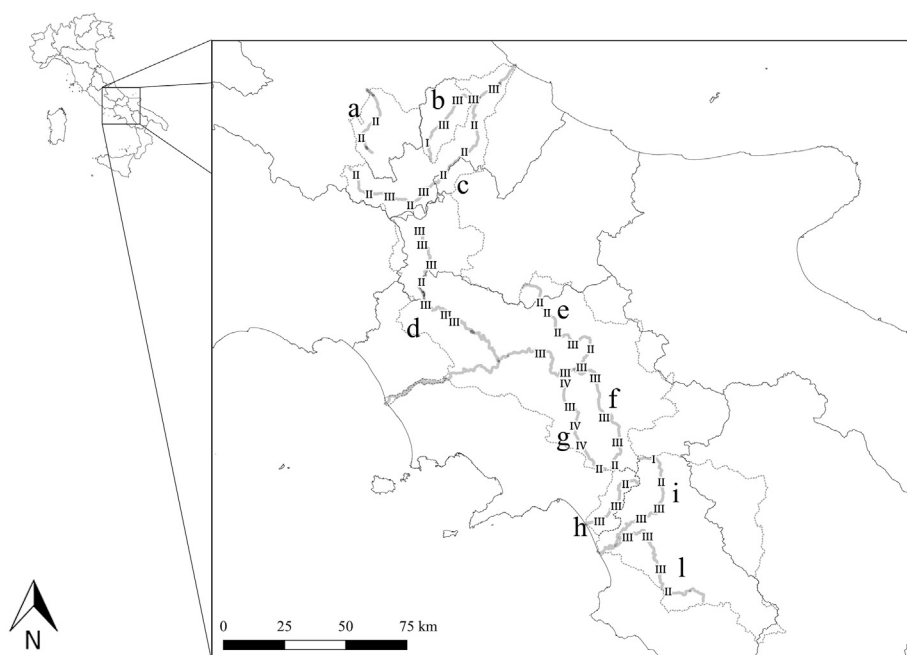


Fig. 2. Fluvial functionality (IFF index) established for ten rivers in Southern and Central Italy. Roman numerals indicate functionality levels: I = Excellent, II = Good, III = Moderate, IV = Poor. Rivers are labelled as follows: a = Sagittario River; b = Aventino River; c = Sangro River; d = Volturno River; e = Tammaro River; f = Calore Irpino River; g = Sabato River; h = Tusciano River; i = Sele River; l = Calore Salernitano River.

Table 1

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

River	R.eur	R.fer	R. hip	M. dau/cap	M. ema	M. m/b	M. nat	unid. <i>Myotis</i>	P. kuh	P. pip	P. pyg/M.sch	Nyc/Ept	H.sav	B.bar	P.aus/aur	T.ten	TOT
Tammaro	1	6	205	3418	68	0	1	242	1516	2911	13	20	237	0	1	40	8679
Tusciano	0	1	99	725	0	1	0	15	1016	200	357	25	151	5	0	4	2599
Sagittario	0	0	2	15	4	10	19	3	107	61	0	7	33	45	5	0	311
Sangro	0	2	158	5539	36	3	245	224	775	3011	247	154	519	19	0	20	10,952
Calore Irpino	4	0	6	1044	1	35	19	32	2196	1154	1046	73	1122	6	1	37	6776
Aventino	0	0	29	1230	24	2	1	845	204	430	4	16	126	2	0	13	2926
Sabato	1	6	9	639	0	0	3	22	1375	1102	1178	24	649	1	1	15	5025
Sele	0	15	13	2118	18	0	38	259	755	329	470	179	236	3	1	4	4438
Volturno	2	29	58	990	13	0	38	3798	651	104	565	39	132	1	1	10	6431
Calore salernitano	0	24	57	317	18	0	0	3724	1674	58	904	40	135	17	3	49	7020
Tot passes	8	83	636	16,035	182	51	364	9164	10,269	9360	4784	577	3340	99	13	192	55,157

Table 2

Effects of river water quality index (STAR_ICMi) and river width on bat absolute and relative activity along 10 rivers (n sampling points = 50) in Central and Southern Italy. *P < 0.05; **P < 0.01; n.s. = not significant.

Scale	Model	R ²	Variable	Estimate ± SE	Z	p
Absolute activity	<i>Myotis daubentonii</i> / <i>M. capaccinii</i>	0.3	STAR_ICMi	-183.0 ± 100.1	-1.8	*
	<i>Miniopterus schreibersii</i> / <i>Pipistrellus pygmaeus</i>	0.4	River width	0.9 ± 1.9	0.5	n.s.
			STAR_ICMi	-108.0 ± 26.4	-4.1	**
	<i>Pipistrellus kuhlii</i>	0.2	River width	1.8 ± 0.5	3.5	**
STAR_ICMi			-105.3 ± 70.8	-1.5	n.s.	
Relative activity	<i>Myotis daubentonii</i> / <i>M. capaccinii</i>	0.3	River width	5.7 ± 1.4	4.147	***
			STAR_ICMi	-17.8 ± 14.3	-1.2	*
	<i>Miniopterus schreibersii</i> / <i>Pipistrellus pygmaeus</i>	0.3	River width	-0.2 ± 0.3	-0.9	n.s.
			STAR_ICMi	-20.4 ± 9.2	-2.2	*
	<i>Nyctalus/Eptesicus serotinus</i>	0.2	River width	0.5 ± 0.2	3.1	n.s.
			STAR_ICMi	4.0 ± 1.6	2.3	**
	<i>Pipistrellus kuhlii</i>	0.4	River width	-0.1 ± 0.0	-2.8	n.s.
			STAR_ICMi	-20.4 ± 9.2	-2.2	n.s.
	River width	0.4 ± 0.2	2.6	*		

Table 3

Effects of river functionality index (IFF) and river width on bat absolute and relative activity, along 10 rivers (n sampling points = 50) in southern Italy. *P < 0.05; n.s. = not significant.

Scale	Model	R ²	Variable	Estimate ± SE	Z	p
Absolute activity	<i>Miniopterus schreibersii</i> / <i>Pipistrellus pygmaeus</i>	0.3	IFF	-0.2 ± 0.1	-2.0	*
			River width	1.7 ± 0.5	3.2	**
	<i>Myotis emarginatus</i>	0.2	IFF	0.0 ± 0.0	2.3	*
			River width	0.0 ± 0.1	0.3	n.s.
	<i>Nyctalus/Eptesicus serotinus</i>	0.2	IFF	0.2 ± 0.0	2.0	*
			River width	-0.1 ± 0.1	-1.8	n.s.
<i>Pipistrellus kuhlii</i>	0.3	IFF	-0.8 ± 0.3	-2.6	n.s.	
		River width	5.1 ± 1.4	3.7	**	
		IFF	-0.0 ± 0.0	-1.5	*	
Relative activity	<i>Miniopterus schreibersii</i> / <i>Pipistrellus pygmaeus</i>	0.2	River width	0.0 ± 0.1	0.0	n.s.
			IFF	-0.2 ± 0.0	-4.4	**
	<i>Pipistrellus kuhlii</i>	0.5	River width	0.4 ± 0.2	2.6	n.s.
			IFF	0.1 ± 0.0	1.7	*
	<i>Pipistrellus pipistrellus</i>	0.2	River width	-0.0 ± 0.1	-0.3	n.s.
			IFF	0.0 ± 0.0	3.0	*
	<i>Myotis emarginatus</i>	0.1	River width	0.0 ± 0.0	0.5	n.s.
			IFF	0.0 ± 0.0	2.1	*
	<i>Myotis nattereri</i>	0.2	River width	-0.1 ± 0.1	-1.7	n.s.
			IFF	0.9 ± 0.0	1.1	*
	<i>Nyctalus/Eptesicus serotinus</i>	0.3	River width	-0.1 ± 0.0	-2.6	n.s.
			IFF	0.0 ± 0.0	2.1	*
			River width	-0.0 ± 0.0	-0.7	n.s.
	<i>Barbastella barbastellus</i>	0.6	IFF	0.0 ± 0.0	2.1	*
			River width	-0.0 ± 0.0	-0.7	n.s.

STAR_ICMi values for *Nyctalus /Eptesicus* (p < 0.01, Table 2). The absolute and relative activity of *Miniopterus schreibersii*/*Pipistrellus pygmaeus* declined with increasing values of IFF (p < 0.05; Table 3). Likewise, the relative activity of *P. kuhlii* declined with increasing IFF values (p < 0.01; Table 3). On the contrary, the absolute and relative activities of *Myotis emarginatus* (both p < 0.05) and *Nyctalus/Eptesicus serotinus* (p < 0.05) increased with increasing IFF values (Table 3). Relative activity was positively related to IFF for *Pipistrellus pipistrellus* (p < 0.05), *Myotis nattereri* (p < 0.05) and *Barbastella barbastellus* (p < 0.05) (Table 3). In all such cases, R² values were ≤ 0.6 (Tables 2 and 3).

3.4. Establishing indicator groups

In the previous analyses, relative activity showed the clearest associations with environmental predictors, so we focused on this only to apply the shopping basket approach. Grouping species together led, again, to R² values ≤ 0.6, but the levels of significance increased (Table 4). We obtained three groups (Fig. 3; Table 4), as follows: 1) *Myotis daubentonii/capaccinii* + *Miniopterus schreibersii*/*Pipistrellus pygmaeus* showed a marked negative response to STAR_ICMi (p < 0.01); 2) *Pipistrellus pipistrellus* + *Myotis emarginatus* + *Myotis nattereri* + *Nyctalus/Eptesicus serotinus* + *Barbastella barbastellus* showed a positive response to IFF (p < 0.001), while 3) *Pipistrellus kuhlii* + *Miniopterus schreibersii*/*Pipistrellus pygmaeus* showed a negative response to IFF (p < 0.001).

Table 4

Generalised linear mixed models testing the effects of STAR_ICMi and IFF indices, and river width on the relative activity (%) of groups of bat species formed according to a shopping basket approach. *P < 0.05; **P < 0.01; ***P < 0.001; n.s. = not significant.

Response by group (Relative activity)	Grouped species	R ²	Variable	Estimate ± SE	Z	p
Negative to IFF	<i>Pipistrellus kuhlii</i> + <i>Miniopterus schreibersii</i> / <i>Pipistrellus pygmaeus</i>	0.6	IFF	-0.2 ± 0.0	-5.1	***
			River width	0.5 ± 0.2	2.7	n.s.
Positive to IFF	<i>Pipistrellus pipistrellus</i> + <i>Myotis emarginatus</i> + <i>Myotis nattereri</i> + <i>Nyctalus/Eptesicus serotinus</i> + <i>Barbastella barbastellus</i>	0.3	IFF	0.1 ± 0.0	3.1	***
			River width	-0.3 ± 0.2	-1.6	*
Negative to STAR_ICMi	<i>Myotis daubentonii</i> / <i>M. capaccinii</i> + <i>Miniopterus schreibersii</i> / <i>Pipistrellus pygmaeus</i>	0.3	STAR_ICMi	-7.5 ± 1.1	-2.0	**
			River width	-0.2 ± 0.3	-0.6	n.s.

4. Discussion

4.1. Associations of foraging bats to structural and environmental characteristics of rivers

Our study shows that assemblages of foraging bats may be used to characterize the environmental status of riverine habitats, in terms of both water quality (a factor we assessed using macroinvertebrates) and river functionality. Instead of relying on published maps or records of river quality, we calculated the indices from field observations, so that their values were the most recent available. Because riverine quality may vary over time, sometimes quickly (Ouyang et al., 2006), our approach may reliably associate bat activity with the quality of the riparian sites we surveyed.

Several studies have detected responses to water pollution by European bats (e.g. Vaughan et al., 1996; Abbott et al., 2009). In Iberia, *M. daubentonii*'s relative abundance, or even presence/absence, were found to be a complementary bioindicator to characterize riparian forest quality but they proved to be insufficient to describe riverine ecosystem health (López-Baucells et al. (2017); but see Langton et al. (2010)). In our case, we found species presence not to be an effective bioindicator, while activity (especially relative activity) of several species or species groups performed well. In a radiotracking study (Biscardi et al., 2007) *M. capaccinii* – a bat species jeopardized by habitat degradation – foraged preferentially at sites of greater quality (as established by benthic macroinvertebrate bioindication) but still

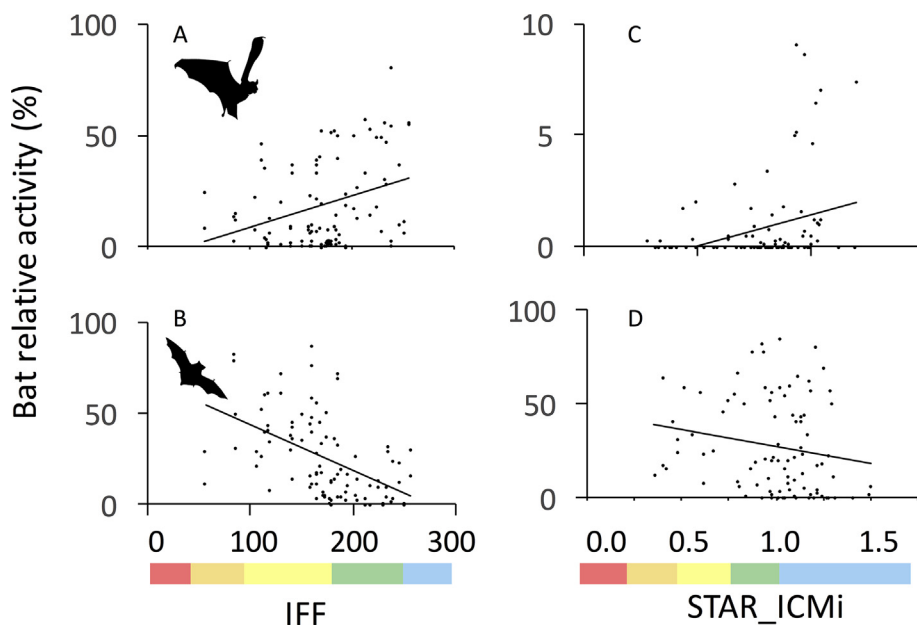


Fig. 3. Relationship between % bat relative activity for species groups and the two bioindication indices considered for this study. “C” is a phonic group including two genera (*Nyctalus* and *Eptesicus*) that we did not discriminate from each other; A, B, and D were established through a “shopping basket” approach. A = *Pipistrellus pipistrellus* + *Myotis emarginatus* + *M. nattereri* + *Nyctalus/Eptesicus serotinus* + *Barbastella barbastellus*; B = *Miniopterus schreibersii*/*Pipistrellus pygmaeus* + *P. kuhlii*; C = *Nyctalus/Eptesicus serotinus*; and D = *M. schreibersii*/*P. pygmaeus* + *M. daubentonii/capaccinii*. Left: Fluvial Functionality Index (IFF); right: STAR_ICMi index (based on macro-invertebrates). Below the plots, we show river-quality ranges (increasing from left to right) according to index values: red = bad, orange = poor, yellow = moderate, green = good, blue = excellent. Linear regression equations and significance values as follows: A) $y = 0.141x - 5.381$, $p < 0.01$; B) $y = 0.247x + 68.354$, $p < 0.01$; C) $y = 2.823x - 1.384$, $p < 0.05$; D) $y = 4.51x + 33.654$, $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

occurred also where quality was low. This, again, suggests that using species presence only may provide poor bioindication performances, while approaches based on activity are more promising (for forests, see Russo et al., 2010, 2016).

Although for all species or species groups, bat relative activity was strongly, positively correlated with absolute activity, the former performed better in models, probably because values were more comparable and less fluctuating across sites. An increase (or decrease) in the relative activity of some species might, in theory, be an artefact generated by corresponding decreases (or increases) in the activity of the remaining species, but we can rule this risk out because of the above-mentioned correlation, showing that relative activity represents a genuine (and responsive) proxy for trends in absolute activity, otherwise no correlation would be found.

Our results confirm the existence of a link between bat activity and water quality (Li and Kalcounis-Rueppell, 2017). We disregarded the possible influence of landscape over bat activity because this was negligible in previous bioindication work that focused on bats (López-Baucells et al., 2017; Li and Kalcounis-Rueppell, 2017). Interbank distance played a minor role, influencing only the activity of *Miniopterus schreibersii*/*Pipistrellus pygmaeus* and *P. kuhlii*. However, this factor was important in other studies, at least for *Myotis* bats (Biscardi et al., 2007; Langton et al., 2010; López-Baucells et al., 2017).

The activity levels of *Miniopterus schreibersii*/*Pipistrellus pygmaeus* and *Myotis daubentonii/capaccinii* were lower for higher STAR_ICMi values, i.e. greater water quality. Biscardi et al. (2007) found an opposite pattern for *M. capaccinii*, but in that case, bats were radiotracked, so their identity was certain. In the present study, based on acoustic surveys, we could not distinguish between *M. capaccinii* and *M. daubentonii*, but since the former is rare, while the latter is widespread in the study area, our sample was mostly made of *M. daubentonii*. Previous work has provided contrasting results about whether *M. daubentonii* activity changes in response to water quality. In Irish rivers, the species was less active downstream from sewage effluents than upstream (Abbott et al., 2009), while in England an opposite pattern was found (Vaughan et al., 1996). Unlike in Italy (Nardone et al., 2015) and other regions, where *M. daubentonii* mainly feeds on chironomids (Taake, 1992, 1993; Beck, 1994; Kokurewicz, 1995; Nardone et al., 2015; Vesterinen et al. 2016), in Ireland this bat species mostly feeds on Trichoptera (caddisfly) adults (Abbott et al., 2009). Chironomids thrive in eutrophic waters, as such *M. daubentonii* might even benefit from the moderate organic pollution of rivers (Kokurewicz, 1995).

Activity of *Miniopterus schreibersii*/*Pipistrellus pygmaeus* also increased with lower values of STAR_ICMi, i.e. with lower water quality. *M. schreibersii* shows opportunistic feeding habits, and mostly feeds on moths (Aizpurua et al., 2018). In our study area, the species occurs frequently only where roost caves are present (D. Russo, pers. obs.), while *P. pygmaeus* is much more widespread (Russo and Jones, 2003). Therefore, for passes we classified as *Miniopterus schreibersii*/*Pipistrellus pygmaeus* most were probably from *P. pygmaeus*. The latter species often feeds on chironomids (Barlow, 1997), so as in Ireland (Abbott et al., 2009), the inverse relationship between its activity and water quality may be due to a large chironomid abundance at polluted sites. In Scotland, eutrophication of river waters had a positive effect on the activity of both *P. pygmaeus* and *M. daubentonii* (Racey et al., 1998). With regards to the *Nyctalus/Eptesicus serotinus* sample, this was probably made mostly of *N. leisleri* passes, since this species is the only one common in our study area. The association between high levels of *Nyctalus/Eptesicus serotinus* activity and high STAR_ICMi values may be due to the fact that these bats often feed on Lepidoptera, Ephemeroptera, and Trichoptera, frequent prey of *N. leisleri* along with small dipterans (Sullivan et al., 1993; Shiel et al., 1998) and often common in high-quality river stretches.

As in the case of STAR_ICMi, *Miniopterus schreibersii*/*Pipistrellus pygmaeus* relative activity was higher where fluvial functionality (IFF) values were lower, i.e. in “less healthy” river stretches, while the opposite was observed for *Nyctalus/Eptesicus serotinus*. *P. kuhlii* relative activity was also associated with lower IFF values. This synurbic species often forages in urbanized sites (Ancillotto et al., 2015a; Russo and Ancillotto, 2015), so it may probably exploit an altered riverine habitat more effectively than other, sensitive species. Noticeably, gleaners or forest species such as *M. emarginatus*, *Myotis nattereri*, and *Barbastella barbastellus* increased relative activity with higher IFF values. Prey suitable for gleaners is common where riparian vegetation is well developed, also characterized by higher IFF values (e.g. Swift and Racey, 2002; Kervyn et al., 2012; Hope et al., 2014). In the study area, both *Myotis* species are often mistnetted at riparian sites with richly wooded banks (D. Russo, pers. obs.). A radiotracking study done in central Italy highlighted the importance of moth-rich riparian vegetation as a foraging habitat for *B. barbastellus* (Ancillotto et al., 2015b). Relative activity of *P. pipistrellus* also increased with higher IFF values: the species was also more active at low-pollution sites in the UK (Vaughan et al., 1996), probably being less tolerant than other pipistrelles, such as *P. kuhlii*, to an altered environmental condition.

4.2. Using bats as bioindicators in riverine ecosystems: methodological considerations

The differences in the direction of the responses found in the bat species we examined is reminiscent of what is also observed in other biological groups, i.e. that trends in one taxon may not indicate similar trends in others (Kotze and Samways, 1999), and it supports the use of a ‘shopping basket’ strategy for monitoring. Pooling species together (or phonic groups) as per a “shopping basket” approach, increased the statistical significance of bat activity responses to IFF and/or STAR_ICMi. This has clear consequences for practical bioindication since such groups are more likely to exhibit clear, unambiguous responses to river quality. It may also make sound analysis more robust, for instance by pooling together similarly calling species, such as e.g. *M. emarginatus* and *M. nattereri*. The relatively limited R^2 values obtained in our models suggest that factors other than those covered by the environmental indices we adopted may influence bat activity. Increasing the number of replications per site would probably reduce “background noise” and improve model fit. However, the values we obtained are comparable to those obtained in other studies that have proposed the use of new bioindicators (Smith et al., 2007), and the levels of significance reached by some bat species or species groups were high enough to draw important conclusions about how changes in bat activity are associated with changes in river environmental conditions.

Total bat activity (i.e. the overall number of bat passes recorded at a site) has proved useful to describe the effects of forestry operations (Cistrone et al., 2015). This is a great advantage in terms of robustness, rapidity of analysis and reliability because assessing total activity requires no species identification – it only requires the counting of bat passes in recordings. In our study, however, total bat activity provided no significant results, so echolocation call identification was necessary. This is not an easy task: it requires experienced operators and does not provide species identity in all cases (Russo and Voigt, 2016; Rydell et al., 2017). Manual identification of bat calls is also time-consuming and subjective, so automated classifiers might speed up the process and make it operator independent, but regrettably we are still far from developing highly reliable classifiers, so human intervention, at least for a final vetting stage, is currently unavoidable (Russo and Voigt, 2016; Rydell et al., 2017). Lumping together species showing similar calls in phonic groups, as done in our study, reduces misclassification risk, yet it might also mask species-specific trends, especially if activity trends differ among members of the same phonic group. In our study, this risk was negligible, because from our knowledge of the local bat fauna we were aware that the phonic groups we considered were dominated by the most common species while the remaining were probably very rare or absent. This may not happen elsewhere, however, and in such a case this would weaken the suitability of using phonic groups for bioindication.

4.3. Using bats as bioindicators in riverine ecosystems: ecological considerations

Our findings represent a significant step towards the use of bats as bioindicators in these habitats. We show that the foraging activity of bat assemblages may reveal changes in the environmental conditions of riverine ecosystems, i.e. bats act as environmental indicators (Paoletti and Bressan, 1996; McGeoch, 1998) that respond predictably to differences, or changes in environmental state. In fact, the high position of bats in trophic webs makes their foraging activity highly responsive to qualitative and quantitative changes in insect food availability. Because different species (or species groups) react differently to variation of riverine quality, and such responses probably relate to differences in prey type or habitat structure, bat assemblages might also act as ecological indicators (Meffe and Carroll, 1994; McGeoch, 1998), i.e. as surrogates for the broader biological community, demonstrating the effects of habitat alteration on the river biota. However, pursuing the

latter challenging goal requires a better understanding of the functional relationships existing between riverine ecosystems and bats, in order to control for geographical differences in species-specific responses to environmental changes. Intraspecific differences in bat diet may lead to diverging responses in different bat populations, which may restrict the geographic scope of bat indicators or require different local interpretations. Our results therefore warrant confirmation in other European regions, and studies following a similar protocol to that which we adopted would greatly aid geographical comparisons.

Another possible limitation to the use of bats as indicators of riverine quality is given by the dependence of some species on the presence of caves used for roosting. In such cases, the absence of a cave-dwelling species might be due to the absence of caves in a certain area rather than being associated with water quality. In our study, cave-dwelling bats were mostly restricted to *M. capaccinii* (rare in the study region) and *M. schreibersii*, whose passes were pooled together with those of *P. pygmaeus*, so we assume this risk to be negligible. In other regions, the availability of caves might be an issue to consider when using bats as bioindicators.

Because no single indicator may provide a comprehensive description of the environmental conditions characterizing a river, we need a complementarity principle in selecting suites of indicators to detect structural, functional and compositional alterations at multiple spatial and temporal scales (Rogers and Biggs, 1999). From this perspective, adding bats to the currently adopted array of indicators of river quality would widen the analysis’ ecological perspective: besides being sensitive to water quality, bats also respond clearly to the status of riparian vegetation, an ecosystem that is undergoing major alterations in many regions (e.g. Smith et al., 2007). Moreover, bats are sensitive to multiple spatial scales, from microhabitats such as roosts (e.g. Russo et al., 2015) or restricted foraging spots (Biscardi et al., 2007; Nardone et al., 2015) up to the landscape level (Li and Kalcounis-Rueppell, 2017), so systematic monitoring of bats may alert land managers of changes occurring at different scales. Unlike macrobenthic organisms, characterized by limited mobility, flying organisms respond rapidly to habitat change and move to seek suitable habitat (Samways et al., 1996; Smith et al., 2007). Bats are long-lived and reproduce slowly, so their demography may fail to react promptly to environmental changes in comparison to short-lived, prolific taxa such as many invertebrates. However, thanks to the above-mentioned mobility, foraging bats react quickly to environmental changes because once a foraging site is altered, bats will immediately move somewhere else to forage. Surveying bat activity for bioindication is therefore a valid approach.

Bats are sensitive to climate change, and both observed (Ancillotto et al., 2016) and predicted (Rebelo et al., 2010) responses suggest that species ranges, as well as altitudinal zones used by different species, are already changing, or will change in the near future. Such changes may lead to novel competition patterns, and possibly rearrangements of bat assemblages, in turn influencing the prospect of using bats as bioindicators. In a quickly changing world, this caveat trespasses the boundaries of bat ecology, for it is probably common to other many other organisms today used for bioindication.

4.4. Synergies between bat conservation and bioindication of river conditions

Employing bats as indicators of river quality would bring about mutual benefits for the conservation of both bats and rivers. The fact that many bat species are at risk endows them with social values that less popular, common organisms such as most invertebrates do not possess: this makes bats excellent candidates to raise public awareness on the state of river ecosystems. On the other hand, increasing the power of bioindication in river ecosystems is an essential step to refine management and ultimately preserve or improve habitat conditions, with obvious positive effects on bats. One point often raised against the use of bats as indicators is the expensiveness of real-time ultrasound

recordings, but this problem will probably be less important as technology develops further and costs decline. Moreover, for conservation reasons, bat monitoring is mandatory in many countries, so carrying it out in riparian habitats, where many bat species forage, could reach the double goal of assessing bat conservation status while evaluating the environmental conditions of rivers, in this way increasing operational and cost effectiveness of monitoring operations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ecolind.2018.08.018>.

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