The challenge of fulfilling the habitats directive's commitments: animal species working group experiences from the project "MONITORARE" in Tuscany

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THE CHALLENGE OF FULFILLING THE HABITATS DIRECTIVE'S COMMITMENTS: ANIMAL SPECIES WORKING GROUP EXPERIENCES FROM THE PROJECT "MONITORARE" IN TUSCANY

Abstract - M. Boschetti, M.A.L. Zuffi, A. Nistri, P. Agnelli, L. Bartolozzi, M. Gennai, G. Innocenti, A. Nocita, S. Rossi De Gasperis, S. Vanni, M. Dellacasa, G. Cananzi, I. Tatini, D. Fontana, D. Barbato, A. Benocci, L. Favilli, S. Piazzini, A. Ugolini, G. Manganelli, G. Petroni, *The challenge of fulfilling the Habitats Directive's commitments: animal species working group experiences from the project "MonitoRARE" in Tuscany.*

The Project "MonitoRARE", underwritten by the Tuscan regional government in 2018 with the Universities of Florence, Pisa, and Siena (Regional Resolution n° 1047, 02/10/17), predicted an interdisciplinary monitoring program for protected animals, plants, and habitats under the Council Directive 92/43/EEC of 21 May 1992 (Habitat Directive) within the regional territory. The Project was structured in two stages, i) a preliminary updating of the distribution maps of the species and habitats included in the Directive, using conventional and grey literature, unpublished data and recent museum records; ii) a consequent field trial aimed to test the applicability as well as the cost-effectiveness/timing of monitoring protocols suggested by ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale) and MATTM (Ministero dell'Ambiente e della Tutela del Territorio e del Mare, now renamed and implemented as Ministero dell'Ambiente e della Sicurezza Energetica, MASE) in their Manuals 140/16, 141/16 and 142/16. In the present manuscript results obtained from bibliographic screening and field monitoring of selected animal species and sites are reported. Moreover, experienced animal field-based monitoring issues in the light of national guidelines aimed at mapping local biodiversity were critically analysed and discussed. Concerning the results of the first project stage (i.e. bibliographic screening), the distribution maps of 61 species (2 molluscs, 10 arthropods, 6 fish, 10 amphibians, 10 reptiles and 23 mammals) were updated with new records fitting to a total of 752 10 × 10 km grid squares, mainly from central and southern Tuscany. Moreover, two records of species never reported up to now in the Tuscan region were included. During the on-field stage, 26 species (2 molluscs, 6 arthropods, 5 fishes, 4 amphibians, 3 reptiles and 6 mammals) and 30 Special Areas of Conservation (SAC - corresponding to about 22.4% of the SACs in the Region), were selected to cover a wide array of ecological features and distribution across the regional territory and to test the reliability of the monitoring protocol. Each species was monitored in a subset of the selected SACs. The application of monitoring protocols permitted to detect the presence of 88.9% of the target species in the selected areas, including 10 species that were not previously reported in 13 out of the selected SACs, together with 30 additional animal species of conservation interest. Obtained results allowed to highlight ongoing pressures on the selected target species as well as potential limits and issues of the suggested monitoring protocols, especially considering the significant needs in term of funding and qualified staff, necessary to perform a simultaneous monitoring plan on numerous taxa and study areas.

Key words - monitoring, fauna, Italy, Tuscany, Habitat Directive

Riassunto - M. Boschetti, M.A.L. Zuffi, A. Nistri, P. Agnelli, L. Bartolozzi, M. Gennai, G. Innocenti, A. Nocita, S. Rossi De Gasperis, S. Vanni, M. Dellacasa, G. Cananzi, I. Tatini, D. Fontana, D. Barbato, A. Benocci, L. Favilli, S. Piazzini, A. Ugolini, G. Manganelli, G. Petroni, *La sfida di adempiere agli obbiettivi della Direttiva Habitat: l'esperienza del progetto "MonitoRARE" per il gruppo di monitoraggio della fauna toscana.*

Il progetto MonitoRARE sottoscritto nel 2018 dal governo regionale della Toscana con le Università di Firenze, Pisa e Siena (Risoluzione Regionale n° 1047, 02/10/17), prevedeva un programma di monitoraggio interdisciplinare del territorio regionale per animali, piante e habitat protetti dalla Direttiva 92/43/EEC del Consiglio d'Europa del 21 maggio 1992 (Direttiva Habitat). Il progetto è stato strutturato in 2 stadi, i) un aggiornamento preliminare delle mappe di distribuzione di specie e habitat inclusi nella direttiva, con l'utilizzo di letteratura grigia, dati non pubblicati e registri museali; ii) una successiva fase di prova su campo per testare l'applicabilità e l'efficienza costo/tempo dei protocolli di monitoraggio suggeriti da ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale) e MATTM (Ministero dell'Ambiente e della Tutela del Territorio e del Mare, ora rinominato e implementato nel Ministero dell'Ambiente e della Sicurezza Energetica, MASE) nei loro Manuali, 140/16, 141/16 e 142/16. Il presente manoscritto racchiude i risultati dell'indagine bibliografica e dei monitoraggi su campo dei siti e delle specie animali selezionati. Inoltre, in seguito all'esperienza sul campo, sono state analizzate e discusse

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le criticità emerse per quel che riguarda le pratiche di monitoraggio indicate nei Manuali. I risultati della prima fase del progetto (i.e. indagine bibliografica) hanno portato all'aggiornamento delle mappe di distribuzione di 61 specie (2 molluschi, 10 artropodi, 6 pesci, 10 anfibi, 10 rettili e 23 mammiferi) con nuove segnalazioni che sono andate a coprire un totale di 752 quadranti di una griglia 10 × 10 km. Inoltre, sono state registrate 2 segnalazioni di specie mai registrate prima in Toscana. Durante la fase di campo, 26 specie (2 molluschi, 6 artropodi, 5 pesci, 4 anfibi, 3 rettili e 6 mammiferi) e 30 Zone Speciali di Conservazione (ZSC o SAC – corrispondenti a circa il 22.4% delle SAC totali della regione), sono state selezionate per coprire una vasta gamma di aspetti ecologici e distributivi attraverso il territorio regionale e testare l'affidabilità dei protocolli di monitoraggio. Ogni specie è stata monitorata in un sottogruppo delle SAC selezionate. L'applicazione dei protocolli di monitoraggio ha consentito di registrare la presenza dell'88.9% delle specie target nelle aree selezionate, incluse 10 specie in precedenza non segnalate (per 13 delle SAC indagate), e 30 altre specie non presenti negli allegati della Direttiva ma di interesse conservazionistico. I risultati ottenuti hanno evidenziato pressioni e rischi a cui sono sottoposte le specie target e le problematiche e i limiti dei protocolli di monitoraggio suggeriti dai Manuali, considerando in particolare le spese e le qualifiche richieste per sostenere un piano di monitoraggio simultaneo su taxa e zone differenti.

Parole chiave - monitoraggio, fauna, Italia, Toscana, Direttiva Habitat

Introduction

The conservation of natural environments is the main, albeit not exclusive, aim for the institution of protected areas (PA), defined as peculiar terrestrial and marine areas subject to adequate management strategies and legislative framework. Italy currently applies two parallel systems of designation of PA *sensu lato*. The Law 394/91 ("Legge quadro sulle aree protette") defines the regulatory framework for PA *sensu stricto*. The Law 157/92 plus DPR (Decree of the President of the Republic) 357/97, modified and integrated by DPR 120/2003, transposes the European Directives Birds (79/409/EEC, amended by 2009/147/EC) and Habitats (92/43/EEC). They concur in establishing the Natura 2000 Network in the national territory. Since 2010, in addition to the pre-existing tools, Italy has also adopted a National Strategy for Biodiversity, an instrument to integrate the principles of biodiversity conservation and of environmental protection into the socio-economic national policies, and to tackle several key issues that emerged in the implementation of Natura 2000 Network (La Posta & Duprè, 2011; Tombolini, 2013).

Natura 2000 sites are not strictly protected reserves where human activities are forbidden; on the contrary, the Habitats Directive aims at making nature conservation compatible with the cultural and socio-economic needs of local populations. The Directive provides precise rules for Natura 2000 sites management: the application of conservation measures for the specific habitats and/or species protected within the site is always required, whereas the establishment of a complete management plan can be facultative under some circumstances. Moreover, it requires the Member States to provide funds (art. 8), to perform monitoring activities and to produce evaluative reports on the implementation of the Directive at national level (art. 11 and 17; European Commission, 2020a). In fact, art. 17 of the Habitats Directive (rearranged into the art. 13 of the DPR 357/97) states that each Member States must provide every six years a National Report, concerning the state of implementation of the Directive provisions adopted by the Member States. The first and the second Italian Reports mainly focused on transposing the conservation status of habitats/species, based on available data and on presumed favourable reference values at national level of the Directive, and on evaluating their conservation status. Furthermore, they highlighted several difficulties, partly resolved with the adoption of the Third Report (2007-2012). With the support provided by the European Commission, the focus was shifted on analysing the evolution of the conservation status by comparing it to the previous Reports. Successively, critical issues and suggestions enabled the preparation of the Fourth Report.

At the regional level, the Tuscan system of PA was established with the L.R. (Legge Regionale, regional law) 49/95, currently disciplined by L.R. 30/2015 "Norme per la conservazione e la valorizzazione del patrimonio naturalistico-ambientale regionale", which coordinated in a single framework the policies of conservation and valorisation of both the regional system of PA *sensu stricto* and the Regional System of Biodiversity (Natura 2000 Network), originally disciplined by the L.R. 56/2000 "Norme per la conservazione e la tutela degli habitat naturali e seminaturali, della flora e della fauna selvatiche".

The entire Natura 2000 Network (SCIs-Sites of Community Importance, SACs-Special Areas of Conservation, and SPAs-Special Protection Areas) covers in Italy approximately 19.35% of the terrestrial national territory and 7.15% of the marine one (MATTM, 2020). In Tuscany, the regional Natura 2000 Network covers two different biogeographical regions, continental and Mediterranean, and includes 158 sites overall, representing 15% of the regional territory and including 10 marine sites, established *sensu* DCR (Regional Council Decision) n. 35/2011 (Regione Toscana, 2020a). It overlaps at 42% with the system of protected areas, established by the national legislation, with which the Network shares a link of mutual functionalities, though aiming at different objectives (Regione Toscana, 2020b). One-hundred thirty-four of the regional Natura 2000 Network sites are nowadays SACs, designated as such in the year 2016, with the D.M. (Ministerial Decree) 24 May 2016 and D.M.

22 December 2016. There are only two remaining SCIs and one marine proposed SCI in the Region that have not been confirmed as SACs (Regione Toscana, 2020b). The transposition of the specific measures of conservation required for SACs and SPAs by Directives 92/43/CEE and 2009/147/CE is disciplined by the following Regional Council resolutions: n. 644, 5 July 2004; n. 454, 16 June 2008; n. 1014, 16 December 2009; n. 1006, 18 November 2014; n. 1223, 15 December 2015 (Regione Toscana, 2020c). The regional Natura 2000 Network comprises additional protected areas called SIR (Siti di Importanza Regionale, i.e., Sites of Regional Interest), created to protect also habitat types and species not included in the European Directive but relevant at regional level. A procedure aiming at converting SIR into SCIs or SPAs is currently ongoing, accordingly to the art. 116 of L.R. 30/2015 (Regione Toscana, 2020b).

During the last decades, the Regional Council of Tuscany arranged specific triennial programs for parks and PA, aiming at building and improving the system of conservation of the natural heritage of the Region. Following regional guidelines, local administrations and Park authorities planned and tried specific socio-economic actions to enhance PA and traditional activities, and to educate the citizens towards the conservation, management, and sustainable use of the environment. Recently, with the approval of the Environmental and Energetic Regional Plan PAER (DCR 10/2015, "Piano Ambientale ed Energetico Regionale"), the Tuscany Region further stated its commitment towards the protection of the environment. Indeed, "the conservation of terrestrial and marine biodiversity and the promotion of usability and sustainable management of the protected areas" were included among the main objectives of the plan (Regione Toscana, 2020a).

Italian law specifically designates natural Parks to guarantee both conservation, protection and restoration of natural environments and continuous, multi- and interdisciplinary scientific research (Palmieri, 2006). Nevertheless, a huge gap often exists between the needs of research on wildlife conservation and effective management of the same, even within protected areas (Sunderland *et al*., 2009; Griffiths & Dos Santos, 2012; Walsh *et al*., 2015).

In this context, the Tuscan Regional Government decided to promote the creation of the Project "MonitoRARE: campionamento e monitoraggio su specie e habitat terrestri di interesse comunitario ai sensi della Direttiva Habitat 92/43/CEE" ("Sampling and monitoring of species and habitats of Community interest under the Council Directive 92/43/ EEC"), thereby Project MonitoRARE. MonitoRARE scheduled a monitoring program in the regional territory for animals, plants and habitats protected by the Habitats Directive in order to meet the Directive commitments and provide all data needed for the Fourth Report, in agreement with Art. 17. The Project, in its preliminary stage held in 2018, was an experiment to establish inter-disciplinary cooperative monitoring for Directive species and habitats, involving academics specialized in zoology, botany and ecology from the Tuscan universities. Therefore, the Tuscan regional government signed the Project with the Universities of Florence, Pisa, and Siena (Regional Resolution n. 1047, 02/10/17).

The Project MonitoRARE was carried out in two stages. The former step (Stage 1) involved the preliminary update of the distribution maps of species and habitats occurring in Tuscany and included in the Directive. The latter one (Stage 2) was a field trial of the monitoring protocols underlined by ISPRA, the Italian Institute for Environmental Protection and Research (ISPRA, Istituto Superiore per la Protezione e la Ricerca Ambientale) and the Ministry of the Environment, Land and Sea (MATTM, Ministero dell'Ambiente e della Tutela del Territorio e del Mare now renamed and implemented as Ministero dell'Ambiente e della Sicurezza Energetica, MASE) (Stoch & Genovesi, 2016). These monitoring protocols are described in three manuals, written and published as "Manuals for the monitoring of species and habitats of Community interest in Italy", dedicated to plant species (Manual 140/16), animal species (Manual 141/16), and habitats (Manual 142/16) (Angelini *et al*., 2016; Commissione Fauna dell'Unione Zoologica Italiana, 2016; Ercole *et al*., 2016; Stoch & Genovesi, 2016). Such Manuals represent essential and valuable steps towards a better standardization of national monitoring plans and better implementation of the Habitats Directive. Nevertheless, the present applicability of the suggested monitoring protocols strongly depends upon local features, such as the availability of pre-existing robust data about occurrence and distribution of target species in the Natura 2000 areas. Such preliminary knowledge is necessary to set up an effective monitoring strategy. However, it might be impaired by knowledge gaps even in a relatively small territory such as a Region, due to remote areas or scarcely investigated species. The main aim of the 2018 field activity was to assess the cost-effectiveness and timing of a potential regional monitoring plan, by testing the standard monitoring protocols in a limited number of Natura 2000 areas. The present work will deal specifically with the part of the Project related to animal species, with results and considerations on the updating of the distribution maps as well as the on-field activities.

Materials and methods

Stage 1

The distribution of 112 animal species (molluscs, arthropods, fish, amphibians, reptiles, mammals) included in the Habitats Directive and occurring in Tuscany was investigated, in order to update their official distribution maps with previously not included records. The current update was aimed at increasing distribution of the species with new records after 2010; as such, it did not include any verifying nor validation processes for the pre-existing reports and previous distribution maps. Once downloaded as shapefiles from the ISPRA portal, the pre-existing distributional maps (ISPRA, 2011) of the target species were updated to include records from 2011 to 2017. Such records were mined from: i) published scientific literature; ii) "grey literature", including technical reports; iii) unpublished personal data (previously collected by members of the MonitoRARE working group); iv) records of Natural History Museum from Tuscany; v) online platforms and forums for biodiversity research and data sharing (limited to those checked and certified by expert assessment). These records were then grouped in categories, named as follow: International articles: articles written in English and published on an ISI/SCOPUS indexed journal; Italian articles/books: articles written in Italian and published on a national journal, or printed volumes with or without ISSN; Reports: official scientific reports, in Italian, addressed to public administrations or local authorities; Websites and citizen-science portals: websites and portals collecting data from citizens' reporting, only if providing experts' validation; Congresses: oral presentations, abstracts, and proceedings of national congresses, either in Italian or in English; Non-IF English articles: articles written in English but published in a non-indexed journal; PhD thesis: doctoral thesis in English.

Data for each species were mapped on a 10 × 10 km regional grid with ETRS89-LAEA Europe as reference system in QGIS software (QGIS, 2009), taking into account only records falling in a previously empty square grid. A shapefile of such new reporting was obtained from the maps.

Subsequently, the Standard Forms of the 134 Tuscan SACs were checked, and in some specific and selected case updated if new species occurrence records were found. In this step corrections of some Standard Forms regarding either erroneous occurrences of a particular species, or nomenclature mistakes, were signalled to the competent authorities.

Stage 2

Expert assessment selected a set of animal species and Natura 2000 sites to test the applicability of the monitoring protocols suggested by ISPRA and MATTM; useful data for further updating the Standard Data Forms of the selected sites were also collected. Target species and sites included 26 species (two molluscs, six arthropods, five fishes, four amphibians, three reptiles and six mammals), and 30 Special Areas of Conservation, corresponding to about 22.4% of the SACs in the Region, both in the Continental and in the Mediterranean sectors of Tuscany (see Tab. 1 and Tab. 2 for a detailed list of the monitored species and sites, selection criteria and applied protocols). Each species was monitored for one year in a subset of SACs, selected on the basis of previous data available, or on suitable habitat conditions. In particular, species and sites selection aimed at representing the widest range of distribution typologies, ecological requirements, animal abundance and technical issues. When present, ongoing pressures and potential threats were also considered and reported to the competent authorities. Species-specific monitoring protocols suggested by Manual 141/16 (Stoch & Genovesi, 2016) or subsequent modifications were applied, depending upon local habitat conditions or personnel's availability, with regard to the allocated funds. In some cases, especially for Chiroptera, it was necessary to select some monitoring areas outside the SACs, because the main colonies of the selected species occurred in non-protected areas.

Table 1. Summary of the methods applied in the operational phase of the project (Stage 2). Including: i) monitored species and their taxonomy (Monitored species and Taxonomical classification); ii) reasons why every species was included in the monitoring (Reasons for species selection); iii) the number of Natura 2000 sites where every species was monitored and their identification codes (N° of investigated SACs and SAC code); iv) description of the used protocol and its eventual deviations from the standard monitoring protocol suggested in the ISPRA Manual 141/16 (Monitoring protocol and Deviations from 141/16).

Monitored species	Taxonomical classification	Reasons for species selection	N° of investigated SACs	SAC code	Monitoring protocol	Deviations from 141/16
Vertigo angustior Jeffreys, 1830	Mollusca, Gastropoda, Vertiginidae	near threatened species; patchy distribution	4	IT51A0025; IT5190006; IT5190002; IT5150002	adapted from 141/16: flood sediment plus litter collection and fractioning, morphological analysis of recovered samples	flood sediments plus litter instead of topsoil surface layer and litter; no hand-harvesting of individuals
Unio mancus Lamarck, 1819/ *Unio elongatulus* Pfeiffer, 1825	Mollusca, Bivalvia, Unionidae	sensitive to high pollution levels	4	IT5180013; IT5170007; IT5190006; IT5190011	as suggested in 141/16: 50 × 1 m linear transects, visual counting plus by net collection; quantitative surveys on 1 × 1 m square in case of sightings	none
Austropotamobius pallipes complex [*A. fulcisianus* Ninni, 1886]	Arthropoda, Malacostraca, Decapoda, Astacidae	endangered species; endemic to Italy	6	IT5110001; IT5120010; IT5120014; IT5130009; IT5140002; IT5180011	adapted from 141/16: from 4 to 8 diurnal plus nocturnal transects; visual counting and by hand collection, plus mtDNA barcoding	reduced transect frequency due to staff availability
Lucanus cervus (Linnaeus, 1758)	Arthropoda, Insecta, Coleoptera, Lucanidae	on-field detectability; wide distribution	2	IT5190002; IT5170002	as suggested in 141/16: 500 m linear transects, on a weekly basis	none
Rosalia alpina (Linnaeus, 1758)	Arthropoda, Insecta, Coleoptera, Cerambycidae	fragmented distribution; rare in Tuscany	2	IT51A0017; Natural Reserve of Pietraporciana (province of Siena)	as suggested in 141/16: transect among suitable trees; by-net catching and photographic CMR of adults	none
Lycaena dispar (Haworth, 1803)	Arthropoda, Insecta, Lepidoptera, Lycaenidae	isolated and declining populations in Italy	1	IT5140010	adapted from 141/16: 800 m linear transect, adult visual counting	transect length adapted to local habitat conditions
Melanargia arge (Sulzer, 1776)	Arthropoda, Insecta, Lepidoptera, Nymphalidae	endemic to Italy; small and isolated populations	2	IT5190012; IT51A0008	adapted from 141/16: 500-1000 m linear transects, adult visual counting	transect length adapted to local habitat conditions
Zerynthia cassandra (Geyer, 1828)	Arthropoda, Insecta, Lepidoptera, Papilionidae	endemic to Italy; small and isolated populations	3	IT5130007; IT5140010; IT5190009	adapted from 141/16: 800-1000 m linear transects, adult visual counting plus counting of eggs/larvae	transect length adapted to local habitat conditions; adult counting associated to eggs/larvae counting due to the persistence of weather conditions unfavourable to adult flight
Padogobius nigricans (Canestrini, 1867)	Chordata, Actinopterygii, Gobiiformes, Gobiidae	endemic to Italy; threatened by competition with alien species	6	IT5190002; IT5180012; IT5180009; IT5150002; IT5150003; IT5150001	as suggested in 141/16: electrofishing with Zippin method	none

Monitored species	Taxonomical classification	Reasons for species selection	N° of investigated SACs	SAC code	Monitoring protocol	Deviations from 141/16
Squalius lucumonis (Bianco, 1983)	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	endemic to Italy; threatened by competition with alien species	3	IT5190002; IT5180012; IT5180009	as suggested in 141/16: electrofishing with Zippin method	none
Barbus tyberinus Bonaparte, 1839	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	endemic to Italy; threatened by competition with alien species	5	IT5190002; IT5180012; IT51A0019; IT5180009; IT5150002	as suggested in 141/16: electrofishing with Zippin method	none
Rutilus rubilio (Bonaparte, 1837)	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	endemic to Italy; threatened by competition with alien species	2	IT5150002; IT5150001	as suggested in 141/16: electrofishing with Zippin method	none
Cottus gobio Linnaeus, 1758	Chordata, Actinopterygii, Perciformes, Cottidae	patchy distribution	3	IT5150003; IT5130009; IT5150001	as suggested in 141/16: electrofishing with Zippin method	none
Telestes muticellus (Bonaparte, 1837)	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	endemic to Italy; requires high quality habitat	6	IT5150003; IT5150002; IT5180009; IT5120019; IT5120010; IT5150001	as suggested in 141/16: electrofishing with Zippin method	none
Salamandrina perspicillata (Savi, 1821)	Chordata, Amphibia, Caudata, Salamandridae	endemic to Italy; highly recognisable; linked to medium-high altitude lotic habitats	9	IT5120010; IT5110001; IT51A0001; IT5190002; IT51A0008; IT5180011; IT5140008; IT5130009; IT5150001	adapted from 141/16: from 1 to 3 200-500 m diurnal transects per SAC; visual counting and by hand collection of adults, visual counting of eggs/larvae	transect length adapted to local habitat conditions; transect frequency adapted to staff availability. It was not possible to monitor all or most reproductive sites due to lack of previous data concerning the number and distribution of such sites within the SACs, and due to staff availability
Rana italica Dubois, 1987	Chordata, Amphibia, Anura, Ranidae	endemic to Italy; highly recognisable; linked to medium-high altitude lotic habitats	9	IT5120010; IT5110001; IT51A0001; IT5190002; IT51A0008; IT5180011; IT5140008; IT5130009; IT5150001	adapted from 141/16: from 1 to 3 200-500 m diurnal transects per SAC; visual counting and by hand collection of adults, visual counting of eggs/larvae	transect length adapted to local habitat conditions; transect frequency adapted to staff availability. It was not possible to monitor all or most reproductive sites due to lack of previous data concerning the number and distribution of such sites within the SACs, and due to staff availability

Monitored species	Taxonomical classification	Reasons for species selection	N° of investigated SACs	SAC code	Monitoring protocol	Deviations from 141/16
Hyla intermedia Boulenger, 1882	Chordata, Amphibia, Anura, Hylidae	endemic to Italy; highly recognisable; linked to lowland lentic habitats	1	IT5170002	adapted from 141/16: from 1 to 3 250 m diurnal transects per SAC; visual counting and by hand collection of adults, visual counting of eggs/larvae	transect length adapted to local habitat conditions; transect frequency adapted to staff availability. It was not possible to monitor all or most reproductive sites due to lack of previous data concerning the number and distribution of such sites within the SACs, and due to staff availability
Bufotes balearicus (Laurenti, 1768)	Chordata, Amphibia, Anura, Bufonidae	highly recognisable; linked to lowland lentic habitats	1	IT5170002	adapted from 141/16: from 1 to 3 250 m diurnal transects per SAC; visual counting and by hand collection of adults, visual counting of eggs/larvae	transect length adapted to local habitat conditions; transect frequency adapted to staff availability. It was not possible to monitor all or most reproductive sites due to lack of previous data concerning the number and distribution of such sites within the SACs, and due to staff availability
Emys orbicularis (Linnaeus, 1758)	Chordata, Reptilia, Testudines, Emydidae	highly recognisable; linked to lowland lentic habitats; fragmented and declining populations	1	IT5170002	adapted from 141/16: CMR method, capturing adults with creels and specific nets	frequency and number of CMR sessions adapted to staff availability
Lacerta bilineata Daudin, 1802	Chordata, Reptilia, Squamata, Lacertidae	highly recognisable; linked to shrub areas and ecotones at low-mid altitude	7	IT5170002; IT5110001; IT5140008; IT5140010; IT5120019; IT5190009; IT5160008	adapted from 141/16: 500-1000 m linear transects, adult visual counting	transect length adapted to local habitat conditions; transect frequency and number adapted to staff availability
Hierophis viridiflavus (Lacépède, 1789)	Chordata, Reptilia, Squamata, Colubridae	highly recognisable; linked to shrub areas and ecotones at low-mid altitude	7	IT5170002; IT5110001; IT5140008; IT5140010; IT5120019; IT5190009; IT5160008	adapted from 141/16: 500-1000 m linear transects, adult visual counting	transect length adapted to local habitat conditions; transect frequency and number adapted to staff availability
Muscardinus avellanarius Linnaeus, 1758	Chordata, Mammalia, Rodentia, Gliridae	highly recognisable; sensitive to habitat loss and fragmentation and deterioration of habitat quality	4	IT5110001; IT5140010; IT5170002; IT5160008	adapted from 141/6: hair-tubes transects (10-20 couples of hair-tubes per transect)	number of hair-tubes adapted to local habitat conditions; one session per year: tubes were positioned *in situ* for 7-15 days

Monitored species	Taxonomical classification	Reasons for species selection	N° of investigated SACs	SAC code	Monitoring protocol	Deviations from 141/16
Rhinolophus ferrumequinum (Schreber, 1774)	Chordata, Mammalia, Chiroptera, Rhinolophidae	highly recognisable; high conservation value; adequate number of known refuges in Tuscany	4	IT5150001; IT5170002; IT5140012; IT51A0025	as suggested in 141/16: monitoring of reproductive and/or wintering refuges (direct counting of individuals, photo- or video-counting according to the specific refuge's conditions)	in addition to the four SACs, it was necessary to monitor 6 additional refuges that, although being outside any protected area, are deemed to be relevant for Chiroptera species in the regional territory
Rhinolophus euryale Blasius, 1853	Chordata, Mammalia, Chiroptera, Rhinolophidae	highly recognisable; high conservation value; adequate number of known refuges in Tuscany	4	IT5150001; IT5170002; IT5140012; IT51A0025	as suggested in 141/16: monitoring of reproductive and/or wintering refuges (direct counting of individuals, photo- or video-counting according to the specific refuge's conditions)	in addition to the four SACs, it was necessary to monitor 6 additional refuges that, although being outside any protected area, are deemed to be relevant for Chiroptera species in the regional territory
Rhinolophus hipposideros (Bechstein, 1800)	Chordata, Mammalia, Chiroptera, Rhinolophidae	highly recognisable; high conservation value; adequate number of known refuges in Tuscany	4	IT5150001; IT5170002; IT5140012; IT51A0025	as suggested in 141/16: monitoring of reproductive and/or wintering refuges (direct counting of individuals, photo- or video-counting according to the specific refuge's conditions)	in addition to the four SACs, it was necessary to monitor 6 additional refuges that, although being outside any protected area, are deemed to be relevant for Chiroptera species in the regional territory
Miniopterus schreibersii (Kuhl, 1817)	Chordata, Mammalia, Chiroptera, Miniopteridae	highly recognisable; high conservation value; adequate number of known refuges in Tuscany	4	IT5150001; IT5170002; IT5140012; IT51A0025	as suggested in 141/16: monitoring of reproductive and/or wintering refuges (direct counting of individuals, photo- or video-counting according to the specific refuge's conditions)	in addition to the four SACs, it was necessary to monitor 6 additional refuges that, although being outside any protected area, are deemed to be relevant for Chiroptera species in the regional territory
Myotis emarginatus (Geoffroy E., 1806)	Chordata, Mammalia, Chiroptera, Vespertilionidae	highly recognisable; high conservation value; adequate number of known refuges in Tuscany	4	IT5150001; IT5170002; IT5140012; IT51A0025	as suggested in 141/16: monitoring of reproductive and/or wintering refuges (direct counting of individuals, photo- or video-counting according to the specific refuge's conditions)	in addition to the four SACs, it was necessary to monitor 6 additional refuges that, although being outside any protected area, are deemed to be relevant for Chiroptera species in the regional territory

Table 2. List of the monitored Natura 2000 sites, with their identification code (SAC code), site name (SAC name) and respective province.

SAC code	SAC name	Province
IT5110001	Valle del Torrente Gordana	Massa-Carrara
IT5120010	Valle del Serra - Monte Altissimo	Lucca-Massa-Carrara
IT5120014	Monte Corchia - Le Panie	Lucca
IT5120019	Monte Pisano	Pisa
IT5130007	Padule di Fucecchio	Firenze - Pistoia
IT5130009	Tre Limentre - Reno	Pistoia
IT5140002	Sasso di Castro e Monte Beni	Firenze
IT5140008	Monte Morello	Firenze
IT5140010	Bosco di Chiusi e Paduletta di Ramone	Firenze-Pistoia
IT5140012	Vallombrosa e Bosco di S. Antonio	Firenze
IT5150001	La Calvana	Firenze-Prato
IT5150002	Monte Ferrato e Monte Iavello	Prato
IT5150003	Appennino pratese	Prato
IT5160008	Monte Calvi di Campiglia	Livorno
IT5170002	Selva Pisana	Pisa
IT5170007	Fiume Cecina da Berignone a Ponteginori	Pisa
IT5180009	Monti Rognosi	Arezzo
IT5180011	Pascoli montani e cespuglieti del Pratomagno	Arezzo
IT5180012	Valle dell'Inferno e Bandella	Arezzo
IT5180013	Ponte a Buriano e Penna	Arezzo
IT5190002	Monti del Chianti	Arezzo-Firenze-Siena
IT5190006	Alta Val di Merse	Siena
IT5190009	Lago di Chiusi	Siena
IT5190011	Crete dell'Orcia e del Formone	Siena
IT5190012	Monte Cetona	Siena
IT51A0001	Cornate e Fosini	Grosseto-Siena
IT51A0008	Monte d'Alma	Grosseto
IT51A0017	Cono vulcanico del Monte Amiata	Grosseto-Siena
IT51A0019	Alto corso del Fiume Fiora	Grosseto
IT51A0025	Monte Argentario, Isolotto di Porto Ercole e Argentarola	Grosseto

Results

Stage 1

Overall, we retrieved 78 documents of interest (i.e., bibliographic sources), which have been grouped in categories (e.g., articles written in English and published on an ISI/SCOPUS indexed journal as international articles). The relative contribution of different categories is reported in Fig. 1. Used bibliographic sources are reported in the bibliography and supplementary materials. Primary data used to draw Fig. 1 are reported in Supplementary Tab. S1.

Retrieved data on species presence and distribution were used to update the ISPRA maps with records concerning 61 species (2 molluscs, 10 arthropods, 6 fishes, 10 amphibians, 10 reptiles, 23 mammals; for details see Supplementary Tab. S2) mainly from central and southern Tuscany, for a total overall increase of 752 grid squares.

Fig. 1 provides details upon the different sources. Remarkably, two records concerned species not reported until 2010 in Tuscany: *Saga pedo* (Pallas, 1771) (common predatory bush-cricket) and *Vespertilio murinus* Linnaeus, 1758 (parti-coloured bat). The highest percentage of new grid squares per group, with respect to the overall number of new grid squares recorded, belonged to Mammals (44.28%), followed by Reptiles (19.55%), Amphibians (16.89%), Arthropods (11.17%), Fish (7.45%) and Molluscs (0.66%).

Stage 2

As mentioned, the target species of stage two included only 26 species. The vast majority of the target species (88.9%) was detected within the chosen study areas, in a range between 25% to 100% of the total SACs selected for each species (Tab. 3). Only three species were not detected in the study areas: *Unio mancus* Lamarck, 1819/*Unio elongatulus* Pfeiffer, 1825 (*Unio* river mussel), *Hyla intermedia* Boulenger, 1882 (Italian tree frog) and *Bufotes balearicus* (Laurenti, 1768) (Balearic green toad). Through the field monitoring, ten target species (corresponding to 37.0% of the overall selected species) were detected in 13 target SACs where their occurrence was not previously signalled, i.e., where they were not reported in the SAC's Standard Form (see Tab. 3). The five most commonly detected ongoing pressures were: i) alteration or destruction of riparian vegetation (insisting on 11 species); ii) changes in the hydrological regime of rivers due to anthropic use and climate change (on 11 species); iii) competition with alien species (on nine species); iv) water pollution (on nine species); v) anthropic disturbance or anthropic-related mortality and habitat loss or degradation (each on eight species). See Fig. 2 for further details and description of the detected pressures. Additionally, we detected other 32 species in 15

different SACs during the monitoring, and we detected three target species in SACs where they were not being actively monitored. Most of these occasional findings happened during observational transects; 20 out of 35 (57.2%) species were species of conservation interest *sensu* Habitats Directive, being included in Annexes II and/or IV and/or V (see Supplementary Tabs S3, S4, S5). For detailed species-by-species results, please see Tab. 3 and Supplementary Tab. S5.

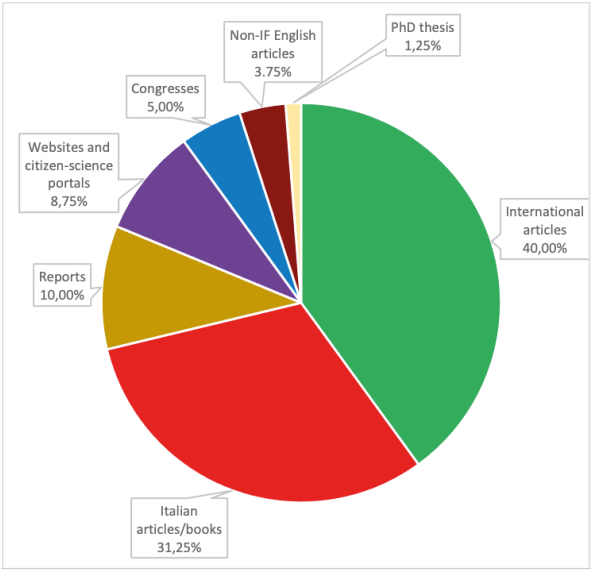

Figure 1. Pie chart showing, in percentage, the different types of sources used for the bibliographic updating. International articles: articles written in English and published on an ISI/SCOPUS indexed journal. Italian articles/books: articles written in Italian and published on a national journal, or printed volumes with or without ISSN. Reports: official scientific reports, in Italian, destined to public administrations or local authorities. Websites and citizen-science portals: websites and portals collecting data from citizens' reporting, only if providing the experts' validation. Congresses: oral presentations, abstracts, and proceedings of national congresses, either in Italian or in English. Non-IF English articles: articles written in English but published in a non-indexed journal. PhD thesis: doctoral thesis in English.

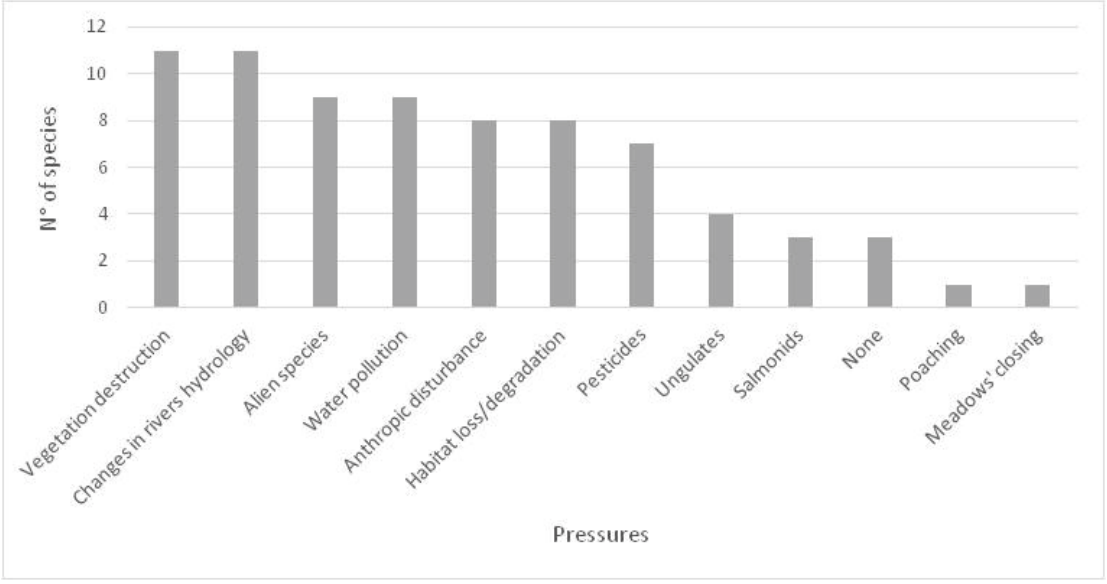

Figure 2. Histogram showing the pressures ongoing against the species in the monitored sites, as detected by expert opinions; on the ordinate, the number of species subject to the pressure is reported (N° of species). Vegetation destruction: Destruction, mowing or alteration of riparian vegetation and/or of nourishing plants. Changes in rivers hydrology: Changes in the hydrological regime of rivers due to anthropic use and climate change. Alien species: Presence of alien species, with ongoing phenomena of competition, predation, genetic pollution against native species. Water pollution: Water pollution deriving from human activities. Anthropic disturbance: Anthropic disturbance against reproductive areas or refuges; anthropic-related non-systematic mortality, like road killing or casual killing during agricultural works; arson episodes. Habitat loss/degradation: Habitat loss and degradation; loss of refuges; environmental alterations of feeding areas. Pesticides: Pollution by pesticides and other chemicals. Ungulates: Excessive density of ungulates, both wild (boars) and domestic (sheep), leading to the destruction of habitats or feeding sources. Salmonids: Presence of introduced salmonids, which seriously prey on eggs/larvae/young specimens. None: During monitoring, no ongoing pressure on the target species was detected. Poaching: During the monitoring, signs of illegal capture and consumption of the target species within the site were detected. Meadows' closing: Reduction of open meadows and grass pastures due to natural succession of shrub vegetation.

Table 3. Summary of the results of the operational phase of the project (Stage 2), including: i) monitored species and their taxonomy (Monitored species and Taxonomical classification); ii) number of Natura 2000 sites where the species was found (N° of positive SACs), compared with those where it was searched (N° of investigated SACs), and percentage of SACs resulted positive for the species' presence (Percentage of positive SACs); iii) number of observed and/or manipulated individuals (N° of detected individuals); iv) ongoing pressures against the species that were recognized by experts' opinion in the site (Detected pressures), in order of importance from the most to the least significant; v) Notes on monitoring, including considerations about effectiveness and criticalities of the applied protocol and, when possible, about population status of the species in the monitored sites; vi) identification codes of the SACs where the species was not recorded in the Standard Form, but was detected during the monitoring (New reports).

Monitored species	Taxonomical classification	N° of positive SACs	N° of investigated SACs	Percentage of positive SACs	Positive SACs codes	N° of detected individuals	Detected pressures	Notes on monitoring	New reports
Vertigo angustior Jeffreys, 1830	Mollusca, Gastropoda, Vertiginidae	3	4	75,00%	IT5190006; IT5190002; IT5150002	16	destruction of riparian vegetation; changes in the hydrological regime of rivers due to anthropic use and climate change	the species seems to occur at very low density at all sites and shows highly patchy distribution. The 141/16 protocol is more suitable for higher densities and more homogeneous distribution. Flood sediment analysis recovers better results than litter analysis	none
Unio mancus Lamarck, 1819/ *Unio elongatulus* Pfeiffer, 1825	Mollusca, Bivalvia, Unionidae	0	4	0,00%	na	0	destruction of riparian vegetation and riverbed works; changes in the hydrological regime of rivers due to anthropic use and climate change; high density of the alien mollusk *Sinanodonta woodiana* (Lea, 1834) in IT5180013	the failure to detect the species could be due either to a real absence, or to low effectiveness of the sampling protocol. The 141/16 protocol can be often impracticable due to logistical difficulties and security related risks	none
Austropotamobius pallipes complex [*A. fulcisianus* Ninni, 1886]	Arthropoda, Malacostraca, Decapoda, Astacidae	2	6	33,33%	IT5140002; IT5180011	1066 (counted); 342 (manipulated)	poaching; alteration of riparian vegetation; changes in the hydrological regime of rivers due to anthropic use and climate change; high presence of bathers within the riverbed	the high transect frequency suggested by 141/16 can be difficult to apply with limited staff, if there are many sites to survey; a lower frequency can be sufficient to detect the specie's occurrence and to give a preliminary, rough estimate of its abundance. Associated mtDNA barcoding, on a small subsample of individuals, can easily confirm specific and subspecific identity of the populations	IT5140002; IT5180011
Lucanus cervus (Linnaeus, 1758)	Arthropoda, Insecta, Coleoptera, Lucanidae	2	2	100,00%	IT5190002; IT5170002	8	none	although present in all monitored SACs, the species seems to occur at very low density at all sites; nevertheless, since it has cryptic habits, it could be more abundant than detected. For exploratory sampling, daily trapping could give more reliable results than transects, though requiring higher sampling efforts	none
Rosalia alpina (Linnaeus, 1758)	Arthropoda, Insecta, Coleoptera, Cerambycidae	1	2	50,00%	Natural Reserve of Pietraporciana (province of Siena)	26	none	transect frequency and duration, as suggested by 141/16, are too high to be followed with limited staff resources; some "key trees", potentially reproductive sites, have been identified during monitoring; there wasn't any recapture of marked individuals	none

Monitored species	Taxonomical classification	N° of positive SACs	N° of investigated SACs	Percentage of positive SACs	Positive SACs codes	N° of detected individuals	Detected pressures	Notes on monitoring	New reports
Lycaena dispar (Haworth, 1803)	Arthropoda, Insecta, Lepidoptera, Lycaenidae	1	1	100,00%	IT5140010	2	mowing of riparian vegetation along the banks, leading to the destruction of nourishing plants	although present in the monitored SAC, the species seems to occur at very low density; being subject to wide fluctuations, a higher number of transects and/or semi-quantitative transects with eggs/larvae counting could be a more suitable monitoring protocol, but it would require greater field effort	none
Melanargia arge (Sulzer, 1776)	Arthropoda, Insecta, Lepidoptera, Nymphalidae	2	2	100,00%	IT5190012; IT51A0008	151	excessive density of ungulates (wild boars), leading to the destruction of nourishing plants; in IT5190012, reduction of open meadows and grass pastures due to natural succession of shrub vegetation	although present in all monitored SACs, the species seems to occur at very low density at all sites; the protocol adapted from 141/16 seems suitable for the species detection and monitoring, but needs to be repeated in the following years in order to detect population trends	IT51A0008
Zerynthia cassandra (Geyer, 1828)	Arthropoda, Insecta, Lepidoptera, Papilionidae	3	3	100,00%	IT5130007; IT5140010; IT5190009	10	mowing of riparian vegetation along the banks and excessive density of ungulates, both wild (boars) and domestic (sheeps), leading to the destruction of nourishing plants	although present in all monitored SACs, the species seems to occur at very low density at all sites; since the species has a short flight period, the persistence of unfavourable weather conditions for adults' flight may impair the effectiveness of the visual transects' monitoring protocol.	IT5130007; IT5140010; IT5190009
Padogobius nigricans (Canestrini, 1867)	Chordata, Actinopterygii, Gobiiformes, Gobiidae	8	6	83,33%	IT5120010; IT5110001; IT51A0001; IT5190002; IT51A0008; IT5180011; IT5140008; IT5130009	149	changes in the hydrological regime of rivers due to anthropic use and climate change; alteration of riparian vegetation; water pollution; competition with alien species	except for the population in IT5150002, the species seems to occur at low density and with unstructured or poorly structured populations at all sites; nevertheless, it would be necessary to monitor a higher number of sites to confirm this result. The 141/16 method confirmed its suitability for the species' detection and monitoring.	None
Squalius lucumonis (Bianco, 1983)	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	3	3	100,00%	IT5190002; IT5180012; IT5180009	78	changes in the hydrological regime of rivers due to anthropic use and climate change; alteration of riparian vegetation; water pollution; competition with alien species	although present in all monitored SACs, the species seems to occur at very low density and with poorly structured populations, probably declining, at all sites; nevertheless, it would be necessary to monitor a higher number of sites to confirm this result. The 141/16 method confirmed its suitability for the species' detection and monitoring.	None

Monitored species	Taxonomical classification	N° of positive SACs	N° of investigated SACs	Percentage of positive SACs	Positive SACs codes	N° of detected individuals	Detected pressures	Notes on monitoring	New reports
Barbus tyberinus Bonaparte, 1839	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	5	5	100,00%	IT5190002; IT5180012; IT51A0019; IT5180009; IT5150002	121	changes in the hydrological regime of rivers due to anthropic use and climate change; alteration of riparian vegetation; water pollution; competition with alien species	although present in all monitored SACs, the species seems to occur at low density and with poorly structured populations, probably declining, at all sites; nevertheless, it would be necessary to monitor a higher number of sites to confirm this result. The 141/16 method confirmed its suitability for the species' detection and monitoring.	IT5150002
Rutilus rubilio (Bonaparte, 1837)	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	1	2	50,00%	IT5150002	217	changes in the hydrological regime of rivers due to anthropic use and climate change; competition with alien species	in IT5150002, the species seems to occur in a good conservation state, with density and biomass higher than expected; the SAC could be relevant for the species' conservation. On the contrary, in the monitored streams in IT5150001, where the species was reported in the standard form, we did not detect fish of any species, suggesting that such streams could have been totally dried up in the recent past. The 141/16 method confirmed its suitability for the species' detection and monitoring.	IT5150002
Cottus gobio Linnaeus, 1758	Chordata, Actinopterygii, Perciformes, Cottidae	3	3	100,00%	IT5150003; IT5130009; IT5150001	167	presence of salmonids, which prey on young specimens; changes in the hydrological regime of rivers and in water temperature/oxygenation due to anthropic use and climate change	the species seems to occur in all monitored SACs in a good conservation state, with density and biomass comparable to previous data. The 141/16 method confirmed its suitability for the species' detection and monitoring.	IT5150001
Telestes muticellus (Bonaparte, 1837)	Chordata, Actinopterygii, Cypriniformes, Cyprinidae	5	6	83,33%	IT5150003; IT5150002; IT5180009; IT5120019; IT5120010	495	changes in the hydrological regime of rivers due to anthropic use and climate change; alteration of riparian vegetation; water pollution; competition with alien species	the species shows different density and biomass in the different SACs; it seems to be abundant, with high density and biomass, in IT5120019 and in IT5120010, although isolated in the latter. On the contrary, in the monitored streams in IT5150001, where the species was reported in the standard form, we did not detect fish of any species, suggesting that such streams could have been totally dried up in the recent past. The 141/16 method confirmed its suitability for the species' detection and monitoring.	IT5120019; IT5120010

Monitored species	Taxonomical classification	N° of positive SACs	N° of investigated SACs	Percentage of positive SACs	Positive SACs codes	N° of detected individuals	Detected pressures	Notes on monitoring	New reports
Salamandrina perspicillata (Savi, 1821)	Chordata, Amphibia, Caudata, Salamandridae	5	9	55,56%	IT5120010; IT5190002; IT5140008; IT5130009; IT5150001	9	presence of salmonids, which prey on eggs and larvae; changes in the hydrological regime of rivers due to anthropic use and climate change; alteration of riparian vegetation; water pollution	the protocol suggested by 141/16 requires high field efforts that might be inapplicable with limited resources; moreover, monitoring all or most reproductive sites would require detailed previous data on the occurrence and distribution of such sites within the SACs, which may often not be available. A simpler protocol still allows detecting or confirming the species' presence.	IT5120010
Rana italica Dubois, 1987	Chordata, Amphibia, Anura, Ranidae	8	9	88,89%	IT5120010; IT5110001; IT51A0001; IT5190002; IT5180011; IT5140008; IT5130009; IT5150001	49	presence of salmonids, which prey on eggs and tadpoles; changes in the hydrological regime of rivers due to anthropic use and climate change; alteration of riparian vegetation; water pollution	the species seems to be common and, in some sites, abundant; its presence was recorded during the whole sampling season (April-July). The protocol suggested by 141/16 requires high field efforts that might be inapplicable with limited resources; moreover, monitoring all or most reproductive sites would require detailed previous data on the occurrence and distribution of such sites within the SACs, which may often not be available. A simpler protocol still allows detecting or confirming the species' presence.	None
Hyla intermedia Boulenger, 1882	Chordata, Amphibia, Anura, Hylidae	0	1	0,00%	na	0	invasive competitor/predator species (*Procambarus clarkii*) in reproductive sites; water pollution and/or depletion	the monitored reproductive sites were known to host the species until 2010-12; further monitoring will be necessary to understand if the missed detection in 2018 was due to a temporary abandon of such sites, maybe for the particular weather conditions of the year, or to an actual local decline or extinction of the species. The protocol suggested by 141/16 requires high field efforts that might be inapplicable with limited resources; a simpler protocol still allows to detect or confirm the species' presence	none

Monitored species	Taxonomical classification	N° of positive SACs	N° of investigated SACs	Percentage of positive SACs	Positive SACs codes	N° of detected individuals	Detected pressures	Notes on monitoring	New reports
Bufotes balearicus (Laurenti, 1768)	Chordata, Amphibia, Anura, Bufonidae	0	1	0,00%	na	0	invasive competitor/predator species (*Procambarus clarkii*) in reproductive sites; water pollution and/or depletion	the monitored reproductive sites were known to host the species until 2010-12; further monitoring will be necessary to understand if the missed detection in 2018 was due to a temporary abandon of such sites, maybe for the particular weather conditions of the year, or to an actual local decline of the species. The protocol suggested by 141/16 requires high field efforts that might be inapplicable with limited resources; a simpler protocol still allows to detect or confirm the species' presence	none
Emys orbicularis (Linnaeus, 1758)	Chordata, Reptilia, Testudines, Emydidae	1	1	100,00%	IT5170002	117	habitat loss and degradation; water pollution; competition with alien species; anthropic disturbance	the species seems to occur at a good density, despite the presence of some criticalities in its habitat. The protocol suggested by 141/16 requires well-trained staff and high field efforts that might be inapplicable with limited resources	none
Lacerta bilineata Daudin, 1802	Chordata, Reptilia, Squamata, Lacertidae	5	7	71,43%	IT5170002; IT5110001; IT5140010; IT5190009; IT5160008	8	arson episodes; habitat loss and degradation; anthropic-related mortality; in IT5140010 e IT5190009, excessive density of wild boars, leading to habitat destruction; in IT5120019, intensive agriculture	due to limited transects' frequency and to species' elusiveness, it was possible to assess only the species' presence; the complete protocol suggested by 141/16 requires high field efforts that might be inapplicable with limited resources	none
Hierophis viridiflavus (Lacépède, 1789)	Chordata, Reptilia, Squamata, Colubridae	5	7	71,43%	IT5110001; IT5140008; IT5140010; IT5120019; IT5160008	5	arson episodes; habitat loss and degradation; anthropic-related mortality; in IT5140010 e IT5120019, excessive density of wild boars, leading to habitat destruction; in IT5190009, intensive agriculture	due to limited transects' frequency and to species' elusiveness, it was possible to assess only the species' presence; the complete protocol suggested by 141/16 requires high field efforts that might be inapplicable with limited resources	IT5110001
Muscardinus avellanarius Linnaeus, 1758	Chordata, Mammalia, Rodentia, Gliridae	2	4	50,00%	IT5160008; IT5110001	na	None	the applied method, chosen among those suggested by 141/16, allows to reliably detect the species' occurrence, as a preliminary data; nevertheless, being an indirect method, is not suitable for estimating species' abundance or population trends. Moreover, it requires quite high field efforts and well-trained staff, and it could be influenced by staff's experience and capability in hair's recognition	IT5160008; IT5110001

Monitored species	Taxonomical classification	N° of positive SACs	N° of investigated SACs	Percentage of positive SACs	Positive SACs codes	N° of detected individuals	Detected pressures	Notes on monitoring	New reports
Rhinolophus ferrumequinum (Schreber, 1774)	Chordata, Mammalia, Chiroptera, Rhinolophidae	3	4	75,00%	IT5150001; IT5170002; IT5140012	247	anthropic disturbance of the refuges and related mortality; loss of refuges; environmental alterations of feeding areas; pesticide pollution	all known bats' monitoring protocols require high resources, specialised staff and safety measures plus cooperation with speleologists (for monitoring caves). Several valuable refuges occur in areas that are not subject to any form of protection, but would deserve one: 199 specimens were detected in out-of-SACs colonies	none
Rhinolophus euryale Blasius, 1853	Chordata, Mammalia, Chiroptera, Rhinolophidae	1	4	25,00%	IT5150001	110	anthropic disturbance of the refuges and related mortality; loss of refuges; environmental alterations of feeding areas; pesticide pollution	all known bats' monitoring protocols require high resources, specialised staff and safety measures plus cooperation with speleologists (for monitoring caves). Several valuable refuges occur in areas that are not subject to any form of protection, but would deserve one: 197 specimens were detected in out-of-SACs colonies	none
Rhinolophus hipposideros (Bechstein, 1800)	Chordata, Mammalia, Chiroptera, Rhinolophidae	1	4	25,00%	IT5150001	5	anthropic disturbance of the refuges and related mortality; loss of refuges; environmental alterations of feeding areas; pesticide pollution	all known bats' monitoring protocols require high resources, specialised staff and safety measures plus cooperation with speleologists (for monitoring caves). Several valuable refuges occur in areas that are not subject to any form of protection, but would deserve one: 35 specimens were detected in out-of-SACs colonies	none
Miniopterus schreibersii (Kuhl, 1817)	Chordata, Mammalia, Chiroptera, Miniopteridae	2	4	50,00%	IT5150001; IT51A0025	239	anthropic disturbance of the refuges and related mortality; loss of refuges; environmental alterations of feeding areas; pesticide pollution	all known bats' monitoring protocols require high resources, specialised staff and safety measures plus cooperation with speleologists (for monitoring caves). Several valuable refuges occur in areas that are not subject to any form of protection, but would deserve one: 1240 specimens were detected in out-of-SACs colonies	none
Myotis emarginatus (Geoffroy E., 1806)	Chordata, Mammalia, Chiroptera, Vespertilionidae	2	4	50,00%	IT5170002; IT5140012	437	anthropic disturbance of the refuges and related mortality; loss of refuges; environmental alterations of feeding areas; pesticide pollution	all known bats' monitoring protocols require high resources, specialised staff and safety measures plus cooperation with speleologists (for monitoring caves). Several valuable refuges occur in areas that are not subject to any form of protection, but would deserve one: 169 specimens were detected in out-of-SACs colonies	none

Discussion and conclusions

Natura 2000 Network's implementation and critical issues

Both in Italy and in Europe, the implementation of Natura 2000 Network faced several key issues during the years. At Community level, despite the existence of EU guidelines and reporting formats for all Member States (European Commission, 2016), the unevenness of data quality for the National Reports is still a major issue that could impair the efficiency of the Reports system itself, due to the delays of some Member States in developing or refining targeted monitoring programmes (de Bello *et al*., 2010; European Commission, 2015; Kallimanis *et al*., 2017; Ellwanger *et al*., 2018). The need to implement a common framework of monitoring both at national and European level, with a more precise definition of targets and methods to be used, is well-known (Cantarello & Newton, 2008; Trochet & Schmeller, 2013; Paliogiannis *et al*., 2019), as well as the direct linking of the scientific knowledge with policy-making and effective conservation actions *in situ* (Pullin *et al*., 2009; Rands *et al*., 2010). Different Member States may indeed still use different methodologies and approaches to assess the conservation status of target species and habitats, with a still significant lack of integration (Kallimanis *et al*., 2017; Painting *et al*., 2020). Even the national representatives, scientists, and monitoring authorities themselves of the different Member States often consider their own national monitoring programmes as not adequate or just partially adequate (Painting *et al*., 2020). A critical analysis of conservation policy at the individual country scale is necessary in order to halt biodiversity loss transnationally (Papageorgiou & Vogiatzakis, 2006); the consequences of a lack of coordination in the implementation of conservation policies would be, in the long term, much more costly than the coordination process itself (Paliogiannis *et al*., 2019). Concerning Italy, it strongly emerged in the last years (Genovesi *et al*., 2014) the necessity to better coordinate and to optimise biodiversity data management and sharing at national level. This awareness decisively contributed to the writing of the series of three "Manuals for the monitoring of species and habitats of Community interest in Italy" (Manual 140/16, 141/16, 142/16). The applicability of their protocols strongly depends upon the adequacy and stability of funds over time and on the availability of experts with a sound competence both on the target species and on the local study areas. Indeed, the inconstancy of funding, budgetary constraints and insufficient financial and human resources may seriously affect the efficiency of a country's conservation efforts in the long term (Trochet & Schmeller, 2013; Paliogiannis *et al*., 2019; Painting *et al*., 2020). Despite the great usefulness of citizen-science forms of data collection that have been developed in the last years (e.g., see Menchetti *et al*., 2021 for what concerns Tuscany), the involvement of experts, specialized in the different taxa and with an adequate training and significant field experience, is necessary for such monitoring programs. Actually, the completeness of the data recorded by the non-experts tends to be inferior, especially for low-density species (Anderson *et al*., 2001; Fitzpatrick *et al*., 2009; Kallimanis *et al*., 2017; Barbato *et al*., 2021).

In 2016, the Natura 2000 network was considered as largely complete on land (European Commission, 2020b), although new objectives have been recently redefined (EU Biodiversity Strategy for 2030). Nevertheless, up to recent years the conservation status of species and habitats has yet to be significantly affected within Natura 2000 sites (European Commission, 2015). It has also been suggested that a large proportion of threatened species could be currently poorly covered by the network. An increase in the number of Natura 2000 sites could be recommended, while preventing the establishment of scarcely effective sites (i.e., containing a low number of protected species, Trochet & Schmeller, 2013), and carefully basing the eventual establishment of new Natura 2000 sites on the real presence of important habitats for the target species and on updated distribution data (Fortuna *et al*., 2018). The present paper is the result of the first attempt of coordinate collaboration among zoologists from the Florence, Pisa, and Siena Universities, to evaluate and monitor species in Habitat Directive on SAC Tuscany territory.

Stage 1

The bibliographic update allowed to significantly widen the distribution maps of several species included in the Habitats Directive on the basis of data obtained after 2010. This increase in the known occupied area can be due to an intensification of regional scientific studies and citizen science reports, with a subsequent improvement in the local knowledge, rather than to an actual range expansion. This could be especially true for poorly mobile or elusive species, such as Arthropods or Molluscs for the first case, and Mammals, especially Chiroptera, for the latter (Barlow *et al*., 2015). Scientific studies came both from the academic world and from technical and administrative authorities, while the increasing of citizen science reports probably benefited from the development and implementation of social media, online dedicated platforms and dissemination activities, and the increasing awareness on conservation topics (Unger *et al*., 2021).

In this first stage, despite taking into consideration that distribution maps may be prone to errors (Trochet & Schmeller, 2013) or may contain errors (Manganelli *et al*., 2020), our approach was conservative:

the data collected allowed us to expand the previously known distributions by adding new grid squares, but, at the present, not to shrink them, since assessing range reductions requires additional evidence based on multi-year monitoring, and has severe consequences in terms of required conservation measures. Just in three selected cases, we notified to the competent authorities the necessity to modify the previous distribution maps by removing some grid squares. In detail, the following data were signalled: i) the report of *Discoglossus sardus* Tschudi, 1837 (Tyrrhenian painted frog) in the grid square E441N215 was surely incorrect and due to an erroneous transposition of the report by Vanni & Nistri (2006), whose distribution maps grid was based on a different coordinates reference system; ii) accurate and recent researches (unpublished data) concerning *Podarcis siculus* (Rafinesque, 1810) (ruin lizard) on the Elba island show that the species is actually extinct on most of the island, except for E434N218 and E435N218 grid squares; iii) the report of *Austropotamobius pallipes* complex [*A. fulcisianus* Ninni, 1886)] (white-clawed crayfish) in E438N229 needs verification but, since that grid square is in the plains and the invasive *Procambarus clarkii* (Girard, 1852) (red swamp crayfish) is reported for the area, most likely this report refers to the latter species. Finally, the presence of *Protochondrostoma genei* in Tuscany has been updated, because, although the species is considered endemic to the Padano-Venetian district, one of the bibliographic records found refers to a locality on the Adriatic side of Tuscany and, as such, falls within the natural range of the species (Falconi *et al.*, 2012).

Stage 2

First-year monitoring field results allowed us to update the Standard Forms of the SACs we monitored, in case we retrieved species not previously reported in a particular Area, and/or to confirm the occurrence of some species in the SACs where they were already known, including species that have in Tuscany the southern limit of their distribution range. We integrated two different approaches in choosing the SACs to be monitored: for some species, whose distribution was already sufficiently known and studied in Tuscany, we chose to strengthen and update already available data by monitoring SACs or areas where the species had already been detected in the past. On the contrary, for other species that lacked basic distribution data especially in the North-West, we chose to investigate poorly studied SACs, which nevertheless showed suitable habitats for the target species and that were close to areas of presence. The different percentage of positive SACs for every species, reported in Tab. 3, could probably depend upon this double selection approach, being higher for species monitored in already known areas. On the contrary, the second approach allowed us to detect the presence of several species in "new" SACs, thus expanding their known distribution and providing new data. Nevertheless, it implied a considerable amount of "ineffective" field work, with several preliminary surveys that did not allow to detect a species' presence in a particular SAC, thus being time-consuming and resource consuming. In three cases, we did not detect a target species in any of the selected SACs, although such areas were previously known to be occupied by the species. In such cases, we consider more probable a missed detection, or a temporary abandon of the specific reproductive sites monitored within the SAC, than an actual extinction from the site. Nevertheless, further monitoring has been scheduled in the prosecution of the project to verify all those cases.

In almost half of the monitored SACs, the applied protocols allowed us to detect also non-target species of conservation interest, and/or protected by national or regional law, and/or endemic. Observational transects were the monitoring protocol that most frequently allowed us to detect by chance the non-target species, thus enriching the local faunal inventory for the study areas. This protocol is low-cost and does not require special equipment; as such, it could be particularly useful to preliminarily improve the local knowledge for poorly studied areas, even though it is time-consuming and requires adequately trained staff, with a sufficient competence for species' detection and recognition.

Data collected in this first year did not allow us to provide any robust consideration about the conservation status of the selected species. Nevertheless, we could detect and notify to the competent authorities some alarming cases of unusually low population densities (e.g., freshwater fish species), and in some cases to detect population trends due to the availability of previous data from the same populations (e.g., bat species). Moreover, the main recorded pressures insisted on several species belonging to different taxa (such as alterations of riparian vegetation or changes in watercourses hydrological regime) or even to different environments (competition with alien species, anthropic disturbance or anthropic-related mortality). As such, this fact opens interesting possibilities, since contrasting or removing such pressures could benefit many species of conservation interest at the same time.

Retrieved major critical issues

1. A significant fraction of useful literature to update distribution maps was represented by "grey literature" whose access was not obvious. The existence of additional, and potentially useful, but not accessible, or difficult to access, records cannot be excluded.

2. The costs of field-monitoring activities across the entire regional territory, both for personnel and for travelling among the different study areas, proved to be considerable. Such costs depended also upon the need for an adequate number of qualified staff, already trained for field working and with expertise for the different target *taxa*, as well as possessing an appropriate knowledge of the local study areas; such technical skills and local experience are functional to optimize both field efforts and species' detection and correct, reliable, identification.
3. The possible variations in the phenology of a species due to the very local habitat and weather conditions, implying the possibility that field-monitoring protocols could suddenly need to be anticipated or postponed, according to specific local situations.
4. The time overlapping of different taxa's field-monitoring activities, which are concentrated for most species in the spring-summer months, increased the number of staff required for operating in parallel and in safety conditions on numerous, different study areas.

Final remarks

As a conclusion we propose that study such as the present one can contribute to:

1. Letting species distribution data from grey literature become available to international community, an issue recently highlighted by Amano *et al.* (2021).
2. Evidencing field-monitoring issues that should be carefully considered in order to set up an appropriate multi-year monitoring plan on the territory of interest, as expected to properly fulfil the requirement of the Habitats Directive.

Contribution of the single authors

M.B. developed the article concept, provided the first draft and contributed to designing the sampling plan and to field surveys for amphibians, arthropods, mammals and reptiles. M.A.L.Z. contributed to designing the sampling plan and to field surveys for amphibians and reptiles, and to text writing and revision. P.A. contributed to designing the sampling plan and to field surveys for mammals. L.B. contributed to designing the sampling plan for insects. G.I. contributed to designing the sampling plan and to field surveys for decapod crustaceans. A.Ni. was the organisational manager for the Natural History Museum (University of Florence) unit, contributed to designing the sampling plan and to field surveys for amphibians and was the corresponding author. A.No. contributed to designing the sampling plan and to field surveys for fish. S.R.D.G. contributed to designing the sampling plan and to field surveys for arthropods. S.V. contributed to designing the sampling plan and to field surveys for amphibians and reptiles. M.D. contributed to designing the sampling plan for arthropods. G.C. and I.T. contributed to data analysis, text writing and revision. D.B. and A.B. contributed to designing the sampling plan and to field surveys for molluscs. L.F. contributed to designing the sampling plan and to field surveys for amphibians, arthropods (Lepidoptera), molluscs, fish and reptiles. S.P. contributed to the field surveys for amphibians, arthropods (Lepidoptera), fish and reptiles. D.F. contributed to the field surveys for amphibians, arthropods, mammals, and reptiles. M.G. was the executive manager of MonitoRARE project. A.U. was the organisational manager for the Department of Biology of the University of Florence. G.M. was the organisational manager for the University of Siena unit, contributed to designing the sampling plan for amphibians, molluscs and fishes and to text revision. G.P. was the organisational manager for the University of Pisa unit, proposed the article concept and contributed to text revision.

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