



Faculté de biologie et de médecine

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**Ecology of the European pond turtle (*Emys orbicularis*, L. 1758) in
Switzerland**

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Prof. Alexandre Reymond

"You're just lucky if you have an obsession, because an obsession is very happiness making. It makes you glad to be there and glad to do it"

Dr. Peter C. H. Pritchard
June 26, 1943 – February 25, 2020
A Turtleman, a friend, a mentor



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Résumé:

La seule tortue d'eau douce de Suisse, la cistude d'Europe (*Emys orbicularis*), est considérée comme « en danger critique » sur la Liste Rouge. Cette espèce vit dans des habitats aquatiques tempérés, riches en végétation (étangs ou marais), et a besoin à la fois d'habitats aquatiques pour se nourrir, se reproduire et d'hiberner, et d'habitats terrestres pour pondre des œufs. Parce que son cycle biologique nécessite la conservation des habitats aquatiques et terrestres, elle est considérée comme une espèce « parapluie ». Aujourd'hui, c'est une espèce prioritaire au niveau de la conservation. Par conséquent, il est essentiel d'acquérir des connaissances sur cette espèce combinant les sciences fondamentales et appliquées.

Deux stratégies de conservation distinctes pourraient être envisagées pour cette espèce : (i) la renaturation des habitats pour améliorer la recolonisation naturelle, ou (ii) le renforcement des populations par des actions de réintroduction dans des sites appropriés. En 1999, le projet Emys a été lancé, un suivi des populations relictuelles a été réalisé et les trois premières réintroductions ont eu lieu (cantons de Genève et Neuchâtel). Après celles-ci, certaines questions ont été soulevées quant à la menace potentielle que cette espèce pourrait avoir sur d'autres espèces menacées (en particulier les amphibiens), ce qui pourrait être un obstacle majeur à l'implantation d'un programme de réintroduction efficace. Nous avons mené une étude pour déterminer son régime alimentaire à l'aide de matières fécales en développant pour la première fois sur cette espèce une nouvelle méthode métabarcodage de l'ADN. Cette approche utilise des amorces de PCR pour identifier l'ADN d'un mélange d'organismes. Avec l'utilisation de l'ADN, la détermination des espèces végétales et animales du régime alimentaire, est possible avec un haut niveau de précision. Nous avons pu démontrer que la cistude d'Europe est une chasseuse opportuniste, se nourrissant principalement de plantes et d'invertébrés et que son impact est marginal sur d'autres espèces.

Il est essentiel de déterminer avec précision la répartition des espèces pour une planification efficace de la conservation, en particulier dans le cas des espèces menacées par la dégradation et la fragmentation de l'habitat. Les modèles de distribution des espèces (Species Distribution Model - SDM) représentent les outils éco-informatiques les plus utilisés pour prédire les changements potentiels dans l'aire géographique des espèces. Sur la base des données d'occurrence et d'un ensemble de prédicteurs environnementaux significatifs, nous avons utilisé des SDM pour prévoir les distributions actuelles et futures pour *E. orbicularis* à deux échelles géographiques : au niveau de l'aire de répartition complète de l'espèce et à l'échelle suisse. Nous avons démontré que la quantité d'habitat augmentera pour cette espèce sous le changement climatique. Cependant, en Suisse, cette augmentation potentielle d'habitats est entravée par la capacité limitée de l'espèce à migrer et la fragmentation de l'habitat.

Enfin, nous avons déterminé que pour être efficace, la conservation de la cistude en Suisse devrait se baser à court terme sur des actions de réintroduction dans des sites favorables. Ensuite, à long terme, des renaturations de l'habitat sont nécessaires, en raison de l'énorme perte de zones humides en Suisse, afin d'augmenter la connectivité de l'habitat afin de minimiser les menaces associées à la fragmentation.

Summary:

The only freshwater turtle of Switzerland, the European pond turtle (*Emys orbicularis*), is ranked as “Critically Endangered” on the Swiss Red List. It lives in temperate aquatic habitats, rich in vegetation such as ponds or marshlands, and needs both aquatic habitats to forage, reproduce and hibernate, and terrestrial habitats for laying eggs and basking. Because its biological cycle requires the conservation of both aquatic and terrestrial habitats, it can be considered an “umbrella” species. *E. orbicularis* is today a priority species for national conservation programs; therefore, it is essential to gain knowledge on the ecology of these species combining both fundamental and applied sciences.

Two distinct conservation strategies, which are not mutually exclusive, could be considered for this species: (i) habitat renaturations to enhance natural recolonization and expansion, or (ii) population reinforcements through reintroduction actions in suitable sites. In 1999, the Emys Project was launched, monitoring of relict populations in Switzerland has been undertaken for several years and the first three reintroductions have taken place in cantons of Geneva and Neuchâtel. After them, some questions were raised about the potential threat that this species could have on other endangered species (especially amphibians), which could be a major obstacle to the implantation of an efficient reintroduction program. We conducted a study to determine the diet of the European pond turtle using faeces by developing for the first time on this species a new long DNA metabarcoding method, which is a method that uses universal or specific PCR primers to identify DNA from a mixture of organisms. With the use of DNA, the determination of plant, invertebrate and vertebrate species, composing the diet, is possible with a high level of precision. We have been able to demonstrate that the European pond turtle is an opportunistic hunter, feeding mainly on plants and invertebrates and that its impact is marginal on other species.

Determining accurately the distribution of species is essential for efficient conservation planning, especially in the case of species that are threatened by human-driven habitat degradation and fragmentation. Species distribution models (SDMs) represent the most used ecoinformatic tools to predict potential changes in species geographic range. Based on species occurrence data and a set of meaningful environmental predictors, we used SDMs to forecast the current and future distributions of potentially suitable areas for *E. orbicularis* at two geographic scales: at the level of the whole range of the species, and at the Swiss scale. We demonstrated that habitat suitability will increase for this species under climate change. However, in Switzerland, this potential increase in suitable habitats could be hampered by the limited capacity of the species to migrate in the landscapes and habitat fragmentation.

Finally, we determined that to be effective, the conservation of the European pond turtle in Switzerland should be based in the short-term on reintroduction actions in favorable sites. Then in the long-term, habitat renaturations are needed, due to the huge loss of wetlands in Switzerland, in order to increase habitat connectivity to minimize the threats associated with fragmentation.

General introduction

The human world's population has almost doubled in fifty years and causes an increased pressure on the natural environment. Numerous species struggle to subsist in a decreased living environment causing a conflict with humans (Lindquist et al., 2012). Indeed, species have less space to live on the planet. Many threats such as destructions of natural habitat, poaching, global warming, or conflicts arising between humans and animals, leading to the gradual disappearance of numerous species. In 1964, as the threat increased, the International Union for Conservation of Nature (IUCN) created a Red List, which is the world's most comprehensive inventory of the global conservation status of plant and animal species (IUCN, 2012). IUCN's Red List comprises threat categories from Least Concern to Extinct. Classification of a species in one of three categories of endangered species (CR, EN, and VU) is fixed through a series of five criteria, which are based on different biological factors associated with extinction risk: population size, rate of decline, geographic distribution, degree of population and distribution fragmentation (IUCN, 2018).

Nowadays, IUCN counts about 31'000 threatened species which represents 27% of all assessed species in the entire world (IUCN, 2018; IPBES, 2019). Some of them depend on conservation projects for their survival; others are considered as vulnerable, endangered, or extinct. Moreover, many species have already disappeared, such as the Chinese white dolphin (*Lipotes vexillifer*) extinct in 2007 or the Tasmania tiger (*Thylacinus cynocephalus*) extinct in 1930 (IUCN, 2013). When a species go extinct, sometimes an entire ecosystem is threatened and consequently results in a high disturbance of ecosystem functioning (Maherali & Klironomos, 2007). For instance, the death of the corals in the Great Barrier Reef due to several aspects (an increase of the temperature, pollution, direct destruction, etc.) could lead to the extinction of thousands

of other endemic species (Berkelmans et al., 2004). Additionally, another threat leading to the disappearance of species is the removal or translocation of species from their natural environment. Indeed, international trades and travels between continents cause unnatural translocations and further colonization in new environments, which promote an unnatural phenomenon: the movement of new species into new environments. Accidental or intentional migration of animal and plant species can seriously disrupt a stable environment. For instance, the introduction of the European fox (*Vulpes vulpes*) in Australia resulted in the disappearance of some marsupial species (Kinnear, 2002).

The order Testudines, which is composed of tortoises, freshwater, and marine turtles, recognized, nowadays, 360 species worldwide (Turtle Taxonomy Working Group 2017; Rhodin et al., 2018; Turtle Conservation Coalition, 2018). These species colonized very contrasting habitats and environments such as oceans, deserts, freshwaters, forests, which are often threatened (Mitchell and Klemens, 2000, Buhlmann et al., 2002). Currently, 187 species are considered as threatened following the IUCN Red List criteria and of these 127 species are ranked endangered or critically endangered (Rhodin et al., 2018). Moreover, seven species and ten taxa have become extinct in modern times, such as the Pinta Giant Tortoise (*Chelonoidis abingdonii*) (Turtle Extinction Working Group, 2018). Thus, chelonians are among the most threatened of the major groups of vertebrates (Lovich et al., 2018; Rhodin et al. 2018). This decline, far from being a natural biological phenomenon, is often directly linked to human exploitation for the pet market, shell trade, food, habitat loss, and competition with exotic species (Gibbons et al., 2000; Mitchell and Klemens, 2000). Moreover, the relatively long time required to attain sexual maturity, the low reproductive output, and high rates of predation of nests and juveniles, limited the ability of turtles and tortoises to recover demographically after a decline

(Iverson, 1991; Congdon et al., 1993). Thus, many turtle and tortoise species are under protection and conservation programs are being implemented for some of them (Ficetola et al., 2005). Several programs combined *in situ* (study and protection of wild populations) and *ex situ* (breeding of species in captivity) measures to better ensure the sustainability of endangered species (Turtle Conservation Fund, 2002). Both measures combined together should make it possible to achieve real success for the conservation of chelonians. However, most of the time a choice is made between these two approaches. Thus, conservation programs tend to focus on birds and mammals but do not consider turtle and tortoise diversities (Roll et al., 2017). Nowadays, the turtle population extinctions mean that their ecological roles and services are greatly decreasing (Lovich et al., 2018). In order to set up efficient conservation plans, we lack field and experimental data on these species, which often leads to a wrong appreciation of the ecology of the species (Seigel and Dodd, 2000; Gibbons & Lovich, 2019). Thus, an improved understanding of turtle ecology is needed in order to optimize conservation plans (Dalton, 2003). Among vertebrates that inhabit aquatic systems, freshwater turtles are especially important for understanding the link between aquatic and terrestrial habitats (Joyal et al., 2001). The existence of an alternating terrestrial (such as egg-laying, basking behaviour, etc.) and aquatic (such as feeding, mating, hibernate, etc.) annual cycle in most freshwater chelonian species make them an attractive and effective model for wetland conservation (Burke & Gibbons, 1995) due to the protection of several types of habitat needed for the completion of their life cycle.

The European pond turtle (*Emys orbicularis*, L. 1758) (Figure 1) lives in aquatic environments rich in vegetation, such as wetlands, marshes and is an emblematic species of wetlands and could be considered as an “umbrella” species because its biological cycle

requires the conservation of both aquatic and terrestrial habitats (Cadi, 2003). Its distribution range is the widest of the Emydidae family and distinct subspecies of pond turtles occur from the Northern Africa Maghreb region, over much of the Southern and Western Europe and a major part of Eastern Europe and to Asia Minor (Fritz, 2003; Fritz et al., 2005). Ponds and marshes have declined sharply over the last two centuries, causing the disappearance of this species in many regions. Moreover, during the past centuries, this species was consumed in large quantities, which had led to the strong decrease of these populations (Devaux et al., 1996; Cheylan, 1998; Vlachos & Delfino, 2016; Daszkiewicz, 2018). Therefore, the European pond turtle is ranked as “near threatened” (NT) through its entire range (Tortoise and Freshwater Turtle Specialist Group, 1996; van Dijk & Sindaco, 2004).



Figure 1: European pond turtles (*Emys orbicularis*) in the natural reserve of Moulin de Vert (Canton of Geneva, Switzerland) ©Olivier Born

In Switzerland, only two native subspecies are found, *Emys orbicularis orbicularis* (L., 1758) for the northern Alps and *Emys orbicularis hellenica* (Valenciennes, 1832) for the Southern Alps (Ticino) (Lenk et al., 1999; Fritz, 2003). The subspecies *E. o. orbicularis* (haplotype II) are currently found in the Danube and Oder rivers catchment basins, in the Balkan Peninsula, Southern France, and Northern Spain. *E. o. hellenica* have a circum-Adriatic distribution. In Switzerland, the species was observed in numerous aquatic habitats, but the large majorities of observations refer to a single or a very few individuals resulting from accidental or voluntary release. In addition to the very recently recreated populations in the Cantons of Geneva and Neuchâtel (composed only by individuals of *E. o. orbicularis*; see below), viable populations of this species are currently limited to the Canton of Geneva, in the natural reserve of Moulin-de-Vert ($46^{\circ}10'46''N$, $6^{\circ}1'42''E$) and Laconnex ($46^{\circ}09'24''N$, $6^{\circ}01'46''E$). These two sites host breeding individuals yet were introduced by humans between the 1950s and the 1980s. Whereas the population of Laconnex is composed only by *E. o. hellenica*, the population of the Moulin-de-Vert reserve is a mix of three subspecies: *E. o. orbicularis*, *E. o. hellenica* and *E. o. galloitalica* (Raemy, 2010). The last is a non-native subspecies originating from mountain stream waters in the Western Apennine peninsula, Sardinia, Corsica, and Southern France (Lenk et al., 1999; Fritz et al., 2005).

In Switzerland, the indigenous status of the European pond turtle has long been questioned. However, remains of shells dating back 7000 years (Mesolithic) have proved the presence of this species in Switzerland (Becker & JohanssOn, 1981; Besse et al., 2003). More recently, several observations of this species have been reported between 1800 and 1930 in the lowlands of Switzerland (Parent, 1968; Hofer et al. 2001). The European pond turtle was considered as “Regionally Extinct” (RE) on the Swiss Red List of threatened and

rare amphibians and reptiles of 1982 and 1994 (Hotz and Broggi, 1982; Duelli, 1994), until its status changed to “Critically Endangered” (CR) on the new Red List of threatened reptiles in Switzerland (Monney and Meyer, 2005). The species is considered today a priority species for national conservation programs. In 1999, the Association « Protection et Récupération des Tortues » (PRT) located in Chavornay (Switzerland), launched the Emys Project in order to protect and promote the only native freshwater turtle species of Switzerland (Ducotterd, 2003). Nowadays, the Swiss coordination center for amphibian and reptile protection (“Centre Suisse de Coordination pour la Protection des Amphibiens et des Reptiles”; karch.ch) is coordinating the project with the help of scientists, breeders, and local authorities. The main goal of this conservation project is to conserve this endangered species and to improve its Swiss Red List status. Different actions were promoted to reach this goal: 1) evaluate the current swiss populations on their size, dynamics, and genetics status; 2) manage and protect favorable site according to the species' requirements (e.g. creation of optimal nesting sites); 3) reintroduce native subspecies (*E. o. orbicularis* and *E. o. hellenica*) in suitable environments (population reinforcement) when the species is not present; 4) promote scientific research on the species in order to improve knowledge on its ecology and improve chances of successful reintroductions (karch, 2014). After numerous monitorings (Nuoffer, 1999; Mosimann, 2002) and genetics studies (Raemy, 2010), reintroduction tests took place in the Cantons of Geneva and Neuchâtel.

Focus on the thesis

In order to pursue the Emys Project, I aimed to set up a clear conservation strategy in Switzerland to ensure the sustainability of the species. Two distinct conservation strategies are commonly used in conservation programs of threatened and endangered

species, which are not mutually exclusive, could be considered for this species: (i) habitat renaturations to enhance natural recolonization and expansion, or (ii) population reinforcements through reintroduction actions in suitable sites. The most critical factor for the success of conservation programs is the maintenance of self-sustaining and free-living populations (Stanford et al., 2020). In this context, it is essential to determine which of the two strategies (habitat renaturation or reintroduction), or if a combination of both, is the most suitable for *E. orbicularis* in Switzerland. In absence of quantitative data on the habitat, behaviour and ecology of this species, most conservation planners have used “expert judgements” to decide which locations are most favourable for reintroductions, or what management actions should be taken to improve habitat for existing populations (Schneider, 2000). Thus, conservation effort could be optimized by improving knowledge on habitat and behaviour of this species. For that fundamental and applied researches are necessary (Standford et al., 2020) and have been implanted to increase our knowledge on the European pond turtle in Switzerland. I identified missing information about the European pond turtle in order to set up efficient studies to allow to set up an efficient conservation strategy in Switzerland.

After implantation of the first reintroduction actions of European pond turtles in Switzerland, some questions were raised about the potential threat that this species could have on other endangered species (especially on endangered amphibians), which could be a major obstacle to the implantation of an efficient reintroduction program. This underlies the importance of having a strong and clear knowledge of the ecology and needs of the reintroduced species. In fact, the knowledge of feeding strategy and food preference is one of the milestones of the natural history of a species and is essential to optimize conservation programs. Indeed, the feeding behaviour of the European pond turtle is still

unclear on the literature, considered in some study as carnivorous, often scavenger (Rollinat, 1934; Lebboroni and Chelazzi, 1991; Kotenko, 2000, Luiselli, 2017) and sometimes vegetarian (Ficetola and De Bernardi, 2006). To our knowledge, studies of *E. orbicularis* diet have dealt with only a few individuals and analyses do not take place during the whole activity period (see Ottonello et al., 2005; Çiçek and Ayaz, 2011; Ottonello et al., 2018). Ottonello et al. (2016) were the first to demonstrate that a shift in diet occurs between the period pre-reproductive and post-reproductive. However, analyzes of the pond turtle faeces were done by direct observations under the microscope, so there is a huge loss of information and difficulty to recognize the type of prey and plant matters present in the sample. In **Chapter 1**, I analyzed the diet of the European pond turtle using faeces by developing a new long DNA metabarcoding method, which is a method that uses universal or specific PCR primers to identify DNA from a mixture of organisms, in our case prey and plant matters present in faecal samples (method developed in **Chapter 1a**). This study is the first to analyze the diet of the European pond turtle by using DNA faeces analysis. With the use of DNA, the determination of plant, invertebrate, and vertebrate species, composing the diet of the European pond turtle, is possible with a high level of precision. Not only I could determine the trophic role of the European pond turtle and its impact on other endangered species but also studied its diet during its whole activity period (April to September) in the natural reserve of Moulin-de-Vert (Canton of Geneva), and determine the difference in food consumption between different populations for the same period of time (July). Furthermore, I wanted to determine if there is a shift in diet during the year and also the difference in food intake between sex and adults/juveniles. Studies on the diet are highly essential to ensure the success of reintroduction (results presented in **Chapter 1b**).

Determining accurately the current and future distribution of wild species is essential for efficient conservation planning and land management, especially in the case of species that are threatened by human-driven habitat degradation and fragmentation (Kumara et al., 2009). Although some species could be able to cope with climate change due to their ability to disperse (Davis et al., 1998; Kubisch et al., 2014), many others, such as reptiles, might not or only partly due to their low dispersal capacity (Halpin, 1997; Gibbons et al. 2000; Pittet, 2017) and therefore are more vulnerable regarding rapid habitat modifications (Mac et al., 1998). The distribution and viability of the European pond turtle were modelled in various regions of Europe (e.g. Italy: Ficetola et al. 2004, Portugal: Segurado & Araujo 2004, Spain: Cordero Rivera and Fernandez 2004, Serbia: Golubovic et al. 2017, Black Sea region: Duysebaeva et al., 2019). However, none of these used dynamic simulations of species dispersal (as proposed by Engler & Guisan 2009; and used e.g. for terrestrial reptiles in Switzerland; Pittet, 2017) and I am not aware of any modeling study for this species in Switzerland.

Therefore, in **Chapter 2**, based on species occurrence data and a set of meaningful environmental predictors, I used species distribution models (SDMs) to forecast the current and future distribution of potentially suitable areas for *E. orbicularis* at two geographic scales: (i) Global, at the level of the whole range of the species, using only climatic variables; and (ii) Regional, at the Swiss scale combining the previous Global climatic model with additional habitat constraints (land cover data such as distance to suitable habitats, roads, railways, building, etc.). Then, I determined the potential impact of climate change on the future distribution of the European pond turtle. For this, I used two different climatic scenarios relative to the representative concentration pathways RCP 4.5 and RCP 8.5, which assume moderate and extreme global warming, respectively (Van Vuuren et al., 2011). I assessed whether the extent of potentially suitable habitat is

projected to increase or decrease under future environmental conditions. Moreover, due to the low dispersal ability of *E. orbicularis*, I further assessed whether habitat fragmentation could slow down or hamper the species natural recolonization or migration under climate change in Switzerland. For this, I used MigClim (Engler & Guisan, 2009), a cellular-automata relating successive SDM predictions (in time) to assess the potential dispersal pathways of the species, accounting for landscape barriers (like fragmentation) and climate change. These models and predictions additionally will provide valuable ecological and biogeographic information that may help target suitable habitats for future reintroductions of the European pond turtles in Switzerland, by identifying regions where the species is currently absent but that share similar environmental conditions to those of current populations.

The final chapter (**Chapter 3**), I reviewed all the current actions, conservation efforts and achievements of the Emys project. This review brings together informative and practical elements and help to better understand the ecology of the European pond turtle by combining both fundamental and applied sciences. Therefore, the multiple studies and the contribution of this thesis (see Chapter 1 and 2) allow me to determine which conservation strategy is the most suitable for the European pond turtle in Switzerland and I designed an integrated framework to plan efficient and essential steps for the conservation of this emblematic species. This final chapter represent an important contribution for the consideration of the species during wetlands management interventions and give useful advice for local authorities who are willing to be involved in the Emys project by reintroducing the European pond turtle in their areas.

Chapter 1

**Determine the diet of the European pond
turtle (*Emys orbicularis*, L. 1758) in
Switzerland by developing a new long DNA
metabarcoding method**

CHAPTER 1a

A powerful long metabarcoding method for the determination of complex diets from faeces analysis evaluated in the European pond turtle (*Emys orbicularis*, L. 1758)

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Author contributions

The first (C.D.) and second (J.C.) author contributed equally to this paper. C.D., S.U. and J.-F.R. designed research, C.D. performed research (sampling and laboratory work) and analyzed data (bioinformatics analyses) and statistics, J.C. set up the methodology (shearing protocol, first upstream method validation, primers selection and design, sequencing and bioinformatics analyses), J.C. and F.L. supervised laboratory work. C.D. wrote the manuscript and all other coauthors revised it.



A powerful long metabarcoding method for the determination of complex diets from faecal analysis of the European pond turtle (*Emys orbicularis*, L. 1758)

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Abstract

High-throughput sequencing has become an accurate method for the identification of species present in soil, water, faeces, gut or stomach contents. However, information at the species level is limited due to the choice of short barcodes and based on the idea that DNA is too degraded to allow longer sequences to be amplified. We have therefore developed a long DNA metabarcoding method based on the sequencing of short reads followed by de novo assembly, which can precisely identify the taxonomic groups of organisms associated with complex diets, such as omnivorous individuals. The procedure includes 11 different primer pairs targeting the COI gene, the large subunit of the ribulose-1,5-bisphosphate carboxylase gene, the maturase K gene, the 28S rRNA and the *trnL-trnF* chloroplastic region. We validated this approach using 32 faeces samples from an omnivorous reptile, the European pond turtle (*Emys orbicularis*, L. 1758). This metabarcoding approach was assessed using controlled experiments including mock communities and faecal samples from captive feeding trials. The method allowed us to accurately identify prey DNA present in the diet of the European pond turtles to the species level in most of the cases (82.4%), based on the amplicon lengths of multiple markers (168–1,379 bp, average 546 bp), and produced by de novo assembly. The proposed approach can be adapted to analyse various diets, in numerous conservation and ecological applications. It is consequently appropriate for detecting fine dietary variations among individuals, populations and species as well as for the identification of rare food items.

KEY WORDS

de novo assembly diet analysis, European pond turtle, faecal analysis, long metabarcoding

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1 | INTRODUCTION

Molecular technologies, such as high-throughput amplicon sequencing (HTS), have become a method of choice to accurately and rapidly characterize complex, multispecies, ecological communities. This approach has the potential to greatly improve the accuracy of diet analysis from faecal samples or stomach contents (Alberdi et al., 2018). HTS has been used to assess the diet composition of a wide taxonomic range of animals. Animals whose diets have been successfully investigated have included mammals (Buglione et al., 2018; De Barba et al., 2014; Esnaola et al., 2018; Robeson et al., 2017), birds (Crisol-Martiniez et al., 2016; Han & Oh, 2018), reptiles (Caut et al., 2019; Kartzinel & Pringle, 2015; Koizumi et al., 2017), fish (Barbato et al., 2019; Harms-Tuohy et al., 2016; Riccioni et al., 2018) and arthropods (Kamenova et al., 2018; Kennedy et al., 2020; Krehenwinkel et al., 2016). For most species, faecal samples, in contrast to stomach contents, can easily be obtained, with a minimal level of interaction and harm inflicted on the studied animal. Using faecal samples is therefore a noninvasive and attractive approach to study dietary patterns (Pompanon et al., 2012; Valentini et al., 2009). This is especially true for endangered species or species whose feeding patterns are difficult to observe in the wild, such as aquatic or nocturnal species (Baamrane et al., 2012; Hibert et al., 2013). Following sampling, laboratory procedures involve total DNA extraction from faecal samples, PCR amplification with either a universal or a specific set of primers corresponding to one or more barcode loci, preparation of DNA libraries and DNA sequencing ultimately terminated by data processing via bioinformatics pipelines (Laudadio et al., 2019).

Previous studies have revealed the difficulty associated with determining operational taxonomic units (OTUs) at the species level, such as has been reported in diet (*Ursus arctos*: De Barba et al., 2014; *Lepus corsicanus*: Buglione et al., 2018) or environmental DNA (eDNA) studies (Lacoursière-Roussel et al., 2018; Lim et al., 2016; Ruppert et al., 2019). Furthermore, obtaining sufficient amplicon length to determine sample identities to the species level can be challenging (De Barba et al., 2014; Deagle et al., 2010). This difficulty is primarily due to the fact that prey DNA in faecal samples is degraded (Deagle et al., 2006). Consequently, primers were selected to target only short and variable prey DNA fragments present in the diet. Long metabarcoding has facilitated the production of long sequencing reads (Godwin et al., 2016). The increased marker length allows a higher taxonomic resolution, with long markers increasing the ability to distinguish closely related species (Singer et al., 2016). Regarding vertebrates and invertebrates, the mitochondrial cytochrome oxidase subunit 1 (COI) is the most frequently used barcode locus, with the most diversified and complete reference database (Jusino et al., 2018). The careful selection and design of amplification primers are essential, as well as the evaluation of primers from close and distant species, using as many DNA sources as possible, in order to characterize primer specificity and/or universality.

Together with primer selection, the parameters of bioinformatic pipelines have an important impact on the identification of OTUs. Indeed, after sequencing, to achieve in-depth analysis of

DNA present in the studied sample, raw sequence data alone are not sufficient (van der Walt et al., 2017). Furthermore, unassembled raw metagenomic sequence data are fragmented, contain errors and/or are affected by unequal sequencing depths (Nagarajan & Pop, 2013), hindering the accuracy when sorting DNA sequences (Nurk et al., 2017). Thus, to accurately analyse metagenomes, larger contiguous segments named contigs can be assembled from raw sequence data (Anantharaman et al., 2016). For this reason, multiple metagenome bioinformatic pipelines have been developed to assemble raw sequences by simply merging paired-end reads or by de novo assembly (van der Walt et al., 2017).

Using a metagenome assembler producing high-quality long contigs (>1,000 bp) will allow for more accurate determination of organisms to the species level using DNA sequences present in the sample (van der Walt et al., 2017). Additionally, control and validation methods are needed for parametrizing bioinformatics pipelines. This can be achieved by creating and sequencing mock communities, which are references for DNA databases and are used as positive controls for HTS (Jusino et al., 2018). Moreover, a captive feeding trial can be conducted to evaluate the applicability of the method and to test whether prey DNA can be reliably detected in faecal samples (Deagle et al., 2005; Nakahara et al., 2015).

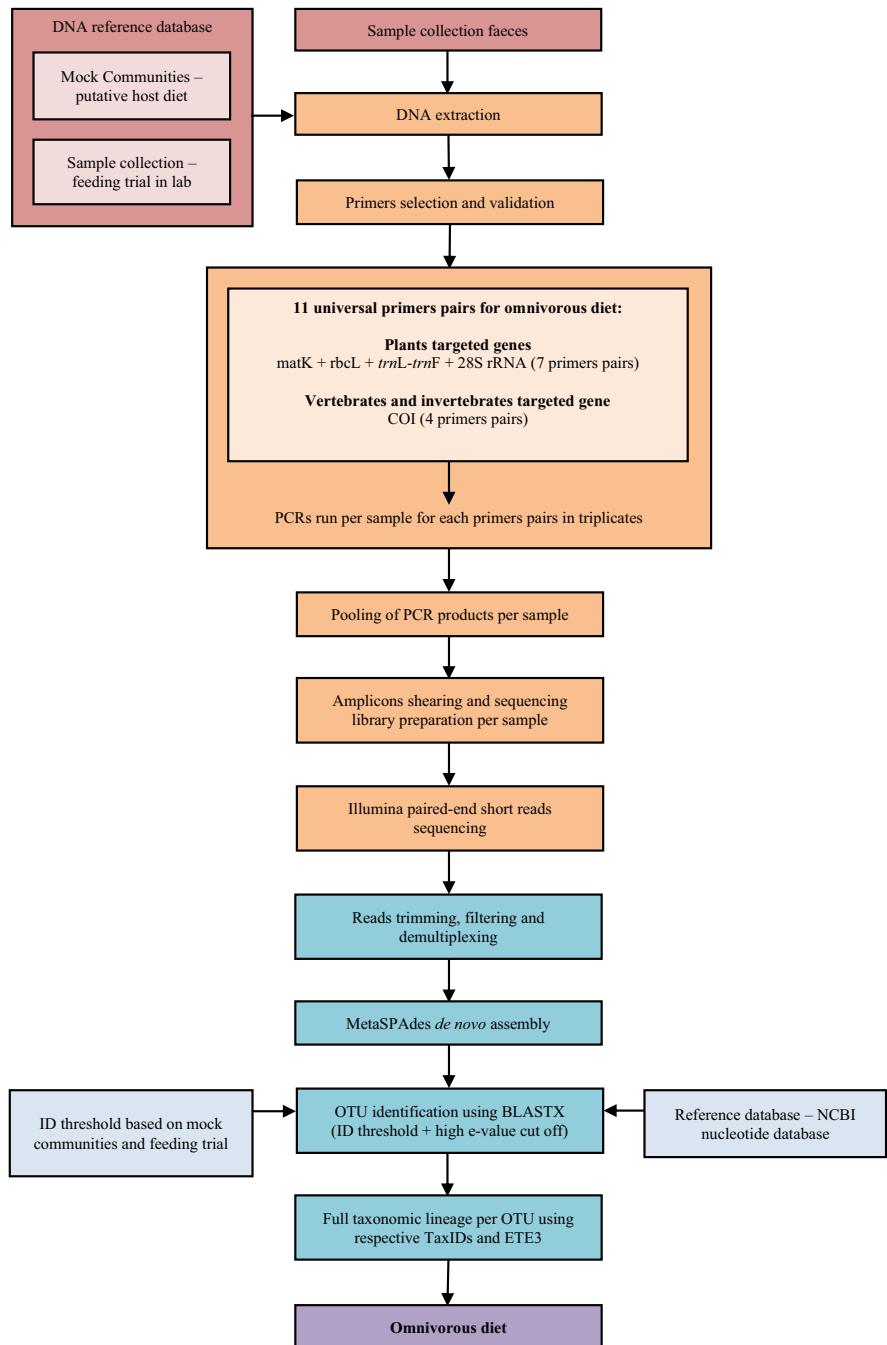
Here, we describe a study of the complex diet of the European pond turtle, using, for the first time in a dietary study, a new long DNA metabarcoding approach. The procedure includes 11 different primers pairs that target a region of the COI gene, the large subunit of the ribulose-1,5-bisphosphate carboxylase gene (*rbcL*), the matrerase K gene (*matK*), the 28S rRNA and the *trnL-trnF* chloroplastic region (the proposed combination of primers covers plants, invertebrates and vertebrates). This will result in the amplification of fragments between 350 and 1,400 bp in length. After sequencing, raw metagenome sequence data are first analysed with the open bioinformatics pipeline, METASPADES version 3.9.0 (Nurk et al., 2017; <http://cab.spbu.ru/software/spades>), which, to our knowledge, is being used for the first time in diet studies using metagenomic approaches, and considered nowadays as the most recommended metagenomic data assembler for high-complexity metagenomes (Forouzan et al., 2018). This new DNA metabarcoding approach is based on the use of multiple primers in order to maximize coverage of species groups and can accurately be used to identify taxa to the species level for plants, vertebrates and invertebrates present in faeces collected in the field. Finally, the accuracy of the method was also validated by using faeces obtained from captive European pond turtles fed using known diets.

2 | MATERIALS AND METHODS

2.1 | General approach for omnivorous diet analysis

We have developed a general method of long DNA metabarcoding for analysis of omnivorous diets (Figure 1), through a diet study of the omnivorous European pond turtle (*Emys orbicularis*, L. 1758).

FIGURE 1 Flowchart summarizing the experimental design for the long DNA metabarcoding approach for omnivorous diet analysis



2.2 | Study species

The European pond turtle is found in wetlands of Europe and North Africa and has been classified as “near threatened” (NT) according to the IUCN Red List. In Switzerland, the species is listed as “critically endangered” (CR) on the Swiss Red List (Monney & Meyer, 2005). Previous studies investigating the feeding behaviour of the species using direct observations and microscopic examination have suggested that the animals have an omnivorous diet (Çicek & Ayaz, 2011; Ottonello et al., 2005, 2016, 2018). However, these techniques have several limitations, including the loss of information due to difficulties identifying prey and plant matter present in

faeces. To our knowledge, no metabarcoding study exploring the European pond turtle diet have been conducted, and metabarcoding studies of reptile diets are scarce (Brown et al., 2012; Kartzinel & Pringle, 2015; Koizumi et al., 2017).

2.3 | Collection of faecal samples and DNA extraction

European pond turtles were captured in April 2017 using conical fishing basket traps placed perpendicular to the banks (Cadi, 2003) in the natural reserve of Moulin de Vert (MDV; 46°10'46"N, 6°1'42"E,

Canton of Geneva, Switzerland). Each trap was observed daily for a week and captured turtles were placed in individual containers without water for the night in order to collect faecal samples. Individuals were sexed, weighed, measured and released at the exact location at which they were captured. To prevent contamination of the samples, each container was cleaned with a 10% bleach solution, followed by 70% denatured ethanol. The captures were conducted after all necessary legal authorizations were acquired (see Acknowledgements). A total of 32 faecal samples were collected in the field. Each sample was stored in a plastic tube, and then placed in the freezer at -80°C until extractions were performed. In the laboratory, the faecal samples were homogenized and ground using liquid nitrogen. The risk of contamination between samples was minimized by decontaminating the mortar and pestle used for grinding in a bath of 10% bleach for 30 min, followed by rinsing with 70% denatured ethanol and UV irradiation for 15 min. Genomic DNA was then extracted from about 100 mg of the resulting powder with the Qiagen QIAamp PowerFecal DNA kit (Qiagen) according to the manufacturer's protocol. Other attempts with adapted CTAB DNA extraction protocols or another commercial kit (i.e., ISOLATE Fecal DNA kit from Bioline) did not achieve the performance of the Qiagen kit in terms of amplification quality of the extracted DNAs (data not shown). DNA quality and concentration were finally assessed with both a NanoDrop 1,000 Spectrophotometer and a Qubit 3.0 Fluorometer (both Thermo Fischer Scientific).

2.4 | Feeding trial

Additionally, six captive European pond turtles were placed in individual containers in the laboratory, and food was withheld for 10 days to empty the digestive systems of the turtles (Devaux et al., 1996). Then, turtles were given diets consisting of a predetermined set of fishes and invertebrates (Table 1). The night after feeding, turtles were placed in dry containers (similar to those used in the field experiment), and multiple faecal samples were collected from each individual and subsequently homogenized. This procedure yielded six faecal samples with known diets, which were separately analysed as defined above.

TABLE 1 Dietary regimes (in g per ingested species) of six captive European pond turtles (*Emys orbicularis*, L. 1758) and their respective compositions determined using long metabarcoding analysis of faecal samples; the reference alignment length (bp) and percentage identity obtained after de novo assembly are provided

Ingested species	Emys_0			Emys_1			Emys_2	
	Amount consumed (g)	Percentage identity match	Reference alignment length (bp)	Amount consumed (g)	Percentage identity match	Reference alignment length (bp)	Amount consumed (g)	Percentage identity match
<i>Esox lucius</i>	20	98.52	323	—	—	—	19	97.83
<i>Oncorhynchus mykiss</i>	—	—	—	—	—	—	5	99.81
<i>Mus musculus</i>	—	—	—	3	98.52	244	6	99.77
<i>Chironomus salinarius</i>	5	97.84	573	8	98.86	636	5	98.71
<i>Gammarus pulex</i>	—	—	—	—	—	—	3	99.32

2.5 | Mock community

A reference DNA database (mock community; MC) was set up using DNAs extracted from known components of the putative diet of the European pond turtle (Appendix S1: S1), which is composed of plants, macro-invertebrates and fishes, according to the literature (Çicek & Ayaz, 2011; Ottonello et al., 2005, 2016, 2018). After individually grinding selected components of the MC in liquid nitrogen, genomic DNA was extracted from samples as described above. In order to highlight the limitations of both PCR amplification and bioinformatic analysis, two different types of mock community were prepared with the same DNA samples. (a) The first mock community (MC1) was a mixture of each DNA sample at a concentration of 10 ng/μl. PCRs were run for each primer set in triplicates with the mock community DNA mixture. (b) For the second mock community (MC2), each DNA sample was first individually amplified in triplicate with each primer set before pooling. For MC1 and MC2, DNA concentrations were determined as described above after purification of their respective pooled PCR products. Then, DNA amplicons from each component of both MCs were submitted to Sanger sequencing at Microsynth to validate species identity.

2.6 | Primer selection and PCR amplification

Previously published primers used for the amplification of the large subunit of the ribulose-1,5-bisphosphate carboxylase gene (*rbcL*), the maturase K gene (*matK*), the 28S rRNA gene, the *trnL-trnF* gene region in plants and a portion of the mitochondrial-encoded cytochrome oxidase subunit I (*COI* or *COX1*) gene in animals were evaluated against DNA samples isolated from the mock community (Table 2). Several other primer pairs were tested (data not shown) but not retained, either because they were unable to amplify or lacked amplification specificity. Moreover, as live microorganisms such as bacteria and fungi are present in high numbers in the digestive systems of hosts, primer sets were also evaluated against DNAs of three bacterial (*Bacillus megaterium*, *Pseudomonas koreensis*, *Erwinia* sp.) and three fungal strains (*Aureobasidium pullulans*, *Trichoderma harzianum*, *Penicillium glabrum*), from our laboratory

DNA collection. Primer pairs which amplified bacterial or fungal genes were then discarded. Primer pairs tested but not used were psbA3f (Sang et al., 1997) and trnHf-05 (Tate & Simpson, 2003), matK 390-F and matK 1326-R (Cuénod et al., 2002), JK11 and JK14 (Aceto et al., 1999), COIF2 and COIR2 (Martinsen et al., 2008), C1-J-2182 (Simon et al., 1994) and TL2-N-3020 (Dobler & Müller, 2000), BF2 and BR2, BF2 and BR1, BF1 and BR2 (Elbrecht & Leese, 2017), ModRepCOI-F and COI-R, VertCOI_7194-F and ModRepCOI-R (Reeves et al., 2018), and Chmf4/Chmr4 (Che et al., 2012). This careful screening finally yielded seven primer pairs targeting three different types of plant genes and four primer pairs specific to *COI* sequences in vertebrates and invertebrates (Table 2). All PCRs were carried out using a 25- μ l reaction volume consisting of 5 μ l MyTaq reaction buffer (Bioline), 2.5 μ l of selected primers (0.5 μ m final concentration), 2 U of MyTaq DNA polymerase (Bioline), 1 μ l of DNA (concentrated at 10 ng/ μ l), and ultrapure sterile water up to completion volume. All PCRs were run in triplicate using the following reaction steps: an initial denaturation step at 95°C for 3 min, followed by 37 cycles of 95°C for 20 s, 52°C for 20 s and 72°C for 20 s, terminated by a final extension step of 20 s at 72°C. The annealing temperature was set to 52°C for all primer pairs except for mICOLintF and jgHCO2198 (54°C), and the primer pair Tab c and Tab f (56°C). These PCR programme conditions were established following the manufacturer's specifications and after optimization with DNAs from MCs, in order to yield the widest coverage at the family/species level while maintaining a high level of specificity at the amplification level. All PCR mixtures were prepared under a Biosan DNA/RNA UV-Cleaner cabinet to avoid any contamination. Positive and negative controls were included.

Furthermore, we designed host-specific blocking primers, but the use of the blocking primer was not compatible with our metabarcoding approach (see explanations in Appendix S1: S2).

2.7 | First upstream method validation

After metabarcode amplification, sequencing and bioinformatics were first validated upstream using in duplicate a synthetic mock community (sMC). In total, four distinct amplified genes from

14 organisms, which included plants, insects, fungi and bacteria (Table 3), were amplified using PCR, controlled using gel electrophoresis and their respective PCR products were pooled and purified with a Wizard Genomic Purification Kit (Promega). After measuring the DNA concentration with the Qubit 3.0 Fluorometer, the two pooled amplicons were diluted to 2 ng/ μ l and then fragmented using a protocol developed to create fragments with a mean size of 290 bp. This protocol utilizes the Covaris S2 focused-ultrasonicator and can be applied to shear amplicons of a variety of lengths, ranging from 350 to 1,400 bp (Figure S3). The quality of sheared products was subsequently evaluated using a Tapestation 2,200 (Agilent).

2.8 | Library and Illumina sequencing

Separate PCR amplifications of each DNA sample for all 11 barcodes (conducted in triplicate) were performed, and 9 μ l of each amplification product was pooled together to create one sequencing library per sample and at the same time allow the analysis of a large number of samples in the most affordable and practical way. The resulting pool corresponding to each sample was then purified with the Wizard SV Gel and PCR Clean-Up System and DNA concentration was assessed with a Qubit 3.0 Fluorometer. Amplicon pools were then diluted to a final concentration of 2 ng/ μ l and were finally fragmented to produce an average fragment size of 290 bp in AFA microtubes (Covaris) using an S2 focused-ultrasonicator (Covaris), and following our established protocol given in Figure S3. Sequencing libraries were created using a TruSeq Nano DNA HT Library Prep Kit (Illumina) following the manufacturer's protocol. All samples were sequenced using an Illumina MiniSeq High Output run at 2 \times 150 bp paired-end read length, which reached a median sequencing depth of 106 Mb per sample.

2.9 | Bioinformatic analysis

Illumina conversion software `BCL2FASTQ2` version 2.20 was automatically run through the MiniSeq local run manager set with

Reference alignment length (bp)	Emys_3			Emys_4			Emys_5		
	Amount consumed (g)	Percentage identity match	Reference alignment length (bp)	Amount consumed (g)	Percentage identity match	Reference alignment length (bp)	Amount consumed (g)	Percentage identity match	Reference alignment length (bp)
366	10	99.78	566	—	—	—	13	100	499
566	—	—	—	—	—	—	—	—	—
567	15	99.13	350	—	—	—	5	99.3	296
588	5	99.6	248	8	98.54	397	—	—	—
264	—	—	—	—	—	—	3	100	275

TABLE 2 Primers used for amplification of the large subunit of ribulose-1,5-bisphosphate carboxylase, maturase K, 28S rRNA, *trnL-trnF* regions (for plants) and mitochondrial-encoded cytochrome oxidase subunit I (for animals) gene

Prey taxon	DNA type	DNA region	Primer name	Forward primer	Reverse primer	Primer sequence 5'-3'	References	Average length of amplified fragment (bp) in this study
Invertebrates	Mitochondrial	COI	mICoIntF jgHCO2198	Forward Reverse	Forward Reverse	GGWACWGWTGAACVGWTW/TAYCCYCC TAIACYTCIGGRTGICCRAARAAYCA	Leray et al. (2013) Leray et al. (2013)	350
Invertebrates	Mitochondrial	COI	ODO_LCO1490d ODO_HCO2198d	Forward Reverse	Forward Reverse	TTTCTTACWAACCAAYAAAGATATTGG TAAACTTCWGGRTGTCCAARAATCA	Dijkstra et al. (2014) Dijkstra et al. (2014)	650
Vertebrates	Mitochondrial	COI	COI-CO2 COI-CO4	Forward Reverse	Forward Reverse	AYTCAAACAAATCATAAAGATATTGG ACYTCRGGRTGACCAAAAAATCA	Che et al. (2012) Che et al. (2012)	600
Vertebrates	Mitochondrial	COI	Mod_RepCOI_F Mod_RepCOI_R	Forward Reverse	Forward Reverse	TNTTYTCMACYAAACCACAAAGA TTCDGGRTGNCCRAARAATCA	Reeves et al. (2018) Reeves et al. (2018)	650
Plants	Plastid	matK	MatK-1RKIM-f MatK-3FKIM-r	Forward Reverse	Forward Reverse	ACCCAGTCATCTGGAAATCTGGTTC CGTACAGTACTTTGTGTTACGAG	K.-J.Kim, pers. comm.	850
Plants	Plastid	matK	MatK-472-f MatK-1248-r	Forward Reverse	Forward Reverse	CCCRTYCATCTGGAAATCTGGTTC GCTRTRATAATGAGAAAAGATTCTGC	Yu et al. (2011) Yu et al. (2011)	730
Plants	Plastid	matK	MatK-5r MatK-xf	Forward Reverse	Forward Reverse	GTTCTAGCACAAGAAAGTCG TAATTTCAGATCAATTCAATT	Ford et al. (2009) Ford et al. (2009)	825
Plants	Chloroplast	rbcl	rbcl_a-F rbcl_a-R	Forward Reverse	Forward Reverse	ATGTCACCAAAACAGGACTAAAGC GTAAAATCAAAGTCCACCRCG	Levin et al. (2003) Kress and Erickson (2007)	520
Plants	Chloroplast	rbcl	rbcl-1F rbcl-724R	Forward Reverse	Forward Reverse	ATGTCACCAAAACAGAAC TCGCATGTACCTGCAGTAGC	Fay et al. (1997) Olmstead et al. (1992)	680
Plants	Chloroplast	28S rRNA	28KJ 28C	Forward Reverse	Forward Reverse	GGCGGTAAATTCCGTC GCTATCCTGAGGGAAACTTC	Cullings (1992) Hamby and Zimmer (1988)	630
Plants	Chloroplast	<i>trnL-trnF</i>	Tab c Tab f	Forward Reverse	Forward Reverse	CGAAATCGGTAGACGCTACG ATTTGAACTGGTGACACGAG	Taberlet et al. (1991) Taberlet et al. (1991)	920

TABLE 3 Species of invertebrates, plants, bacteria and fungi used as the synthetic mock community (sMC) for upstream method validation, with the primers used for amplification of ITS/5.8S rRNA, 16S, the *tRNA*-*trnF* region and the mitochondrial-encoded cytochrome oxidase subunit 1 gene

Phylum	Species	Amplified gene	Primer name	Primer sequence 5'-3'	Reference	Median amplicon length (bp)	Post-assembly median recovered contig length (bp)
Ascomycota	<i>Aureobasidium pullulans</i>	ITS1, 5.8S, ITS2	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)	550	649
Ascomycota	<i>Trichoderma harzianum</i>	ITS1, 5.8S, ITS2	ITS4/5	GGAAAGTAAAAGTCGTAACAAAGG	White et al. (1990)	550	669
Ascomycota	<i>Penicillium glabrum</i>	ITS1, 5.8S, ITS2	ITS4/5		White et al. (1990)	550	645
Firmicutes	<i>Bacillus megaterium</i>	16S	27F/1492R	AGAGTTTGATCCTGGCTCAG CTACGGCTACCTTGTACGA	Hudson et al. (1998) Hudson et al. (1998)	1,400	1,410
Proteobacteria	<i>Pseudomonas koreensis</i>	16S	27F/1492R		Hudson et al. (1998)	1,400	1,396
Proteobacteria	<i>Erwinia</i> sp.	16S	27F/1492R		Hudson et al. (1998)	1,400	1,400
Streptophyta	<i>Platanus occidentalis</i>	<i>tRNA</i> - <i>trnF</i>	Tabc/f	CGAAATCGGTAGACCGCTACG ATTGAACTGGTGACACGAG	Taberlet et al. (1991) Taberlet et al. (1991)	1,200	1,245
Streptophyta	<i>Chisquea culou</i>	<i>tRNA</i> - <i>trnF</i>	Tabc/f		Taberlet et al. (1991)	1,200	1,196
Arthropoda	<i>Aeshna juncea</i>	ITS1, 5.8S, ITS2	ITS4/5		Spadaro et al. (2012)	750	834
Arthropoda	<i>Aeshna cyanea</i>	ITS1, 5.8S, ITS2	ITS4/5		Spadaro et al. (2012)	750	837
Arthropoda	<i>Aeshna caerulea</i>	ITS1, 5.8S, ITS2	ITS4/5		Spadaro et al. (2012)	750	842
Arthropoda	<i>Aeshna juncea</i>	COI	ODO_LCO1490d ODO_HCO2198d	TTTCTACWAACCAVAAAAGATATTGG TAAACACTTCWGGRGTCCAARAATCA	Dijkstra et al. (2014) Dijkstra et al. (2014)	650	720
Arthropoda	<i>Aeshna cyanea</i>	COI	ODO_LCO1490d/ ODO_HCO2198d		Dijkstra et al. (2014)	650	674
Arthropoda	<i>Aeshna caerulea</i>	COI	ODO_LCO1490d/ ODO_HCO2198d		Dijkstra et al. (2014)	650	717

default parameters, in order to trim Illumina adapters and to demultiplex samples based on their respective index. The sequencing quality of the MiniSeq run was high, with 93% of the sequencing reads above the quality Phred score of 30. Nevertheless, cleaned reads were further evaluated for quality and adaptor contamination using FASTQC (Andrews, 2010). An additional quality trimming of raw Illumina reads with TRIMOMATIC 0.32 (Bogler et al., 2014) was evaluated on 10 samples, with stringent settings for base quality filtering, and was found not to be conclusive based on post de novo assembly results. Moreover, METASPADES, the used de novo assembly software, comes with an “error correction read” process prior to contig assembly—i.e., “BayesHammer error correction tool,” which uses Bayesian subclustering to correct sequencing reads (Nikolenko et al., 2013). Following trimming and demultiplexing, cleaned sequencing reads were downloaded from the Illumina Basespace account. De novo assembly of sequencing data was separately carried out for each sample using the genome assembly software SPADES 3.11 (Nurk et al., 2017), with the metagenome assembly option (“metaSPAdes”) which includes the “error correction read” process prior to contig assembly. The parameters used in the software are described in Figure S4. Contigs smaller than 150 bp were removed, which represented between 0.17% and 10.34% of all contigs across all samples with an average of 2.86%. The resulting contigs files were analysed with the Basic Local Alignment Search Tool (BLAST) using BLAST+ (Camacho et al., 2009), searching the complete NCBI nucleotide (nt) database (command lines using BLAST + are described in Figure S3). Only the sequences of eukaryotes were conserved. Following the BLAST search characterized by a strong e-value cut off (E-value 1e-20) (Truelove et al., 2019), the five most significant matches (max_target_seq5) to the reference database for each of the query sequences were recorded. If only a single taxon was present in the top five and above 97.6% identity (see below for the level applied), the query was assigned directly to this taxon. If more than one reference taxon was present in the top five and above 97.6% identity, the query was assigned to the lowest taxonomic level that was shared by all taxa. In these specific cases (i.e., multiple taxa shared for a query sequence), the species identity was if possible confirmed without any ambiguity, thanks to the knowledge of biologists or botanists specialized in these studied sites. Finally, query sequences for which the best BLAST hit had less than 97.6% identity to any sequence were simply not considered. This threshold was determined following analysis of the sequences from both the mock communities and the captive feeding trials. The complete taxonomy of each species identity assignment per contig was completed using its respective TaxID and the ETE3 toolkit software (Huerta-Cepas et al., 2016). Finally, read abundance per contigs was determined using BOWTIE2 (Langmead & Salzberg, 2012) and SAMTOOLS (Li et al., 2009) sequencing read alignment tools, plus an additional Perl script from multi-metagenome (Albertsen et al., 2012) (command lines are described in Figure S4).

3 | RESULTS

3.1 | Upstream method validation

After sequencing, 14 amplicons (550–1400 bp in length) of the two MCs were entirely de novo assembled and all micro-organism identities were successfully retrieved using NCBI BLAST. This confirmed that the proposed method could be performed on several different samples with DNA sequences (barcodes/amplicons) of different sizes, up to 1,400 bp and more.

3.2 | Mock community

Two different mock communities with different amplification procedures (MC1 and MC2) were used to determine whether the differences in sequencing data were observed when DNAs were pooled before amplification (MC1) versus. amplified individually (MC2) (Table 4). All MC members within MC1 were identified to the species level, except for *Nymphaea alba*, which was assigned at the genus level only (*Nymphaea* sp.). The average contig length for the pooled sample was 628 bp. For MC2, all MC members were also assigned to the species level and the average contig length was 425 bp. This experiment validated that the threshold was met for the determination of prey DNA from faeces to the species level. The sequence similarity requirement for species determination was >97.6% identity; below this threshold, analysis of the two MCs revealed false positives. This confirmed that if DNAs are pooled together (as in both MC1 and faecal samples), the established PCR configuration allows for the amplification of all the species present within the DNA mixture.

3.3 | Captive feeding trial

Results of the analysis of faecal samples obtained by captive feeding trials demonstrated that every prey given to the European pond turtle was amplified and correctly assigned down to the species level. This produced a minimum identity threshold of 97.8% for the exact determination of prey species from DNA extracted from faeces and allowed for the allocation of identity to the species level. The average length of the reference alignment was 422 bp (Table 1).

3.4 | Blocking primers

Without the use of any host-specific blocking primers (that would have prevented host COI gene amplification), *Emys orbicularis* was formerly identified in only 12.5% of the samples, namely four out of 32. Across these four samples, we found that between 0.74% and

TABLE 4 Species composition of the two mock communities (MC1 and MC2)

MC_ID	Species	Percentage identity match	Reference alignment length (bp)
MC1	<i>Aeshna cyanea</i>	99.12	340
MC1	<i>Baetis rhodani</i>	99.34	603
MC1	<i>Bufo viridis</i>	99.85	709
MC1	<i>Caenis sp.</i>	99.72	476
MC1	<i>Chironomus salinarius</i>	98.63	709
MC1	<i>Cloeon dipterum</i>	98.42	657
MC1	<i>Esox lucius</i>	99.43	572
MC1	<i>Gammarus pulex</i>	100.00	709
MC1	<i>Iris pseudacorus</i>	100.00	1,065
MC1	<i>Lycopus europaeus</i>	100.00	981
MC1	<i>Mentha spicata</i>	97.70	957
MC1	<i>Mus musculus</i>	99.84	682
MC1	<i>Notonecta glauca</i>	100.00	259
MC1	<i>Nuphar lutea</i>	100.00	642
MC1	<i>Nymphaea sp.</i>	99.38	161
MC1	<i>Potamogeton perfoliatus</i>	100.00	495
MC1	<i>Radix balthica</i>	98.31	233
MC1	<i>Tinca tinca</i>	99.85	710
MC1	<i>Utricularia australis</i>	99.23	967
MC2	<i>Aeshna cyanea</i>	98.74	440
MC2	<i>Baetis rhodani</i>	99.18	540
MC2	<i>Bufo viridis</i>	99.84	709
MC2	<i>Caenis sp.</i>	100	285
MC2	<i>Chironomus salinarius</i>	98.69	306
MC2	<i>Cloeon dipterum</i>	100	667
MC2	<i>Esox lucius</i>	99.28	279
MC2	<i>Gammarus pulex</i>	100	520
MC2	<i>Iris pseudacorus</i>	100	319
MC2	<i>Lycopus europaeus</i>	100	669
MC2	<i>Mentha spicata</i>	—	—
MC2	<i>Mus musculus</i>	99.31	436
MC2	<i>Notonecta glauca</i>	100	413
MC2	<i>Nuphar lutea</i>	100	342
MC2	<i>Nymphaea alba</i>	100	210
MC2	<i>Potamogeton perfoliatus</i>	100	226
MC2	<i>Radix balthica</i>	97.6	242
MC2	<i>Tinca tinca</i>	99.8	519
MC2	<i>Utricularia australis</i>	100	524

Note: Contig sequences (recovered amplicons) were obtained by de novo assembly with METASPADES (Nurk et al., 2017) and queried using the Basic Local Alignment Search Tool (BLAST) and BLAST+ (Camacho et al., 2009), against the complete NCBI nucleotide (nt) database.

3.35% of raw sequencing paired-end reads per sample aligned to the *Emys COI* gene contig, with an average of 1.78%. Considering the 32 samples totally, the average drops to 0.22% (Appendix S1: S5).

3.5 | Qualitative analysis: Diet

Metabarcoding analyses showed that all samples contained plant DNA, 46.9% of the samples contained vertebrate DNA and 84.4% of the samples contained macro-invertebrate DNA. Most of the OTUs identified were assigned to a particular prey species (192 out of 233 OTUs; 82.4%); one invertebrate was determined to the order level only, another invertebrate to the family level and finally six plants to the genus level. In some cases, ecological information for Switzerland was available, and we were able to manually assign contig sequences to species known to be present in the area. For example, *Cladum* sp. was assigned to the species *Cladum mariscus* (Pohl, 1809) because it is known to be the only *Cladum* species living in Switzerland. Regarding *Betula* sp., the single *Betula* species found in this area and assigned in other samples was *Betula pubescens* (Ehrh, 1791), so we assigned this sequence to this species. For other genera (*Salix* sp., *Quercus* sp., *Picea* sp., *Carex* sp.), and for the family (Cecidomyiidae sp.) and order identified (Hemiptera sp.), it was not possible to identify the organism at a further precise level. Overall, this new long metabarcoding approach allowed us to allocate 82.4% of the amplified organisms to a precise species level. Thus, 34 different Arthropoda species were identified (with average amplicon length of 462 ± 204 bp), as well as three Mollusca species (365 ± 178 bp), three species of Chordata (318 ± 178 bp) and 28 plant species (598 ± 291 bp). A total of 68 different species were characterized within these results (mean 547 ± 274 bp) (Figure 2; Appendix S1: S6). No contamination was detected in the positive and negative controls.

3.6 | Quantitative analysis: Read abundance

Finally, we determined the read abundance of all prey and plant species identified per sample, including the two MCs and feeding trial samples, in order to conclude whether we could use this information as a quantification indicator of metabarcoding diet (Appendix S1: S6). For instance, regarding plant species identified in our study, *Phragmites australis* was identified within the same sample MDV02 with three different barcode markers, namely *trnL-trnF*, *matK* and *rbcL*, with 4.18%, 1.14% and 10.25% respectively of the total mapping reads. Another example, in the EMYS2 feeding control sample, we fed the individual with 19 g of *Esox lucius* and an average of 5 g of *Oncorhynchus mykiss*, *Mus musculus*, *Chironomus salinarius* and *Gammarus pulex*. Read abundance of *Esox lucius* was not significantly higher than the read abundance of the other prey taxa and was surprisingly even lower (Appendix S1: S5).

4 | DISCUSSION

It has typically been assumed that prey DNA fragments in faecal samples were short and degraded as a result of the digestion by the host. This causes a major difficulty for the taxonomic identification of prey taxa using methods based on the analysis of DNA sequences within faecal samples (Deagle et al., 2006). For these reasons, previous protocols have used barcodes that target very short amplicons (<100 bp) (De Barba et al., 2014). However, the rate of DNA degradation in faeces may vary according to the identity of the ingested species. Indeed, in the present study, we found that plant parts (including seeds), bones and insect parts (such as legs and elytra) are only partially digested in the faecal samples. These observations suggest that prey DNA may not always be highly degraded and therefore enable the amplification of longer amplicons, thereby facilitating their identification down to the species level. Furthermore, the feeding trial experiment also demonstrated that the proposed long metabarcoding method can detect the DNA sequences of vertebrates and macro-invertebrates fed to captive European pond turtles. In this case, prey could not be identified using direct observation or microscopy, because they were entirely digested. The method has been shown to be appropriate for the analysis of the diet of wild European pond turtle, and probably other species as well. DNA in faecal samples was not overly degraded, produced reliable results and allowed for the recovery of long amplicon sequences after de novo assembly. However, to accurately elucidate the real diet of the European pond turtle, the feeding trial should have contained plants (this information was unknown before this study). Use of mock communities, combined with samples obtained

through captive feeding trials, proved to be essential to produce positive controls and validation data for parametrizing (threshold set up) bioinformatics pipelines. Finally, the various tests performed on samples collected throughout feeding trials and MCs enabled us to set a relatively high detection threshold at almost 98% identity. Without using any host-specific blocking primers, we demonstrated that on average only 0.22% of raw sequencing paired-end reads per sample aligned to *Emys* contigs. This is relatively weak and questions the usefulness of blocking primers related to the host DNA in metabarcoding diet studies from faeces. Indeed, it seemed, at least in the specific case of *Emys orbicularis*, that cell loss following cell renewal throughout the intestinal lumen of the host generates only a little or no DNA of amplifiable quality, as the *COI* gene of the host DNA was only identified in four out of 32 samples.

Regarding the qualitative analysis of faecal samples, the recovered amplicon lengths following de novo assembly (through contigs) varied between 168 and 1,379 bp (average of 546 bp); taxonomic resolution to the species level was reached for most sequences (82.4%). In previous diet analyses, taxonomic identification to the species level did not reach such high levels. For example, species were assigned for 75% of the fragments analysed in a study of red-headed wood pigeon diet (Ando et al., 2013), for ≥60% when examining the diet of a bear (De Barba et al., 2014), and dietary analyses of the black wheatear detected the presence of animal DNA in 94 samples out of 112 using 18S, thus yielding 91 taxa from 21 orders of which 10% were assigned to the genus or species level (da Silva et al., 2019). Obtaining longer amplicons provides a well-known advantage for our long metabarcoding approach because it enhances the precision of taxonomic identification (Heeger et al., 2018; Jamy et al., 2020;

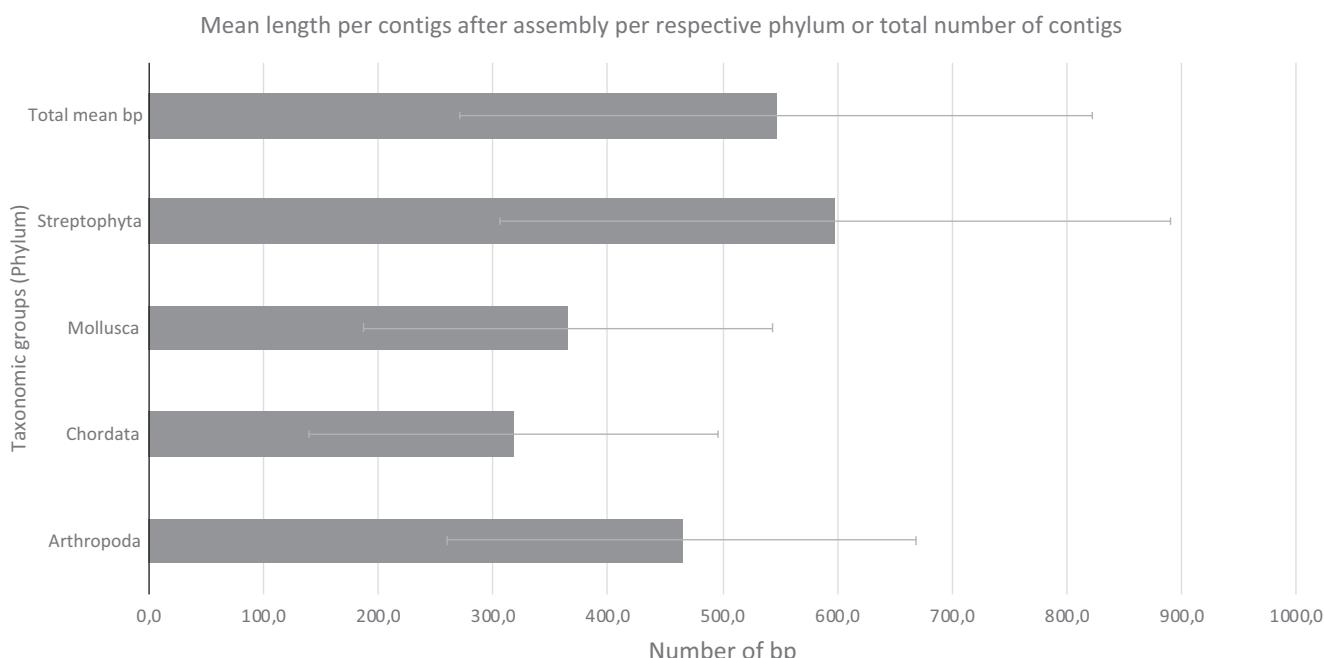


FIGURE 2 Mean length (bp; with SD) of the de novo assembly amplicons (contigs) assembled following long metabarcoding analysis of the 32 faecal samples of European pond turtles (*Emys orbicularis*). Total mean was calculated from a total of 233 contigs (each contig per sample is attributed to a unique OTU), whereas the other means were calculated per phylum

Liu et al., 2020; Piper et al., 2019; Porter & Golding, 2011). Furthermore, the approach developed here not only allowed us to amplify both barcodes of short to medium size, in cases of degraded DNA, but also and especially of long size (>500–650 bp and more), which is believed to increase the taxonomic affiliation at the species level. Indeed, shorter DNA fragments (e.g., 100 bp or less) are more likely to be sequenced, while longer DNA fragments provide a better taxonomic identification and resolution (Liu et al., 2020). Analyses made on degraded DNA samples demonstrated that few very informative barcodes such as *COI*, of shorter size between 135 bp (Hajibabaei et al., 2006) and 250 bp (Meusnier et al., 2008), can reliably identify animal species, if they target an appropriate placement within the larger barcode region (Elbrecht et al., 2019). Thus, longer amplicons generally increase the level of taxonomic assignment, and especially elevate the veracity and power of the results (absence of false positives; Piper et al., 2019). This method of long metabarcoding by short-read sequencing (Illumina Platform) and de novo assembly has other advantages compared to the long-read Pacific Bioscience sequencing platform used by Heeger et al. (2018) and Jamy et al. (2020). The method is more affordable and provides a higher level of sequencing depth. Moreover, our approach combining the use of different specific and universal primer pairs targeting both the same and unique gene (or gene portion) and several different genes as well, coupled with different targeted amplicon sizes (both long and short amplicons in case of highly degraded DNA), allowed us to amplify a large spectrum of species richness (da Silva et al., 2019) and to confirm some of the identified plant OTUs with redundancy of identification using many genes (*matK*, *rbcL* and 28S genes). Usually, species-specific primers are used to amplify DNAs within a particular diet from faecal samples or gut contents (Leal et al., 2014; Pumarino et al., 2011; Wallinger et al., 2012). However, this approach is only useful if prior information regarding the diet of the studied animal is available and if the range of the diet is not too large (Moorhouse-Gann et al., 2018). Thus, the use of multiple specific and universal primers in this study is an optimal method to determine complex diets, such as the diets of omnivorous animals such as the European pond turtle.

Nevertheless, the set of multiple primers that we developed cannot be considered as the optimal one for any diet study. In future research, depending on the diet or eDNA sample studied, additional primer pairs could be needed, to reach a higher level of discrimination at the species level (especially for plants), and to target longer amplicons. We hope this new approach can be an inspiration, and further developed and improved in many other diversity studies. The important sequencing depth reached with this approach (Illumina short-read sequencing) allowed us to target a large number of different amplicons.

This approach maximizes taxonomic coverage and ensures that all potential target DNAs of prey species are amplified and correctly identified at the highest possible taxonomic level. However, even if the taxonomic assignment reached the species level for the majority of the samples, some plant taxa, such as the genera *Salix*, *Quercus*,

Picea and *Carex*, were not identified to the species level despite the number of amplified genes produced and the seemingly adequate length of DNA sequences. Indeed, even by successfully coupling the identification of the genus *Carex* with several different amplified genes (i.e., *rbcL*, *trnL-trnF*, *matK* and 28S), and long amplicons (>800–1,200 bp), taxonomic assignment at the species level was not possible and the final identification remained at the genus level, “*Carex* sp.” This may be caused by the difficulty associated with discriminating between closely related species or, more probably, by the incomplete nature of the NCBI database. Indeed, many species have not yet been added to this database (Kennedy et al., 2020). To identify the aforementioned plants to a higher taxonomic level, we recommend the elaboration of a local DNA sequence database, containing the plant species representing the known botanical diversity of the studied areas.

Unfortunately, one of the limitations of metabarcoding approaches is related to the status of the prey; indeed, it is not possible to determine whether adults, juveniles and/or eggs were consumed or if individuals were dead or alive. Moreover, plant fragments present in our samples could also be derived from prey. Indeed, some prey species of the European pond turtle are known to consume plants as a typical part of their own diets. The identification of certain plants may also be due to pollen contamination of the turtle's food. However, DNA present in faecal samples is usually degraded (Deagle et al., 2006), meaning that food items eaten by both prey and, subsequently, the predator, were degraded twice, making it unlikely that DNA fragments from this source could be detected. Furthermore, plant matter and seeds were present in large quantities in faecal samples, which confirmed the importance of combining direct observation with metabarcoding to validate the sequencing results.

Regarding the quantitative analysis, the amount of sequencing reads is a debatable indicator in the quantification of metabarcoding diets (Deagle et al., 2019). Indeed, the correlation between composition of the sample and sequence reads varies from none to strong. It remains to be shown whether biomass may be linked to read abundance as previously shown in copepods (Clarke et al., 2017; Hirai et al., 2015), in nematode communities (Schenk et al., 2019) and in below-ground plants (Matesanz et al., 2019), while others failed to assess this link such as in zooplankton assemblages (Harvey et al., 2017). Given that metabarcoding relies primarily on barcode amplification, and that pairs of primers have different affinities for the multitude of targeted gene species amplified within a sample, variable amplification level completely biases the putative quantification of the identified species. In our samples, large differences in abundance are found for the same OTU within the same sample depending on the amplified barcode/gene, *rbcL* vs *matK*, *matK* vs *trnL-trnF*, etc. For instance, in the MDV02 sample, the plant species *Phragmites australis* was identified with three contigs, namely *trnL-trnF*, *matK* and *rbcL* with respectively 4.18%, 1.14% and 10.25% of the total mapping reads. Quantification of any dietary abundance data was therefore impossible with the

current methodology. The abundance results of the species in the two MC samples confirmed this observation; they display large disparities depending on whether the DNAs were assembled before amplification or amplified separately in an equimolar manner. Additionally, according to the feeding controls, the metabarcoding analysis also revealed that quantification was not possible, as the relative ingested biomass does not correlate with the abundance of respective reads of each ingested prey. For instance, in EMYS2, we fed the individual with 19 g of *Esox lucius* and an average of 5 g of *Oncorhynchus mykiss*, *Mus musculus*, *Chironomus salinarius* and *Gammarus pulex*. Read abundance was not higher for *Esox lucius* as expected, but even lower compared to the other prey. Finally, a certain number of false negatives due to the lack of amplification of all the present species by the multiple pairs of primers used (both universal and specific) would also modify the proportion of biomass ingested and the numbers of reads.

Similarly, the efficiency of the digestion could have an impact on the detected DNA. In the present study, that is a diet analysis based on the extraction of DNAs from faeces, and verified by visual analysis (see also Ottonello et al., 2018), it was established that the DNA of some prey are excreted with higher integrity than others (e.g., intact seeds, bones, elytra of some beetles). Consequently, a prey can represent 95% of the food intake but its DNA, after extraction, would only represent 1% of the total faecal DNA, compared to a less digestible vegetable food (e.g., intact seeds) representing 5% of the food intake, which would represent 99% of the faecal DNA sample after extraction.

Finally, an additional bias preventing any relative quantitative evaluation when different barcodes are used is the number of copies of a barcoded gene within a targeted organism genome, and whether it is of nuclear, mitochondrial or chloroplast origin. When studying an omnivorous diet by metabarcoding analysis on stool samples, and with our current knowledge, it would be inappropriate to estimate a putative quantification of the prey ingested. Only qualitative analysis is reliable in this particular case.

The large species richness identified using the long metabarcoding approach proposed here is congruent with other molecular studies, which yielded high resolutions and an even greater richness regarding prey consumption, compared to histological analyses of the same samples (Soininen et al., 2015; Ando et al., 2013). To our knowledge, this is the first time that the METASPades software (Nurk et al., 2017) has been used for a long metabarcoding analysis, especially within a dietary study. We showed that this metagenome assembler is able to retrieve amplicons with a high confidence level and consequently provides an accurate taxonomic assignment.

Finally, this new long metabarcoding method with a short-read sequencing approach, combining the use multiple primers pairs (da Silva et al., 2019) and de novo assembly, could be used as a universal, standardized method for studying complex diets, as well as in other complex eDNA analyses. Its high level of precision allows for improvements in studies of biodiversity assessments and trophic interactions, which would enhance our understanding of community ecology and ecosystem functioning.

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AUTHOR CONTRIBUTIONS

The first (C.D.) and second (J.C.) author contributed equally to this paper. C.D., S.U. and J.-F.R. designed research, C.D. performed research (sampling and laboratory work) and analysed data (bioinformatics analyses), J.C. set up the methodology (shearing protocol, first upstream method validation, primer selection and design, sequencing and bioinformatics analyses), and J.C. and F.L. supervised laboratory work. C.D. wrote the manuscript and all other coauthors revised it.

DATA AVAILABILITY STATEMENT

All raw sequencing reads for 40 metabarcodes (32 faecal samples from wild European pond turtles, six faecal samples from the feeding trial and two MCs) were registered in the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) under Bioproject accession PRJNA546135 and the SRA accessions SRR9317490 to SRR9317499, SRR9317517 to SRR9317526, SRR9317540 to SRR9555, SRR9317564, SRR9317567 to SRR9317574 and SRR9317645.

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REFERENCES

- Aceto, S., Caputo, S., Cozzolino, S., Gaudio, L., & Moretti, A. (1999). Phylogeny and evolution of Orchis and allied genera based on ITS DNA variation: Morphological gaps and molecular continuity. *Molecular Phylogenetics and Evolution*, 13, 67–76.
- Alberdi, A., Aizpurua, O., Bohmann, K., Gopalakrishnan, S., Lynggaard, C., Nielsen, M., & Gilbert, M. T. P. (2018). – Promises and pitfalls of using high-throughput sequencing for diet analysis. *Molecular Ecology Resources*, 19(2), 327–348. <https://doi.org/10.1111/1755-0998.12960>
- Albertsen, M., Hugenholtz, P., Skarszewski, A., Nielsen, K. L., Tyson, G. W., & Nielsen, P. H. (2012). Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. *Nature Biotechnology*, 31, 533–538. <https://doi.org/10.1038/nbt.2579>
- Anantharaman, K., Brown, C. T., Hug, L. A., Sharon, I., Castelle, C. J., Probst, A. J., Thomas, B. C., Singh, A., Wilkins, M. J., Karaoz, U., Brodie, E. L., Williams, K. H., Hubbard, S. S., & Banfield, J. F. (2016). Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. *Nature Communications*, 7, 13219. <https://doi.org/10.1038/ncomms13219>
- Ando, H., Setsuko, S., Horikoshi, K., Suzuki, H., Umehara, S., Inoue-Murayama, M., & Isagi, Y. (2013). Diet analysis by next-generation sequencing indicates the frequent consumption of introduced plants

- by critically endangered red-headed wood pigeon (*Columba janthina nitens*) in oceanic island habitat. *Ecology and Evolution*, 3(12), 4057–4069.
- Andrews, S. (2010). FASTQC: A quality-control tool for high throughput sequences data. Babraham Institute.
- Baamrane, M. A. A., Shehzad, W., Ouhammou, A., Abbad, A., Naimi, M., Coissac, E., & Znari, M. (2012). Assessment of the food habits of the Moroccan Dorcas Gazelle in M'Sabih Talaa, West Central Morocco, using the trnL approach. *PLoS One*, 7(4), e35643.
- Barbato, M., Kovács, T., Coleman, M. A., Broadhurst, M. K., & De Bruyn, M. (2019). Metabarcoding for stomach-content analyses of Pygmy devil ray (*Mobula kuhlii* cf. *eregoidotoenkee*): Comparing tissues and ethanol preservative-derived DNA. *Ecology and Evolution*, 9(5), 2678–2687.
- Bogler, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible read trimming tool for Illumina NGS data. *Bioinformatics*, 30(15), 2114–2120.
- Brown, D. S., Jarman, S. N., & Symondson, W. O. C. (2012). Pyrosequencing of prey DNA in reptile faeces: Analysis of earthworm consumption by slow worms. *Molecular Ecology Resources*, 12, 259–266. <https://doi.org/10.1111/j.1755-0998.2011.03098.x>
- Buglione, M., Maselli, V., Rippa, D., De Filippo, G., Trapanese, M., & Fulgione, D. (2018). A pilot study on the application of DNA metabarcoding for non-invasive diet analysis in the Italian hare. *Mammalian Biology*, 88, 31–42. <https://doi.org/10.1016/j.mambio.2017.10.010>
- Cadi, A. (2003). *Écologie de la cistude d'Europe (Emys orbicularis): Aspects spatiaux et démographiques, application à la démographie* (pp. 1–350). Université Bernard Lyon.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, 10, 421. <https://doi.org/10.1186/1471-2105-10-421>
- Caut, S., Francois, V., Bacques, M., Guiral, D., Lemaire, J., Lepoint, G., Marquis, O., & Sturaro, N. (2019). The dark side of the black caiman: Shedding light on species dietary ecology and movement in Agami Pond, French Guiana. *PLoS One*, 14(6), e0217239. <https://doi.org/10.1371/journal.pone.0217239>
- Che, J., Chen, H.-M., Yang, J.-X., Jin, J.-Q., Jiang, K. E., Yuan, Z.-Y., Murphy, R. W., & Zhang, Y.-P. (2012). Universal COI primers for DNA barcoding amphibians. *Molecular Ecology Resources*, 12(2), 247–258. <https://doi.org/10.1111/j.1755-0998.2011.03090.x>
- Çicek, K., & Ayaz, D. (2011). Food composition of the European pond turtle (*Emys orbicularis*) in Lake Sülüklü (Western Anatolia, Turkey). *Journal of Freshwater Ecology*, 26, 571–578.
- Clarke, L. J., Beard, J. M., Swadling, K. M., & Deagle, B. E. (2017). Effect of marker choice and thermal cycling protocol on zooplankton DNA metabarcoding studies. *Ecology and Evolution*, 7(3), 873–883. <https://doi.org/10.1002/ece3.2667>
- Crisol-Martiniez, E., Moreno-Moyano, L. T., Wormington, K. R., Brown, P. H., & Stanley, D. (2016). Using next-generation sequencing to contrast the diet and explore pest-reduction services of sympatric bird species in macadamia orchards in Australia. *PLoS One*, 11, e0150159. <https://doi.org/10.1371/journal.pone.0150159>
- Cuénod, P., Savolainen, V., Chatrou, L. W., Powell, M., Grayer, R. J., & Chase, M. W. (2002). Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB* and *matK* DNA sequences. *American Journal of Botany*, 89(1), 132–144. <https://doi.org/10.3732/ajb.89.1.132>
- Cullings, K. (1992). Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology*, 1, 233–240. <https://doi.org/10.1111/j.1365-294X.1992.tb00182.x>
- Da Silva, L. P., Mata, V. A., Lopes, P. B., Pereira, P., Jarman, S. N., Lopes, R. J., & Beja, P. (2019). Advancing the integration of multi-marker metabarcoding data in dietary analysis of trophic generalists. *Molecular Ecology Resources*, 19(6), 1–13. <https://doi.org/10.1111/1755-0998.13060>
- De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, 14, 306–323. <https://doi.org/10.1111/1755-0998.12188>
- Deagle, B. E., Chiaradia, A., McInnes, J., & Jarman, S. N. (2010). Pyrosequencing faecal DNA to determine diet of little penguins: Is what goes in what comes out? *Conservation Genetics*, 11, 2039–2048. <https://doi.org/10.1007/s10592-010-0096-6>
- Deagle, B. E., Everson, J. P., & Jarman, S. N. (2006). Quantification of damage in DNA recovered from highly degraded samples – a case study on DNA in faeces. *Frontiers in Zoology*, 3, 11. <https://doi.org/10.1186/1742-9994-3-11>
- Deagle, B. E., Thomas, A. C., McInnes, J. C., Clarke, L. J., Vesterinen, E. J., Clare, E. L., & Eveson, J. P. (2019). Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? *Molecular Ecology*, 28(2), 391–406.
- Deagle, B. E., Tollit, D. J., Jarman, S. N., Hindell, M. A., Tristes, A. W., & Gales, N. J. (2005). Molecular scatology as a tool to study diet: Analysis of prey DNA in scats from a captive Steller sea lion. *Molecular Ecology*, 14, 1831–1842.
- Devaux, B., Bonin, F., & Dupré, A. (1996). *Toutes les tortues du monde. Les encyclopédies naturalistes*. Dijkstra, K. B., Kalkman, V. J., Dow, R. A., Stokvis, F. R., & Van Tol, J. (2014). Redefining the damselfly families: A comprehensive molecular phylogeny of Zygoptera (Odonata). *Systematic Entomology*, 39, 68–96.
- Dobler, S., & Müller, J. K. (2000). Resolving phylogeny at the family level by mitochondrial cytochrome oxidase sequences: Phylogeny of carrion beetles (Coleoptera, Silphidae). *Molecular Phylogenetics and Evolution*, 13, 149–158. <https://doi.org/10.1006/mpev.1999.0765>
- Elbrecht, V., Braukmann, T. W. A., Ivanova, N. V., Prosser, S. W. J., Hajibabaei, M., Wright, M., Zakharov, E. V., Hebert, P. D. N., & Steinke, D. (2019). Validation of COI metabarcoding primers for terrestrial arthropods. *PeerJ*, 7, e7745. <https://doi.org/10.7717/peerj.7745>
- Elbrecht, V., & Leese, F. (2017). Validation and development of freshwater invertebrate metabarcoding COI primers for environmental impact assessment. *Frontiers in Environmental Sciences*, 5, 1–11.
- Esnaola, A., Arrizabalaga-Escudero, A., Gonzalez-Esteban, J., Elosegi, A., & Aihartza, J. (2018). Determining diet from faeces: Selection of metabarcoding primers for the insectivore Pyrenean desman (*Galemys pyrenaicus*). *PLoS One*, 13(12), e0208986. <https://doi.org/10.1371/journal.pone.0208986>
- Fay, M. F., Swensen, S. M., & Chase, M. W. (1997). Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bulletin*, 52, 111–120. <https://doi.org/10.2307/4117844>
- Ford, C. S., Ayres, K. L., Toomey, N., Haider, N., Van Alphen, Stahl J., Kelly, L. J., Wikström, N., Hollingsworth, P. M., Duff, R. J., Hoot, S. B., Cowan, R. S., Chase, M. W., & Wilkinson, M. J. (2009). Selection of candidate DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society*, 159(1), 1–11.
- Forouzan, E., Shariati, P., Maleki, M. S. M. M., Karkhane, A. A., & Yakhchali, B. (2018). Practical evaluation of 11 *de novo* assemblers in metagenome assembly. *Journal of Microbiological Methods*, 151, 99–105. <https://doi.org/10.1016/j.mimet.2018.06.007>
- Godwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics*, 17, 333–351. <https://doi.org/10.1038/nrg.2016.49>
- Hajibabaei, M., Smith, M. A., Janzen, D. H., Rodriguez, J. J., Whitfield, J. B., & Hebert, P. D. N. (2006). A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes*, 6, 959–964. <https://doi.org/10.1111/j.1471-8286.2006.01470.x>
- Hamby, R. K., & Zimmer, E. A. (1988). Ribosomal RNA sequences for inferring phylogeny within the grass family (Poaceae). *Plant Systematics and Evolution*, 160, 29–37. <https://doi.org/10.1007/BF00936707>

- Han, S. H., & Oh, H. S. (2018). Genetic identification of prey birds of the Endangered peregrine falcon (*Falco peregrinus*). *Mitochondrial DNA Part A*, 29, 175–180.
- Harms-Tuohy, C. A., Schizas, N. V., & Appeldoorn, R. S. (2016). Use of DNA metabarcoding for stomach content analysis in the invasive lionfish *Pterois volitans* in Puerto Rico. *Marine Ecology Progress Series*, 558, 181–191. <https://doi.org/10.3354/meps11738>
- Harvey, J. B. J., Johnson, S. B., Fisher, J. L., Peterson, W. T., & Vrijenhoek, R. C. (2017). Comparison of morphological and next generation DNA sequencing methods for assessing zooplankton assemblages. *Journal of Experimental Marine Biology and Ecology*, 467, 113–126.
- Heeger, F., Bourne, E. C., Baschien, C., Yurkov, A., Bunk, B., Spröer, C., Overmann, J., Mazzoni, C. J., & Monaghan, M. T. (2018). Long-read DNA metabarcoding of ribosomal RNA in the analysis of fungi from aquatic environments. *Molecular Ecology Resources*, 18, 1500–1514. <https://doi.org/10.1111/1755-0998.12937>
- Hibert, F., Taberlet, P., Chave, J., Scotti-Saintagne, C., Sabatier, D., & Richard-Hansen, C. (2013). Unveiling the diet of elusive rainforest herbivores in next generation sequencing era? The tapir as a case study. *PLoS One*, 8(4), e60799. <https://doi.org/10.1371/journal.pone.0060799>
- Hirai, J., Kuriyama, M., Ichikawa, T., Hidaka, K., & Tsuda, A. (2015). A metagenetic approach for revealing community structure of marine planktonic copepods. *Molecular Ecology Resources*, 15(1), 68–80. <https://doi.org/10.1111/1755-0998.12294>
- Hudson, J. A., Morvan, B., & Jobline, K. N. (1998). Hydration of linoleic acid by bacteria isolated from ruminants. *FEMS Microbiology Letters*, 169, 277–282. <https://doi.org/10.1111/j.1574-6968.1998.tb13329.x>
- Huerta-Cepas, J., Serra, F., & Bork, P. (2016). ETE 3: Reconstruction, analysis and visualization of phylogenomic data. *Molecular Biology Evolution*, 33(6), 1635–1638. <https://doi.org/10.1093/molbev/msw046>
- Jamy, M., Foster, R., Barbera, P., Czech, L., Kozlov, A., Stamatakis, A., Burki, F. (2020). Long-read metabarcoding of the eukaryotic rDNA operon to phylogenetically and taxonomically resolve environmental diversity. *Molecular Ecology Resources*, 20(2), 429–443. <https://doi.org/10.1101/627828>
- Jusino, M. A., Banik, M. T., Palmer, J. M., Wray, A. K., Xiao, L., Pelton, E., Barber, J. R., Kawahara, A. Y., Gratton, C., Peery, M. Z., & Lindner, D. L. (2018). An improved method for utilizing high-throughput amplicon sequencing to determine the diets of insectivorous animals. *Molecular Ecology Resources*, 19(1), 176–190. <https://doi.org/10.1111/1755-0998.12951>
- Kamenova, S., Mayer, R., Rubbmark, O. S., Coissac, E., Plantegenest, M., & Traugott, M. (2018). Comparing three types of dietary samples for prey DNA decay in an insect generalist predator. *Molecular Ecology Resources*, 18, 966–973. <https://doi.org/10.1111/1755-0998.12775>
- Kartzinel, T. R., & Pringle, R. M. (2015). Molecular detection of invertebrate prey in vertebrate diets: Trophic ecology of Caribbean island lizards. *Molecular Ecology Resources*, 15(4), 903–914. <https://doi.org/10.1111/1755-0998.12366>
- Kennedy, S. R., Prost, S., Overcast, I., Rominger, A. J., Gillespie, R. G., & Krehenwinkel, H. (2020). High-throughput sequencing for community analysis: The promise of DNA barcoding to uncover diversity, relatedness, abundances and interactions in spider communities. *Development Genes and Evolution*, 230, 185–201. <https://doi.org/10.1007/s00427-020-00652-x>
- Koizumi, N., Mori, A., Mineta, T., Sawada, E., Watabe, K., & Takemura, T. (2017). Plant species identification using fecal DNAs from red-eared slider and Reeves' pond turtle in agricultural canals for rural ecosystem conservation. *Paddy and Water Environment*, 15(4), 723–730. <https://doi.org/10.1007/s10333-016-0576-5>
- Krehenwinkel, H., Kennedy, S., Pekar, S., & Gillespie, R. G. (2016). A cost-efficient and simple protocol to enrich prey DNA from extractions of predatory arthropods for large-scale gut content analysis by Illumina sequencing. *Methods in Ecology and Evolution*, 8, 126–134.
- Kress, W. J., & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: The coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoS One*, 2(6), e508. <https://doi.org/10.1371/journal.pone.0000508>
- Lacoursière-Roussel, A., Howland, K., Normandeau, E., Grey, E. K., Archambault, P., & Deiner, K., & Bernatchez, L. (2018). eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. *Ecology and Evolution*, 8, 7763–7777.
- Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, 9(4), 357–359. <https://doi.org/10.1038/nmeth.1923>
- Laudadio, I., Fulci, V., Stronati, L., & Carissimi, C. (2019). Next-generation metagenomics: Methodological challenges and opportunities. *OMICS: A Journal of Integrative Biology*, 23(7), 327–333. <https://doi.org/10.1089/omi.2019.0073>
- Leal, M., Ferrier-Pagès, C., Calado, R., Thompson, M. E., Frischer, M. E., & Nejstgaard, J. C. (2014). Coral feeding on microalgae assessed with molecular trophic markers. *Molecular Ecology*, 23(15), 3870–3876. <https://doi.org/10.1111/mec.12486>
- Leray, M., Agudelo, N., Mills, S. C., & Meyer, C. P. (2013). Effectiveness of annealing blocking primers versus restriction enzymes in the gut contents of two coral reef fish species. *PLoS One*, 8(4), e58076.
- Levin, R. A., Wagner, W. L., Hoch, P. C., Nepokroeff, M., Pires, J. C., Zimmer, E. A., & Sytsma, K. J. (2003). Family-level relationships of Onagraceae based on chloroplast *rbcL* and *ndhF* data. *American Journal of Botany*, 90, 107–115.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., & Durbin, R. (2009). 1000 Genome Project Data Processing Subgroup, The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079.
- Lim, N. K. M., Tay, Y. C., Srivathsan, A., Tan, J. W. T., Kwik, J. T. B., Baloğlu, B., Meier, R., & Yeo, D. C. J. (2016). Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. *The Royal Society Open Science*, 3, 160635. <https://doi.org/10.1098/rsos.160635>
- Liu, M., Clarke, L. J., Baker, S. C., Jordan, G. J., & Burridge, C. P. (2020). A practical guide to DNA metabarcoding for entomological ecologists. *Ecological Entomology*, 45(3), 373–385.
- Martinsen, E. S., Perkins, S. L., & Schall, J. J. (2008). A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): Evolution of life-history traits and host switches. *Molecular Phylogenetics and Evolution*, 47, 261–273. <https://doi.org/10.1016/j.ympev.2007.11.012>
- Matesanz, S., Pescador, D. S., Pías, B., Sánchez, A. M., Chacón-Labolla, J., Illuminati, A., Cruz, M., López-Angulo, J., Marí-Mena, N., Vizcaíno, A., & Escudero, A. (2019). Estimating belowground plant abundance with DNA metabarcoding. *Molecular Ecology Resources*, 19(5), 1265–1277. <https://doi.org/10.1111/1755-0998.13049>
- Meusnier, I., Singer, G. A. C., Landry, J.-F., Hickey, D. A., Hebert, P. D. N., & Hajibabaei, M. (2008). A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics*, 9, 204. <https://doi.org/10.1186/1471-2164-9-214>
- Monney, J.-C., & Meyer, A. (2005). *Liste rouge des espèces menacées en Suisse, Reptile*. : Office fédéral de l'environnement, des forêts et du paysage (OFEV), Centre de coordination des amphibiens et reptiles de Suisse (Karch), 46 p.
- Moorhouse-Gann, R. J., Dunn, J. C., de Verre, N., Goder, M., Cole, N., Hipperson, H., & Symondson, W. O. (2018). New universal ITS2 primers for high-resolution herbivore analyses using DNA metabarcoding in both tropical and temperate zones. *Scientific Reports*, 8, 8542.
- Nagarajan, N., & Pop, M. (2013). Sequence assembly demystified. *Nature Reviews Genetics*, 14(3), 157–167. <https://doi.org/10.1038/nrg3367>
- Nakahara, F., Ando, H., Ito, H., Murakami, A., Morimoto, N., Yamasaki, M., Takayanagi, A., & Isagi, Y. (2015). The applicability of DNA barcoding for dietary analysis of sika deer. *DNA Barcodes*, 3(1), 200–206. <https://doi.org/10.1515/dna-2015-0021>

- Nikolenko, S. I., Korobeynikov, A. I., & Alekseyev, M. A. (2013). BayesHammer: Bayesian clustering for error correction in single-cell sequencing. *BMC Genomics*, 14, S7. <https://doi.org/10.1186/1471-2164-14-S1-S7>
- Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. (2017). metaSPADEs: A new versatile de novo metagenomic assembler. *Genome Research*, 27(5), 824–834.
- O'Rorke, R., Lavery, S., & Jeffs, A. (2012). PCR enrichment techniques to identify the diet of predators. *Molecular Ecology Resources*, 12, 5–17. <https://doi.org/10.1111/j.1755-0998.2011.03091.x>
- Olmstead, R. G., Michaels, H. J., Scott, K. M., & Palmer, J. D. (1992). Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequence of rbcL. *Annals of the Missouri Botanical Garden*, 79, 249–265.
- Ottanello, D., D'Angelo, S., Oneto, F., Malavasi, S., & Zuffi, M. A. L. (2016). Feeding ecology of the Silican pond turtle *Emys trinacris* (Testudines, Emydidae) influenced by seasons and invasive aliens' species. *Ecological Researches*, 32, 71–80.
- Ottanello, D., Oneto, F., Vignone, M., Rizzo, A., & Salvadio, S. (2018). Diet of a restocked population of the European pond turtle *Emys orbicularis* in NW Italy. *Acta Herpetologica*, 13, 89–93.
- Ottanello, D., Salvadio, S., & Rosecchi, E. (2005). Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). *Amphibia-Reptilia*, 26, 562–565. <https://doi.org/10.1163/156853805774806241>
- Piper, A. M., Batovska, J., Cogan, N. O. I. C., Weiss, J., Cunningham, J. P., Rodoni, B. C., & Blacket, M. J. (2019). Prospects and challenges of implementing DNA metabarcoding for high-throughput insect surveillance. *GigaScience*, 8, giz092. <https://doi.org/10.1093/gigascience/giz092>
- Pompanon, F., Deagle, B. E., Symondson, W. O. C., Brown, D. S., Jarman, S. N., & Taberlet, P. (2012). Who is eating what: Diet assessment using next generation sequencing? *Molecular Ecology Resources*, 21(8), 1931–1950. <https://doi.org/10.1111/j.1365-294X.2011.05403.x>
- Porter, T. M., & Golding, G. B. (2011). Are similarity – or phylogeny – based methods more appropriate for classifying internal transcribed spacer (ITS) metagenomic amplicons? *New Phytologist*, 192, 775–782. <https://doi.org/10.1111/j.1469-8137.2011.03838.x>
- Pumarino, L., Alomar, O., & Agusti, N. (2011). Development of specific ITS markers for plant DNA identification within herbivorous insects. *Bulletin of Entomology Resources*, 101, 271–276. <https://doi.org/10.1017/S0007485310000465>
- Reeves, L. E., Gillett-Kaufman, J. L., Kawahara, A. Y., & Kaufman, P. E. (2018). Barcoding blood meals: New vertebrate-specific primer sets for assigning taxonomic identities to host DNA from mosquito blood meals. *PLoS Neglected Tropical Diseases*, 12(8), e0006767. <https://doi.org/10.1371/journal.pntd.0006767>
- Ricciioni, G., Stagioni, M., Piccinetti, C., & Libralato, S. (2018). A metabarcoding approach for the feeding habits of European hake in the Adriatic Sea. *Ecology and Evolution*, 8, 10435–10447. <https://doi.org/10.1002/ece3.4500>
- Robeson, M. S. II, Khanipov, K., Golovko, G., Wisley, S. M., White, M. D., Bodenbach, M., & Piaggio, A. J. (2017). Assessing the utility of metabarcoding for diet analyses of the omnivorous wild pig (*Sus scrofa*). *Ecology and Evolution*, 8, 185–196.
- Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods monitoring, and application of global eDNA. *Global Ecology and Conservation*, 17, e00547.
- Sang, T., Crawford, D. J., & Stuessy, T. F. (1997). Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany*, 84, 1120–1136.
- Spadaro, D., Amatulli, M. L., Gullino, A., & Garibaldi, A. (2012). Quantitative real-time PCR for *Fusarium fujikuroi* and *Fusarium proliferatum* on rice. *Journal of Plant Pathology*, 94(S4), 80.
- Schenk, J., Geisen, S., Kleinböting, N., & Traunspurger, W. (2019). Metabarcoding data allow for reliable biomass estimates in the most abundant animals on earth. *Metabarcoding & Metagenomics*, 3, 117–126. <https://doi.org/10.3897/mbmg.3.46704>
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flok, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of Entomological Society of America*, 87(6), 651–701. <https://doi.org/10.1093/aesa/87.6.651>
- Singer, E., Bushnell, B., Coleman-Derr, D., Bowman, B., Bowers, R. M., Levy, A., & Woyke, T. (2016). High-resolution phylogenetic microbial community profiling. *The ISME Journal*, 10, 2020–2032.
- Soininen, E. M., Gauthier, G., Bilodeau, F., Berteaux, D., Gielly, L., Taberlet, P., & Yoccoz, N. G. (2015). Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS One*, 10(1), e0115335.
- Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17, 1105–1109. <https://doi.org/10.1007/BF00037152>
- Tate, J. A., & Simpson, B. B. (2003). Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany*, 28, 723–773.
- Truelove, N. K., Andruszkiewicz, E. A., & Block, B. A. (2019). A rapid environmental DNA method for detecting white sharks in the open ocean. *Methods in Ecology and Evolution*, 10, 1128–1135. <https://doi.org/10.1111/2041-210X.13201>
- Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in Ecology & Evolution*, 24, 110–117.
- Van der Walt, A. J., van Goethem, M. W., Ramond, J.-B., Makhalanyane, T. P., Reva, O., & Cowan, D. A. (2017). Assembling metagenomes, one community at a time. *BioMed Central Genomics*, 18, 521. <https://doi.org/10.1186/s12864-017-3918-9>
- Vestheim, H., & Jarman, S. N. (2008). Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology*, 5, 12. <https://doi.org/10.1186/1742-9994-5-12>
- Wallinger, C., Staudacher, K., Schallhart, N., Peter, E., Dresch, P., Juen, A., & Traugott, M. (2012). The effect of plant identity and the level of plant decay on molecular gut content analysis in an herbivorous soil insect. *Molecular Ecology Resources*, 13(1), 75–83.
- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes from phylogenetics. In M. A. Innis, H. Gelfand, J. S. Sninsky, & T. J. White (Eds.), *PCR Protocols: A guide to methods and applications* (pp. 315–322). Academic Press.
- Yu, J., Xue, J., & Zhou, S. (2011). New universal *matK* primers for DNA barcoding angiosperms. *Journal of Systematics and Evolution*, 49, 176–181. <https://doi.org/10.1111/j.1759-6831.2011.00134.x>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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MOLECULAR ECOLOGY RESOURCES

Supplemental Information for:

A powerful long metabarcoding method for determination of complex diets from faecal analysis of the European pond turtle (*Emys orbicularis*, L. 1758)

Charlotte Ducotterd, Julien Crovadore, François Lefort, Jean-François Rubin, Sylvain Ursenbacher

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Figure S1. Species comprising the putative diet of the European pond turtle (*Emys orbicularis*) were included in the Mock communities MC1 and MC2. The species have been selected according to the literature (Ottonello et al. 2005; Çiçek & Ayaz, 2011; Ottonello et al. 2016; Ottonello et al. 2018).

Phylum	Family	Species	English name
Magnoliphyt			
a	Iridaceae	<i>Iris pseudacorus</i>	Yellow iris
Magnoliphyt	Potamogetonacea	<i>Potamogeton perfoliatus</i>	Gypswort
Magnoliphyt			
a	Nymphaeaceae	<i>Nuphar lutea</i>	Yellow water-lily
Magnoliphyt			European white water-lily
a	Nymphaeaceae	<i>Nymphaea alba</i>	
Magnoliphyt			
a	Lamiaceae	<i>Lycopus europaeus</i>	Perfoliate pondweed
Magnoliphyt			
a	Lentibulariaceae	<i>Utricularia australis</i>	Bladderwort
Magnoliphyt			
a	Lamiaceae	<i>Mentha aquatica</i>	Aquatic mint
Arthropoda	Aeshnidae	<i>Aeshna cyanea</i>	Blue hawker
Arthropoda	Baetidae	<i>Baetis rhodani</i>	Mayflies
Arthropoda	Baetidae	<i>Cloeon dipterum</i>	Mayflies
Arthropoda	Caenidae	<i>Caenis horaria</i>	Mayflies
Arthropoda	Notonectoidea	<i>Notonecta glauca</i>	Backswimmer
Arthropoda	Chironomidae	<i>Chironomus salinarius</i>	Midges larvae
Arthropoda	Gammaridae	<i>Gammarus pulex</i>	Amphipod crustacean
Mollusca	Lymnaeoidea	<i>Radix balthica</i>	Wandering snail
Chordata	Cyprinidae	<i>Tinca tinca</i>	Tench
Chordata	Esocidae	<i>Esox lucius</i>	Northern pike
Chordata	Bufonidae	<i>Bufo viridis</i>	European green toad
Chordata	Muridae	<i>Mus musculus</i>	Grey mouse

Figure S2. Host-specific blocking primer

Faecal samples contain degraded prey DNA but also contained high levels of high-quality host DNA. (Deagle et al., 2006). Predator DNA amplification may bias or prevent amplification of rare DNA sequences (Vestheim and Jarman, 2008; O'Rorke et al., 2012; Leray et al., 2013). Previous analyses (Roberson II et al., 2017) demonstrated that metabarcoding sequencing of faeces without a blocking primer, which prevents host DNA amplification, resulted in a high relative abundance of the host COI amplicons compare to target prey COI sequences.

Preliminary tests demonstrated that three of the four selected primers pairs targeting the COI region were perfectly amplifying *E. orbicularis* COI DNA. The seven selected primers pairs targeting plants were also tested against pond turtle DNA but did not produce amplicons as expected.

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Consequently, based on an alignment of reference COI sequences of the European pond turtle, different blocking primers specific to *E. orbicularis* were developed, according to Vestheim and Jarman (2008). The aim was to block or limit amplification of COI sequences of the focal species. Turtle blocking primers overlapped with the 3' end of the forward universal sequence, extending into *E. orbicularis*-specific sequences modified with a Spacer C3 at the 3' end (Vestheim and Jarman, 2008). As two forward primers (ODO_LC01490d and COI-C02) were overlapping the same region of the COI sequence, a single blocking primer was needed for both. Sequences of the blocking primers were as follows: (1) COI-blkEmys1-3'c3 5'-ATAAAGATATTGGTACCCCTATCT-C3-3'; (2) COI-blkEmys2-3'c3 5'-TGTATACCCCCGCTAGCCGGAAAC-C3-3'. Turtle blocking primers were then rigorously evaluated against *E. orbicularis* DNA. COI-blkEmys1-3'c3 had proven to be effective by blocking 100% of the amplification of host DNA. On the other hand, COI-blkEmys2-3'c3 was totally ineffective despite numerous tests, even at a concentration a hundred times higher than its target primer pair in the reaction mixture. Finally, both were assessed against animal DNA from MC. Unfortunately, it turned out that under our established PCR conditions, COI-blkEmys1-3'c3 also partially inhibited the amplification of some DNA of target organisms. Therefore, the use of the blocking primer was not compatible with our metabarcoding approach.

Figure S3. Covaris S2 290 bp median size shearing protocol, applicable to a mixture of amplicons ranging from 350 to 1400 bp.

Amplicon length	350 to 1400 bp
Median Target size	290 bp
Duty Factor	10
Intensity	50
Peak/Display Power	23
Cycles/Burst	200
Mode	Frequency sweeping
T°C	5.5
Time (sec)	140

Figure S4. Bioinformatics workflow and command lines used with the software metaSPAdes v3.9.0 (Nurk et al., 2017; <http://cab.spbu.ru/software/spades>), BOWTIE2 (Langmead & Salzberg, 2012), SAMTOOLS (Li et al., 2009) and multi-metagenome (Albertsen et al. 2012) Perl script.

Step 1: Adapters removal and demultiplexing using automatic Illumina bcl2fastq2 conversion Software v2.20 through MiniSeq local run manager.

Step 2: Quality and adaptor contamination check using FastQC (Andrews, 2010).

Step 3: De novo assembly of the paired end reads using metaSPAdes.

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```
./spades.py -o MetaSPAdesSampleX -meta -m 500 -t 72 -pe1-1 SampleX_R1.fastq.gz -pe1-2 SampleX_R2.fastq.gz
```

Step 4: Contigs mapping to NCBI server complete nucleotide database (nr/nt), using "BLAST+" [Camacho et al., 2009]

```
./blastn -db nt -query contigsSampleX.fasta -out resultscontigsSampleX.out -remote -outfmt '6 qseqid sseqid pident evalue staxids sscinames scomnames sskingdoms stitle std' -max_target_seqs 5 -evalue 1e-20
```

Step 5: Identified species full taxonomy completion based on their respective TaxID and using ETE toolkit software (Huerta-Cepas et al., 2016).

```
./export PATH=~/anaconda_ete/bin:$PATH  
./ete3 ncbiquery --search (TaxIDs numbers list) -info
```

Step 6: Determination of the reads abundance per contig/identified OTU

BOWTIE2 :

Index creation

```
bowtie2-build contigsSampleX.fasta contigsSampleX.btindex
```

Mapping

```
bowtie2 -x contigsSampleX.btindex -1 SampleX_R1.fastq -2 SampleX_R2.fastq -S SampleXbt2Map.sam
```

SAMTOOLS :

File conversion

```
samtools view -bS SampleXbt2Map.sam > SampleXbt2Map.bam
```

Alignment toward reference order

```
samtools sort SampleXbt2Map.bam -o SampleXbt2Map.sorted.bam
```

Obtaining "Depth" file

```
samtools depth SampleXbt2Map.sorted.bam > depthSampleX.txt
```

multi-metagenome

Convert file in .csv

```
perl calc.coverage.in.bam.depth.pl -i depthSampleX.txt -o coverage.lengthSampleX.csv
```

MOLECULAR ECOLOGY

RESOURCES

Figure S5. Analysis of faecal sample ($N = 32$) from the European pond turtle (*Emys orbicularis*), two mock communities and feeding trial samples. Sample ID, along with genus/species of each identified ingested preys and plants, targeted genes, contig length (bp), total reads number per sample, percentage of mapping reads and its respective number of reads.

Sample ID	Species	Targeted gene	Length (bp)	Total number of reads	% of mapping reads	Number of reads
EMYS0	<i>Chironomus salinarius</i>	COI	573	396575	0,45	1791
EMYS0	<i>Esox lucius</i>	COI	323	396575	2,03	8042
EMYS1	<i>Chironomus salinarius</i>	COI	636	338965	0,09	293
EMYS1	<i>Mus musculus</i>	COI	244	338965	0,55	1875
EMYS2	<i>Esox lucius</i>	COI	366	317877	0,03	93
EMYS2	<i>Chironomus salinarius</i>	COI	588	317877	0,03	106
EMYS2	<i>Gammarus pulex</i>	COI	221	317877	0,18	557
EMYS2	<i>Mus musculus</i>	COI	567	317877	0,05	156
EMYS2	<i>Mus musculus</i>	COI	218	317877	0,64	2045
EMYS2	<i>Oncorhynchus mykiss</i>	COI	566	317877	0,53	1698
EMYS3	<i>Chironomus salinarius</i>	COI	410	400717	0,27	1095
EMYS3	<i>Esox lucius</i>	COI	566	400717	1,70	6801
EMYS3	<i>Mus musculus</i>	COI	350	400717	0,02	96
EMYS4	<i>Chironomus salinarius</i>	COI	397	327146	0,37	1218
EMYS5	<i>Esox lucius</i>	COI	499	338415	0,57	1929
EMYS5	<i>Gammarus pulex</i>	COI	275	338415	0,65	2188
EMYS5	<i>Mus musculus</i>	COI	296	338415	0,01	49
MC1	<i>Iris pseudacorus</i>	trnL - trnF	1065	355086	0,62	2208
MC1	<i>Iris pseudacorus</i>	matK	989	355086	2,90	10307
MC1	<i>Iris pseudacorus</i>	rbcL	261	355086	0,34	1219
MC1	<i>Utricularia australis</i>	trnL - trnF	967	355086	0,08	298
MC1	<i>Mentha spicata</i>	matK	957	355086	0,88	3135
MC1	<i>Mentha spicata</i>	rbcL	957	355086	0,73	2583
MC1	<i>Tinca tinca</i>	COI	710	355086	0,62	2187
MC1	<i>Chironomus salinarius</i>	COI	709	355086	2,06	7309
MC1	<i>Gammarus pulex</i>	COI	709	355086	0,27	945
MC1	<i>Bufo bufo</i>	COI	709	355086	0,07	246
MC1	<i>Mus musculus</i>	COI	682	355086	0,09	327
MC1	<i>Cloeon dipterum</i>	COI	657	355086	0,04	126
MC1	<i>Nuphar lutea</i>	rbcL	270	355086	0,16	566
MC1	<i>Nuphar lutea</i>	matK	636	355086	4,58	16265
MC1	<i>Baetis rhodani</i>	COI	603	355086	0,01	24

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MC1	<i>Esox lucius</i>	COI	572	355086	0,03	108
MC1	<i>Potamogeton perfoliatus</i>	trnL - trnF	1120	355086	0,50	1793
MC1	<i>Potamogeton perfoliatus</i>	rbcL	635	355086	4,89	17368
MC1	<i>Potamogeton perfoliatus</i>	matK	588	355086	0,74	2625
MC1	<i>Caenis horaria</i>	COI	476	355086	0,01	37
MC1	<i>Aeshna cyanea</i>	COI	340	355086	0,01	23
MC1	<i>Notonecta glauca</i>	COI	259	355086	0,21	735
MC1	<i>Radix balthica</i>	COI	233	355086	0,00	9
MC2	<i>Aeshna cyanea</i>	COI	440	374942	0,27	997
MC2	<i>Baetis rhodani</i>	COI	540	374942	0,02	77
MC2	<i>Bufoates viridis</i>	COI	709	374942	0,56	2100
MC2	<i>Caenis horaria</i>	COI	285	374942	0,04	151
MC2	<i>Chironomus salinarius</i>	COI	306	374942	2,09	7847
MC2	<i>Cloeon dipterum</i>	COI	667	374942	0,29	1086
MC2	<i>Esox lucius</i>	COI	279	374942	0,21	804
MC2	<i>Gammarus pulex</i>	COI	520	374942	1,61	6029
MC2	<i>Iris pseudacorus</i>	matK	391	374942	7,86	29460
MC2	<i>Iris pseudacorus</i>	rbcL	230	374942	1,46	5467
MC2	<i>Iris pseudacorus</i>	trnL - trnF	229	374942	2,98	11169
MC2	<i>Lycopus europaeus</i>	trnL - trnF	669	374942	4,93	18486
MC2	<i>Lycopus europaeus</i>	matK	560	374942	4,02	15089
MC2	<i>Mus musculus</i>	COI	436	374942	0,36	1352
MC2	<i>Notonecta glauca</i>	COI	413	374942	0,05	181
MC2	<i>Nuphar lutea</i>	matK	166	374942	8,22	30838
MC2	<i>Nymphaea alba</i>	rbcL	166	374942	3,41	12781
MC2	<i>Potamogeton perfoliatus</i>	trnL - trnF	226	374942	1,26	4726
MC2	<i>Potamogeton perfoliatus</i>	rbcL	197	374942	2,05	7692
MC2	<i>Radix balthica</i>	COI	242	374942	0,02	59
MC2	<i>Tinca tinca</i>	COI	519	374942	0,93	3474
MC2	<i>Utricularia australis</i>	matK	524	374942	4,76	17840
MDV01	Host - <i>Emys orbicularis</i>	COI	712	282083	0,74	2085
MDV05	Host - <i>Emys orbicularis</i>	COI	366	351453	3,35	11777
MDV23	Host - <i>Emys orbicularis</i>	COI	709	253462	1,37	3468
MDV35	Host - <i>Emys orbicularis</i>	COI	365	677104	1,68	11369
MDV01	<i>Bufo bufo</i>	COI	217	564166	1,07	6009
MDV01	<i>Endochironomus tendens</i>	COI	706	564166	0,01	65
MDV01	<i>Phaenopsectra punctipes</i>	COI	233	564166	0,01	44
MDV01	<i>Phragmites australis</i>	trnL - trnF	1041	564166	0,45	2566
MDV01	<i>Phragmites australis</i>	rbcL	751	564166	0,99	5587
MDV01	<i>Streblotrichum convolutum</i>	rbcL	650	564166	2,46	13866
MDV01	<i>Limnephilus flavigornis</i>	COI	709	564166	0,06	335

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MDV02	<i>Alnus glutinosa</i>	rbcL	421	636104	4,11	26174
MDV02	<i>Alnus glutinosa</i>	trnL - trnF	1055	636104	0,06	367
MDV02	<i>Betula pubescens</i>	matK	834	636104	0,23	1492
MDV02	<i>Populus alba</i>	rbcL	584	636104	6,45	41018
MDV02	<i>Populus nigra</i>	rbcL	281	636104	7,45	47395
MDV02	<i>Juncus effusus</i>	28S	364	636104	2,15	13703
MDV02	<i>Juncus effusus</i>	28S	272	636104	3,28	20855
MDV02	<i>Phragmites australis</i>	trnL - trnF	1041	636104	4,18	26606
MDV02	<i>Phragmites australis</i>	matK	889	636104	1,14	7258
MDV02	<i>Phragmites australis</i>	rbcL	751	636104	10,25	65208
MDV03	<i>Alnus alnobetula</i>	18S	548	705948	7,96	56197
MDV03	<i>Ilyocoris cimicoides</i>	COI	366	705948	0,09	635
MDV03	<i>Asellus aquaticus</i>	COI	365	705948	0,35	2470
MDV03	<i>Populus alba</i>	rbcL	307	705948	0,38	2655
MDV03	<i>Brachytron pratense</i>	COI	385	705948	3,56	25151
MDV03	<i>Pyrrhosoma nymphula</i>	COI	706	705948	0,01	102
MDV03	<i>Carex sp.</i>	28S	285	705948	6,63	46833
MDV03	<i>Carex sp.</i>	rbcL	318	705948	5,20	36707
MDV03	<i>Juncus effusus</i>	28S	312	705948	7,38	52113
MDV03	<i>Phragmites australis</i>	trnL - trnF	257	705948	0,00	10
MDV03	<i>Phragmites australis</i>	rbcL	426	705948	6,92	48830
MDV03	<i>Phragmites australis</i>	matK	891	705948	0,48	3356
MDV03	<i>Castor fiber</i>	COI	259	705948	0,01	105
MDV03	<i>Limnephilus flavicornis</i>	COI	709	705948	1,23	8675
MDV04	<i>Potamogeton perfoliatus</i>	trnL - trnF	1056	717992	0,03	196
MDV04	<i>Bufo bufo</i>	COI	328	717992	0,15	1081
MDV04	<i>Alnus alnobetula</i>	28S	472	717992	1,97	14166
MDV04	<i>Betula pubescens</i>	matK	547	717992	0,01	67
MDV04	<i>Betula pubescens</i>	trnL - trnF	889	717992	0,10	701
MDV04	<i>Quercus sp.</i>	trnL - trnF	922	717992	0,05	371
MDV04	<i>Quercus sp.</i>	rbcL	627	717992	3,56	25575
MDV04	<i>Quercus sp.</i>	matK	889	717992	0,15	1056
MDV04	<i>Ilyocoris cimicoides</i>	COI	727	717992	5,41	38826
MDV04	<i>Salix sp.</i>	28S	492	717992	4,04	29017
MDV04	<i>Salix sp.</i>	matK	594	717992	8,42	60458
MDV04	<i>Phragmites australis</i>	matK	922	717992	0,13	927
MDV04	<i>Phragmites australis</i>	rbcL	738	717992	6,89	49443
MDV04	<i>Phragmites australis</i>	trnL - trnF	1040	717992	1,51	10859
MDV04	<i>Limnephilus flavicornis</i>	COI	707	717992	0,03	180
MDV05	<i>Caenis horaria</i>	COI	233	702906	0,00	33
MDV05	<i>Betula pubescens</i>	rbcL	587	702906	2,01	14150

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MDV05	<i>Ilyocoris cimicoides</i>	COI	365	702906	0,01	50
MDV05	<i>Anax imperator</i>	COI	365	702906	0,01	45
MDV05	<i>Phragmites australis</i>	matK	891	702906	0,71	4978
MDV05	<i>Phragmites australis</i>	rbcL	751	702906	6,02	42323
MDV05	<i>Phragmites australis</i>	trnL - trnF	1379	702906	1,60	11220
MDV05	<i>Limnephilus flavicornis</i>	COI	709	702906	0,02	115
MDV06	<i>Alnus alnobetula</i>	28S	548	750912	9,19	68979
MDV06	<i>Betula pubescens</i>	trnL - trnF	901	750912	0,04	335
MDV06	<i>Ilyocoris cimicoides</i>	COI	366	750912	0,01	77
MDV06	<i>Carex sp.</i>	rbcL	426	750912	2,58	19342
MDV06	<i>Phragmites australis</i>	rbcL	580	750912	6,39	47949
MDV06	<i>Phragmites australis</i>	matK	898	750912	0,62	4619
MDV06	<i>Phragmites australis</i>	trnL - trnF	1041	750912	0,71	5318
MDV06	<i>Limnephilus vittatus</i>	COI	316	750912	0,04	295
MDV07	<i>Alnus subcordata</i>	28S	176	765398	2,35	17990
MDV07	<i>Hypnum cupressiforme</i>	rbcL	599	765398	0,62	4712
MDV07	<i>Populus alba</i>	matK	370	765398	7,47	57165
MDV07	<i>Populus nigra</i>	rbcL	455	765398	0,00	30
MDV07	<i>Carex sp.</i>	28S	541	765398	5,41	41402
MDV07	<i>Phragmites australis</i>	rbcL	774	765398	12,29	94065
MDV07	<i>Phragmites australis</i>	trnL - trnF	1041	765398	0,23	1750
MDV08	<i>Gammarus fossarum</i>	COI	365	745676	0,03	191
MDV08	<i>Chironomus pallidivittatus</i>	COI	423	745676	0,01	45
MDV08	<i>Endochironomus tendens</i>	COI	706	745676	0,02	133
MDV08	<i>Alnus alnobetula</i>	28S	696	745676	7,15	53330
MDV08	<i>Asellus aquaticus</i>	COI	387	745676	0,00	18
MDV08	<i>Carex sp.</i>	trnL - trnF	451	745676	0,00	32
MDV08	<i>Carex sp.</i>	rbcL	398	745676	1,19	8902
MDV08	<i>Phragmites australis</i>	matK	890	745676	0,31	2307
MDV08	<i>Phragmites australis</i>	rbcL	591	745676	5,34	39839
MDV08	<i>Phragmites australis</i>	trnL - trnF	1040	745676	1,25	9357
MDV08	<i>Barbula unguiculata</i>	rbcL	579	745676	1,05	7795
MDV08	<i>Limnephilus flavicornis</i>	COI	706	745676	0,60	4486
MDV09	<i>Bufo bufo</i>	COI	207	708078	0,00	10
MDV09	<i>Alnus alnobetula</i>	28S	579	708078	3,21	22749
MDV09	<i>Quercus sp.</i>	matK	266	708078	0,00	16
MDV09	<i>Quercus sp.</i>	trnL - trnF	915	708078	0,05	360
MDV09	<i>Populus alba</i>	rbcL	284	708078	0,60	4225
MDV09	<i>Carex sp.</i>	rbcL	543	708078	1,31	9261
MDV09	<i>Phragmites australis</i>	rbcL	548	708078	6,08	43052
MDV09	<i>Phragmites australis</i>	matK	891	708078	0,55	3917

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MDV09	<i>Pleurochaete squarrosa</i>	trnL - trnF	504	708078	0,54	3790
MDV09	<i>Pottiopsis caespitosa</i>	rbcL	620	708078	3,00	21229
MDV10	<i>Cloeon dipterum</i>	COI	706	752412	0,03	254
MDV10	<i>Alnus glutinosa</i>	rbcL	265	752412	8,70	65449
MDV10	<i>Betula pubescens</i>	matK	889	752412	0,27	2039
MDV10	<i>Radix auricularia</i>	COI	263	752412	0,01	53
MDV10	<i>Pheosia tremula</i>	COI	212	752412	0,00	22
MDV10	<i>Salix sp.</i>	rbcL	282	752412	4,74	35654
MDV11	<i>Equisetum hyemale</i>	trnL - trnF	275	791092	0,01	45
MDV11	<i>Alnus glutinosa</i>	rbcL	416	791092	10,16	80345
MDV11	<i>Alnus glutinosa</i>	trnL - trnF	961	791092	1,37	10825
MDV11	<i>Ilyocoris cimicoides</i>	COI	709	791092	0,16	1227
MDV11	<i>Salix sp.</i>	trnL - trnF	232	791092	0,00	18
MDV11	<i>Salix sp.</i>	rbcL	338	791092	8,20	64850
MDV11	<i>Calopteryx virgo</i>	COI	712	791092	0,01	52
MDV11	<i>Coenagrion pulchellum</i>	COI	710	791092	0,77	6096
MDV11	<i>Carex sp.</i>	28S	591	791092	0,38	3033
MDV11	<i>Phragmites australis</i>	matK	888	791092	0,03	209
MDV11	<i>Phragmites australis</i>	rbcL	743	791092	6,40	50639
MDV11	<i>Phragmites australis</i>	trnL - trnF	1041	791092	0,02	143
MDV11	<i>Tortella tortuosa</i>	rbcL	502	791092	0,75	5944
MDV11	<i>Pleurochaete squarrosa</i>	trnL - trnF	635	791092	0,02	132
MDV11	<i>Limnephilus flavicornis</i>	COI	712	791092	10,21	80764
MDV12	<i>Cyprinus carpio</i>	COI	300	653550	7,95	51952
MDV12	<i>Alnus glutinosa</i>	rbcL	421	653550	1,25	8139
MDV12	<i>Betula pubescens</i>	trnL - trnF	497	653550	0,03	168
MDV12	<i>Notonecta glauca</i>	COI	266	653550	0,00	18
MDV12	<i>Bithynia tentaculata</i>	COI	364	653550	0,00	26
MDV12	<i>Carex sp.</i>	trnL - trnF	269	653550	0,00	23
MDV12	<i>Carex sp.</i>	rbcL	676	653550	2,76	18010
MDV12	<i>Phragmites australis</i>	trnL - trnF	1050	653550	0,66	4292
MDV12	<i>Phragmites australis</i>	matK	890	653550	0,53	3451
MDV12	<i>Phragmites australis</i>	rbcL	790	653550	8,40	54887
MDV12	<i>Pleurochaete squarrosa</i>	trnL - trnF	502	653550	0,09	600
MDV12	<i>Tortella tortuosa</i>	trnL - trnF	669	653550	2,07	13560
MDV12	<i>Limnephilus flavicornis</i>	COI	436	653550	0,18	1174
MDV14	<i>Cricotopus bicinctus</i>	COI	229	673182	0,01	45
MDV14	<i>Quercus sp.</i>	28S	543	673182	15,10	101677
MDV14	<i>Quercus sp.</i>	trnL - trnF	376	673182	0,01	70
MDV14	<i>Sympetrum striolatum</i>	COI	364	673182	0,01	72
MDV14	<i>Carex sp.</i>	28S	282	673182	5,50	37017

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MDV14	<i>Carex sp.</i>	rbcL	655	673182	14,79	99531
MDV14	<i>Cladium mariscus</i>	trnL - trnF	870	673182	0,03	207
MDV14	<i>Juncus effusus</i>	28S	31	673182	3,02	20340
MDV14	<i>Phragmites australis</i>	trnL - trnF	975	673182	0,23	1522
MDV14	<i>Phragmites australis</i>	rbcL	426	673182	14,79	99534
MDV15	<i>Alnus alnobetula</i>	28S	474	758966	3,76	28512
MDV15	<i>Quercus sp.</i>	rbcL	601	758966	4,54	34472
MDV15	<i>Quercus sp.</i>	trnL - trnF	336	758966	0,00	36
MDV15	<i>Phragmites australis</i>	rbcL	818	758966	7,89	59882
MDV15	<i>Phragmites australis</i>	trnL - trnF	1041	758966	0,19	1466
MDV15	<i>Limnephilus flavicornis</i>	COI	271	758966	0,00	34
MDV16	<i>Bufo bufo</i>	COI	708	775452	0,02	144
MDV16	<i>Salix sp.</i>	rbcL	347	775452	6,09	47254
MDV16	<i>Phragmites australis</i>	trnL - trnF	1041	775452	0,06	443
MDV16	<i>Phragmites australis</i>	rbcL	785	775452	5,27	40833
MDV16	<i>Limnephilus flavicornis</i>	COI	709	775452	0,03	264
MDV17	<i>Cricotopus triannulatus</i>	COI	709	821384	0,04	289
MDV17	<i>Equisetum ramosissimum</i>	rbcL	234	821384	0,00	17
MDV17	<i>Betula pubescens</i>	trnL - trnF	823	821384	0,19	1520
MDV17	<i>Quercus sp.</i>	rbcL	922	821384	0,03	264
MDV17	<i>Asellus aquaticus</i>	COI	365	821384	0,01	109
MDV17	<i>Salix sp.</i>	rbcL	306	821384	1,00	8210
MDV17	<i>Carex sp.</i>	rbcL	232	821384	0,24	1954
MDV17	<i>Phragmites australis</i>	trnL - trnF	1040	821384	3,63	29848
MDV17	<i>Limnephilus flavicornis</i>	COI	709	821384	0,27	2195
MDV18	<i>Potamogeton perfoliatus</i>	trnL - trnF	984	730360	0,02	145
MDV18	<i>Agrilus angustulus</i>	COI	179	730360	9,99	72990
MDV18	<i>Alnus incana</i>	28S	493	730360	10,81	78926
MDV18	<i>Ilyocoris cimicoides</i>	COI	365	730360	9,28	67759
MDV18	<i>Bithynia tentaculata</i>	COI	281	730360	0,01	46
MDV18	<i>Salix sp.</i>	28S	241	730360	0,00	20
MDV18	<i>Carex sp.</i>	rbcL	255	730360	7,14	52140
MDV18	<i>Cladium sp.</i>	rbcL	868	730360	0,69	5011
MDV18	<i>Phragmites australis</i>	rbcL	429	730360	8,49	62036
MDV18	<i>Phragmites australis</i>	matK	890	730360	0,09	662
MDV18	<i>Phragmites australis</i>	trnL - trnF	975	730360	1,21	8822
MDV18	<i>Tortella tortuosa</i>	28S	560	730360	1,75	12772
MDV19	<i>Phragmites australis</i>	trnL - trnF	1042	677986	2,06	13937
MDV19	<i>Phragmites australis</i>	matK	889	677986	0,28	1910
MDV19	<i>Phragmites australis</i>	rbcL	784	677986	10,73	72761
MDV19	<i>Limnephilus flavicornis</i>	COI	709	677986	0,06	416

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MDV21	<i>Alnus incana</i>	28S	579	766000	6,67	51104
MDV21	<i>Armadillidium nasatum</i>	COI	224	766000	0,80	6145
MDV21	<i>Populus alba</i>	rbcL	262	766000	3,02	23158
MDV21	<i>Phragmites australis</i>	rbcL	385	766000	21,98	168331
MDV21	<i>Phragmites australis</i>	trnL - trnF	252	766000	0,71	5427
MDV22	<i>Gammarus fossarum</i>	COI	263	539366	0,01	29
MDV22	<i>Bufo bufo</i>	COI	208	539366	0,01	28
MDV22	<i>Donacia clavipes</i>	COI	213	539366	0,00	18
MDV22	<i>Betula sp.</i>	trnL - trnF	634	539366	1,43	7726
MDV22	<i>Hypnum cupressiforme</i>	trnL - trnF	249	539366	0,03	187
MDV22	<i>Populus alba</i>	rbcL	744	539366	1,07	5752
MDV22	<i>Phragmites australis</i>	trnL - trnF	1041	539366	1,66	8939
MDV22	<i>Phragmites australis</i>	matK	889	539366	0,08	414
MDV22	<i>Phragmites australis</i>	rbcL	745	539366	12,33	66501
MDV23	<i>Potamogeton perfoliatus</i>	matK	793	506924	0,10	526
MDV23	<i>Caenis horaria</i>	COI	288	506924	0,00	19
MDV23	<i>Alnus alnobetula</i>	28S	579	506924	9,54	48346
MDV23	<i>Betula pubescens</i>	rbcL	308	506924	3,43	17380
MDV23	<i>Bithynia tentaculata</i>	COI	709	506924	0,09	435
MDV23	<i>Coenagrion pulchellum</i>	COI	715	506924	0,48	2433
MDV23	<i>Carex sp.</i>	rbcL	398	506924	6,43	32591
MDV23	<i>Phragmites australis</i>	trnL - trnF	547	506924	6,49	32891
MDV23	<i>Phragmites australis</i>	rbcL	271	506924	0,00	10
MDV23	<i>Pleurochaete squarrosa</i>	trnL - trnF	274	506924	0,00	15
MDV23	<i>Limnephilus flavidus</i>	COI	365	506924	0,07	376
MDV24	<i>Alnus alnobetula</i>	28S	729	667950	11,09	74053
MDV24	<i>Quercus sp.</i>	rbcL	344	667950	1,76	11750
MDV24	<i>Bithynia tentaculata</i>	COI	365	667950	0,01	40
MDV24	<i>Populus alba</i>	rbcL	637	667950	7,74	51722
MDV24	<i>Carex sp.</i>	rbcL	398	667950	10,93	72983
MDV24	<i>Phragmites australis</i>	rbcL	639	667950	10,45	69825
MDV25	<i>Phragmites australis</i>	rbcL	1040	492756	0,29	1445
MDV25	<i>Limnephilus flavidus</i>	COI	622	492756	0,00	10
MDV26	<i>Populus alba</i>	rbcL	629	758900	11,24	85303
MDV26	<i>Carex sp.</i>	rbcL	398	758900	8,55	64906
MDV26	<i>Carex sp.</i>	trnL - trnF	241	758900	0,00	18
MDV26	<i>Phragmites australis</i>	trnL - trnF	1041	758900	1,00	7581
MDV26	<i>Phragmites australis</i>	rbcL	399	758900	11,62	88180
MDV26	<i>Pleurochaete squarrosa</i>	trnL - trnF	503	758900	1,61	12189
MDV26	<i>Tortella tortuosa</i>	trnL - trnF	601	758900	3,95	29988
MDV27	<i>Dendroctonus ponderosae</i>	COI	220	723572	0,00	13

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MDV27	<i>Salix sp.</i>	28S	503	723572	8,42	60924
MDV27	<i>Carex sp.</i>	rbcL	318	723572	0,94	6772
MDV27	<i>Phragmites australis</i>	trnL - trnF	1041	723572	1,84	13314
MDV27	<i>Phragmites australis</i>	matK	890	723572	0,38	2748
MDV27	<i>Phragmites australis</i>	rbcL	630	723572	7,89	57115
MDV27	<i>Deroferas laeve</i>	COI	211	723572	0,00	14
MDV27	<i>Limnephilus flavicornis</i>	COI	414	723572	0,01	38
MDV29	<i>Baetis rhodani</i>	COI	706	764060	0,04	319
MDV29	<i>Betula pubescens</i>	matK	889	764060	0,11	845
MDV29	<i>Asellus aquaticus</i>	COI	365	764060	0,01	56
MDV29	<i>Populus nigra</i>	matK	957	764060	0,01	82
MDV29	<i>Brachytron pratense</i>	COI	709	764060	8,62	65895
MDV29	<i>Carex sp.</i>	trnL - trnF	392	764060	0,00	33
MDV29	<i>Phragmites australis</i>	trnL - trnF	1062	764060	2,58	19678
MDV29	<i>Phragmites australis</i>	matK	892	764060	0,24	1817
MDV29	<i>Pleurochaete squarrosa</i>	trnL - trnF	502	764060	2,49	19049
MDV30	<i>Pericoma blandula</i>	COI	713	827212	4,89	40467
MDV30	<i>Alnus glutinosa</i>	trnL - trnF	1055	827212	4,38	36269
MDV30	<i>Ilyocoris cimicoides</i>	COI	352	827212	0,04	332
MDV30	<i>Salix sp.</i>	28S	241	827212	0,00	9
MDV30	<i>Juncus effusus</i>	28S	250	827212	1,72	14205
MDV30	<i>Phragmites australis</i>	matK	889	827212	0,25	2035
MDV30	<i>Phragmites australis</i>	trnL - trnF	520	827212	0,39	3240
MDV30	<i>Chionoloma tenuirostre</i>	COI	624	827212	1,66	13692
MDV30	<i>Pleurochaete squarrosa</i>	trnL - trnF	521	827212	4,37	36173
MDV32	<i>Gammarus pulex</i>	COI	709	722194	0,05	372
MDV32	<i>Cecidomyiidae sp.</i>	COI	365	722194	0,01	39
MDV32	<i>Alnus glutinosa</i>	matK	455	722194	0,01	50
MDV32	<i>Alnus glutinosa</i>	trnL - trnF	343	722194	0,01	66
MDV32	<i>Quercus sp.</i>	rbcL	343	722194	1,75	12638
MDV32	<i>Hemiptera sp.</i>	COI	233	722194	0,02	133
MDV32	<i>Populus nigra</i>	trnL - trnF	1042	722194	0,28	2003
MDV32	<i>Populus nigra</i>	matK	987	722194	0,46	3356
MDV32	<i>Phragmites australis</i>	trnL - trnF	991	722194	4,690746	33876
MDV32	<i>Phragmites australis</i>	matK	973	722194	0,65	4708
MDV32	<i>Phragmites australis</i>	rbcL	582	722194	7,98	57654
MDV32	<i>Pleurochaete squarrosa</i>	trnL - trnF	312	722194	0,24	1721
MDV33	<i>Nymphaea alba</i>	rbcL	676	662592	96,64	640352
MDV34	<i>Ilyocoris cimicoides</i>	COI	364	752330	1,54	11554
MDV34	<i>Notonecta glauca</i>	COI	214	752330	0,00	16
MDV34	<i>Carex sp.</i>	rbcL	425	752330	5,95	44741

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MDV34	<i>Juncus effusus</i>	28S	345	752330	7,34	55233
MDV34	<i>Juncus effusus</i>	28S	272	752330	6,52	49054
MDV34	<i>Phragmites australis</i>	trnL - trnF	1049	752330	8,44	63533
MDV34	<i>Phragmites australis</i>	rbcL	542	752330	9,92	74616
MDV35	<i>Alnus alnobetula</i>	28S	523	677104	12,07	81749
MDV35	<i>Betula pubescens</i>	rbcL	971	677104	11,69	79132
MDV35	<i>Betula pubescens</i>	rbcL	683	677104	10,21	69105
MDV35	<i>Parapoynx stratiotata</i>	COI	269	677104	0,00	21
MDV35	<i>Phragmites australis</i>	trnL - trnF	1026	677104	0,07	468
MDV35	<i>Phragmites australis</i>	matK	890	677104	0,15	1006
MDV35	<i>Phragmites australis</i>	rbcL	640	677104	9,96	67412
MDV35	<i>Pleurochaete squarrosa</i>	trnL - trnF	318	677104	4,62	31300
MDV35	<i>Athripsodes aterrimus</i>	COI	216	677104	0,00	17
MDV36	<i>Salix sp.</i>	rbcL	276	625446	0,00	13
MDV36	<i>Nymphaea alba</i>	rbcL	598	625446	9,08	56813
MDV36	<i>Phragmites australis</i>	matK	213	625446	0,00	13
MDV36	<i>Phragmites australis</i>	rbcL	637	625446	8,66	54152
MDV36	<i>Phragmites australis</i>	trnL - trnF	1040	625446	0,05	338

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1 **Figure S6.** Faecal samples ($N = 32$) from European pond turtle (*Emys orbicularis*) analyzed using our long metabarcoding approach and
 2 *de novo* assembly. Sample and turtle identities, along with sex (M = Male, F = female and U = Undetermined), maturity (A = Adult, J =
 3 Juvenile) of each individual as well as genus/species of each prey DNA fragment sequenced and identified have been included. The
 4 reference alignment length, corresponding to the number of bp of each contig (amplicon assembly), and respective identity matches have
 5 been given using the NCBI nucleotide database BLAST search.

Turtle_ID	Sex	Maturity	Sample_ID	Class	Order	Species	% identity match	Reference alignment length (in bp)
141	M	A	MDV01	Amphibia	Anura	<i>Bufo bufo</i>	99.4	217
141	M	A	MDV01	Insecta	Diptera	<i>Endochironomus tendens</i>	98.7	706
141	M	A	MDV01	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	100.0	709
141	M	A	MDV01	Insecta	Hemiptera	<i>Ovatus crataegarius</i>	100.0	239
141	M	A	MDV01	Insecta	Lepidoptera	<i>Phaenopsectra punctipes</i>	97.0	233
141	M	A	MDV01	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
141	M	A	MDV01	Bryopsida	Pottiales	<i>Streblotrichum convolutum</i>	99.2	650
437	U	J	MDV02	Magnoliopsida	Fagales	<i>Alnus glutinosa</i>	99.7	1055
437	U	J	MDV02	Magnoliopsida	Fagales	<i>Betula pubescens</i>	98.3	834
437	U	J	MDV02	Liliopsida	Poales	<i>Carex sp.</i>	99.8	438
437	U	J	MDV02	Liliopsida	Poales	<i>Juncus effusus</i>	99.3	272
437	U	J	MDV02	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
437	U	J	MDV02	Magnoliopsida	Malpighiales	<i>Populus alba</i>	100.0	584
437	U	J	MDV02	Magnoliopsida	Malpighiales	<i>Populus nigra</i>	98.6	278
437	F	A	MDV03	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	98.9	548
437	F	A	MDV03	Malacostraca	Isopoda	<i>Asellus aquaticus</i>	98.2	365
437	F	A	MDV03	Insecta	Odonata	<i>Brachytron pratense</i>	98.3	385
437	F	A	MDV03	Liliopsida	Poales	<i>Carex sp.</i>	100.0	318
437	F	A	MDV03	Mammalia	Rodentia	<i>Castor fiber</i>	98.1	259

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437	F	A	MDV03	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	98.8	366
437	F	A	MDV03	Liliopsida	Poales	<i>Juncus effusus</i>	99.3	285
437	F	A	MDV03	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	100.0	709
437	F	A	MDV03	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	257
437	F	A	MDV03	Magnoliopsida	Malpighiales	<i>Populus alba</i>	99.7	307
437	F	A	MDV03	Insecta	Odonata	<i>Pyrrhosoma nymphula</i>	99.8	706
212	M	A	MDV04	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	98.9	472
212	M	A	MDV04	Magnoliopsida	Fagales	<i>Betula pubescens</i>	99.9	889
212	M	A	MDV04	Amphibia	Anura	<i>Bufo bufo</i>	98.8	328
212	M	A	MDV04	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	99.8	727
212	M	A	MDV04	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	99.8	707
212	M	A	MDV04	Liliopsida	Poales	<i>Phragmites australis</i>	99.8	1040
212	M	A	MDV04	Liliopsida	Alismatales	<i>Potamogeton perfoliatus</i>	99.7	1056
212	M	A	MDV04	Magnoliopsida	Fagales	<i>Quercus sp.</i>	99.3	889
212	M	A	MDV04	Magnoliopsida	Malpighiales	<i>Salix sp.</i>	99.7	594
431	M	A	MDV05	Insecta	Odonata	<i>Anax imperator</i>	99.4	365
431	M	A	MDV05	Magnoliopsida	Fagales	<i>Betula pubescens</i>	99.7	587
431	M	A	MDV05	Insecta	Ephemeroptera	<i>Caenis horaria</i>	98.0	233
431	M	A	MDV05	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	99.1	365
431	M	A	MDV05	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	100.0	709
431	M	A	MDV05	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1379
431	M	A	MDV05	Magnoliopsida	Malpighiales	<i>Populus alba</i>	99.5	987
95	M	A	MDV06	Equisetopsida	Fagales	<i>Alnus alnobetula</i>	98.9	548
95	M	A	MDV06	Magnoliopsida	Fagales	<i>Betula pubescens</i>	99.8	901
95	M	A	MDV06	Liliopsida	Poales	<i>Carex sp.</i>	100.0	426
95	M	A	MDV06	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	100.0	366

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95	M	A	MDV06	Insecta	Trichoptera	<i>Limnephilus vittatus</i>	99.2	316
95	M	A	MDV06	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
95	M	A	MDV06	Magnoliopsida	Malpighiales	<i>Populus nigra</i>	100.0	256
198	F	A	MDV07	Magnoliopsida	Fagales	<i>Alnus subcordata</i>	98.3	176
198	F	A	MDV07	Liliopsida	Poales	<i>Carex sp.</i>	96.5	541
198	F	A	MDV07	Bryopsida	Hypnales	<i>Hypnum cupressiforme</i>	99.7	599
198	F	A	MDV07	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
198	F	A	MDV07	Magnoliopsida	Malpighiales	<i>Populus alba</i>	98.9	370
198	F	A	MDV07	Magnoliopsida	Malpighiales	<i>Populus nigra</i>	99.8	455
297	M	A	MDV08	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	98.3	696
297	M	A	MDV08	Malacostrcra	Isopoda	<i>Asellus aquaticus</i>	98.4	387
297	M	A	MDV08	Bryopsida	Pottiales	<i>Barbula unguiculata</i>	98.1	579
297	M	A	MDV08	Liliopsida	Poales	<i>Carex sp.</i>	100.0	398
297	M	A	MDV08	Insecta	Diptera	<i>Chironomus pallidivittatus</i>	99.0	423
297	M	A	MDV08	Insecta	Diptera	<i>Endochironomus tendens</i>	98.1	706
297	M	A	MDV08	Malacostrcra	Amphipoda	<i>Gammarus fossarum</i>	97.2	365
297	M	A	MDV08	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	100.0	706
297	M	A	MDV08	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1040
297	M	A	MDV08	Malacostrcra	Isopoda	<i>Proasellus coxalis</i>	99.2	387
75	M	A	MDV09	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	99.1	579
75	M	A	MDV09	Amphibia	Anura	<i>Bufo bufo</i>	98.0	207
75	M	A	MDV09	Liliopsida	Poales	<i>Carex sp.</i>	100.0	379
75	M	A	MDV09	Liliopsida	Poales	<i>Phragmites australis</i>	99.7	891
75	M	A	MDV09	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	99.6	504
75	M	A	MDV09	Magnoliopsida	Malpighiales	<i>Populus alba</i>	99.3	284
75	M	A	MDV09	Bryopsida	Pottiales	<i>Pottiopsis caespitosa</i>	98.7	620

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75	M	A	MDV09	Magnoliopsida	Fagales	<i>Quercus</i> sp.	99.1	915
75	M	A	MDV09	Bryopsida	Pottiales	<i>Tortella tortuosa</i>	99.6	504
123	F	A	MDV10	Magnoliopsida	Fagales	<i>Alnus glutinosa</i>	100.0	265
123	F	A	MDV10	Magnoliopsida	Fagales	<i>Betula pubescens</i>	99.4	889
123	F	A	MDV10	Insecta	Ephemeroptera	<i>Cloeon dipterum</i>	99.8	706
123	F	A	MDV10	Insecta	Lepidoptera	<i>Pheosia tremula</i>	98.9	212
123	F	A	MDV10	Gastropoda	Hygrophila	<i>Radix auricularia</i>	99.2	263
123	F	A	MDV10	Magnoliopsida	Malpighiales	<i>Salix</i> sp.	100.0	282
438	U	J	MDV11	Magnoliopsida	Fagales	<i>Alnus glutinosa</i>	99.8	961
438	U	J	MDV11	Insecta	Odonata	<i>Calopteryx virgo</i>	98.9	712
438	U	J	MDV11	Liliopsida	Poales	<i>Carex</i> sp.	95.9	591
438	U	J	MDV11	Insecta	Odonata	<i>Coenagrion pulchellum</i>	99.8	710
438	U	J	MDV11	Polypodiopsida	Equisetales	<i>Equisetum hyemale</i>	100.0	275
438	U	J	MDV11	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	99.8	709
438	U	J	MDV11	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	100.0	712
438	U	J	MDV11	Liliopsida	Poales	<i>Phragmites australis</i>	99.9	1041
438	U	J	MDV11	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	99.8	502
438	U	J	MDV11	Magnoliopsida	Malpighiales	<i>Salix</i> sp.	99.1	338
438	U	J	MDV11	Bryopsida	Pottiales	<i>Tortella tortuosa</i>	98.1	635
131	M	A	MDV12	Magnoliopsida	Fagales	<i>Alnus glutinosa</i>	98.1	421
131	M	A	MDV12	Magnoliopsida	Fagales	<i>Betula pubescens</i>	100.0	497
131	M	A	MDV12	Gastropoda	Littorinimorpha	<i>Bithynia tentaculata</i>	98.5	364
131	M	A	MDV12	Liliopsida	Poales	<i>Carex</i> sp.	99.6	269
131	M	A	MDV12	Actinoptergii	Cypriniformes	<i>Cyprinus carpio</i>	99.7	300
131	M	A	MDV12	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	99.7	436
131	M	A	MDV12	Insecta	Hemiptera	<i>Notonecta glauca</i>	98.1	266

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131	M	A	MDV12	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1050
131	M	A	MDV12	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	99.6	502
131	M	A	MDV12	Bryopsida	Pottiales	<i>Tortella tortuosa</i>	98.0	669
400	M	A	MDV14	Liliopsida	Poales	<i>Carex sp.</i>	99.3	282
400	M	A	MDV14	Liliopsida	Poales	<i>Cladium mariscus</i>	100.0	870
400	M	A	MDV14	Insecta	Diptera	<i>Cricotopus bicinctus</i>	98.3	229
400	M	A	MDV14	Liliopsida	Poales	<i>Juncus effusus</i>	99.3	282
400	M	A	MDV14	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	975
400	M	A	MDV14	Magnoliopsida	Fagales	<i>Quercus sp.</i>	99.8	543
400	M	A	MDV14	Insecta	Odonata	<i>Sympetrum striolatum</i>	98.8	364
U	M	A	MDV15	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	98.9	474
U	M	A	MDV15	Amphibia	Anura	<i>Bufo bufo</i>	99.1	708
U	M	A	MDV15	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	99.6	271
U	M	A	MDV15	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
U	M	A	MDV15	Magnoliopsida	Fagales	<i>Quercus sp.</i>	99.7	601
322	F	A	MDV16	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	98.8	709
322	F	A	MDV16	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
322	F	A	MDV16	Magnoliopsida	Malpighiales	<i>Populus alba</i>	100.0	168
322	F	A	MDV16	Magnoliopsida	Malpighiales	<i>Salix sp.</i>	99.4	347
441	U	J	MDV17	Malacostrca	Isopoda	<i>Asellus aquaticus</i>	98.5	365
441	U	J	MDV17	Magnoliopsida	Fagales	<i>Betula pubescens</i>	99.9	823
441	U	J	MDV17	Liliopsida	Poales	<i>Carex sp.</i>	100.0	232
441	U	J	MDV17	Polypodiopsida	Equisetales	<i>Equisetum ramosissimum</i>	99.1	234
441	U	J	MDV17	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	100.0	709
441	U	J	MDV17	Insecta	Diptera	<i>Cricotopus triannulatus</i>	100.0	709
441	U	J	MDV17	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1040

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441	U	J	MDV17	Magnoliopsida	Fagales	<i>Quercus sp.</i>	99.7	922
441	U	J	MDV17	Magnoliopsida	Malpighiales	<i>Salix sp.</i>	98.4	306
170	F	A	MDV18	Insecta	Coleoptera	<i>Agrilus angustulus</i>	100.0	179
170	F	A	MDV18	Magnoliopsida	Fagales	<i>Alnus incana</i>	99.8	493
170	F	A	MDV18	Gastropoda	Littorinimorpha	<i>Bithynia tentaculata</i>	97.5	281
170	F	A	MDV18	Liliopsida	Poales	<i>Carex sp.</i>	99.2	255
170	F	A	MDV18	Liliopsida	Poales	<i>Cladium sp.</i>	100.0	868
170	F	A	MDV18	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	99.1	365
170	F	A	MDV18	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	975
170	F	A	MDV18	Liliopsida	Alismatales	<i>Potamogeton perfoliatus</i>	99.7	984
170	F	A	MDV18	Magnoliopsida	Malpighiales	<i>Salix sp.</i>	100.0	241
170	F	A	MDV18	Bryopsida	Pottiales	<i>Tortella tortuosa</i>	98.9	560
309	F	A	MDV19	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	99.8	709
309	F	A	MDV19	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1042
1	F	A	MDV21	Magnoliopsida	Fagales	<i>Alnus incana</i>	99.3	579
1	F	A	MDV21	Malacostrca	Isopoda	<i>Armadillidium nasatum</i>	99.1	224
1	F	A	MDV21	Liliopsida	Poales	<i>Phragmites australis</i>	99.7	385
1	F	A	MDV21	Magnoliopsida	Malpighiales	<i>Populus alba</i>	100.0	262
105	M	A	MDV22	Magnoliopsida	Fagales	<i>Betula sp.</i>	99.5	634
105	M	A	MDV22	Amphibia	Anura	<i>Bufo bufo</i>	98.0	208
105	M	A	MDV22	Insecta	Coleoptera	<i>Donacia clavipes</i>	97.3	213
105	M	A	MDV22	Malacostrca	Amphipoda	<i>Gammarus fossarum</i>	98.5	263
105	M	A	MDV22	Bryopsida	Hypnales	<i>Hypnum cupressiforme</i>	100.0	249
105	M	A	MDV22	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
105	M	A	MDV22	Magnoliopsida	Malpighiales	<i>Populus alba</i>	99.9	744
69	F	A	MDV23	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	99.1	579

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69	F	A	MDV23	Magnoliopsida	Fagales	<i>Betula pubescens</i>	100.0	308
69	F	A	MDV23	Gastropoda	Littorinimorpha	<i>Bithynia tentaculata</i>	99.7	709
69	F	A	MDV23	Insecta	Ephemeroptera	<i>Caenis horaria</i>	98.1	288
69	F	A	MDV23	Liliopsida	Poales	<i>Carex sp.</i>	100.0	398
69	F	A	MDV23	Insecta	Odonata	<i>Coenagrion pulchellum</i>	99.8	715
69	F	A	MDV23	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	98.5	365
69	F	A	MDV23	Liliopsida	Poales	<i>Phragmites australis</i>	99.8	547
69	F	A	MDV23	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	98.5	274
69	F	A	MDV23	Liliopsida	Alismatales	<i>Potamogeton perfoliatus</i>	99.6	528
69	F	A	MDV23	Bryopsida	Pottiales	<i>Tortella tortuosa</i>	98.5	274
442	M	A	MDV24	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	99.3	729
442	M	A	MDV24	Magnoliopsida	Fagales	<i>Betula pubescens</i>	100.0	307
442	M	A	MDV24	Gastropoda	Littorinimorpha	<i>Bithynia tentaculata</i>	97.2	365
442	M	A	MDV24	Liliopsida	Poales	<i>Carex sp.</i>	100.0	398
442	M	A	MDV24	Liliopsida	Poales	<i>Phragmites australis</i>	99.7	639
442	M	A	MDV24	Pinopsida	Pinales	<i>Picea sp.</i>	99.5	601
442	M	A	MDV24	Magnoliopsida	Malpighiales	<i>Populus alba</i>	99.6	637
442	M	A	MDV24	Magnoliopsida	Fagales	<i>Quercus sp.</i>	100.0	344
267	M	A	MDV25	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	100.0	622
267	M	A	MDV25	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1040
60	M	A	MDV26	Liliopsida	Poales	<i>Carex sp.</i>	100.0	398
60	M	A	MDV26	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
60	M	A	MDV26	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	99.6	503
60	M	A	MDV26	Magnoliopsida	Malpighiales	<i>Populus alba</i>	99.8	629
60	M	A	MDV26	Bryopsida	Pottiales	<i>Tortella tortuosa</i>	99.3	601
216	M	A	MDV27	Liliopsida	Poales	<i>Carex sp.</i>	100.0	318

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216	M	A	MDV27	Insecta	Coleoptera	<i>Dendroctonus ponderosae</i>	99.5	220
216	M	A	MDV27	Gastropoda	Stylommatophora	<i>Deroceras laeve</i>	99.1	211
216	M	A	MDV27	Insecta	Trichoptera	<i>Limnephilus flavicornis</i>	98.5	414
216	M	A	MDV27	Malacostrca	Isopoda	<i>Philoscia muscorum</i>	99.1	211
216	M	A	MDV27	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1041
216	M	A	MDV27	Pinopsida	Pinales	<i>Picea sp.</i>	100.0	370
216	M	A	MDV27	Magnoliopsida	Fagales	<i>Quercus sp.</i>	99.8	415
216	M	A	MDV27	Magnoliopsida	Malpighiales	<i>Salix sp.</i>	99.8	503
157	M	A	MDV29	Malacostrca	Isopoda	<i>Asellus aquaticus</i>	97.5	365
157	M	A	MDV29	Insecta	Ephemeroptera	<i>Baetis rhodani</i>	99.5	706
157	M	A	MDV29	Magnoliopsida	Fagales	<i>Betula pubescens</i>	99.6	889
157	M	A	MDV29	Insecta	Odonata	<i>Brachytron pratense</i>	100.0	709
157	M	A	MDV29	Liliopsida	Poales	<i>Carex sp.</i>	99.2	392
157	M	A	MDV29	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1062
157	M	A	MDV29	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	99.6	502
157	M	A	MDV29	Magnoliopsida	Malpighiales	<i>Populus nigra</i>	99.5	957
157	M	A	MDV29	Magnoliopsida	Fagales	<i>Quercus sp.</i>	97.8	503
166	M	A	MDV30	Magnoliopsida	Fagales	<i>Alnus glutinosa</i>	99.9	1055
166	M	A	MDV30	Liliopsida	Poales	<i>Carex sp.</i>	99.2	250
166	M	A	MDV30	Bryopsida	Pottiales	<i>Chionoloma tenuirostre</i>	99.2	624
166	M	A	MDV30	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	99.7	352
166	M	A	MDV30	Liliopsida	Poales	<i>Juncus effusus</i>	99.2	250
166	M	A	MDV30	Insecta	Diptera	<i>Pericoma blandula</i>	98.2	713
166	M	A	MDV30	Liliopsida	Poales	<i>Phragmites australis</i>	99.7	889
166	M	A	MDV30	Pinopsida	Pinales	<i>Picea sp.</i>	99.8	998
166	M	A	MDV30	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	100.0	521

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166	M	A	MDV30	Magnoliopsida	Malpighiales	<i>Salix sp.</i>	99.2	241
92	M	A	MDV32	Magnoliopsida	Fagales	<i>Alnus glutinosa</i>	99.3	455
92	M	A	MDV32	Insecta	Diptera	<i>Cecidomyiidae sp.</i>	99.4	365
92	M	A	MDV32	Malacostrcra	Amphipoda	<i>Gammarus pulex</i>	99.8	709
92	M	A	MDV32	Insecta	Hemiptera	<i>Hemiptera sp.</i>	100.0	233
92	M	A	MDV32	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	991
92	M	A	MDV32	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	100.0	312
92	M	A	MDV32	Magnoliopsida	Malpighiales	<i>Populus alba</i>	100.0	340
92	M	A	MDV32	Magnoliopsida	Malpighiales	<i>Populus nigra</i>	99.6	1042
92	M	A	MDV32	Magnoliopsida	Fagales	<i>Quercus sp.</i>	100.0	343
415	M	A	MDV33	Magnoliopsida	Nymphaeales	<i>Nymphaea alba</i>	99.2	676
U	U	J	MDV34	Liliopsida	Poales	<i>Carex sp.</i>	99.8	425
U	U	J	MDV34	Insecta	Hemiptera	<i>Ilyocoris cimicoides</i>	98.2	364
U	U	J	MDV34	Liliopsida	Poales	<i>Juncus effusus</i>	99.3	272
U	U	J	MDV34	Insecta	Hemiptera	<i>Notonecta glauca</i>	99.5	214
U	U	J	MDV34	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1049
306	M	A	MDV35	Magnoliopsida	Fagales	<i>Alnus alnobetula</i>	99.0	523
306	M	A	MDV35	Insecta	Trichoptera	<i>Athripsodes aterrimus</i>	97.7	216
306	M	A	MDV35	Magnoliopsida	Fagales	<i>Betula pubescens</i>	99.9	971
306	M	A	MDV35	Insecta	Lepidoptera	<i>Parapoynx stratiotata</i>	96.2	269
306	M	A	MDV35	Liliopsida	Poales	<i>Phragmites australis</i>	99.3	1026
306	M	A	MDV35	Bryopsida	Pottiales	<i>Pleurochaete squarrosa</i>	99.4	315
306	M	A	MDV35	Magnoliopsida	Fagales	<i>Quercus sp.</i>	99.4	314
306	M	A	MDV35	Bryopsida	Pottiales	<i>Tortella tortuosa</i>	100.0	243
436	U	J	MDV36	Liliopsida	Poales	<i>Phragmites australis</i>	100.0	1040
436	U	J	MDV36	Magnoliopsida	Malpighiales	<i>Salix sp.</i>	100.0	276

CHAPTER 1b

The feeding behaviour of the European pond turtle is not a threat for other endangered species

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Author contributions

C.D., S.U. and J.-F.R. designed research, C.D. performed research (sampling and laboratory work) and analyzed data (bioinformatics analyses) and statistics, J.C. set up the methodology (shearing protocol, first upstream method validation, primers selection and design, sequencing and bioinformatics analyses), J.C. and F.L. supervised laboratory work. C.D. wrote the manuscript and all other coauthors revised it.

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Original Research Article

The feeding behaviour of the European pond turtle (*Emys orbicularis*, L. 1758) is not a threat for other endangered species



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ABSTRACT

Molecular technologies, such as metabarcoding, have become powerful tools for conservation purposes. Here, we present a non-invasive study analyzing the diet of one population of European pond turtle (*Emys orbicularis*) during its whole activity period and of four other populations during the same period, based on faecal sample, and using for the first time on this species, a long metabarcoding approach. *Emys orbicularis* is an emblematic freshwater species of wetlands in Europe. In several countries, this species is endangered and, in Switzerland, *Emys orbicularis* is ranked as critically endangered on the Swiss Red List. A national conservation program was created to reintroduce this species and raised the question if this reintroduced species could be a threat for other endangered species. We developed a new method of long metabarcoding analysis, using universal PCR primers to determine prey species occurrence in the faeces. The analysis conducted on 174 faeces collected on 142 individuals revealed 1153 preys from 270 species. *Emys orbicularis* consumed plants throughout the year with a more diverse diet during the reproduction period (April–June). This study therefore not only determines precisely the omnivorous and opportunistic diet of the *Emys orbicularis*, but also shows that this species is not a threat to its environment, as 85.5% of the consumed species were not listed on the Swiss Red List. Moreover, it also demonstrated that the genetic analyses of faeces could be an efficient tool to determine trophic interaction with a high level of precision, yielding promising perspectives for food web ecology.

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1. Introduction

The quantification of interactions and fluxes in food webs is of prime importance for the understanding of ecosystem functioning. Deciphering a diet composition provides the relative contribution of different food sources and therefore how predators (or consumers) can switch between resources. This knowledge is a crucial step for determining the potential impacts of a predator on its prey populations (Jedlicka et al., 2016; Krauel et al., 2018). Diet studies are essential as bases for conservation measures aiming to maintain optimal species interactions in the ecosystems (Soulé et al., 2003). This is particularly relevant in the current context of anthropogenic alterations of climate and ecosystems, which could probably result in the modification of the distribution, the availability and the abundance of food resources for many wild animal populations (Sanderson et al., 2002; Thuiller et al., 2001), thus leading to a severe biodiversity collapse (Global IPBES 2019 report). Conducting diet analyses can however be challenging with omnivorous predators feeding on a wide diversity of plant and animal species (De Barba et al., 2014). Furthermore, such predators are often opportunistic, and their feeding behaviour may vary temporally and/or spatially depending on prey availability, nutritional needs and environmental conditions; individuals of a predator species are also able to exhibit food choice variation (Williams et al., 2004; Kratina et al., 2012).

DNA metabarcoding is a powerful tool for conservation purpose, for studying food chains (King et al., 2008) and determine predator diet (Taberlet et al., 2012). DNA metabarcoding combined with next-generation sequencing (NGS) technologies (Shendure and Ji, 2008; Glenn, 2011) makes the taxonomic identification of DNAs present in soil, water, faecal, gut and stomach samples possible, by simultaneously sequencing in parallel thousands of DNA molecules corresponding to short DNA barcodes amplified by universal and/or specific primers (Valentini et al., 2009; Taberlet et al., 2012; De Barba et al., 2014). Nowadays, long metabarcoding provided much longer sequencing reads (Godwin et al., 2016), with longer markers increasing the ability to distinguish closely related species, allowing a higher taxonomic resolution (Singer et al., 2016). They advantageously allow the rapid and accurate identification and assignment of the taxonomic identity of preys, when the remains are degraded or when no hard parts are available.

Furthermore, samples, such as faeces, are obtained in ways that minimize interaction with animals and do not harm them (Pompanon et al., 2012; De Barba et al., 2014). Moreover, species-specific DNA sequences being easier to identify, these methods are among the most accurate approaches available to understand feeding behaviours in ecosystem (Valentini et al., 2009) and are also generally better at making species-level identifications compared to other biomarker methods such as stable isotopes, signature lipids and antigen detection (Symondson, 2002).

One emblematic species for which such new diet analysis approach is particularly beneficial is the European pond turtle (*Emys orbicularis*, L. 1758), a species occurring in the wetlands of Europe and North Africa and ranked as “near threatened” (NT) on the IUCN Red List. In Switzerland, this species is native and listed as “critically endangered” (CR) on the Swiss Red List (Monney and Meyer, 2005). In 1999, the *Emys* project was developed to protect and reintroduce this species in Switzerland (<http://www.karch.ch/karch/home/reptilien/reptiliarten-der-schweiz/europaische-sumpfshildkrote/wiederansiedlung-sumpfshildkrot.html>). Since then, three successful reintroductions took place in the country, but raised a critical question of whether this reintroduced species would feed on other threatened species, such as amphibian species (e.g. *Bufo bufo*, considered as VU in Switzerland, or *Rana temporaria*, NT) in their new environment and thus add a new threat to them.

Indeed, for a long time the diet composition of the European pond turtle remained unclear, since it was successively considered as carnivorous, often scavenger (Rollinat, 1934; Lebboroni and Chelazzi, 1991; Kotenko, 2000; Luiselli, 2017), sometimes vegetarian (Ficetola and De Bernardi, 2006), and finally, in more recent years, omnivorous (Ottanello et al., 2005, 2016, 2018; Çiçek and Ayaz, 2011). Up until now, methods used to determine food intake by *E. orbicularis* included direct observation and microscopic examinations (Ottanello et al., 2005, 2016, 2018; Çiçek and Ayaz, 2011). However, these techniques have several limitations, such as a loss of information and the difficulty in recognizing the type of prey (plants and animals) in the faeces. Here, we present a diet study conducted for the European pond turtle with the aim to support its reintroduction and conservation without harming other threatened species. To our knowledge, no metabarcoding study has yet explored the diet of this species and only a few metabarcoding studies analyzed the diet of reptiles, such as slow worm (Brown et al., 2012), Caribbean island lizard (Kartzinel and Pringle, 2015), red-eared slider and Reeve's pond turtle (Koizumi et al., 2017). Although metabarcoding approach allows a precise determination of the species, this method does not allow the identification of the species stage (i.e. larva or adults, or roots, seeds and leaves). Moreover, the technique does not give information on the number or biomass of ingested items but only on the diversity of species consumed.

Currently, one of the drawbacks of metabarcoding approach is the limited length of the amplicons that are too short to allow assignment to the species level (Valentini et al., 2016). However, we recently developed a new method based on a long metabarcoding approach using primer redundancy and *de novo* assembly in order to efficiently and accurately identify taxon to the species level for plants, vertebrates and invertebrates present in faeces collected in the field (Ducotterd et al., submitted). In the present study, we aim to determine if *E. orbicularis* could be a threat for other endangered species (such as amphibians) by determining its diet using a universal and standardized method for a molecular and non-invasive diet assessment. We were interested assessing the overall variation in diet consumption over time, across locations and among individuals. In particular, we wanted to test whether differences in the diet of *E. orbicularis* exist between (i) month (temporal diet), (ii) populations (spatial diet), (iii) males and females and (iv) adults and juveniles; early studies suggest that the diet of juvenile and adult turtles from Emydidae family are different, with juvenile turtles being more carnivorous (Clark and Gibbons, 1969; Hart, 1983; Ottanello et al., 2005). Thus, we hypothesized that juveniles were more carnivorous than

adults. Ultimately, based on previous findings, we wanted to determine if threatened species could be found in the diet of the European pond turtle and in which individuals and populations.

2. Materials and methods

2.1. Study sites

Individuals of *E. orbicularis* were sampled in four different areas (Fig. 1), all sites are mature and stable ponds rich in vegetation and biodiversity. This type of habitats corresponds to the habitat used by the European pond turtle in the central and northern range of the species:

- (1) The natural reserve of Moulin de Vert (MDV; 46°10'46"N, 6°1'42"E) located in the canton of Geneva (Switzerland) downstream of the Verbois dam, on the left bank of the Rhone River. This habitat harbours the largest known population in Switzerland (about 180 adult individuals; S. Ursenbacher & M. Raemy, pers. comm.).
- (2) The natural reserve of Laconnex (LAC; 46°09'24"N, 6°01'46"E) located in the canton of Geneva (Switzerland). A population of about 150 individuals inhabit this area (C. Ducotterd, pers. obs.).
- (3) The natural reserve of Jussy (JUS; 46°15'04"N, 6°16'40"E) located in the canton of Geneva (Switzerland). In 2009, renaturation works were conducted, and first reintroduction took place in 2010; with a total of 52 ponds turtles being released there.

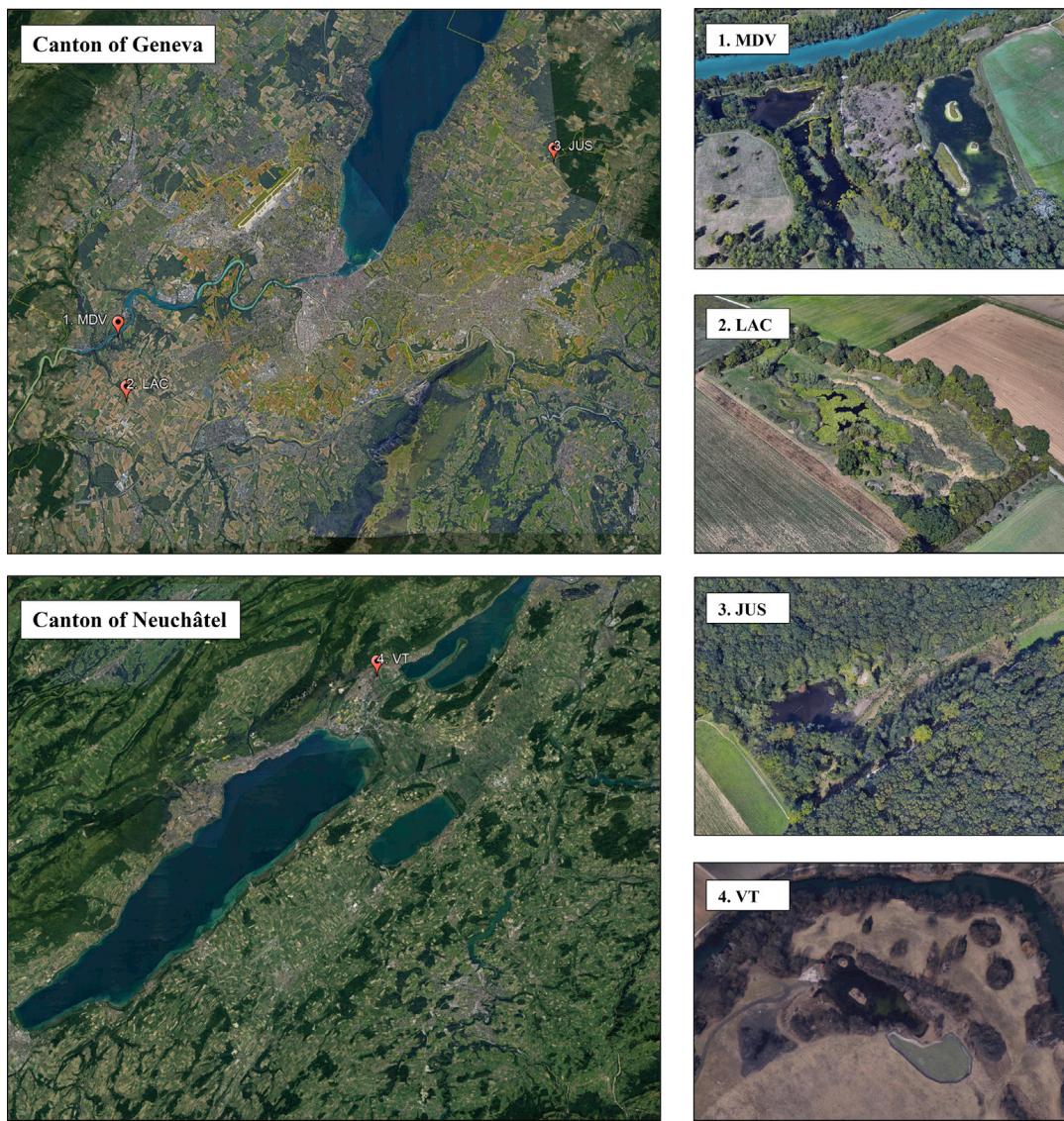


Fig. 1. Studied sites in cantons of Geneva and Neuchâtel (Switzerland). The diet of the European pond turtle was studied in four natural reserves (MDV = Moulin de Vert; LAC = Laconnex; JUS = Jussy and VT=La Vieille Thielle).

- (4) The natural reserve of La Vieille Thielle (VT; 47°2'57"N, 7°2'51"E) located in the canton of Neuchâtel (Switzerland). After some renaturation work conducted in 2009, the principal pond and the ancient oxbow lake of the Thielle River became the ideal habitat for the European pond turtle. Reintroduction took place in 2013, 2015 and 2019, a total of 27 European pond turtles were released until now.

2.2. Faecal samples collection

In order to determine their diet throughout their active season, European pond turtles were captured, with legal authorization, using conical fishing basket traps placed perpendicularly to the banks ([Cadi, 2003](#)) from April to September 2017 at MDV. Capture sessions took place each month and lasted for a week. Each trap was controlled every day and the captured pond turtles were placed in individual containers without water for the night, in order to collect faecal samples. Individuals were identified by notches made on marginal scales during previous monitoring studies, some others were not identified due to the absence of new monitoring studies (principally juveniles) and released at the exact location where they were captured. In order to compare diets at the same period in different location, European pond turtles were also captured using the same method during July 2017 in the JUS, LAC and VT sites. To prevent contamination of the samples, each container was cleaned with 10% bleach solution (NaOCl), followed by 70% denatured ethanol.

In total, 174 faecal samples from 142 individuals were collected in the field. All samples were stored in a plastic tube annotated with the individual's number, location and date of collection and then kept frozen at -80 °C. In the laboratory, before grinding the samples with liquid nitrogen in order to proceed to DNA extraction, feaces were examined in order to determine visible prey items on the basis of their morphology, such as seeds, bones, shell, elytra, etc. This information was used as positive control for the metabarcoding approaches.

2.3. Metabarcoding approach

We developed a new method based on long DNA metabarcoding, primers redundancy and *de novo* assembly to identify different taxonomic group of organisms from complex diets ([Ducotterd et al., submitted](#)). We used previously published primers for the amplification of the large subunit of the ribulose-1,5-bisphosphate carboxylase gene (rbcL), the maturase K gene (matK), the 28S rRNA gene, the trnL-trnF gene region in plants, as well as a portion of the mitochondrial-encoded cytochrome oxidase subunit I (COI or COX1) gene in animals in order to amplify prey DNA extracted from faecal samples (Supplementary Material S1).

All PCR reactions were carried out in 25 µl reaction volumes, consisting of 5 µl MyTaq™ Reaction Buffer (Bioline GmbH, Germany), 2.5 µl of selected versatile primers (0.5 µM final concentration), 2 U of Bioline MyTaq™ DNA Polymerase (Bioline GmbH, Germany), 1 µl of DNA (concentrated at 10 ng/µl), and completed up to the final volume with ultrapure sterile water. Each PCR was run under the following conditions: an initial denaturation step at 95 °C for 3 min, followed by 37 cycles of 95 °C for 20 s, 52 °C (annealing temperature for all primer sets except for the pairs mICoIntF/jgHCO2198, at 54 °C, and Tab c/Tab f at 56 °C) for 20 s and 72 °C for 20 s, and terminated by a final extension step of 20 s at 72 °C.

Then, 9 µl of PCR products amplified in triplicates with each distinctive primer pair were pooled per respective sample. Pooled PCR products were purified with the Wizard® SV Gel and PCR Clean-Up System (Promega) and diluted at 2 ng/µl final concentration. Pooled DNA amplicons were fragmented to an average fragment size of 290 bp in AFA microtubes (Covaris, USA) using a S2 focused-ultrasonicator (Covaris) following our established protocol. Sequencing libraries were created using the TruSeq® Nano DNA HT Library Prep Kit (Illumina Inc., USA) following manufacturer Prep Guide. All samples were sequenced using two Illumina MiniSeq High Output run at 2 x 151 bp paired-end reads length, reaching a median sequencing depth of 106 Mb per sample.

Finally, cleaned sequencing reads were downloaded from the lab Illumina Basespace account. *De novo* assembly of amplicons per respective sample sequencing data was performed using the genome assembly open software "SPAdes 3.11" ([Nurk et al., 2017](#)), with the metagenome assembly option « metaSPAdes ». Sequences under 150 bp were deleted and resulting contigs files were then blasted on the server of the National Center for Biotechnology Information (NCBI) using the BLAST + suite of command line tools ([Camacho et al., 2009](#)), against the complete NCBI nucleotide (nr/nt) collection. Resulting sequences other than prokaryotes and fungi, and with identity >97.6% (this threshold was determined from the analyses of the mock communities and the captive feeding trials analyses) were selected to represent the prey consumed by the European pond turtles. More details about the whole methodology can be found in [Ducotterd et al. \(submitted\)](#).

2.4. Statistical analysis

All statistical analyses were run on the software RStudio ([RStudio Team, 2015](#)). In our statistical analyses, we selected non-parametric test as the sample distribution was asymmetric. In order to compare the distribution of plants, invertebrates and vertebrates in the diet of *E. orbicularis* and to determine differences with respect to sex (female or male), maturity (adult or juvenile), month (temporal diet), and sites (spatial diet), we used a proportion test, the Kruskall Wallis test and a factorial analysis (Multiple Correspondence Analysis-MCA) which used binary (presence/absence) data, in order to test the variation in

consumption of plants, vertebrates and invertebrates. Indeed, only the diversity of species and no information of the quantity (biomass and volume) of prey consumed are available with metabarcoding approaches.

More precisely in the MDV population, we studied differences by sex, periods and months in the number of species found in the diet. We measured the species richness within a group (sex, period or month). Additionally, we also calculated the dissimilarity in diet between groups, i.e. the β -diversity (change in composition).

The species richness, which the number of prey species, was simply the sum of our incidence (presence/absence) variable at the species level, based on the number of species detected in the diet of each turtle.

Then to determine the β -diversity, which is a measure of dissimilitude between observations, we used the Jaccard index, which is the most common index used for assessment of β -diversity (Jaccard, 1912; Gianni et al., 2011; Ricardo and Francisco, 2011) and defined as follows:

$$S^j = \frac{a}{a + b + c}$$

where a is the overall richness, b is the number of species which appeared only in sample B and c is the number of species which appeared only in the sample C. Jaccard's dissimilarity index is $1 - S^j$. This index ranges between 0 and 1; if the index equals to 0 then all species observed appear in both samples, if the index is 1, and, all species are different. The β -diversity computes the distance in terms of species in the diet between two observations; we compared pairs for sex, pairs of Female-Female, Male-Male and Male-Female, as well as for period combinations for both April–June, both from July–September and one from each period. For that, we computed statistics for sets of individuals belonging to the same category and for those belonging to different categories.

To compare statistically the distribution of prey species (species richness) by sex and by period (April–June vs. July–September), we did a univariate analysis using a non-parametric test (Wilcoxon signed-rank test). Furthermore, in order to compare prey species between months, we used a Kruskall-Wallis rank sum test comparing all month by pairs. Finally, Dunn's tests were used to compare for the number of species by months and to determine difference in β -diversity between sex, period and months.

3. Results

In 174 faeces from 142 individuals collected on the field, a total of 1153 preys were consumed from 270 different OTU, of which 86.1% could be identified to species level, while the others were determined only at the genus/family level (Supplementary Material S2). In our sample, pseudo-replications are low as very few turtles are observed for multiple months. More in details, we recaptured twice 13 individuals and four times 2 individuals. Thus, it would not be possible to take into account individual effect as we could with a balanced panel (with all the individual replicated on each time period). Moreover, note that as the juvenile turtle is missing an identification number, it is impossible to track which juvenile turtle has been recaptured. Moreover, the fact that p-values are far below the 5% threshold strengthens the idea that rare pseudo-replications would not affect the conclusion of this paper.

3.1. Moulin de Vert (MDV) population - Temporal diet assessment

In the natural reserve of MDV, 146 faecal samples were collected from 114 individuals (83 marked and 31 non-marked individuals). Samples were collected on 71 females, 45 males (females were overrepresented), and 28 juveniles between April and September (80.6% adults and 19.4% juveniles). The DNA sequencing of all these faecal samples revealed that 97% of the faeces contained plants, 81% macro-invertebrates and 15% vertebrates.

Differences in the consumption of plants, vertebrates and invertebrates were not significant between females and males (Kruskall Wallis test; plant: p-value = 0.680; vertebrates: p-value = 0.444; invertebrates: p-value = 0.526; Fig. 2a).

Regarding maturity, no significant difference was found in the proportion of plants (Kruskall Wallis test; p-value = 0.319), invertebrates (Kruskall Wallis test; p-value = 0.774) and vertebrates (Kruskall Wallis test; p-value = 0.820) in the regime of juveniles versus adults (Fig. 2b).

Our results showed a significant difference in vertebrate consumption between the reproduction period – April to June, corresponding to mating and egg laying period – and the post reproduction period – July to September – with higher consumption during the reproduction period (Kruskall Wallis test; p-value = 0.008). For invertebrates, the difference was not significant (Kruskall Wallis test; p-value = 0.139) and the proportion of plant ingestion did not change between the two periods (p-value = 0.293). In other words, consumption of plant and invertebrates were similar through the whole activity period, only the consumption of vertebrates decreased in summer (Fig. 2c).

The Multiple Correspondence Analysis (MCA) confirmed this relationship (see Fig. 3a and b). The European pond turtle seems to diversify its diet during the reproduction and egg laying period by eating more vertebrates.

3.1.1. Number of species – species richness

Overall, the number of species in the diet went from 1 to 24 with a median of 6 and a mean slightly higher than 6.9 (Fig. 4a; Table 1). We noticed that the number of species in the diet was lower for the post-reproduction period (July–September) with

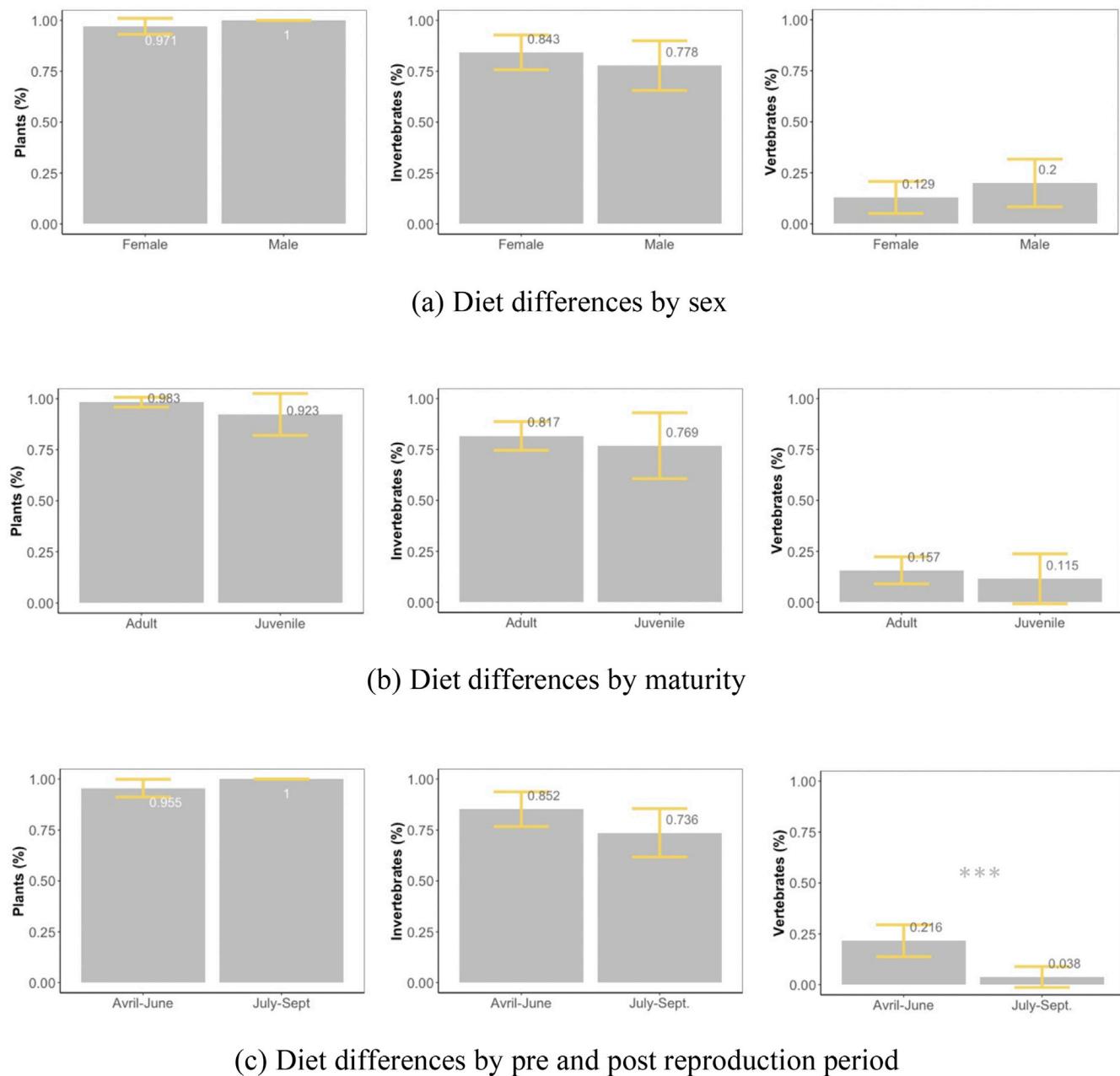


Fig. 2. Temporal diet (April to September) of the European pond turtle population of the natural reserve of Moulin de Vert (MDV; Geneva, Switzerland). (a) Difference between males and females in plants, invertebrates and vertebrates' consumption, (b) difference between adults and juveniles in diet and (c) variation in diet between the pre-reproduction period (April–June) and the post-reproduction period.

a mean of 3.4, a median of 3.0 and a maximum of only 7, while for the reproduction period (April–June), the mean and median were 9.0 with a maximum of 24 (Fig. 4b). The period was statistically significant, influencing the number of species consumed ($p\text{-value} < 0.001$; Fig. 5a). The mean and the median were higher for males (7.0 and 7.6 respectively) compared to females (5.0 and 6.5 respectively). However, this difference may be the result of an over-representation of males during April to June (35 observations) compared to July to September (only 10 observations; Fig. 4c).

Using a non-parametric test, we cannot say that sex influenced the number of species consumed (Wilcoxon signed-rank test; $p\text{-value} = 0.068$, Fig. 5a). Furthermore, the dietary habits of the turtles were clearly split in two periods: April–June (pre-reproduction) and July–September (post-reproduction). The comparison of all months by pairs reflected this fact as all statistically significant differences (Kruskall-Wallis rank sum test; $p\text{-value} < 0.001$) were between months from April to June with months from July to September (Fig. 5a).

3.1.2. β -diversity

The β -diversity demonstrated that the difference by period was highly significant, which corroborated our previous findings (Dunn's test; $p\text{-value} < 0.001$) with 95.5% of the species consumed between the reproduction period (April–June) and the other months (July–September) (β -diversity = 0.955, Table 1 and Fig. 5b).

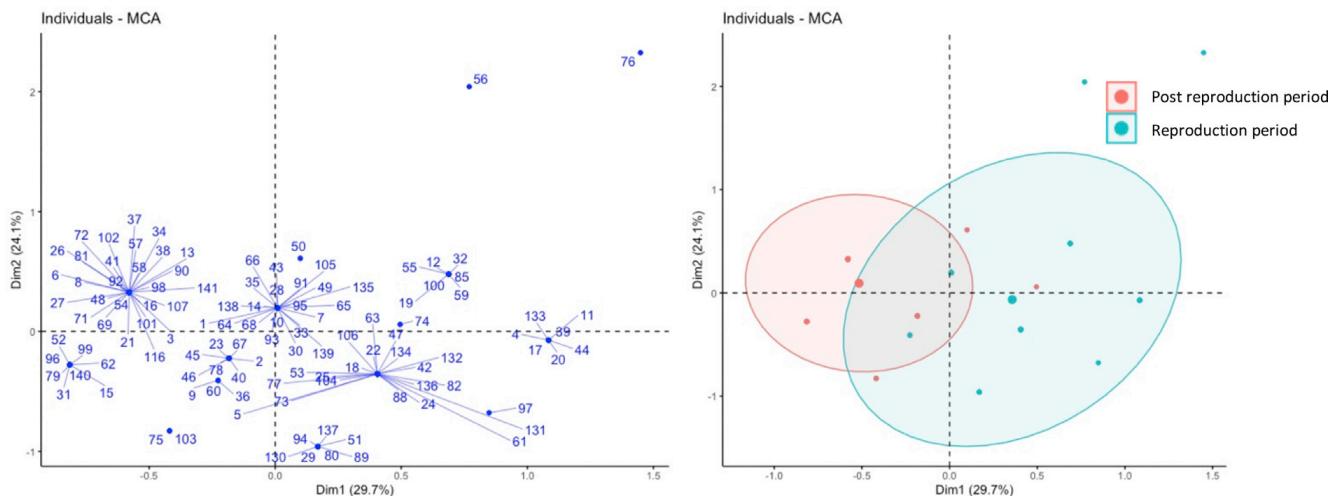


Fig. 3. Multiple Correspondence Analysis (MCA) of the composition (plants, invertebrates and vertebrates) of faeces obtained from European pond turtle in the natural reserve of Moulin de Vert (MDV; Canton of Geneva, Switzerland). (a) Individuals samples (blue points) demonstrated a massive overlap of two clusters of individuals (numbers connected by segments). (b) demonstrated the same clustering without labels ID but with a color code (Reproduction period between April–June). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

β -diversity sex showed no statistical difference between the distribution of Female-Female and Male-Male pairs (Dunn's test; p-value = 0.095). However, the pairs Female-Male were statistically different (Dunn's test; p-value < 0.001) showing that indeed the dissimilitude is larger between female and male with 90.5% (β -diversity = 0.905) of the species consumed being different (Table 1 and Fig. 5b).

3.2. Comparison between four populations - Spatial diet assessment

In July 2017, samples were collected in four different populations of *E. orbicularis*. During this period, 56 faecal samples were collected on 56 individuals represented by 30 females, 13 males and 13 juveniles (76.8% adults and 23.2% juveniles). The metabarcoding DNA sequencing of the 56 faecal samples revealed that 100% faeces contained plants, 12.5% vertebrates and 85.7% macro-invertebrates (Fig. 6). Unfortunately, only two European pond turtles were captured in VT due to the very low number of turtles present in this population ($n = 18$). Therefore, the number of samples was too small to allow a relevant statistical analysis on this population.

No significant differences were found between sites (MDV, LAC and JUS) regarding consumption of the three groups of organisms evaluated (Kruskall Wallis test, p-value = 0.220).

3.3. Endangered species consumption?

Three species of amphibian were found through the metabarcoding DNA sequencing of faeces; the common toad (*Bufo bufo*, L., 1758; found in 13 occurrences), the green frog (*Pelophylax lessonae*, Camerano, 1882; 1 occurrence) and one sample containing bones of which DNA analysis allowed us to determine the common frog (*Rana temporaria*, L., 1758) (see Table 1). Some other species ranked as near threatened or vulnerable in the Swiss Red Lists were also consumed by the European pond turtles, especially a moss, *Pleurochaete squarrosa* (Limpr., 1888; 20 occurrences), a fish *Cyprinus carpio* (L., 1758; 8 occurrences) an odonatan, *Gomphus pulchellus* (Selys, 1840; 4 occurrences), and a slug *Deroceras laeve* (Müller, 1774; 4 occurrences). More anecdotally, we found the presence of one odonatan species, *Coenagrion pulchellum* (Vander Linden, 1825), a butterfly species, *Polyptoca ridens* (Fabricius, 1787), a caddisfly, *Limnephilus vittatus* (Fabricius, 1798), a snail *Zonitoides nitidus* (Müller, 1774) and a plant *Typha angustifolia* (L. 1753), which were also considered threatened on the Swiss Red Lists. Some plants, such as *Nymphaea alba* (L., 1753) and *Utricularia australis* (R. Br., 1810) were more frequently found, with 90 and 18 occurrences, respectively (Table 1). Many undigested seeds were also found in the faecal samples and species were determined based on their morphology and then confirmed with our metabarcoding approaches. These results precisely showed that 13 endangered species were consumed by the European pond turtle. Therefore, its diet was composed of the 270 different species, meaning that 85.5% of them were not considered as threatened on the Swiss Red Lists or were listed as "data insufficient".

4. Discussion

We presented results from a new molecular approach to assess the diet of a reintroduced species to better understand the potential impact of its feeding habits on other species. Using long metabarcoding approach to determine dietary composition and richness in a sample of any kind (water, faeces, gut content, soil, etc.) allowed us to widen the pre-existing knowledge of

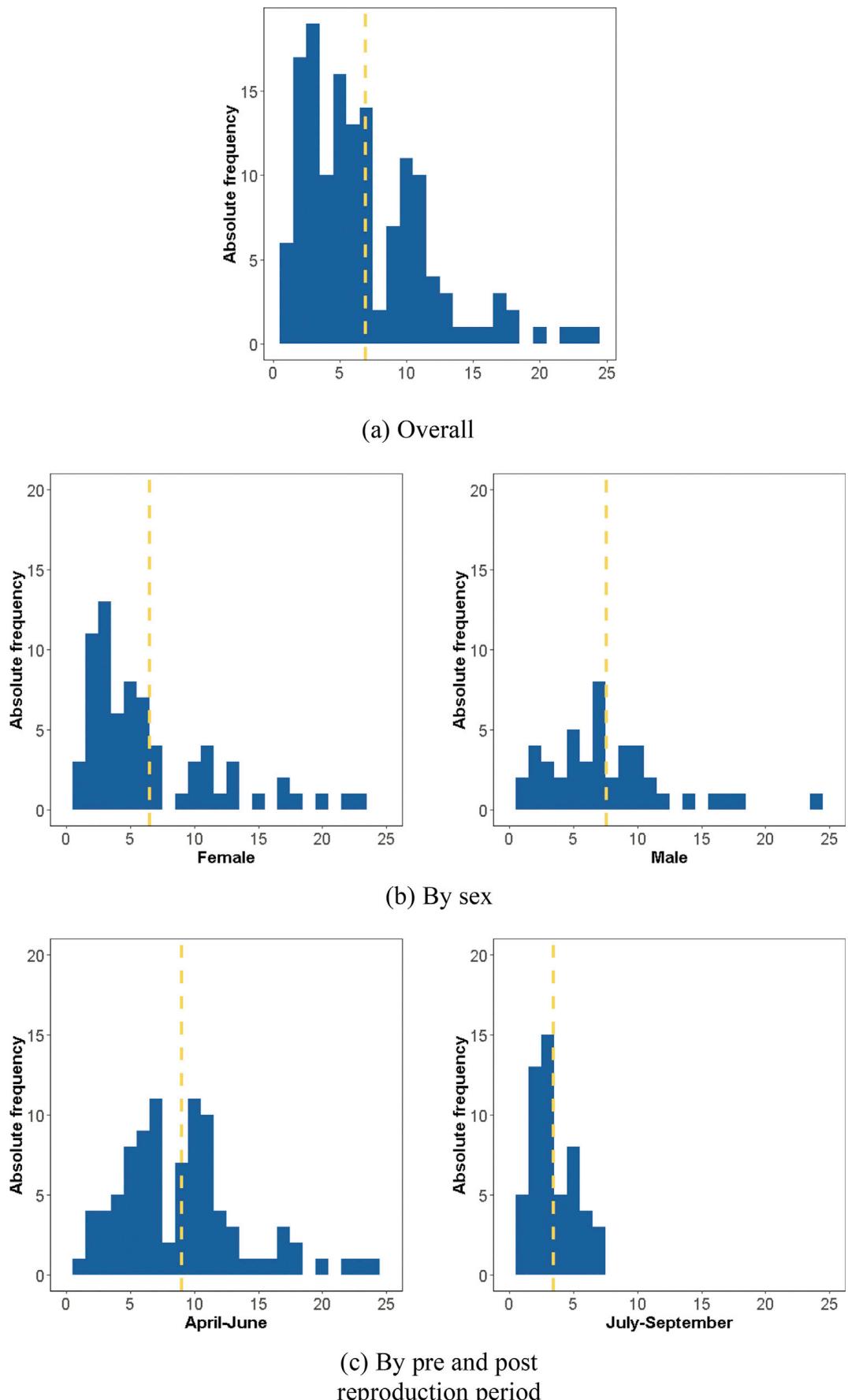
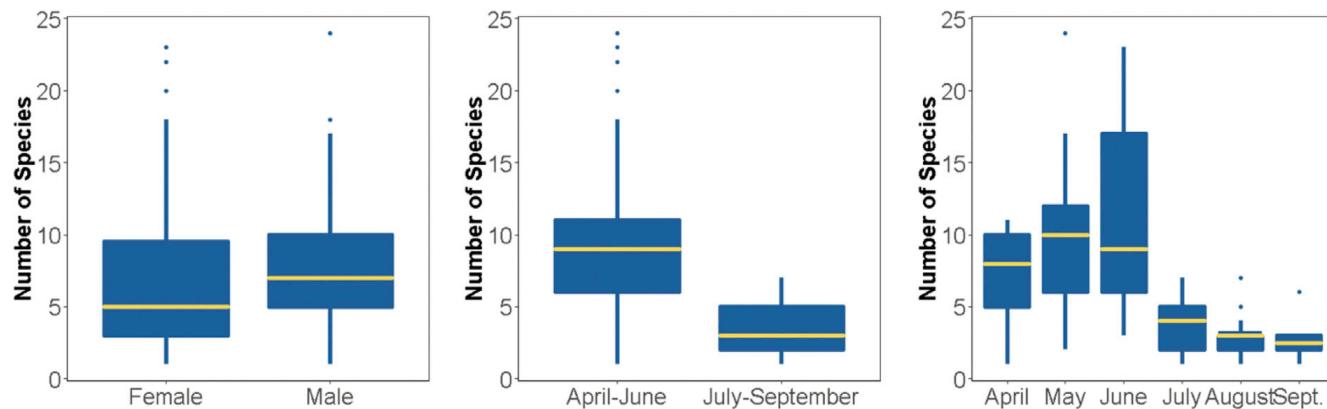


Fig. 4. Distribution of the number of species consumed of the European pond turtle (a) overall; (b) separated by sexes; and (c) differences between the pre- and post-reproduction period.

Table 1

Summary statistics of the number of species, the β -diversity of the diet of the European pond turtle during its whole activity period (April to September) in the population of Moulin de Vert (Geneva, Switzerland).

	Mean	Median	SD	Min.	Max.
Number of species (richness)					
Overall	6.944	6	4.792	1	24
Sex					
Female	6.535	5	5.253	1	23
Male	7.578	7	4.76	1	24
Period					
April–June	9	9	4.823	1	24
July–September	3.415	3	1.669	1	7
β-diversity (Jaccard)					
Overall	0.907	0.933	0.115	0	1
Sex					
Female, Female	0.897	0.933	0.129	0	1
Male, Male	0.89	0.917	0.114	0	1
Male, Female	0.905	0.929	0.12	0	1
Period					
Both April–June	0.901	0.917	0.088	0	1
Both July–September	0.756	0.8	0.148	0	1
Not in the same period	0.955	1	0.081	0	1



(a) Number of prey species in the diet

(b) β -diversity (Jaccard index)

Fig. 5. Box plot of the number of species (species richness) and β -diversity (Jaccard index) in the diet of the European pond turtle during its whole activity period (April–September). (a) Difference in number of species found between male and female, pre- and post-reproduction period and between each month, (b) β -diversity in the diet by sex and pre- and post-reproduction period (F = female, M = male, 1 = pairs from April–June; 2 = pairs from July–September and 3 = pairs with one observation from April–June and the other from July–September).

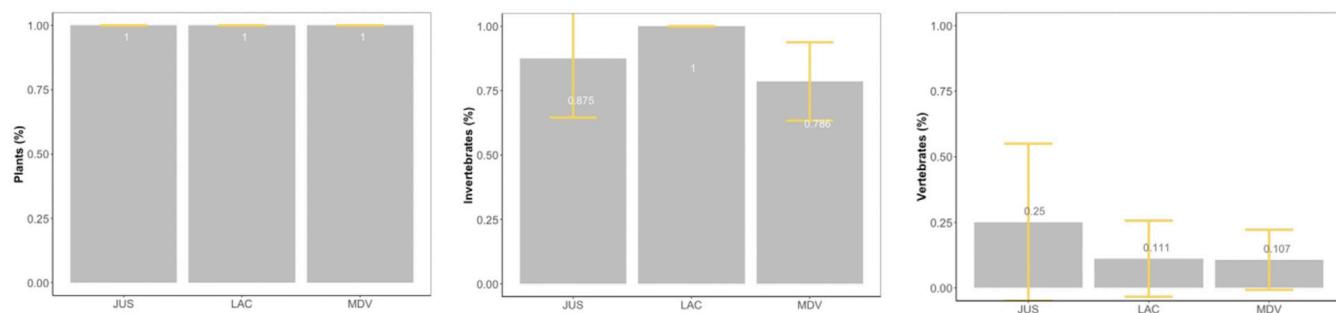


Fig. 6. Spatial variation in the diet of the European pond turtle in three different population of Switzerland (JUS = Jussy; LAC = Laconnex and MDV = Moulin de Vert, see Fig. 1) during the month of July. Proportion of plants, invertebrates and vertebrates consumed among populations.

the studied species' ecology and behaviour. Indeed, the higher level of ingested species richness found with the new molecular approach compared to histological analyses of the same sample, corroborated the result of other studies on different species (Soinien et al., 2015; Ando et al., 2013). Our results demonstrated a great precision, since 86,1% of the preys were identified to the species level and revealed a large species richness in the diet of the European pond turtle. Previous studies on the diet of this species, which were only made via direct observation or using microscope (Ottonello et al., 2005, 2016, 2018; Çiçek and Ayaz, 2011), only yielded the identification of preys, in most cases to the order level, or in some cases to the family level, and very often, plants were defined as “unidentified organic matter” or “plant fragments”. This resulted in a huge loss of ecological information.

In the present study, we revealed the presence of 1153 prey items from 270 different species of vertebrates, invertebrates and plants, consumed by the European pond turtle. Consequently, assessing diets only via the visual identification of ingested prey results in a wide underestimation of taxonomic diversity. The integration of new metabarcoding approaches is therefore essential to provide a more precise knowledge of diet and feeding strategy, even if this approach does not allow the evaluation of the amount of each prey.

Our results show that the European pond turtle feeding strategy in Switzerland follows an opportunistic and omnivorous pattern. Moreover, almost all sampled turtles consumed plants in their diet and throughout their activity period, suggesting that aquatic plants are a key component of the diet of *E. orbicularis*, in contradiction with previous studies which stated that plant matters were accidentally ingested together with animal preys, and did not represent a primary food item (Lindeman, 1996). This actually addresses the question whether the plant fragments found in our samples could come from animal preys, as some of these are plant consumers. In this regard, the DNA present in faecal sample is usually degraded (Deagle et al., 2006), meaning that food items eaten by preys, itself eaten by the predator, were degraded twice, making unlikely the possible detection of plant DNA ingested by the preys. Moreover, a large proportion (97.9%) of the analyzed faeces contained plants. Consequently, our findings are corroborating previous results (Ficetola and De Bernardi, 2006; Ottonello et al., 2016) showing the presence of large pieces of plant matter such as leaves from *Typha* or *Phragmites* and seeds from *Nymphaea*. These results likely support that the European pond turtles ingested these plants voluntarily as food item (Ayres et al., 2010).

Concerning the difference between adults and juveniles in the diet of the Emydid turtle family, previous studies (*Trachemys scripta elegans*: Clark and Gibbons, 1969; Hart, 1983; *Emys orbicularis*: Ottonello et al., 2005) suggested that adult turtles feed more frequently on plants than juveniles, and proposed that turtles shift to a more herbivorous diet as they grow. Surprisingly, our results did not corroborate these views and rather demonstrate a lack of difference in plant consumption between juveniles and adults. However, our analyses are based on the number of species and not on proportions of ingested items or their volumes or biomass. Thus, the morphological and molecular methods should be considered as complementary approaches in order to determine both species and the volume or biomass consumed. In the case of juveniles, their feaces are extremely small and therefore visual determination of prey items are very difficult or impossible.

Moreover, previous studies reported that the consumption of plants increased during the post breeding period, suggesting a diet shift throughout the year (Ottonello et al., 2005; Ayres et al., 2010). Yet, our study demonstrated that the plant consumption did not change through the year. Indeed, we demonstrated that the number of species (species richness) was larger from April to June, meaning that plants species were not just replaced by invertebrates and vertebrates, but that the diet was indeed more varied in spring. Furthermore, we showed that the dissimilarity between females and males was strongly significant, meaning that both sexes consumed different species. Regarding the period, the average Jaccard β -diversity was 0.955, meaning that the European pond turtle has a completely different diet between April–June and July–September with on average 95.5% difference in the species being consumed by turtles between periods. Therefore, this result confirms the opportunistic and omnivorous diet of the European pond turtle in Switzerland.

We demonstrated that 97.9% of the faeces contained plants. This result combined with those of species turnover (β -diversity) suggests that the plant species being consumed varied greatly through time, meaning that plant-based regime is not constant through months, but most certainly evolves over time.

Another significant change in diet was related to vertebrate's consumption which was higher during the reproduction period (April–June). We can hypothesize that the European pond turtle consumes less vertebrates after the reproduction period due to the fact that its energy need, and thus its hunting activity, is reduced.

The diversity in diet might therefore be due to the temporal availability of preys, as some species are only present in the pond for short periods. Amphibians are, for instance, dense in spring with *Bufo bufo* and *Rana temporaria* breeding early, whereas tadpoles remain available until the beginning of summer, except for *Pelophylax* species. The European pond turtle thus seems to behave opportunistically and to target preys that are the easiest to obtain, in congruence with the optimal foraging theory (MacArthur and Pianka, 1966). Observing the abundance of species present in the environment through months would allow observing a potential variation of availability between the April–June and July–September periods.

In our study, the ponds inhabited by the European pond turtle are mature and stable and correspond to the habitat in the central and northern range of the species, therefore we could hypothesize that the diet and trophic niche would be very similar to the population in Switzerland. However, as the European pond turtle seems to have an opportunistic behavior, the species can locally eat completely different prey depending on their microhabitat.

In future studies, comparison with subpopulations of the same species, such as *E. orbicularis galloitalica*, which live in rivers in Corsica, or *E. orbicularis persica* from Jelilabad, Azerbaijan, which is considered as carnivorous (Luiselli, 2017) or with populations living in Mediterranean ponds, with high seasonal variations in water level and water temperature, would provide additional insights into the general ecology of this species across its range.

4.1. Is the European pond turtle a threat for other endangered species?

The European pond turtle is considered a vulnerable species at the European level and has even disappeared in numerous regions. Consequently, several reintroductions occurred in Western Europe (Fritz and Chiari, 2013). One of the crucial questions before any reintroduction has always related to its potential danger to other threatened species.

Our study, however, demonstrates that the European pond turtle mainly eats plants. Regarding the consumption of threatened plants such as *Nymphaea alba* (NT) and *Utricularia australis* (NT), we could deduce from the high quantity of seeds, which were determined by morphological observations and DNA analyses, present in faeces that the European pond turtle consumed mostly their fruits (see Table 2). This turtle species might therefore also participate in the dissemination of their seeds, together with other turtle species (Kimmens and Moll, 2010; Padgett et al., 2018). Moreover, *Nymphaea* seeds were also shown to germinate better after transiting in the digestive system of the European pond turtle (Calvino-Cancela et al., 2007; Ayres et al., 2010). Regarding the consumption of threatened invertebrates and vertebrates, some of these were only occasionally eaten. *Bufo bufo* was the most consumed vertebrates (present in 13 out of 174 samples), and only during the European pond turtle reproduction period, which corresponds to the presence of tadpoles in the pond (Table 2). Unfortunately, one of the limitations of metabarcoding approaches is related to the status of the prey; indeed, it is impossible to determine whether adults, juveniles, larva, or/and eggs were consumed, and if individuals were dead or alive when eaten. In our case, we could only hypothesize that the European pond turtle consumed tadpoles in spring. To conclude, the variety of preys consumed by the European pond turtle strongly suggests that this species is an opportunistic hunter and its impact on other endangered species is rather marginal.

Table 2

Threatened species found in the diet of the European pond turtle in Switzerland.

Phylum	Class	Order	Family	Genus	Species	Statut on the Swiss Red List	Found in x samples on 174
Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	VU	13
Chordata	Amphibia	Anura	Ranidae	Rana	<i>Rana temporaria</i>	NT	1
Chordata	Amphibia	Anura	Ranidae	Pelophylax	<i>Pelophylax lessonae</i>	NT	1
Chordata	Actinopterygii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	NT	8
Plantae	Spermatophyta	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	NT	90
Plantae	Equisetopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	NT	18
Plantae	Tracheophyta	Poales	Thypaceae	Typha	<i>Typha angustifolia</i>	NT	3
Plantae	Equisetopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	VU	20
Mollusca	Gastropoda	Stylommatophora	Agriolimacinae	Deroceras	<i>Deroceras laeve</i>	NT	4
Mollusca	Gastropoda	Stylommatophora	Gastropontidae	Zonitoides	<i>Zonitoides nitidus</i>	NT	1
Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus vittatus</i>	VU	1
Arthropoda	Insecta	Lepidoptera	Drepanidae	Polyploca	<i>Polyploca ridens</i>	VU	1
Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion	<i>Coenagrion pulchellum</i>	NT	3
Arthropoda	Insecta	Odonata	Aeshnoidea	Gomphus	<i>Gomphus pulchellus</i>	VU	4

5. Conclusion

Our study demonstrated the use of metabarcoding as a powerful tool for conservation purpose, allowing to provide precise answers to ecological questions about specific diets. As the European pond turtle benefits from a national conservation program in Switzerland, the key question was to determine if its reintroduction in new locations might threaten other endangered species. The answer provided by long metabarcoding rather suggests a very marginal impact. Using metabarcoding further made it possible to considerably improve our understanding of the feeding behaviour of the European pond turtle and the diversity of preys consumed, at a level never reached before. Our approach and findings also offer a great perspective in future studies of food web and trophic interactions. As next possible steps, comparisons of these results with other *Emys* populations living in different environment would greatly improve knowledge of the whole genus, found in very diverse environments with diverse food opportunities.

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Author contribution

CD, SU and JFR designed the study, CD conducted the sampling, the laboratory and the bioinformatics analyses, JC set up the methodology (primers selection and design, sequencing and bioinformatics analyses), JC and FL supervised the laboratory work. CD wrote the manuscript and the other coauthors revised it.

Data accessibility

All raw sequencing reads for 182 metabarcodes were registered in the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) under the Bioproject accession PRJNA546135.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gecco.2020.e01133>.

References

- Ando, H., Setsuko, S., Horikoshi, K., Suzuki, H., Umehara, S., Inoue-Murayama, M., et al., 2013. Diet analysis by next-generation sequencing indicates the frequent consumption of introduced plants by critically endangered red-headed wood pigeon (*Columba janthina nitens*) in oceanic island habitat. *Ecol. Evol.* 29, 4057–4069.
- Ayres, C., Calvino-Cancela, M., Cordero-Rivera, A., 2010. Water lilies, *Nymphaea alba*, in the summer diet of *Emys orbicularis* in the Northwestern Spain: use of emergent resources. *Chelonian Conserv. Biol.* 9 (1), 128–131.
- Brown, D.S., Jarman, S.N., Symondson, W.O.C., 2012. Pyrosequencing of prey DNA in reptile faeces: analysis of earthworm consumption by slow worms. *Mol. Ecol. Res.* 12, 259–266.
- Cadi, A., 2003. PhD Thesis. Écologie de la Cistude d'Europe (*Emys orbicularis*): aspects spatiaux et démographiques, applications à la conservation, vol. 1. Université de Lyon, Lyon, p. 310.
- Calvino-Cancela, M., Ayres-Fernandez, C., Cordero-Rivera, A., 2007. European pond turtles (*Emys orbicularis*) as alternative dispersers of « water-dispersed » waterlily (*Nymphaea alba*). *Ecoscience* 14 (4), 529–534.
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. *BMC Bioinf.* 10, 421.
- Çicek, K., Ayaz, D., 2011. Food composition of the European pond turtle (*Emys orbicularis*) in lake Sülüklü (Western Anatolia, Turkey). *J. Freshw. Ecol.* 26, 571–578.
- Clark, D.B., Gibbons, J.W., 1969. Dietary shift in the turtle *Pseudemys scripta* (Schoepff) from youth to maturity. *Copeia* 704–706.
- De Barba, M., Miquel, C., Boyer, F., Mercier, D., Rioux, D., Coissac, E., Taberlet, P., 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. *Mol. Ecol. Res.* 14, 306–323.
- Deagle, B.E., Everson, J.P., Jarman, S.N., 2006. Quantification of damage in DNA recovered from highly degraded samples – a case study on DNA in faeces. *Front. Zool.* 3, 11.
- Ducotterd, C., Crovadore, J., Lefort, F., Rubin, J.-F., Ursenbacher, S., submitted. A Powerful and Deeply Resolutive Metabarcoding Method for the Determination of Omnivorous Diet from Faeces Analysis Evaluated in the European Pond Turtle (*Emys orbicularis*, L.1758).
- Ficetola, G.F., De Bernardi, F., 2006. Is the European pond turtle *Emys orbicularis* strictly aquatic and carnivorous? *Amphibia-Reptilia* 27, 445–447.

- Fritz, U., Chiari, Y., 2013. Conservation actions for the European pond turtles – a summary of current efforts in distinct European countries. *Herpet. Notes* 5, 105.
- Gianni, Q.H., Francisco, J.C., Andivan, C.F.M., 2011. Species diversity of myrmecofauna and araneofauna associated with agroecosystem and forest fragments and their interaction with Carabidae and Staphylinidae. *Fla. Entomol.* 94 (1), 500–509.
- Glenn, T.C., 2011. Field guide to next-generation sequencers. *Mol. Ecol. Resour.* 11, 759–769.
- Godwin, S., McPherson, J.D., McCombie, W.R., 2016. Coming of age: Ten years of next-generation sequencing technologies. *Nat. Rev. Genet.* 17, 333–351.
- Hart, D.R., 1983. Dietary and habitat shifts of red-eared turtles (*Pseudemys scripta*) in southern Louisiana population. *Herpeto* 39, 285–290.
- Jaccard, P., 1912. The distribution of the flora in the Alpine zone. *The Phytologist* 11 (2), 37–50.
- Jedlicka, J.A., Vo, A.T.E., Almeida, R.P., 2016. Molecular scatology and high throughput sequencing reveal predominately herbivorous insects in the diets of adult and nestling Western Bluebirds (*Sialia mexicana*) in California vineyards. *Auk* 134, 116–127.
- Kartzinel, T.R., Pringle, R.M., 2015. Molecular detection of invertebrate prey in vertebrate diets: trophic ecology of Caribbean island lizards. *Mol. Ecol. Res.* 15 (4), 903–914.
- Kimmons, J.B., Moll, D., 2010. Seed dispersal by red-eared sliders (*Trachemys scripta elegans*) and common snapping turtles (*Chelydra serpentina*). *Chelonian Conserv. Biol.* 9 (2), 289–294.
- King, R.A., Read, D.S., Traugott, M., Symondson, W.O.C., 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. *Mol. Ecol.* 17 (4), 947–963.
- Koizumi, N., Mori, A., Mineta, T., Sawada, E., Watabe, K., Takemura, T., 2017. Plant species identification using fecal DNAs from red-eared slider and Reeve's pond turtle in agricultural canals for rural ecosystem conservation. *Paddy Water Environ.* <https://doi.org/10.1007/s10333-016-0576-5>.
- Kotenko, T.I., 2000. The European pond turtle (*Emys orbicularis*) in the Steppe Zone of the Ukraine. In: Hödl, W., Rössler, M. (Eds.), *Die Europäische Sumfschildkröte*, Staphia, vol. 68, pp. 87–106.
- Kratina, P., LeCraw, R.M., Ingram, T., Anholt, B.R., 2012. Stability and persistence of food webs with omnivory: is there a general pattern? *Ecosphere* 3, 1–18.
- Krauel, J.J., Brown, V.A., Westbrook, J.K., Mccracken, G.F., 2018. Predator-prey interaction reveals local effects of high-altitude insect migration. *Oecologia* 186, 49–58.
- Lebboroni, M., Chelazzi, G., 1991. Activity patterns of *Emys orbicularis* L. (*Chelonia Emydidae*) in central Italy. *Ethol. Ecol. Evol.* 3, 257–268.
- Lindeman, P.V., 1996. Comparative life history of painted terrapins (*Chrysemys picta*) in two habitats in the inland Pacific Northwest. *Copeia* 1996, 114–130.
- Luiselli, L., 2017. Food habits, habitat use and density of *Emys orbicularis persica* from Jelilabad, Azerbaijan. *Herpetol. J.* 27, 245–251.
- MacArthur, R.H., Pianka, E.R., 1966. On the optimal use of patchy environment. *Am. Nat.* 100, 603–609.
- Monney, J.-C., Meyer, A., 2005. Liste rouge des espèces menacées en Suisse, Reptile. Office fédéral de l'environnement, des forêts et du paysage (OFEV). Centre de coordination des amphibiens et reptiles de Suisse (Karch), Berne, p. 46.
- Ottonello, D., Salvidio, S., Rosecchi, E., 2005. Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). *Amphibia-Reptilia* 26, 562–565.
- Nurk, Meleshko, Korobeynikov, Pevzner, 2017. metaSPAdes: a new versatile de novo metagenomic assembler. *Genome Res.* 27 (5), 824–834.
- Ottonello, D., D'Angelo, S., Oneto, F., Malavasi, S., Zuffi, M.A.L., 2016. Feeding ecology of the Silican pond turtle *Emys trinacris* (Testudines, Emydidae) influenced by seasons and invasive aliens species. *Ecol. Res.* 32 (1), 71–80.
- Ottonello, D., Oneto, F., Vignone, M., Rizzo, A., Salvidio, S., 2018. Diet of a restocked population of the European pond turtle *Emys orbicularis* in NW Italy. *Acta Herpetol.* 13, 89–93.
- Padgett, D.J., Joyal, M., Quirk, S., Laubi, M., Surasinghe, T.D., 2018. Evidence of aquatic plant seed dispersal by eastern painted turtles (*Chrysemys picta picta*) in Massachusetts USA. *Aquat. Bot.* 149, 40–45.
- Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. *Mol. Ecol.* 21, 1931–1950.
- Ricardo, J.P., Francisco, J.P.F., 2011. Diversity and community structure of opiinae (Hymenoptera: Braconidae) in the forest estate of Artikutza (Spain). *Fla. Entomol.* 94 (1), 472–479.
- Rollinat, R., 1934. *La vie des reptiles de la France centrale*. Delagrave, Paris, p. 337.
- RStudio Team, 2015. RStudio. Integrated Development for R. RStudio, Inc., Boston, MA. URL <http://www.rstudio.com/>.
- Sanderson, E.W., Jaiteh, M., Levy, M.A., et al., 2002. The human footprint and the last of the wild: the human footprint is a global map of human influence on the land surface, which suggests that human beings are stewards of nature, whether we like it or not. *Bioscience* 52, 891–904.
- Shendure, J., Ji, H., 2008. Next generation DNA sequencing. *Nat. Biotechnol.* 26, 1135–1145.
- Singer, E., Bushnell, B., Coleman-Derr, D., Bowman, B., Bowers, R.M., Levy, A., et al., 2016. High-resolution phylogenetic microbial community profiling. *ISME J.* 10, 2020–2032.
- Soininen, E.M., Gauthier, G., Bilodeau, F., Berteaux, D., Gielly, L., Taberlet, P., et al., 2015. Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS One* 10, e0115335.
- Soulé, M.E., Estes, J.A., Berger, J., Del Rio, C.M., 2003. Ecological effectiveness: conservation goals for interactive species. *Conserv. Biol.* 17, 1238–1250.
- Symondson, W.O.C., 2002. Molecular identification of prey in predator diets. *Mol. Ecol.* 11 (4), 627–641.
- Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA barcoding. *Nucleic Acids Res.* 35, e14.
- Thuiller, W., Lavergne, S., Roquet, C., et al., 2001. Consequences of climate change on the tree of life in Europe. *Nature* 470, 531–534.
- Valentini, A., Pompanon, F., Taberlet, P., 2009. DNA barcoding for ecologists. *Trends Ecol. Evol.* 24, 110–117.
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J., Peroux, T., Crivelli, A.J., Olivier, A., Acqueberge, M., Brun, M.L., Möller, P.R., Willerslev, E., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.* 25, 929e942.
- Williams, T.M., Estes, J.A., Doak, D.F., Springer, A.M., 2004. Killer appetites: assessing the role of predators in ecological communities. *Ecol.* 85, 3373–3384.

Supplementary Material S1: Primers used for amplification of the ribulose-1,5-bisphosphate carboxylase gene, the maturase K genes, the 28S rRNA gene, the *trnL-trnF* gene region (for plants) and the mitochondrial-encoded cytochrome oxidase subunit I gene (for animals).

Prey taxon	DNA type	DNA region	Primer name	Forward/reverse primer	Primers sequence 5'-3'	Reference	Average length of amplified fragment (bp) in this study
Invertebrates	Mitochondrial	COI	mICOLintF	Forward	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al. (2013)	350
			jgHCO2198	Reverse	TAIACYTCIGGRTGICCRAARAAYCA	Leray et al. (2013)	
Invertebrates	Mitochondrial	COI	ODO_LCO1490d	Forward	TTTCTACWAACCAYAAAGATATTGG	Dijkstra et al. (2014)	650
			ODO_HCO2198d	Reverse	TAAACTTCWGGRTGTCAAARAATCA	Dijkstra et al. (2014)	
Vertebrates	Mitochondrial	COI	COI-CO2	Forward	AYTCAACAAATCATAAAGATATTGG	Che et al. (2012)	600
			COI-CO4	Reverse	ACYTCRGGRTGACCAAAAAATCA	Che et al. (2012)	
Vertebrates	Mitochondrial	COI	Mod_RepCOI_F	Forward	TNTTYTCMACYACCACAAAGA	Reeves et al. (2018)	650
			Mod_RepCOI_R	Reverse	TTCDGGRTGNCCRAARAATCA	Reeves et al. (2018)	
Plants	Plastid	maturase K	MatK-1RKIM-f	Forward	ACCCAGTCCATCTGAAATCTTGGTC	K.-J. Kim, pers. comm.	850
			MatK-3FKIM-r	Reverse	CGTACAGTACTTTGTGTTACGAG	K.-J. Kim, pers. comm.	
Plants	Plastid	maturase K	MatK-472-f	Forward	CCCRTYCATCTGAAATCTTGGTC	Yu et al. (2011)	730
			MatK-1248-r	Reverse	GCTTRTRATAATGAGAAAGATTCTGC	Yu et al. (2011)	
Plants	Plastid	maturase K	MatK-5r	Forward	GTTCTAGCACAGAAAGTCG	Ford et al. (2009)	825
			MatK-xf	Reverse	TAATTTACGATCAATTTCATTTC	Ford et al. (2009)	
Plants	Chloroplast	rbcL	rbcL a-F	Forward	ATGTCACCACAAACAGAGACTAAAGC	Levin et al. (2003)	520
			rbcL a-R	Reverse	GTAAAATCAAGTCCACCRG	Kress and Erickson (2007)	
Plants	Chloroplast	rbcL	rbcL-1F	Forward	ATG TCA CCA CAA ACA GAA AC	Fay et al. (1997)	680
			rbcL-724R	Reverse	TCG CAT GTA CCT GCA GTA GC	Olmstead et al. (1992)	
Plants	Chloroplast	28S rRNA	28KJ	Forward	GGCGGTAAATTCCGTCC	Cullings (1992)	630
			28C	Reverse	GCTATCCTGAGGGAAACTTC	Hamby and Zimmer (1988)	
Plants	Chloroplast	<i>trnL-trnF</i>	Tab c	Forward	CGAAATCGGTAGACGCTACG	Taberlet et al. (1991)	920
			Tab f	Reverse	ATTTGAACCTGGTGACACGAG	Taberlet et al. (1991)	

Supplementary Material S2: Diet of the European pond turtle during its whole activity period in the natural reserve of Moulin de Vert (MDV) as well as three other populations of Switzerland, which are Laconnex (LAC), Jussy (JUS) and La Vieille Thielle (VT).

Turtle_ID	Sex	Maturity	Weight	Sample_ID	Location	Month	Kingdom	Phylum	Class	Order	Family	Genus	Species	% identical matches	Reference alignment length
U	U	J	71.3	JUS1	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.6	679
U	U	J	71.3	JUS1	JUS	July	Viridiplantae	Streptophyta	Liliopsida	Poales	Typhaceae	Typha	<i>Typha latifolia</i>	99.9	819
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Agriphila	<i>Agriphila vulgivagella</i>	100.0	189
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Diptera	Syrphidae	Cheilosia	<i>Cheilosia barbata</i>	96.3	229
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Enochrus	<i>Enochrus testaceus</i>	99.1	223
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Helochares	<i>Helochares obscurus</i>	100.0	431
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrochara	<i>Hydrochus carinatus</i>	99.6	327
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Noteridae	Noterus	<i>Noterus clavicornis</i>	99.4	558
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Hemiptera	Pleidae	Plea	<i>Plea minutissima</i>	100.0	399
U	U	J	71.3	JUS1	JUS	July	Metazoa	Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	<i>Ranatra linearis</i>	99.5	319
U	U	J	71.3	JUS1	JUS	July	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	98.2	283
U	U	J	71.3	JUS1	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.9	801
7492	U	J	242.7	JUS2	JUS	July	Metazoa	Arthropoda	Diplopoda	Glomerida	Glomeridae	Glomeris	<i>Glomeris marginata</i>	98.1	688
7492	U	J	242.7	JUS2	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	100.0	232
7492	U	J	242.7	JUS2	JUS	July	Metazoa	Arthropoda	Insecta	Hymenoptera	Vespidae	Vespula	<i>Vespula vulgaris</i>	100.0	223
7492	U	J	242.7	JUS2	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	234
6505	U	J	151	JUS3	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Dytiscidae	Cybister	<i>Cybister lateralimarginalis</i>	100.0	268
6505	U	J	151	JUS3	JUS	July	Arthropoda	Arthropoda	Insecta	Diptera	Fanniidae	Fannia	<i>Fannia fuscitibia</i>	97.1	182
6505	U	J	151	JUS3	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrochara	<i>Hydrochara caraboides</i>	98.0	247
6505	U	J	151	JUS3	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrochara	<i>Hydrochara flavipes</i>	98.5	189
6505	U	J	151	JUS3	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrochara	<i>Hydrochara obtusata</i>	98.6	222
6505	U	J	151	JUS3	JUS	July	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	98.7	261
6505	U	J	151	JUS3	JUS	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus occultus</i>	100.0	216
6505	U	J	151	JUS3	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	431
9164	F	A	614	JUS4	JUS	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	100.0	280
9164	F	A	614	JUS4	JUS	July	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion	<i>Coenagrion puella</i>	99.5	518
9164	F	A	614	JUS4	JUS	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Cladotanytarsus	<i>Cladotanytarsus pallidus</i>	100.0	258
9164	F	A	614	JUS4	JUS	July	Metazoa	Arthropoda	Insecta	Lepidoptera	Geometridae	Plagodis	<i>Plagodis dolabraria</i>	100.0	581
9164	F	A	614	JUS4	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	590
2795	U	J	221	JUS5	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	761
7499	U	J	183	JUS6	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.9	233
7499	U	J	183	JUS6	JUS	July	Metazoa	Arthropoda	Insecta	Hemiptera	Issidae	Issus	<i>Issus coleoptratus</i>	100.0	226
7499	U	J	183	JUS6	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	459

6510	U	J	189	JUS7	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	100.0	477
6510	U	J	189	JUS7	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Chrysomeloidea	Donacia	<i>Donacia vulgaris</i>	98.5	365
6510	U	J	189	JUS7	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrochara	<i>Hydrochara caraboides</i>	96.8	759
6510	U	J	189	JUS7	JUS	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Micropsectra	<i>Micropsectra notescens</i>	99.7	709
6510	U	J	189	JUS7	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Noteridae	Noterus	<i>Noterus clavicornis</i>	100.0	709
6510	U	J	189	JUS7	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	904
6510	U	J	189	JUS7	JUS	July	Viridiplantae	Streptophyta	Liliopsida	Poales	Typhaceae	Typha	<i>Typha angustifolia</i>	100.0	601
6509	U	J	209	JUS8	JUS	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis luctuosa</i>	98.9	216
6509	U	J	209	JUS8	JUS	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Baetidae	Cloeon	<i>Cloeon dipterum</i>	100.0	388
6509	U	J	209	JUS8	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	99.3	395
6509	U	J	209	JUS8	JUS	July	Metazoa	Arthropoda	Insecta	Coleoptera	Noteridae	Noterus	<i>Noterus clavicornis</i>	100.0	303
6509	U	J	209	JUS8	JUS	July	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	97.4	209
6509	U	J	209	JUS8	JUS	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	471
6509	U	J	209	JUS8	JUS	July	Viridiplantae	Streptophyta	Liliopsida	Poales	Typhaceae	Typha	<i>Typha angustifolia</i>	99.4	794
U	F	A	670	LAC01	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	100.0	211
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	99.2	474
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Culicidae	Culicidae	<i>Culicinae sp.</i>	100.0	276
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Drosophila	Drosophila	<i>Drosophila sp.</i>	100.0	228
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Forcipomyia	<i>Forcipomyia bipunctata</i>	100.0	212
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Insecta	Hymenoptera	Charipinae	Phaenoglyphis	<i>Phaenoglyphis villosa</i>	100.0	216
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Psychodidae	Psychoda	<i>Psychoda alternata</i>	99.2	249
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Sarcophagidae	Sarcophaga	<i>Sarcophaga variegata</i>	99.7	287
U	F	A	670	LAC01	LAC	July	Metazoa	Arthropoda	Insecta	Coleoptera	Corylophidae	Sericoderus	<i>Sericoderus lateralis</i>	97.1	218
U	F	A	670	LAC01	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	504
U	F	A	733	LAC02	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	99.4	169
U	F	A	733	LAC02	LAC	July	Metazoa	Ectoprocta	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	98.0	575
U	F	A	733	LAC02	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	100.0	214
U	F	A	733	LAC02	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes	<i>Glyptotendipes glaucus</i>	99.3	255
U	F	A	733	LAC02	LAC	July	Metazoa	Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	<i>Ranatra linearis</i>	99.3	266
U	F	A	733	LAC02	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	97.9	261
U	F	A	733	LAC02	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	309
U	M	A	432	LAC03	LAC	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Baetidae	Cloeon	<i>Cloeon dipterum</i>	99.1	235
U	M	A	432	LAC03	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	97.5	228
U	M	A	432	LAC03	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus nuditarsis</i>	98.3	234
U	M	A	432	LAC03	LAC	July	Metazoa	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	<i>Gammarus fossarum</i>	100.0	226
U	M	A	432	LAC03	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes	<i>Glyptotendipes glaucus</i>	97.7	541
U	M	A	432	LAC03	LAC	July	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Lasius	<i>Lasius niger</i>	100.0	249
U	M	A	432	LAC03	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	235

U	F	A	812	LAC04	LAC	July	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.1	235
U	F	A	812	LAC04	LAC	July	Metazoa	Ectoprocta	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	99.0	530
U	F	A	812	LAC04	LAC	July	Metazoa	Arthropoda	Insecta	Coleoptera	Latridiidae	Cortinicara	<i>Corticaria gibbosa</i>	100.0	258
U	F	A	812	LAC04	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Culicidae	Culex	<i>Culex pipiens</i>	100.0	226
U	F	A	812	LAC04	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	406
U	F	A	812	LAC04	LAC	July	Viridiplantae	Streptophyta	Liliopsida	Poales	Typhaceae	Typha	<i>Typha angustifolia</i>	100.0	226
U	F	A	812	LAC04	LAC	July	Metazoa	Arthropoda	Insecta	Odonata	Corduliidae	Somatochlora	<i>Somatochlora arctica</i>	100.0	225
U	M	A	402	LAC05	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.9	210
U	M	A	402	LAC05	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	100.0	231
U	M	A	402	LAC05	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Calliphoridae	Bellardia	<i>Bellardia vulgaris</i>	98.9	706
U	M	A	402	LAC05	LAC	July	Metazoa	Arthropoda	Insecta	Hemiptera	Aphidiidae	Rhopalosiphum	<i>Rhopalosiphum nymphaeae</i>	100.0	624
U	M	A	402	LAC05	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	243
U	M	A	364	LAC06	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus pallidivittatus</i>	98.9	401
U	M	A	364	LAC06	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.2	261
U	M	A	364	LAC06	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.7	302
U	F	A	634	LAC07	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.8	225
U	F	A	634	LAC07	LAC	July	Metazoa	Ectoprocta	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	99.3	686
U	F	A	634	LAC07	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus annularius</i>	100.0	469
U	F	A	634	LAC07	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia muelleri</i>	100.0	255
U	F	A	634	LAC07	LAC	July	Metazoa	Chordata	Amphibia	Anura	Ranidae	Rana	<i>Rana temporaria</i>	100.0	299
U	F	A	634	LAC07	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	99.0	199
U	F	A	634	LAC07	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	516
U	U	J	160	LAC08	LAC	July	Metazoa	Ectoprocta	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	100.0	220
U	U	J	160	LAC08	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	99.8	637
U	U	J	160	LAC08	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus sp.</i>	97.4	252
U	U	J	160	LAC08	LAC	July	Metazoa	Arthropoda	Insecta	Hemiptera	Aphrophoridae	Lepyronia	<i>Lepyronia coleoptrata</i>	100.0	283
U	U	J	160	LAC08	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	422
U	U	J	125	LAC09	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes	<i>Glyptotendipes glaucus</i>	100.0	272
U	U	J	125	LAC09	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	872
U	U	J	133	LAC10	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	100.0	266
U	U	J	133	LAC10	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Culicidae	Culex	<i>Culex pipiens</i>	100.0	429
U	U	J	133	LAC10	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Sarcophagidae	Sarcophaga	<i>Sarcophaga lehmanni</i>	99.3	297
U	U	J	133	LAC10	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	393
U	M	A	394	LAC11	LAC	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Baetidae	Cloeon	<i>Cloeon dipterum</i>	99.7	438
U	M	A	394	LAC11	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.6	277
U	M	A	394	LAC11	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.8	204
U	M	A	394	LAC11	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	98.3	574
U	M	A	394	LAC11	LAC	July	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Lasius	<i>Lasius niger</i>	100.0	770

U	M	A	394	LAC11	LAC	July	Metazoa	Arthropoda	Arachnida	Araneae	Lycosidae	Pirata	<i>Pirata piraticus</i>	100.0	709
U	M	A	394	LAC11	LAC	July	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	99.0	496
U	M	A	394	LAC11	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	750
U	M	A	231	LAC12	LAC	July	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Ischnura	<i>Ischnura elegans</i>	100.0	319
U	M	A	231	LAC12	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.9	217
U	M	A	231	LAC12	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes	<i>Glyptotendipes glaucus</i>	99.0	265
U	M	A	231	LAC12	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	99.5	257
U	M	A	231	LAC12	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	262
U	F	A	722	LAC13	LAC	July	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.0	229
U	F	A	722	LAC13	LAC	July	Metazoa	Ectoprocta	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	98.0	496
U	F	A	722	LAC13	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	406
U	F	A	722	LAC13	LAC	July	Metazoa	Arthropoda	Insecta	Hemiptera	Aphidiae	Rhopalosiphum	<i>Rhopalosiphum nymphaeae</i>	99.8	588
U	F	A	722	LAC13	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.3	924
U	M	A	475	LAC14	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.3	605
U	M	A	475	LAC14	LAC	July	Metazoa	Arthropoda	Insecta	Hymenoptera	Braconidae	Binodoxys	<i>Binodoxys acalephae</i>	99.8	493
U	M	A	475	LAC14	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Calliphoridae	Calliphoridae	<i>Calliphoridae sp.</i>	98.6	491
U	M	A	475	LAC14	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Campnocladius	<i>Campnocladius stercorarius</i>	99.5	533
U	M	A	475	LAC14	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Forcipomyia	<i>Forcipomyia bipunctata</i>	99.2	446
U	M	A	475	LAC14	LAC	July	Metazoa	Arthropoda	Insecta	Hymenoptera	Charipinae	Phaenoglyphis	<i>Phaenoglyphis villosa</i>	99.7	644
U	M	A	475	LAC14	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Psychodidae	Psychoda	<i>Psychoda alternata</i>	100.0	300
U	M	A	475	LAC14	LAC	July	Metazoa	Arthropoda	Insecta	Hemiptera	Aphidiae	Rhopalosiphum	<i>Rhopalosiphum nymphaeae</i>	99.8	559
U	M	A	475	LAC14	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	571
U	F	A	758	LAC15	LAC	July	Metazoa	Ectoprocta	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	99.6	607
U	F	A	758	LAC15	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	98.3	225
U	F	A	758	LAC15	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus sp.</i>	97.2	294
U	F	A	758	LAC15	LAC	July	Metazoa	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	<i>Gammarus fossarum</i>	100.0	291
U	F	A	758	LAC15	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	538
U	F	A	873	LAC16	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Muscidae	Musca	<i>Musca domestica</i>	100.0	612
U	F	A	873	LAC16	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.9	211
U	F	A	873	LAC16	LAC	July	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	439
U	F	A	873	LAC16	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Culicidae	Culex	<i>Culex pipiens</i>	99.4	207
U	F	A	873	LAC16	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	97.4	281
U	F	A	873	LAC16	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Parachironomus	<i>Parachironomus monochromus</i>	97.4	237
U	F	A	873	LAC16	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	643
U	M	A	440	LAC17	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	99.2	367
U	M	A	440	LAC17	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus sp.</i>	100.0	254
U	M	A	440	LAC17	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	254
U	U	J	171	LAC18	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	100.0	203

U	U	J	171	LAC18	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	100.0	229
U	U	J	171	LAC18	LAC	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	99.0	226
U	U	J	171	LAC18	LAC	July	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes	<i>Glyptotendipes glaucus</i>	99.2	250
U	U	J	171	LAC18	LAC	July	Metazoa	Arthropoda	Insecta	Hemiptera	Corixidae	Sigara	<i>Sigara striata</i>	100.0	709
U	U	J	171	LAC18	LAC	July	Metazoa	Annelida	Clitellata	Crassiclitellata	Spanganophiloidea	Spanganophilus	<i>Spanganophilus tamesis</i>	99.5	280
U	U	J	171	LAC18	LAC	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	100.0	172
U	U	J	171	LAC18	LAC	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	563
141	M	A	390	MDV01	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	709
141	M	A	390	MDV01	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
141	M	A	390	MDV01	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Streblotricum	<i>Streblotrichum convolutum</i>	99.2	650
141	M	A	390	MDV01	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus tendens</i>	98.7	706
141	M	A	390	MDV01	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Aphididae	Ovatus	<i>Ovatus crataegarius</i>	100.0	239
141	M	A	390	MDV01	MDV	April	Metazoa	Arthropoda	Insecta	Lepidoptera	Chironomidae	Phaenosectra	<i>Phaenopsectra punctipes</i>	97.0	233
141	M	A	390	MDV01	MDV	April	Metazoa	Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	99.4	217
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.7	1055
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus incana</i>	100.0	287
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	98.3	834
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	<i>Juncus effusus</i>	99.3	272
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	100.0	584
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus nigra</i>	98.6	278
437	U	J	171	MDV02	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus sp.</i>	100.0	584
437	U	J	170	MDV02	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.8	438
437	F	A	501	MDV03	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	98.9	548
437	F	A	501	MDV03	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Brachytron	<i>Brachytron pratense</i>	98.3	385
437	F	A	501	MDV03	MDV	April	Metazoa	Chordata	Mammalia	Rodentia	Castoridae	Castor	<i>Castor fiber</i>	98.1	259
437	F	A	501	MDV03	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	<i>Juncus effusus</i>	99.3	285
437	F	A	501	MDV03	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	709
437	F	A	501	MDV03	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	257
437	F	A	501	MDV03	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus sp.</i>	99.7	307
437	F	A	501	MDV03	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Pyrrhosoma	<i>Pyrrhosoma nymphula</i>	99.8	706
437	F	A	501	MDV03	MDV	April	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	98.2	365
437	F	A	501	MDV03	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	318
437	F	A	501	MDV03	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	98.8	366
212	M	A	300	MDV04	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	98.9	472
212	M	A	300	MDV04	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.9	889
212	M	A	300	MDV04	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	99.8	707
212	M	A	300	MDV04	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.8	1040

212	M	A	300	MDV04	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	Potamogeton	<i>Potamogeton perfoliatus</i>	99.7	1056
212	M	A	300	MDV04	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.3	889
212	M	A	300	MDV04	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.7	594
212	M	A	300	MDV04	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.8	727
212	M	A	300	MDV04	MDV	April	Metazoa	Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	98.8	328
431	M	A	271	MDV05	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Anax	<i>Anax imperator</i>	99.4	365
431	M	A	271	MDV05	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.7	587
431	M	A	271	MDV05	MDV	April	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.0	233
431	M	A	271	MDV05	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	709
431	M	A	271	MDV05	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1379
431	M	A	271	MDV05	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	99.5	987
431	M	A	271	MDV05	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.1	365
95	M	A	312	MDV06	MDV	April	Viridiplantae	Streptophyta	Equisetopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	98.9	548
95	M	A	312	MDV06	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.8	901
95	M	A	312	MDV06	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
95	M	A	312	MDV06	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus nigra</i>	100.0	256
95	M	A	312	MDV06	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus sp.</i>	100.0	256
95	M	A	312	MDV06	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	426
95	M	A	312	MDV06	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	100.0	366
95	M	A	312	MDV06	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus vittatus</i>	99.2	316
198	F	A	636	MDV07	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus subcordata</i>	98.3	176
198	F	A	636	MDV07	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Hypnaceae	Hypnum	<i>Hypnum cupressiforme</i>	99.7	599
198	F	A	636	MDV07	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
198	F	A	636	MDV07	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	98.9	370
198	F	A	636	MDV07	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus nigra</i>	99.8	455
198	F	A	636	MDV07	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	96.5	541
297	M	A	265	MDV08	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	98.3	696
297	M	A	265	MDV08	MDV	April	Metazoa	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	<i>Gammarus fossarum</i>	97.2	365
297	M	A	265	MDV08	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	706
297	M	A	265	MDV08	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
297	M	A	265	MDV08	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Syntichia	<i>Syntichia laevipila</i>	98.3	579
297	M	A	265	MDV08	MDV	April	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	98.4	387
297	M	A	265	MDV08	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Barbula	<i>Barbula unguiculata</i>	98.1	579
297	M	A	265	MDV08	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	398
297	M	A	265	MDV08	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus pallidivittatus</i>	99.0	423
297	M	A	265	MDV08	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus tendens</i>	98.1	706
297	M	A	265	MDV08	MDV	April	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Proasellus	<i>Proasellus coxalis</i>	99.2	387
75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pottiosis	<i>Pottiosis caespitosa</i>	98.7	620

75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	99.1	579
75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	891
75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	99.3	284
75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.1	915
75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	99.6	504
75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	379
75	M	A	334	MDV09	MDV	April	Metazoa	Chordata	Amphibia	Anura	Bufoidae	Bufo	<i>Bufo bufo</i>	98.0	207
75	M	A	334	MDV09	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.6	504
123	F	A	701	MDV10	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	100.0	265
123	F	A	701	MDV10	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.4	889
123	F	A	701	MDV10	MDV	April	Metazoa	Arthropoda	Insecta	Ephemeroptera	Baetidae	Cloeon	<i>Cloeon dipterum</i>	99.8	706
123	F	A	701	MDV10	MDV	April	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	<i>Radix auricularia</i>	99.2	263
123	F	A	701	MDV10	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	100.0	282
123	F	A	701	MDV10	MDV	April	Metazoa	Arthropoda	Insecta	Lepidoptera	Notodontidae	Pheosia	<i>Pheosia tremula</i>	98.9	212
438	U	J	138	MDV11	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.8	961
438	U	J	138	MDV11	MDV	April	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	100.0	275
438	U	J	138	MDV11	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	712
438	U	J	138	MDV11	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.9	1041
438	U	J	138	MDV11	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.1	338
438	U	J	138	MDV11	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	98.1	635
438	U	J	138	MDV11	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Calopterygidae	Calopteryx	<i>Calopteryx virgo</i>	98.9	712
438	U	J	138	MDV11	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	95.9	591
438	U	J	138	MDV11	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoridae	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.8	709
438	U	J	138	MDV11	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion	<i>Coenagrion pulchellum</i>	99.8	710
438	U	J	138	MDV11	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.8	502
131	M	A	338	MDV12	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	98.1	421
131	M	A	338	MDV12	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	100.0	497
131	M	A	338	MDV12	MDV	April	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	98.5	364
131	M	A	338	MDV12	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	99.7	436
131	M	A	338	MDV12	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1050
131	M	A	338	MDV12	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	98.0	669
131	M	A	338	MDV12	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.6	269
131	M	A	338	MDV12	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Notonectidae	Notonecta	<i>Notonecta glauca</i>	98.1	266
131	M	A	338	MDV12	MDV	April	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	99.7	300
131	M	A	338	MDV12	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.6	502
400	M	A	280	MDV14	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	<i>Juncus effusus</i>	99.3	282
400	M	A	280	MDV14	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	975
400	M	A	280	MDV14	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.8	543

400	M	A	280	MDV14	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.8	364
400	M	A	280	MDV14	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.3	282
400	M	A	280	MDV14	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	<i>Cricotopus bicinctus</i>	98.3	229
400	M	A	280	MDV14	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Cladium	<i>Cladium mariscus</i>	100.0	870
U	M	A	293	MDV15	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	98.9	474
U	M	A	293	MDV15	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	99.6	271
U	M	A	293	MDV15	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
U	M	A	293	MDV15	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.7	601
U	M	A	293	MDV15	MDV	April	Metazoa	Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	99.1	708
322	F	A	525	MDV16	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	98.8	709
322	F	A	525	MDV16	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
322	F	A	525	MDV16	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	100.0	168
322	F	A	525	MDV16	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.4	347
441	U	J	131	MDV17	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.9	823
441	U	J	131	MDV17	MDV	April	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum ramosissimum</i>	99.1	234
441	U	J	131	MDV17	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	709
441	U	J	131	MDV17	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
441	U	J	131	MDV17	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.7	922
441	U	J	131	MDV17	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	98.4	306
441	U	J	131	MDV17	MDV	April	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	98.5	365
441	U	J	131	MDV17	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	232
441	U	J	131	MDV17	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	<i>Cricotopus triannulatus</i>	99.2	709
441	U	J	131	MDV17	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Orthocladiinae	<i>Cricotopus triannulatus</i>	100.0	709
170	F	A	609	MDV18	MDV	April	Metazoa	Arthropoda	Insecta	Coleoptera	Buprestidae	Agrius	<i>Agrius angustulus</i>	100.0	179
170	F	A	609	MDV18	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus sp.</i>	99.8	493
170	F	A	609	MDV18	MDV	April	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	97.5	281
170	F	A	609	MDV18	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	975
170	F	A	609	MDV18	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	Potamogeton	<i>Potamogeton perfoliatus</i>	99.7	984
170	F	A	609	MDV18	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	100.0	241
170	F	A	609	MDV18	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	98.9	560
170	F	A	609	MDV18	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.2	255
170	F	A	609	MDV18	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoridae	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.1	365
170	F	A	609	MDV18	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Cladium	<i>Cladium mariscus</i>	100.0	868
309	F	A	564	MDV19	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	99.8	709
309	F	A	564	MDV19	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1042
1	F	A	564	MDV21	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus sp.</i>	99.3	579
1	F	A	564	MDV21	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	385
1	F	A	564	MDV21	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	100.0	262

1	F	A	564	MDV21	MDV	April	Metazoa	Arthropoda	Malacostrca	Isopoda	Armadillidiidae	Armadillidium	<i>Armadillidium nasatum</i>	99.1	224
105	M	A	335	MDV22	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.5	634
105	M	A	335	MDV22	MDV	April	Metazoa	Arthropoda	Malacostrca	Amphipoda	Gammaridae	Gammarus	<i>Gammarus fossarum</i>	98.5	263
105	M	A	335	MDV22	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Hypnaceae	Hypnum	<i>Hypnum cypresiforme</i>	100.0	249
105	M	A	335	MDV22	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
105	M	A	335	MDV22	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	99.9	744
105	M	A	335	MDV22	MDV	April	Metazoa	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Donacia	<i>Donacia clavipes</i>	97.3	213
105	M	A	335	MDV22	MDV	April	Metazoa	Chordata	Amphibia	Anura	Bufoidae	Bufo	<i>Bufo bufo</i>	98.0	208
69	F	A	510	MDV23	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	99.1	579
69	F	A	510	MDV23	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	100.0	308
69	F	A	510	MDV23	MDV	April	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.7	709
69	F	A	510	MDV23	MDV	April	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.1	288
69	F	A	510	MDV23	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	98.5	365
69	F	A	510	MDV23	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.8	547
69	F	A	510	MDV23	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	Potamogeton	<i>Potamogeton perfoliatus</i>	99.6	528
69	F	A	510	MDV23	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	98.5	274
69	F	A	510	MDV23	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	398
69	F	A	510	MDV23	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion	<i>Coenagrion pulchellum</i>	99.8	715
69	F	A	510	MDV23	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	98.5	274
442	M	A	261	MDV24	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	99.3	729
442	M	A	261	MDV24	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	100.0	307
442	M	A	261	MDV24	MDV	April	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	97.2	365
442	M	A	261	MDV24	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	639
442	M	A	261	MDV24	MDV	April	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.5	601
442	M	A	261	MDV24	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	99.6	637
442	M	A	261	MDV24	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	100.0	344
442	M	A	261	MDV24	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	398
267	M	A	358	MDV25	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	622
267	M	A	358	MDV25	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
60	M	A	358	MDV26	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
60	M	A	358	MDV26	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	99.8	629
60	M	A	358	MDV26	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	99.3	601
60	M	A	358	MDV26	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	398
60	M	A	358	MDV26	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.6	503
216	M	A	408	MDV27	MDV	April	Metazoa	Arthropoda	Insecta	Coleoptera	Curculionidae	Dendroctonus	<i>Dendroctonus ponderosae</i>	99.5	220
216	M	A	408	MDV27	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus flavicornis</i>	98.5	414
216	M	A	408	MDV27	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
216	M	A	408	MDV27	MDV	April	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	100.0	370

216	M	A	408	MDV27	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.8	415
216	M	A	408	MDV27	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.8	503
216	M	A	408	MDV27	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	318
216	M	A	408	MDV27	MDV	April	Metazoa	Arthropoda	Malacostraca	Isopoda	Philosciidae	Philoscia	<i>Philoscia muscorum</i>	99.1	211
216	M	A	408	MDV27	MDV	April	Metazoa	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	Deroberas	<i>Deroberas laeve</i>	99.1	211
157	M	A	460	MDV29	MDV	April	Metazoa	Arthropoda	Insecta	Ephemeroptera	Asellidae	Baetis	<i>Baetis rhodani</i>	99.5	706
157	M	A	460	MDV29	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.6	889
157	M	A	460	MDV29	MDV	April	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Brachytron	<i>Brachytron pratense</i>	100.0	709
157	M	A	460	MDV29	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1062
157	M	A	460	MDV29	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus sp.</i>	99.5	957
157	M	A	460	MDV29	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	97.8	503
157	M	A	460	MDV29	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiacae	Tortella	<i>Tortella tortuosa</i>	99.6	502
157	M	A	460	MDV29	MDV	April	Metazoa	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	97.5	365
157	M	A	460	MDV29	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.2	392
157	M	A	460	MDV29	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiacae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.6	502
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.9	1055
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiacae	Chionoloma	<i>Chionoloma tenuirostre</i>	99.2	624
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	<i>Juncus effusus</i>	99.2	250
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	889
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.8	998
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.2	241
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.2	250
166	M	A	315	MDV30	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.7	352
166	M	A	315	MDV30	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Psychodidae	Pericomma	<i>Pericomma blandula</i>	98.2	713
166	M	A	315	MDV30	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiacae	Pleurochaete	<i>Pleurochaete squarrosa</i>	100.0	521
92	M	A	361	MDV32	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.3	455
92	M	A	361	MDV32	MDV	April	Metazoa	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	<i>Gammarus pulex</i>	99.8	709
92	M	A	361	MDV32	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	991
92	M	A	361	MDV32	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	100.0	340
92	M	A	361	MDV32	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus nigra</i>	99.6	1042
92	M	A	361	MDV32	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	100.0	343
92	M	A	361	MDV32	MDV	April	Metazoa	Arthropoda	Insecta	Diptera	Cecidomyiidae	Cecidomyiidae	<i>Cecidomyiidae sp.</i>	99.4	365
92	M	A	361	MDV32	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Hemiptera	Hemiptera	<i>Hemiptera sp.</i>	100.0	233
92	M	A	361	MDV32	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiacae	Pleurochaete	<i>Pleurochaete squarrosa</i>	100.0	312
415	M	A	256	MDV33	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.2	676
U	U	J	188	MDV34	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	<i>Juncus effusus</i>	99.3	272
U	U	J	188	MDV34	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1049
U	U	J	188	MDV34	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.8	425

U	U	J	188	MDV34	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	98.2	364
U	U	J	188	MDV34	MDV	April	Metazoa	Arthropoda	Insecta	Hemiptera	Notodontidae	Notonecta	<i>Notonecta glauca</i>	99.5	214
306	M	A	307	MDV35	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	99.0	523
306	M	A	307	MDV35	MDV	April	Metazoa	Arthropoda	Insecta	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	97.7	216
306	M	A	307	MDV35	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.3	294
306	M	A	307	MDV35	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.9	971
306	M	A	307	MDV35	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.3	1026
306	M	A	307	MDV35	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.4	314
306	M	A	307	MDV35	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	100.0	243
306	M	A	307	MDV35	MDV	April	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoxyn	<i>Parapoxyn stratiotata</i>	96.2	269
306	M	A	307	MDV35	MDV	April	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.4	315
436	U	J	134	MDV36	MDV	April	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
436	U	J	134	MDV36	MDV	April	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	100.0	276
8	F	A	642	MDV37	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Phryganeidae	Agrypnia	<i>Agrypnia varia</i>	99.8	444
8	F	A	642	MDV37	MDV	May	Metazoa	Chordata	Mammalia	Rodentia	Muridae	Apodemus	<i>Apodemus flavicollis</i>	99.6	282
8	F	A	642	MDV37	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	98.9	709
8	F	A	642	MDV37	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	99.8	709
8	F	A	642	MDV37	MDV	May	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	99.0	393
8	F	A	642	MDV37	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephiloidea	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	710
8	F	A	642	MDV37	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
8	F	A	642	MDV37	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.7	999
8	F	A	642	MDV37	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	1035
8	F	A	642	MDV37	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoxyn	<i>Parapoxyn stratiotata</i>	100.0	241
8	F	A	642	MDV37	MDV	May	Metazoa	Arthropoda	Malacostraca	Isopoda	Philosciidae	Philoscia	<i>Philoscia muscorum</i>	97.6	709
8	F	A	642	MDV37	MDV	May	Metazoa	Annelida	Clitellata	Haplotauxida	Spanganophilidae	Spanganophilus	<i>Spanganophilus tamesis</i>	100.0	706
8	F	A	642	MDV37	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus pallidicornis</i>	99.1	709
8	F	A	642	MDV37	MDV	May	Metazoa	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	Deroceras	<i>Deroceras laeve</i>	99.8	709
8	F	A	642	MDV37	MDV	May	Metazoa	Mollusca	Gastropoda	Stylommatophora	Gastrodontidae	Zonitoides	<i>Zonitoides nitidus</i>	99.5	228
8	F	A	642	MDV37	MDV	May	Metazoa	Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	99.7	353
8	F	A	642	MDV37	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Aeshnoidea	Gomphus	<i>Gomphus pulchellus</i>	99.1	365
177	F	A	626	MDV38	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Sapindales	Aceraceae	Acer	<i>Acer pseudoplatanus</i>	100.0	889
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Buprestidae	Agrius	<i>Agrius angustulus</i>	99.4	178
177	F	A	626	MDV38	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.9	1056
177	F	A	626	MDV38	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	99.2	709
177	F	A	626	MDV38	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.7	712
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	100.0	709
177	F	A	626	MDV38	MDV	May	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	98.9	745
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephiloidea	Limnephilus	<i>Limnephilus flavicornis</i>	98.5	227

177	F	A	626	MDV38	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.9	744
177	F	A	626	MDV38	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	<i>Salix sp.</i>	<i>Salix sp.</i>	99.9	1117
177	F	A	626	MDV38	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	<i>Barbula</i>	<i>Barbula unguiculata</i>	99.3	5944
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Calopterygidae	Calopteryx	<i>Calopteryx virgo</i>	99.4	715
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Maladera	<i>Maladera holosericea</i>	99.7	713
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoynx	<i>Parapoynx stratiotata</i>	99.7	709
177	F	A	626	MDV38	MDV	May	Metazoa	Platyhelminthes	Turbellaria	Tricladida	Dugesiidae	Schmidtea	<i>Schmidtea polychroa</i>	96.8	225
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus pallidicornis</i>	99.3	710
177	F	A	626	MDV38	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Aeshnoidea	Gomphus	<i>Gomphus pulchellus</i>	99.2	414
282	F	A	640	MDV39	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus luridus</i>	99.0	779
282	F	A	640	MDV39	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus riparius</i>	99.2	709
282	F	A	640	MDV39	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Paratanytarsus	<i>Paratanytarsus laccophilus</i>	99.7	710
282	F	A	640	MDV39	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	<i>Polypedilum tritum</i>	98.5	706
23	M	A	352	MDV40	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Sapindales	Aceraceae	Acer	<i>Acer campestre</i>	100.0	889
23	M	A	352	MDV40	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.7	698
23	M	A	352	MDV40	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Asparagales	Iridaceae	Iris	<i>Iris pseudacorus</i>	100.0	942
23	M	A	352	MDV40	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	890
23	M	A	352	MDV40	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	100.0	340
23	M	A	352	MDV40	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	162
444	M	A	274	MDV41	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.8	899
444	M	A	274	MDV41	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
444	M	A	274	MDV41	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	<i>Salix sp.</i>	<i>Salix sp.</i>	99.8	503
444	M	A	274	MDV41	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.6	895
444	M	A	274	MDV41	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	100.0	465
141	M	A	386	MDV42	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	Potamogeton	<i>Potamogeton perfoliatus</i>	99.5	600
141	M	A	386	MDV42	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	97.2	281
141	M	A	386	MDV42	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoridea	Ilyocoris	<i>Ilyocoris cimicoides</i>	98.8	364
141	M	A	386	MDV42	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.7	595
38	M	A	292	MDV43	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	100.0	287
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Baetidae	Cloeon	<i>Cloeon dipterum</i>	97.9	221
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion	<i>Coenagrion puella</i>	98.2	365
38	M	A	292	MDV43	MDV	May	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	99.3	277
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephiloidea	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	580
38	M	A	292	MDV43	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	975
38	M	A	292	MDV43	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	<i>Salix sp.</i>	<i>Salix sp.</i>	100.0	361
38	M	A	292	MDV43	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Typhaceae	Typha	<i>Typha latifolia</i>	99.1	975
38	M	A	292	MDV43	MDV	May	Arthropoda	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	99.2	712
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Calopterygidae	Calopteryx	<i>Calopteryx virgo</i>	100.0	306

38	M	A	292	MDV43	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	Carex sp.	99.1	1035
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Collembola	Bourletiellidae	Deuterosminthurus	Deuterosminthurus sp.	100.0	318
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Geometridae	Operophtera	Operophtera brumata	99.9	709
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	Polypedilum sp.	97.9	365
38	M	A	292	MDV43	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Procladius	Procladius sp.	97.4	706
38	M	A	292	MDV43	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	Pleurochaete squarrosa	100.0	465
290	F	A	594	MDV44	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	Betula pubescens	99.9	900
290	F	A	594	MDV44	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	Phragmites australis	98.0	601
U	U	J	226	MDV45	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	Alnus alnobetula	99.3	292
U	U	J	226	MDV45	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Hypnaceae	Hypnum	Hypnum cupressiforme	99.1	459
U	U	J	226	MDV45	MDV	May	Metazoa	Annelida	Clitellata	Haplotaxida	Lumbricidae	Lumbricus	Lumbricus terrestris	97.1	365
U	U	J	226	MDV45	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	Populus alba	99.8	540
U	U	J	226	MDV45	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	Salix sp.	99.5	804
U	U	J	226	MDV45	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	Carex sp.	99.1	1101
U	U	J	226	MDV45	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	Ilyocoris cimicoides	98.2	366
U	U	J	226	MDV45	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	Tantytarsus pallidicornis	99.3	365
U	U	J	226	MDV45	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	Utricularia australis	98.5	797
U	U	J	140	MDV46	MDV	May	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	Equisetum arvense	100.0	601
U	U	J	140	MDV46	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	Juncus effusus	98.3	293
U	U	J	140	MDV46	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	Phragmites australis	99.8	602
U	U	J	140	MDV46	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	Carex sp.	98.6	293
U	U	J	140	MDV46	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	Nymphaea alba	99.5	608
U	U	J	140	MDV46	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	Utricularia australis	98.6	530
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	Alnus alnobetula	98.7	731
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	Betula pubescens	99.6	905
U	M	A	294	MDV47	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephiloidea	Limnephilus	Limnephilus flavicornis	98.2	365
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	Phragmites australis	99.7	889
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	Quercus sp.	99.3	425
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	Salix sp.	100.0	265
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Syntichia	Syntichia ruralis	98.6	515
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	Tortella tortuosa	100.0	242
U	M	A	294	MDV47	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Donacia	Donacia clavipes	97.9	251
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	Utricularia australis	99.8	889
U	M	A	294	MDV47	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	Pleurochaete squarrosa	99.4	319
U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	Alnus alnobetula	99.7	335
U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	Betula pubescens	99.5	366
U	F	A	300	MDV48	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	Bithynia tentaculata	99.5	709
U	F	A	300	MDV48	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephiloidea	Limnephilus	Limnephilus flavicornis	100.0	706

U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.3	297
U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	976
U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Typhaceae	Typha	<i>Typha latifolia</i>	99.2	945
U	F	A	300	MDV48	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Calopterygidae	Calopteryx	<i>Calopteryx virgo</i>	98.5	341
U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.8	426
U	F	A	300	MDV48	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus sp.</i>	99.8	706
U	F	A	300	MDV48	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Geometridae	Operophtera	<i>Operophtera brumata</i>	99.4	329
U	F	A	300	MDV48	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Polydipedium	<i>Polydipedium sordens</i>	98.5	365
U	F	A	300	MDV48	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tanytarsus pallidicornis</i>	99.1	706
U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.4	798
U	F	A	300	MDV48	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	99.3	977
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	98.6	349
U	F	A	453	MDV49	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Atripsodes	<i>Atripsodes aterrimus</i>	99.8	709
U	F	A	453	MDV49	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.4	709
U	F	A	453	MDV49	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	99.8	706
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus nigra</i>	99.5	1041
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.7	348
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	98.5	700
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	162
U	F	A	453	MDV49	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Donacia	<i>Donacia clavipes</i>	97.1	365
U	F	A	453	MDV49	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus tendens</i>	98.9	369
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	100.0	527
U	F	A	453	MDV49	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	100.0	464
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus sp.</i>	100.0	317
U	M	A	282	MDV50	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Anax	<i>Anax imperator</i>	99.2	709
U	M	A	282	MDV50	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Atripsodes	<i>Atripsodes aterrimus</i>	99.5	225
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.4	538
U	M	A	282	MDV50	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.4	404
U	M	A	282	MDV50	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	99.4	709
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus alba</i>	96.6	559
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.0	288
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	99.3	602
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Bryales	Bryaceae	Bryum	<i>Bryum argenteum</i>	98.8	601
U	M	A	282	MDV50	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Parachironomus	<i>Parachironomus sp.</i>	96.6	320
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	97.4	894
U	M	A	282	MDV50	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.4	315

148	F	A	641	MDV51	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	100.0	228
148	F	A	641	MDV51	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	98.1	365
148	F	A	641	MDV51	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	97.1	365
148	F	A	641	MDV51	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	601
148	F	A	641	MDV51	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	97.1	209
148	F	A	641	MDV51	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Chrysomelidae	Donacia	<i>Donacia clavipes</i>	98.5	537
148	F	A	641	MDV51	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Formicidae	<i>Formicidae sp.</i>	97.9	365
148	F	A	641	MDV51	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Noteridae	Noterus	<i>Noterus clavigornis</i>	99.4	365
148	F	A	641	MDV51	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.5	601
148	F	A	641	MDV51	MDV	May	Metazoa	Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	98.3	364
U	U	J	130	MDV52	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	99.4	315
U	U	J	130	MDV52	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	99.1	364
U	U	J	130	MDV52	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.6	316
U	U	J	130	MDV52	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Asparagales	Iridaceae	Iris	<i>Iris pseudacorus</i>	99.8	438
U	U	J	130	MDV52	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.5	602
U	U	J	130	MDV52	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.5	750
U	U	J	130	MDV52	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	98.8	364
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Hypnaceae	Hypnum	<i>Hypnum cupressiforme</i>	99.4	339
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.9	743
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.8	1003
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	100.0	299
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.4	635
U	U	J	138	MDV53	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.7	230
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	99.5	608
U	U	J	138	MDV53	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	<i>Ranatra linearis</i>	99.4	365
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.4	863
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	99.2	945
U	U	J	138	MDV53	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.6	504
U	U	J	80	MDV54	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	99.1	214
U	U	J	80	MDV54	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	100.0	626
U	U	J	80	MDV54	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.9	709
U	U	J	80	MDV54	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephiloidea	Limnephilus	<i>Limnephilus flavicornis</i>	97.9	365
U	U	J	80	MDV54	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.8	1008
U	U	J	80	MDV54	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus sp.</i>	99.1	632
U	U	J	80	MDV54	MDV	May	Arthropoda	Arthropoda	Malacostraca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	97.9	365
U	U	J	80	MDV54	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Dryopidae	Dryops	<i>Dryops liridus</i>	99.1	368
U	U	J	80	MDV54	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Stratiomyidae	Oplodontha	<i>Oplodontha viridula</i>	98.5	370
U	U	J	80	MDV54	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	<i>Ranatra linearis</i>	99.1	365

U	U	J	80	MDV54	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tanytarsus medius</i>	98.5	365
U	U	J	80	MDV54	MDV	May	Metazoa	Arthropoda	Collembola	Entomobryomorpha	Entomobryidae	Willowsia	<i>Willowsia nigromaculata</i>	98.9	343
U	U	J	154	MDV55	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	99.1	364
U	U	J	154	MDV55	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	96.5	365
U	U	J	154	MDV55	MDV	May	Metazoa	Arthropoda	Malacostrca	Amphipoda	Gammaridae	Gammarus	<i>Gammarus pulex</i>	99.7	706
U	U	J	154	MDV55	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
U	U	J	154	MDV55	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Psectrocladius	<i>Psectrocladius sp.</i>	96.8	365
U	U	J	27	MDV56	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	99.0	688
U	U	J	27	MDV56	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.3	569
U	U	J	27	MDV56	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnophilus	<i>Limnophilus decipiens</i>	99.1	365
U	U	J	27	MDV56	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.8	1063
U	U	J	27	MDV56	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.8	1026
U	U	J	27	MDV56	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.0	364
U	U	J	27	MDV56	MDV	May	Arthropoda	Arthropoda	Malacostrca	Isopoda	Asellidae	Asellus	<i>Asellus aquaticus</i>	99.2	709
U	U	J	27	MDV56	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	96.7	674
U	U	J	27	MDV56	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Helochares	<i>Helochares obscurus</i>	99.4	365
U	U	J	27	MDV56	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Stratiomyidae	Oplodontha	<i>Oplodontha viridula</i>	98.2	369
U	F	A	462	MDV58	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Gerridae	Gerris	<i>Gerris argentatus</i>	98.9	300
U	F	A	462	MDV58	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.7	890
U	F	A	462	MDV58	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	Enochrus	<i>Enochrus testaceus</i>	99.8	594
U	F	A	462	MDV58	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Formicidae sp.	<i>Formicidae sp.</i>	97.3	716
U	F	A	462	MDV58	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.8	709
U	F	A	462	MDV58	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Stratiomyidae	Oplodontha	<i>Oplodontha viridula</i>	97.3	365
U	F	A	462	MDV58	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.5	798
209	M	A	406	MDV59	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Athripsodes	<i>Athripsodes aterrimus</i>	99.2	713
209	M	A	406	MDV59	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	98.7	365
209	M	A	406	MDV59	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	99.8	709
209	M	A	406	MDV59	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.9	956
209	M	A	406	MDV59	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Barbula	<i>Barbula unguiculata</i>	97.9	589
209	M	A	406	MDV59	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	214
209	M	A	406	MDV59	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tanytarsus lactescens</i>	98.3	309
U	M	A	316	MDV60	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	98.4	366
U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Malacostrca	Amphipoda	Gammaridae	Gammarus	<i>Gammarus fossarum</i>	98.0	365
U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnophilus	<i>Limnophilus decipiens</i>	99.2	262
U	M	A	316	MDV60	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.9	1104
U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Lestidae	Sympecma	<i>Sympecma fusca</i>	98.2	365
U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.1	364
U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus pallidivittatus</i>	99.8	706

U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Formicidae	<i>Formicidae sp.</i>	97.3	716
U	M	A	316	MDV60	MDV	May	Metazoa	Annelida	Clitellata	Hirudinida	Glossiphoniidae	Hemiclepsis	<i>Hemiclepsis marginata</i>	95.9	316
U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Psectrocladius	<i>Psectrocladius sp.</i>	96.8	364
U	M	A	316	MDV60	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus pallidicornis</i>	99.2	705
U	M	A	316	MDV60	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	100.0	606
128	F	A	565	MDV61	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Phryganeidae	Agrypnia	<i>Agrypnia varia</i>	100.0	462
128	F	A	565	MDV61	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.2	889
128	F	A	565	MDV61	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Atripsodes	<i>Atripsodes aterrimus</i>	99.8	709
128	F	A	565	MDV61	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.5	709
128	F	A	565	MDV61	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	99.8	709
128	F	A	565	MDV61	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.8	510
128	F	A	565	MDV61	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	Picea	<i>Picea sp.</i>	99.8	1003
128	F	A	565	MDV61	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.8	883
128	F	A	565	MDV61	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.6	745
128	F	A	565	MDV61	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Paratanytarsus	<i>Paratanytarsus bituberculatus</i>	98.2	709
128	F	A	565	MDV61	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus lactescens</i>	98.0	281
128	F	A	565	MDV61	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	99.4	945
U	U	J	176	MDV62	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus alnobetula</i>	99.4	317
U	U	J	176	MDV62	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.6	565
U	U	J	176	MDV62	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	100.0	262
U	U	J	176	MDV62	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	100.0	262
U	U	J	176	MDV62	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
U	U	J	176	MDV62	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.8	437
U	U	J	176	MDV62	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	99.1	703
U	U	J	176	MDV62	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	398
U	U	J	176	MDV62	MDV	May	Metazoa	Annelida	Clitellata	Hirudinida	Glossiphoniidae	Hemiclepsis	<i>Hemiclepsis marginata</i>	98.1	322
U	U	J	176	MDV62	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Hymenoptera	Hymenoptera	<i>Hymenoptera sp.</i>	98.0	716
U	U	J	176	MDV62	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Stratiomyidae	Oplodontha	<i>Oplodontha viridula</i>	97.9	366
U	U	J	176	MDV62	MDV	May	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	99.7	315
U	U	J	163	MDV63	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Atripsodes	<i>Atripsodes aterrimus</i>	99.4	706
U	U	J	163	MDV63	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.3	709
U	U	J	163	MDV63	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Didymodon	<i>Didymodon rigidulus</i>	97.1	278
U	U	J	163	MDV63	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.7	793
U	U	J	163	MDV63	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	98.9	602
U	U	J	163	MDV63	MDV	May	Metazoa	Annelida	Clitellata	Hirudinida	Glossiphoniidae	Hemiclepsis	<i>Hemiclepsis marginata</i>	96.0	718
U	U	J	163	MDV63	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Hemiptera	Hemiptera	<i>Hemiptera sp.</i>	99.0	221
U	U	J	163	MDV63	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Corixidae	Micronecta	<i>Micronecta scholtzi</i>	99.6	255
U	U	J	163	MDV63	MDV	May	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	98.0	381

U	U	J	163	MDV63	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	100.0	312
135	F	A	637	MDV64	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	<i>Alnus sp.</i>	99.3	762	
135	F	A	637	MDV64	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	<i>Betula pubescens</i>	99.9	901	
135	F	A	637	MDV64	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	<i>Bithynia tentaculata</i>	99.4	335	
135	F	A	637	MDV64	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	<i>Sympetrum striolatum</i>	98.7	236	
135	F	A	637	MDV64	MDV	May	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	<i>Armadillidium nasatum</i>	97.0	365	
135	F	A	637	MDV64	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	<i>Carex sp.</i>	99.7	603	
135	F	A	637	MDV64	MDV	May	Metazoa	Annelida	Clitellata	Haplotaxida	Lumbriidae	<i>Eiseniella tetraedra</i>	98.2	365	
135	F	A	637	MDV64	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	<i>Endochironomus albipennis</i>	99.1	365	
135	F	A	637	MDV64	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Noctuidae	<i>Orthosia populeti</i>	98.5	416	
135	F	A	637	MDV64	MDV	May	Metazoa	Arthropoda	Malacostraca	Isopoda	Philosciidae	<i>Philoscia muscorum</i>	98.5	365	
135	F	A	637	MDV64	MDV	May	Metazoa	Chordata	Amphibia	Anura	Bufoidae	<i>Bufo bufo</i>	97.7	362	
224	F	A	564	MDV65	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Phryganeidae	<i>Agrypnia varia</i>	99.8	706	
224	F	A	564	MDV65	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	<i>Bithynia tentaculata</i>	100.0	415	
224	F	A	564	MDV65	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	<i>Caenis horaria</i>	99.8	709	
224	F	A	564	MDV65	MDV	May	Metazoa	Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Gammarus fossarum</i>	99.1	709	
224	F	A	564	MDV65	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	<i>Ischnura elegans</i>	99.8	706	
224	F	A	564	MDV65	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	<i>Phragmites australis</i>	97.1	212	
224	F	A	564	MDV65	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	<i>Salix sp.</i>	98.4	561	
224	F	A	564	MDV65	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	<i>Tortella tortuosa</i>	99.3	743	
224	F	A	564	MDV65	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	<i>Chironomus sp.</i>	99.8	706	
224	F	A	564	MDV65	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	<i>Tantytarsus pallidicornis</i>	99.8	709	
U	U	J	23	MDV66	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	<i>Caenis horaria</i>	96.5	366	
U	U	J	23	MDV66	MDV	May	Metazoa	Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Gammarus pulex</i>	99.4	709	
U	U	J	23	MDV66	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	<i>Ischnura elegans</i>	98.7	248	
U	U	J	23	MDV66	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	<i>Parapoxyn stratiotata</i>	99.7	709	
U	U	J	23	MDV66	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Notodontidae	<i>Pheosia tremula</i>	99.8	706	
U	U	J	23	MDV66	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	<i>Rhizotrogus aestivus</i>	98.8	365	
U	M	A	257	MDV67	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	<i>Betula pubescens</i>	99.6	889	
U	M	A	257	MDV67	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	<i>Phragmites australis</i>	100.0	974	
U	M	A	257	MDV67	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	<i>Picea sp.</i>	99.8	1002	
U	M	A	257	MDV67	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	<i>Quercus sp.</i>	98.8	328	
U	M	A	257	MDV67	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	<i>Carex sp.</i>	99.6	750	
U	M	A	257	MDV67	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	<i>Utricularia australis</i>	100.0	363	
433	U	J	162	MDV68	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	<i>Phragmites australis</i>	99.7	889	
433	U	J	162	MDV68	MDV	May	Viridiplantae	Streptophyta	Pinopsida	Pinales	Pinaceae	<i>Picea sp.</i>	99.8	1003	
433	U	J	162	MDV68	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Culicidae	<i>Anopheles sp.</i>	100.0	247	
433	U	J	162	MDV68	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	<i>Carex sp.</i>	96.1	539	

433	U	J	162	MDV68	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Formicidae	Formicidae sp.	98.3	709	
433	U	J	162	MDV68	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.8	709	
433	U	J	162	MDV68	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Mesoveliidae	Mesovelia	<i>Mesovelia furcata</i>	99.5	223	
433	U	J	162	MDV68	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Phyllopertha	<i>Phyllopertha horticola</i>	98.8	365	
433	U	J	162	MDV68	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tanytarsus mendax</i>	100.0	713	
433	U	J	162	MDV68	MDV	May	Metazoa	Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	97.7	364	
433	U	J	162	MDV68	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.6	502	
313	F	A	655	MDV69	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus sp.</i>	99.3	896	
313	F	A	655	MDV69	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	Potamogeton	<i>Potamogeton perfoliatus</i>	98.4	243	
313	F	A	655	MDV69	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.8	401	
313	F	A	655	MDV69	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	99.7	312	
313	F	A	655	MDV69	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Hymenoptera	Hymenoptera	<i>Hymenoptera sp.</i>	96.5	310	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Buprestidae	Agrius	<i>Agrius angustulus</i>	100.0	159	
166	M	A	324	MDV70	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus sp.</i>	99.9	1055	
166	M	A	324	MDV70	MDV	May	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	98.4	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Cerambycinae	Clytus	<i>Clytus arietis</i>	99.7	709	
166	M	A	324	MDV70	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1049	
166	M	A	324	MDV70	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.4	804	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.0	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.0	365	
166	M	A	324	MDV70	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Typhaceae	Typha	<i>Typha latifolia</i>	99.9	792	
166	M	A	324	MDV70	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Sapindales	Aceraceae	Acer	<i>Aelia acuminata</i>	100.0	317	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	Armadillidium	<i>Armadillidium nasatum</i>	98.3	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Mollusca	Gastropoda	Stylommatophora	Limacoidea	Boettgerilla	<i>Boettgerilla pallens</i>	99.1	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus sp.</i>	100.0	541	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Tenthredinidae	Dolerus	<i>Dolerus asper</i>	99.1	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Einfeldia	<i>Einfeldia sp.</i>	99.1	728	
166	M	A	324	MDV70	MDV	May	Metazoa	Annelida	Clitellata	Haplotauxida	Lumbricidae	Eiseniella	<i>Eiseniella tetraedra</i>	98.5	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Formicidae	<i>Formicidae sp.</i>	97.3	715	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Malacostraca	Isopoda	Trichoniscidae	Hyloniscus	<i>Hyloniscus riparius</i>	98.5	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Malacostraca	Isopoda	Philosciidae	Philoscia	<i>Philoscia muscorum</i>	97.5	405	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Malacostraca	Arachnida	Araneae	Lycosidae	Pirata	<i>Pirata piraticus</i>	98.2	217
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Carabidae	Pterostichus	<i>Pterostichus madidus</i>	100.0	706	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Cantharidae	Rhagonycha	<i>Rhagonycha fulva</i>	98.8	376	
166	M	A	324	MDV70	MDV	May	Metazoa	Arthropoda	Malacostraca	Isopoda	Trachelipodidae	Trachelipus	<i>Trachelipus rathkii</i>	98.5	365	
166	M	A	324	MDV70	MDV	May	Metazoa	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	Deroberas	<i>Deroberas laeve</i>	98.1	216	
169	M	A	291	MDV71	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.8	601	
169	M	A	291	MDV71	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Streblotricum	<i>Streblotrichum convolutum</i>	97.7	223	

169	M	A	291	MDV71	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.5	602
U	U	J	149	MDV72	MDV	May	Metazoa	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	Deroceras	<i>Deroceras reticulatum</i>	100.0	407
U	U	J	149	MDV72	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.3	269
U	U	J	149	MDV72	MDV	May	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Aphodius	<i>Aphodius granarius</i>	97.7	244
U	U	J	149	MDV72	MDV	May	Metazoa	Arthropoda	Insecta	Hymenoptera	Hymenoptera	<i>Hymenoptera sp.</i>	97.2	379	
U	U	J	149	MDV72	MDV	May	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.4	365
U	U	J	149	MDV72	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	100.0	601
U	M	A	350	MDV73	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1042
U	M	A	350	MDV73	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	99.2	594
U	M	A	350	MDV73	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.7	638
U	M	A	350	MDV73	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	98.9	889
U	M	A	350	MDV73	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	99.6	503
U	U	J	163	MDV74	MDV	May	Arthropoda	Arthropoda	Hexapoda	Trichoptera	Leptoceroidea	Atripsodes	<i>Atripsodes aterrimus</i>	99.2	709
U	U	J	163	MDV74	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	100.0	338
U	U	J	163	MDV74	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.3	709
U	U	J	163	MDV74	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	<i>Limnephilus decipiens</i>	98.5	223
U	U	J	163	MDV74	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.1	441
U	U	J	163	MDV74	MDV	May	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	Quercus	<i>Quercus sp.</i>	99.7	344
U	U	J	163	MDV74	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	96.9	358
U	U	J	163	MDV74	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Noctuidae	Orthosia	<i>Orthosia cerasi</i>	98.1	267
U	U	J	163	MDV74	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoxyn	<i>Parapoxyn stratiotata</i>	99.7	709
U	U	J	163	MDV74	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Drepanidae	Polyploca	<i>Polyploca ridens</i>	98.8	365
U	U	J	163	MDV74	MDV	May	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	95.9	507
8	F	A	630	MDV75	MDV	May	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.5	366
8	F	A	630	MDV75	MDV	May	Metazoa	Arthropoda	Insecta	Trichoptera	Limnephiloidea	Limnephilus	<i>Limnephilus flavicornis</i>	100.0	709
8	F	A	630	MDV75	MDV	May	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.5	601
8	F	A	630	MDV75	MDV	May	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoxyn	<i>Parapoxyn stratiotata</i>	98.8	274
8	F	A	630	MDV75	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus mendax</i>	100.0	709
8	F	A	630	MDV75	MDV	May	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus pallidicornis</i>	99.1	709
8	F	A	630	MDV75	MDV	May	Metazoa	Chordata	Amphibia	Anura	Ranidae	Pelophylax	<i>Pelophylax lessonae</i>	98.8	407
138	F	A	628	MDV76	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Cerambycinae	Clytus	<i>Clytus arietis</i>	98.1	242
138	F	A	628	MDV76	MDV	June	Metazoa	Annelida	Clitellata	Haplotauxida	Lumbricidae	Lumbricidae sp.	<i>Lumbricidae sp.</i>	97.3	366
138	F	A	628	MDV76	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
138	F	A	628	MDV76	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Populus	<i>Populus sp.</i>	99.1	926
138	F	A	628	MDV76	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	98.9	272
138	F	A	628	MDV76	MDV	June	Metazoa	Arthropoda	Insecta	Lepidoptera	Noctuidae	Egira	<i>Egira conspicillaris</i>	97.6	364
138	F	A	628	MDV76	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Miridae	Leptopterna	<i>Leptopterna dolabrata</i>	99.1	235
138	F	A	628	MDV76	MDV	June	Metazoa	Arthropoda	Insecta	Lepidoptera	Noctuidae	Orthosia	<i>Orthosia populeti</i>	100.0	709

138	F	A	628	MDV76	MDV	June	Metazoa	Arthropoda	Malacostrcia	Isopoda	Philosciidae	Philoscia	<i>Philoscia muscorum</i>	100.0	709
138	F	A	628	MDV76	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	<i>Polypedilum sp.</i>	98.4	214
138	F	A	628	MDV76	MDV	June	Metazoa	Arthropoda	Malacostrcia	Isopoda	Trachelipodidae	Trachelipus	<i>Trachelipus rathkii</i>	98.3	706
40	F	A	731	MDV77	MDV	June	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	98.4	365
40	F	A	731	MDV77	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.9	365
40	F	A	731	MDV77	MDV	June	Metazoa	Chordata	Amphibia	Anura	Bufoidae	Bufo	<i>Bufo bufo</i>	96.9	363
93	F	A	790	MDV78	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Sapindales	Aceraceae	Acer	<i>Acer pseudoplatanus</i>	100.0	421
93	F	A	790	MDV78	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	100.0	531
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.3	709
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Ischnura	<i>Ischnura elegans</i>	100.0	708
93	F	A	790	MDV78	MDV	June	Metazoa	Annelida	Clitellata	Haplotauxida	Lumbricidae	Lumbricus	<i>Lumbricus terrestris</i>	97.3	365
93	F	A	790	MDV78	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	552
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Curculionidae	Polydrusus	<i>Polydrusus formosus</i>	99.8	709
93	F	A	790	MDV78	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.7	1060
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	97.8	365
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	Armadillidium	<i>Armadillidium nasatum</i>	97.5	365
93	F	A	790	MDV78	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	98.8	250
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Cecidomyiidae	Cecidomyiidae	<i>Cecidomyiidae sp.</i>	100.0	709
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus albipennis</i>	98.3	365
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes	<i>Glyptotendipes glaucus</i>	98.5	258
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Scirtoidea	Microcara	<i>Microcara testacea</i>	97.3	212
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Notonectidae	Notonecta	<i>Notonecta glauca</i>	99.1	364
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Probezzia	<i>Probezzia seminigra</i>	96.8	365
93	F	A	790	MDV78	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Rhizotrogus	<i>Rhizotrogus aestivus</i>	99.8	709
U	U	J	44	MDV79	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.5	1101
U	U	J	44	MDV79	MDV	June	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Cristellidae	Cristella	<i>Cristella mucedo</i>	98.2	218
U	U	J	44	MDV79	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Palpomyia	<i>Palpomyia lineata</i>	98.8	712
U	U	J	44	MDV79	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Phaenobezzia	<i>Phaenobezzia rubiginosa</i>	98.8	710
U	U	J	44	MDV79	MDV	June	Metazoa	Arthropoda	Arachnida	Sarcoptiformes	Steganacaridae	Steganacarus	<i>Steganacarus magnus</i>	100.0	709
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Buprestidae	Agrilus	<i>Agrilus angustulus</i>	96.8	403
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Ephemeroptera	Asellidae	Baetis	<i>Baetis rhodani</i>	97.7	309
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Baetidae	Cloeon	<i>Cloeon dipterum</i>	99.7	706
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Malacostrcia	Amphipoda	Gammaridae	Gammarus	<i>Gammarus fossarum</i>	97.6	245
15	M	A	313	MDV80	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lamiaceae	Lycopus	<i>Lycopus europaeus</i>	99.4	563
15	M	A	313	MDV80	MDV	June	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	<i>Radix auricularia</i>	97.9	235
15	M	A	313	MDV80	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	98.6	1060
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	Armadillidium	<i>Armadillidium nasatum</i>	99.7	706
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus tendens</i>	98.1	706

15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	98.4	244
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Mesoveliiidae	Mesovelia	<i>Mesovelia furcata</i>	99.4	709
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoxyn	<i>Parapoxyn stratiotata</i>	98.4	208
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Phyllopertha	<i>Phyllopertha horticola</i>	99.8	709
15	M	A	313	MDV80	MDV	June	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	99.0	317
15	M	A	313	MDV80	MDV	June	Metazoa	Mollusca	Gastropoda	Stylommatophora	Succineidae	Succineidae sp.	<i>Succineidae sp.</i>	95.9	492
15	M	A	313	MDV80	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus lactescens</i>	100.0	706
15	M	A	313	MDV80	MDV	June	Metazoa	Chordata	Amphibia	Anura	Bufo	Bufo	<i>Bufo bufo</i>	99.5	706
28	F	A	549	MDV81	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Sapindales	Aceraceae	Acer	<i>Acer pseudoplatanus</i>	99.4	792
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Anax	<i>Anax imperator</i>	97.8	365
28	F	A	549	MDV81	MDV	June	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.7	709
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Baetidae	Cloeon	<i>Cloeon simile</i>	100.0	709
28	F	A	549	MDV81	MDV	June	Metazoa	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	Deroberas	<i>Deroberas reticulatum</i>	99.8	706
28	F	A	549	MDV81	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	<i>Juncus effusus</i>	98.8	250
28	F	A	549	MDV81	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1040
28	F	A	549	MDV81	MDV	June	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	<i>Radix auricularia</i>	98.8	703
28	F	A	549	MDV81	MDV	June	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	<i>Radix balthica</i>	99.7	706
28	F	A	549	MDV81	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.5	957
28	F	A	549	MDV81	MDV	June	Metazoa	Chordata	Aves	Passeriformes	Turdidae	Turdus	<i>Turdus merula</i>	98.4	365
28	F	A	549	MDV81	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	100.0	232
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Cladotanytarsus	<i>Cladotanytarsus pallidus</i>	99.4	365
28	F	A	549	MDV81	MDV	June	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Cristellidae	Cristella	<i>Cristatella mucedo</i>	99.1	365
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Dasyhelea	<i>Dasyhelea notata</i>	99.1	709
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Dryopidae	Dryops	<i>Dryops luridus</i>	98.2	365
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus tendens</i>	98.1	465
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Lasius	<i>Lasius niger</i>	100.0	709
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Meloidae	Meloe	<i>Meloe rugosus</i>	99.3	300
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoxyn	<i>Parapoxyn stratiotata</i>	99.7	709
28	F	A	549	MDV81	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Rhizotrogus	<i>Rhizotrogus aestivus</i>	100.0	709
28	F	A	549	MDV81	MDV	June	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	99.4	317
28	F	A	549	MDV81	MDV	June	Metazoa	Chordata	Amphibia	Anura	Bufo	Bufo	<i>Bufo bufo</i>	99.5	403
51	F	A	606	MDV82	MDV	June	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	99.3	595
51	F	A	606	MDV82	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Juncaceae	Juncus	<i>Juncus effusus</i>	99.4	367
51	F	A	606	MDV82	MDV	June	Metazoa	Annelida	Clitellata	Haplotaxida	Lumbricidae	Lumbricidae	<i>Lumbricidae sp.</i>	98.5	709
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.1	365
51	F	A	606	MDV82	MDV	June	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Syntichia	<i>Syntichia ruralis</i>	98.8	595
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	Armadillidium	<i>Armadillidium nasatum</i>	99.7	708
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Arachnida	Araneae	Linyphiidae	Bathyphantes	<i>Bathyphantes gracilis</i>	100.0	253

51	F	A	606	MDV82	MDV	June	Metazoa	Mollusca	Gastropoda	Stylommatophora	Limacoidea	Boettgerilla	<i>Boettgerilla pallens</i>	99.8	706
51	F	A	606	MDV82	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.5	1101
51	F	A	606	MDV82	MDV	June	Metazoa	Annelida	Oligochaeta	Opisthopora	Lumbricidae	Dendrobaena	<i>Dendrobaena octaedra</i>	100.0	238
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Insecta	Hymenoptera	Tenthredinidae	Dolerus	<i>Dolerus asper</i>	98.5	366
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Dryopidae	Dryops	<i>Dryops luridus</i>	99.5	411
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Malacostreca	Isopoda	Trichoniscidae	Hyloniscus	<i>Hyloniscus riparius</i>	100.0	709
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Arachnida	Araneae	Tetragnathidae	Pachygynatha	<i>Pachygynatha clercki</i>	99.5	243
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Sciomyzidae	Pherbellia	<i>Pherbellia schoenherri</i>	100.0	320
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Malacostreca	Isopoda	Philosciidae	Philoscia	<i>Philoscia muscorum</i>	98.6	710
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Arachnida	Araneae	Lycosidae	Pirata	<i>Pirata piraticus</i>	99.8	706
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Insecta	Collembola	Entomobryidae	Pseudosinella	<i>Pseudosinella octopunctata</i>	100.0	322
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Cantharidae	Rhagonycha	<i>Rhagonycha fulva</i>	100.0	709
51	F	A	606	MDV82	MDV	June	Metazoa	Arthropoda	Arachnida	Araneae	Gnaphosidae	Zelotes	<i>Zelotes subterraneus</i>	99.2	271
332	U	J	203	MDV83	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Anax	<i>Anax imperator</i>	98.9	710
332	U	J	203	MDV83	MDV	June	Metazoa	Arthropoda	Insecta	Ephemeroptera	Asellidae	Baetis	<i>Baetis rhodani</i>	99.4	340
332	U	J	203	MDV83	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Pediciidae	Dicranota sp.	<i>Dicranota sp.</i>	96.0	227
332	U	J	203	MDV83	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoridea	Ilyocoris	<i>Ilyocoris cimicoides</i>	98.8	365
199	M	A	292	MDV84	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus sp.</i>	98.1	890
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Anax	<i>Anax imperator</i>	97.9	365
199	M	A	292	MDV84	MDV	June	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.7	712
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Baetidae	Cloeon	<i>Cloeon simile</i>	100.0	706
199	M	A	292	MDV84	MDV	June	Metazoa	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	Deroceras	<i>Deroceras reticulatum</i>	99.8	703
199	M	A	292	MDV84	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1041
199	M	A	292	MDV84	MDV	June	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	<i>Radix balthica</i>	99.3	70
199	M	A	292	MDV84	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.7	915
199	M	A	292	MDV84	MDV	June	Metazoa	Chordata	Aves	Passeriformes	Turdidae	Turdus	<i>Turdus merula</i>	98.4	364
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	Armadillidium	<i>Armadillidium nasatum</i>	97.5	365
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Cladotanytarsus	<i>Cladotanytarsus pallidus</i>	99.1	365
199	M	A	292	MDV84	MDV	June	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Cristellidae	Cristella	<i>Cristella mucedo</i>	99.1	365
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Dasyhelea	<i>Dasyhelea notata</i>	99.1	709
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Dryopidae	Dryops	<i>Dryops luridus</i>	98.2	366
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Lasius	<i>Lasius niger</i>	99.9	711
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoynx	<i>Parapoynx stratiotata</i>	99.7	709
199	M	A	292	MDV84	MDV	June	Metazoa	Chordata	Amphibia	Anura	Bufonidae	Bufo	<i>Bufo bufo</i>	99.4	401
199	M	A	292	MDV84	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Aeshnoidea	Gomphus	<i>Gomphus pulchellus</i>	98.8	271
31	F	A	536	MDV86	MDV	June	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.4	223
31	F	A	536	MDV86	MDV	June	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	98.3	709
31	F	A	536	MDV86	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Ischnura	<i>Ischnura elegans</i>	99.1	364

31	F	A	536	MDV86	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	98.5	471
31	F	A	536	MDV86	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.5	1102
31	F	A	536	MDV86	MDV	June	Viridiplantae	Streptophyta	Charophyceae	Charales	Characeae	<i>Chara braunii</i>	<i>Chara braunii</i>	96.9	225
31	F	A	536	MDV86	MDV	June	Metazoa	Arthropoda	Insecta	Lepidoptera	Drepanidae	<i>Polyploca</i>	<i>Polyploca rideans</i>	100.0	709
21	F	A	528	MDV87	MDV	June	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	<i>Caenis</i>	<i>Caenis horaria</i>	97.1	365
21	F	A	528	MDV87	MDV	June	Metazoa	Arthropoda	Malacostraca	Amphipoda	Gammaridae	<i>Gammarus</i>	<i>Gammarus pulex</i>	100.0	706
21	F	A	528	MDV87	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	1043
21	F	A	528	MDV87	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.6	365
21	F	A	528	MDV87	MDV	June	Viridiplantae	Streptophyta	Bryopsida	Bryales	Bryaceae	<i>Bryum</i>	<i>Bryum argenteum</i>	99.5	603
21	F	A	528	MDV87	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	<i>Chironomus</i>	<i>Chironomus curabilis</i>	99.8	709
21	F	A	528	MDV87	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	<i>Ilyocoris</i>	<i>Ilyocoris cimicoides</i>	97.9	364
21	F	A	528	MDV87	MDV	June	Metazoa	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	<i>Polydesmus</i>	<i>Polydesmus angustus</i>	99.1	365
21	F	A	528	MDV87	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Rhizotrogus	<i>Rhizotrogus aestivus</i>	97.0	228
124	F	A	726	MDV89	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Alismataceae	<i>Alisma</i>	<i>Alisma plantago-aquatica</i>	100.0	1001
124	F	A	726	MDV89	MDV	June	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiacae	<i>Barbula</i>	<i>Barbula unguiculata</i>	98.2	569
124	F	A	726	MDV89	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.5	1094
U	U	J	149	MDV90	MDV	June	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Amblystegiaceae	Cratoneuron	<i>Cratoneuron filicinum</i>	98.8	603
U	U	J	149	MDV90	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Curculionidae	Dendroctonus	<i>Dendroctonus ponderosae</i>	99.3	278
U	U	J	149	MDV90	MDV	June	Mollusca	Mollusca	Gastropoda	Stylommatophora	Discidae	Discus	<i>Discus rotundatus</i>	95.3	365
U	U	J	149	MDV90	MDV	June	Metazoa	Annelida	Clitellata	Haplotaxida	Lumbricidae	Lumbricidae	<i>Lumbricidae sp.</i>	98.8	347
U	U	J	149	MDV90	MDV	June	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	<i>Lasius</i>	<i>Lasius platythorax</i>	99.8	745
U	U	J	149	MDV90	MDV	June	Metazoa	Arthropoda	Chilopoda	Lithobiomorpha	Lithobiidae	<i>Lithobius</i>	<i>Lithobius tricuspis</i>	99.7	314
U	U	J	149	MDV90	MDV	June	Metazoa	Arthropoda	Malacostraca	Isopoda	Oniscidae	<i>Oniscus</i>	<i>Oniscus asellus</i>	98.6	365
U	U	J	149	MDV90	MDV	June	Metazoa	Arthropoda	Malacostraca	Isopoda	Philosciidae	<i>Philoscia</i>	<i>Philoscia muscorum</i>	100.0	709
U	U	J	149	MDV90	MDV	June	Metazoa	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	<i>Polydesmus</i>	<i>Polydesmus angustus</i>	98.2	365
U	U	J	149	MDV90	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	<i>Tantytarsus signatus</i>	98.3	709
168	F	A	561	MDV91	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	<i>Aeshna</i>	<i>Aeshna cyanea</i>	97.5	307
168	F	A	561	MDV91	MDV	June	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	<i>Bithynia</i>	<i>Bithynia tentaculata</i>	98.0	244
168	F	A	561	MDV91	MDV	June	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	<i>Equisetum</i>	<i>Equisetum hyemale</i>	98.4	253
168	F	A	561	MDV91	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	<i>Potamogeton</i>	<i>Potamogeton perfoliatus</i>	98.7	453
168	F	A	561	MDV91	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Fagaceae	<i>Quercus</i>	<i>Quercus sp.</i>	99.7	932
168	F	A	561	MDV91	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Lestidae	Sympecma	<i>Sympecma fusca</i>	99.0	234
168	F	A	561	MDV91	MDV	June	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	<i>Armadillidium</i>	<i>Armadillidium nasatum</i>	99.7	709
168	F	A	561	MDV91	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.7	1253
168	F	A	561	MDV91	MDV	June	Metazoa	Mollusca	Gastropoda	Stylommatophora	Euconulidae	<i>Euconulus</i>	<i>Euconulus praticola</i>	97.3	219
168	F	A	561	MDV91	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Hydrophilidae	<i>Helochares</i>	<i>Helochares lividus</i>	100.0	264
168	F	A	561	MDV91	MDV	June	Metazoa	Arthropoda	Malacostraca	Isopoda	Philosciidae	<i>Philoscia</i>	<i>Philoscia muscorum</i>	100.0	399
168	F	A	561	MDV91	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Cantharidae	<i>Rhagonycha</i>	<i>Rhagonycha fulva</i>	96.5	363

168	F	A	561	MDV91	MDV	June	Metazoa	Arthropoda	Malacostrcia	Isopoda	Trachelipodidae	Trcahelipus	Trachelipus sp.	96.7	706
191	F	A	614	MDV92	MDV	June	Metazoa	Mollusca	Gastropoda	Stylommatophora	Arionidae	Arion	Arion distinctus	98.1	440
191	F	A	614	MDV92	MDV	June	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	Bithynia tentaculata	99.7	712
191	F	A	614	MDV92	MDV	June	Viridiplantae	Streptophyta	Equisetopsida	Hypnales	Hylocomiaceae	Ctenidium	Ctenidium molluscum	98.2	394
191	F	A	614	MDV92	MDV	June	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	Equisetum hyemale	100.0	577
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Ischnura	Ischnura elegans	100.0	295
191	F	A	614	MDV92	MDV	June	Metazoa	Annelida	Clitellata	Haplotaxida	Lumbricidae	Lumbricus	Lumbricus terrestris	98.0	364
191	F	A	614	MDV92	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	Phragmites australis	100.0	1087
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Curculionidae	Polydrusus	Polydrusus cervinus	98.5	709
191	F	A	614	MDV92	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	Potamogeton	Potamogeton perfoliatus	98.7	793
191	F	A	614	MDV92	MDV	June	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	Radix auricularia	98.8	706
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	Armadillidium	Armadillidium nasatum	99.6	709
191	F	A	614	MDV92	MDV	June	Arthropoda	Arthropoda	Malacostrcia	Isopoda	Asellidae	Asellus	Asellus aquaticus	99.2	712
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Cecidomyiidae	Cecidomyiidae	Cecidomyiidae sp.	98.5	365
191	F	A	614	MDV92	MDV	June	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Cristellidae	Cristella	Cristatella mucedo	99.1	710
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Arachnida	Trombidiformes	Eupodidae	Eupodidae	Eupodidae sp.	99.1	365
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Malacostrcia	Isopoda	Trichoniscidae	Hyloniscus	Hyloniscus riparius	100.0	710
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Hymenoptera	Tenthredinidae	Monsoma	Monsoma pulveratum	99.7	712
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmus	Polydesmus angustus	100.0	728
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	Polypedilum sordens	98.5	365
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Cantharidae	Rhagonycha	Rhagonycha fulva	99.2	277
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Scarabaeidae	Rhizotrogus	Rhizotrogus aestivus	97.7	266
191	F	A	614	MDV92	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	Tantytarsus lactescens	99.3	315
U	M	A	287	MDV93	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	Phragmites australis	100.0	1041
U	M	A	287	MDV93	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Alismatales	Potamogetonaceae	Potamogeton	Potamogeton perfoliatus	98.7	792
U	M	A	287	MDV93	MDV	June	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	Radix auricularia	98.4	706
U	M	A	287	MDV93	MDV	June	Metazoa	Arthropoda	Insecta	Hymenoptera	Tenthredinidae	Monsoma	Monsoma pulveratum	99.7	733
U	M	A	287	MDV93	MDV	June	Metazoa	Arthropoda	Insecta	Lepidoptera	Crambidae	Parapoxyn	Parapoxyn stratiota	99.1	365
U	M	A	287	MDV93	MDV	June	Metazoa	Arthropoda	Diplopoda	Polydesmida	Polydesmidae	Polydesmus	Polydesmus angustus	100.0	709
U	M	A	287	MDV93	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	Tantytarsus lactescens	99.1	239
107	M	A	388	MDV94	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	Salix sp.	100.0	416
107	M	A	388	MDV94	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	Sympetrum striolatum	99.7	365
107	M	A	388	MDV94	MDV	June	Metazoa	Annelida	Clitellata	Arhynchoobdellida	Erpobdellidae	Erpobdella	Erpobdella sp.	100.0	331
107	M	A	388	MDV94	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Hemiptera	Hemiptera	Hemiptera sp.	98.0	244
107	M	A	388	MDV94	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoridea	Ilyocoris	Ilyocoris cimicoides	99.8	709
107	M	A	388	MDV94	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tantytarsus	Tantytarsus lactescens	99.4	378
107	M	A	388	MDV94	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	Nymphaea alba	99.5	378
290	F	A	600	MDV95	MDV	June	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	Equisetum hyemale	99.6	247

290	F	A	600	MDV95	MDV	June	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Scardinus	<i>Scardinus erythrophthalmus</i>	98.6	365
290	F	A	600	MDV95	MDV	June	Metazoa	Chordata	Aves	Passeriformes	Sturnidae	<i>Sturnus vulgaris</i>	97.8	365	
290	F	A	600	MDV95	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	<i>Sympetrum striolatum</i>	98.3	364
290	F	A	600	MDV95	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	99.8	712
290	F	A	600	MDV95	MDV	June	Metazoa	Arthropoda	Insecta	Hemiptera	Notonectidae	Notonecta	<i>Notonecta glauca</i>	98.2	393
U	U	J	124	MDV96	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.2	397
U	U	J	124	MDV96	MDV	June	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Anax	<i>Anax imperator</i>	99.5	710
U	U	J	124	MDV96	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	99.2	713
177	F	A	624	MDV97	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	99.9	1055
177	F	A	624	MDV97	MDV	June	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	98.2	640
177	F	A	624	MDV97	MDV	June	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Pylaisiaceae	Pylaisia	<i>Pylaisia polyantha</i>	100.0	514
177	F	A	624	MDV97	MDV	June	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Streblotricum	<i>Streblotrichum convolutum</i>	99.2	246
177	F	A	624	MDV97	MDV	June	Metazoa	Arthropoda	Insecta	Isopoda	Armadillidiidae	Armadillidium	<i>Armadillidium nasatum</i>	99.7	711
177	F	A	624	MDV97	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.5	1101
177	F	A	624	MDV97	MDV	June	Metazoa	Arthropoda	Arachnida	Araneae	Clubionidae	Clubiona	<i>Clubiona phragmitis</i>	100.0	709
177	F	A	624	MDV97	MDV	June	Metazoa	Arthropoda	Malacostraca	Isopoda	Trichoniscidae	Hyloniscus	<i>Hyloniscus riparius</i>	100.0	709
177	F	A	624	MDV97	MDV	June	Metazoa	Arthropoda	Insecta	Diptera	Ceratopogonidae	Palpomyia	<i>Palpomyia lineata</i>	98.1	564
177	F	A	624	MDV97	MDV	June	Metazoa	Arthropoda	Malacostraca	Isopoda	Philosciidae	Philoscia	<i>Philoscia muscorum</i>	96.8	365
177	F	A	624	MDV97	MDV	June	Metazoa	Arthropoda	Arachnida	Araneae	Lycosidae	Pirata	<i>Pirata piraticus</i>	99.2	706
177	F	A	624	MDV97	MDV	June	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	99.7	296
177	F	A	624	MDV97	MDV	June	Metazoa	Mollusca	Gastropoda	Stylommatophora	Agriolimacidae	Deroceras	<i>Deroceras laeve</i>	98.5	366
56	F	A	609	MDV98	MDV	June	Metazoa	Arthropoda	Insecta	Coleoptera	Dytiscidae	Cybister	<i>Cybister lateralimarginalis</i>	99.0	223
56	F	A	609	MDV98	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	98.2	219
56	F	A	609	MDV98	MDV	June	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Rhytidiaeae	Rhytidium	<i>Rhytidium rugosum</i>	99.2	602
56	F	A	609	MDV98	MDV	June	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	98.9	633
56	F	A	609	MDV98	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.5	744
56	F	A	609	MDV98	MDV	June	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	100.0	583
140	F	A	485	MDV99	MDV	July	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	100.0	247
140	F	A	485	MDV99	MDV	July	Metazoa	Annelida	Clitellata	Haplotaxida	Lumbricidae	Eiseniella	<i>Eiseniella tetraedra</i>	100.0	223
140	F	A	485	MDV99	MDV	July	Metazoa	Arthropoda	Insecta	Hymenoptera	Formicidae	Lasius	<i>Lasius platythorax</i>	99.6	258
140	F	A	485	MDV99	MDV	July	Metazoa	Arthropoda	Insecta	Lepidoptera	Geometridae	Lycia	<i>Lycia hirtaria</i>	100.0	709
140	F	A	485	MDV99	MDV	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Trachelipodidae	Trachelipus	<i>Trachelipus sp.</i>	96.7	238
298	F	A	422	MDV110	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	543
168	F	A	529	MDV112	MDV	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	97.8	443
168	F	A	529	MDV112	MDV	July	Viridiplantae	Streptophyta	Polypodiopsida	Equisetales	Equisetaceae	Equisetum	<i>Equisetum hyemale</i>	99.4	326
168	F	A	529	MDV112	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	466
168	F	A	529	MDV112	MDV	July	Metazoa	Arthropoda	Insecta	Odonata	Aeshnoidea	Gomphus	<i>Gomphus pulchellus</i>	100.0	262
4	M	A	446	MDV113	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.8	210

4	M	A	446	MDV113	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	494
102	F	A	598	MDV114	MDV	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	100.0	224
102	F	A	598	MDV114	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	100.0	182
102	F	A	598	MDV114	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	612
102	F	A	598	MDV114	MDV	July	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	100.0	301
102	F	A	598	MDV114	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	468
56	F	A	652	MDV115	MDV	July	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Cristellidae	Cristella	<i>Cristatella mucedo</i>	100.0	263
56	F	A	652	MDV115	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	481
56	F	A	652	MDV115	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	440
322	F	A	574	MDV116	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	100.0	224
322	F	A	574	MDV116	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.4	270
322	F	A	574	MDV116	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	997
422	F	A	441	MDV117	MDV	July	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	100.0	208
422	F	A	441	MDV117	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.5	572
422	F	A	441	MDV117	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	98.8	169
422	F	A	441	MDV117	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	188
422	F	A	441	MDV117	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.6	604
227	F	A	533	MDV118	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Alnus	<i>Alnus glutinosa</i>	100.0	363
227	F	A	533	MDV118	MDV	July	Metazoa	Chordata	Actinoptergii	Cypriniformes	Cyprinidae	Cyprinus	<i>Cyprinus carpio</i>	98.6	511
227	F	A	533	MDV118	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	300
227	F	A	533	MDV118	MDV	July	Metazoa	Arthropoda	Insecta	Diptera	Calliphoridae	Lucilia	<i>Lucilia cuprina</i>	98.6	370
178	F	A	704	MDV119	MDV	July	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Cristellidae	Cristella	<i>Cristatella mucedo</i>	100.0	292
178	F	A	704	MDV119	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	235
164	M	A	334	MDV120	MDV	July	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.5	316
164	M	A	334	MDV120	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	298
96	F	A	654	MDV122	MDV	July	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	100.0	233
96	F	A	654	MDV122	MDV	July	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	98.7	584
96	F	A	654	MDV122	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	564
96	F	A	654	MDV122	MDV	July	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	100.0	321
96	F	A	654	MDV122	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.1	232
9	M	A	427	MDV123	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	468
89	F	A	523	MDV124	MDV	July	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Plumatellidae	Plumatella	<i>Plumatella repens</i>	99.5	324
89	F	A	523	MDV124	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	337
89	F	A	523	MDV124	MDV	July	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	100.0	230
89	F	A	523	MDV124	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	465
U	F	A	516	MDV125	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Fagales	Betulaceae	Betula	<i>Betula pubescens</i>	99.8	812
U	F	A	516	MDV125	MDV	July	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiales	Chionoloma	<i>Chionoloma tenuirostre</i>	99.5	252
U	F	A	516	MDV125	MDV	July	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiales	Tortella	<i>Tortella tortuosa</i>	99.5	252

U	F	A	516	MDV125	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	280
U	F	A	516	MDV125	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	99.0	244
124	F	A	722	MDV126	MDV	July	Metazoa	Arthropoda	Malacostraca	Isopoda	Cylisticidae	Cylisticus	<i>Cylisticus convexus</i>	100.0	247
124	F	A	722	MDV126	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.6	216
124	F	A	722	MDV126	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.4	673
124	F	A	722	MDV126	MDV	July	Metazoa	Arthropoda	Insecta	Hemiptera	Naucoroidea	Ilyocoris	<i>Ilyocoris cimicoides</i>	100.0	607
124	F	A	722	MDV126	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	355
8	F	A	601	MDV127	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	98.7	263
207	F	A	664	MDV128	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	99.2	217
207	F	A	664	MDV128	MDV	July	Metazoa	Bryozoa	Phylactolaemata	Plumatellida	Cristellidae	Cristella	<i>Cristatella mucedo</i>	99.1	365
207	F	A	664	MDV128	MDV	July	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.4	228
207	F	A	664	MDV128	MDV	July	Metazoa	Arthropoda	Insecta	Diptera	Psychodidae	Psychoda	<i>Psychoda alternata</i>	100.0	428
207	F	A	664	MDV128	MDV	July	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	442
41	F	A	805	MDV129	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	833
41	F	A	805	MDV129	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	1022
338	F	A	496	MDV130	MDV	August	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	97.2	240
338	F	A	496	MDV130	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	499
338	F	A	496	MDV130	MDV	August	Metazoa	Arthropoda	Insecta	Psocodea	Psyllipsocidae	Doryteryx	<i>Doryteryx domestica</i>	100.0	519
338	F	A	496	MDV130	MDV	August	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.8	624
338	F	A	496	MDV130	MDV	August	Metazoa	Arthropoda	Insecta	Hemiptera	Aphididae	Hyalopterus	<i>Hyalopterus pruni</i>	98.9	303
338	F	A	496	MDV130	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	498
338	F	A	496	MDV130	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	100.0	587
50	F	A	754	MDV131	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nuphar	<i>Nuphar lutea</i>	99.0	228
50	F	A	754	MDV131	MDV	August	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus tendens</i>	98.3	304
50	F	A	754	MDV131	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	527
150	F	A	498	MDV132	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	98.0	215
150	F	A	498	MDV132	MDV	August	Metazoa	Mollusca	Gastropoda	Hygrophila	Physidae	Physella	<i>Physella acuta</i>	99.5	330
150	F	A	498	MDV132	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	438
404	M	A	444	MDV133	MDV	August	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	100.0	208
404	M	A	444	MDV133	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	413
110	F	A	536	MDV134	MDV	August	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Brachytron	<i>Brachytron pratense</i>	100.0	217
110	F	A	536	MDV134	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	98.2	235
110	F	A	536	MDV134	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	452
102	M	A	416	MDV136	MDV	August	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	100.0	649
102	M	A	416	MDV136	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Malpighiales	Salicaceae	Salix	<i>Salix sp.</i>	98.8	385
102	M	A	416	MDV136	MDV	August	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	654
102	M	A	416	MDV136	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	448
20	M	A	442	MDV138	MDV	August	Metazoa	Arthropoda	Insecta	Odonata	Aeshnidae	Brachytron	<i>Brachytron pratense</i>	100.0	399

20	M	A	442	MDV138	MDV	August	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.3	619
20	M	A	442	MDV138	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	507
242	F	A	589	MDV139	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	394
U	U	J	136	MDV140	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Cyperaceae	Carex sp.	<i>Carex sp.</i>	99.6	234
U	U	J	136	MDV140	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	245
U	U	J	136	MDV140	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	100.0	161
U	U	J	136	MDV140	MDV	August	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Pleurochaete	<i>Pleurochaete squarrosa</i>	100.0	209
140	F	A	610	MDV141	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	283
140	F	A	610	MDV141	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	418
7	F	A	679	MDV142	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.3	707
7	F	A	679	MDV142	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	1037
U	U	J	18	MDV143	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Asparagales	Iridaceae	Iris	<i>Iris pseudacorus</i>	99.8	774
U	U	J	18	MDV143	MDV	August	Viridiplantae	Streptophyta	Bryopsida	Hypnales	Plagiotheciaceae	Plagiothecium	<i>Plagiothecium piliferum</i>	99.5	200
U	U	J	18	MDV143	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	212
212	M	A	309	MDV144	MDV	August	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	98.1	217
212	M	A	309	MDV144	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	99.0	416
212	M	A	309	MDV144	MDV	August	Metazoa	Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	<i>Ranatra linearis</i>	99.0	207
212	M	A	309	MDV144	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	466
212	M	A	309	MDV144	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Lamiales	Lentibulariaceae	Utricularia	<i>Utricularia australis</i>	100.0	292
216	F	A	743	MDV146	MDV	August	Metazoa	Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	<i>Caenis horaria</i>	99.5	307
216	F	A	743	MDV146	MDV	August	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	<i>Chironomus sp.</i>	98.5	257
216	F	A	743	MDV146	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	273
290	F	A	582	MDV147	MDV	August	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	827
290	F	A	582	MDV147	MDV	August	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	<i>Endochironomus tendens</i>	98.9	221
290	F	A	582	MDV147	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	824
8	F	A	612	MDV148	MDV	August	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	<i>Radix auricularia</i>	98.9	432
8	F	A	612	MDV148	MDV	August	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	642
8	F	A	612	MDV148	MDV	August	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	277
U	U	J	14	MDV149	MDV	September	Metazoa	Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Radix	<i>Radix auricularia</i>	98.3	560
U	U	J	14	MDV149	MDV	September	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	464
U	U	J	14	MDV149	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	244
171	F	A	534	MDV150	MDV	September	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	817
171	F	A	534	MDV150	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	842
337	F	A	508	MDV153	MDV	September	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion	<i>Coenagrion pulchellum</i>	100.0	556
337	F	A	508	MDV153	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	866
135	F	A	624	MDV154	MDV	September	Viridiplantae	Streptophyta	Liliopsida	Poales	Poaceae	Phragmites	<i>Phragmites australis</i>	100.0	256
135	F	A	624	MDV154	MDV	September	Viridiplantae	Streptophyta	Bryopsida	Pottiales	Pottiaceae	Tortella	<i>Tortella tortuosa</i>	100.0	235
135	F	A	624	MDV154	MDV	September	Metazoa	Arthropoda	Arachnida	Araneae	Amaurobiidae	Amaurobius	<i>Amaurobius similis</i>	100.0	648

135	F	A	624	MDV154	MDV	September	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	100.0	471
135	F	A	624	MDV154	MDV	September	Metazoa	Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	<i>Ranatra linearis</i>	99.1	224
135	F	A	624	MDV154	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	865
U	U	J	25	MDV156	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	99.0	216
8	F	A	642	MDV157	MDV	September	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Ephydatia	<i>Ephydatia fluviatilis</i>	99.6	706
8	F	A	642	MDV157	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	500
167	F	A	610	MDV158	MDV	September	Metazoa	Porifera	Demospongiae	Spongillida	Spongillidae	Spongilla	<i>Spongilla lacustris</i>	100.0	249
167	F	A	610	MDV158	MDV	September	Metazoa	Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsus	<i>Tanytarsus lactescens</i>	99.3	251
167	F	A	610	MDV158	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	262
U	F	A	588	MDV159	MDV	September	Metazoa	Mollusca	Gastropoda	Littorinimorpha	Bithyniidae	Bithynia	<i>Bithynia tentaculata</i>	99.2	249
U	F	A	588	MDV159	MDV	September	Metazoa	Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion	<i>Coenagrion puella</i>	98.3	232
U	F	A	588	MDV159	MDV	September	Viridiplantae	Streptophyta	Magnoliopsida	Nymphaeales	Nymphaeaceae	Nymphaea	<i>Nymphaea alba</i>	100.0	332

Chapter 2

Determine the potential distribution and dispersion of the European pond turtle (*Emys orbicularis*, L. 1758) using Species Distributions Models

CHAPTER 2

Current and future distributions of the threatened European pond turtle (*Emys orbicularis*) – impact of limited dispersal and habitat fragmentation

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Author contributions

C.D., S.U., O.B., J.-F.R. and A.G. designed the study, C.D. conducted the species distribution models analyses, O.B. and A.G. supervised the modelization work. C.D. wrote the manuscript and the other coauthors revised it.

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Abstract

Aim: The European pond turtle (*Emys orbicularis*) is an emblematic reptile of wetlands in Europe and threatened though its entire range. The aim is twofold: (i) to forecast the current and future (RCP 4.5 and 8.5) distributions of *E. orbicularis* through its whole range and in Switzerland; (ii) to determine whether habitat fragmentation could slow down or hamper the species natural recolonization or migration under climate change.

Location: Global range of the European pond turtle (Europe and north Africa) and local range (Switzerland).

Methods: To forecast current and future *Emys* distributions, we used a hierarchical approach combining ensembles of species distribution models at two geographic scales; global (whole range) with only climatic variables, and regional at the Swiss scale with habitat constraints (landcover data). Within Switzerland, the species' dispersal was additionally constrained by using the MigClim simulation tool.

Results: The ensemble models performed very well (Global: maxTSS=0.706 and AUC=0.917; Regional: maxTSS=0.849 and AUC=0.977), indicating high environmental suitability for *E. orbicularis* around the Mediterranean and Black Sea and, in Switzerland (combined model), over the Swiss Plateau, the Rhône valley and the canton of Ticino, strongly matching the known occurrence patterns in both cases. Under climate change, the global model predicts a clear shift of the species' range to the North of its original distribution and a gain of potential suitable habitat throughout Switzerland. However, the dispersal simulations indicate that the species would only be able to colonize 11.9% of newly suitable habitats in Switzerland.

Main conclusions: Using a promising hierarchical, dispersal-constrained modeling approach, we provided the first detailed maps of the predicted potential distributions of a threatened species - *E. orbicularis* - in Europe and over Switzerland. We further

demonstrated that to conserve this species in Switzerland, translocation or reintroduction programs will be essential to counterbalance the limited dispersal ability.

KEYWORDS

climate change, conservation, *Emys orbicularis*, endangered species, habitat fragmentation, species distribution model.

1 | INTRODUCTION

Nowadays, the Earth system is undergoing enormous changes due to anthropogenic pressures (Barnosky et al., 2011; Lewis & Maslin, 2015), with climate and land use changes having a huge impact on species, ecosystems and biodiversity (Buckley & Jetz, 2007; Sexton et al., 2009; Chen et al., 2011). Species distribution models (SDMs; e.g. Guisan et al. 2017) currently represent the most used ecoinformatic tools to predict potential changes in species geographic range under anthropogenic changes (Guisan & Thuiller, 2005; Elith & Leathwick, 2009; Franklin 2010; Peterson et al., 2011). They can accordingly play a critical role in spatial conservation planning to support the implementation of efficient conservation actions (Margules & Pressey, 2000; Addison et al., 2013; Guisan et al., 2013), yet have been so far underused in real conservation planning (Tulloch et al., 2016). Furthermore, SDM provide a means to understand and predict species responses to environmental changes in dynamics and fragmented environments (Cianfrani et al., 2018; Uusitalo et al., 2019). Determining accurately the distribution of wild species is essential for efficient conservation planning and land management, especially in the case of species that are threatened by human-driven habitat degradation and fragmentation (Kumara et al., 2009).

Although some species could be able to cope with climate change due to their ability to disperse (Davis et al., 1998; Kubisch et al., 2014), many others, such as reptiles, might not or only partly due to their low dispersal capacity (Halpin, 1997; Gibbons et al. 2000; Pittet, 2017) and therefore are more vulnerable regarding rapid habitat modifications (Mac et al., 1998). However, only a few studies so far evaluated and modeled future effects of climate change on reptiles' distributions (Ceia-Hasse et al., 2014; Gonçalves et al., 2016; Alvarez et al., 2017; Javed et al., 2017; Salas et al., 2017) and even less by considering dispersal constraints (Pittet 2017). This is particularly the case for

aquatic and semi-aquatic reptile species, such as the European pond turtle (*Emys orbicularis*, L. 1758), which are also particularly dependent on healthy wetlands, streams, and rivers for their survival.

Emys orbicularis is a species occurring in wetlands of Europe and North Africa, and is already ranked today as “near threatened” (NT) on the IUCN Red List. It is the only endemic freshwater turtle species in Switzerland (with two distinct subspecies are *Emys orbicularis orbicularis* (northern Alps) and *Emys orbicularis hellenica* (southern Swiss (Ticino)), where it is listed as “critically endangered” (CR; Swiss Reptile Red List; Monney & Meyer, 2005). This freshwater turtle species is a good candidate for habitat suitability modelling and viability analyses, due to its direct dependence on abiotic environmental conditions (Ficetola et al. 2004, Segurado & Araujo 2004, Golubovic et al. 2017) and its sensitivity to human activities (Cordero Rivera and Fernandez 2004). It lives in temperate aquatic habitats, rich in vegetation such as ponds or marshlands, and needs both aquatic habitats to forage, reproduce and hibernate, and terrestrial habitats for laying eggs and basking. Because its biological cycle requires the conservation of both aquatic and terrestrial habitats, it can be considered an “umbrella” species (Cadi, 2003). As most other aquatic reptiles, it stays in water for long periods to forage, reproduce or escape predators, and thermoregulation is generally achieved by aerial basking (Krawchuck and Brooks, 1998) by exploiting the warmest aquatic habitats for activity (Picard et al., 2011), such as warm surface waters (Manning and Grigg, 1997). Through its dependence on water, this aquatic species is thus particularly susceptible to be impacted by climate change, such as warming and increased drought frequency (Gibbons et al., 2000). Previous studies demonstrated negative impacts of warming temperatures on turtles, including earlier ages of maturity (Frazer et al., 1993), faster growth rates (Stearns & Koella, 1986), and a huge decreased of male offspring by temperature-dependent species

(Janzen, 1994; Jensen et al., 2018). Even if increased drought frequency could have a negative impact on juvenile and adult individuals, on the other hand, an increased substrate-humidity expands the embryonic development in emydid turtles (Paukstis et al., 1984), especially bad, cool and humid weather are known to delay embryonic development (Ewert, 1991). Moreover, embryonic development in *E. orbicularis* is disturbed by temperatures exceeding 33°C (Moll & Legler, 1971; Vasse, 1983).

Based on such knowledge, the distribution and viability of the species was modelled in various regions of Europe (e.g. Italy: Ficetola et al. 2004, Portugal: Segurado & Araujo 2004, Spain: Cordero Rivera and Fernandez 2004, Serbia: Golubovic et al. 2017, Black Sea region: Duysebaeva et al., 2019). Yet, none of these used dynamic simulations of species dispersal (as proposed by Engler & Guisan 2009; and used e.g. for terrestrial reptiles in Switzerland; Pittet 2017) and we are not aware of any modelling study for this species in Switzerland. Furthermore, regional modelling of species with distribution range that expands into other geographic areas, likely encompassing different environmental conditions, bears the risk of capturing only a limited part of the species' environmental niche (Pearson et al. 2004). This 'niche truncation' issue is particularly likely to become a problem when the model fitted regionally is transferred into other environmental conditions, in space or time, as typically done temporally when assessing the impacts of climate change (Thuiller et al. 2004; Barbet-Massin et al. 2010). In such cases, a hierarchical approach combining models at regional and global scales is necessary (Pearson et al. 2004; Petitpierre et al. 2016, Mateo et al. 2019).

Here, based on species occurrence data and a set of meaningful environmental predictors, we used SDMs to forecast the current and future distribution of potentially suitable areas for *E. orbicularis* at two geographic scales: (i) Global, at the level of the whole range of the species, using only climatic variables; and (ii) Regional, at the swiss

scale combining the previous Global climatic model with additional habitat constraints (land cover data such as distance to suitable habitats, roads, railways, building etc.). We then used this combined SDM to determine the potential impact of climate change on the future distribution of the European pond turtle in Switzerland. For this, we used two different climatic scenarios relative to the representative concentration pathways RCP 4.5 and RCP 8.5, which assume moderate and extreme global warming, respectively (Van Vuuren et al., 2011). We assessed whether the extent of potential suitable habitat is projected to increase or decrease under future environmental conditions at both regional and global scales. Moreover, due to the low dispersal ability of *E. orbicularis*, we further assessed whether habitat fragmentation could slow down or hamper the species natural recolonization or migration under climate change in Switzerland. For this, we used MigClim (Engler & Guisan, 2009), a cellular-automata relating successive SDM predictions (in time) to assess the potential dispersal pathways of the species, accounting for landscape barriers (like fragmentation). These models and predictions additionally provide valuable ecological and biogeographic information that may help target suitable habitats for future reintroductions of the European pond turtles in Switzerland, by identifying regions where the species is currently absent but that share similar environmental conditions to those of current populations.

Based on the existing knowledge on *E. orbicularis*, we expect that climatic variable such annual temperature and precipitations will have a high contribution in our model. Moreover, we hypothesize that *E. orbicularis*' distribution will shift northward and higher in elevation due to climate change and that suitable habitat should increase at least in Switzerland and possibly also throughout its whole range, with however growing concern for the persistence of its southern populations. Finally, we expect habitat fragmentation

and the low dispersal capacity of the species to be a limit for its natural colonization of new habitats under climate change.

2 | METHODS

2.1 | Analytical framework

Habitat suitability models were developed for *E. orbicularis* at the Global and Swiss scale using ensemble modelling implemented in the R package BIOMOD2 (Thuiller et al., 2013) following the ODMAP (Overview, Data, Methods, Assessments and Predictions) protocol proposed by Zurell et al. (2020). The models developed at the Global level (Europe and North Africa) used only climatic variables at a 1 km resolution, while land cover variable at 25 m were used for the models at Swiss scale. Then, a hierarchical approach was used to combine the model predictions at the two scales, allowing to take into account the climatic conditions encountered by the species over the whole species range (Europe) and fine scale habitat requirements of the species over Switzerland. This approach was used to avoid biased model projections in the future due to climatic niche truncation (Pearson et al. 2004; Petitpierre et al. 2016, Mateo et al. 2019). We projected future distribution for the average time period 2061-2080 using two representative concentration pathways models; RCP 4.5 (moderate global warming) and RCP 8.5 (extreme global warming) (see Fig. 1). For Switzerland, we further used the R package MigClim, to simulate the colonization of sites not only environmentally suitable but also accessible through dispersal (Engler et al., 2009; Engler et al., 2012).

2.2 | *E. orbicularis* occurrence data

The *E. orbicularis* global occurrence data used in the present study were downloaded from the EMYSystem (<http://emys.geo.orst.edu/>). Even if the Sicilian lineage described as

Emys trinacris by Fritz et al. (2005) is still under wide debate and should be considered according to Speybrowck et al. (2020) as *E. o. trinacris*, we removed one occurrence from this island (see also Vamberger & Fritz, 2018). We also removed two occurrence points, which were located in the Atlantic Ocean. At the Swiss level, *E. orbicularis* occurrence data with a resolution possible between 1 to 250 m were obtained from the Swiss data and competence center for species observations (<https://www.infospecies.ch/>). After checking all the *Emys* observations, we did not remove or correct any Swiss occurrence.

2.3 | Climatic data

To account for the impact of climatic factors on the distribution of *E. orbicularis*, bioclimatic variables at 1 km resolution known to have an impact on the distribution of the European pond turtle were extracted from the Chelsa 1.2 database (Karger et al., 2017). Seven predictor variables (bio1, bio3, bio7, bio10, bio12, bio13, bio16; see Table 1) with direct impact (Austin et al., 2006; Peterson & Nakazawa, 2008; Petitpierre et al., 2016) on the species distribution were favored (Duysebaeva et al., 2019). These bioclimatic variables are calculated from average monthly minimum and maximum temperature and precipitation for 1979-2013. The same set of variables following the representative concentration pathways RCP 4.5 and RCP 8.5 averaged for 2061-2080 were also extracted from the Chelsa 1.2 database.

2.4 | Distance to habitats of importance

We first selected data representing habitats regularly used by the European pond turtle: aquatic habitats (lakes, rivers, ponds, marshes; data.geo.admin.ch/ch.bafu.ren-feuchtgebiete) and terrestrial habitats (dry meadows; data.geo.admin.ch/ch.bafu.ren-trockenstandorte) from The National Ecological Network (REN). Additionally, other

habitats potentially impacting negatively the distribution of the species (forests; data.geo.admin.ch/ch.bafu.ren-wald from REN) and human land uses (agriculture area, buildings, roads, railways; shop.swisstopo.admin.ch/fr/products/landscape//tgm3D) from swissTLM3D were also implemented. Then, the distances to these habitats were calculated and mapped at 25 m resolution in ArcMap 10.7 and used in the regional model to account for the impact of local habitats on the distribution of *E. orbicularis* in Switzerland.

2.5 | Modeling approach

The global potential distribution (using only climatic variables at a resolution of 1 km²) and the regional Swiss distribution (using only land cover data at a resolution of 25 m²) were estimated based on an ensemble species distribution modeling (SDM), in the BIOMOD2 R package (Thuiller et al., 2013) (see Fig. 1). We produced ensemble SDMs with two different modelling techniques: general linear models (GLM) with linear and quadratic terms and general additive models (GAM) with smoothing splines. Both with a binomial probability distribution family and logit link, requiring presence and absence data to determine the range of suitable conditions for the species. As explicit *E. orbicularis* absence data were not available, we defined instead a set of 10'000 random pseudo-absences (also called background points; Barbet-Massin et al., 2012; Phillips & Elith, 2013) within the biomes occupied by the species (Olsen et al., 2001; see Fig. 4).

In order to evaluate the performance of each model, we ran 25 iterations of repeated split-sample cross-validation, randomly splitting our data set into 2 subsets each containing 80% and 20% of the data respectively, one for model training and one for model testing (Guisan et al., 2017). The maximization approach of the true skill statistic (maxTSS) and the area under the receiver operating characteristics curve (AUC) were

used to assess model performance (Allouche et al., 2006; Shabani et al., 2018; see Guisan et al., 2017 for max TSS). The ensemble model was then used to predict the Global (including the Swiss) potential distribution of the species over space and time (using the BIOMOD_Projection function). At this Global level, we predicted the current (1979-2013) and future (RCP 4.5 and 8.5 for 2061-2080) potential distributions of suitable habitat for *E. orbicularis* at 1 km resolution.

Then, at the Swiss level, in order to create more precise maps of potential habitat distributions, we used a hierarchical approach combining climatic predictions by the Global model (over the whole species range) with the regional Swiss model using only land cover data over Switzerland at a spatial resolution of 25 m², both for current and future predictions. In order to combine predictions at the Global and Swiss scale, we projected the current and future predictions of the Global model into the Swiss geographic coordinate system (CH1903) and resampled them using a bilinear interpolation (with the function resample of the R package raster) to downscale them from a resolution of 1 km to 25 m. We then multiplied the Global predictions with the Swiss predictions pixel by pixel and rescaled the final values to a maximum of 1. This approach ensures that at the Swiss scale, values of high suitability are obtained only when climatic conditions at coarse scale and habitats at local scale are both suitable. To demonstrate the importance of using a hierarchical approach, we then compared the predicted potential distribution of the combined hierarchical model (Swiss land cover data and Global climatic prediction) with the prediction of the Swiss model based on local habitat data only.

Finally, using the MigClim R package (Engler et al., 2012), a cellular automaton that enables the implementation of species-specific dispersal constraints into projections of species distribution models under environmental change (Engler & Guisan, 2009), we simulated the gradual dispersal of *E. orbicularis* in the Swiss landscapes as climate

changes across the 20th century. For this, we first calculated maps of suitability for every 10 year period between 2020 and 2060 using the mean of current predictions and future predictions weighted by the time between the focal year and the two reference periods (e.g. if the suitability of a pixel for 1979-2013 (average year = 1996) is 1 and for 2060-2080 (average year = 2070) the value is 0.2, the suitability for year 2050 is $(1-(2050-1996)/(2070-1996)) * 1 + (1-(2070-2050)/(2070-1996)) * 0.2 = 0.4162$). This series of suitability maps were then used as inputs to run the MigClim simulations, using a dispersal kernel based on the literature (Lebboroni & Chelazzi, 1991; Rovero & Chelazzi, 1996; Lebboroni & Chelazzi, 2000; Cadi et al., 2004) and expert knowledge (CD and SU) (see Appendix S1 in Supporting Information).

3 | RESULTS

3.1 | Model evaluation and importance of environmental variables

The ensemble models performed well, with values of maxTSS=0.706 and AUC=0.917 for the Global model and maxTSS=0.849 and AUC=0.977 for the hierarchical Regional (Swiss) model. For the evaluations of the Global model, maxTSS was > 0.70, which was considered as good accuracy and for the hierarchical Swiss model maxTSS was > 0.80, which was considered as excellent accuracy based on the classification of Ben Rais Lasram et al. (2010). Both AUC values were higher than 0.90, which was considered as good accuracy based on the classification of Swets (1998). Regarding the Global model, we assessed the importance of climatic variables: in the GAM model, annual precipitation (bio12) had the highest overall contribution, followed by temperature annual range (bio7), annual mean temperature (bio1), isothermality (bio3), mean temperature of warmest quarter (bio10), precipitation of wettest month (bio13) and finally precipitation of wettest quarter (bi016; Fig. 2a). In the GLM model, annual mean temperature (bio1) and precipitation of wettest

quarter (bio16) had the highest overall contribution, followed by temperature annual range (bio7), mean temperature of warmest quarter (bio10), precipitation of wettest month (bio13), annual precipitation (bio12) and then isothermality (bio3; Fig. 2b).

Concerning the importance of landcover in the Regional model, the distance to wetlands had by far the highest overall contribution in both GLM and GAM models (Fig. 3a and 3b).

3.2 | Predicted geographical distribution of *E. orbicularis*

3.2.1 | Global distribution

The spatial predictions from the global ensemble model under current conditions (Fig. 4) indicates high environmental suitability for *E. orbicularis* around the Mediterranean and Black Sea, strongly matching most known occurrence patterns. Under the two different climatic pathways RCP 4.5 and 8.5, the global model predicts a clear shift of the species' range to the North of its original distribution and an increase of 63.8% of suitable habitats under RCP 4.5 and of 71.3% under RCP 8.5 (Fig. 4). The northward shift is visible by contrasting the current and future spatial predictions: under current conditions, the Continental (e.g. Germany, Poland, Ukraine, Belarus, Russia, Denmark, Estonia, Lithuania, Leetonia) and Atlantic (e.g. Great Britain, Belgium, Holland, North of France) regions are not predicted to be as favorable as region around the Mediterranean and Black Sea (Fig. 4a). However, these Continental and Atlantic regions become favorable under RCP 4.5 (suitability >0.75; Fig. 4b) and highly favorable under RCP 8.5 (suitability =1; Fig. 4c). Moreover, the current most suitable condition in the South, such as central Spain and North Africa (Morocco, Tunisia, Algeria), become markedly less favorable under climate change (Fig. 4b and 4c).

3.2.2 / Swiss distribution

Importance of hierarchical approach

The comparison of the hierarchical model combining Swiss land cover data (Fig. 5a) and Global climatic predictions and the Swiss model using only land cover data (fig. 5b) demonstrated an increase of 70.2% of potential suitable habitat in the land cover data model (Fig. 5c), which did not match most known occurrence patterns and the current distribution of *Emys orbicularis* in Switzerland (under 500 m).

Current and future distributions

The hierarchical spatial predictions from the ensemble model at the Swiss scale under current conditions indicate high climatic suitability for *E. orbicularis* over the Swiss Plateau, the Rhône valley and the canton of Ticino, strongly matching most known occurrence patterns (Fig. 6). However, this model also predicts a wide range of suitable habitats in places where the species was never recorded or has probably disappeared. Under the climatic pathways RCP 4.5 and 8.5, predictions suggest a gain of potential suitable habitat for *E. orbicularis* throughout Switzerland (Fig. 6). Indeed, *E. orbicularis* is projected to gain on average 50.6% and 64.6% of new potential habitats under the climatic pathways RCP 4.5 and RCP 8.5 respectively. The species normally occurs in the Plateau and Ticino regions (see occurrence points in Fig. 6) at elevations below 500 m, however the spatial predictions from the ensemble model under RCP 4.5 and 8.5 suggest not only new suitable habitats on the Plateau (central and north of Switzerland) and Ticino regions but also at higher elevations, for instance around the lake of Joux (Canton of Vaud) at an average altitude of 1000 m.

3.3 | Dispersal simulations

Even if habitat suitability of *E. orbicularis* is predicted to increase under climate change in Switzerland, the MigClim dispersal simulations indicate that the species would be capable of colonizing only 11.9% of current and newly suitable habitats from the current locations where the species was observed since 1997, on average, relative to its current distribution (Fig. 7).

4 | DISCUSSION

Our study provides the first detailed maps of the predicted potential distributions of *E. orbicularis* in Europe and, at a fine resolution, in Switzerland using a hierarchical ensemble modeling approach combining models at the two scales, i.e. complementing predictions by the Global climatic model over the whole species range (Europe) with land cover data over Switzerland, and accounting for dispersal limitations in future predictions.

Using a hierarchical approach is essential to predict climatic distributions accurately while accounting for landuse and landcover as filters (Pearson et al. 2004). Such hierarchical approach had only been used so far in a limited number of studies, mostly for birds and plants (Pearson et al. 2004, Thuiller et al. 2004, Barbet-Massin et al. 2010, Regos et al. 2018, Mateo et al. 2019). On the other hand, studies integrating dispersal and connectivity into SDMs have been numerous (see Vasudev et al. 2015), but hierarchical models had never been constrained by dispersal simulations, as done here. Such combined hierarchical-dispersal approach should prove essential for species of high local conservation concern (Mateo et al. 2019) and with low dispersal capacity in fragmented landscapes (Cushman, 2006). This is the case of the European pond turtle in Switzerland, for which patches playing an important connectivity role must be identified

and highlighted as of conservation and/or monitoring priority (Pereira et al., 2011), but where knowledge of the species' climatic suitability outside Switzerland matters particularly (being ectotherm) for drawing future predictions.

Our ensemble models performed well at the two scales (Global model: maxTSS=0.706 and AUC=0.917; Swiss model: maxTSS=0.849 and AUC=0.977), indicating a good ability to distinguish between suitable and unsuitable habitat (Allouche et al., 2006). Moreover, we identified several important factors to predict the distribution of *E. orbicularis*. The factor importance was quite different for GLM model versus the GAM model (see Fig.2). Indeed, most response are asymmetrical, thus better captured by the GAM model, which provide a better fit and predictive power overall. The models were largely driven by temperatures and precipitations, with annual mean temperature (bio1), temperature annual range (bio7), annual precipitation (bi012) and precipitation of wettest quarter (bio16) being the major determinants of the species' distribution. The results seem to follow the ecological behavior of *E. orbicularis*; indeed, if the humidity in nesting sites substrate increases or if the weather is cold and humid, the embryonic development is prolonged (Paukstis et al., 1984; Ewert, 1991). Moreover, a too high temperature in the soil (exceeding 33°C) was shown to disturb the embryonic development (Moll & Legler, 1971; Vasse, 1983). In our Regional model, the contribution of land cover to the model was low for both GLM and GAM except for the distance to the wetlands, which is the main habitat of *E. orbicularis* in Switzerland. Interestingly, the predictor importances are much more similar, here than in the Global model, for GLM model versus GAM model, which demonstrated that binary classes of landuse and landcover would be more similarly accounted by the GLM and GAM models. In the present study, the climatic variables were obtained from Chelsa 1.2, which is the most widely used climatic data source when construction SDMs (Fick & Hijmans, 2017). However, the

database is known to have some limitation (Deblauwe et al., 2016); indeed Chelsa 1.2 has average monthly climate data from minimum, mean and maximum temperature and precipitations only between 1979-2013, which may not accurately reflect the current climatic conditions for our species. Alternative climatic databases, such as WorldClim (Hijmans et al., 2005) or CliMond (Kriticos et al., 2011) could also be tested, or used in complement (e.g. ensemble models based on different climatic data sets; Araujo & New 2007), in future studies.

At the Global scale, the model predicted a clear shift of the potential distribution northwards (Continental and Atlantic regions), with a large overall gain of potential habitat (+63.8% under RCP 4.5 and +71.3% under RCP 8.5). For instance, the British coasts would become potentially suitable for *E. orbicularis*, although currently the European pond turtle is presumed not to occur naturally there, and has probably been absent since the last glaciation (as suggested by fossil remains; Gent, 2013). However, the species seems unlike to be able to disperse toward all news habitats due to the high degree of habitat fragmentation caused by transportation, urban and agriculture infrastructures (Jaeger et al., 2016). As a result, populations from the southern part of the range (North Africa and central Spain) might suffer from too high drought and/or eggs incubation temperatures to allow hatchling and/or producing a bias sex ratio toward females. Indeed, according to the literature on *E. orbicularis* (Pieau & Dorizzi, 1981; Vasse, 1983) and other Emydidae species (Moll & Legler, 1971; Mahmoud et al., 1973), embryo develops successfully between 18 and 33°C, and sex is determined by average temperature of 28°C for males and 29°C for females between the 30th and 40th day of incubation (Pieau & Dorizzi, 1981).

At the Swiss level, suitable habitats for *E. orbicularis* are also predicted to increase under climate change (+50.6% under RCP 4.5 and +64.6% under RCP 8.5). However, this

potential increase in suitable habitats may not translate into a larger realized distribution through colonization of new habitats because of the limited capacity of the species to migrate in the landscapes, as shown by the MigClim simulations. Moreover, the *Emys* occurrence points used for latter are sometimes made from the observation of one individual and occasionally individuals are non-native subspecies such as *Emys orbicularis galloitalica* (Raemy, 2010) and may not constitute viable populations from which new colonization could occur. Indeed, viable populations are occurring only in the Canton of Geneva (karch, 2014), which means that the species would not be able to recolonize suitable habitats naturally and, in order to ensure persistent and perennial populations of this emblematic endangered species, reintroduction actions are necessary. Indeed, habitat degradation (such as for *E. orbicularis* destruction (e.g. through road and urban developments) of water and nesting habitats (Trakimas & Sidaravičius, 2008; Drobekov, 2014)) leads to habitat fragmentation (e.g. roads and railways) and is definitively considered as strong barriers to the species' dispersal. Habitat degradations and fragmentations are a well-known primary threats to global diversity (IPBES 2019; <https://www.IPBES.org/>) and are shown to be more important than climate change at regional and local scales (Dirnboöck et al., 2003).

In order to minimize the threats associated with fragmentation, landscape connectivity should be enhanced, for example, by protecting corridors between suitable areas (Fahrig and Merriam, 1994; Pascual-Hortal & Saura, 2006; Arponen et al., 2013; Vasudev et al., 2015) and by building efficient ecological networks to facilitate the movements of species under future environmental conditions (Devictor et al., 2007; Alagador et al., 2016) or by translocation of individuals or reintroduction when natural recolonization is not achievable (Guisan et al., 2013).

5 | CONCLUSION

This study was the first to use a combined hierarchical-dispersal approach to predict the distribution of a species of major conservation importance, providing essential maps of *E. orbicularis'* potential habitats for the present and the future under two of the most recent climate change scenarios. The study further highlighted key habitats of conservation and monitoring priority at the Swiss scale, this way also providing a major contribution to help implement efficient conservations actions in the field. We further demonstrated that even if *E. orbicularis* might gain suitable habitats under future climate, the colonization of new suitable habitat from currently occupied sites could be hampered by the species' low dispersal capacity and by habitat fragmentation. Therefore, in order to conserve this species in Switzerland, and probably also in its European entire range, reintroduction programs should prove essential.

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DATA AVAILABILITY STATEMENT

The data that support the finding of this study are available on request from info fauna, Switzerland.

REFERENCES

- Addison, P. F. E., Rumpff, L., Bau, S. S., Carey, J. M., Chee, Y. E., Jarrad, F. C., McBride, M. F., & Burgman, M. A. (2013). Practical solutions for making models indispensable in conservation decision-making. *Diversity and Distributions*, 19, 490-502.
- Alagador, D., Cerdeira, J. O., & Araújo, M. B. (2016). Climate change, species range shifts and dispersal corridors: an evaluation of spatial conservation models. *Methods in Ecology and Evolution*, 7, 853–866.
- Allouche, O., Tsoar, A., & Kadmon, R. (2006). Assessing the accuracy of species distribution models: Prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology*, 43, 1223-1232.
- Alvarez, G., Salas, E. A. L., Harnings, N. M., & Boykin, K. G. (2017). Projections of future suitable bioclimatic conditions of parthenogenetic whiptails. *Climate*, 5,34.
- Araujo, M.B. & New, M. (2007). Ensemble forecasting of species distributions. *Trends in Ecology & Evolution*, 22, 42-47.
- Arponen, A., Heikkinen, R. K., Paloniemi, R., Poyry, J., Simila, J., & Kuussaari, M. (2013). Improving conservation planning for semi-natural grasslands: Integrating connectivity into agri-environment schemes. *Biological Conservation*, 160, 234-241.
- Austin, M. P., Belbin, L., Meyers, J. A., Doherty, M. D., & Luoto, M. (2006). Evaluation of statistical models for predicting plant species distributions: role of artificial data and theory. *Ecological Modelling*, 199, 197-216.
- Barbet-Massin, M., Thuiller, W., & Jiguet, F. (2010). How much do we overestimate future local extinction rates when restricting the range of occurrence data in climate suitability model? *Ecography*, 33, 878-886.

Barbet-Massin, M., Jiguet, F., Albert, C. H., & Thuiller, W. (2012). Selecting pseudo-absences for species distribution models: how, where and how many? *Methods in Ecology and Evolution*, 3, 327-338.

Barnosky, A. D., Matzke, N., Tomaia, S., Wogan, G. O. U., Swartz, B., Quental, T. B., Marshall, C., McGuire, J. L., Lindsey, E. L., Maguire, K. C., Mersey, B., & Ferrer, E. A. (2011). Has the Earth's sixth mass extinction already arrived? *Nature*, 471, 51-57.

Ben Rais Lasmar, F., Guilhaumon, F., Albouy, C., Somot, S., Thuiller, W., & Mouillot, D. (2010). The Mediterranean Sea as a 'cul-de-sac' for endemic fishes facing climate change. *Global Change Biology*, 16, 3233-3245.

Buckley, L. B., & Jetz, W. (2007). Environmental and historical constraints on global patterns of amphibian richness. *Proceedings of the Royal Society B: Biological Sciences*, 274, 1167-1173.

Cadi, A. (2003). Écologie de la cistude d'Europe (*Emys orbicularis*) : Aspects spatiaux et démographiques, application à la démographie. Lyon, Université Claude-Bernard 1: 350p.

Cadi, A., Nemoz, M., Thienpont, S., & Joly, P. (2004). Home range, movements, and habitat use of the European pond turtle (*Emys orbicularis*) in the Rhône-Alpes region, France. *Biologia*, 59(14), 89-94.

Ceia-Hasse, A., Sinervo, B., Vicente, L., & Pereira, H.M. (2014). Integrating ecophysiological models into species distribution projections of European reptile range shifts in response to climate change. *Ecography*, 37, 679-688.

Chen, I. C., Hill, J. K., Ohlemuller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, 333, 1024-1026.

Cianfrani, C., Broennimann, O., Loy, A., & Guisan, A. (2018). More than range exposure: Global otter vulnerability to climate change. *Biological Conservation*, 221, 103-113.

- Cordero Rivera, C., & Fernandez, C. (2004). A management plan for the European pond turtle (*Emys orbicularis*) populations of the Louro river basin (Northwest Spain). *Biologia, Bratislava*, 59(14), 161-171.
- Cushman, S.A. (2006). Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation*, 128(2), 231-240.
- Davis A. J., Jenkison, L. S., Lawton, J. H., Shorrocks, B., & Wood, S. (1998). Making mistakes when predicting shifts in species range in response to global warming. *Nature*, 391, 783-786.
- Deblauwe, V., Droissart, V., Bose, R., Sonké, B., Blach-Overgaard, A., Svenning J. C., Wieringa, J. J., Ramesh, B. R., Stévart, T., & Couvreur, T. L. P. (2016). Remotely sensed temperature and precipitation data improve species distribution modelling in the tropics. *Global Ecology and Biogeography*, 25(4), 443-454.
- Devictor, V., Godet, L., Julliard, R., Couvet, D., & Jiguet, F. (2007). Can common species benefit from protected areas? *Biological Conservation*, 139, 29-36.
- Dirnböck, T., Dullinger, S., & Grabbherr, G. (2003). A regional impact assessment of climate and land-use change on alpine vegetation. *Journal of Biogeography*, 30, 401-417.
- Drobenkov, S.M. (2014). Current state, anthropogenic threats and conservation the European pond turtle (*Emys orbicularis*) in Belarus. *Acta Bioliga*, 14(1), ISSN 1407-9853
- Duysebaeva, T. N., Doronin, I. V., Malakhov, D. V., Kukushkin, A. G., & Bakiev, A. G. (2019). GIS analysis of the distribution and habitation conditions of *Emys orbicularis orbicularis* (Testudines, Emydidae): Methodical aspects. *Natural Sciences*, 1(25).
- Elith, J., & Leathwick, J. R. (2009). Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systematics*, 40, 677-697.

- Engler, R., & Guisan, A. (2009). MigClim: Predicting plant distribution and dispersal in a changing climate. *Diversity and Distributions*, 15, 590-601.
- Engler, R., Hordijk, W., & Guisan, A. (2012). The MIGCLIM R package – seamless integration of dispersal constraints into projections of species distribution models. *Ecography*, 35, 872-878.
- Ewert, M. A. (1991). Cold torpor, diapause, delayed and aestivation in reptiles and birds. Eggs incubation: its effects on embryonic developments in birds and reptiles, pp. 173-191. In: Deeming, D. C., Ferguson M. W. J. (eds) Eggs incubation: Its effects on embryonic developments in birds and reptiles. Cambridge University Press, Cambridge.
- Fahrig, L., & Merriam, G. (1994). Conservation of fragmented populations. *Conservation Biology* 8: 50-59.
- Ficelota, G. F., Padoa-Schioppa, E. P., Monti, A., Massa, R., De Bernardi, F., & Bottoni, L. (2004). The importance of aquatic and terrstral habitat for the European pond turtle (*Emys orbicularis*): implication for conservation planning and management. *Revue Canadienne de Zoologie*, 82(11), 1704-1712.
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*; 37, 4302-4315.
- Franklin, J. (2010). Mapping species distribution: Spatial interference and prediction. Cambridge University Press, Cambridge.
- Frazer, N. B., Greene, J. L., & Gibbons, J. W. (1993). Temporal variation in growth rate and age at maturity of male Painted Turtles, *Chrysemys picta*. *The American Midland Naturalist*; 130, 314-324.

- Fritz, U., Fattizzo, T., Guicking, D., Tripepi, S., Pennisi, M. G., Lenk, P., Joger, U., & Wink, M. (2005). A new cryptic species of pond turtle from southern Italy, the hottest spot in the range of the genus *Emys*. *Zoologica Scripta*, 34, 351-371.
- Gent, T. (2013). Historical and current situation of the European pond turtle (*Emys orbicularis*) in the United Kingdom. *Herpetology Notes*: 6, 117-118.
- Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., & Green, J. L. (2000). The Global decline of Reptiles, déjà vu amphibians. *BioScience*, 50, 653-666.
- Golubović, A., Grabovac, D., & Popović, M. (2017). Actual and potential distribution of the European pond turtle, *Emys orbicularis* (L., 1758) in Serbia, with conservation implications. *Acta Zoologia Bulgaria*, 10, 49-56.
- Gonçalves, J., Honrado, J. P., Vicente, J. R., & Civantos, E. (2016). A model-based framework for assessing the vulnerability of low dispersal vertebrates to landscape fragmentation under environmental change. *Ecological Complexity*, 28, 174-186.
- Guisan, A., & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitats models. *Ecological Letters*, 8, 993-1009.
- Guisan, A., Tingley, R., Baumgartner, J. B., Naujokaitis-Lewis, I., Sutcliffe, P. R., Tulloch, A. I. T, Tracey, J. R., et al. (2013). Predicting species distributions for conservation decisions. *Ecology Letters*, 16, 1424-1435.
- Guisan, A., Thuiller, W., & Zimmermann, N. E. (2017). Habitat suitability and distribution model with applications in R. *Ecology, Biodiversity and Conservation*, Cambridge.
- Halpin, P. N. (1997). Global climate change and natural-area protection: management responses and research directions. *Ecological Application*, 7, 828-843.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surface for global land areas. *International Journal of Climatology*, 25, 1965-1978.

IPBES (2019). Global assessment report on biodiversity and ecosystem services. Science and Policy for People and Nature.

IPCC (2019). Climate change and land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems [P. R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.-O. Pörtner, D. C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M. Belkacemi, J. Malley, (eds.)]. In press.

Jaeger, J. A. G., Soukup, T., Schwick, C., Madriñán, L. F., & Kienast, F. (2016). Landscape fragmentation in Europe. In: Jan Feranec, Tomas Soukup, Gerard Hazeu, Gabriel Jaffrain (Eds), European Landscape Dynamics: CORINE Land Cover Data, Chapter 20, 42 pp.

Javed, S. M. M., Raj, M., & Kumar, S. (2017). Predicting potential habitat suitability for an endemic gecko *Calodactylodes aureus* and its conservation implications in India. *Tropical Ecology*, 58(2), 271-282.

Janzen, F. J. (1994). Climate change and temperature dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences*, 91, 7487-7490.

Jensen, M. P., Allen, C. D., Eguchi, T., Bell, I. P., LaCasella, E. L., Hilton, W. A., Hof, C. A. M., & Dutton, P. H. (2018). Environmental warming and feminization of one of the largest sea turtle populations in the world. *Current Biology*, 28(1), 154-159.

Karch. (2014). Lignes directrices du karch pour la conservation de la Cistude d'Europe (*Emys orbicularis*) en Suisse. Centre Suisse de Coordination pour la Protection des Amphibiens et Reptiles (karch).

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kref, H., Soria-Auza, R. W., Zimmermann, N. E., Linder, H. P., & Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, 4, 170122.

Krawchuck, M. A., & Brooks, R. J. (1998). Basking behavior as a measure of reproductive cost and energy allocation in the painted turtle, *Chrysemys picta*. *Herpetology*, 51, 217-224.

Kriticos, D. J., Webber, B. L., Leriche, A., Ota, N., Macadam, I., Bathols, J., & Scott, J. K. (2011). CliMond: global high-resolution historical and future scenario climate surface for bioclimatic modelling. *Methods in Ecology and Evolution*, 3(1), 53-64.

Kubisch, A., Holt, R. D., Poethke, H. J., & Fronhofer, E. A. (2014). Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos*, 123, 5-22.

Kumara, H. N., Irfan-Ullah, M., & Kumar, S. (2009). Mapping potential distribution of Slender Loris subspecies in peninsular India. *Endangered Species Research*, 7, 29-38.

Lebboroni, M., & Chelazzi, G. (1991). Activity patterns of *Emys orbicularis* L. (Chelonia Emydidae) in central Italy. *Ethology Ecology & Evolution*, 3(3), 257-268.

Lebboroni, M., & Chelazzi, G. (2000). Waterward orientation and homing after experimental displacement in the European pond turtle, *Emys orbicularis*. *Ethology Ecology & Evolution*, 12(1), 83-88.

Lewis, S. L., & Maslin, M. A. (2015). Defining the Anthropocene. *Nature*, 519, 171-180.

Mac, M. J., Opler, P. A., Puckett Heacker, C. E., & Doran, P. D. (1998). Status and trends of the nation's biological resources. U.S. Department of the Interior, U.S. Geological Survey, Minneapolis, Minnesota, USA.

Mahmoud, I. Y., Hess, G. L., & Klicka, J. (1973). Normal embryonic stages of western painted turtles, *Crysemys picta bellii*. *Journal of Morphology*, 141, 269-279.

Manning, B., & Grigg, G. C. (1997). Basking is not of thermoregulation significance in the "basking" freshwater turtle *Emydura signata*. *Copeia*, 1997, 579-585.

Margules, C. R., & Pressey, R. L. (2000). Systematic conservation planning. *Nature*, 405, 243-253.

Mateo, R. G., Gastón A., Aroca Fernández M. J., Broennimann O., Guisan A., Saura S., & García Viñas J. I. (2019). Hierarchical species distributions models in support of vegetation conservation at the landscape scale. *Journal of Vegetation Science*, 30(2), 386-396.

Moll, E. O., & Legler, J. M. (1971). The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff) in Panamal, *Bulletin of Los Angeles county Museum of Natural History*, 11, 1-102.

Monney, J.-C., & Meyer, A. (2005). Liste rouge des espèces menacées en Suisse, Reptile. Office fédéral de l'environnement, des forêts et du paysage (OFEV), Centre de coordination des amphibiens et reptiles de Suisse (Karch). Berne, 46 p.

National ecological network (REN) 2004. Final report. A vision for a nationwide networked living space. Environmental SRU series. 131 p. Federal Office for the Environment FOEN.

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., D'Amico, J. A., Itoua, I., Strand, H. E., Morrison, J. C., Loucks, C. J., Allnutt, T. F., Ricketts, T. H., Kura, Y., Lamoreux, J. F., Wettengel, W. W., Hedao, P., &

Kassem, K. R. (2001). Terrestrial ecoregions of the world: a new map of life on Earth.

Bioscience, 51(11), 933-938.

Pascual-Hortal, L., & Saura, S. (2006). Comparison and development of new graph-based landscape connectivity indices: towards the prioritization of habitat patches and corridors for conservation. *Landscape Ecology*, 21(7), 959-967.

Paukstis, G. L., Gutzke, W. H. N., & Packard, G. C. (1984). Effects of substrate water potential and fluctuating temperatures on sex ratios of hatchling painted turtles (*Chrysemys picta*). *Canadian Journal of Zoology*, 62, 1491-1494.

Pearson, R. G., Dawson, T. P., & Liu, C. (2004). Modelling species distributions in Britain: a hierarchical integration of climate and land-cover data. *Ecography*, 27, 285-298.

Pereira, M., Segurado, P., & Neves, N. (2011). Using spatial network structure in landscape management and planning: A case study with pond turtles. *Landscape and Urban Planning*, 100, 67-76.

Peterson A. T., & Nakazawa, Y. (2008). Environmental data sets matter in ecological niche modelling: an example with *Sphenopsis invicta* and *Sphenopsis richteri*. *Global Ecology and Biogeography*, 17, 135-144.

Peterson, A. T., Soberón, J., Pearson, R. G., Anderson, R. P., Martinez-Meyer, E., Nakamura, M., & Araújo, M. B. (2011). Ecological niches and geographic distributions. Princeton University Press, Princeton, USA.

Petitpierre, B., Broennimann, O., Kueffer, C., Daehler, C., & Guisan, A. (2016). Selecting predictors to maximize the transferability of species distribution models: lessons from cross-continental plant invasions. *Global Ecology & Biogeography*, 26(3), 275-287.

- Petitpierre, B., McDougall., K., Seipel, T., Broennimann, O., Guisan, A., & Kueffer, C. (2016). Will climate change increase the risk of plant invasions into mountains? *Ecological Applications*, 26, 530-544.
- Phillips, S. J., & Elith, J. (2013). On estimating probability of presence from use-availability or presence-background data. *Ecology*, 94, 1409-1419.
- Piccard, G., Carrière, M. A., & Blouin-Demers, G. (2011). Common musk turtles (*Sternotherus odoratus*) select habitats of high thermal quality at the northern extrem of their range. *Amphibia-Reptilia*, 32, 83-92.
- Pieau, C., & Dorizzi, M. (1981). Determination of temperature sensitive stages for sexual differentiation of the gonads in embryos of the turtle, *Emys orbicularis*. *Journal of Morphology*, 170, 373-382.
- Pittet, M. (2017). Impact of global warming on the distribution and dispersal of reptiles in Western Swiss Alps. Master Thesis. Master in Behaviour, Evolution, Conservation (BEC), Department of Ecology & Evolution, University of Lausanne, Switzerland.
- Rego, A., Clavero, M., D'Amen, M., Guisan, A., & Brotons, L. (2018). Wildfire-vegetation dynamics affect predictions of climate change impact on bird communities. *Ecography*, 41(6), 982-995.
- Rovero, F., & Chelazzi, H. (1996). Nesting migrations in a population of the European pond turtle *Emys orbicularis* (L.) (Chelonia Emydidae) from central Italy. *Ethology Ecology & Evolution*, 8(3), 297-304.
- Salas, E. A. L., Seamster, V. A., Harings, N. M., Boykin, K. G., Alvarez, G., & Dixon, K. W. (2017). Projected future bioclimate-envelope suitability for reptile and amphibian species of concern in South Central USA. *Herpetological Conservation and Biology*, 12(2), 522-547.

Segurado, P., & Araújo, A. P. R. (2004). Coexistence of *Emys orbicularis* and *Mauremys leprosa* in Portugal at two spatial scales: is there evidence of spatial segregation? *Biologia, Bratislava*, 59(14), 61-72.

Sexton, J.P., McIntyre, P.J., Angert, A.L., Rice, K.J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics* 40: 415-436.

Shabani, F., Kumar, L., & Ahmadi, M. (2018). Assessing accuracy methods of species distribution models: AUC, specificity, sensitivity and the true skill statistics. *Global Journal of Human-Social Science*, 18.

Speybroeck, J., Beukema, W., Dufresnes, C., Fritz, U., Jablonski, D., Lymberakis, P., Martinez-Solano, I., Razzetti, E., Vamberger, M., Vences, M., Vörös, J., & Crochet, P. A. (2020). Species list of the European herpetofauna – 2020 update by Taxonomic Committee of the Societas Europaea Herpetologica. *Amphibia-Reptilia*, 41, 139-189.

Stearns, S. C., & Koella, J. C. (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution*, 40, 893-913.

Swets, J.A. (1998). Measuring the accuracy of diagnostic systems. *Science*, 240, 1285-1293.

Swisstopo. 2020. swissTLM3D. Available online: <https://shop.swisstopo.admin.ch/de/products/landscape/tlm3d>

Thuiller, W., Brotons, L. Araujo, M. B., & Lavorel, S. (2004). Effects of restricting environmental range of data to project current and future species distributions. *Ecography*, 27, 165-172.

Thuiller, W., Georges, D., & Engler, R. (2013). Biomod2: Ensemble platform for the species distribution. R package version, 2, r560.

Tulloch, A. I. T., Sutcliffe, P., Naujokaitis-Lewis, I., Tingley, R., Brotons, L., Ferraz, K., et al. (2016). Conservation planners tend to ignore improved accuracy of modelled

species distributions to focus on multiple threats and ecological processes.

Biological Conservation, 199, 157-171.

Trakimas, G., & Sidaravičius, J. (2008). Road mortality threatens small northern populations of the European pond turtle, *Emys orbicularis*. *Acta Herpetologica*, 3(2), 161-166.

Uusitalo, R., Siljander, M., Culverwell, C. L., Mutai, N. C., Forbes, K. M., Vapalahti, O., & Pellikka, P. K. E. (2019). Predictive mapping of mosquito distribution based on environmental and anthropogenic factors in Taita Hills, Kenya. *International Journal of Applied Earth Observation and Geoinformation*, 76, 84-92.

Vamberger, M., & Fritz, U. (2018). Big data can cause big mistakes: using the Societas Europaea Herpetologica atlas by Sillero et al. (2014), the distribution of *Emys orbicularis* will be misunderstood. Letter to the Editor. *Biologia*.

Van Vuuren, D. P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G. C., Kram, T., Lamarque, J. F., Masui, T., Meinshausen, M., Nakicenovic, N., Smith, S. J., & Rose, S. K. (2011). The representative concentration pathways: an overview. *Climatic Change*, 109, 5.

Vasudev, D., Fletcher Jr. R. J., Goswami, V. R., & Krishnadas, M. (2015). From dispersal constraints to landscape connectivity: lessons from species distribution modeling. *Ecography*, 38(10), 967-978.

Vasse, J. (1983). Transplantation of turtle embryonic thymus into quail embryo: colonization by quail cells. *Journal of Embryology and Experimental Morphology*, 77, 309-322.

Zurell D, Franklin J, König C, Bouchet PJ, Serra-Diaz JM, Dormann CF, Elith J, Fandos Guzman G, Feng X, Guillera-Arroita G, Guisan A, Leitão PJ, Lahoz-Monfort JJ, Park DS, Peterson AT, Rapacciulo G, Schmatz DR, Schröder B, Thuiller W, Yates KL,

Zimmermann NE, Merow C (2020) A standard protocol for describing species distribution models. *Ecography*. DOI: 10.1111/ecog.04960.

Tables and Figures

Table 1: Bioclimatic variables used for the species distribution model (SDM) of the European pond turtle (*Emys orbicularis*). These variables were extracted from Chelsa version 1.2 database (Karger et al., 2017).

Bioclimatic variable - Global Model	
bio1	Annual Mean Temperature
bio3	Isothermality (Mean Diurnal Range / Temperature Annual Range) (*110)
bio7	Temperature Annual Range (Max Temperature of Warmest Month - Min Temperature of Coldest Month)
bio10	Mean Temperature of Warmest Quarter
bio12	Annual Precipitation
bio13	Precipitation of Wettest Month
bio16	Precipitation of Wettest Quarter

Table 2: Land cover data used for the species distribution model (SDM) of the European pond turtle (*Emys orbicularis*) at the Regional scale (Switzerland). Data were download from GeoAdmin (<https://maps.geo.admin.ch/>).

Land cover data - Regional Model	
Aquatic	Distance to aquatic habitats
Wetlands	Distance to wetlands
Meadows	Distance to meadows (nesting sites)
Forest	Distance to forests
Buildings	Distance to building (villages, cities)
Roads	Distance to roads (large of 6 to 10 m)
Railways	Distance to railways
Agriculture	Distance to extensive agriculture area
Reserve	Distance to natural reserves

Figure 1: Ensemble modeling implemented in the R package BIOMOD2 (Thuiller et al., 2013) to model habitat suitability models for *E. orbicularis* at the Global and Swiss scale.

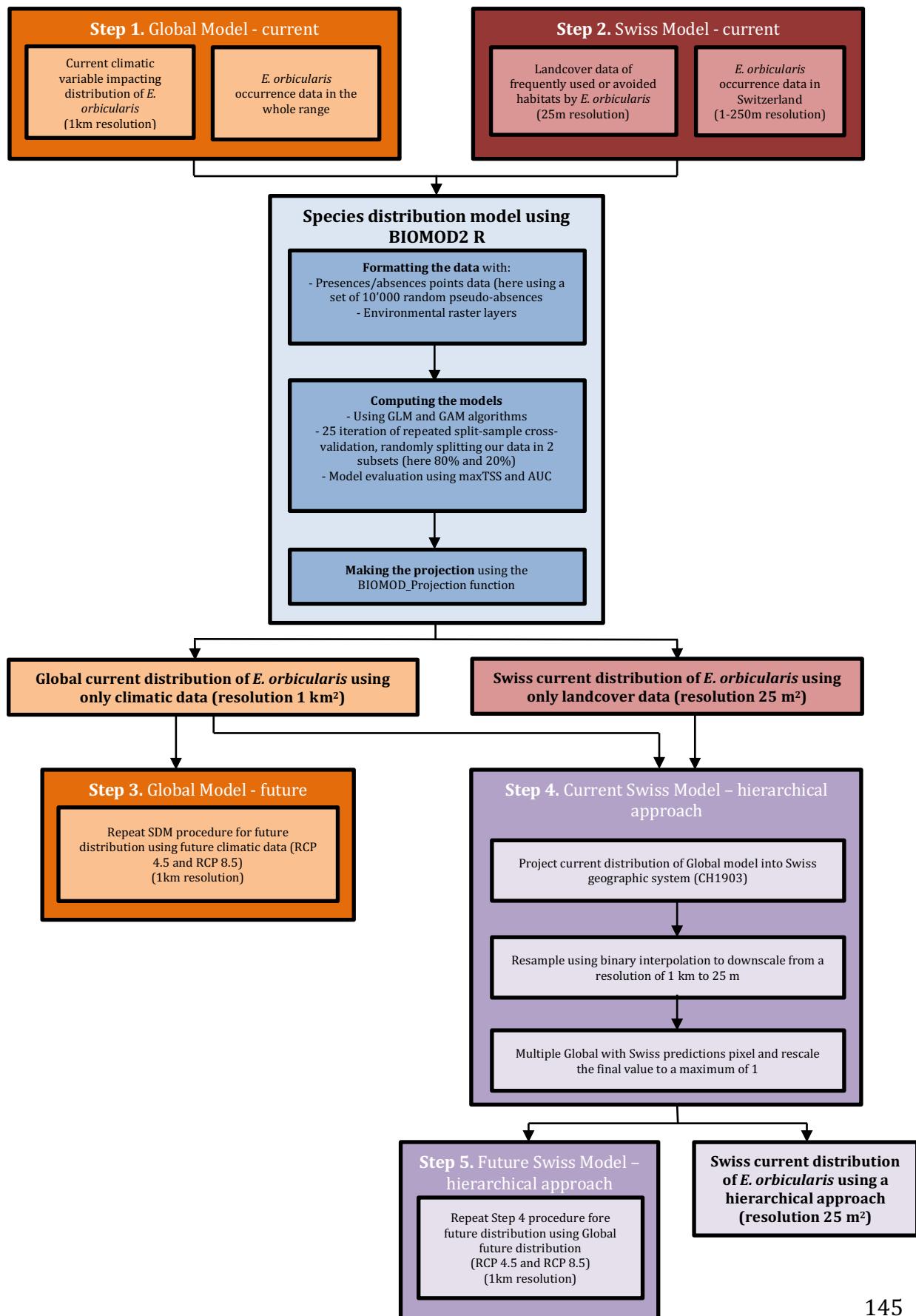


Figure 2: Boxplot illustrating the importance of bioclimatic variables from Chelsa 1.2 (Karger et al., 2017) over the 25 prediction models perform for the species model distribution of *Emys orbicularis*. The bioclimatic variables are annual mean temperature (bio1), isothermality (bio3), temperature annual range (bio7), mean temperature of warmest quarter (bio10), annual precipitation (bio12), precipitation of wettest month (bio13) and precipitation of wettest quarter (bio16).

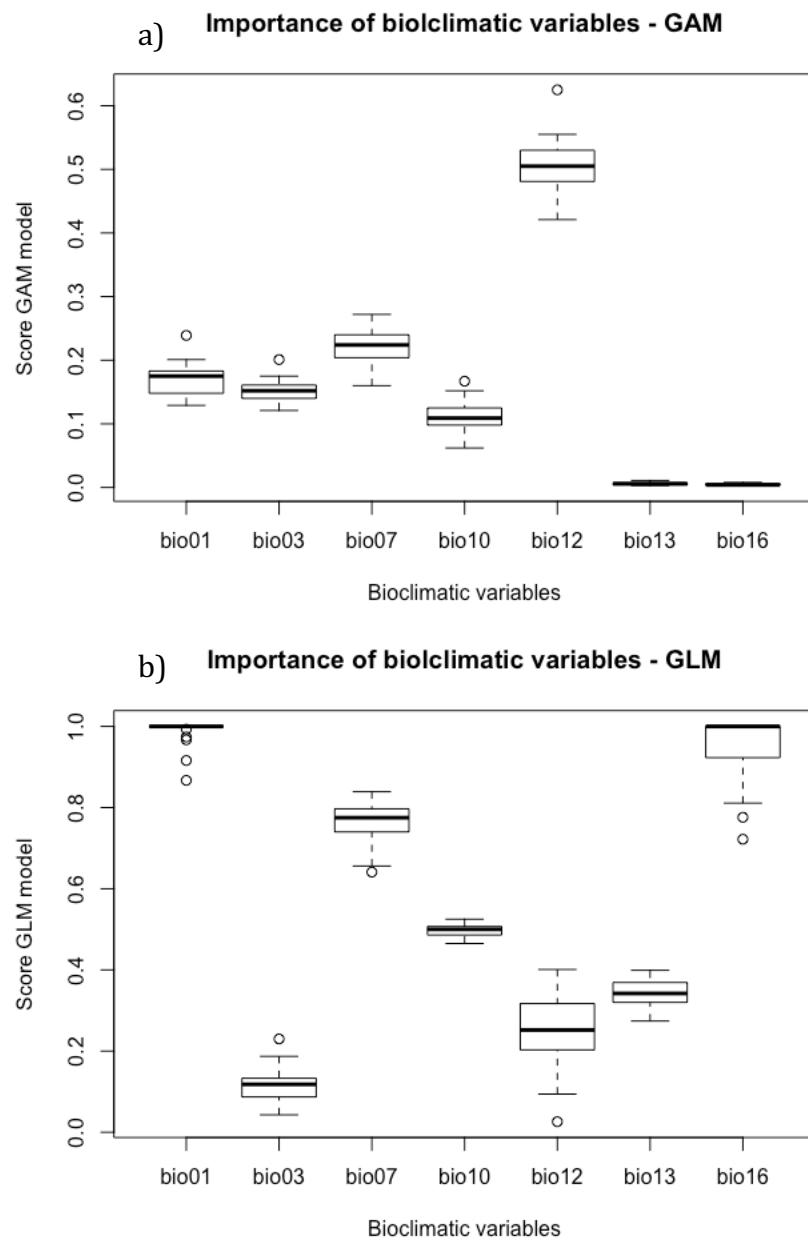


Figure 3: Boxplot illustrating the importance of distance to these landcover classes from GeoAdmin (<https://maps.geo.admin.ch/>) over the 25 prediction models perform for the species model distribution of *Emys orbicularis* in Switzerland.

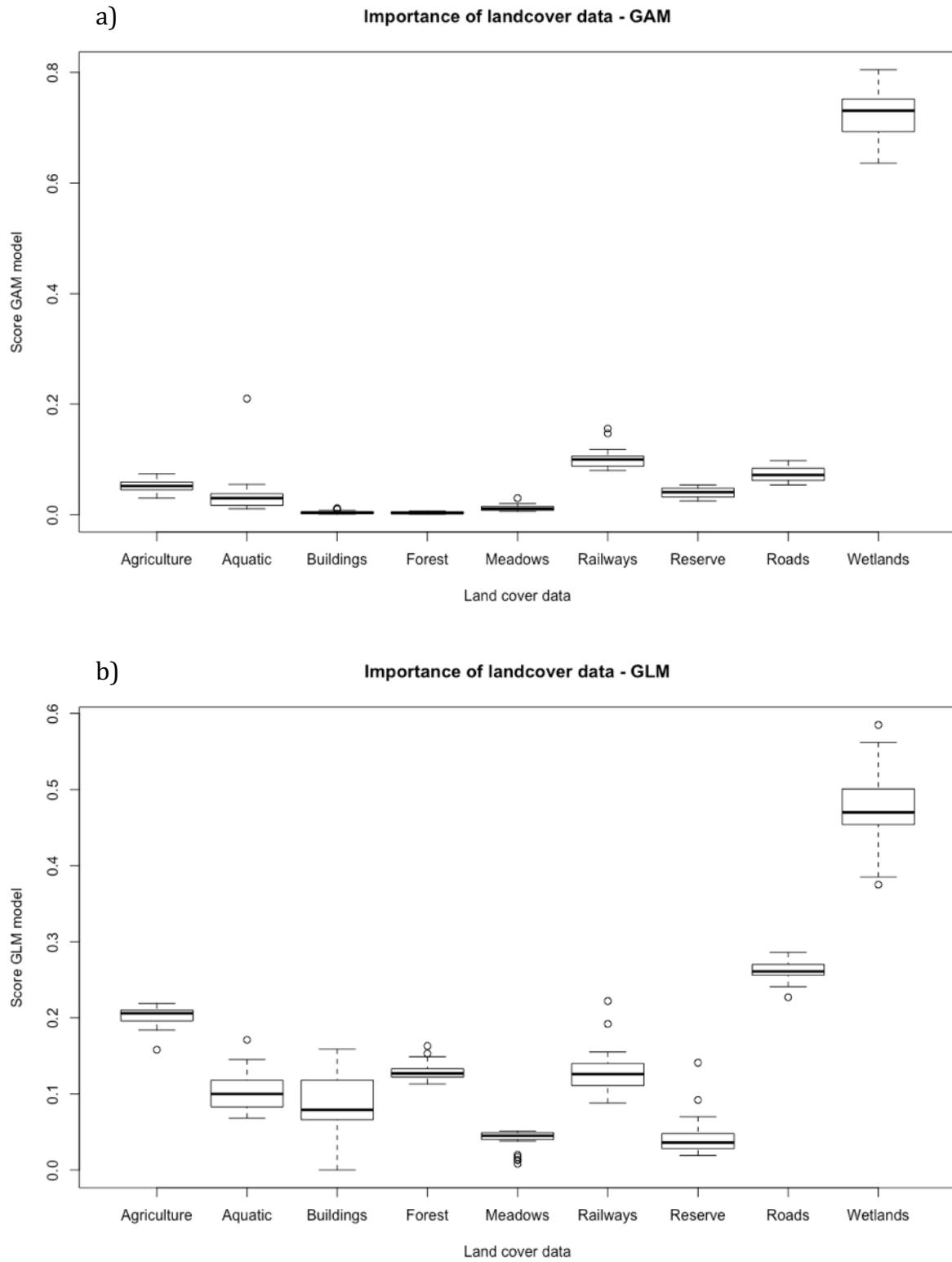


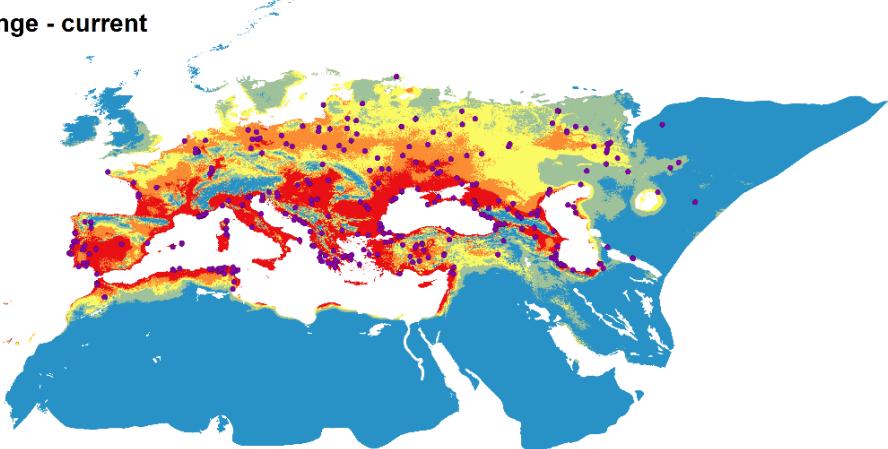
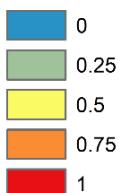
Figure 4: Predicted current potential distribution of *Emys orbicularis* throughout its whole range (a) and under climatic datasets relative to the representative concentration pathways (b) RCP 4.5 and (c) RCP 8.5 (2061-2080). The scale indicates less suitable environment (cooler colors) and most suitable environment (warmer colors).

(a)

- Emys orbicularis occurrence points

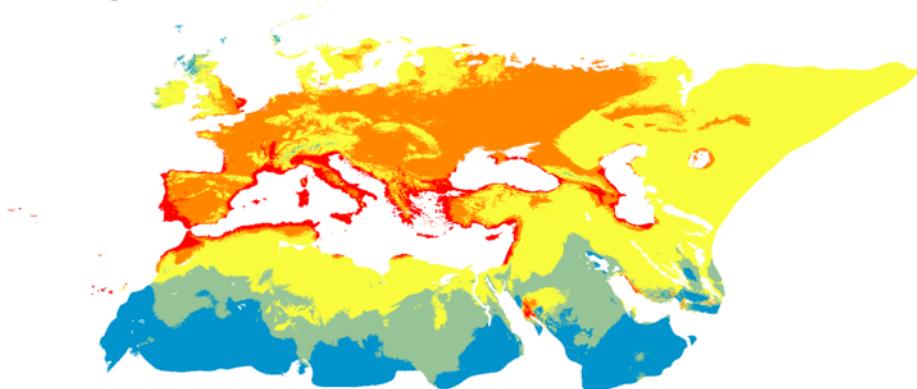
Suitability for Emys orbicularis occurrence

Global range - current



(b)

Global range - RCP 4.5



(c)

Global range - RCP 8.5

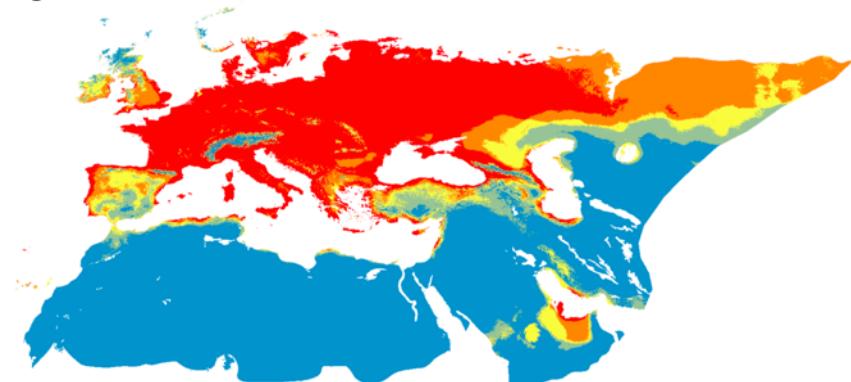


Figure 5: Comparison between predicted current potential distributions of *Emys orbicularis* using (a) only land cover data, (b) combining land cover data and Global climatic model. The scale indicates less suitable environment (cooler colors) and most suitable environment (warmer colors). (c) The land cover data model predicted more potential suitable habitats (70.17% - yellow color) than the model combining land cover data and Global climatic model (green color).

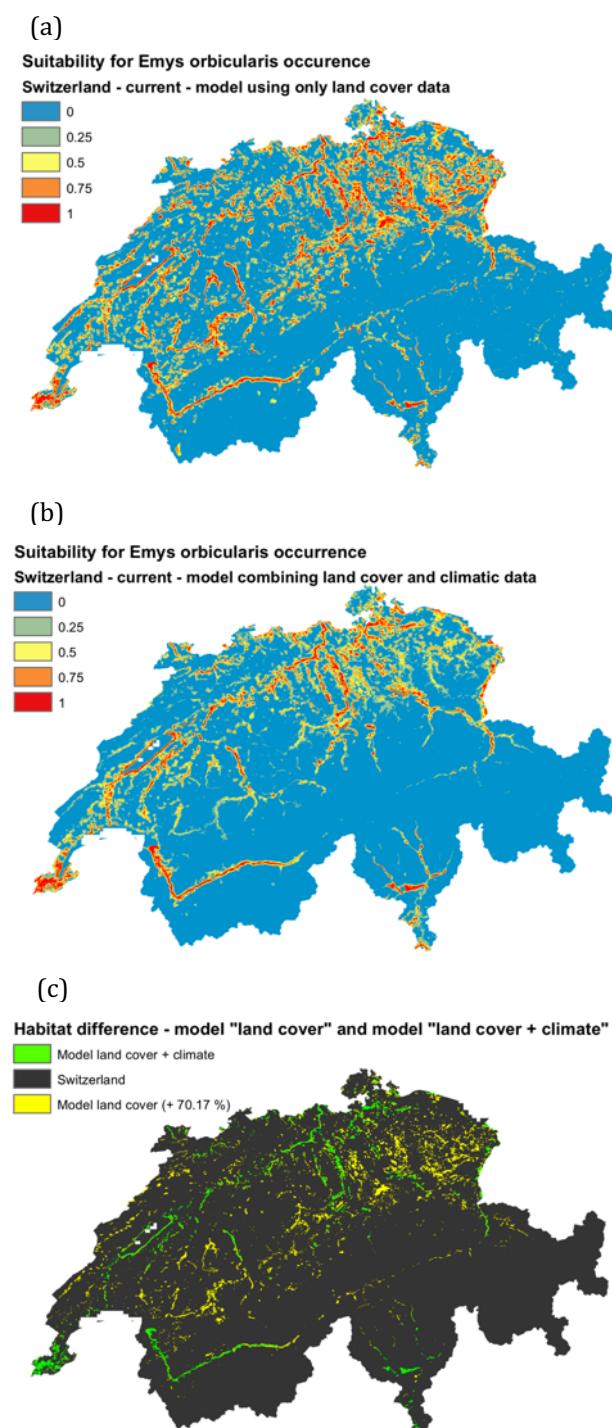


Figure 6: Predicted current potential distribution of *Emys orbicularis* in Switzerland (a) and under climatic datasets relative to the representative concentration pathways (b) RCP 4.5 and (c) RCP 8.5 (2061-2080) based on the combined model. The scale indicates less suitable environment (cooler colors) and most suitable environment (warmer colors). The occurrence points represent the most recent observations (1997-2019) of the species in Switzerland. These observations are not all viable population but could be isolated and genetically not adapted individuals

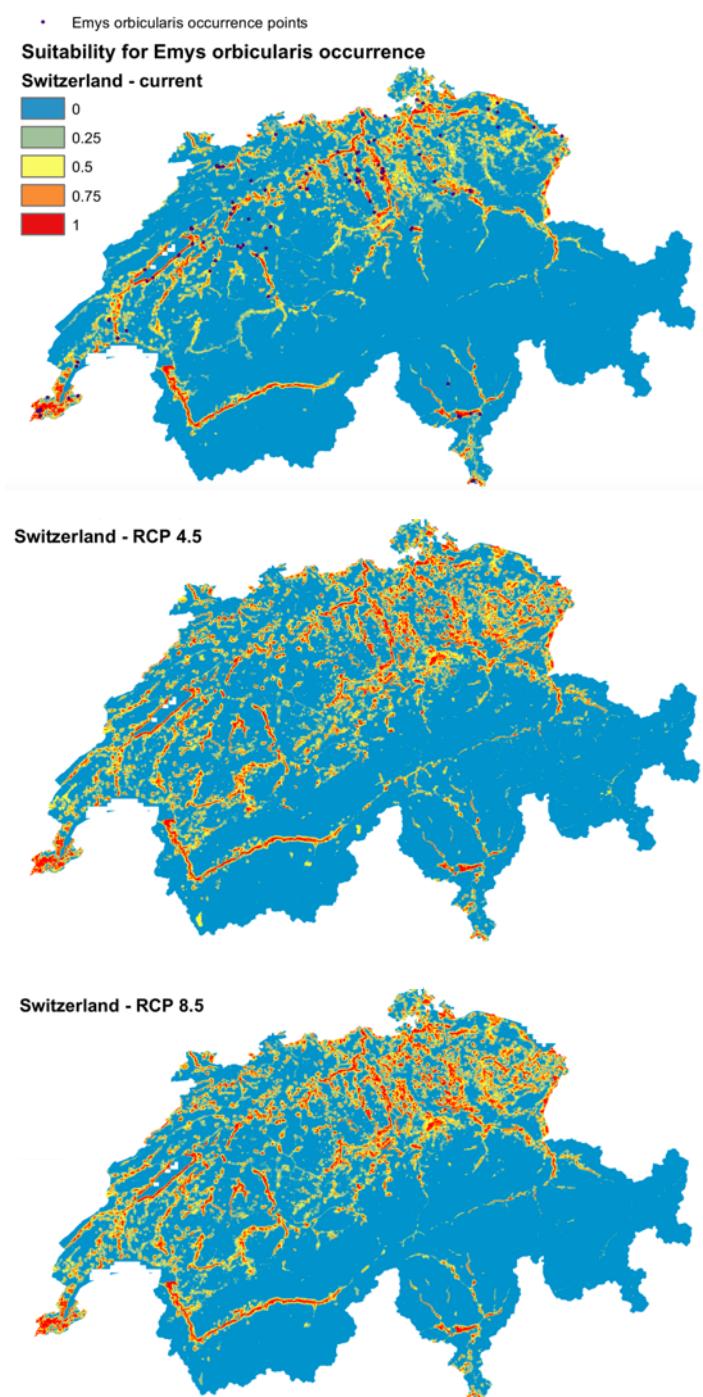
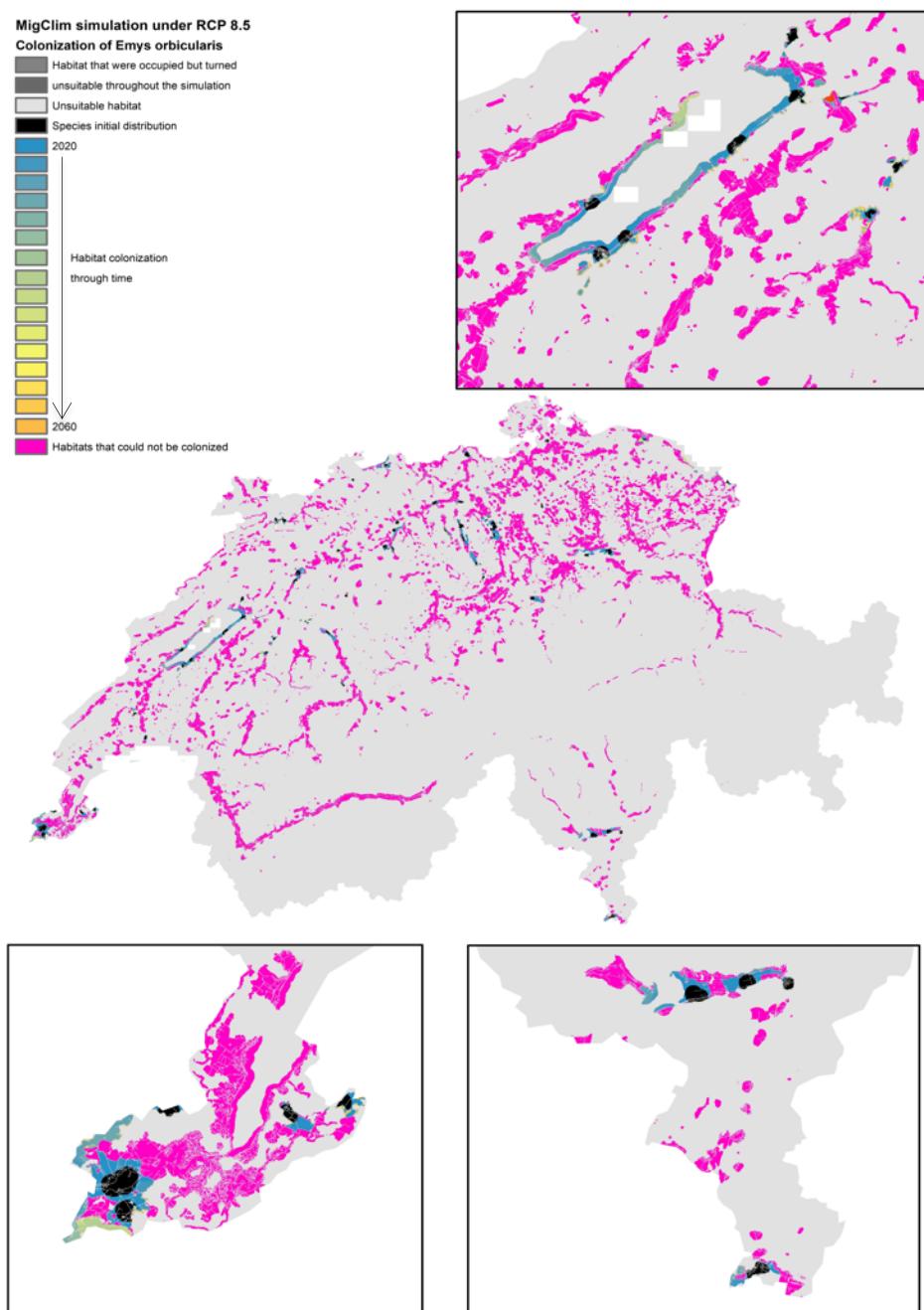
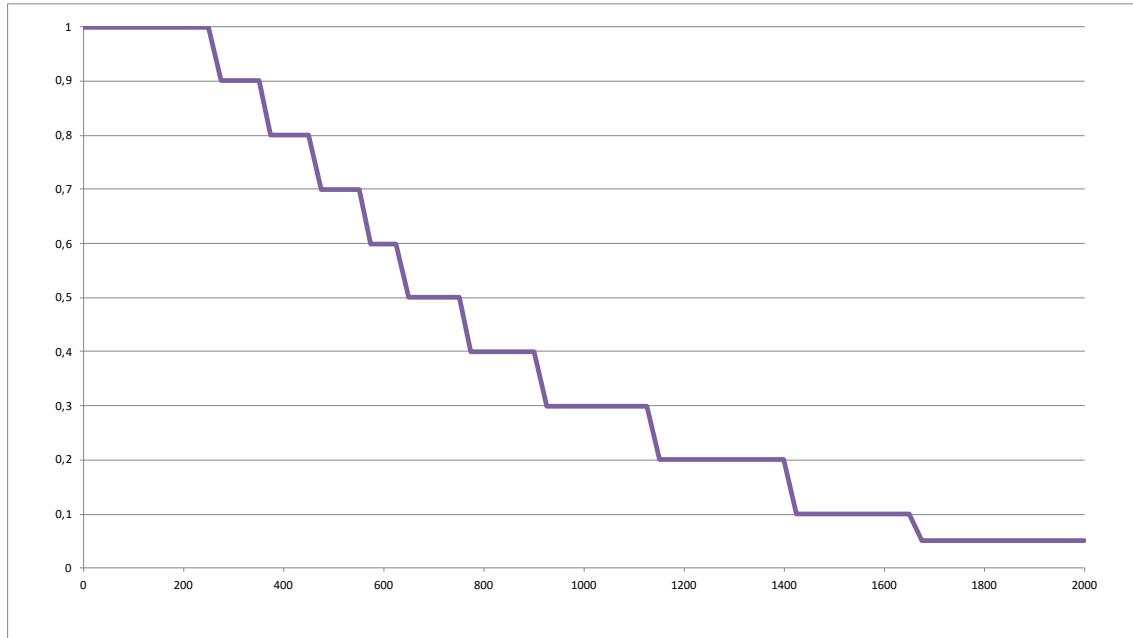


Figure 7: Dispersal simulation, using the MigClim R package (Engler et al. 2012), from recent observations (black color) of *Emys orbicularis* in Switzerland. The scale (from cooler to warmer color) indicate the colonization of new habitat by the species though times (current to 2060) and pink color are habitats that could not be colonized. The migration is limited by habitat fragmentation and urbanization (roads and railways) and by the low ability of the species to disperse.



Supplementary Material

Figure S1: Dispersal kernel of the European pond turtle (*Emys orbicularis*) based on the literature (Lebboroni & Chelazzi, 1991; Rovero & Chelazzi, 1996; Lebboroni & Chelazzi, 2000; Cadi et al., 2004) and expert knowledge (com. pers. S. Ursenbacher and C. Ducotterd).



Chapter 3

**What is next for the European pond turtle in
Switzerland?**

**Conservation strategy of European pond
turtles (*Emys orbicularis*, L. 1758)**

CHAPTER 3

Conservation actions for the European pond turtles (*Emys orbicularis*, L.1758) – current efforts, integrated framework and future perspectives in Switzerland

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C.D. wrote the manuscript and the other coauthors revised it.

Abstract

Turtle and tortoise species are in decline globally, and some species are already extinct. This decline has led to the development of numerous conservation programmes to ensure long-term viable populations of turtles and tortoises. The European pond turtle (*Emys orbicularis*) is a freshwater turtle emblematic of wetlands and is considered an “umbrella” species because its biological cycle requires the conservation of both aquatic and terrestrial habitats. In Switzerland, this species is ranked as “critically endangered” on the Red List. Two distinct conservation strategies, which are not mutually exclusive, could be considered for this species: (i) habitat renaturations to enhance natural recolonization and expansion and (ii) population reinforcements through reintroduction actions in suitable sites. In 1999, the Emys Project was launched to conserve this species. Until 2019, multiple studies combining both fundamental and applied sciences (e.g., population monitoring, ecological studies) enabled the determination of an integrated framework. Thus, to be effective, in the short term, the conservation of the European pond turtle in Switzerland should be based on the reinforcement conservation strategy through reintroduction actions in favourable sites. In the long term, due to the large-scale loss of wetlands in Switzerland, habitat renaturations are needed to increase habitat connectivity between existing *Emys* populations and between reintroduced populations and new habitats, as well as potentially increasing the natural expansion of *E. orbicularis*. Moreover, this species could be employed as a flagship species and may play an important role in raising public awareness regarding the highly important function and vulnerability of wetlands.

Keywords: Endangered species, *Emys orbicularis*, conservation, reintroduction, Switzerland

1. Introduction

Currently, 360 turtle and tortoise species are recognized worldwide (Turtle Taxonomy Working Group 2017; Rhodin et al., 2018), colonizing very diverse habitats, such as oceans, deserts, freshwater, and forests, many of which are often threatened (Mitchell & Klemens, 2000, Buhlmann et al., 2002). Approximately 60% of turtle and tortoise species are considered to be endangered, are the subject of conservation concerns or have become extinct in modern times, making them the most threatened of the major large groups of vertebrates (Lovich et al., 2018). Currently, turtle and tortoise populations are in decline globally, and some species are already extinct due to urbanization, habitat loss and fragmentation, pet trade, and food consumption, meaning their ecological roles and services are decreasing on a large scale (Lovich et al., 2018; Rhodin et al. 2018). In terrestrial and aquatic ecosystems, the order Testudines (tortoises, freshwater and marine turtles) plays an underestimated role, extending from seed dispersal to mineral cycling and carbon storage (Lovich et al., 2018). The overall aim of the conservation programmes is to ensure long-term viable populations (Stanford et al., 2020). In current efforts to conserve and protect these species, there is a lack of field and experimental data necessary to gain knowledge on the life history and ecology of turtles and tortoise around the world (Gibbons & Lovich, 2019), which can lead to an incorrect understanding of their behaviour and ecological roles and requirements, and can also lead, consequently, to their removal from ecosystems (Lovich, 2018). As a result, most stakeholders and/or naturalists still have a rather poor understanding of the dynamics of turtle and tortoise populations and the underlying mechanisms underlying these dynamics (Seigel et Dodd 2000). Efficiently conserving turtle and tortoise populations around the world and implementing successful conservation programmes requires an improvement in our knowledge field on the ecology, demography, habitat use, and

genetics of these species (Dirzo et al., 2014; Stanford et al., 2020), as well as the impact of changes due to land use and climate change (Stanford et al., 2020).

The European pond turtle (*Emys orbicularis*, L. 1758) is the freshwater turtle species with the widest distribution range of the Emydidae family and does not only occur in Europe, as is suggested by its common name (Fritz & Chiari, 2013). Distinct subspecies of European pond turtles occur from the Northern African Maghreb region, over much of Southern and Western Europe and a major part of Eastern Europe, to Asia Minor (Fritz, 2003; van Dijk & Sindaco, 2004; Fritz et al., 2005). This freshwater turtle is an emblematic species of wetlands and could be considered an “umbrella” species because its biological cycle requires the conservation of both aquatic and terrestrial habitats (Cadi, 2003). According to the IUCN Red List, habitat loss due to anthropogenic activities is considered among the main reasons for the European pond turtle decline (Tortoise and Freshwater Turtle Specialist Group, 1996). Indeed, throughout its whole range, the areas of ponds and marshes have declined sharply over the last two centuries due to intensification of agricultural practices and urbanization, causing the disappearance of this species in many regions. In addition, this species also suffers from a direct impact, as individuals of the species were consumed in large numbers during prehistorical times and the Middle Ages (Cheylan, 1998; Vlachos & Delfino, 2016). Moreover, the Romans were direct actors in the dissemination of species across Europe; thus, they contributed to the dispersal of the European pond turtle, which they used as a source of meat or as pets (Beisel & Léveque, 2010). More recently, a drastic decline in the *Emys* population occurred at the end of the 18th century and in the first half of the 19th century (Daszkiewicz, 2018) due to the use of this species for meat consumption and as medicine, and due to the destruction of its habitat (Devaux et al., 1996; Daszkiewicz, 2018). Accordingly, the European pond turtle

is now ranked as “Near Threatened” (NT) throughout its entire range (Tortoise and Freshwater Turtle Specialist Group, 1996; van Dijk & Sindaco, 2004).

In Switzerland, two subspecies of the European pond turtle are currently recognized as native: *Emys orbicularis orbicularis* in the Northern Alps (Swiss Plateau region) and *Emys orbicularis hellenica* in the Southern Alps (Ticino) (Lenk et al., 1999; Fritz, 2003). The subspecies *E. o. orbicularis* (haplotype II) is currently found in the Danube and Oder River basins, the Balkan Peninsula, southern France and northern Spain. *E. o. hellenica* has a circum-Adriatic distribution. Both subspecies inhabit wetlands, marshlands and oxbow lakes rich in aquatic vegetation. Habitat requirements in Switzerland for *E. orbicularis* include both aquatic and terrestrial habitats. Aquatic habitats are wetlands rich in vegetation composed of large ponds, where water is persistent (Ficetola et al., 2005), and with submerged trunks ideal for aerial basking (thermoregulation) (Vignoli et al., 2015). Terrestrial habitat represents open areas with soft soil and good sun exposure for nesting sites (Chelazzi et al., 2000), which are an important factor in population establishment (Monney, 2009; Schaffner & Kützli, 2010; Ducotterd, 2015). Indeed, gravid females can travel great distances over terrestrial areas to locate the most favourable nesting sites (Steen et al., 2012), and road networks create barriers between wetlands (foraging area) and nesting sites (Joyal et al., 2001; Steen et al., 2006), resulting in an additive and unnatural mortality of adults (Trakimas & Sidaravičius, 2008). Moreover, *E. orbicularis* may occupy different types of wetlands at different life stages; hatchlings require shallower water than do adults to avoid the risk of drowning, and ditches and ponds can be used during migration (Rovero & Chelazzi, 1996).

In Switzerland, the species was observed in numerous aquatic habitats, but the large majority of observations refer to a single individual or a very few individuals, resulting from accidental or voluntary release. In addition to the very recently recreated

populations in the cantons of Geneva and Neuchâtel (composed only of individuals of *E. o. orbicularis*; see below), viable populations of this species are currently limited to the canton of Geneva in the natural reserve of Moulin-de-Vert (46°10'46"N, 6°1'42"E) and Laconnex (46°09'24"N, 6°01'46"E). These two sites host breeding individuals that were introduced by humans between the 1950s and the 1980s. Whereas the population of Laconnex is composed of only *E. o. hellenica*, the population of the Moulin-de-Vert reserve is a mix of three subspecies: *E. o. orbicularis*, *E. o. hellenica* and *E. o. galloitalica* (Raemy, 2010). The latter is a non-native subspecies originating from mountain stream waters in the western Apennine Peninsula, Sardinia, Corsica and southern France (Lenk et al., 1999; Fritz et al., 2005). Under normal conditions, native and non-native pond turtle subspecies do not meet in contact zones. When such meetings happen, as can be caused by conservation programmes when locally threatened populations are restocked with genetically incompatible individuals, risks such as a decrease in fitness due to hybridization can arise for the pond turtles (Fritz and Chiari, 2013).

In Switzerland, the indigenous status of the European pond turtle has long been questioned. However, remains of shells dating back to 7000 years BP (Mesolithic) have proven the ancient presence of this species in Switzerland (Becker & Johansson, 1981; Besse et al., 2003), and several observations of this species were further reported between 1800 and 1930 in the lowlands of Switzerland (Parent, 1968; Hofer et al. 2001). However, the European pond turtle was considered Regionally Extinct (RE) on the Swiss Red List of threatened and rare amphibians and reptiles in 1982 and 1994 (Hotz and Broggi, 1982; Duelli, 1994); its status changed to “critically endangered” (CR) on the last Red List of threatened reptiles in Switzerland (Monney and Meyer, 2005). As the European pond turtle is considered today a priority species for national conservation programmes, in this study, we aim to summarize all conservation expertise that has been

gathered and all actions that have been conducted, and to highlight the potential actions that could be developed in the future to improve the protection of this emblematic species in Switzerland. In this report, we present two possible and nonexclusive conservation strategies (reintroduction and population reinforcement, and habitat renaturation to favour natural expansion and recolonization) that the Emys Project could consider for the European pond turtle in Switzerland.

2. Reintroduction actions or habitat renaturation waiting for natural recolonization? – two possible and non-exclusive,conservation strategies

In 1999, the association “Protection et Récupération des Tortues” (PRT) located in Chavornay (Switzerland) launched the Emys Project to protect the only native freshwater turtle species in Switzerland (Ducotterd, 2003). Currently, the Swiss coordination centre for amphibian and reptile protection (“Centre Suisse de Coordination pour la Protection des Amphibiens et des Reptiles”; KARCH.ch) is coordinating the project with the help of scientists, breeders and local authorities. The primary goal of this conservation project is to conserve this endangered species and improve its Swiss Red List status. Different actions were promoted to reach this goal: 1) evaluate the size, dynamics and genetics status of current Swiss populations of European pond turtles; 2) manage and protect favourable habitat sites according to the species' requirements (e.g., creation of optimal nesting sites); 3) reintroduce native subspecies (*E. o. orbicularis* and *E. o. hellenica*) in suitable environments (population reinforcement) where the species is not present; and 4) promote scientific research on the species to improve knowledge on its ecology and improve the chances of successful reintroductions (see details of all actions in Ducotterd et al., 2004; Raemy, 2010; Raemy et al., 2013; KARCH, 2014; Ducotterd, 2019). In addition, a group of *Emys* experts and breeders created the association SwissEmys in 2012, with

the objective of breeding native subspecies of the European pond turtle (*E. o. orbicularis*, haplotype IIa), to be able to supply young animals for official reintroduction projects.

To pursue the Emys Project, we attempted to establish a clear conservation strategy in Switzerland to ensure the sustainability of the species. Two distinct conservation strategies, which are not mutually exclusive, are commonly employed in conservation programmes for threatened and endangered species and could be considered for this species: (i) habitat renaturations to enhance natural recolonization and expansion, and (ii) population reinforcements through reintroduction actions in suitable sites. A conservation strategy can be successful for one taxon, such as river renaturation to enhance the natural density of fish (Belliard et al., 2009; Keckeis, 2014) or population reinforcement through reintroduction actions (Bretagnolle & Inchausti, 2005; Schaub et al, 2009; Krammer, 2013) but a complete failure for other taxa. For instance, regarding migratory fish, some populations progressively collapsed due to canalization, pollution, and obstructions to migration (for *Salmo salar*: Lenders, 2016; for *Alosa alosa*: Belliard et al., 2009; for *Prochilodus argenteus* and *Prochilodus castatus*: Arantes et al., 2010). The ecological rehabilitation of rivers through habitat renaturations and improvements is an efficient conservation strategy for these species and enhances the natural recolonization of the habitat (for *Alosa alosa*: Belliard et al., 2009; Keckeis, 2014). Indeed, fish restocking is useless if the habitat to which they are reintroduced is still unsuitable (due to such factors as obstacles to migration and pollution) (Caudron et al., 2006; Caudron et al., 2010; OFEV, 2018). On the other hand, after population collapses or extinctions, population reinforcements and reintroductions attempt to re-establish species within their historical range by releasing wild or captive individuals (IPBES, 2019); such was the case for the Alpine ibex (*Capra ibex ibex*, Krammer, 2013), the little

bustard (*Tetrax tetrax*, Bretagnolle & Inchausti, 2005), or the bearded vulture (*Gypaetus barbatus*, Schaub et al, 2009). Moreover, Howell et al. (2019) demonstrated during a 30-year time frame that protection of critical habitat was not enough to ensure the persistence of the endangered spotted turtle (*Clemmys guttata*). These examples underline the need to ensure that a chosen conservation strategy is the most appropriate approach (Fisher & Lindenmayer, 2000) and that habitats selected for reintroduction actions are the most suitable depending on the species needs (Moorhouse et al., 2009). These examples also highlight the importance of gaining complete ecological knowledge on the selected species by improving field and experimental studies (Dirzo et al., 2014; Stanford et al., 2020), of ensuring serious monitoring after reintroduction, and of publishing scientific research outlining conservation steps (Fisher & Lindenmayer, 2000).

In this context, it is essential to determine which of the two strategies (habitat renaturation or reintroduction), or a combination of both, is the most suitable for *E. orbicularis* in Switzerland. Fundamental and applied studies are necessary (Stanford et al., 2020) and have been implemented to increase our knowledge of the European pond turtle in Switzerland. Indeed, to favour natural recolonization, we needed to study the current and future habitat suitability and dispersal capacities of the species (see Chapter 2) using species distribution models (SDMs) to determine population dynamics (Nuoffer, 1999; Mosiman, 2002) and population genetics (Raemy, 2010) from relict populations. To promote reintroduction strategies, it was also essential to monitor the remaining populations (Nuoffer, 1999; Mosiman, 2002) and determine their genetic status and possible hybridization (Raemy, 2010). Moreover, we needed to set up reintroduction tests at selected sites to determine whether this approach could be successful. In reintroduction programs, individuals could be translocated from other wild populations nearby (wild individuals) or reintroduced from breeding stations (captive individuals). In

the case of captive individuals, it is essential to determine their genetic status and parasitic load (Schönbächer et al., in prep). For translocated individuals, analyses are similar to those for captive individuals, but additionally, the impact of removing individuals on the dynamics of the source population(s) should be evaluated. In our case, reintroductions were made with captive-born individuals, as current wild populations were not suitable (see below). Therefore, breeding stations were created. Moreover, in a conservation program, it is important to understand the ecology and behaviour of the studied species in a specific place. We analyzed the diet of the European pond turtle using newly developed DNA approaches to evaluate the trophic niche of the species and determine if indigenous species could be threatened by reintroduction (see Chapter 1a and Chapter 1b or Ducotterd et al. 2020a, b). Hereafter, we present and discuss the main findings gathered through this project and many others.

3. Emys project (1999-2020) – What are the achievements of the project?

3.1 Monitoring of relict populations

The two single populations of European pond turtles in Switzerland that are considered viable are both located in the canton of Geneva (Moulin-de-Vert and Laconnex populations). In the Moulin-de-Vert reserve, the species was reintroduced between the 1950s and the 1980s, with individuals from various origins. Eco-ethological studies carried out in 1998 and 2001 revealed regular and natural reproduction of reintroduced individuals and a stable population (Nuoffer, 2000; Mosimann, 2002; Mosimann & Cadi 2004; Ursenbacher & Raemy, 2013). Using traps, the population size was estimated to be 306 ± 10.5 individuals, which represents a good population density of 64 individuals/ha (Mosimann, 2002; Mosimann & Cadi 2004). Ayaz et al. (2007) estimated that a density of 93 (41.5 – 124.5) individuals/ha represented an intact thriving population. Monitoring of

the Moulin-de-Vert population continued until 2011, and there was a slight increase in the adult population from 143 adults in 2002 to 186 adults in 2011 (Ursenbacher & Raemey, 2013). Genetic analyses of the individuals from the Moulin-de-Vert population detected the occurrence of three different subspecies and a large proportion of hybrids (Raemy, 2010). The second viable population (Laconnex) of the European pond turtle is composed of *E. o. hellenica* but is located north of the Alps, confirming reintroduction (Laconnex population: report Ursenbacher, 2018). Consequently, the Moulin-de-Vert population could not be used for natural recolonization or active translocation, whereas individuals identified as pure *E. o. hellenica* (haplotype IVa) in the Laconnex population could only be translocated to the south of the Alps (Ticino).

3.2 Dispersal capacity

Currently, habitat degradation and fragmentation have a large impact on species, ecosystems and biodiversity (Chen et al., 2011). While some species, due to their high dispersal capacities, could be able to respond and adapt to habitat modifications (Kubisch et al., 2014), many others, such as reptiles, might not or might only partly be able to, due to their low ability to disperse (Halpin, 1997; Gibbons et al. 2000; Pittet, 2017) and therefore are more vulnerable to the effects of rapid habitat modifications (Mac et al., 1998). Currently, the climatically optimal sites for the European pond turtle are limited to the Northern Alps (Swiss Plateau) and to the Southern Alps (Ticino) at elevations below 500 m (KARCH, 2014), where most human activities, such as agriculture, settlements and transport routes, are present. As a result, the natural habitats (wetlands) and suitable nesting sites of the European pond turtle have been severely altered; indeed, since 1800, 90% of wetlands have been destroyed in Switzerland (Broggi & Schelge, 1989). In Chapter 2, we used species distribution models (SDMs) to analyse the dispersal capacity of the

European pond turtle in Switzerland and demonstrated that colonization of new habitats by *E. orbicularis* would be limited due to its low dispersal ability, habitat fragmentation and urbanization (see Chapter 2).

3.3 Site selection for reintroductions

As previously demonstrated, natural recolonization seems to be impossible in Switzerland due to habitat fragmentation and the current genetic status of relict populations. Therefore, reintroduction seems to be the most appropriate approach for improving the distribution (thereby lowering the threat level) of this species in Switzerland. To determine if and where reintroduction of the European pond turtle could be possible in Switzerland, several criteria that enable the selection of suitable sites for reintroduction were utilized (KARCH, 2014):

- Elevation \leq 500 m
- At least one optimal nesting site with no barrier between aquatic habitats and nesting sites
- Large reintroduction sites (20-100 ha)
- Habitat connectivity that allows further colonization in other ponds and wetlands

Based on these criteria, three areas were selected and validated by the KARCH and by experts of the species: one area in the canton of Geneva (natural reserve of Près-Bordon and Teppes-de-Verbois), one area in the canton of Neuchâtel (La-Vieille-Thielle) and, finally, one area in the canton of Ticino (Bolle-di-Magadino) (see Figure 1) (KARCH, 2014). The reintroduction to these locations was considered to be a testing phase before any other reintroduction.

At each site, individuals were tested genetically and marked with specific notches on marginal scales of the shell or with microchips before being released. To date, no studies have determined the suitable number of released *Emys* individuals to ensure long-term persistence and avoid consanguinity. Based on the minimum viable population size (MPV), defined as the smallest population size necessary for a species to become persistent (Shaffer, 1981), we chose to release approximately 50 individuals in each site from different breeding stations. Of course, these theoretical values must in no case be considered “magic numbers” guaranteeing the viability of the species (Reed et al., 2003). Regarding the sex ratio, Girondot et al. (1998) demonstrated that if females of *E. orbicularis* are released in a larger number than males, the sex ratio in the long term will be male biased as a result of the selection of masculinizing factors, and consequently, the population size will decrease. Therefore, a balanced sex ratio should be chosen (Girondot et al., 1998). In captive-born individuals, eggs are incubated at different temperatures to select the sex of the future hatchlings.

3.4 Breeding stations

To reintroduce indigenous subspecies into favourable environments, multiple breeding stations were developed in Switzerland. For haplotype IIa subspecies (*E. o. orbicularis*), three different breeding stations are currently operational: 1) the SwissEmys group (<http://www.swissemys.ch>) with 41 genitors (24 females and 17 males) split among different locations; 2) Papiliorama in Kerzers (<https://papiliorama.ch/fr/news/operation-de-sauvetage-des-cistudes-deurope/>) with 16 genitors (11 females and 5 males); and 3) Tierpark Dählötzli in Berne. Tierpark Dählötzli in Berne is also operational for the haplotype IVa (*E. o. hellenica*) with 13 genitors (10 females and 3 males). For haplotype IVa (*E. o. hellenica*) occurring only south

of the Alps, one station was created at the Botanical Garden on Brissago Island in Ticino (<https://ptsi.webnode.com/progetto-emys-ticino/>) with 12 genitors (8 females and 4 males).

3.5 Reintroduction tests of *E. orbicularis*

By the end of 2019, a total of 109 European pond turtles had been reintroduced in the cantons of Geneva and Neuchâtel (Table 1). Reintroductions in the canton of Ticino will be planned in coming years. In 2017, during the trapping session allowing for the annual monitoring of the population of Près-Bordon (Geneva), three juveniles born in the wild in 2016 were discovered, thereby attesting to natural reproduction events at this site. However, we do not know if these juveniles are the descendants of a single female or of several females. Monitoring of released populations is thus essential to attest to the success of reintroduction. The four main objectives of successful reintroduction are (1) survival of the individuals after release, (2) settlement of the animals into the release area, (3) successful reproduction in the release site (Teixeira et al., 2007), and (4) persistence of the established population (Seddon, 1999; Armstrong & Seddon, 2008).

3.6 Monitoring of reintroduced populations

The aim of any reintroduction programme is to recreate wild populations that are viable in the long term. Therefore, it is essential to implement scientific monitoring to evaluate the acclimation of reintroduced individuals and their survival and growth rates in order to assess the potential success of reintroduction programmes. To this end, we use telemetry (with transmitters glued on the shells of the turtles) and capture-recapture each year. These monitoring programmes are essential to detect potential, partial, or complete failures, to enable continuous re-evaluations and adaptations of the project, and

to implement appropriate management and conservation actions at the levels of both the reintroduction sites and the individuals.

Monitoring post-reintroduction was conducted on the populations of Près-Bordon (Geneva) and La-Vieille-Thielle using telemetry (Australia 26K, Tetley Scientific, Ballina, Australia) and transmitters (R1100 reptile body implants) glued on the shell. The transmitters weighed 5 gr and represented less than 5% of the total weight of the released individuals. Telemetry allowed for the investigation of habitat use and demonstrated that individuals reintroduced in Près-Bordon showed significantly more exploratory behaviour than individuals from La-Vieille-Thielle (Près-Bordon: max 1000 m and La-Vieille-Thielle: max 200 m) (Raemy & Ursenbacher, 2012; Ducotterd, 2015), although the growth rates were similar in both populations (Ducotterd, 2015). We could hypothesize that reintroduced individuals in La-Vieille-Thielle had less exploratory behaviour than individuals from Près-Bordon due to the quality of the habitat. Indeed, the pond in La-Vieille-Thielle was a mature and stable pond; in contrast, ponds in Près-Bordon were freshly renatured, and *Emys* individuals may have had to travel a greater distance to find food (Ducotterd, 2015). Moreover, to ensure the survival of released individuals and to study population dynamics, capture-recapture of the released populations using conical nets is performed each year.

3.7 Species ecology studies

3.7.1 Mapping the species' current and future habitats suitability in support of conservation planning

Currently, to implement efficient conservation actions, such as habitat renaturation to favour natural recolonization or habitat expansion, it is essential to take into account the climatic changes that have large impacts on species, ecosystems and

biodiversity (Chen et al., 2011). Using species distribution models (SDMs), which are the most-used ecoinformatic tools to predict potential changes in species habitat suitability under anthropogenic changes (Guisan & Thuiller, 2005), we predicted that in Switzerland, suitable habitats for the European pond turtle will increase under climate change (representative concentration pathways 4.5 and 8.5, which assume moderate and extreme global warming, respectively) (see Chapter 2). We provided the first map highlighting key locations of conservation priority for *E. orbicularis* in Switzerland (Figure 2), a major contribution to help implement efficient conservation actions in the field. Information could also come from studying past distributions as keys for the present and future spreads (Waterson et al., 2016), but this approach was not used in Chapter 2. The suitability assessment can help determine regions and areas that are favourable to this species but not occupied to date, e.g., due to competition with other species, native (Segurado et al., 2012) or invasive (Ficetola et al. 2009). These suitability analyses could also be refined by testing how the species-environment relationship and the species' relation to other species are influenced by the geographic neighbourhood and extent investigated at different scales (Pellet et al., 2004; Segurado et al., 2012; Scherrer et al., 2019). Field work and local analyses by experts (e.g., the members of the Emys project) of potential reintroduction sites are necessary for the evaluation of habitat suitability; for example, scientists can study nesting site temperatures to assess whether specific habitat restoration actions are needed (e.g., nesting sites were built in La-Vieille-Thielle prior to reintroductions following expert advice).

3.7.2 Optimal nesting sites as limiting factors

Due to the current climate conditions in Switzerland, it is essential to determine, before reintroduction, whether the thermal conditions necessary for successful

hatchlings are obtained in nesting sites. Multiple potential nesting sites have accordingly been studied throughout Switzerland, using iButtons® (Maxim Integrated Products, Inc.) to measure and compare the temperatures of known and potential nesting sites. These dataloggers were placed in the soil at a depth of 8-10 cm, which corresponds to the average depth of nests, between late May and late September in 2009 and 2014. On average, the incubation period of European pond turtles is 98-117 days with an average temperature in the nest of 22.6°C (Fritz, 2001). Schneeweiss (2004) demonstrated that temperatures below 18°C stop embryonic development. Temperature sums calculated following the method used by Schneeweiss (2004) demonstrated that above an elevation of 500 m the temperature sums did not reach the optimum value for hatchlings, meaning that recommendations to reintroduce European pond turtles below 500 m altitude were necessary to ensure successful reproduction (Schaffer & Kutzli, 2010; Ducotterd, 2015).

3.7.3 Use of eDNA to help detect the potential presence of E. orbicularis

At a time of steeply declining biodiversity, efficient detection methods are needed for the optimal management of endangered species (IPBES, 2019), especially for species with low population densities. Currently, molecular approaches using environmental DNA (eDNA) have become efficient tools for species detection in water environments (Wilcox et al., 2013; Sigsgaard et al., 2015). Indeed, traditional population monitoring (e.g., with traps) is time-consuming, may be difficult to set up and could lead to incorrect detection rates (Jerde et al., 2011). Moreover, the monitoring activities themselves could have a negative impact on the habitats by disturbing fauna and flora (Tyre et al., 2003; Bider, 2011). Considering European pond turtle detection, visual observations could be an alternative option to active monitoring, but this method usually leads to an underestimation of population size (Raemy & Ursenbacher, 2018). Therefore, it is

essential to develop other potential methods of detection, e.g., the use of eDNA (Lacoursière-Roussel et al., 2018). Raemy & Ursenbacher (2018) demonstrated that the detection of *Emys* through eDNA was possible in both artificial and natural ponds, with particularly higher detection rates being observed in the former due to small water volumes. Moreover, eDNA concentration could not be correlated with the number of individuals of European pond turtles (Raemy & Ursenbacher, 2018). To summarize, the latter study demonstrated that eDNA detection surveys are a promising tool for conservation but might be less suitable for low-secreting species, such as aquatic reptiles. Indeed, eDNA detection rates are lower for aquatic reptiles than for fish or amphibians (which are considered high-secreting species), which may be due to the presence in turtles of scutes, rather than mucus or epithelial cells, and to their different excretion systems (Kelly et al. 2014). Therefore, eDNA approaches should only be employed to complement traditional monitoring methods (Raemy & Ursenbacher, 2018).

3.7.4 Determining the diet – Is the European pond turtle a threat for other species?

After implantation of the first reintroduction actions of European pond turtles in Switzerland, some questions were raised about the potential threat that this species could have on other endangered species (especially endangered amphibians), which could be a major obstacle to the reimplantation of an extirpated species. This possibility underlines the importance of having strong and clear knowledge of the ecology and needs of the reintroduced species. Indeed, the feeding behaviour of European pond turtles was poorly understood for a long time; this species was first considered to be a carnivorous, often scavenging (Rollinat, 1934; Lebboroni and Chelazzi, 1991; Kotenko, 2000, Luiselli, 2017), sometimes vegetarian (Ficetola and De Bernardi, 2006) and, more recently, an

omnivorous species (Ottonello et al., 2005; Çiçek and Ayaz, 2011; Ottonello et al., 2016; Ottonello et al., 2018). Using molecular technologies, a new method of long metabarcoding analysis was developed using universal PCR primers to determine the species occurring in the faeces of European pond turtles (Chapter 1a). This study therefore not only precisely determined the omnivorous and opportunistic diet of the European pond turtle but also showed that this species has a marginal impact on the other native species (such as amphibians), as very few threatened species were consumed, with 85.5% of eaten species not listed on the Swiss Red List (Chapter 1b or Ducotterd et al., 2020b). This study was essential to demonstrate that the European pond turtle is not a threat for other endangered species; therefore, the species could be reintroduced to new suitable habitats in Switzerland without impacting the local biodiversity. The completion of other studies on *Emys* populations living in different environments with diverse food opportunities using the same DNA method would greatly improve knowledge on the species and determine if every *Emys* subspecies are opportunistic predators and/or scavengers. Moreover, this new method of DNA metabarcoding demonstrated that the analysis of faeces can be an efficient tool for conservation purposes by considerably improving our understanding of trophic interactions and food webs with a high level of precision (Chapter 1b or Ducotterd et al., 2020b). Furthermore, this method could be used not only to study the diet of a species using faecal samples but also for analysing stomach contents and in eDNA water analyses to determine the species present in the environment.

3.7.5 Parasitic charge analysis

Until 2019, no routine medical examinations were carried out on the turtles prior to their release. When Tierpark Dählötzli (Berne) decided to join the Emys Project, they

recommended health assessments before release. To this end, a study was launched that aimed to provide a comprehensive health assessment and to compare the results between free-ranging and captive animals (Schönbächler et al., submitted). A total of 141 European pond turtles were captured from different breeding facilities and reintroduction sites in summer 2019. All animals were examined according to a standardized examination protocol, which included an extensive clinical examination, swab collection for microbiological analysis of three major chelonian pathogens, blood analysis, faecal parasitology and diagnostic imaging (Schönbächler et al., submitted).

The results demonstrated that 133/141 turtles (94%) were healthy according to the protocol, even though during microbiological investigation, the presence of an Emydidae Mycoplasma with an estimated prevalence of 40% could be detected for the first time in this species. No Ranavirus or herpesvirus was detected. Furthermore, reference intervals for haematology and selected blood chemistry parameters as well as guidelines for ultrasound and X-ray examinations were compiled with this study (Schönbächler et al., submitted). During the study time, two cases of fatal infestation with a spirorchiid trematode parasite occurred in two breeding institutions, which led to the decision to deworm all animals with a specific anti-parasitic treatment protocol before reintroduction. This study was the first broad health assessment performed for European pond turtles. The examination protocol developed will be adopted and constantly updated for the selection of suitable animals to be reintroduced in Switzerland, and further studies on the major pathogens of European pond turtles are underway (Schönbächler et al., submitted).

4. Based on the achievement of the Emys Project, what is the best conservation strategy for *E. orbicularis* in Switzerland?

As demonstrated by Ducotterd et al. (in prep) (Chapter 2), natural recolonization of the most suitable habitats by *E. orbicularis* is impossible due to strong fragmentation and low dispersal ability. Indeed, even if habitat suitability increases in Switzerland for *E. orbicularis* under climate change (+ 64.6% under RCP 8.5), the European pond turtle would be able to colonize only 11.9% of the suitable habitats until 2060. Furthermore, we based our dispersal scenarios on occurrence data of the European pond turtle in Switzerland, which are composed of small populations, isolated individuals or genetically non-native subspecies (Chapter 2). Moreover, in European pond turtle populations, sexual maturity is late (approximately 8-12 years), fertility and longevity are extended, reproductive output is low, and the rate of nest predation and juvenile mortality is high (Congdon et al., 1993). Therefore, the measures of conservation success are different for Cheloniants than for most other animals (Stanford et al., 2020). Consequently, perennial populations take a long time to be established, and it may take decades to determine if a healthy and viable population has been successfully reintroduced (Stanford et al., 2020); e.g., the first individuals in the population of Moulin-de-Vert were reintroduced in the 1950s, and this population is still slightly growing, as demonstrated by monitoring studies conducted between 2002 and 2011 (Ursenbacher & Ramey, 2013). Furthermore, the only two viable populations are mixtures of three different subspecies, and the three reintroduced populations (native subspecies: *E. o. orbicularis*) are composed of only approximately 109 individuals (+ several naturally born individuals in Près-Bordon), which is not sufficient to ensure the sustainability of the species. Moreover, regarding the geographical localization of the reintroduced populations (cantons of Geneva and Neuchâtel), the sites are separated by more than 100 km, which makes it impossible to

connect these populations due to the high density of humans and agriculture between these sites. Furthermore, isolated individuals are found in the wild, and some are reproducing; unfortunately, we do not know their current genetic status (Raemy, 2010). Thus, to be effective, the conservation of the European pond turtle in Switzerland must combine *in situ* actions (the study and protection of wild turtle population, habitat improvements) with *ex situ* measures (reintroductions). Therefore, we strongly recommend choosing in the short term the reinforcement conservation strategy through reintroduction actions in favourable sites to increase populations of this emblematic species (see Figure 2 to determine future reintroduction sites). Then, in the long term, habitat renaturations are needed, due to the huge loss (destruction and fragmentation) of wetlands in Switzerland, to increase habitat connectivity between *Emys* populations, or between a reintroduced population and new habitats, and potentially to increase the natural expansion of *E. orbicularis*. We could integrate connectivity on decision-making conservation plans and determine which are the most essential habitats to reconnect using a hybrid approach based on network analysis and empirical suitability models; such analyses have been performed by Pereira et al., 2011. Therefore, it is essential to reconnect habitat and preserve large areas of natural habitat, including both aquatic and terrestrial habitats (Cadi, 2003; Ficetola et al., 2005), with open areas with soft soil and good sun exposure for nesting sites (Chelazzi et al., 2000).

4.1 What is next for the European pond turtle? - Defining an integrated framework

Numerous population monitoring and ecological studies on the European pond turtle in Switzerland demonstrated that to choose and implant an efficient conservation programme for a species, it is essential to combine both fundamental and applied sciences with multiple approaches (Stanford et al., 2020). However, these approaches are still

rarely combined with a conservation plan for the same species (Salafsky et al., 2002; Stanford et al. 2020).

An integrated framework is needed to determine the essential steps to plan and consider when a conservation programme is launched for an endangered species (Possingham et al., 1993; Groves et al., 2000; 2002; Himes Boor, 2013). Such a framework should allow for the identification of conservation targets, the collection and identification of informational gaps in species ecology, the establishment of conservation goals (such as reintroduction or habit renaturation), the selection of priority conservation areas (e.g., using a species distribution model, (Chapter 2)), and the evaluation of conservation strategy success (Groves et al., 2000; 2002). Based on the literature (Possingham et al., 1993; Groves et al., 2000; 2002; Himes Boor, 2013; Bonfim et al., 2018), we propose here a general integrated framework for the conservation of endangered species depending on the chosen conservation strategy (Figure 3). Our framework determines the most suitable conservation strategy by studying the habitat suitability and dispersal capacity, ecology, and key factors of the selected species and planning suitable actions depending on the chosen strategy (Figure 3). From this general integrated framework, based on the knowledge gained during the *Emys* project and on *Emys* literature (Cadi, 2003; Ficetola et al., 2004; Rivera & Ayres, 2004; Fritz & Chiari, 2013), we could adapt a suitable and precise strategy for the conservation of the European pond turtle in Switzerland (see Figure 4) when a cantonal authority is willing to take actions for conservation of this species in natural habitats. This framework will define the best conservation strategy by taking into account whether the habitat suitability of the species (here *E. orbicularis*) was established; whether the species was already reintroduced or not; whether the reintroduced population is healthy or infected by some diseases (as here, potentially by *Mycoplasmas*); whether there are other turtle

species present, both native and invasive; and other possible criteria (Guisan et al., 2013). To implement suitable and long-term conservation programmes for the European pond turtle in Switzerland, meetings with governmental and cantonal institutions and stakeholders need to be organized to identify potential future reintroduction sites and explain to these conservation actors the habitat requirements and species protection measures that matter for this emblematic species. In this study, we discuss a potential step that is necessary to continue the *Emys* project with (i) in the short term, the reintroduction strategy and (ii) in the long-term, habitat renaturation following the integrated framework outlined in Figure 4.

5. Short term – Reintroduction strategy – Essential steps

5.1 New reintroductions in suitable sites and population reinforcements of already reintroduced *E. orbicularis*

Based on the habitat suitability map provided by Ducotterd et al. (in prep) (Chapter 2), we suggest prioritizing new areas/regions for the reintroduction of *E. orbicularis* to increase the number of wild individuals across the country. When a region is selected, it is essential to assess the suitability of aquatic and terrestrial habitats in the field with experts (e.g., members of the Emys project), such as the presence of close optimal nesting sites, aquatic habitats rich in vegetation, and low anthropogenic pressure. Indeed, to ensure population establishment and reproduction, it is essential to assess the site quality prior to reintroduction, as demonstrated by the unsuccessful reintroduction of *Mauremys leprosa* in the Ebre Delta (Catalonia, northeast Spain) (Bertolero & Oro, 2009).

Then, when a site is considered favourable, it is essential to ensure the total absence of *E. orbicularis* individuals (genetically non-native individuals) or other

potential invasive turtle species (such as *Trachemys*, see below) by implementing a protocol for research and detection of species on the sites (Cadi, 2003). If *Emys* individuals are found, individuals must be genetically tested to determine their subspecies (native or not); if individuals are native, they will remain in the reintroduction site, and non-native or hybrid individuals will be placed in awareness ponds in zoos and nature centres to raise public awareness of the Emys Project. In the case of capturing exotic species, individuals will be taken to the turtle rescue at Emys Center (Association de Protection et Récupération des Tortues), or, if capture and transfer to the rescue centre is not possible, individuals will be killed.

Population reinforcements must be made on already-reintroduced populations to attain the number of 50 reintroduced individuals per site necessary to enhance genetic diversity.

5.2 Enhance genetic diversity to avoid consanguinity

Currently, 4 breeding stations are implemented in Switzerland. To enhance genetic diversity, a new breeding station is under construction for *E. o. orbicularis* at the turtle rescue at Emys Center (Association de Protection et Récupération des Tortues) with 9 adults (4 females and 5 males) and 15 juveniles from the Brenne region, France. To date, the 3 breeding stations for *E. o. orbicularis* produce approximately 100 juveniles per year. Therefore, individuals released in the wild (50 individuals per site) come from diverse breeding stations and are thus genetically different. Another way to diversify reintroduced populations is to exchange genitors with other *E. orbicularis* breeding programmes from other countries. An international studbook for genitors of *E. orbicularis* is being created by Jan Vermeer (Parc Animalier de Sainte Croix, 57810 Rhodes).

However, conservation collaborations between countries can be complicated and require additional logistical efforts (Kark et al., 2009).

5.3 Health of reintroduced populations

Schönbächer et al. (submitted) demonstrated that some breeding stations and reintroduced individuals are positive for *Mycoplasma* parasites; therefore, individuals infected with parasites should be released in populations where these parasites are already present. Additionally, parasite-free populations should welcome only parasite-free individuals. Using *Emys* populations where *Mycoplasmas* are present, we should use this opportunity to determine if the presence of the parasite has an impact on the growth and survival rate of reintroduced populations. Future monitoring will provide useful insight for the management of populations with parasites.

5.3 Setting up reintroduction tests of juveniles

To date, only reintroduction of subadults has been conducted in Switzerland to allow for the marking and monitoring (with pit tags or transmitters) of the released individuals. Due to the presence of *Mycoplasmas* in some breeding stations, we suggest reintroducing turtles directly after hatching because *Mycoplasmas* are not transmitted from infected females to eggs (Schumacher et al., 1999). By reintroducing juveniles, we could monitor and health-check these juveniles following the methodology used by Schönbächer et al. (submitted). Moreover, studies have demonstrated great success with reintroduction based only on juveniles (e.g., for *Macrochelys temminckii*, Anthony et al., 2015), or at least have demonstrated the same survival rate as with adults (for *Emys orbicularis*, Canessa et al., 2016), suggesting that releasing juvenile individuals can provide positive outcomes while reducing management costs (Canessa et al., 2016).

However, Mitrus (2005) suggested that conservation programmes involving reintroduction of juveniles for species that mature late are difficult to evaluate because the first visible outcomes could arrive after 20 years or more. Consequently, we suggest identifying three potential sites (Figure 2) based on previous suitability measures to use as test sites for the reintroduction of juveniles (hatchlings) in the upcoming years. The criteria for these sites are slightly different because sites need to be small to facilitate monitoring by capture-recapture.

5.4 Population monitoring to assess reintroduction success – When to end releases in reintroduction programmes?

Canessa et al., 2016 demonstrated that population growth of *E. orbicularis* would be slow even under optimal conditions. Moreover, Paul (2004) also suggested the requirement for long-term (> 20 years) conservation to ensure population stability. Furthermore, the Moulin-de-Vert population reintroduced between 1950 and 1980 is still slightly growing (Ursenbacher & Raemy, 2013), demonstrating that perennial populations take a long time to settle. We suggest monitoring all reintroduced *E. orbicularis* populations using capture-recapture approaches: for subadult reintroduced populations, once every two or three years; and for juvenile reintroduced populations, every year. We could determine if the reintroduction actions were successful by the persistence and signs of reproduction of the established population (Seddon, 1999; Armstrong & Seddon, 2008). Therefore, we strongly suggest estimating vital rates (survival and fecundity), providing decisive insight into management (Beissinger & Westphal, 1998) and estimating a more accurate viable population size (MVP), which is often underestimated (Reed et al., 2002; Reed et al., 2003). Then, with increased knowledge on vital rates and MVP, we could determine if additional reinforcements are

needed to help the perennation of the reintroduced population or if we can cease reintroduction actions after approximately 50 individuals have been reintroduced in each selected site. Indeed, Howell et al. (2019) demonstrated the importance of long-term studies in monitoring population changes of turtle species and determined that these species could persist for a long time despite having little reproduction and a declining population trajectory. The conservation of *E. orbicularis* in Switzerland has the potential to become an effective long-term conservation action in the wild.

5.5 Turtle invasions: an increasing threat to anticipate and manage

The introduction of invasive species is a huge contribution to global biodiversity loss (IPBES, 2019). The Invasive Species Specialist Group (ISSG) published a list of the 100 most problematic invasive species in the world, and slider turtles (*Trachemys scripta*) are on the list (Lowe et al., 2000). Slider turtles are native to the north and central United States (Iverson, 1992; Painter & Christman, 2000), and the species most commonly imported as pets, was the red-eared slider (*Trachemys scripta elegans*, Wied 1839) until the end of the 1990s (Telecky, 2001). At that time, the European Union and Switzerland prohibited the importation of *T. s. elegans* (Règlement (UE) 1143/2014 – espèces exotiques envahissantes), unfortunately leading to the importation of other species of freshwater turtles such as the yellow-bellied slider (*T. s. scripta*, Schoepff 1792) as well as at least fifteen other species, some being more aggressive than the slider species, such as the common snapping turtle (*Chelydra serpentina*, L. 1758). Even if illegal, these turtles are often released by their owners into natural ecosystems (Cadi et al. 2004). The introduction of invasive slider turtles has occurred in Europe (Warwick, 1991), Africa (Newberry, 1984) and Asia (Warwick, 1991; Moll, 1995; Chen & Lue, 1998). The main factor limiting successful invasion by these species is reproduction (Cadi et al., 2004), as

reproductive success is present only in Southern and Central Europe. Indeed, successful hatchlings of slider turtles have been observed in numerous sites in Europe: in South France (Cadi et al, 2004), Corsica (com. pers. M. Cheylan), Italy (Luiselli et al., 1997), Austria (Kleewein, 2014), and Switzerland (Ticino and Constance Lake regions).

The spread and impact of *T. s. elegans* on native species has been the subject of several studies (e.g., Ficetola et al., 2009; Rodder et al., 2009 for spread) which have demonstrated that this species is more aggressive in semi-captivity than *E. orbicularis* (Cadi & Joly, 2004) and has a strong impact on the number of *Emys marmorata* in California. Lambert et al. (2019) demonstrated that after removing a large number of *T. s. elegans*, the native species (*E. marmorata*) was able to improve its body condition. Moreover, slider turtles have an omnivorous diet (Prévot-Julliard et al., 2007; Works & Olson, 2018) and therefore could compete with *E. orbicularis* for prey items. However, Ducotterd et al. (2020a, b) demonstrated a huge loss of information when studying diet only with direct observation under a microscope. Therefore, to determine diet competition between the *Trachemys* invasive species and *E. orbicularis*, we suggest comparing their respective diets in a population where both species live together and determining the impact of *Trachemys* on potential threatened species using the new metabarcoding method developed by Ducotterd et al. (2020a) (Chapter 1a).

In addition to the possible impact of *Trachemys scripta* on local biodiversity, slider turtles also carry pathogens that can affect a large number of animal species. Indeed, studies in France and Spain demonstrated that the parasites of *Trachemys* have been transmitted to the two local turtle species (*Emys orbicularis* and *Mauremys leprosa*) and have had a strong impact on native turtle populations (Domènech et al., 2015; Meyer et al., 2015). Occasionally, *Spirorchis elegans*, a parasite from North America probably introduced by slider turtles, caused significant mortality in a population of *E. orbicularis*.

in Galicia (Iglesias et al., 2015). Therefore, we suggest conducting veterinary studies of the parasitic load (e.g., Mycoplasmas, herpesvirus, Ranavirus, and nematodes) of turtle individuals in the wild and in captivity (shelters and pet stores).

Until last decade, the problem of slider turtles and other exotic turtle species was considered secondary in Switzerland because the presence of individuals in the wild was only linked to isolated release, and many of these exotic turtles were transferred to the Emys Center (Association Protection et Récupération des Tortues) when their owners wanted to get rid of them. Thus, it was thought that individuals encountered in nature would naturally disappear without human intervention. However, now that natural reproduction has been detected in Switzerland, the problem has become serious and deserves to be evaluated and treated. Therefore, in the near future, it will be necessary to determine (i) the most appropriate approaches for the detection of invasive aquatic turtles, (ii) the most suitable capture methods for invasive turtle species, (iii) an appropriate approach to detect turtle nests (for instance, with dogs), (iv) the impact (diet competition, pathogen transmissions, etc.) of invasive species on the only native freshwater turtle (*E. orbicularis*), (v) the areas at high risk of presence and reproduction of *Trachemys scripta* (and other exotic turtles) in Switzerland, and, finally, (vi) a directly applicable procedure for the detection, capture and management of these exotic and invasive turtles in Switzerland.

An approach that could be used for the determination of high risks of presence, reproduction and dispersal abilities of invasive species is the SDM method used in Ducotterd et al. (in prep) for the detection of potential suitable habitats for the European pond turtle. This approach would allow for the determination of potential suitable places for invasive turtle species (Ficetola et al, 2009; Rodder et al., 2009) and can be used as a tool to support their control and eradication and anticipate future invasions (Guisan et al.,

2013). Another method could be the use of citizen science for the detection of invasive turtle individuals or populations as well as relict individuals or populations of *E. orbicularis*. Citizen science is defined as the engagement of citizens in a scientific project (McKinley et al., 2017). After information is disseminated, the public can transmit reliable information on the localization of turtle species to info species (www.infospecies.ch), which is the Swiss Species Information Center, and, from these observations, captures could be conducted.

6. Long term – Habitat renaturation strategy – Essential steps

6.1 Habitat renaturation

In the long-term conservation strategy of *E. orbicularis*, it is essential to undertake renaturation work. Indeed, most wetlands in Switzerland have been destroyed over the past centuries due to human activities (Broggi & Schelge, 1898). Moreover, Ducotterd et al. (Chapter 2) demonstrated that dispersal of *E. orbicularis* was limited mainly due to habitat fragmentation. Habitat fragmentation and degradation, principally due to human activities, is a major factor causing the decrease or extinction of species (IPBES, 2019). Habitat connectivity (such as streams) is essential to allow the species to move among ponds and favourable habitats and is therefore crucial for the persistence of the European pond turtle (Pereira et al., 2011). Similar results have been found for other turtle species (for *Chrysemys picta*: Browne et al. 2006; for *Clemmys guttata* and *Emydoidea blandigii*: Lisa et al., 2001) and other species. Habitat renaturation could enhance connectivity between *Emys* populations, and between populations and new colonizable suitable sites, and could thus increase the dispersal capacity of the species (see Figure 3: Conservation Strategy – habitat renaturation for natural recolonization). The success of habitat renaturation would aid in the assessment of whether *E. orbicularis* successfully recolonizes the habitat and if the

population settles and reproduces. Moreover, the European pond turtle is considered an “umbrella” species because its biological cycle requires both aquatic and terrestrial habitats (Cadi, 2003); thus, renaturation would favour a multitude of other species.

6.2 Raising public awareness of wetlands using a charismatic species

Wetlands are habitats with a high biodiversity of plants and animals and are among the most productive environments (Adhikari et al., 2009). Indeed, wetlands play an extremely important role as hydrologic systems in the water cycle (Bacon, 1999). However, these fragile ecosystems are one of the most rapidly disappearing habitat types and have been degraded and transformed by anthropogenic activities (e.g., drainage, farming, and urbanization) due to the lack of understanding of their natural and social value (Polajnar, 2008). In Switzerland, wetlands have been severely destroyed and fragmented since 1800 (Hintermann, 1992). These habitats have been reduced by 90% (Broggi & Schlegel, 1989), leading to the reduction and extinction of plant and animal species (Lienert et al., 2002; Lienert & Fischer, 2003). The first worldwide intergovernmental agreement on the conservation and sustainable use of wetlands, the Ramsar Convention, was signed in 1971 and aimed for the global protection and revitalization of wetlands (Ramsar Convention Secretariat, 2003). The European pond turtle is an emblematic species of wetlands in Europe and Switzerland (Fritz & Chiari, 2013), and, as turtles and tortoises are the only reptiles that are universally loved by people (Lovich et al., 2018), this species could be used as a flagship species. The European pond turtle could consequently play an important role in raising public awareness about the Ramsar Convention and the extremely important role and vulnerability of wetlands and therefore could enhance habitat renaturation.

7. Conclusion

The numerous population monitoring and ecological studies conducted on *E. orbicularis* in Switzerland underlined the fact that to choose and implant an efficient conservation programme, it is essential to combine multiple studies using both fundamental and applied approaches (Stanford et al., 2020). The case of the European pond turtle in Switzerland is special; indeed, even if successful reintroduction of the species (natural reproduction), for example, in the natural reserve of Près-Bordon, represents in some way an expansion of its distributional range in the country, natural expansion seems impossible due to the low dispersal capacity of the species and habitat fragmentation (Ducotterd et al. in prep or Chapter 2). Therefore, in the short term, reintroduction actions are necessary if we want to encourage the expansion of this reptile in Switzerland.

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References

- Adhikari, S., Bajracharaya, R.M., Sitaula, B.K. (2009). A review of carbon dynamics and sequestration in wetlands. *Journal of Wetlands Ecology* 2: 42-46.
- Anthony, T. Riedle, D., East, M.B., Fillmore, E., Ligon, D.B. (2015). Monitoring of a reintroduced population of juvenile alligator snapping turtles. *Chelonian Conservation and Biology* 14(1): 43-48.
- Arantes, F.P., Dos Santos, H.B., Rizzo, E., Sato, Y., Bazzoli, N. (2010). Collapse of the reproductive process of two migratory fish (*Prochilodus argenteus* and *Prochilodus costatus*) in the Três Marias Reservoir, São Francisco River, Brazil. *Journal of Applied Ichthyology* 27: 847-853.
- Armstrong, D.P., Seddon, P.J. (2008). Directives in reintroduction biology. *Trends in Ecology and Evolution* 23:20-25.
- Ayaz, D., Fritz, U., Tok, C.V., Mermmer, A., Tosunlu, M., Afsar, M., Ciçek , K. (2007). Population estimates and body size of European pond turtles (*Emys orbicularis*) from Pazaragac (Afyonkarahisar/Turkey). *Biologia, Bratislava* 62(2): 225-227.
- Bacon, P.R. (1999), The role of wetlands in the water cycle. "People and Wetlands: The Vital Link", 7th Meeting of the Conference of the Contracting Parties to the Convention on Wetland (Ramsar, Iran, 1971), San José, Costa Rica, 10-18 May 1999.

- Becker, C., Johansson, F. (1981). Tierknochenfunde, Zweiter Bericht. Die neolithischen Ufersiedlungen von Twann 11, Bern.
- Beisel, J.N., Lévêque, C. (2010). Introductions d'espèces dans les milieux aquatiques. Faut-il avoir peur des invasions biologique ? Éditions Quae ISSN : 1777-4624.
- Belliard, J., Marchal, J., Ditche, J.-M., Tales, E., Sabatié, R., Baglinière, J.-L. (2009). Return of adult anadromous allis shad (*Alosa alosa* L.) in the river Seine, France: a sign of river recovery? River Research and Application 25(6): 788-794.
- Bertolero, A., Oro, D. (2009). Conservation diagnosis of reintroducing Mediterranean pond turtle: what is wrong? Animal Conservation 12: 581-591.
- Besse, M., Stahl Gretsch, L.-I., Curdy, P. (2003). ConstellaSion. Hommage à Alain Gally. Lausanne : cahiers d'archéologie romande (Cahiers d'archéologie romande ; 95).
- Biber, E. (2011). The problem of environmental monitoring. University of Colorado Law Review 83:1-82.
- Bonfim, F.C.G., Cordeiro, P.H.C., Peres, C.A., Canale, G.R., Bernardo, C.S.S. (2018). Combining modeling tools to identify conservation priority areas: A case study of the last large-bodied avian frugivore in the Atlantic Forest. Global Ecology and Conservation 15 e00426
- Bretagnolle, V., Inchausti, P. (2005). Modelling population reinforcement at a large spatial scale as a conservation strategy for the declining little bustard (*Tetrax tetrax*) in agricultural habitats. Animal Conservation 8: 59-68.
- Broggi, M.F., Schlegel, H. (1989). Mindestbedarf an naturnahen Flächen in der Kulturlandschaft. Nationales Forschungsprogramm "Nutzung des Bodens in der Schweiz", Bericht 31. Liebefeld-Bern, Switzerland.

Browne, D.R., Bowers, M.A., Hines, J.E. (2006). Connectivity in an agricultural landscape as reflected by interpond movements of a freshwater turtle. *Conservation Biology* 20(3): 780-791.

Buhlmann K.A., Thomas S.B., Iverson J.B., Karapatakis D., Mittermeier R.A., Georges A., Rhodin A.G.J., van Dijk P.P., Gibbons J.W. (2009): A global analysis of tortoise and freshwater turtle distributions with identification of priority conservation areas. *Chelonian Conservation Biology* 8: 116-149.

Cadi, A. (2003). Écologie de la cistude d'Europe (*Emys orbicularis*) : Aspects spatiaux et démographiques, application à la démographie. Lyon, Université Claude-Bernard 1: 350p.

Cadi, A., Delmas, V., Prevot-Julliard, A.C., Joly, P., Pieau, C., Girondot, M. (2004). Successful reproduction of the introduced slider turtle (*Trachemys scripta elegans*) in the south France. *Aquatic Conservation: Marine and Freshwater* 14: 237-246.

Cadi, A., Joly, P. (2004). Impact of the introduction of red-eared slider (*Trachemys scripta elegans*) on survival rates of the European pond turtle (*Emys orbicularis*). *Biodiversity and Conservation* 13: 2511-2518.

Canessa, S., Genta, P., Jesu, Riccardo, Lamagni, L., Oneto, F., Salvido, S., Ottonello, D. (2016). Challenges of monitoring reintroduction outcomes: Insights from the conservation breeding program of an endangered turtle in Italy. *Biological Conservation* 204: 128-133.

Caudron, A., Champigneule, A., Guyomard, R. (2006). Assessment of restocking as a strategy for rehabilitating a native population of brown trout *Salmo trutta* L. in a fast-flowing mountain stream in the northern French Alps. *Journal of Fish Biology* 69: 127-139.

- Caudron, A., Champigneule, A., Guyomard, R., Largiadèr, C.R. (2010). Assessment of three strategies practiced by fishery managers for restoring native brown trout (*Salmo trutta*) population in Northern French Alpine Streams. *Ecology of Freshwater Fish* doi: 10.1111/j.1600-0633.2010.00458.x.
- Chelazzi, G., Lebboroni, M., Tripepi, S., Utzeri, C., Zuffi, M.A.L. (2000). A primer on the conservation of the European pond turtle, *Emys orbicularis*, of Italy. *Chelonii* 2: 101-104.
- Chen, I.C., Hill, J.K., Ohlemuller, R., Roy, D.B., Thomas, C.D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science* 333: 1024-1026.
- Chen, T.-H., Lue, K.-Y. (1998). Ecological notes on feral populations of *Trachemys scripta elegans* in northern Taiwan. *Chelonian Conservation and Biology* 3: 87-90.
- Cheylan, M. (1998). Evolution of the distribution of the European pond turtle in the French Mediterranean area since the post-glacial. *Mertensiella* 10: 47-65.
- Çicek, K., Ayaz, D. (2011). Food composition of the European pond turtle (*Emys orbicularis*) in Lake Sülüklü (Western Anatolia, Turkey). *Journal of Freshwater Ecology* 26: 571-578.
- Congdon, R.D., Dunham, A.E., Van Loben Sels, R.C. (1993). Delayed sex maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. *Conservation Biology* 7: 826-833.
- Daszkiewicz, P. (2018). The forgotten trade of european pond turtle *Emys orbicularis* in central Europe in the 18th and 19th centuries. An essential introduction to historical and economic investigation. *Studia Historiae Oeconomicae* 36(1): 99-103.
- Devaux, B., Bonin, F., Dupré, A. (1996). *Toutes les tortues du monde*. Ed. Delachaux et Niestlé, Paris, 254p.

- Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B., Collen, B. (2014). Defaunation in the Anthropocene. *Science* 345: 401-406.
- Domènech, F., Marquina, R., Soler, L., Valls, L., Aznar, F.J., Fernandez, M., Navarro, P., Lluch, J., (2015). Helminth fauna of the invasive American red-eared slider *Trachemys scripta* in eastern Spain: potential implications for the conservation of native terrapins. *Journal of Natural History* DOI:10.1080/00222933.2015.1062931.
- Ducotterd, J.M. (2003). Association Suisse de Protection et Récupération des Tortues in Chavornay. *Testudo* 12(3): 23-27.
- Ducotterd, J.M., Mosimann, D., Monney, J.C., Cadi, A. (2004). European pond turtle (*Emys orbicularis*) conservation program in Switzerland. International Congress of Chelonians Conservation, Saly, Sénégal.
- Ducotterd, C. (2015). Habitat use, body temperatures of the European pond turtle, *Emys orbicularis*, in four different locations of Switzerland using telemetry and temperatures of potential nesting sites. Travail de Master, Université de Neuchâtel. 72p.
- Ducotterd, C. (2019). Dans la carapace de notre tortue suisse. *Chéloniens* 48, 18-23.
- Ducotterd, C., Crovadore, J., Lefort, F., Rubin, J.F., Ursenbacher, S. (2020). A powerful long metabarcoding method for the determination of complex diets from faeces analysis evaluated in the European pond turtle (*Emys orbicularis*, L. 1758). *Molecular Ecology Resources*.
- Ducotterd, C., Crovadore, J., Lefort, F., Ursenbacher, S., Rubin, J.F. (2020). The European pond turtle's feeding behaviour is not a threat for other endangered species. *Global Ecology and Conservation* 23: e01133.
- Ducotterd, C., Brönniman, O., Rubin, J.F., Ursenbacher, S., Guisan, A. (in prep). SDM EMYS.

- Duelli, P. (1994). Liste Rouge des espèces animales menacées en Suisse. Office Fédéral de l'Environnement, des Forêts et du Paysage (OFEFP), Berne, 97p.
- Ficetola, G.F., De Bernardi, F. (2006). Is the European pond turtle *Emys orbicularis* strictly aquatic and carnivorous? *Amphibia-Reptilia* 27: 445-447.
- Ficetola, G.F., Padoa-Schioppa, E., Monti, A., De Bernardi, F., Bottone, L. (2005). The importance of aquatic and terrestrial habitat for the European pond turtle (*Emys orbicularis*): implications for conservation planning and management. *Canadian Journal of Zoology* 82: 1704-1712.
- Ficetola, G.F., Thuiller, W., Padoa-Shioppa, E. (2009). From introduction to the establishment of alien species: bioclimatic difference between presence and reproduction localities in the slider turtle. *Diversity and Distributions* 15: 108-116.
- Fischer, J., Lindenmayer, D.B. (2000). An assessment of the published results of animal relocations. *Biological Conservation* 96: 1-11.
- Fritz, U. (2001). Handbuch der reptilian und amphibian, Europas Schildkröten, 456-461.
- Fritz, U. (2003). Die Europäische Sumpfschildkröte, Ed. Laurenti Verlag, 224p.
- Fritz, U., Cadi, A., Cheylan, M., Coïc, C., Détaing, M., Olivier, A., Rosecchi, E., Guicking, D., Lenk, P., Joger, U., Wink, M. (2005). Distribution of mtDNA haplotypes (cyt b) of *Emys orbicularis* in France and implications for post-glacial recolonization. *Amphibia-Reptilia* 26: 231-238.
- Fritz, U., Chiari, Y. (2013). Conservation actions for European pond turtles – a summary of current effort in distinct European countries. *Herpetology Notes* 6.
- Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Green, J.L. (2000). The Global decline of Reptiles, déjà vu amphibians. *BioScience* 50: 653-666.
- Gibbons, J.W., Lovich, J.E. (2019). Where has turtle ecology been, and where is it going? *Herpetologica* 75(1): 4-20.

Girondot, M., Fouillet, H., Pieau, C. (1998). Feminizing turtle embryos as a conservation tool. *Conservation Biology* 2: 353-362.

Groves C, Valutis L, Vosick D, Neely B, Wheaton K, Touval J, Runnels B. 2000. Designing a Geography of Hope: A Practitioner's Handbook for Ecoregional Conservation Planning. Arlington (VA): The Nature Conservancy. (20 January 2002; www.conservonline.org)

Groves, C.R., Jensen, D.B., Valutis, L.L., Redford, K.H., Shaffer, M.L., Scott, J.M., Baumgartner, J.V., Higgins, J.V., Beck, M.W., Anderson, M.G. (2002). Planning for biodiversity conservation: Putting conservation sciences into practice. *BioScience* 52: 499-512.

Guisan, A., Thuiller, W. (2005). Predicting species distribution: offering more than simple habitats models. *Ecological Letters* 8: 993-1009.

Guisan, A., Tingely, R., Baumgartner, J.B., Naujokaitis-Lewis, I., Sutcliffe, P.R., Tulloch, A.I.T., Regan, T.J., Brotons, L., et al. (2013). Predicting species distributions for conservation decisions. *Ecology Letters* 16(2): 1424-1435.

Halpin, P.N. (1997). Global climate change and natural-area protection: management responses and research directions. *Ecological Application* 7: 828-843.

Himes Boor, G.K.H. (2013). A framework for developing objective and measurable recovery criteria for threatened and endangered species. *Conservation Biology* 00(0): 1-11.

Hintermann, U. (1992). Inventar der Moorlandschaften von besonderer Schönheit von nationaler Bedeutung. Schlussbreicht, Schriftenreihe Umwelt Nummer 168. BUWAL (Bundesamt für Umwelt, Wald und Landschaft), Bern, Switzerland.

Hofer, U., Monney, J.-C., Dusej, G. (2001). Les reptiles de Suisse. Répartition, habitats, protection. Birkhäuser Verlag. Bâle. 202p.

Hotz, H., Broggi, M.F. (1982). Liste Rouge des espèces d'amphibiens et de reptiles menacés et rares en Suisse. Ligue Suisse pour la Protection de la Nature, Bâle, 112p.

Howell, H.J., Legere, R.H.Jr., Holland, D.S., Seigel, R.A. (2019). Long-term turtle declines: Protected is a verb, not an outcome. *Copeia* 107: 493-501.

Iglesias, R., García-Estévez JM, Ayres C, Acuña A., Cordero-Rivera, A. (2015). First reported outbreak of severe spirochiidiasis in *Emys orbicularis*, probably resulting from a parasite spillover event. *Diseases of Aquatic Organisms* 113: 75-80.

IPBES (2019). Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. E. S. Brondizio, J. Settele, S. Díaz, and H. T. Ngo (editors). IPBES secretariat, Bonn, Germany. XXX pages.

Iverson, J.B. (1992). A revised checklist with distribution maps of the turtles of the world. Green Nature Books: Richmond, IN: privately printed.

Jerde, C.L., Mahon, A.R., Chadderton, W.L., Lodge, D.M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA: eDNA surveillance of rare aquatic species. *Conservation Letter* 4: 150-157.

Joyal, L.A., McCollough, M., Hunter, M.I.Jr. (2001). Landscape ecology approaches to wetlands species conservation: a case study of two turtle species in southern Maine. *Conservation Biology* 15: 1755-1762.

Karch. (2014). Lignes directrices du karch pour la conservation de la Cistude d'Europe (*Emys orbicularis*) en Suisse. Centre Suisse de Coordination pour la Protection des Amphibiens et Reptiles (karch).

Keckes, H. (2014). Short-term effects of inshore restoration measures on early stages, benthic species, and the sublittoral fish assemblage in a large river (Danube, Austria). *Hydrobiologia* 729: 61-76.

- Kelly, R.P., Port, J.A., Yamahara, K.M., Crowder, L.B. (2014). Using environmental DNA to census marine fishes in a large mesocosm. PLoS One 9: e86175.
- Kleewein, A. (2014). Natural reproduction of *Trachemys scripta troostii* (Holbrook, 1836) x *Trachemys scripta scripta* (Schoepff, 1792) in Austria. Herpetozoa 26: 183-185.
- Klemens, M.W. (2000). Turtle Conservation, Smithsonian Institution, 330p.
- Krammer, M. (2013). Le Bouquetin des Alpes (*Carex ibex ibex*) en Provenance-Alpes-Côte d'Azur : passé, présent et avenir. Faune-PACA Publication 30 : 35p.
- Krak, S., Levin, N., Grantham, H.S., Possingham, H.P. (2009). Between-country collaboration and consideration of costs increase conservation planning efficiency in the Mediterranean Basin. PNAS 106(36).
- Kotenko, T.I. (2000). The European pond turtle (*Emys orbicularis*) in the Steppe Zone of the Ukraine. In Hödl W. and Rössler M. (eds), Die Europäische Sumfschlidkröte, Staphia 68: 87-106.
- Kubisch, A., Holt, R.D., Poethke, H.J., Fronhofer, E.A. (2014). Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. Oikos 123: 5-22.
- Lacoursière-Rousseel, A., Howland, K., Normandeau, E., Grey, E.K., Archambault, P., Deiner, K., Lodge, D.M., Hernandez, C., Leduc, N., Bernatchez, L. (2018). eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. Ecology and Evolution 8(16): 7763-7777.
- Lebboroni, M. Chelazzi, G. (1991). Activity patterns of *Emys orbicularis* L. (Chelonia Emydidae) in central Italy. Ethology Ecology & Evolution 3: 257-268.
- Lembert, M.R., McKenzie, J.M., Screen, R.M., Clause, A.G., Johnson, B.B., Mount, G.G., Shaffer, H.B., Pauly, G.B. (2019). Experimental removal of introduced slider turtles offers new insight into competition with native, threatened turtle. PeerJ 7: e444.

Lenders, H.J.R., Chamuleau, T.P.M., Hendriks, A.J., Lauwerier, R.C.G., Leuven, R.S.E.W., Verberk, W.C.E.P. (2016). Historical rise of waterpower initiated the collapse of salmon stocks. *Scientific Reports* 6: 292669.

Lenk, P., Fritz, U., Jogger, U., Wink, M. (1999). Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis*, (Linnaeus, 1758). *Molecular Ecology* 8: 1911-1922.

Lienert, J., Fischer, M., Diemer, M. (2002). Local extinctions of the wetland specialist *Swertia pereennis* L. (Gentianaceae) in Switzerland: a revisit study based on herbarium records. *Biological Conservation* 103: 65-67.

Lienert, J., Fischer, M. (2003). Habitat fragmentation affects the common wetland specialist *Primula farinosa* in the north-east Switzerland. *Journal of Ecology* 91: 587-599.

Lisa, A.J., McCollough, M., Malcolm, L.H. (2001). Landscape ecology approaches to wetland species conservation: a case study of two turtle species in southern Maine. *Conservation Biology* 15(6): 1755-1762.

Lovich J.E., Ennen J.R., Agha M., Gibbons W. (2018): Where have all the turtles gone, and why does it matter? *BioScience*, doi:10.1093/biosci/biy095.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M. (2000). 100 of the world's worst invasive alien species: A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp. First published as special lift-out in Aliens 12. Updated and reprinted version: November 2004.

Luiselli, L., Capula, M., Capizzi, D., Filippi, E., Jesus, V.T., Ainbaldi, C. (1997). Problems for conservation of pond turtles (*Emys orbicularis*) in central Italy: is the introduced

redeared turtle (*Trachemys scripta*) a serious threat? Chelonian Conservation and Biology 2: 417-419.

Luiselli, L. (2017). Food habits, habitat use and density of *Emys orbicularis persica* from Jelilabad, Azerbaijan. Herpetological Journal 27: 245-251.

Mac, M.J., Opler, P.A., Puckett Heacker, C.E., Doran, P.D. (1998). Status and trends of the nation's biological resources. U.S. Department of the Interior, U.S. Geological Survey, Minneapolis, Minnesota, USA.

McKinley, D.C., Miller-Rushing, A.J., Ballard, H.L., Bonney, R., Brown, H., Cook-Patton, C.C., Evans, D.M., French, R.A., Parrish, J.K., Phillips, T.B., Ryan, S.F., Shanley, L.A., Shirk, J.L., Stepenuck, K.F., Weltzin, J.F., Wiggins, A., Boyle, O.D., Briggs, R.D., Chapin III, S.F., Hewitt, D.A., Preuss, P.W., Soukup, M.A. (2017). Citizen science can improve conservation science, natural resource management, and environmental protection. Biological Conservation 208: 15-28.

Meyer, A., Zumbach, S., Schmidt, B., Monney, J.-C. (2009). Les amphibiens et reptiles de Suisse. Haupt, 336 pp.

Meyer, L., Du Preez, L., Bonneau, E., Héritier, L., Franch Quintana, M., Valdeón, A., Sadaoui, A., Kechemir-Issad, N., Palacios, C., Verneau, O. (2015). Parasite host-switching from the invasive American red-eared slider, *Trachemys scripta elegans*, to the native Mediterranean pond turtle, *Mauremys leprosa*, in natural environments. Aquatic Invasions 10: 79-91.

Mitchell J.C., Klemens M.W. (2000): Primary and secondary effects of habitat alternation. In: Turtle Conservation, p. 5-32. Klemens M.W., Ed., Smithsonian Institution Press, Washington D.C.

Mitrus, S. (2005). Headstarting in European pond turtle (*Emys orbicularis*): Does it work? Amphibia-Reptilia 26:333-341.

- Moll, E.O. (1995). The turtle *Trachemys scripta* and the pet trade. Aliens 2: 3.
- Monney, J.C., Meyer, A. (2005). Liste Rouge des espèces menacés de Suisse, Reptiles. Office Fédéral de l'Environnement, des Forêts et du Paysage (OFEFP) et Centre Suisse de Coordination pour la Protection des Amphibiens et Reptiles (karch), Berne. 46p.
- Moorhouse, T.P., Gelling, M., Macdonald, D.W. (2009). Effects of habitat quality upon reintroduction success in water voles: Evidence from a replicated experiment. Biological Conservation 142: 53-60.
- Mosimann, D. (2002). État d'une population de cistude d'Europe, *Emys orbicularis* (Linneaus, 1758), 50 ans après les premières (ré) introduction au Moulin-de-Vert (Genève, Suisse). Travail de diplôme. Université de Neuchâtel. 107p.
- Mosimann, D., Cadi, A. (2004). On the occurrence and viability of the European pond turtle (*Emys orbicularis*) in Moulin-de-Vert (Geneva, Switzerland): 50 years after first introduction. Biologia, Bratislava 59(14): 109-112.
- Nuoffer, F. (2000). Situation de la cistude d'Europe *Emys orbicularis* (Emydidae, Chelonia) dans le canton de Genève et données éco-éthologiques sur la population du Moulin-de-Vert. Mémoire de certificat. Université de Neuchâtel. 71p.
- Newberry, R. (1984). The American red-eared terrapin in South Africa. African Wildlife 38: 186-189.
- OFEV (2018). Repeuplement durable des cours d'eau. Conditions-cadres et principes. Office fédéral de l'environnement, Berne. Connaissance de l'environnement n° 1832 : 42 p.
- Ottonello, D., Salvidio, S., Rosecchi, E. (2005). Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). Amphibia-Reptilia 26: 562-565.

Otonello, D., D'Angelo, S., Oneto, F., Malavasi, S., Zuffi, M.A.L. (2016). Feeding ecology of the Silican pond turtle *Emys trinacris* (Testudines, Emydidae) influenced by seasons and invasive aliens species. Ecological Research 32(1): 71-80.

Otonello, D., Oneto, F., Vignone, M., Rizzo, A., Salvidio, S. (2018). Diet of a restocked population of the European pond turtle *Emys orbicularis* in NW Italy. Act. Herpetologica 13: 89-93.

Painter, C.W., Christman, B.L. (2000). Geographic distribution. *Trachemys scripta*. Herpetological Review 31: 253.

Parent, G.-H. (1968). Contribution à la connaissance du peuplement herpétologique de la Belgique. Note 4. La question controversée de l'indigénat de la cistude d'Europe, *Emys orbicularis* (Linné), en Lorrain, au Benelux et dans les territoires adjents. Bulletin de l'Institut royal des Sciences naturelles de Belgique 44 : 29.

Pellet, J., Guisan, A., Perrin, N. (2004). A concentric analysis of the impact of urbanization on the threatened European tree frog in an agricultural landscape. Conservation Biology 18: 1599-1606.

Pereira, M., Segurado, P., Neves, N. (2011). Using spatial network structure in landscape management and planning: A case study with pond turtles. Landscape and Urban Planning 100: 67-76.

Pittet, M. (2017). Impact of global warming on the distribution and dispersal of reptiles in Western Swiss Alps. Master Thesis. Master in Behaviour, Evolution, Conservation (BEC), Department of Ecology & Evolution, University of Lausanne, Switzerland.

Polajnar, K. (2008). Public awareness of wetlands and their conservation. Acta Geographica Slovenia 48(1): 121-146.

Possingham, H.P., Lindemayer, D.B., Norton, T.W. (1993). A framework for the improved management of threatened species based on Population Viability Analysis (PVA). *Pacific Conservation Biology* 1: 39-45.

Prévôt-Julliard, A.C., Gousset, E., Archinard, C., Cadi, A., Girondot, M. (2007). Pets and invasion risks: is the Slider turtle strictly carnivorous? *Amphibia-Reptilia* 28: 139-143.

Raemy, M. (2010). Hybridization and genetics of the Swiss *Emys orbicularis* populations. Master Thesis, University of Neuchâtel, Switzerland. 51p.

Raemy, M., Ursenbacher, S. (2012). Bilan du suivi 2010-2012 de la population de cistude d'Europe (*Emys orbicularis* L. 1758) réintroduite dans les étangs de Près-Bordon et des Rapports. Unpublished report.

Raemy, M., Monney, J.C., Ursenbacher, S. (2013). Conservation activities for European pond turtles (*Emys orbicularis*) in Switzerland. *Herpetology Notes* 6: 111-112.

Raemy, M., Ursenbacher, S. (2018). Detection of the European pond turtle (*Emys orbicularis*) by environmental DNA: is eDNA adequate for reptiles? *Amphibia-Reptilia* 39(2): 135-143.

Ramsar Convention Secretariat. (2013). The Ramsar Convention Manual: a guide to the conservation on Wetlands (Ramsar, Iran, 1971), 6th ed. Ramsar Conservation Secretariat, Gland, Switzerland.

Reed, D.H., O'Grady, J.O., Brook, B.W., Ballou, J.D., Frankham, R. (2003). Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. *Biological Conservation* 113: 23-34.

Reed, M., Mills, L.S., Dunning, Jr., J.B., Menges, E.S., McKelvey, K.S., Frye, R., Beissinger, S.R., Anstett, M.-C., Miller, P. (2002). Emerging issues in population viability analysis. *Conservation Biology* 16: 1-7.

Rivera, A.C., Ayres Fernandez, C. (2004). A management plan for the European pond turtle (*Emys orbicularis*) populations of the Louro river basin (Northwest Spain). *Biologia, Bratislava* 59(14) : 161-171

Rhodin, A.G.J., Stanford, C.B., van Dijk, P.P., Eisemberg, C., Luiselli, L., Mittermeir, R.A., Hudson, R., Horne, B.D., Goode, E.V., Kuchling, G., et al. (2018). Global conservation status of turtles and tortoises (Order Testudines). *Chelonian Conservation Biology* 17: 135-161.

Rodder, D., Schmidlein, S., Vwith, M., Lotters, S. (2009). Alien invasive slider turtle in unpredicted habitat: A matter of niche shift or of predictors studied? *PLoS One*, 4.

Rollinat, R. (1934). *La vie des reptiles de la France centrale*. Delagrave, Paris, 337p.

Rovero, F., Chelazzi, G. (1996). Nesting migrations in a populations of the European pond turtle *Emys orbicularis* (L.) from central Italy. *Ethology, Ecology, and Evolution* 8: 297-304.

Salafsky, N., Margoluis, R., Redford, K.H., Robinson, J.G. (2002). Improving the practice of conservation: a conceptual framework and research agenda for conservation science. *Conservation Biology* 16: 1469-1479.

Schaffner H.P., Kützli, M. (2010). Nistplätze der Europäischen Sumpfschildkröte, *Emys orbicularis*, in der Schweiz. SwissEmys, Switzerland.

Schaub, M., Zink, R., Beissmann, H., Sarrazin, F., Arlettaz, R. (2009=). When to end releases in reintroduction programmes: demographic rates and population viability analysis of bearded vultures in the Alpes. *Journal of Applied Ecology* 46: 92-100.

Scherrer, D., Christe, P., Guisan, A. (2019) Modelling bat distributions and diversity in a mountain landscape using focal predictors in ensemble of small models. *Diversity and Distributions* 25: 770-782

Schneeweiss, N. (2004). Climatic impact on reproductive success of *Emys orbicularis* at the northern border of the species' range (Germany). *Biologia, Bratislava* 59(14): 131-137.

Schumacher, I.M., Rostal, D.C., Yates, R.A., Brown, D.R., Jacobson, E.R., Kelin, P.A. (1999). Persistence of maternal antibodies against *Mycoplasma agassizii* in desert tortoise hatchlings. *American Journal of Veterinary Research* 60(7): 826-831.

Seddon, P.J. (1999). Persistence without intervention: assessing success in wildlife reintroductions. *Trends in Ecology and Evolution* 14: 503

Segurado, P., Kunin, W.E., Filipe, A.F., Araujo, M.B. (2012). Patterns of coexistence of two species of freshwater turtles are affected by spatial scale. *Basic and Applied Ecology* 13: 371-379.

Shaffer, M.L. (1981). Minimum population sizes for species conservation. *BioScience* 31: 131-134.

Siegel, R.A., Dodd, J.R. (2000). Manipulation of populations for conservation. In *Turtle Conservation*, Klemens M.W. (ed.), Smithsonian Institution, pp 218-238.

Sigsgaard, E.E., Carl, H., Møller, P.R., Thomsen, P.F. (2015). Monitoring the near-extinct European weather loach in Denmark based on environmental DNA water samples. *Biological Conservation* 183: 46-52.

Stanford, C.B., Iverson, J.B., Rhodin, A.G.J., van Dijk, P.P., Mittermeier, R.A., Kuchling, G., Berry, K.H., et al. (2020). Turtle and tortoise are in trouble. *Current Biology* 30, R721-R735.

Steen, D.A., Aresco, M.J., Belike, S.G., Compton, B.W., Condon, E.P., Dodd, K.C., et al. (2006). Relative vulnerability of female turtles to road mortality. *Animal Conservation* 9: 269-273.

- Steen, D.A, Gibbs, J.P., Buhlmann, K.A., Carr, J.L., Compton, B.W., Congdon, J.D et al. (2012). Terrestrial habitat requirements of nesting freshwater turtles. *Biological Conservation* 150: 121-128.
- Teixeira, C.P., Schnetinil de Azevedo, C., Mendl, M. Cipreste, C.F., Young, R.J. (2007). Revisiting translocation and reintroduction programmes: the importance of considering stress. *Animal Behaviour* 73: 1-13.
- Telecky, T.M. (2001). United States import and export of live turtles and tortoises. *Turtle and Tortoise Newsletter* 4: 8-13.
- Tortoise and Freshwater Turtle Specialist Group. (1996). *Emys orbicularis*. The IUCN Red List of Threatened Species 1996.
- Trakimas, G., Sidaravičius, J. (2008). Road mortality threatens small northern populations of the European pond turtle, *Emys orbicularis*. *Acta Herpetologica* 3(2): 161-166.
- Turtle Taxonomy Working Group [Rhodin AGJ, Iverson JB, Bour R, Fritz U, Georges A, Shaffer HB, van Dijk PP]. 2017. *Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status*, 8th ed. Monograph 7 of Rhodin AGJ, Iverson JB, van Dijk PP, Saumure RA, Buhlmann KA, Pritchard PCH, Mittermeier RA, eds. *Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*. Chelonian Research Monographs.
- Tyre, A.J., Tenhumberg, B., Field, S.A., Niejalke, D., Parris, K., Possingham, H.P. (2003). Improving precision and reducing bias in biological survey: estimating false-negative error rates. *Ecological Applications* 13: 1790-1801.
- Ursenbacher, S. (2018). Rapport: analyses génétiques des échantillons de cistude d'Europe (*Emys orbicularis*) de la réserve de Laconnex. Department of

Environmental Science, Section of Conservation Biology, University of Basel,
unpublished report.

Ursenbacher, S., Raemy, M. (2013). Etat des connaissances actuelles de la population de
Cistudes d'Europe (*Emys orbicularis*, L. 1758) de la réserve du Moulin-de-Vert (GE).
Unpublished report.

Vlachos, E., Delfino, M. (2016). Food for thought: Sub-fossil and fossil chelonian remains
form Franchthi Cave and Megalopolis confirm a glacial refuge for *Emys orbicularis*
in Peloponnesus (S. Greece). Quaternary Science Reviews 150: 158-171.

Van Dijk, P.P., Sindaco, R. (2004). *Emys orbicularis*. The IUCN Red List of Threatened
Species 2004.

Vignoli, L., Bologna, M.A., Manzini, S., Rugiero, L., Luiselli, L. (2015). Attributes of basking
sites of the European pond turtle (*Emys orbicularis*) in central Italy. Amphibia-
Reptilia DOI:10.1163/15685381-00002988.

Warwick, C. (1991). Conservation of red-eared terrapins *Trachemys scripta elegans*:
Threats from international pet and culinary markets. Testudo 3: 34-44.

Waterson, A.M., Schmidt, D.N., Valdes, P.J., Holroyd, P.A., Nicholson, D.B., Fransworth, A.,
Barret, P.M. (2016) Modelling the climatic niche of turtles: a deeptime perspective.
Proceedings of the Royal Society of B-Biological Sciences 283: 9.

Wilcox, T.M., McKelvey, K.S., Young, M.K., Jane, S.F., Lowe, W.H., Whiteley, A.R., Schwartz,
M.K. (2013). Robust detection of rare species using environmental DNA: the
importance of primer specificity. PLoS One 8: e59520.

Works, A.J., Olson, D.H. (2018). Diets of two nonnative freshwater turtle species
(*Trachemys scripta* and *Pelodiscus sinensis*) in Kawai Nui March, Hawai. Journal of
Herpetology 52: 445-453.

TABLE AND FIGURES

Table 1: Reintroduction actions (year and number of individuals released) of *Emys orbicularis* in different cantons of Switzerland.

All selected sites were suitable or became suitable after renaturation works according to the ecology and needs of the European pond turtle.

		1st reintroduction		2nd reintroduction		3rd reintroduction		4th reintroduction		
Canton	Site	Year	Number of individuals	Total individuals released						
Geneva	Près-Bordon	2010	14	2011	8	2016	19	2018	11	52
	Teppes-de-Verbois	2017	20	2018	10	2019	10	-	-	30
Neuchâtel	La-Vieille-Thielle	2013	10	2015	9	2019	8	-	-	27
Ticino	Bolle-di-Magadino	-	-	-	-	-	-	-	-	-

Figure 1: Reintroduction sites for the European pond turtle (*Emys orbicularis*) in Switzerland, chosen to determine if reintroduction is successful in Switzerland. Three regions were selected and validated by the KARCH and by experts of the species: in the canton of Geneva (natural reserve of Près-Bordon and Teppes-de-Verbois), in the canton of Neuchâtel (La-Vieille-Thielle) and, finally, in the canton of Ticino (Bolle-di-Magadino) (KARCH, 2014).

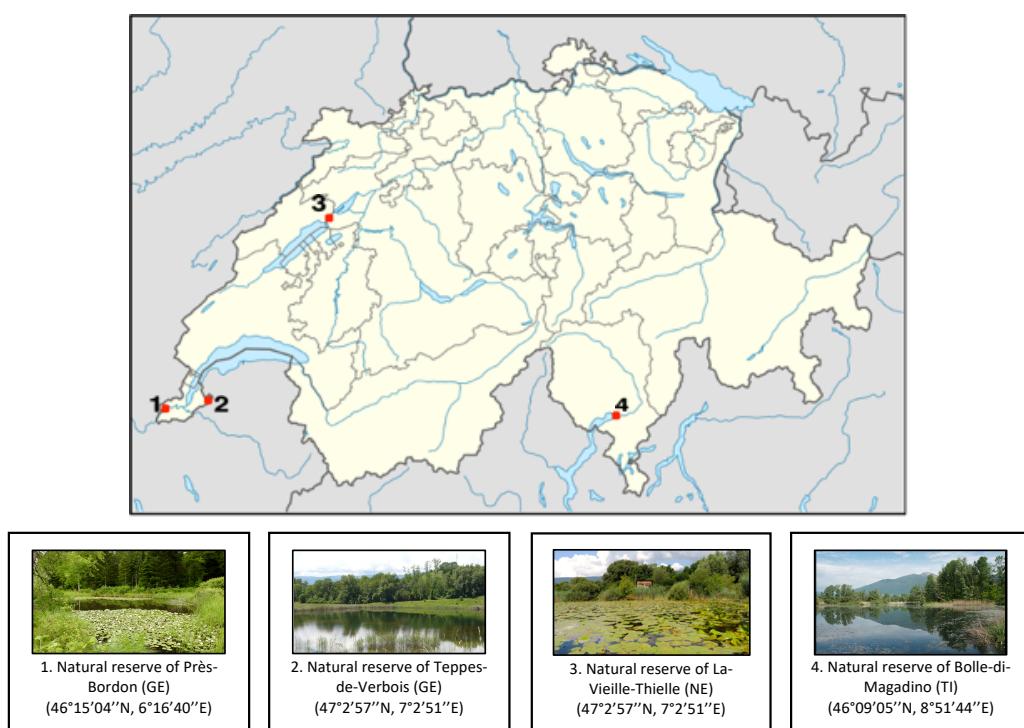


Figure 2: Predicted current potential distribution of *Emys orbicularis* in Switzerland. The scale indicates a less suitable environment (cooler colours – 0 to 0.25) and more suitable environment (warmer colours – 0.75 to 1). The occurrence points represent the most recent observations (1997-2019) of the species in Switzerland (Chapter 2). Most of these observations are made of isolated individuals or of genetically non-native individuals.

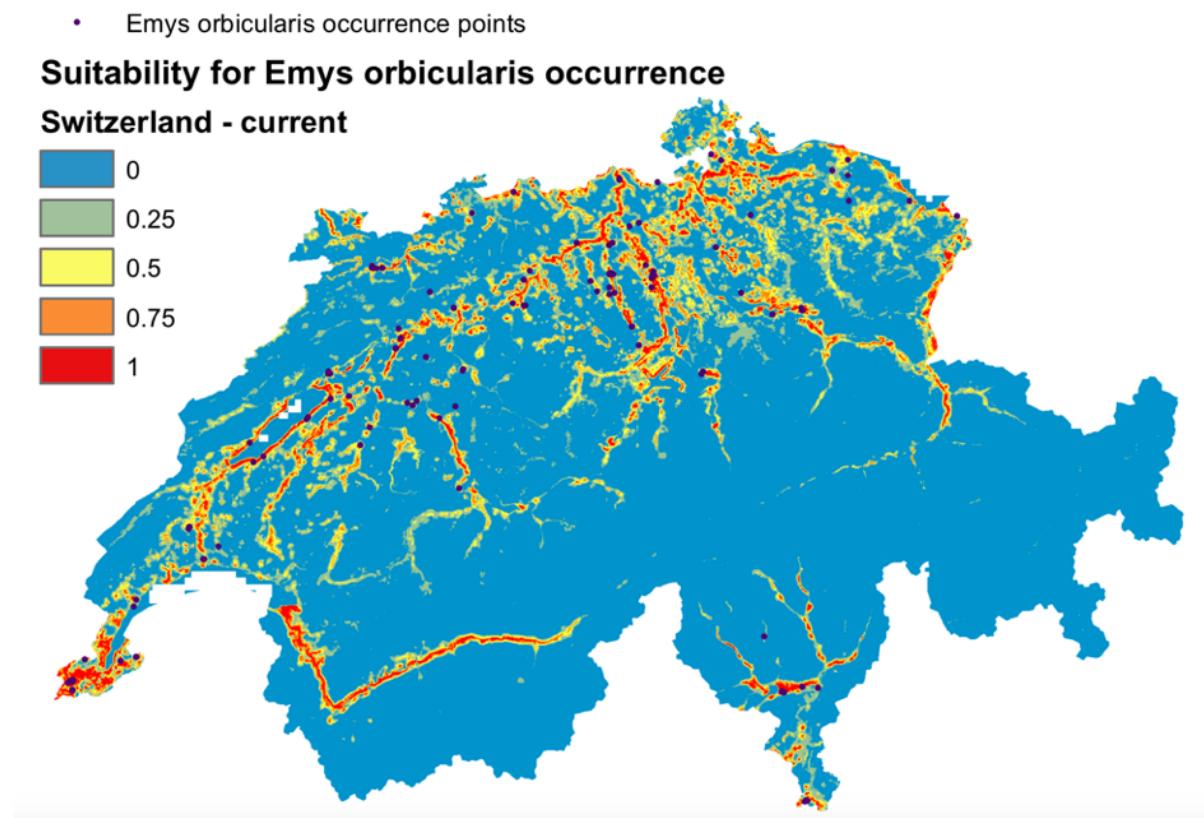


Figure 3: Based on the literature (Possingham et al., 1993; Groves et al., 2000; 2002; Himes Boor, 2013; Bonfim et al., 2018), we propose a general integrated framework for the conservation of endangered species to determine the essential steps to plan and consider when a conservation program is launched.

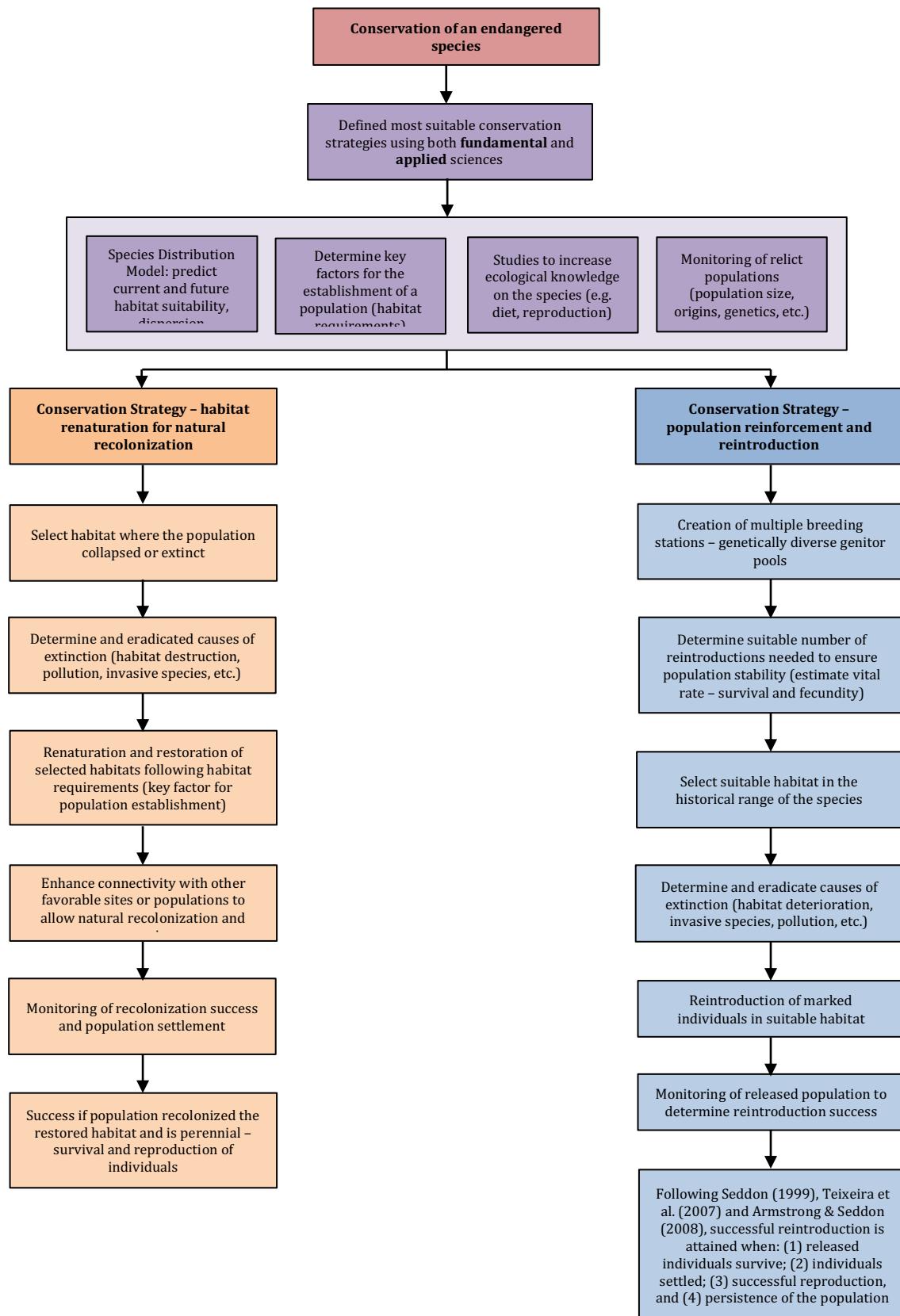
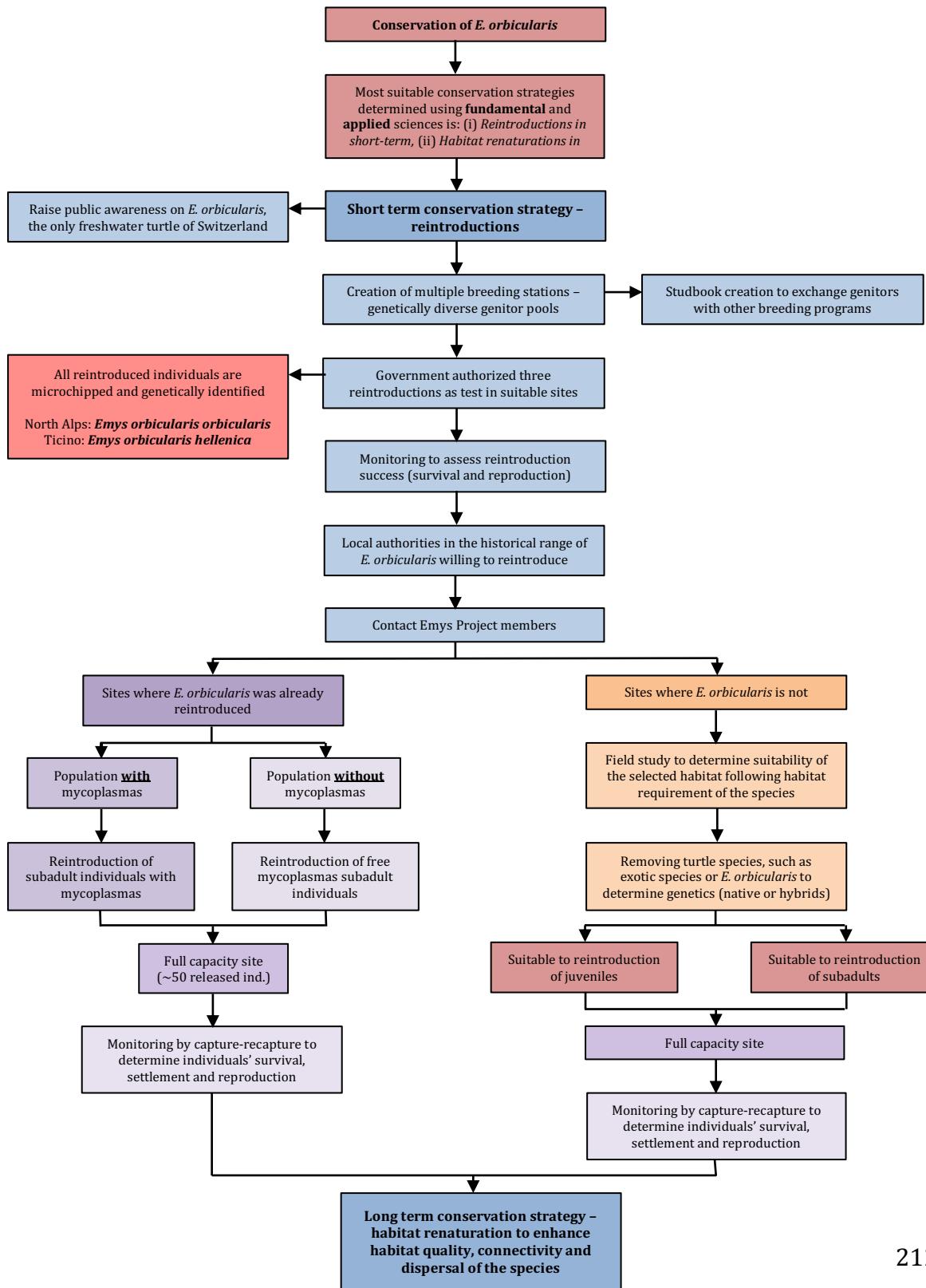


Figure 4: Integrated framework, based on the knowledge gained during the *Emys* Project and from the *Emys* literature (Cadi, 2003; Ficetola et al., 2004; Rivera & Ayres, 2004; Fritz & Chiari, 2013), to determine the essential steps to plan and consider for the conservation of the European pond turtle (*Emys orbicularis*) in Switzerland.



References:

- Allendorf, F.W., Leary, R.F., Sprunell, P., Wenburg, J.K. (2001): The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution*, **16**(11): 613-622.
- Beaudry, F., De Maynadier, P.G., Hunter, M.L.Jr. (2010): Nesting movements and the use of anthropogenic nesting sites by spotted turtles (*Clemmys guttata*) and Blanding's turtles (*Emydoidea blandingii*). *Herpetological Conservation Biology*, **50**: 514-517.
- Becker, C., Johansson, F. (1981): Tierknochenfunde, Zweiter Bericht. Die neolithischen Ufersiedlungen von Twann 11, Bern.
- Berkelmans, R., De'ath, G., Kininmonth, S., Skirving, W.J. (2004): A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation, patterns, and predictions. *Coral Reefs*, **23**(1):74-83.
- Besse, M., Stahl Gretsch, L.-I., Curdy, P. (2003): ConstellaSion. Hommage à Alain Gally. Lausanne : cahiers d'archéologie romande (Cahiers d'archéologie romande ; 95).
- Buhlmann, K.A., Thomas, S.B., Iverson, J.B., Karapatakis, D., Mittermeier, R.A., Georges, A., Rhodin, A.G.J., van Dijk, P.P., Gibbons, J.W. (2009): A global analysis of tortoise and freshwater turtle distributions with identification of priority conservation areas. *Chelonian Conservation Biology*, **8**: 116-149.
- Burke, V.J., Gibbons, J.W. (1995): Terrestrial buffer zones and wetlands conservation: A case study of freshwater turtles in a Carolina bay. *Conservation Biology*, **9**: 1365-1369.
- Bulté, G., Blouin-Demers, G. (2010): Estimating the energetic significance of basking behaviour in a temperate-zone turtle. *Ecoscience*, **17**: 387-393.
- Cadi, A. (2003): Écologie de la cistude d'Europe (*Emys orbicularis*): Aspects spatiaux et démographiques, application à la démographie. Lyon, Université Claude Bernard Lyon 1:350 pp.

Cheyelan, M. (1998): Evolution of the distribution of the European pond turtle in the French Mediterranean area since the post-glacial. *Mertensiella*, **10**: 47-65.

Çicek, K., Ayaz, D. (2011): Food composition of the European pond turtle (*Emys orbicularis*) in Lake Sülüklü (Western Anatolia, Turkey). *Journal of Freshwater Ecology*, **26**: 571-578.

Congdon, J.D., Dunham, A.E., Van Loben Sels, R.C (1993): Delayed sex maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. *Conservation Biology*, **7**: 826-833.

Cordero Rivera, C., Fernandez, C. (2004). A management plan for the European pond turtle (*Emys orbicularis*) populations of the Louro river basin (Northwest Spain). *Biologia, Bratislava*, **59**(14): 161-171.

Dalton, R. (2003): Mock turtles. *Nature* (Lond.), **423**: 219-220.

Davis A.J., Jenkison, L.S., Lawton, J.H., Shorrocks, B., Wood, S. (1998): Making mistakes when predicting shifts in species range in response to global warming. *Nature*, **391**: 783-786.

Daszkiewicz, P. (2018): The forgotten trade of European pond turtle *Emys orbicularis* in central Europe in the 18th and 19th centuries. An essential introduction to historical and economic investigation. *Studia Historiae Oeconomicae*, **36**(1): 99-103.

Devaux, B., Bonin, F., Dupré, A. (1996): Toutes les tortues du Monde. Ed. Delachaux et Niestlé, 254p. Paris.

Ducotterd, J.M. (2003): Association Suisse de Protection et Récupération des Tortues in Chavornay. *Testudo*, **12**(3): 23-27.

Ducotterd, J.M., Mosimann, D., Monney, J.C., Cadi, A. (2004): European pond turtle (*Emys orbicularis*) conservation program in Switzerland. *International Congress of Chelonians Conservation*, Saly, Sénégal.

Duelli P. (1994): Liste rouge des espèces animales menacées en Suisse. OFEFP, Bern :97pp.

Duysebaeva, T.N., Doronin, I.V., Malakhov, D.V., Kukushkin, A.G., Bakiev, A.G. (2019): GIS analysis of the distribution and habitation conditions of *Emys orbicularis orbicularis* (Testudines, Emydidae): Methodical aspects. *Natural Sciences*, **1**(25).

Engler, R., Guisan, A. (2009). MigClim: Predicting plant distribution and dispersal in a changing climate. *Diversity and Distributions*, **15**: 590-601.

Eskew, E.A., Price, S.J., Dorcas, M.E. (2010a): Survivorship and population densities of painted turtles (*Chrysemys picta*) in recently modified suburban landscapes. *Chelonian Conservation Biology*, **9**: 244-249.

Eskew E.A., Price S.J., Dorcas M.E. (2010b): Survival and recruitment of semi-aquatic turtles in an urbanized region. *Urban Ecosystems*, **265**: 81-95.

Ficetola, G.F., Padoa-Schioppa, E., Monti, A., Massa, R., De Bernardi, F., Bottoni, L. (2004): The importance of aquatic and terrestrial habitat for the European pond turtle (*Emys orbicularis*): implications for conservation planning and management. *Canadian Journal of Zoology*, **82**: 1704-1712.

Ficetola, G. F., De Bernardi, F. (2006): Is the European pond turtle *Emys orbicularis* strictly aquatic and carnivorous? *Amphibia-Reptilia*, **27**: 445-447

Fritz, U. (2003): Die Europäische Sumpfschildkröte, edn. Laurent Verlag, 224p.

Fritz, U., Cadi, A., Cheylan, M., Coïc, C., Détaigne, M., Olivier, A., Rosecchi, E., Guicking, D., Lenk, P., Joger, U., Wink, M. (2005): Distribution of mtDNA haplotypes (cyt b) of *Emys*

orbicularis in France and implications for post glacial recolonization. *Amphibia-Reptilia*, **26**: 213-238.

Fritz, U., Chiari, Y. (2013): Conservation action for European pond turtles – a summary of current efforts in distinct European countries. *Herpetology Notes*, **6**.

Gibbons, J.W., Scott, D.E., Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S., Winne, C.T. (2000): The global decline of reptiles, Déjà Vu amphibians. *BioScience*, **50**: 653-666.

Gibbons, J.W., Lovich, J.E. (2019): Where has turtle ecology been, and where is it going? *Herpetologica*, **75**(1): 4-20.

Golubović, A., Grabovac, D., Popović, M. (2017): Actual and potential distribution of the European pond turtle, *Emys orbicularis* (L., 1758) in Serbia, with conservation implications. *Acta Zoologia Bulgaria*, **10**: 49-56.

Hofer, U., Monney, J-C., Dušej, G. (2001): Les reptiles de Suisse. Répartition, habitats, protection. Birkhäuser Verlag AG: 202p.

Hotz, H., Broggi, M. F. (1982): Liste rouge des espèces d'amphibiens et de reptiles menacés et rares en Suisse. Ligue Suisse pour la Protection de la Nature, Bâle: 112p.

Hoobs, R.J., Higgs, E., Harris, J.A. (2009): Novel ecosystems: implications for conservation and restoration. *Trends in Ecology and Evolution*, **24**: 599-605.

Iverson, J.B., Jablonska, S. (1998): Patterns of survivorship in turtles (order Testudines). *Canadian Journal of Zoology*, **69**: 385-391.

IPBES (2019): Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. E. S. Brondizio, J. Settele, S. Díaz, and H. T. Ngo (editors). IPBES secretariat, Bonn, Germany. XXX pages.

IUCN (2013): Liste rouge mondiale 2013 : les plus anciennes et les plus grandes espèces du monde sont en déclin. Comité français de l'IUCN - 26 rue Geoffroy Saint Hilaire - 75005 Paris.

IUCN (2019): IUCN 70 years: International Union for Conservation of Nature annual report 2018. Gland, Switzerland. IUCN-2019-007.

Joyal, L.A., McCollough, M., Hunter, M.L. (2001): Landscape ecology approaches to wetlands species conservation: a case study of two turtle species in Southern Maine. *Conservation Biology*, **15**(6): 1755-1762.

Karch. (2014): Lignes directrices du karch pour la conservation de la Cistude d'Europe (*Emys orbicularis*) en Suisse. Centre Suisse de Coordination pour la Protection des Amphibiens et Reptiles (karch).

Kinnear, J.E., Sumner, N.R., Onus, M.L. (2002): The red fox in Australia – an exotic predator turned biocontrol agent. *Biological Conservation*, **108**(3):335-359.

Kiviat, E., Stevens, G., Brauman, R., Hoeger, S., Petokas, P.J., Hollands, G.G. (2000): Restoration of wetland and upland habitat for the Blanding's turtle, *Emydoidea blandingii*. *Chelonian Conservation Biology*, **3**: 650-657.

Klemens, M.W. (2000): Turtle Conservation, Smithsonian Institution, 330p.

Kotenko, T.I. (2000): The European pond turtle (*Emys orbicularis*) in the Steppe Zone of the Ukraine. In Hödl W. and Rössler M. (eds), Die Europäische Sumfschlidkröte, Staphia **68**: 87-106.

Kubisch, A., Holt, R.D., Poethke, H.J., Fronhofer, E.A. (2014): Where am I and why? Synthesizing range biology and the eco-evolutionary dynamics of dispersal. *Oikos*, **123**: 5-22.

Kumara, H.N., Irfan-Ullah, M., Kumar, S. (2009): Mapping potential distribution of Slender Loris subspecies in peninsular India. *Endangered Species Research*, **7**: 29-38.

- Lebboroni, M., Chelazzi, G. (1991): Activity patterns of *Emys orbicularis* L. (Chelonia Emydidae) in central Italy. *Ethology, Ecology and Evolution*, **3**: 257-268.
- Lenk, P., Fritz, U., Joger, U., Wink, M. (1999): Mitochondrila phylogeography of the European pond turtle, *Emys orbicularis*, (Linneaus, 1759). *Molecular Ecology*, **8**: 1911-1922.
- Lindquist, E. J., D'Annunzio, R., Gerrand, A. (2012) – Global forest land-use change 1990-2005. Food and agriculture organization of the United Nation, Rome.
- Lovich, J.E., Ennen, J.R., Agha, M., Gibbons, W. (2018): Where have all the turtles gone, and why does it matter ? *BioScience*, doi:10.1093/biosci/biy095.
- Luiselli, L. (2017): Food habits, habitat use and density of *Emys orbicularis persica* from Jelilabad, Azerbaijan. *Herpetological Journal*, **27**: 245-251.
- Mac, M.J., Opler, P.A., Puckett Heacker, C.E., Doran, P.D. (1998): Status and trends of the nation's biological resources. U.S. Department of the Interior, U.S. Geological Survey, Minneapolis, Minnesota, USA.
- Maherali, H., Klironomos, J.N. (2007). Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, **316**: 1746-1748.
- Marchand, M.N., Litvaitis, J.A. (2004): Effects of landscape composition, habitat features, and nest distribution on predation rates of simulated turtle nest. *Biological Conservation*, **117**: 243-251.
- Mitchell, J.C., Klemens, M.W. (2000): Primary and secondary effects of habitat alternation. In: *Turtle Conservation*, p. 5-32. Klemens M.W., Ed., Smithsonian Institution Press, Washington D.C.
- Monney, J. C., Meyer, A. (2005): Liste rouge des espèces menacées en Suisse, Reptiles. Office fédéral de l'environnement, des forêts et du paysage (OFEFP), Centre de Coordination des amphibiens et reptiles de Suisse (KARCH). Berne, 46p.

Mosimann, D. (2002): État d'une population de cistude d'Europe, *Emys orbicularis* (Linneaus, 1758), 50 ans après les premières (ré) introduction au Moulin-de-Vert (Genève, Suisse). Travail de diplôme. Université de Neuchâtel. 107p.

Nuoffer, F. (2000): Situation de la cistude d'Europe *Emys orbicularis* (Emydidae, Chelonia) dans le canton de Genève et données éco-éthologiques sur la population du Moulin-de-Vert. Mémoire de certificat. Université de Neuchâtel. 71p.

Ottanello, D., Salvidio, S., Rosecchi, E. (2005): Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). *Amphibia-Reptilia*, **26**: 562-565.

Ottanello, D., D'Angelo, S., Oneto, F., Malavasi, S., Zuffi, M.A.L. (2016): Feeding ecology of the Silican pond turtle *Emys trinacris* (Testudines, Emydidae) influenced by seasons and invasive aliens species. *Ecological Resources*, **32**(1): 71-80.

Ottanello, D., Oneto, F., Vignone, M., Rizzo, A., Salvidio, S. (2018): Diet of a restocked population of the European pond turtle *Emys orbicularis* in NW Italy. *Acta Herpetologica*, **13**: 89-93.

Parent, G.-H. (1968) : Contribution à la connaissance du peuplement herpétologique de la Belgique. Note 4. La question controversée de l'indigénat de la cistude d'Europe, *Emys orbicularis* (Linné), en Lorrain, au Benelux et dans les territoires adjoints. *Bulletin de l'Institut royal des Sciences naturelles de Belgique*, **44**: 29.

Paterson, J.E., Steinberg, B.D., Litzgus, J.D. (2013): Not just any old pile of dirt: evaluating the use of artificial nesting mounds as conservation tools for freshwater turtles. *Fauna & Flora International, Oryx*, **47**(4): 607-615.

Pittet, M. (2017): Impact of global warming on the distribution and dispersal of reptiles in Western Swiss Alps. Master Thesis. Master in Behaviour, Evolution, Conservation (BEC), Department of Ecology & Evolution, University of Lausanne, Switzerland.

Raemy, M. (2010): Hybridization and genetics of the Swiss *Emys orbicularis* populations.

Master Thesis, University of Neuchâtel, Switzerland. 51p.

Rhodin, A.G.J., Stanford, C.B., van Dijk, P.P., Eisemberg, C., Luiselli, L., Mittermeier, R.A.,

Hudson, R., Horne, B.D., Goode, E.V., Kuchling, G., et al. (2018). Global conservation status of turtles and tortoises (Order Testudines). *Chelonian Conservation Biology*, **17**: 135–161.

Roll U., et al. (2017): The global distribution of tetrapod's reveals a need for targeted reptile conservation. *Nature Ecology and Evolution*, **1**:1677-1682.

Rollinat, R. (1934): La vie des reptiles de la France centrale. Delagrave, Paris, 337p.

Schneider, P. (2000): Possibilities offered by the woerr site in the framework of the plan to reintroduce the European pond terrapin (*Emys orbicularis*, L.) in the Rhine plain. *Chelonii*, **2**: 208-111.

Segurado, P., Araújo, A.P.R. (2004): Coexistence of *Emys orbicularis* and *Mauremys leprosa* in Portugal at two spatial scales: is there evidence of spatial segregation? *Biologia, Bratislava*, **59**(14): 61-72.

Siegel, R.A., Dodd, J.R. (2000): Manipulation of populations for conservation. In Turtle Conservation, Klemens, M.W. (ed.), Smithsonian Institution, pp 218-238.

Stanford, C.B., Iverson, J.B., Rhodin, A.G.J., van Dijk, P.P., Mittermeier, R.A., Kuchling, G., Berry, K.H., et al. (2020): Turtle and tortoise are in trouble. *Current Biology*, **30**: R721-R735.

Tortoise and Freshwater Turtle Specialist Group. (1996): *Emys orbicularis*. The IUCN Red List of Threatened Species 1996.

Turtle Conservation Fund (2002): A global action plan for conservation of tortoises and freshwater turtles. Strategy and funding prospectus 2002-2007. Washington D.C.: *Conservation International and Chelonian Research Foundation*, 30pp.

Turtle Conservation Coalition [Stanford, C.B, Rhodin, A.G.J., van Dijk, P.P., Horne, B.D., Black, T., Goode, E.V., Hudson, R., Mittermeier, R.A., Currylow, A., Eisemberg, *et al.*] (2018). Turtle in Trouble: The top 25+ most endangered tortoises and freshwater turtle - 2018 (Ojai, CA: IUCN SSC Tortoise and Freshwater Turtle Specialist Group, Turtle Conservancy, Turtle Survival Alliance, Turtle Conservation Fund, Chelonian Research Foundation, Conservation International, Wildlife Conservation Society, and Global Wildlife Conservation), p. 80.

Turtle Extinctions Working Group [Rhodin, A.G.J., Thomson, S., Georgalis, G.L., Karl, H.-V., Danilov, I.G., Takahashi, A., de la Fuente, M.S., Bourque, J.R., Delfino, M., Bour, R., *et al.*] (2015): Turtles and tortoises of the world during the rise and global spread of humanity: first checklist and review of extinct Pleistocene and Holocene chelonians. Chelonian Research Monographs, **5**: 000e.1-000e66.

Turtle Taxonomy Working Group [Rhodin A.G.J., Iverson, J.B., Bour, R., Fritz, U., Georges, A., Shaffer, H.B., van Dijk, P.P.]. (2017): Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status, 8th ed. Monograph 7 of Rhodin, A.G.J., Iverson, J.B., van Dijk, P.P., Saumure, R.A., Buhlmann, K.A., Pritchard, P.C.H., Mittermeier, R.A., eds. Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs, **7**: 1-292.

Vlachos, E., Delfino, M. (2016): Food for thought: Sub-fossil and fossil chelonian remains from Franchthi Cave and Megalopolis confirm a glacial refuge for *Emys orbicularis* in Peloponnesus (S. Greece). *Quaternary Science Reviews*, **150**: 158-171.

Van Dijk, P.P., Sindaco, R. (2004): *Emys orbicularis*. The IUCN Red List of Threatened Species 2004.

Van Vuuren, D.P., Edmonds, J., Kainuma, M., Riahi, K., Thomson, A., Hibbard, K., Hurtt, G.C., Kram, T., Lamarque, J.F., Masui, T., Meinshausen, M., Nakicenovic, N., Smith, S.J., Rose, S.K. (2011): The representative concentration pathways: an overview. *Climatic Change*, **109**, 5.

Charlotte Ducotterd



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Goals

Work for an association of protection and conservation of the environment and species. Participate to conservation programs and reintroduction programs of endangered species in Switzerland and/or aboard.



Education

PhD in Life Sciences

2016 – 2020

University of Lausanne – Ecology of the European pond turtle (*Emys orbicularis*) in Switzerland – Diet analysis using metabacoding, species distribution model (EU and CH) under climate change.

Animal experimentation and wild animals

October 2016

University of Lausanne and La Maison de la Rivière – Course to become an animal experimenter: Module 1.

Master in Biology specialization Parasitology and Ethology

2012 – 2015

University of Neuchâtel, Switzerland – Master project on the European pond turtle (*Emys orbicularis*), reintroduction and monitoring by telemetry of this endangered species in the natural reserve of La Vieille Thielle in the canton of Neuchâtel, Switzerland.

Bachelor in Biology

2008 - 2011

University of Neuchâtel, Switzerland.

Expertise

Info fauna / karch

September 2020 - Today

- Coordination Center for the Protection of Reptiles and Amphibians in Switzerland, based in Neuchâtel, Switzerland.
- Tasks: Management of invasive turtle species in Switzerland, detection, capture method.

Turtle Sanctuary and Conservation Center

September 2019 - Today

- NGO based in Paris, France.
- Tasks: Wildlife Ecologist. Development and application of field studies and scientific research in Asian country, especially Vietnam.

HEPIA, HES-SO

August 2019 – February 2021

- Research assistant at the University of Applied Sciences and Arts Western Switzerland, Jussy, Geneva, Switzerland.

Le Village des Tortues

May 2019

- Mandated by Turtle-Sanctuary-Conservation Center for a rescue mission to the Village des Tortues of Ifaty, Madagascar after the record seizure by Malagasy authorities of 11'000 radiated turtles (*Astrochelys radiata*).
- Tasks: Quarantine animals, veterinary care (hydration, antibiotics, etc.), construction of 800m² of new enclosures.

Oceans Research

October 2015 – November 2015

- Research internship on wild life at Oceans Research, Mossel Bay, South Africa.



- Tasks: Learn how to safely approach animals, while being conscious of their comfort zones. Tracking of free roaming cheetah with telemetry device to monitor their position and determine if a kill was made. Capture small mammals (with setting live traps) to establish habitat preference. Studying giraffes and rhinos behaviour in the presence or absence of free roaming predator. Identify and estimate bird abundances visually using different survey techniques (i.e. point surveys, transect survey etc.).

La Maison de la Rivière

March 2015 - August 2015

- Environmental education at La Maison de la Rivière of Tolochenaz, Vaud, Switzerland.
- Tasks: Animations for groups of children and adults; promotional work and organization of various events. Research work on whitefish scales (scalimetry) and Alizarin-marked otoliths to determine if restocking in the lake of Joux was effective or not.

Guyana Marine Turtle Conservation Society

April 2013

- Volunteer to the conservation of Sea turtle programs on Shell Beach, Guyana.
- Tasks: Night patrol on the beach to count and protect nests of Leatherback turtles (*Dermochelys coriacea*) and Loggerhead turtles (*Caretta caretta*). Tagging and measuring individuals.

“Direction générale de la nature et du paysage” (DGNP)

April - September 2012

- Internship at the DGNP in the canton of Geneva, Switzerland, which aims to the protection and conservation of the fauna and flora in the canton.
- Tasks: Monitoring by telemetry of the reintroduced European pond turtles (*Emys orbicularis*), released in 2010 in the natural reserve of Jussy/Près-Bordon in the canton of Geneva, Switzerland.

Chelonian Research Institute

Mars - April 2011

- Internship at the Chelonian Research Institute, Oviedo, Florida, USA, under the direction of Dr. Peter C. H. Pritchard.
- Tasks: Reconstruction of a Galapagos tortoise skeleton, classification of turtle and tortoise's bones and shells.

Association of “La Grande Cariçaie”

June - August 2010

- Internship at the association of “La Grande Cariçaie” in Champ-Pittet, Switzerland.
- Tasks: Monitoring of populations of the Dusky Large Blue (*Phengaris nausithous*), Dryad (*Minois dryas*) and European Fire Ants (*Myrmica rubra*).

***Emys* Project**

August 2001 - Today

- The *Emys* project aims to protect and reintroduce the European pond turtle (*Emys orbicularis*) in Switzerland.
- Tasks: Monitoring of relict populations in Switzerland. Between 2010 and today, reintroduction in the natural reserve of Jussy/Près-Bordon, Les Teppes de Verbois (Geneva) and in the natural reserve of La Vieille Thielle (Neuchâtel). Monitoring of reintroduced animals. Scientific coordination.

Competences

Languages

- French: Mother tongue
- English: Excellent knowledge – First Certificate in English (obtained in December 2012 at Kaplan College, Cairns, Queensland, Australia)
- German: Basic knowledge

Computer skills

- Office: MS Word, Excel, PowerPoint
- Statistical analysis: R, RStudio, SIG



Laboratory skills

- Molecular biology: DNA extraction, PCR, sequencing library, next generation sequencing, metabarcoding

Interests

- Diving (Advanced Open Water Diver), travel, nature, drawing, yoga and reading
- Volunteer at the Emys Center of Chavornay (Association Protection et Récupération des Tortues), Switzerland

Conferences

Cercles de Sciences Nyon - La Côte March 2016

- UICN, Gland, Suisse
- Title: « Dans la carapace de notre tortue Suisse »

Seminar University of Basel December 2017

- Title: « Diet of the European pond turtle »

EAZA meeting April 2018

- European Association of Zoos and Aquariums, Aquatius, Lausanne, Suisse
- Title: « In the Shell of the Swiss turtle (*Emys orbicularis*) »

Congress de la FFEPT Mai 2018

- Fédération Francophone pour l'Elevage et la Protection des tortues, Bessières, France
- Title: « Conservation de la cistude d'Europe en Suisse »

Natural History Museum September 2018

- Temporary exhibition on turtles and tortoises, Porrentruy, Suisse
- Title: « Dans la carapace de notre tortue Suisse »

European Studbook Foundation June 2019

- Links between *in situ* and *ex situ* measures
- Title: « Reintroduction program in Switzerland for the European pond turtle (*Emys orbicularis*) »

SEFS11 June 2019

- Symposium of European Freshwater Sciences 11, Zagreb, Croatia
- Title: « Metabarcoding as a tool to determine feeding behavior - is the European pond turtle a threat for other endangered species »

SEH 2019 2-8th September 2019

- 20th European Congress of Herpetology, Milano, Italy
- Title: « Metabarcoding as a tool to determine feeding behavior - Is the European pond turtle a threat for other endangered species»

47^{me} congrès de la SHF 10-12th October 2019

- Congress of Hepetological Society of France, Saint-Girons, France
- Title: « Le métabarcodage en tant qu'outil de détermination du régime alimentaire - Est-ce que la cistude d'Europe est une menace pour les autres espèces en danger ? »



TSA meeting

15-16th November 2019

- Turtle Survival Alliance meeting, Chelmsfield, United Kingdom
- Title: « Reintroduction program in Switzerland for the European pond turtle (*Emys orbicularis*, L. 1758) »

WCH9

5-10th January 2020

- 9th World Congress of Herpetology, Dunedin, New Zealand
- Title: « Metabarcoding as a tool to determine feeding behavior - is the European pond turtle a threat for other endangered species? »

TSA online meeting

6th August-24th September 2020

- 18th Annual Symposium on the Conservation and Biology of Tortoise and Freshwater Turtle, Turtle Survival Alliance USA, online presentation due to the Covid-19 pandemic
- Oral presentation: « The feeding behaviour of the European pond turtle is not a threat for other endangered species »
- Poster presentation: « Field survey uncover unexpected forest turtle diversity in hill forests in northern Vietnam»

Publications

Journal of Nature Conservation

June 2020

- A new locality of presence of the world's rarest turtle (*Rafetus swinhoei*) gives new hope for its survival
- Le Duc, O., Pham Van, T., Zuklin, T., Bordes, C., Leprince, B., Ducotterd, C., Quang Luu, V., Luiselli, L.

Global Ecology and Conservation

September 2020

- The feeding behavior of the European pond turtle (*Emys orbicularis*, L. 1758) is not a threat for other endangered species
- Ducotterd, C., Crovadore, J., Lefort, F., Guisan, A., Ursenbacher, S., Rubin, J.-F.

Molecular Ecology Resources

October 2020

- A powerful long metabarcoding method for the determination of complex diets form faecal analysis of the European pond turtle (*Emys orbicularis*, L. 1758)
- Ducotterd, C., Crovadore, J., Lefort, F., Rubin, J.-F., Ursenbacher, S.

Biodiversity and Conservation

December 2020

- Unexpected high forest turtle diversity in hill forests in northern Vietnam
- Pham Van, T., Le Duc, Leprince, B., O., Bordes, C., Zuklin, T., Ducotterd, C., Quang Luu, V., Van Oanh, L., Nguyen Thi Tam, A., Fa, J.E., Luiselli, L.

Oryx

March 2021

- Female wanted: prioritization of sites with potential wild survival of the world's rarest turtle (*Rafetus swinhoei*)
- Pham Van, T., Le Duc, O., Bordes, C., Leprince, B., Ducotterd, C., Zuklin, T., Quang Luu, V., Ha Dinh, D., Luiselli, L.