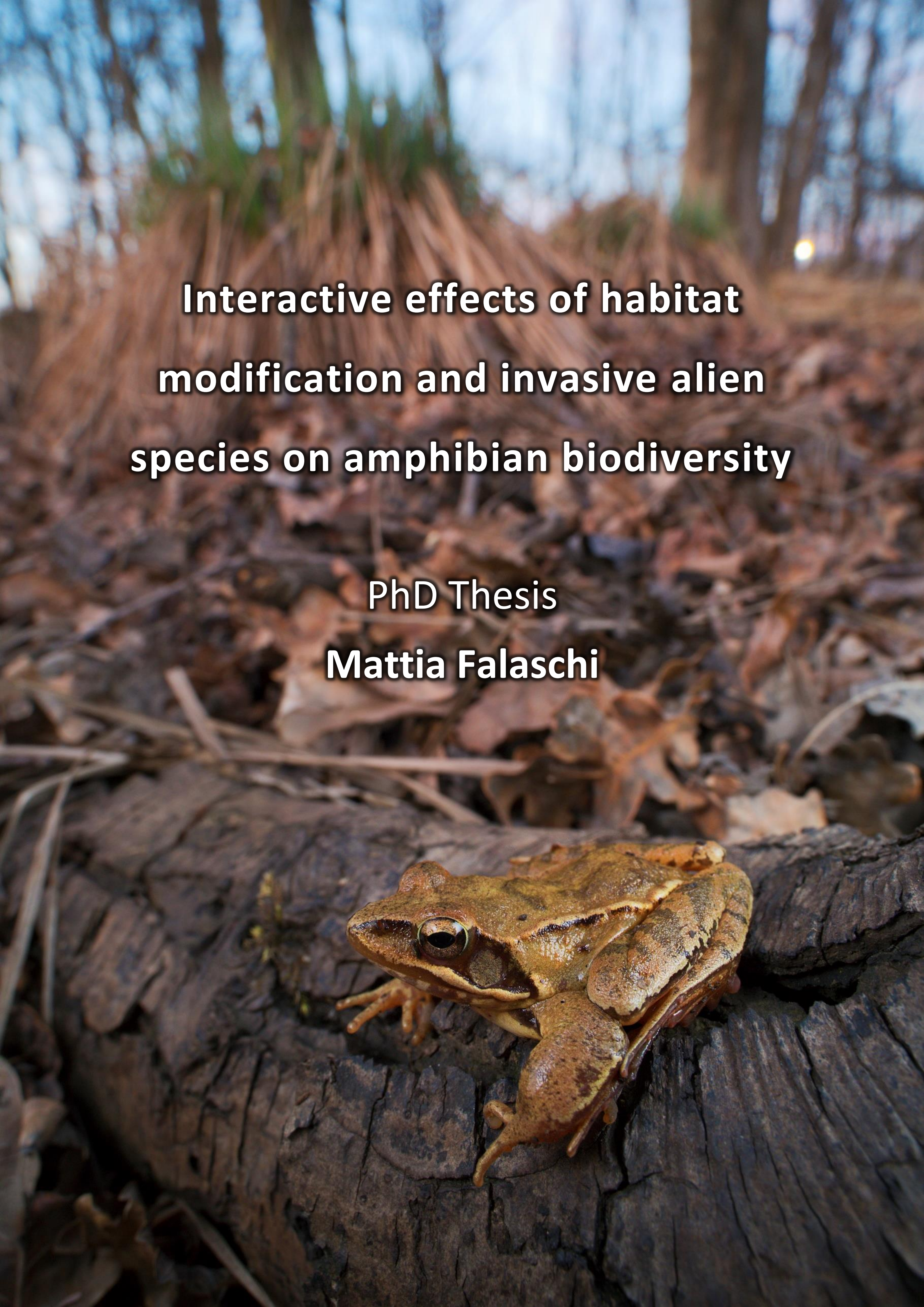

Interactive effects of habitat modification and invasive alien species on amphibian biodiversity

PhD Thesis

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UNIVERSITÀ DEGLI STUDI DI MILANO

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Front cover: an agile frog (*Rana dalmatina*) at
one of the study sites of Chapters 4 and 5.

CONTENTS

ABSTRACT

The biodiversity of our planet is facing a severe crisis. Habitat alteration, climate change, the spread of alien species, and overexploitation are major drivers of biodiversity loss. These global change drivers often act jointly or even synergistically on population declines. To identify priorities for conservation, it is thus essential to compare the relative impact of different threats on biodiversity. Amphibians are the vertebrates showing both the largest proportion of threatened species and the highest rate of decline. Out of the ~6,800 amphibian species assessed by the IUCN, more than 4,000 species are threatened by at least one stressor, and more than 2,000 by at least two stressors. Habitat modification (alteration and/or destruction) and alien species are the two factors threatening the largest number of amphibian species. For this reason, this thesis investigates the role of habitat modification and invasive alien species in determining the temporal dynamics of amphibian populations. The role of these stressors is examined at different spatial scales and jointly with other factors such as microhabitat, landscape, or climatic variables.

The first study (Chapter 2) is a continental-scale analysis, which evaluates the combined effects of alien species, habitat availability, habitat changes, and climate change in determining population trends of European amphibian (and some reptile) species over the last 45 years. The results showed that populations declined more often in areas with a high number of alien species and where climate change has caused a loss of climatic suitability. The effect of habitat availability was not relevant when averaged across species; however, when excluding the two commonest species, habitat loss was the main correlate of negative population trends for the remaining species. Furthermore, I observed a strong interaction between habitat availability and the richness of alien species, which indicated that the negative impact of alien species was particularly strong for populations living in landscapes with less suitable habitat

Then, the thesis focuses on a specific system: the invasion of the red swamp crayfish *Procambarus clarkii* in Italy, and its effect on the population dynamics of amphibians. The second study (Chapter 3) presents the updated distribution of the red swamp crayfish in Italy. Thirty years after its first record in Italy in 1989, the red swamp crayfish has invaded 80% of Italian provinces. The invasion is particularly extensive in central and northern Italy, where this invasive crayfish is present in 100% and 96% of provinces, respectively. Given the strong negative effects that the red swamp crayfish can exert on native amphibians, its widespread

distribution raises concern about the future status of conservation of the Italian batrachofauna.

Chapter 4 analyses site occupancy data of newts in northern Italy, in an area suffering the introduction of the invasive crayfish, in order to investigate the role of microhabitat, landscape change, and invasive species in determining the decline of two newt species through time. Initial occupancy of newts was negatively affected by landscape alteration (i.e. urban and agricultural cover) and by the presence of fish. Both species suffered a strong decline, with a net loss of site occupancy of 25%–36% along the study period. After the crayfish invasion, the main drivers of population dynamics sharply shifted, and occupancy changes were not determined by landscape or microhabitat alterations, as the strongest predictor of local extinctions was the colonization of wetlands by invasive crayfish.

The last study (Chapter 5) focuses on the effect of habitat characteristics and invasive crayfish in determining the temporal dynamics of the amphibian community in a large number of wetlands in northern Italy. Since connectivity among wetlands is fundamental for the dynamics of spatially structured populations, we considered two measures of connectivity acting on each focal wetland: incidence of the focal species, and incidence of invasive crayfish. Survival and colonization of local populations were jointly determined by factors acting at different scales. Survival probability was positively related to the area and the permanence of wetlands, while it showed a negative relationship with the occurrence of fish. Colonization probability was highest in semi-permanent wetlands and in sites with a high incidence of the focal species in nearby sites. Furthermore, the incidence of invasive crayfish emerged as one of the strongest drivers of colonization probability.

In this thesis, I showed that invasive alien species are a major driver of the decline of European amphibians. The negative effect of invasive alien species acted differently across species and spatial scales. While the site-level impact was evident only for a subset of species, the landscape-level effect of invasive crayfish was ubiquitous across the whole amphibian community. Habitat modification showed a less evident effect, but invasive alien species and habitat modification can strongly interact: the negative effect of alien species is exacerbated in highly modified landscapes. It is thus essential to directly prevent and manage the spread of alien species, while simultaneously preserving natural landscapes. This can help in mitigating the negative effects of invasives, even when habitat alteration is not the main threatening factor.

CHAPTER 1 | INTRODUCTION

1 | THE BIODIVERSITY CRISIS

The biodiversity of our planet is facing a severe crisis (IPBES 2018). The rate of species extinction over the last century was so high that we can assert to be experiencing the sixth mass extinction (Ceballos et al. 2015). Several human-induced changes are pushing Earth's ecosystems beyond their limits (Sala et al. 2000; Ripple et al. 2017). Among these drivers of change, we find habitat alteration, climate change, the spread of alien species, and overexploitation (Sala et al. 2000; Ducatez & Shine 2017). Global change drivers often act jointly or even synergistically on population declines (Didham et al. 2007; Brook et al. 2008). For instance, terrestrial vertebrates considered threatened by the International Union for Conservation of Nature (IUCN) are affected on average by more than one threat (Ducatez & Shine 2017). In order to identify priorities for conservation, it is thus essential to compare the relative impact of different threats on biodiversity.

The conservation status of the world's biodiversity keeps deteriorating and, within vertebrates, amphibians are the group showing both the largest proportion of threatened species and the highest rate of decline (Hoffmann et al. 2010). Nearly 41% of amphibian species are listed as threatened by the IUCN, compared to 26% of mammals, 21% of reptiles, and 13% of birds (https://www.iucnredlist.org/statistics; accessed on 27 October 2020). Out of the ~6,800 amphibian species assessed by the IUCN, more than 4,000 species are menaced by at least one stressor, and more than 2,000 by at least two stressors (Green et al. 2020). Habitat modification (alteration and/or destruction) and alien species are the two factors threatening the largest number of amphibian species (Stuart et al. 2004, 2008; Ducatez & Shine 2017; Green et al. 2020). For this reason, this thesis investigates the role of habitat modification and invasive alien species in determining the temporal dynamics of amphibian populations.

2 | HABITAT MODIFICATION

Habitat modification (i.e. the destruction or alteration of natural habitats) is by far the major driver of ongoing global biodiversity loss (Sala et al. 2000; Foley et al. 2005). Among threatened vertebrates, the vast majority of species is menaced by habitat modifications, including ~60% of reptiles,

~80% of birds and mammals, and ~85% of amphibians (Ducatez & Shine 2017).

Human activities have deeply modified the Earth's surface for thousands of years (Goldewijk et al. 2011). The conversion of primary vegetation to other land-use types can cause a reduction or even the complete disappearance of some habitats, and this often has heavy detrimental effects on local biodiversity and consequently on essential ecosystem services (Cardinale et al. 2012; Dirzo et al. 2014; Newbold et al. 2015). For example, mountaintop removal mining can have severe negative impacts on the population dynamics of stream salamanders by reducing both abundance and vital rates (Price et al. 2018). The composition of the landscape matrix surrounding breeding sites is an important parameter for pond-breeding amphibians. Cultivated or urban areas can negatively affect survival during seasonal migrations, by increasing the risk of mortality due to dehydration or predation, leading to a higher probability of local or even regional extinction (Joly et al. 2001; Cayuela et al. 2020).

The effect of habitat modification can vary both among species and across space. Some species are more sensitive than others because of different ecological traits such as body size, heat tolerance, or diet (Newbold et al. 2013; Nowakowski et al. 2018). Furthermore, habitat modification in the past was not constant across space or time. Land conversion from primary vegetation to cropland or pastures has ancient roots in Eurasia and Africa, while it is more recent in the Americas (Goldewijk et al. 2011). When humans colonize a natural environment, natural ecosystems are progressively substituted with land uses which provide immediate benefits to the resident populations (Foley et al. 2005). For instance, European forests have undergone large reductions for hundreds of years, with heavy impacts on biodiversity (Foley et al. 2005; Goldewijk et al. 2011; Newbold et al. 2015). However, land abandonment over the last decades resulted in some areas regaining forest cover (Falcucci et al. 2007; Hansen et al. 2013).

Future projections of land-use change suggest that without specific interventions, anthropogenic habitat modification will continue in the future, with catastrophic consequences for biodiversity (Stehfest et al. 2014; Molotoks et al. 2018). While in regions such as Europe or northern Asia, amphibians are expected to be less affected by habitat modification in the future, land-use is likely to be particularly severe in tropical regions.

(Hof et al. 2011). Here, amphibian biodiversity is usually high, and tropical amphibians are expected to experience an extensive aggravation of the conservation status because of habitat modification (Powers & Jetz 2019). Additionally, synergistic effects of climate change could exacerbate the detrimental effects of habitat modification (Powers & Jetz 2019).

3 | INVASIVE ALIEN SPECIES [1]

Invasive alien species (IAS) are a major threat to biodiversity. Of about 800 animal extinctions that have been recorded since 1,500, IAS have been implicated in 33% of them (Blackburn et al. 2019). For amphibians, IAS have been the cause of about one third of extinctions, and ca. 16% of extant species currently are threatened by IAS (Stuart et al. 2008; Blackburn et al. 2019). Nevertheless, biological invasions are a complex process (see Box 1 for definitions and conceptual framework), and the impact of IAS on biodiversity is highly heterogeneous both among habitats and geographic areas. Amphibians living on islands and in freshwater are disproportionately affected by invasive species (Stuart et al. 2008; Strayer 2010; Spatz et al. 2017).

IAS can affect amphibians through a broad range of pathways (Fig. 1; Bucciarelli et al. 2014; Nunes et al. 2019) including predation (Kats & Ferrer 2003), competition (Richter-Boix et al. 2013), hybridization (Dufresnes et al. 2016), habitat alteration (Matsuzaki et al. 2009), and the spread of disease (Miaud et al. 2016).

3.1 | Predation

Amphibians with aquatic life-history stages are particularly sensitive to the introduction of alien predators. This is because many amphibians breed in freshwater ecosystems such as isolated ponds or headwater streams where large predators normally are scarce (Cox & Lima 2006). Even though several amphibian species have evolved mechanisms to co-exist with some predacious fishes (Van Buskirk 2003), invasive predators can drive local populations to decline, or even extinction, because they directly reduce the abundance of eggs, larvae, or adults (Kats & Ferrer 2003; Bucciarelli et al. 2014; Nunes et al. 2019). Fish are probably the most frequently introduced large predators in freshwaters and have caused massive loss of amphibian breeding sites in

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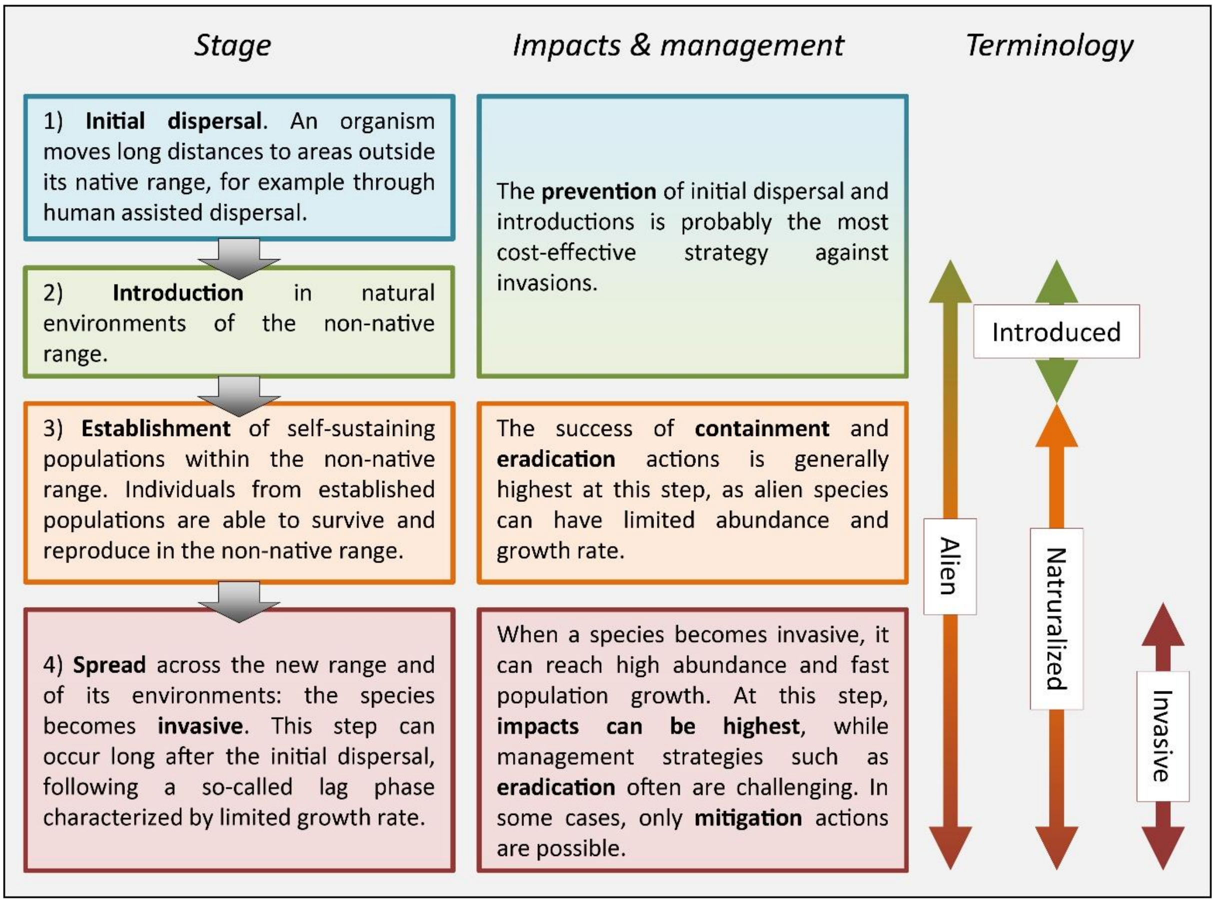
Box 1 - From introduced to invasive species: How to define a multi-step process.

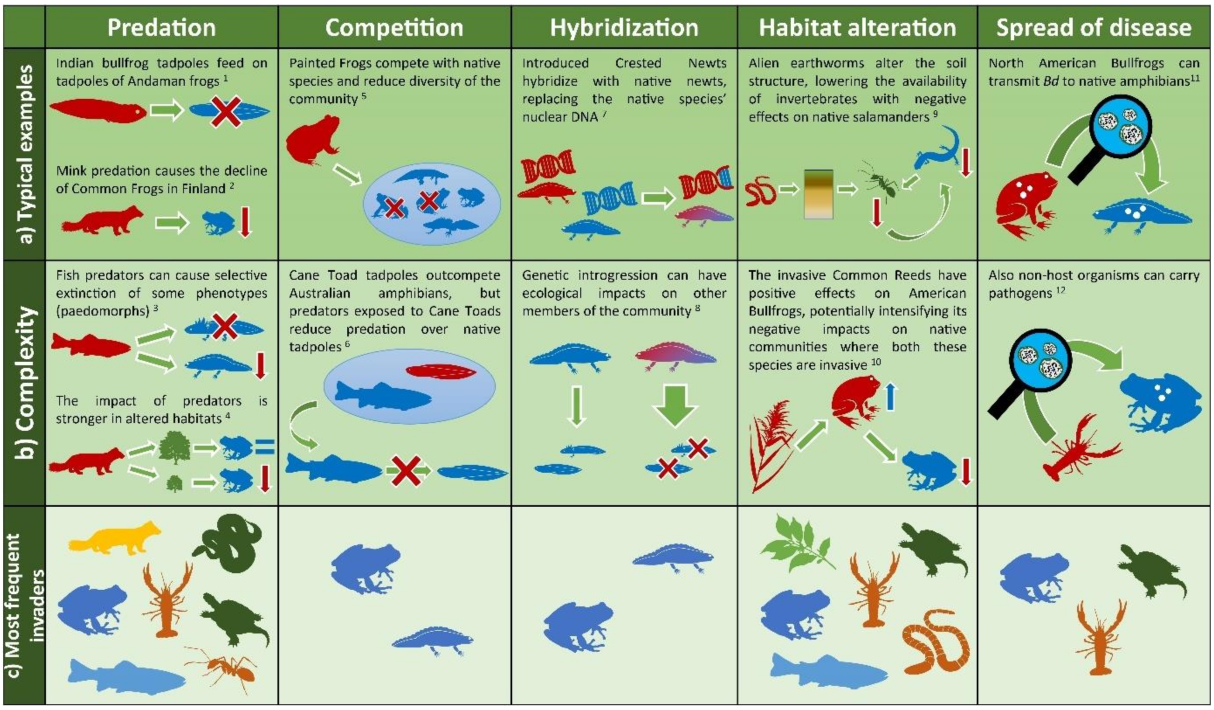
Describing invasions as a multi-step processes, comprising four major phases, allows a better understanding of invasions, provides a unified terminology, and helps management, given that different approaches can have different efficiency and feasibility across stages (modified after Blackburn et al. 2011). In this review, we generally focus on invasive species, i.e. the category causing the strongest impacts on biodiversity. Nevertheless, when discussing conservation strategies, we also consider the early stages, due to the efficiency of management actions against them (Puth & Post 2005).



Stage	*Impacts & management*
1) **Initial dispersal**. An organism moves long distances to areas outside its native range, for example through human assisted dispersal.	The **prevention** of initial dispersal and introductions is probably the most cost-effective strategy against invasions.
2) **Introduction** in natural environments of the non-native range.	
3) **Establishment** of self-sustaining populations within the non-native range. Individuals from established populations are able to survive and reproduce in the non-native range.	The success of **containment** and **eradication** actions is generally highest at this step, as alien species can have limited abundance and growth rate.
4) **Spread** across the new range and of its environments: the species becomes **invasive**. This step can occur long after the initial dispersal, following a so-called lag phase characterized by limited growth rate.	When a species becomes invasive, it can reach high abundance and fast population growth. At this step, **impacts can be highest**, while management strategies such as **eradication** often are challenging. In some cases, only **mitigation** actions are possible.

all the continents (e.g. Knapp & Matthews 2000; Knapp 2005; Tiberti & Von Hardenberg 2012). Large fishes such as trout as well as small-sized fishes are efficient amphibian predators (Remon et al. 2016; Miró et al. 2018). Besides fishes, many other taxa can exert heavy predation pressure. For instance, carnivorous tadpoles of Indian Bullfrogs, *Hoplobatrachus tigerinus*, rapidly prey upon native tadpoles, hampering the survival of larvae of frogs endemic to the Andaman archipelago (Mohanty & Measey 2019).

The effects of alien predators can go well beyond simple declines in abundance at invaded sites. When predators invade a large number of sites, they can cause declines over broad regional or national scales. For example,



	Predation	Competition	Hybridization	Habitat alteration	Spread of disease
a) Typical examples	Indian bullfrog tadpoles feed on tadpoles of Andaman frogs [1] Mink predation causes the decline of Common Frogs in Finland [2]	Painted Frogs compete with native species and reduce diversity of the community [5]	Introduced Crested Newts hybridize with native newts, replacing the native species' nuclear DNA [7]	Alien earthworms alter the soil structure, lowering the availability of invertebrates with negative effects on native salamanders [9]	North American Bullfrogs can transmit *Bd* to native amphibians[11]
b) Complexity	Fish predators can cause selective extinction of some phenotypes (paedomorphs) [3] The impact of predators is stronger in altered habitats [4]	Cane Toad tadpoles outcompete Australian amphibians, but predators exposed to Cane Toads reduce predation over native tadpoles [6]	Genetic introgression can have ecological impacts on other members of the community [8]	The invasive Common Reeds have positive effects on American Bullfrogs, potentially intensifying its negative impacts on native communities where both these species are invasive [10]	Also non-host organisms can carry pathogens [12]
c) Most frequent invaders					

FIGURE 1 The main processes through which invasive species affect amphibians. (a) Examples of direct impacts. (b) Examples of complex impacts, often mediated via interactive effects. (c) Examples of the most frequent taxonomic groups involved in each process. Native species are depicted in blue. Invasive species are depicted in red. Drawings by Mattia Falaschi, Gentile Francesco Ficetola, B. Comix, and N. Sinegina and obtained from http://www.supercoloring.com/ under a Creative Commons 4.0 License. Key references: 1) Mohanty & Measey 2019; 2) Ahola et al. 2006; 3) Denoël et al. 2019; 4) Salo et al. 2010; 5) Richter-Boix et al. 2013; 6) Nelson et al. 2010; 7) Dufresnes et al. 2016; 8) Ryan et al. 2009; 9) Ransom 2017; 10) Rogalski & Skelly 2012; 11) Miaud et al. 2016; 12) Brannelly et al. 2015.

many amphibians live in networks of spatially structured subpopulations (e.g. metapopulations) and invasive predators often may cause a loss of fitness at the invaded sites (Ficetola et al. 2011). This can lead to local declines but also to reductions in the number of juvenile amphibians that may disperse to nearby populations. This will result in negative effects on the long-term dynamics of the whole metapopulation network, and a negative impact at a regional scale even in non-invaded wetlands (Manenti et al. 2020). Furthermore, alien predators do not affect just species occurrence and abundance, they can also influence intra-specific variation. Paedomorphosis is an example of intra-specific variation, in which metamorphosing individuals coexist with fully aquatic, paedomorphic conspecifics that do not metamorphose, which has important consequences for adaptation and evolution of the species (Denoël et al. 2005). Fish introductions were the main determinant of extirpation of paedomorphs of two newt species (*Ichthyosaura alpestris* and *Lissotriton graecus*) in Montenegro (Denoël et al. 2019). The abundance declined at a much faster rate among paedomorphic populations than among metamorphic populations. The paedomorphic populations have declined by over 80% in less than 70 years, whereas metamorphic newts have lost ca. 50% of populations during the same period.

3.2 | Competition

When an invader's ecological niche overlaps that of a native species, the resulting competition can lead to the native species' decline or even extirpation (Mooney & Cleland 2001). Many studies on interspecific competition have focused on interaction between native and alien amphibians. For example, Painted Frogs, *Discoglossus pictus*, are invasive in Spain, where they have the potential to reduce fitness in native Spanish toads and modify the composition of native anuran communities because its larvae can out-compete larvae of the native species (Richter-Boix et al. 2013). Native toads now avoid laying eggs in ponds where Painted Frog larvae are present, producing a complex pattern of species co-occurrence at the landscape scale due to the interaction between competition and breeding preferences (Richter-Boix et al. 2013; Pujol-Buxó et al. 2019).

The broader the ecological niche of an invasive species, the more likely it will compete with native species. American Bullfrogs, *Lithobates* (*Aquarana*) *catesbeiana*, are typical of IAS with broad ecological niches and are among the most problematic of invasive amphibians. American Bullfrogs, which are generalist predators and have a broad climatic tolerance, strongly impact many native amphibians where they have been introduced (Ficetola et al. 2007b; D'Amore 2012; Bissattini et al. 2019). Because of their large size and voracious behavior, American Bullfrog tadpoles and adults often become the dominant amphibian competitors in freshwater communities (D'Amore 2012). As they may also be vectors of diseases, American Bullfrogs may alter therefore environmental processes in complex ways (D'Amore 2012; Measey et al. 2016).

3.3 | Hybridization

Hybridization of native species with alien species can lead to loss of fitness in the native taxa and, in some cases, to extirpation (Mooney & Cleland 2001). For example, Italian Crested Newts, *Triturus carnifex*, which were introduced in Switzerland at the beginning of the 20th century, hybridize with native Great Crested Newts, *T. cristatus*. In places where *T. carnifex* was introduced, newt populations showed a high rate of genetic introgression, sometimes leading to the complete elimination of pure *T. cristatus* (Dufresnes et al. 2016). Via hybridization, natural selection may favor the rapid spread of some genes of an invasive lineage across the range of native species, resulting in genetic pollution that may accelerate the replacement of native lineages (Fitzpatrick et al. 2010). This form of genetic introgression may thus have indirect effects on other components of the invaded ecological community. Compared to native Tiger Salamanders, introgressed Tiger Salamanders will drastically decrease recruitment of native amphibians due to their higher predation rates (Ryan et al. 2009).

Hybridization with invasive species is particularly problematic among the hybridogenetic European water frogs of the genus *Pelophylax.* In several areas of Central and Western Europe, native Pool Frogs, *P. lessonae,* and Edible Frogs, *P. esculentus,* naturally form an L–E hybridogenetic system. *Pelophylax esculentus* is a klepton between Marsh Frogs, *P. ridibundus,* and Pool Frogs, *P. lessonae*, that eliminates the *lessonae* genome during gametogenesis and clonally transmits the *ridibundus* genome (Vorburger & Reyer 2003; Holsbeek & Jooris 2010). *Pelophlyax ridibundus* frogs native to Eastern Europe, however, are commonly traded for human consumption throughout Europe. In Eastern Europe, *P. ridibundus,* and *P. esculentus* form an R–E hybridogenetic system whereby the *ridibundus* genome in *P. esculentus* is eliminated during gametogenesis and the *lessonae* genome is clonally transmitted. When introduced to an L–E system, R–E *P. ridibundus* mate with both native frogs, producing *P. esculentus* offspring with *P. lessonae*, and *P. ridibundus* offspring with *P. esculentus*. Thus, in several areas of Europe, invasive *P. ridibundus* are rapidly spreading at the expense of both native taxa (Vorburger & Reyer 2003; Holsbeek & Jooris 2010). The situation is further complicated because multiple *Pelophylax* species are actually traded throughout Europe. Because the hybridization with *P. ridibundus* can produce sterile or fertile hybrids depending on geographic origin, morphological identification of species is very difficult (Vorburger & Reyer 2003; Holsbeek & Jooris 2010; Quilodrán et al.

2015). Management of this situation is a major challenge because of the risk of rapid elimination of the native species (Quilodrán et al. 2018).

3.4 | Spread of Diseases

Hundreds of amphibian species are threatened by emerging infectious diseases, which are often spread by IAS (Stuart et al. 2008; Martel et al. 2014; Scheele et al. 2019). Worldwide, over 500 amphibian species have declined because of chytridiomycosis, the disease caused by two chytrid fungi: *Batrachochytrium dendrobatidis* and *B. salamandrivorans* (Fisher et al. 2009; Scheele et al. 2019). Invasive amphibians have been implicated as vectors of the chytrid pathogens and some may even show resistance to the disease (Garner et al. 2006). American Bullfrogs and African Clawed Frogs, *Xenopus laevis*, which can be resistant to chytridiomycosis, are thought to be able to transmit the pathogens to native amphibians (Miaud et al. 2016), although crayfish (Brannelly et al. 2015) and mosquitoes (Gould et al. 2019) could also be vectors. Chytrids may have spread in Europe via infected amphibians in the commercial pet trade, possibly leading to dramatic declines in some populations of European salamanders (Martel et al. 2014; Fitzpatrick et al. 2018). Given the high impact that novel diseases may pose to amphibians, any efforts to prevent the spread of pathogens by the monitoring and control of trade, and the application of strict sanitary protocols are worth considering (see also Bienentreu & Lesbarrères 2020).

3.5 | Habitat Alteration

IAS that become keystone species and ecosystem engineers can cause major habitat alterations, with strong impacts on native amphibians. Changes in habitat structure that alter base levels of wind and solar radiation and thereby modify the thermal landscape can have particularly strong impacts on ectothermic vertebrates (Watling et al. 2011; Garcia & Clusella-Trullas 2019). Some invasive plants are capable of severely modifying both terrestrial and freshwater habitats. For example, Amur Honeysuckles, *Lonicera maackii*, form a dense shrub layer in invaded forests that results in a decrease of species richness and produces shifts in amphibian community composition (Watling et al. 2011). The invasive earthworm, *Octolasion tyrtaeum*, which modifies the soil by reducing the organic layer, reduces the abundance of Red-Backed Salamanders, *Plethodon cinereus*

(Ransom 2017). Because the majority of amphibians spend their adult lifetimes in terrestrial environments, and many species, especially in tropical areas, are fully terrestrial, the impact of invasive terrestrial plants and other organisms on amphibians is probably underestimated (Nunes et al. 2019).

3.6 | Indirect and Context-Dependent Impacts of Invasive Species

It is increasingly evident that multiple biotic and abiotic factors often act in concert, with synergistic effects between IAS with other stressors such as habitat loss and climate change. Such indirect effects can account for a large part of biodiversity changes (Menge 1995; Didham et al. 2007). Joint and indirect effects are evident at multiple scales. In several cases, the negative effect of alien species is magnified by habitat loss and landscape alteration (Salo et al. 2010). In South Carolina, for example, forest harvesting increased the local abundance of invasive Fire Ants, *Solenopsis invicta*, leading to higher predation pressure on native salamanders (Todd et al. 2008). At a broader scale, negative effects of IAS on population trends on European amphibians and reptiles are stronger in landscapes providing less suitable habitat to native species (Falaschi et al. 2019).

Indirect biotic interactions are also frequent, and can both amplify or limit the impact of IAS (White et al. 2006; Nelson et al. 2010; Rogalski & Skelly 2012). For example, invasive Japanese Stilt Grasses, *Microstegium vimineum*, led to an increased abundance of native lycopsid spiders in Georgia, which resulted in increased predation on small arthropods, leading to diminished food resources for native American Toads, *Anaxyrus americanus*, and a decline in their abundance (DeVore & Maerz 2014). Such connections and chains of causality are important components of overall the impact of IAS on native populations (Brook et al. 2008; Bucciarelli et al. 2014).

4 | AIMS OF THE THESIS

This thesis investigates the joint role of invasive alien species and habitat modification in driving temporal changes in amphibian populations. The role of these stressors is examined at different spatial scales and taking into account other factors such as microhabitat or climatic variables.

The first study (Chapter 2) is a continental-scale analysis evaluating the effect of four candidate drivers in determining population trends from seventeen European amphibian (and reptile) species over the last 45 years. I investigated the joint effects of alien species, habitat availability, habitat change, and climate change in driving trends of population abundance over time, also testing for possible interactions among drivers

After this continental-scale analysis, the thesis focuses on a specific system: the invasion of the red swamp crayfish *Procambarus clarkii* in Italy, and its effect on population dynamics of amphibians. This invasive alien species can exert strong negative impacts on native amphibians (Cruz & Rebelo 2005; Cruz et al. 2008; Ficetola et al. 2011; Manenti et al. 2020). For this reason, the second study (Chapter 3) presents the updated distribution of the red swamp crayfish in Italy, thirty years after its first record in Italy.

The last two studies (Chapters 4 and 5) focus on the temporal dynamics of amphibian populations in northern Italy. Chapter 4 analyses 22 years of site occupancy data in 63 wetlands suffering the introduction of the invasive crayfish, in order to investigate the role of microhabitat, landscape change, and invasive species, in determining the decline of two newt species through time. In this study, I investigated whether the relative importance of the factors driving occupancy changed before and after the introduction of the invasive crayfish in the study area.

The last study (Chapter 5) focuses on the effect of habitat characteristics and invasive crayfish, in determining the temporal dynamics of the amphibian community in 202 wetlands in northern Italy. I assessed the relative importance of factors acting at different scales, from local to landscape-scale, in determining survival and colonization probability of local populations, across ten amphibian species. Since connectivity among wetlands is fundamental for the dynamics of spatially structured populations, I considered two measures of connectivity acting on each focal wetland: incidence of the focal species, and incidence of invasive crayfish. Given the high interspecific variability, results were summarized through meta-analysis, to evaluate which are the most influential factors for the whole amphibian community.

FIGURE 3 A green toad (*Bufotes viridis*) at one of the study sites of Chapter 5.

CHAPTER 2 | Continental-scale determinants of population trends in European amphibians and reptiles

Mattia Falaschi, Raoul Manenti, Wilfried Thuiller, Gentile Francesco Ficetola

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ABSTRACT

The continuous decline of biodiversity is determined by the complex and joint effects of multiple environmental drivers. Still, a large part of past global change studies reporting and explaining biodiversity trends have focused on a single driver. Therefore, we are often unable to attribute biodiversity changes to different drivers, since a multi-variable design is required to disentangle joint effects and interactions. In this work, we used a meta-regression within a Bayesian framework to analyze 843 time-series of population abundance from seventeen European amphibian and reptile species over the last 45 years. We investigated the relative effects of climate change, alien species, habitat availability, and habitat change in driving trends of population abundance over time, and evaluated how the importance of these factors differs across species. A large number of populations (54%) declined, but differences between species were strong, with some species showing positive trends. Populations declined more often in areas with a high number of alien species, and in areas where climate change has caused loss of suitability. Habitat features showed small variation over the last 25 years, with an average loss of suitable habitat of 0.1% / year per population. Still, a strong interaction between habitat availability and the richness of alien species indicated that the negative impact of alien species was particularly strong for populations living in landscapes with less suitable habitat. Furthermore, when excluding the two commonest species, habitat loss was the main correlate of negative population trends for the remaining species. By analyzing trends for multiple species across a broad spatial scale, we identify alien species, climate change, and habitat changes as the major drivers of European amphibian and reptile decline.

1 | INTRODUCTION

Biodiversity is declining at an unprecedented rate (Butchart et al. 2005; IPBES 2018). Understanding the main causes of these changes is a major endeavor for the scientific community, should we want to anticipate and mitigate future impacts. Climate change, land-use change, spread of alien species, atmospheric CO_2 increase, anthropogenic nitrogen deposition, and spread of disease are all drivers known to strongly influence the structure and distribution of biodiversity (Gallardo et al. 2015; Bateman et al. 2016; Tracewski et al. 2016). These drivers do not affect biodiversity independently, rather they act in synergistic or antagonistic ways. For instance, in a global study comprising multiple taxa, Mantyka-Pringle, Martin, & Rhodes (2012) found that the negative effects of habitat loss and fragmentation on species abundance and diversity were magnified in areas where average rainfall has decreased in the past. Multi-variable studies, taking into account more than one driver of global change, are thus essential to disentangle the relative importance of different threats (Didham et al. 2007). Nevertheless, a large part of past global change studies focused on one single driver, perhaps because it is difficult to retrieve standardized data across broad spatial extents, or because integrating multiple factors can result in overly complex models. In the last years, attention is growing toward the importance of disentangling the effects of multiple drivers (e.g. Campbell Grant et al., 2016; Northrup, Rivers, Yang, & Betts, 2019), because knowing the relative impact of different drivers on the different biodiversity facets is essential to identify conservation priorities and management strategies (Brook et al. 2008).

Global change drivers impact populations in multiple ways and can, for instance, impact morphology, breeding success, survival, and abundance (Menzel et al. 2006; Saino et al. 2011; Ficetola & Maiorano 2016; Ficetola et al. 2016). Trends of population abundance are connected to extinction risk and are commonly used to evaluate the conservation status of species, thus population trends are one of the key demographic parameters to assess the effects of global change drivers on biodiversity (IUCN 2012; Flesch et al. 2017). However, studies on population abundance are generally local, thus limiting the possibility of drawing broad-scale, generalizable inference. Quantitative analyses of the results of multiple studies (meta-regressions) can alleviate this issue, as they allow to summarize information from a broad range of sources. Meta-regressions showed excellent performance in the analysis of multiple demographic time series and helped to obtain general inference on patterns of global change (Hadfield & Nakagawa 2010; Bonardi et al. 2011; Gurevitch et al. 2018).

Amphibians and reptiles are two vertebrate groups particularly threatened by global changes (Hoffmann et al. 2010; Böhm et al. 2013). Amphibian populations are declining at a greater pace than the other vertebrates, and their decline is determined by the combined effects of multiple threats, notably land-use change, climate change, and alien species (Stuart et al. 2008; Hof et al. 2011). While the global reptile assessment has not been completed yet, land-use change, climate change, and alien species are listed as major threats also for reptiles (Todd et al. 2010). Furthermore, climate change is expected to have a particularly strong impact on ectothermic vertebrates, because it can affect essential life-history processes that depend on the characteristics of the environment (Buckley et al. 2012; Flesch et al.

2017). Finally, the response of local populations to global drivers vary across taxa and geographic areas. It is thus important to assess whether different species show heterogeneous responses, in order to understand the generality of patterns of change (Muths et al. 2017).

In this study, we used meta-regression to quantify the relative importance of different global change drivers on population trends of European amphibians and reptiles (Fig. 1). In particular, we tested four hypotheses:

Hypothesis 1 *Population trends are determined by changes in climatic suitability*

Climatic suitability is a measure of how much the climate of an area is suitable for a particular species (Araújo et al. 2011). Suitability can provide a better measure of the impact of climate change compared to climatic velocity since it accounts for the geographic position of a population. For instance, in a situation of poleward shift of the geographic range of a species, populations nearest to the pole can gain suitability, while the farthest ones often lose suitable space (Parmesan et al. 1999). We thus predict that a decrease in climatic suitability negatively affects population trends and vice versa;

Hypothesis 2 *Population trends are negatively influenced by alien species*

Alien species exert multiple impacts on native biodiversity (Gallardo et al. 2015). Negative impacts increase with the abundance and richness of alien species (Vilà et al. 2010), thus we predict a negative relationship between the richness of alien species and population trends;

Hypothesis 3 *Population trends are determined by habitat availability*

The amount of available habitat in the landscape is a key parameter influencing species distribution and population dynamics (Flesch 2017; Seibold et al. 2017). For example, a low amount of suitable habitat leads to reduced carrying capacity and vital rates, and to a decrease in the amount of source populations that could provide migrants from the surrounding landscape (Hodgson et al. 2009). Because these processes can affect the long-term dynamics of populations and their probability of persistence, we predict a positive relationship between the amount of suitable habitat and population trends;

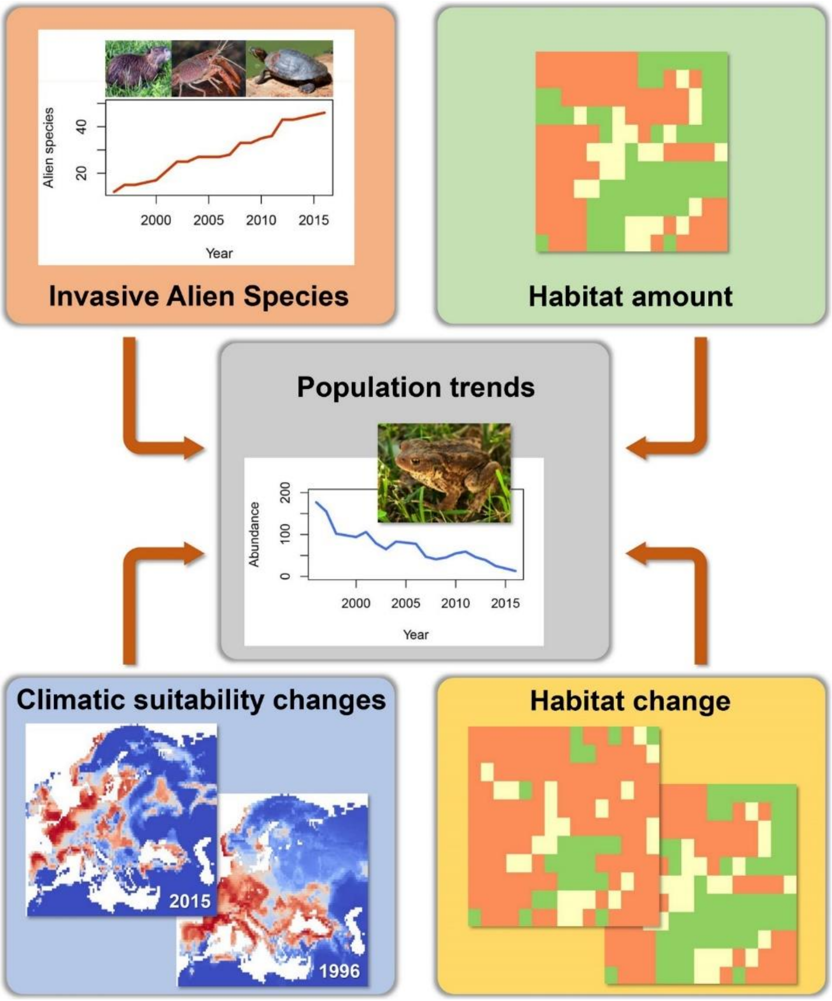

FIGURE 1 General framework of the study. We assessed the relative importance of multiple global change drivers on population trends of European amphibians and reptiles.

Hypothesis 4 *Population trends are determined by changes in habitat availability*

Land-use has undergone heavy changes in the last decades, with the conversion of natural vegetation to urban or agricultural land-use in some areas and forest gain in others (Hansen et al. 2013). Habitat loss is considered the main cause of decline for the vast majority of vertebrates, hence we predict a negative relationship between the decrease in habitat availability and population trends (Hoffmann et al. 2010).

We used data on long-term population abundance of reptiles and amphibians to evaluate the support of these hypotheses and also investigated possible interactions among drivers. Furthermore, we appraised whether the response of populations to these drivers was heterogeneous across taxa.

2 | MATERIALS AND METHODS

2.1 | Abundance data

The study area included Europe plus Anatolia, with eastern limit in the Ural Mountains and the Caucasus. We performed a literature search in February 2017. Published data on abundance for reptile and amphibian species were collected by searching in the ISI Web of Science for keywords “demography”, “population”, and “decline”, associated with “reptile”, and “amphibian” keywords. We reviewed the text and selected data of populations with at least 4 years of sampling. Shorter time series were discarded to ensure more relevant estimates of temporal trends. We also analyzed the data associated to the Houlahan, Findlay, Schmidt, Meyer, & Kuzmin (2000) paper on amphibian population trends, and retrieved all the time series from the study area, for which enough information was available to reconstruct the population locality. We also added two unpublished population times series for which we directly collected data for the period 2010-2016 (Manenti R., unpublished data). We obtained a total of 16 studies, comprising time-series for 843 populations of 17 different species (see Supporting Information Table S1 for a complete list of references).

From these time series, we derived population trends by calculating, for each population, the Pearson’s correlation (*r*) between years of sampling and log-transformed population abundance. We then used Fisher’s *Z* to calculate the effect size of temporal trends and the associated variance for each population (Ficetola & Maiorano 2016). To identify the drivers of population trends, we then assessed the relationships between population trends (effect sizes) and four drivers: (i) climatic suitability changes, (ii) alien species richness, (iii) habitat availability, (iv) changes in habitat availability.

2.2 | Climatic suitability changes

We used species distribution models (SDM) to assess changes in climatic suitability through time for each population. To build SDM, species presence was derived from the European Herpetological Atlas (Sillero et al. 2014), at a resolution of 0.5°. All the frogs of the hybridogenic complex *Pelophylax* spp. were modeled as a single taxon; the distribution range of the grass snake *Natrix natrix* extends outside the boundaries of the European Herpetological Atlas, thus presences for this species were integrated with points obtained from the global biodiversity information facility (https://www.gbif.org/); The distribution records of newts (*Triturus cristatus* and *Lissotriton vulgaris*) were taken from Wielstra et al., (2018) and Wielstra, Sillero, Vörös, & Arntzen (2014).

SDM were calibrated on the climatic conditions in the last 51 years (averaged from 1966 to 2016), obtained from an updated version of the Climatic Research Unit dataset

(Harris et al. 2014) (CRU TS v. 4.01), which reports monthly values of precipitation and temperature for the time-period 1901-2016. We used four climatic variables: mean annual temperature, total annual precipitation, annual temperature standard deviation, annual precipitation coefficient of variation. Minimum annual temperature and maximum annual temperature, and minimum / maximum temperature during breeding seasons, are additional variables important for tolerance and activity of ectotherms but are strongly correlated to mean temperature and standard deviation (Appendix S1). We thus also re-ran the SDMs using min/max values instead of mean and standard deviation of temperature and for subsequent analyses we used models with higher performance values (see results). Models were built within the biomod2 R package (Thuiller et al. 2016), running an ensemble of the following models: boosted regression trees, generalized additive models, classification tree analysis, multivariate adaptive regression splines, and random forests. For each species, we selected 3,000 pseudo-absence points within a radius of 1,000 km from the species distribution range. To get a meaningful evaluation of the models and to avoid over-fitting, models were repeated five times to perform cross-validation, and for each run we used a random sample of 67% of the initial occurrence data to calibrate the models and the remaining 33% for evaluation. Models performance was assessed using the True Skill Statistic (TSS) and the Area Under the receiver operating characteristic Curve (AUC) (Liu et al. 2011). Finally, we obtained an ensemble model through a weighted sum of the probability of occurrence, proportional to the cross-validated TSS.

Subsequently, to assess changes in climatic suitability through time, the overall bioclimatic SDM for each species (calibrated for 1966-2016) was projected on the climatic conditions for the years for which information on population abundance was available. This allowed obtaining time series of climatic suitability for each population. Specifically, we calculated the trend of SDM suitability for each population by calculating the correlation between years of sampling and climatic suitability. Correlation coefficients were then transformed to Fisher's *Z* to obtain comparable measures of effect size. The trend of climatic suitability was considered as independent variable to measure the effect of changes of climatic suitability.

2.3 | Alien species

The richness of alien species was obtained from the Global Alien Species First Record Database (Seebens et al. 2017), which reports the first year of detection of alien species at the regional level resolution. At this scale, alien species richness can be a good proxy to measure negative effects on native biodiversity, because the number of impacts is higher in areas with more alien species (Vilà et al. 2010; Latombe et al. 2017). For each population, we extracted the total number of alien species starting from 1901 to the last year of sampling by summing the total number of vertebrates and crustaceans, as these taxa are known to have a major impact on amphibians and reptiles (Kats & Ferrer 2003; Ficetola et al. 2011). The database reports the occurrences of alien species at the regional level, but the considered regions had a coarser resolution than the localities used for our analyses. Therefore, the number of alien species obtained using this approach probably overestimates the actual number of alien species at a given locality. Nevertheless, the Global Alien Species First Record Database has the advantage that the alien species records are temporally explicit, thus allowing analyses of temporal processes. Furthermore, the number of alien species is strongly related to economic and political factors of territories, thus we expect a strong correlation between regional-level and local abundance of alien species (Pyšek et al. 2010). For each time series, the total number of alien species from the year 1901 to the last year of the time series was considered as independent variable to measure the effect of alien species.

2.4 | Habitat availability and habitat changes

For each species, we identified a list of suitable land-cover classes in order to calculate the extent of suitable habitat (ESH) by integrating the habitat preferences obtained from the IUCN Red List with land-use information (see Table S2 for details; Rondinini et al., 2011). Land-use information was obtained from the time series of the European Space Agency Climate Change Initiative Land Cover project (https://www.esa-landcover-cci.org/). This map is available from 1992 and does not cover the whole 1972-2016 period. Hence, analyses which considered land use variables were limited to populations sampled in the period 1992-2016 (N = 705 populations). Previous analyses showed that ESH maps, built on the basis of the occurrence of suitable land-use classes, allow a good representation of the actual habitat that can be exploited by species, and provide useful information to estimate species trends (Rondinini et al. 2011; Ficetola et al. 2015; Tracewski et al. 2016). We used ESH to calculate the habitat availability at the beginning of the study period, and the trend of suitable habitat during the study period. Habitat variables were calculated within the 9 × 9 km cell surrounding each population; we selected this resolution because about 90% of amphibian species have a maximum dispersal ability of ~4.5 km (Smith & Green 2005), and because it matched well the accuracy of population localities in our dataset.

2.4.1 | Habitat availability at the beginning of the period

We extracted the percentage cover of ESH in the first year of monitoring within the 9 × 9 km cell surrounding each population. We considered cover at the beginning of time series since we aimed at testing whether initial habitat amount can affect subsequent abundance changes within that landscape. We also repeated analyses using ESH at the end of the period and obtained very consistent results.

2.4.2 | Habitat changes

To assess the impact of the change of ESH on population trends, we calculated the ESH within the 9 × 9 km cell surrounding each population in each year of monitoring, obtaining a time series of ESH. Hence, we calculated the Fisher's *Z* of the correlation between years of sampling and ESH, and considered this value as the trend of ESH (hereafter habitat change).

2.5 | Statistical analyses

We used meta-regression to identify the most influential drivers of population trends (Gurevitch et al. 2018). Global change drivers were related to population trends through Bayesian generalized linear mixed models, using the effect size of population trends (Fisher's *Z*) as the dependent variable. First of all, the overall trend averaged across all the populations was assessed by performing a model of the mean (i.e. a meta-regression model including the intercept and without independent variables) (Kéry 2010). We also ran a separate model of the mean for each species, in order to assess the average species trend. Then, four separate meta-regressions were run to assess the single-variable relationships between population trends at each locality and: (*i*) trend of climatic suitability, (*ii*) richness of alien species, (*iii*) habitat availability at the beginning of the period, (*iv*) trend of habitat availability (Fig. 1). Independent variables used, and time period considered for each single-variable model are described in Table S3. Finally, we performed a multi-variable model including all four independent variables, for the period 1992-2016. We also tested pairwise interactions between the four variables and, in the final model, we considered only interactions with 95% credible intervals (CIs) not overlapping zero. The biological rationale of tested interactions is listed in Table S4. Our multi-variable meta-regression included data from all the species, in order to evaluate the overall pattern. Subsequently, to assess if the effects were consistent across species, we re-run the meta-regression separately for the two commonest species (the common toad *Bufo bufo* and the common frog *Rana temporaria*) and then considering all the species except common toad and common frog.

Before the analysis, we tested the collinearity among the global change drivers and found no strong correlations ($|r| < 0.4$, Table S5). In meta-regressions we included as random effects: the study source of the data; species, family, order, and class, fitted as nested random intercepts, to consider the phylogeny; the id of the 0.5° cell, to take into account the non-independence of nearby populations (i.e. populations within the same cell). Furthermore, for models including alien species, we included region identity as an additional random effect because alien species data are derived from a regional-level database (Seebens et al. 2017). To take into account different variances of Fisher's *Z* among studies, we weighted the records by using the "mev" argument in the MCMCglmm function, considering 1 / variance of *Z* as weight (following Hadfield & Nakagawa, 2010). All models were run for 2,000,000 iterations, with 1,000,000 burn-in and a thinning of 250. The number of alien species was log-transformed and all variables were scaled (mean = 0, SD = 1) before analyses to allow comparison of their estimated effects. Finally, we used respectively Moran's I and Pagel's lambda, to assess whether the residuals of meta-regressions showed spatial or phylogenetic correlation. To test the phylogenetic signal, we used a phylogeny tree including all the 17 species, derived from the phylogenetic tree of the European tetrapods (Roquet et al. 2014).

All analyses were performed in the R environment (R Core Team 2018), using the packages compute.es (del Re 2013) to compute population trends effect sizes and variance, raster (Hijmans 2019) and rgeos (Bivand & Rundel 2019) to process maps, biomod2 (Thuiller et al. 2016) to create SDMs, MCMCglmm (Hadfield 2010) to perform Bayesian generalized linear mixed models, EcoGenetics (Roser et al. 2017) to test spatial autocorrelation, and caper (Orme et al. 2018) to test phylogenetic autocorrelation.

3 | RESULTS

Overall, we obtained 843 time-series for 17 species, covering 11 European countries across the period 1972-2016 (Fig. S1; Appendix S2). 705 time-series were included in the period for which land cover information was available (after 1992, Appendix S3).

3.1 | General trend

Out of the 843 populations, 458 (54%) showed negative population trends, 383 (45%) positive trends and two remained stable ($|r| < 0.01$). The averaged population trend (effect size), obtained from the models of the mean of the different species, was negative for ten species and positive for seven species (Fig. 2). 95% CIs of the estimates of population trends did not overlap zero in seven out of 17 species (41%), however, there were strong differences of trends across populations (Fig. 2). The population trend averaged across the populations of all species, was negative but credible intervals overlapped zero (mean = -0.084; 95% CI = -0.284 / 0.152).

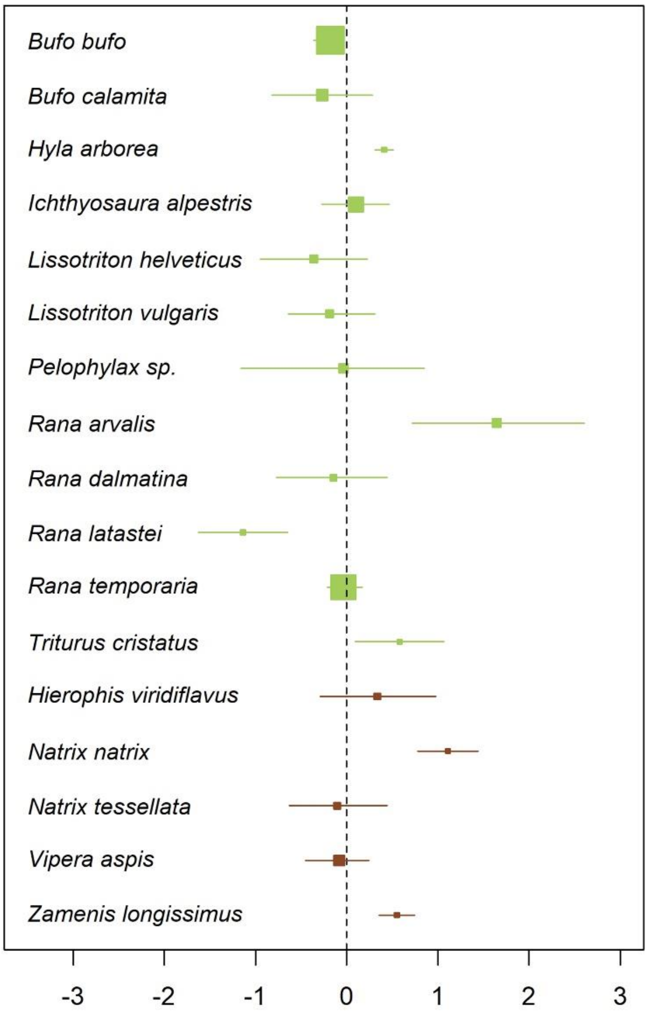

FIGURE 2 Average population trend of species, with 95% credible intervals. Point size is proportional to the number of populations considered in this study. Amphibians are shown in green, reptiles in brown.

3.2 | Single-variable relationships

3.2.1 | Climatic suitability changes

All bioclimatic models showed very good or excellent performance (Fig. S2). The models built with mean annual temperature and annual temperature standard deviation showed higher TSS and AUC values than models including minimum and maximum annual temperature (Table S6) and were hence used to calculate climatic suitability.

Climatic suitability increased through time for 309 populations, decreased for 520 populations, and remained stable for 14 populations. For the period 1972-2016, the average change of climatic suitability/year was -0.15% (SD = 2.4%). The relationship between climatic suitability and population trends was positive, indicating more positive trends in populations experiencing improvement of suitability. Nevertheless, 95% CIs slightly overlapped zero (effect size = 0.046; 95% CI = -0.021 / 0.107). The pattern was similar when we limited analyses to the period 1992-2016, even though the effect size of the relationship between climatic suitability and population trends was slightly weaker (effect size = 0.038).

3.2.2 | Alien species

The average number of alien species per region was 45 (SD = 24), and population trends declined in regions with more alien species. For the period 1972-2016, the 95% CIs of this relationship slightly overlapped zero, while 90% CIs did not (effect size = -0.061; 95% CI = -0.125 / 0.004; 90% CI = -0.112 / -0.008). When we limited analyses to 1992-2016, the effect size was significantly lower than zero (effect size = -0.093, 95% CI = -0.166 / -0.022).

3.2.3 | Habitat availability

Average cover of suitable habitat was 29% (SD = 20%), and population trends were more positive in landscapes with high habitat availability (effect size = 0.069; 95% CI = 0.002 / 0.130; Table S7a).

3.2.4 | Habitat change

Cover of suitable habitat increased for 33 populations, decreased for 396 populations, and remained stable for 276 populations (|r| < 0.01); the average absolute value of habitat change across all the populations was 0.17% / year (SD = 0.39%). The single-variable relationship between habitat change and population trends was weak, with CIs broadly overlapping zero (Table S7a).

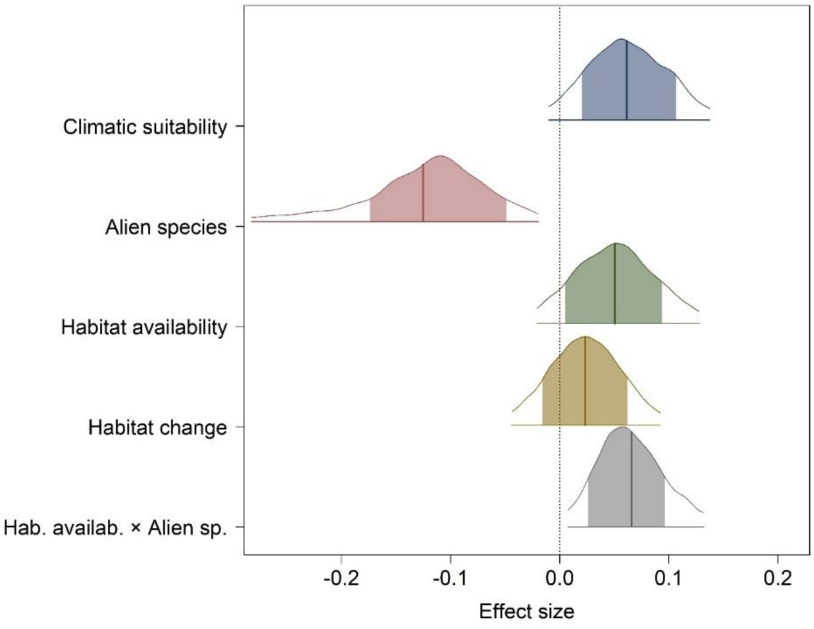

FIGURE 3 Density plots of the posterior distribution for the relationships between trends of 705 populations of amphibians and reptiles and the candidate drivers (from top to bottom: trend of climatic suitability, richness of alien species, initial habitat availability, habitat change through time, interaction between initial habitat availability and richness of alien species). Thick vertical lines represent the average effect size, outer lines represent the 95% credible interval, inner colors represent the 75% credible interval. The y-axis indicates the frequency of posterior distributions and it is consistent for the five plots.

3.3 | Multi-variable analysis

Out of the six possible paired interactions among the four candidate drivers, only the interaction between the richness of alien species and the initial habitat availability showed 95% CIs not overlapping zero and was included in the meta-regression analysis including all the predictors and all the populations (full model; N = 705 populations). The effect sizes of predictors were nearly identical between the models and without the interaction (Table S7b, S7c).

The full model confirmed that population trends were negatively related to the richness of alien species (Fig. 3 and 4b). There was a positive relationship between the trend of climatic suitability and population trends (Fig. 4a) and, even though

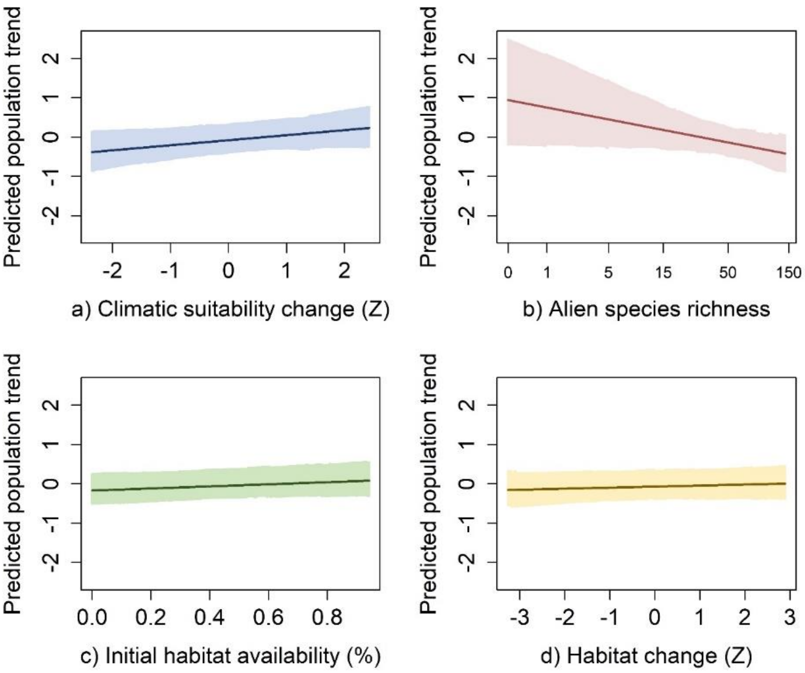

FIGURE 4 Relationship between the four independent variables and population trends, as predicted by the full model. In each plot the dark line shows the predicted value of population trends (Fisher's *Z*) and the shaded area indicates the 95% credible interval.

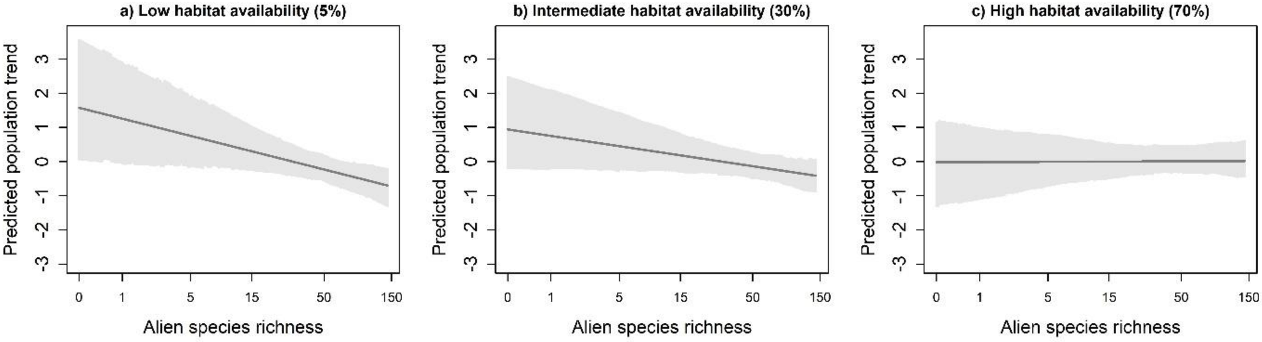

FIGURE 5 Interaction between habitat availability and alien species. Plots show the relationship between the richness of alien species and the population trends predicted by the full model at different levels of habitat availability: a) habitat availability = 5%, b) habitat availability = 30%, c) habitat availability = 70%. In each plot the dark line shows the predicted value of population trends and the shaded area indicates the 95% credible interval.

the 95% CIs slightly overlapped zero, the 90% CIs did not (Fig. 3; Table S7c). Population trends were positively related to habitat availability and to the habitat trend (Fig. 4c and 4d), but credible intervals overlapped zero for both variables (Fig. 3). Furthermore, there was a strong interaction between habitat availability and richness of alien species, showing that the negative impact of alien species was particularly strong in landscapes with a low amount of suitable habitat (Fig. 5). Values of random intercepts for the multiple regression model are listed in Table S8. The residuals of the model showed no significant spatial or phylogenetic autocorrelation (Moran's *I* = 0.001; 95% CIs = -0.009 – 0.010; Pagel's Lambda = 0; 95% CIs = 0 – 0.503).

3.4 | Robustness to interspecific variation

When we repeated meta-regression including only common toad populations, results were generally consistent with the full analysis. Common toad population trends were more negative in sites with more alien species, were positively related to the trend of climatic suitability and were more positive in landscapes with higher habitat availability, even though 95% intervals were broader than in the analysis including all the species. Conversely, trends were unrelated to habitat change and to the interaction between habitat availability and richness of alien species (Fig. 6a). Common frog populations showed a different pattern compared to the full analysis, as population trends only showed a weak positive relationship with habitat change (Fig. 6b).

Results remained partially consistent when we repeated analyses excluding the two most common species (the common toad and the common frog), even though credible intervals were much broader than in the full analysis. Population trends were positively related to climatic suitability trend and negatively related to alien species. While the effect size of habitat availability was close to zero, this analysis confirmed the interaction between habitat availability and richness of alien species (Fig. 6c). Moreover, in this analysis we observed a strong positive effect of habitat changes, indicating that population trends were more positive in landscapes were the extent of suitable habitat increased through time (Fig. 6c).

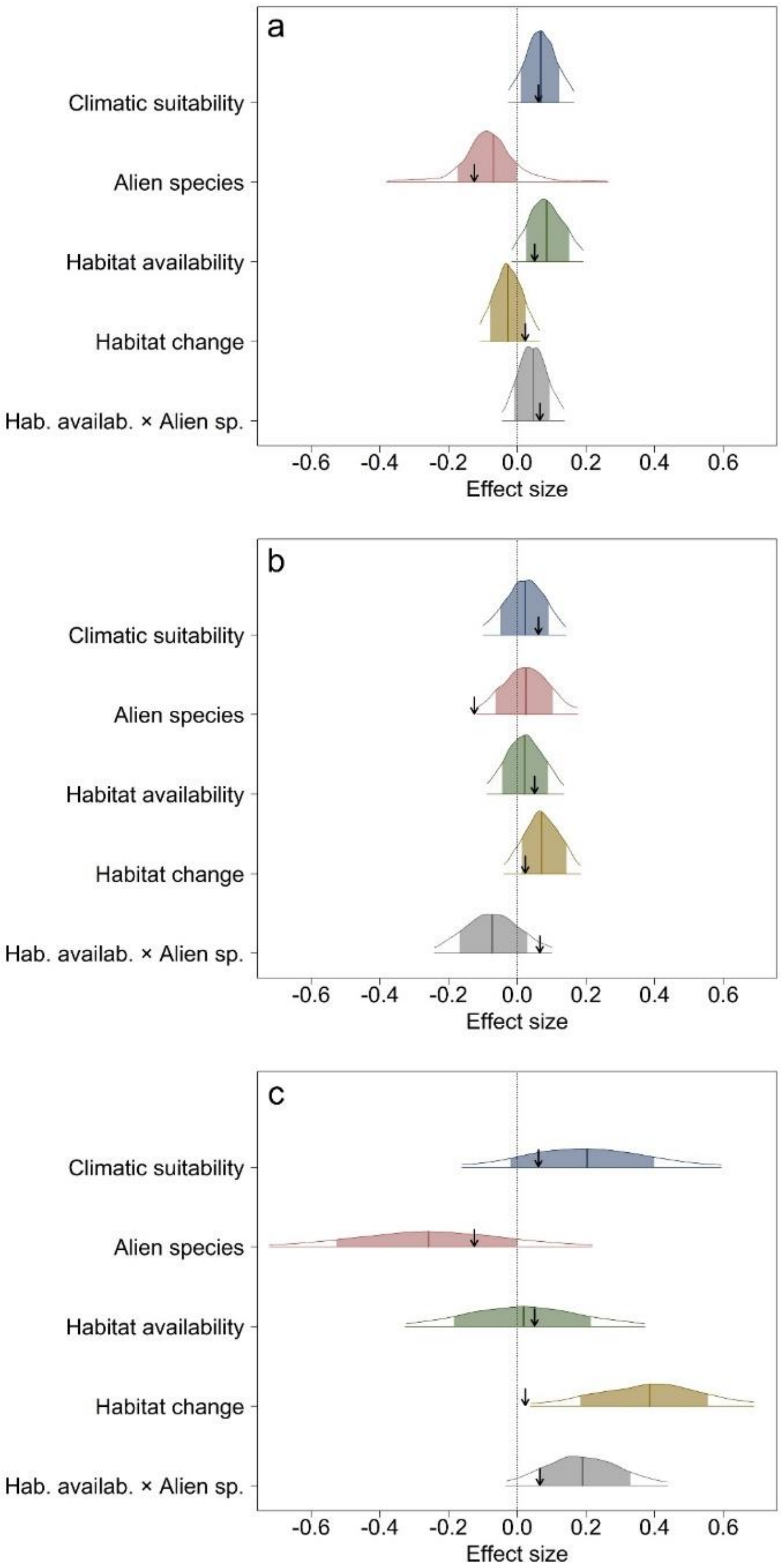

FIGURE 6 Density plots of the posterior distribution for the relationships between population trends and the candidate drivers (from top to bottom: trend of climatic suitability, richness of alien species, initial habitat availability, habitat change through time, interaction between initial habitat availability and richness of alien species), considering a) common toad populations only, b) common frog populations only and c) all data except for common toad and common frog populations. Thick vertical lines represent the average effect size, outer lines represent the 95% credible interval, inner colors represent the 75% credible interval. Arrows represent the mean effect size of the analysis including all the species. The y-axis indicates the frequency of posterior distributions and it is consistent for all the plots.

4 | DISCUSSION

Our study provides one of the first broad-scale and long-term assessments of the impact of multiple global change drivers on population trends of amphibians and reptiles. Despite amphibians and reptiles having a major functional role in ecosystems, these vertebrates remain underrepresented in population trend analyses, and they only account for a tiny part of studies even in global databases of species abundance (Dornelas et al. 2018; Santini et al. 2018). Our work summarized the trends of multiple European amphibian and reptile populations through 45 years and showed that climate change, alien species, habitat availability, and habitat change have complex impacts on their dynamics, even though their importance differed among taxa (Fig. 3 and 6).

Population trends of amphibians and reptiles were jointly determined by multiple drivers. In the multi-variable analysis, alien species showed the largest effect, followed by climate change and habitat availability (Fig. 3), indicating that they might be among the most influential drivers of population trends for many amphibians and reptiles. Alien species have a major impact on the European native fauna (Vilà et al. 2010); unfortunately, the number of alien species is quickly growing in all the continents, stressing the urgency of prevention and mitigation actions in order to limit the intensification of impacts in the future (Vilà et al. 2010; Seebens et al. 2017). Nevertheless, the effect of alien species differed across taxa, as they showed a negative effect on the common toad and on other amphibians and reptiles, while were unrelated to the trends of common frogs (Fig. 6). Other studies on population trends detected heterogeneous responses to broad-scale environmental stressors (Grant et al. 2016; Flesch et al. 2017; Muths et al. 2017). For instance, Muths et al. (2017) analyzed the demographic response of amphibian populations to climate and observed that the magnitude and direction of the response were highly heterogeneous across taxa and even within species. This confirms the importance of studies including species with various ecological tolerances, in order to disentangle the heterogeneous effects of global changes on natural populations.

Global change scenarios suggest that climate change will have a growing impact on biodiversity (Intergovernmental Panel on Climate Change 2015). However, the impact of climate change can be heterogeneous among species and even among populations within a given species. For instance, in the northern hemisphere, climatic warming can determine the extinction of populations in southern portions of species ranges, while can have positive effects on northern populations

(Parmesan et al. 1999). To assess the impact of climate change it is thus important to develop appropriate measures of how climatic variation influences populations in different areas of the species' range. Climatic suitability can provide information on the actual effect of climatic variation on populations because it considers the differences that can occur across distant geographic areas. Therefore, in our analyses we considered climatic suitability instead of raw temperature/precipitation change. Despite 95% CIs slightly overlapping zero, the effects of suitability changes were consistent with our predictions (Fig. 3, 4 and 6) with negative changes in climatic suitability corresponding to negative population trends. Studies relating the trends of amphibians and reptiles to climate change obtained mixed results. For instance, Ficetola and Maiorano (2016) found that changes in precipitation can have a significant impact on amphibian trends, but did not detect a clear effect of changes in temperature. Conversely, when using climatic suitability, we found a consistent pattern across species (Fig. 3 and 6). Changes in climatic suitability are often used to explore potential impacts of future climate change on biodiversity (Araújo et al. 2011; Thuiller et al. 2011), while fewer studies have used this approach to understand the impact of changes occurring in the past (e.g. Bateman et al., 2016; Fouquet, Ficetola, Haigh, & Gemmell, 2010). Our analysis suggests that suitability can provide a measure of the impact of climate change more comparable across species. The moderate effect of suitability change on population trends can also be related to the ability of populations to adapt in response to climate change (Seebacher et al. 2015). The integration of ongoing adaptive changes is a challenge for global change research and could allow to better understand the responses of populations and to make better predictions (Hoffman & Sgró 2011).

While the effects of alien species and climate were generally consistent across taxa, the effects of habitat availability and habitat change were more complex. Even though habitat loss is described as the factor threatening the largest number of amphibians and reptiles (Stuart et al. 2008), relationships between changes in habitat availability and population trends were only detected in a subset of analyses. Population trends were more positive in landscapes with more habitat (Table S7a), but the effects of habitat availability were weak when taking into account also other factors and potential interactions (Fig. 3; Table S7b). Several factors can explain the limited effects of habitat variables. First, a significant interaction between habitat availability and the richness of alien species indicates that the impact these two variables can be context dependent, complicating the detection of their effects. Second, we assessed habitat change on the basis of broad-scale land cover maps, which do not provide measures of the specific resources and conditions needed by different species. Obtaining accurate measures of habitats is particularly complex for small vertebrates, which often exploit specific microhabitats (Mendenhall et al. 2011; Ficetola et al. 2018a). For instance, agricultural mechanization can determine loss of suitable micro-habitats (e.g. hedgerows, ditches) even in areas with a stable amount of agricultural lands, thus impacting species that can exploit semi-natural landscapes. Third, average rates of habitat change were extremely low during the study period (average: ~0.1% / year). Such a limited variation is characteristic of broad areas of Europe (Fig. S3) but reduces the possibility to detect relationships and can explain the weak effect of this driver. Furthermore, population declines often do not occur immediately after environmental pressures (Dullinger et al. 2013), thus we might experience the legacy of present anthropogenic pressures in the next decades. Nevertheless, habitat change showed a clear effect when we removed the commonest species (common toad and common frogs) from our dataset, with more positive population trends in landscapes where the amount of suitable habitat increased through time. Common toad and common frog are widespread, generalist species that can exploit a very wide range of habitats (Table S2), therefore it may be more difficult detecting their response to habitat change, compared to habitat specialists. This further stresses the need of monitoring a wide range of species in order to obtain generalizable information of the effects of global changes on biodiversity loss and highlights the importance of comparing the responses of both widespread and specialized species.

Habitat availability showed a strong interaction with the richness of alien species, as the negative impact of alien species was particularly strong in landscapes with less habitat availability (Fig. 5). The importance of interactions among different drivers is increasingly recognized by global change studies, as interactive effects can both magnify and mitigate the impact of stressors (Blaustein & Kiesecker 2002; Mantyka-Pringle et al. 2012). Alien species show complex relationship with the availability of natural habitats, which can strongly modify their impact. For instance, invasive species can be more abundant in human-modified landscapes, thus native populations living in landscape with a less natural habitat can suffer a stronger impact by invasives

(Blaustein & Kiesecker 2002; Didham et al. 2007; Quinn et al. 2011). The complex interactions between alien species and habitat availability further stress the importance of conservation actions targeting multiple threats and also considering synergies among drivers of decline in order to mitigate biodiversity loss (Didham et al. 2007; Brook et al. 2008).

Despite the broad temporal and geographic extent, our analyses have some limitations. Most of the data are from amphibian populations, and one species (the common toad, *Bufo bufo*) accounted for more than half of populations (Table S9). This occurs because the common toad is one of the most abundant amphibians in Europe and is regularly monitored by many citizen science programs. Common amphibians have a major role in ecosystem functioning and nutrient transfer (Beard et al. 2003; Kyek et al. 2017). Several studies have shown negative trends in toad populations (e.g. Bonardi et al., 2011; Petrovan & Schmidt, 2016) and understanding the factors underlying a common species decline is extremely important to maintain ecosystem functioning (Gaston & Fuller 2008). Alien species and climate change showed a similar effect across most of the taxa: despite broader credible intervals, effect sizes pointed in the same direction even if we removed the most common species (Fig. 6c), suggesting that uneven sample size across species did not bias our conclusions. In our dataset we collected fewer data regarding reptiles than amphibians. This is a recurrent pattern in the herpetological literature and hampered separate analyses of these taxa. Estimating the abundance of reptiles is usually harder compared to amphibians, because reptiles often have low detection probability, and estimating their abundance requires effort-demanding survey methods (e.g. capture mark recapture instead of repeated counts) (Ficetola et al. 2018b). Increasing the monitoring efforts toward reptile populations is urgently required to better assess the drivers of the decline of this group and guide future conservation efforts. Finally, our analyses were limited both in space and in time by the availability of population and land-cover data. Continuous series of land-cover data are only available since 1992, thus preventing us from assessing the effects of habitat availability during previous periods, when the velocity of habitat change in Europe was probably stronger than in recent years (Falcucci et al. 2007). Furthermore, the majority of our data came from just two European countries, potentially limiting the spatial representativeness of our dataset (Fig. S1). Despite not spanning the whole Europe, analyzed localities provide good coverage of the features occurring through Europe for habitat availability and changes (Fig. S4), richness of alien species (Fig. S5), and climatic features (with the exception of coldest climates; Fig. S6). Overall, the frequency of declining populations in our dataset was similar to previous broad-scale estimates of trends of herps in Europe (e.g. Houlahan et al., (2000); 53% negative and 43% positive trends). This suggests that our analyses can provide an accurate picture of patterns occurring throughout most of Europe.

Population trends of European reptiles and amphibians are driven by the combined effects of alien species, climate change, habitat features, and habitat changes, with complex joint and interactive effects among factors. Even though we identified general patterns in the response to some environmental drivers, when retrieving broad-scale patterns it is important to consider that the same factors can act differently among taxonomic groups (Grant et al. 2016; Muths et al. 2017). For instance, habitat change showed a contrasting effect across species and its crucial role was only evident for a subset of them. Understanding the impact of global change drivers is the first step for management. This requires drawing general syntheses of the combined effects of multiple drivers but also considering how responses can be different across species.

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

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CHAPTER 3 | Thirty years of invasion: the distribution of the invasive crayfish *Procambarus clarkii* in Italy

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ABSTRACT

The presence of the red swamp crayfish *Procambarus clarkii* in Italy is documented since 1989, but no comprehensive data are available on its spread through time at the national scale. New confirmed records for *Procambarus clarkii* are continuously arising in recent years across the country. By reviewing the scientific and grey literature, we obtained an up-to-date map of the species invasion in Italy. This information can help to monitor and understand the spread of this highly invasive crayfish and to implement more effective management measures.

1 | INTRODUCTION

Intentional and inadvertent introductions of alien species have become more frequent than ever in the last decades, due to the effects of globalization, particularly the increase of international trade (Westphal et al. 2008; Hulme 2009). Although the difficulty of understanding the effect of invasives may lead to the underestimation of their impacts (Williamson & Fitter 1996; Graves & Shapiro 2003; Ricciardi & Atkinson 2004; Rodriguez 2006), alien species are one of the greatest threats to global biodiversity (Gallardo et al. 2015; Doherty et al. 2016). An alien species can represent a threat to native species through various mechanisms, such as the increase of predation rate (Gherardi et al. 2001; Wanless et al. 2007), competition for resources (Brown et al. 2002; Cadi & Joly 2003), spread of pathogens (Diéguez-Uribeondo & Söderhäll 1993), and food web alterations (Ficetola et al. 2012a).

Biological invasions in freshwater habitats can cause degradation to various levels of biological organization, negatively affecting the entire ecosystem (Vilà & García-Berthou 2010; Gallardo et al. 2015). Inland waters are particularly susceptible to biological invasions and, in extreme cases, alien species can become a relevant part of freshwater communities, both in terms of the number of species and biomass (Strayer 2010). This vulnerability is due to several factors, such as the high dispersal ability of some freshwater organisms along with the interconnected hydrographic network and the strong impacts of human disturbance on these environments (Gherardi et al. 2009). Furthermore, many freshwater species are specialists to environments with a limited set of predators and can be lacking adequate antipredator responses (Cox & Lima 2006). Accidental and deliberate introductions of alien freshwater animals or plants are usually linked to aquaculture, fishing activities, passive transport, and ornamental uses of exotic species (Vilà & García-Berthou 2010). The red swamp crayfish *Procambarus clarkii* Girard, 1852 is a crayfish native from the United States and northern Mexico and, due to its relevance for aquaculture, in the last decades, it was introduced in all the continents except for Antarctica and Oceania (Holdich 1993; Capinha et al. 2011; Gonçalves Loureiro et al. 2015). This crayfish has a generalist diet (Barbaresi & Gherardi 2000; Gonçalves Loureiro et al. 2015) and can tolerate extreme variations in oxygen level, water salinity and acidity, and even prolonged droughts (Claire & Wroiten 1978; Barbaresi & Gherardi 2000; Casellato & Masiero 2011; Gonçalves Loureiro et al. 2015). Its tolerance and its fecundity allowed this species to rapidly spread and successfully invade a large variety of habitats (Gherardi 2006; Aquiloni & Gherardi 2008; Siesa et al. 2014). Introduced red swamp crayfish populations are known to have a negative impact on several members of freshwater communities. For instance, *Procambarus clarkii* has a negative impact on the abundance and distribution of many amphibians (Cruz & Rebelo 2005; Cruz et al. 2008; Ficetola et al. 2011; Manenti et al. 2020), aquatic insects (Siesa et al. 2014), and on macrophytes biomass and biodiversity (Matsuzaki et al. 2009; Carreira et al. 2014). Moreover, *Procambarus clarkii* is a vector for the oomycete *Aphanomyces astaci*, causing the so-called crayfish plague, which is lethal for freshwater European crayfish species and already determined several local extinctions of native crayfish (Diéguez-Uribeondo & Söderhäll 1993; Holdich 1993; Aquiloni et al. 2011; Gonçalves Loureiro et al. 2015; Bonelli et al. 2017; Manenti et al. 2019).

In Italy, the red swamp crayfish was observed for the first time in the wild in the Turin province in 1989 (Delmastro 1992), but it rapidly spread, originally in northern and central Italy and more recently also in the southern portion of the peninsula (Morpurgo et al. 2010; Aquiloni et al. 2011; Cilenti et al. 2017). However, so far no study has described the pattern and the rate of invasion by this species in Italy. The aim of this work is to provide updated information on the distribution of *Procambarus clarkii* in Italy using both scientific and grey literature such as management plans of protected natural areas, local fishing maps, biodiversity-dedicated websites, online forums, and local newspaper articles.

2 | MATERIALS AND METHODS

The Italian territory is divided into 20 administrative regions, each one composed of a variable number of institutional bodies called “provinces” (Italian: “province”) or “metropolitan cities” (Italian: “città metropolitane”), hereinafter collectively referred to as “provinces” (total: 107 provinces). We focused on the distribution of *Procambarus clarkii* at the provincial level. We searched for distribution data of the red swamp crayfish through multiple search engines using the keywords “*Procambarus clarkii*”, “gambero rosso”, and “gambero killer” (the Italian common names for the red swamp crayfish) followed by the name of each Italian province. Since some provinces are no longer administratively valid, and some sources do not explicitly indicate the name of the province, we also performed searches using the name of each Italian region. The literature search was performed between December 2019 and February 2020. Searches for scientific literature were performed using Web of Science, Scopus, and Google Scholar, while searches for grey literature (newspaper articles, fishing maps, and management plans) and online platforms (forums, and social networks) were performed using Google. Furthermore, we searched for *P. clarkii* records in the ClimCKmap database, a georeferenced database for the Italian fauna (Marta et al. 2019).

Collected data included: the occurrence of the species, the year and, when available, locality of first observation in the province, and the consulted source (Appendix S1). When the date of the first observation was not specified, *Procambarus clarkii* is considered present in the province since the date of publication of the oldest source assessing its presence. The locality of first observation, whether specified in the sources, is reported as the name of the municipality, natural protected area, stream, river, or lake where the red swamp crayfish was found. It must be stressed that the absence of occurrence data of the species in a province does not necessarily imply the absence of the species but only the lack of records.

We created a distribution map of *Procambarus clarkii* in Italy at the province resolution, with an indication of the first year of detection for each province (Fig. 1, appendix S1). The distribution map was processed using QGIS 3.4.13 (https://qgis.org/) and the list and boundaries of the Italian provinces were retrieved from the Italian National Institute of Statistics (https://www.istat.it/it/archivio/222527, accessed on 18 February 2020).

3 | RESULTS

We found records of *Procambarus clarkii* in 86 out of 107 Italian provinces (80.4%; Fig. 1). The highest concentration of invaded provinces is in central Italy (Lazio, Marche, Tuscany, and Umbria regions), where the red swamp crayfish is present in all the provinces. In north-western Italy (Aosta Valley, Liguria, Lombardy, and Piedmont regions) it is present in 24 out of 25 provinces (96%) and in north-eastern Italy (Emilia-Romagna, Friuli-Venezia Giulia, Trentino-Alto Adige/Südtirol, and Veneto regions) it is recorded in 20 out of 22 provinces (90.9%). In insular Italy (Sardinia and Sicily) fewer records are available and crayfish was detected in 9 out of 14 provinces (64.3%). Finally, in Southern Italy (Abruzzo, Apulia, Basilicata, Calabria, Campania, and Molise) the species was detected in 11 out of 24 provinces (45.8%). From 1989 to 1999 *Procambarus clarkii* was observed in 22 provinces; between 2000 and 2009 it invaded 38 new provinces (+172.7% compared to the previous decade); in the last decade (2010-2019) the red swamp crayfish has been recorded in 26 additional provinces (+43.3%) (Fig. 2).

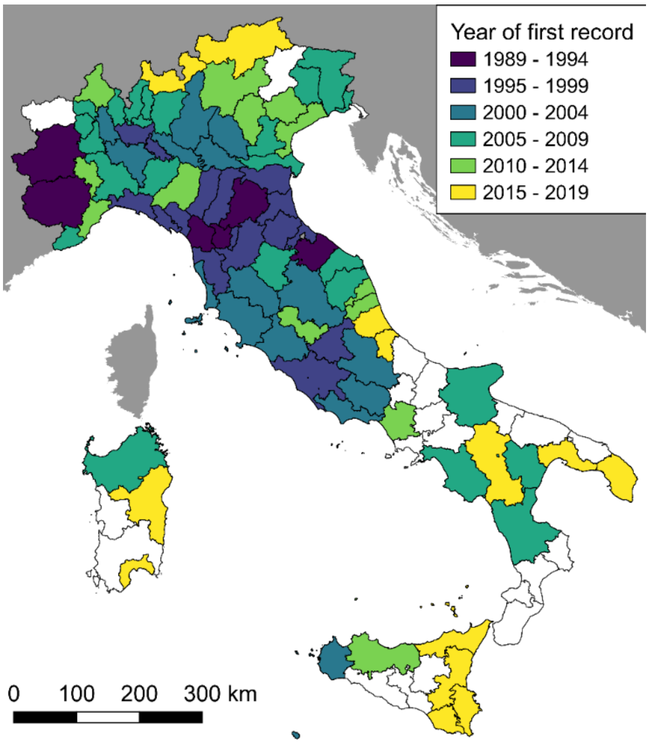

FIGURE 1 Distribution map of *Procambarus clarkii* at the provincial level. The colors represent the different periods of first observation of the species. Grey represent territories outside the Italian boundaries.

4 | DISCUSSION

The range of *Procambarus clarkii* underwent an impressive expansion in Italy since its first introduction thirty years ago (Fig. 1). This is probably due to both natural dispersal and multiple deliberate or accidental introductions (Gherardi et al. 2001; Gherardi 2006). Given the major impacts of the crayfish on biodiversity, the high number of invaded provinces in northern and central Italy raises major concerns for the conservation of wetlands in these areas. Looking at the number of invaded provinces over time (Fig. 2), it seems that the crayfish spread was fastest during the second decade after the introduction and then slowed down in recent years. However, this deceleration is due to the fact that the more the range of this invasive species expands, the fewer provinces remain not invaded and therefore colonizable (Fig. 2).

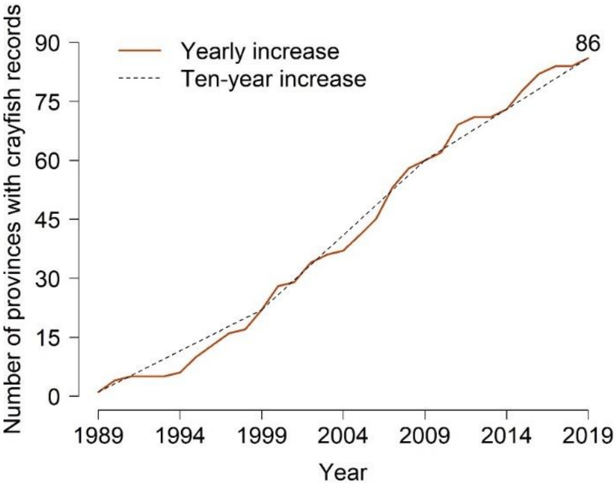

FIGURE 2 Cumulative number of invaded Italian provinces since the first observation of *Procambarus clarkii* in the wild.

We observed sharp differences between northern/central regions, where almost all provinces are invaded, and southern/insular regions, where fewer records are available (Fig. 3). Several non-exclusive reasons can determine this spatial pattern. First, the difference could be caused by different habitat availability for the crayfish across the country, for instance, because northern and central regions have a larger extent of inland waters compared to the southern/insular regions (Allen & Pavelsky 2018). Second, introductions of alien species are more frequent in areas with high human density (Stohlgren et al. 2006; Silva-Rocha et al. 2019), and human population is particularly dense in some of the northern/central regions. Third, differences could be caused by historical reasons. The oldest observations of *Procambarus clarkii* in Italy occurred in northern and central regions, while the first records in southern and insular Italy are more recent. Despite available data are not enough to unravel the reason behind the differences, this last explanation is supported by the rates of invaded provinces across the Italian territory: in north-western, north-eastern, and central Italy the rate of invaded provinces was fastest until 2009, followed by a plateau during the last decade. Conversely, southern and insular Italy showed no records until 1999, a slow increase in the second decade, and a fast increase in the last decade (Fig. 3).

Therefore, in the last decade, the invasion rate in southern and insular regions seems to follow a pattern similar to the one previously occurred in northern and central regions. Finally, we cannot exclude the role of sampling bias, given that for several taxonomic groups fewer distribution data are available from southern Italy (e.g. Sindaco et al. 2006).

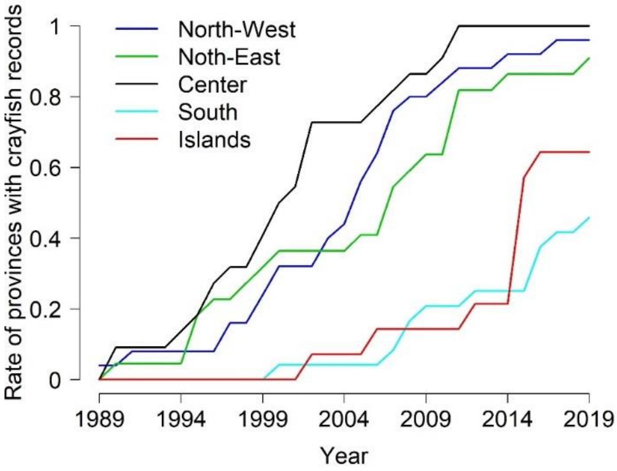

FIGURE 3 Proportion of provinces invaded by *Procambarus clarkii* in five areas of Italy. Regions included in each area: North-West = Aosta Valley, Liguria, Lombardy, Piedmont; North-East = Emilia-Romagna, Friuli-Venezia Giulia, Trentino-Alto Adige, Veneto; Center = Lazio, Marche, Tuscany, Umbria; South = Abruzzo, Apulia, Basilicata, Calabria, Campania, Molise; Islands = Sardinia, Sicily.

To achieve a national-wide representation of the invasion, this study only reports the first records at the provincial level. However, more detailed data can help to improve our understanding of crayfish spread and impact. First, data at a finer spatial grain are needed to understand whether, in a given administrative area, the crayfish has invaded the whole territory or is limited to specific areas/habitats. Second, the impact of invasive species generally increases with their abundance (Leung et al. 2012). Standardized monitoring efforts, providing measures of abundance, would be extremely important to identify the areas where the crayfish impact can be highest. Finally, distribution data can be combined with information on environmental features and species dispersal to identify the drivers of the invasion and help to prevent the invasion of the environments that remain crayfish-free (Siesa et al. 2011; Hefley et al. 2017; Falaschi et al. 2018).

This update of *Procambarus clarkii* distribution in Italy underlines the importance of monitoring invasive species of concern, in order to fill distribution gaps and to map the species distribution in more detail. Given the strong negative impacts of this crayfish on native biodiversity, its current widespread distribution stresses the urgency of rapidly implementing efficient containment strategies to avoid further spread and limit the negative impacts (Falaschi et al. 2020). Predation upon the red swamp crayfish is known for some native species, such as the European eel *Anguilla anguilla* (Aquiloni et al. 2010), the European pond turtle *Emys orbicularis* (Ottonello et al. 2005), the European otter *Lutra lutra* (Prigioni et al. 2009), the red fox *Vulpes vulpes* (Correia 2001), and many birds (Correia 2001; Gherardi 2006; Delsinne et al. 2013). However, predation, particularly by birds, does not seem to negatively affect the persistence of *Procambarus clarkii* populations (Correia 2001). Containment is probably the only way to preserve the remaining non-invaded territories since the eradication of the red swamp on a large scale is currently not feasible due to the huge amount of economic resources needed and to the lack of techniques able to effectively remove crayfish without impacting on native freshwater species (Peay 2001; Aquiloni & Gherardi 2008; Aquiloni et al. 2010; Cecchinelli et al. 2012).

SUPPORTING INFORMATION

Supporting Information is available at: https://escholarship.org/uc/item/94d0z05n#supplemental

CHAPTER 4 | Invasive species override habitat change in determining decline of newts at the regional scale

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Manuscript under review

ABSTRACT

The current biodiversity crisis is caused by the joint effects of multiple processes such as habitat modifications, climate change, spread of diseases, and introduction of invasive species. The relative importance of these drivers can vary through time, but the long-term studies needed to identify changes of threats through time remain scarce. In this work, we analysed 22 years of site occupancy data (from 1996 to 2017) in 63 wetlands suffering the introduction of invasive crayfish, in order to investigate the role of microhabitat, landscape change, and invasive species, in determining the decline of two newt species through time. We performed repeated monitoring to assess the changes in occupancy by newts (Italian crested newts and smooth newts) in Northern Italy. Initial occupancy of newts was negatively affected by landscape alteration (i.e. urban and agricultural cover), and by the presence of fish. Both species suffered a strong decline, with a net loss of site occupancy of 25%–36% along the study period. In 2009 the red swamp crayfish *Procambarus clarkii* was introduced in the study area. After the crayfish invasion, the main drivers of populations dynamics sharply shifted, and occupancy changes were not determined by landscape or microhabitat alterations, as the strongest predictor of local extinctions was the colonization of wetlands by invasive crayfish. If we want to properly identify conservation priorities under continuous environmental changes, we need long-term data on the occurrence of both species and threats, but we also have to consider how the main threatening factors can change over time.

1 | INTRODUCTION

Biodiversity is facing the strongest crisis since the last mass extinction, with extinction rates estimated to be 100 times higher than background rates (Ceballos et al. 2015). The current biodiversity crisis is caused by multiple processes such as habitat modifications, climate change, overexploitation, spread of diseases, atmospheric CO2 increase, anthropogenic nitrogen deposition, and introduction of invasive species (Stuart et al. 2008). However, these drivers do not influence populations independently, but can act together or agonistically at multiple levels complicating the identification of key processes affecting populations and species survival (Didham et al. 2007; Falaschi et al. 2019). Furthermore, the relative importance of different drivers can change through time. For instance, human persecution has been a key driver of the decline of large carnivores in Europe for centuries. However, in the last decades, legal protection has limited direct human impact, and improvements in habitat and in prey availability are now the main determinants of population trends (Chapron et al. 2014).

Quantifying the determinants of population changes over time is therefore a challenging task because it would require long term data on both populations and drivers. The potential drivers are often assessed in snapshot correlative studies, in which species distribution at a given time is related to the spatial variation of candidate stressors. Unfortunately, such snapshot studies can be unable to identify the main factors determining temporal dynamics, and similar studies can even yield strongly contrasting results. For instance, Ficetola et al. (2011) found a negative correlation between the abundance of larval amphibians and the presence of alien crayfish, while similar analyses performed in a different area did not detect strong negative relationships (Bélouard et al. 2019). However, both these studies only considered short periods, while long-term data would be required to understand whether crayfish actually causes a decline of native species. Long-term data, reporting detailed information on both populations and potential stressors over broad scale are urgently needed to identify the key drivers of population changes (Jetz et al. 2019).

Amphibians are an exemplary case of the current biodiversity crisis, with >40% of species recognised as threatened by the International Union for Nature Conservation (Hoffmann et al. 2010). Land use change and alien species are listed among their main threats (Hof et al. 2011). Habitat loss is the threatening factor affecting the largest number of amphibian species, so we might expect it to be the strongest driver of populations trends. However, the intensity of land-use change can vary through space and time. Some areas of the world suffered strong habitat loss through the last centuries, which has been partially compensated by recent rewilding trends (Falcucci et al. 2007; Goldewijk et al. 2011; Hansen et al. 2013). On the other hand, invasive alien species (IAS) are a growing issue (Falaschi et al. 2020). The number of IAS is exponentially increasing at the global scale (Seebens et al. 2017), and IAS now exert heavy impact even in areas with well-conserved habitat (Denoël et al. 2019).

Given that major threats to species can change over time, planning current conservation actions based on stressors that affected populations dynamics decades ago would result in wasted conservation efforts. Hence, there is a need to understand whether the pressure of different drivers is stable or is changing through time. This can be achieved with a long-term monitoring of natural populations and constantly updated assessments of the relative importance of the drivers of decline.

Here we assessed the importance of multiple factors in determining site occupancy of newts at the regional scale between 1996 and 2017, by performing multiple surveys before and after the introduction of an invasive species. We focused on the invasive crayfish, *Procambarus clarkii*, which is among the "100 worst" alien species in the world, and that was introduced in the study area few years after the beginning of our study (Lowe et al. 2000; Fea et al. 2006; Nentwig et al. 2018). First, we identified the determinants of species distribution in the past, evaluating the role of landscape alteration and microhabitat. Second, we tested whether the recent introduction of invasive crayfish modified the relative importance species distribution drivers. Amphibians are particularly sensitive to predation by aquatic alien predators, so the introduction of an invasive crayfish can cause a shift in the main drivers of species occurrence (Cox & Lima 2006; Falaschi et al. 2020). Specifically we predict that, before the introduction of the invasive crayfish, landscape features and microhabitat were the main drivers of newt occurrence (Denoel & Ficetola 2008). However, after the introduction of invasive crayfish, we expect it to become one of the main drivers of changes in occupancy.

2 | MATERIALS AND METHODS

2.1 | Study system

The study area (Lombardy region, Northern Italy) is a human-dominated region, with prevalence of agricultural and urban landscapes (Fig. 1). The southern part of the study area includes suburbs and agricultural landscapes, where wooded areas are small and fragmented. The northern part of the study area, while still dominated by suburbs, is characterised by the presence of several lakes and wooded hilly areas (Fig. 1).

We focused on two native amphibians: the Italian crested newt (*Triturus carnifex*) and the smooth newt (*Lissotriton vulgaris*). Newts spend their reproductive period and the larval phase in the aquatic environment, even if in some cases they remain in the water through the year. During the terrestrial phase, they live in small natural or semi-natural microhabitats nearby breeding sites (Joly et al. 2001; Schabetsberger et al. 2004; Denoel & Ficetola 2008). The Italian crested newt is declining because of landscape anthropization, climate change, intensive farming, and introduction of aquatic predators (Ficetola & De Bernardi 2004; Romano et al. 2009). The smooth newt is more widespread, still it is sensitive to aquatic predators and landscape alteration (Denoël 2012).

The red swamp crayfish *Procambarus clarkii* is native of North America and was

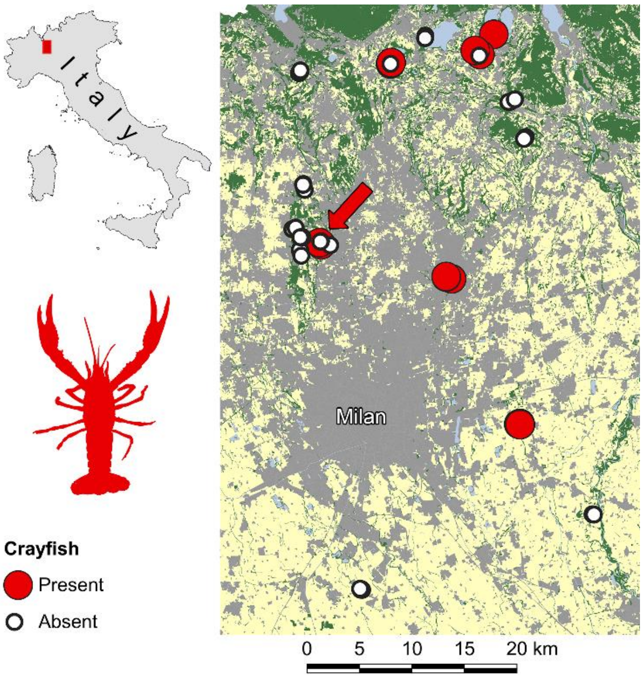

FIGURE 1 Distribution of alien crayfish (*Procambarus clarkii*) in the study area in 2017, with indication of the first site of detection (red arrow).

introduced in a few wetlands considered in this study in 2009. Natural and human-driven dispersal events allowed the crayfish to quickly spread, and the crayfish has now invaded many wetlands across the whole study area (Siesa et al. 2011). Field activities were performed between 1996 and 2017 and, during this time frame, we monitored 63 ponds and ditches (hereafter: wetlands). All the wetlands were monitored at least in two different sampling seasons (i.e. in two different years). Each site was monitored at least once before 2009, and at least once after 2009 (average number of sampling seasons per site = 2.9; SD = 1.0). The timespan between the first and the last sampling season ranged from 6 to 21 years (average ± SD = 13.3 ± 4.5 years). Surveys took place between February and September and, because of the limited detectability of newts, in each sampling season we carried out multiple surveys in each wetland (2 – 7 field surveys per year; average = 4.5 ± 0.9). During field surveys, we recorded the detection/non-detection of the newts and of the crayfish using visual encounter surveys and dip-netting (Halliday 2006).

2.2 | Microhabitat characteristics and landscape features

In each sampling season, we recorded three microhabitat characteristics of the surveyed wetlands: presence / absence of fish, maximum depth, and hydroperiod (permanent / temporary; a site was considered permanent if it never dried up throughout the sampling season), following the protocols described in Ficetola et al. (2011). The occurrence of the invasive crayfish in the wetlands was considered as an additional potential driver of newt distribution. The detection probability of the crayfish is high, and previous studies showed that two surveys allow to ascertain presence / absence of the crayfish with reliability >99% (Manenti et al. 2019). We extracted two variables representing landscape composition from a land use map of Lombardy region (http://www.cartografia.regione.lombardia.it

; ground resolution: 3m), in order to assess the impact of the modification of terrestrial environments. The land use map covers the whole regions, is regularly updated by local authorities and is available for years 2000, 2005, 2009, 2012, and 2015. For each sampling season, landscape composition was calculated based on the nearest temporal update of the map. For each wetland and sampling season, we calculated the percentage cover of agricultural and urban areas, as specified in the level-1 classification of the land use map, within a radius of 400m from each wetland, as many amphibians require a buffer zone of ~ 400m of terrestrial habitat (Joly et al. 2001; Ficetola et al. 2009).

2.3 | Statistical analyses

Analyses were carried out in two steps. First, we assessed which environmental characteristics were the main drivers of the presence / absence of newts in each wetland in the first sampling season, which always took place before the introduction of the alien crayfish in the study area. Second, we examined whether the changes in environmental characteristics and the colonization of crayfish determined the changes in newt occupancy over time.

In habitat selection studies, including sites that could not be reached because of dispersal limitations can bias the results of analyses (Godsoe 2010). For instance, relationships between species occurrence and environmental characteristics can be masked by the absence of a species from suitable sites due to dispersal but not ecological reasons (Godsoe 2010). Therefore, for each species, we excluded wetlands where the focal species was never detected throughout the whole sampling period and that were too isolated to be reached by dispersing individuals. For each species, we only considered wetlands that were within 1200 m from wetlands where the target species has been detected at least once during the study periods. This distance roughly corresponds to the maximum dispersal ability of newts (Glandt 1986; Smith & Green 2005). As the two species were not always present in the same network of wetlands, the analyses were carried out on slightly different sets of sites (48 wetlands for Italian crested newts and 59 wetlands for smooth newts, fig. S1). This procedure does not influence the outcome of the analysis of changes in occupancy, since we excluded wetlands where the species is always absent, while it allows to avoid biases in the analysis of the drivers of presence / absence during the first sampling season.

2.4 | Dynamic occupancy models

Species present in an area can remain undetected, thus we used dynamic occupancy modelling to identify the environmental features related to newt occupancy at the beginning of the study period, and to estimate their rate of extinction and colonization (MacKenzie et al. 2003). Dynamic occupancy models are suitable for estimating population dynamics in species for which detection probability is less than one, because they explicitly consider both detection probability and the processes underlying site occupancy (Kéry et al. 2013). We implemented the models in the R package unmarked (Fiske & Chandler 2011) using a dynamic approach that estimates site occupancy probability in a given year as the sum of (1) the probability that a site was occupied in the previous year and the species did not become locally extinct, plus (2) the probability that a site was not occupied in the previous year and the species colonised it. This model accounts for imperfect detection, by modelling each series of detection/non-detection from occupied sites as a Bernoulli trial in order to estimate the detection probability (Kéry et al. 2013).

In our analyses, we used dynamic occupancy models to: (i) assess extinction and colonization rates of the two species, and (ii) evaluate the factors determining newt occupancy in the first year of sampling. Because sampling took place in different years for different areas, the first year of monitoring is not the same for every wetland, but it corresponds to the year of the first sampling performed at each site, which always occurred before the introduction of invasive crayfish.

Extinction and colonization rates were used to evaluate the status of conservation of newts in the study area, in order to better understand the temporal dynamics of the decline of these species. In dynamic occupancy models a species is not considered as present or absent, instead, a probability of presence at each site is calculated. Changes in the probability of presence are used to obtain estimates of extinction and colonization rates. Adding possible covariates influencing detection probability can allow obtaining more reliable estimates of the probability of occurrence and, consequently, more reliable extinction and colonization rates. In occupancy models, we related detection probability to three variables: the date (expressed as Julian Day), considering both the linear and the quadratic term, and hour of the survey.

Initial occupancy, i.e. the probability of occurrence in the first sampling season, was related to the three microhabitat (fish presence, depth, and hydroperiod) and two landscape (agricultural and urban cover) variables. We did not detect strong correlation between independent variables (Pearson's correlation: $|r| < 0.3$ for all variables; supplementary tables S1 and S2), and in all models, variance-inflation factor values were <1.2, suggesting limited multicollinearity issues. Water depth was log-transformed to improve normality. For each species, we built models including all the possible combination of the five independent variables determining occupancy and of the three predictors determining detection probability. We then used the corrected Akaike Information Criterion (AICc) to rank the models: the model with the lowest AICc value is the one that explains more variation with fewest variables and is hence considered the best models (Burnham & Anderson 2002). AICc can select overly complex models, therefore we considered a complex model as a candidate only if it showed AICc values lower than the AICc of all its simpler nested models (Richards et al. 2011). We used a likelihood ratio test (χ^2 approximation) to estimate the significance of variables included in the best-AICc models. For each species, we compared the likelihood of best-AIC model to the one of models with the same structure but missing one independent variable at time (Venables & Ripley 2002) (Table S3).

2.5 | Drivers of changes in occupancy

Dynamic occupancy models can measure the relationships between the changes in occupancy and the changes in environmental characteristics (i.e. microhabitat characteristics and landscape features) only when data from at least three sampling seasons are available (Kéry M. pers. comm.; see https://groups.google.com/forum/#!msg/unmarked/ySBIbcg9ch8/iNfeStRZBAAJ). Some wetlands were monitored in just two sampling seasons, thus we performed a separate analysis to identify the environmental factors determining the extinctions / colonizations of newts. In so doing, for each species, we calculated the occupancy probability at each site in each sampled year, as estimated by the dynamic occupancy model. Subsequently, we calculated the changes in occupancy probability between a sampling season and the previous one as follows:

$$\text{(eq. 1) } \Delta\psi_i = \psi_{i,k} - \psi_{i,k-1}$$

where ψ indicates the occupancy, i indicates the site, and k indicates the year of monitoring. The resulting $\Delta\psi$ was used as response variable in mixed models. Then, for each site, we calculated the changes in each environmental characteristic between each sampling season and the previous one and included these as independent variables. For each species, we only considered environmental features included in the best dynamic occupancy models. Additionally, we considered a variable representing crayfish invasion at each site. Site identity was included as random effect, resulting in the model structures described in eq. 2 for Italian crested newts, and in eq. 3 for smooth newts.

$$\text{(eq. 2) } \Delta\psi \sim \Delta_{Urban} + \Delta_{Crayfish} + 1|site$$

$$\text{(eq. 3) } \Delta\psi \sim \Delta_{Urban} + \Delta_{Agricultural} + \Delta_{Fish} + \Delta_{Crayfish} + 1|site$$

where $\Delta\psi$ indicates the changes in occupancy, the other Δs indicate the changes in environmental features, and $1|site$ is the identity of the site included as a random effect.

Finally, we calculated the effect size of the variables related to initial occupancy (based on likelihood ratio values) and to changes in occupancy (based of the t-values of mixed models), using Fisher's *z*. Analyses were performed in the R environment (R Core Team 2018), using the packages unmarked for building dynamic occupancy models (Fiske & Chandler 2011), MuMIn for model selection (Bartoń 2018), nlme to build mixed models (Pinheiro et al. 2018), and compute.es to calculate effect sizes (del Re 2013).

3 | RESULTS

3.1 | Dynamics of newt occupancy

The Italian crested newt was detected in 22 wetlands in the period 1996-2009, and in 22 wetlands in 2010-2017 (Fig. 2). Occupied wetlands in the first and the second period were different in many cases, with several local extinctions and colonizations. The detection probability of Italian crested newts was related to the date of the survey with a quadratic relationship (Tables 1a and S4a), indicating that detection probability was highest in surveys carried out in early June (Fig. S2). According to the best-AICc model, the initial occupancy of Italian crested newts was negatively related to urban cover within 400 m from the wetland (Tables 1a and S4b, Fig. 3a). The average local extinction rate of Italian crested newts was 21% (95% confidence intervals, 95% CIs: 13 / 32%), while the average colonization rate was 8% (95% CIs: 4 / 17%). Overall, estimated occupancy declined from 53% in 1996-2009, to 40% in the last survey.

The smooth newt was detected in 27 wetlands in the period 1996-2009, and in 23 wetlands in 2010-2017. The detection probability of smooth newts showed a positive relationship with the hour and the date of the survey (Tables 1b and S4b), indicating that detection probability was highest in surveys carried out late in the night and in summer (Fig. S3). According to the best-AICc model, the initial occupancy of smooth newts was related to three variables: urban cover and agricultural cover within 400 m from the wetland, and fish presence (Tables 1b and S4b, Fig. 3c). The occupancy probability of smooth newts was negatively related with agricultural cover (Table 1b), indicating that this newt is more frequent in natural landscapes. Furthermore, smooth newts were more frequent in fishless wetlands (Table 1b). Smoot newt occupancy was also negatively related to urban cover, but this relationship was not significant (*P* = 0.07; Table 1b). The average extinction rate of smooth newts in sub-populations was 21% (95% CIs: 13% / 31%), while the average colonization rate was 5% (95% CIs: 2% / 12%). Overall, occupancy declined from 53% in 1996-2009, to 34% in the last survey.

3.2 | Land-use changes and the invasion of *Procambarus clarkii*

Urban cover within 400m from the study wetlands showed little change over time, with average cover ~20%. The agricultural cover showed a slow decrease, from ~34% in 2000 to ~30% in 2015 (see Fig. S4).

While the introduction of the invasive crayfish in Lombardy region dates back to 1997 (Lo Parrino et al. 2020), it was first detected within the study wetlands in 2009. This first detection occurred in wetlands in the Groane Regional Park, in the western part of the study area (Fig. 1). The crayfish quickly

TABLE 1 Estimated parameters and standard errors for the best occupancy models for s) Italian crested newt, and b) Smooth newt. For independent variables related to detection and occupancy probabilities, the table reports χ^2 and significance values obtained with likelihood ratio tests (see Table S3 a list of the compared models).

a) Italian crested newt

process / variable	*B* (logit scale)	SE	χ²	*P*
Initial occupancy				
Intercept	0.88	0.47		
Urban	-12.94	6.12	7.63	0.006
Colonization				
Intercept	-2.37	0.39		
Extinction				
Intercept	-1.32	0.29		
Detection				
Intercept	-0.53	0.13		
Day	1.23	0.14	102.40	< 0.001
Day^2	-0.40	0.09	31.81	< 0.001

b) Smooth newt

process / variable	*B* (logit scale)	SE	χ²	*P*
Initial occupancy				
Intercept	1.50	0.67		
Agricultural	-4.05	1.98	4.82	0.028
Fish	-1.79	0.93	4.28	0.039
Urban	-6.66	3.88	3.28	0.070
Colonization				
Intercept	-2.90	0.45		
Extinction				
Intercept	-1.34	0.29		
Detection				
Intercept	-1.640	0.36		
Day	0.237	0.09	6.39	0.011
Hour	0.434	0.14	11.05	0.001

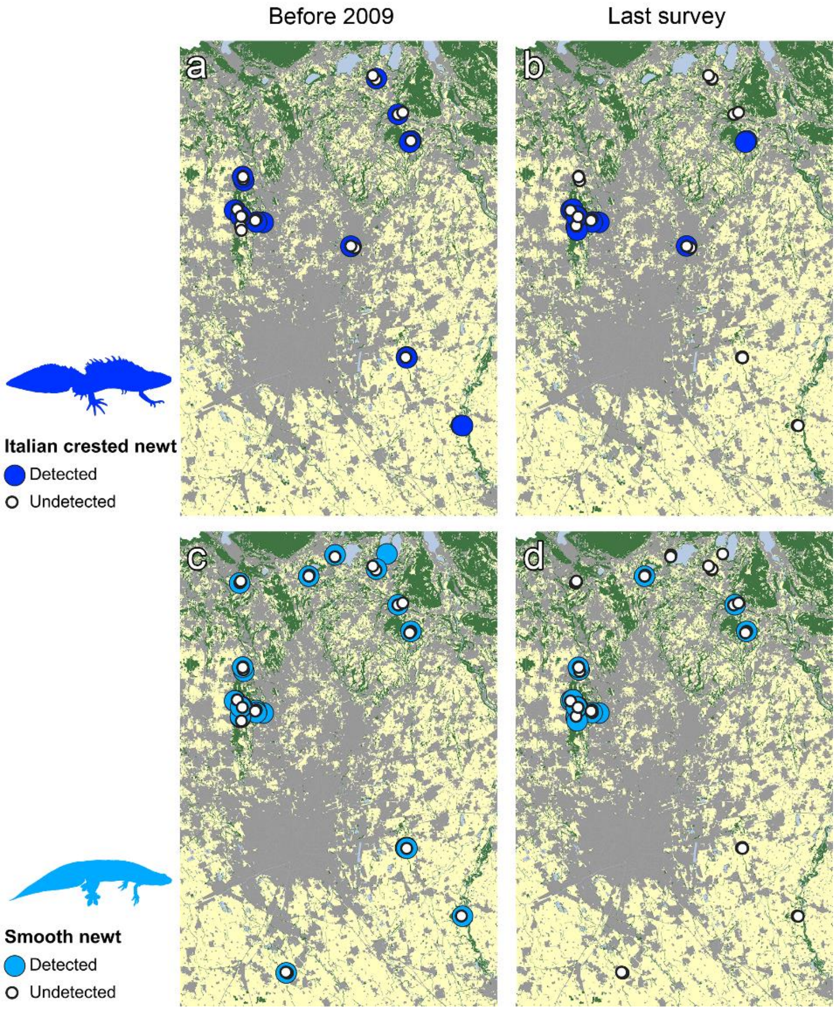

FIGURE 2 Distribution of newts, before and after the introduction of the invasive crayfish. Distribution of (a, b) Italian crested newts before the introduction of invasive crayfish in the study area and in the last survey, and distribution of (c, d) smooth newts before the introduction of invasive crayfish in the study area and in the last survey.

TABLE 2 Drivers of changes in the probability of presence of (a) Italian crested newts and (b) smooth newt through time. *B* = coefficient of the relationship between the independent variable and changes in occupancy; SE = standard error; df = degrees of freedom; *t* = *t*-value.

a) **Italian crested newt**

Variable	***B***	**SE**	**df**	***t***	***P***
Urban expansion	-0.76	0.47	1, 53	-1.56	0.12
Crayfish colonization	-0.56	0.17	1, 53	-3.37	0.001

b) **Smooth newt**

Variable	***B***	**SE**	**df**	***t***	***P***
Urban expansion	-0.19	0.73	1, 48	-0.26	0.80
Agricultural expansion	-0.42	0.65	1, 48	-0.65	0.52
Fish colonization	0.004	0.14	1, 48	0.03	0.98
Crayfish colonization	-0.44	0.17	1, 48	-2.62	0.01

spread due to both natural and human-driven dispersal events, and in 2017 it was detected in 27% of wetlands (Fig. 1).

3.3 | Drivers of occupancy changes

For Italian crested newts, occupancy changes were negatively related to changes in crayfish occurrence (Table 2a, Fig. 3b), i.e. newt extinctions occurred in wetlands colonised by the crayfish. Although there was a negative relationship between changes in occupancy and changes in urban cover, this relationship was not statistically significant (Table 2a, Fig. 3b).

For smooth newts, occupancy changes were negatively related to changes in crayfish occurrence (Table 2b, Fig. 3d), while they were unrelated to either landscape changes or changes of fish presence (Table 2b, Fig. 3d).

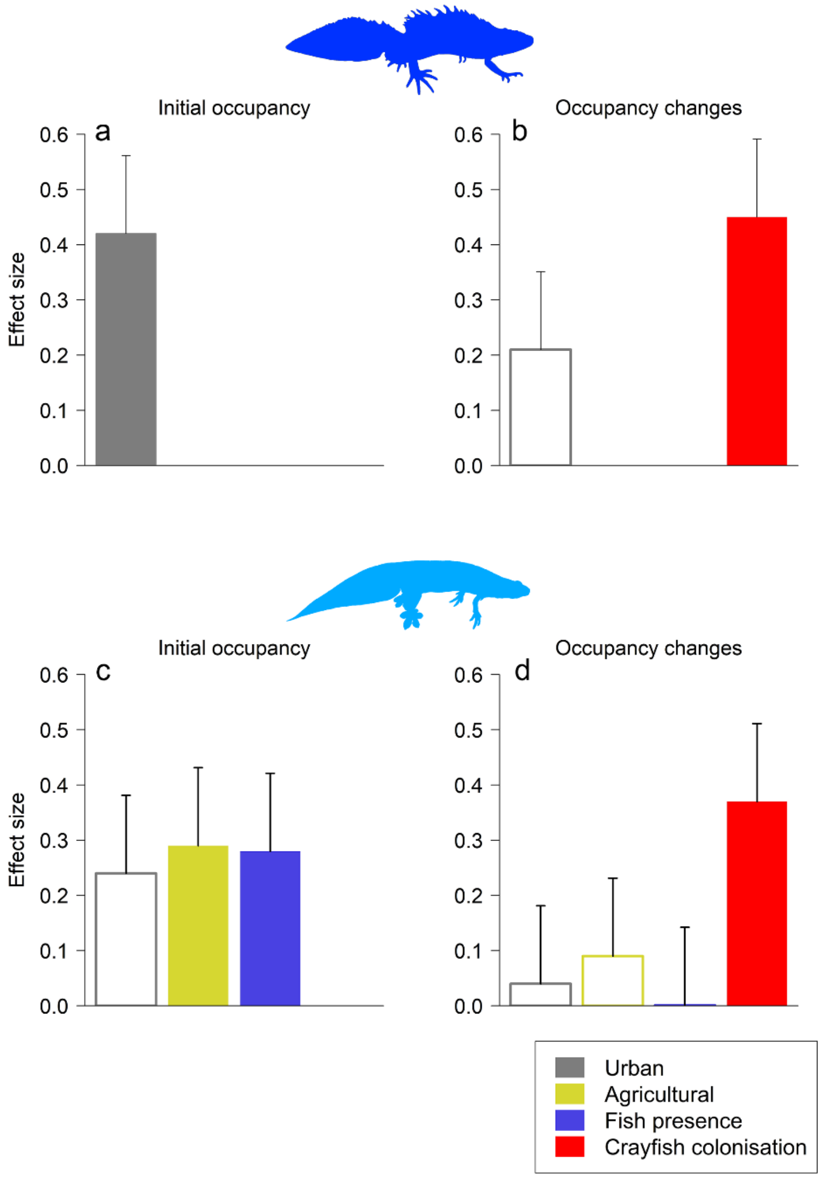

FIGURE 3 Effect size (Fisher's *z*) of the relationships between independent variables and newt distribution, before and after the introduction of the invasive crayfish. Effect size for (a, b) Italian crested newts before the introduction of invasive crayfish in the study area and in the last survey, and effect size for (c, d) smooth newts before the introduction of invasive crayfish in the study area and in the last survey. Filled bars represent significant relationships, empty bars represent non-significant relationships, error bars are the standard deviation of *z*.

4 | DISCUSSION

Assessing the drivers of population occurrence and extirpation is a key challenge of conservation biology, still this task can be difficult when new threats arise and the relative importance of stressors changes through time. Amphibians are vulnerable to multiple threatening factors occurring both in aquatic and in terrestrial environments and are thus a main target for conservation research (Blaustein & Kiesecker 2002; Beebee & Griffiths 2005; Gardner et al. 2007; Ficetola 2015; Falaschi et al. 2019). Despite human alterations of landscape and wetlands were the key drivers of newt occupancy in the last decades, our study shows that recently introduced invasive species are overriding these impacts, heavily accelerating the rate of population loss.

Over the last 22 years, Italian crested newt and smooth newt populations showed a clear decline, with local extinction rates significantly higher than colonization rates (crested newt: extinction rate 21%, colonization rate 8%; smooth newt: extinction rate 21%, colonization rate 5%). Unfortunately, newts are declining across the whole Europe both in human-dominated and in relatively natural landscapes (Curado et al. 2011; Denoël 2012; Denoël et al. 2019), at rates similar to the ones observed here. This net loss of local populations stresses the urgency of understanding the drivers of local extinctions in order to allow effective conservation planning.

At the beginning of the surveys, newt occupancy was strongly related to both microhabitat characteristics and landscape features. Italian crested newts were associated with the less urbanised landscapes, while the distribution of smooth newts was negatively related to agricultural cover and fish occurrence. Many amphibians require aquatic habitats for breeding and terrestrial habitats for the post-breeding activities, hence the features of terrestrial landscapes surrounding wetlands are essential to complete their life cycle. Human modifications of the landscape, such as urbanization and agriculture, have negative impacts on both terrestrial and aquatic habitats exploited by newts, therefore landscape alterations are increasingly recognised as major determinants of the distribution of amphibians (Denoel & Ficetola 2007, 2008; Hartel et al. 2010; Denoel et al. 2013). Urbanization can clear large portions of available habitats and increase isolation (Hamer & McDonnell 2008). Similarly, agriculture can cause the loss of reproductive sites and of terrestrial shelters that are necessary for the amphibian life cycle (Denoel & Ficetola 2008). Moreover, the runoff of pesticides used in agriculture into wetlands can strongly impact the aquatic life history stages, determining breeding failure (Boone & Semlitsch 2001; Relyea et al. 2005; Ortiz-Santaliestra et al. 2009). The distribution of smooth newt was also negatively related to fish occurrence. Our study focused on small wetlands, most of which are naturally fishless. Humans often introduce fish in these wetlands for angling or ornamental purposes. Newt larvae are nektonic and require a long time to complete development, thus they are highly exposed to fish predation, and our study confirms the heavy impact of introduced fish on these animals (Denoel & Ficetola 2008; Denoel et al. 2013).

In this analysis we found less variables related to newt distribution, compared to previous studies performed in the same area. For instance, previous works observed relationships between the distribution of newts and wetland features (hydroperiod and depth) (Ficetola & De Bernardi 2004; Ficetola et al. 2011), that were not observed here. Compared to these studies, here we considered a lower number of sites, because we focused on wetlands that received multiple surveys before and after the introduction of alien crayfish. This probably reduced the power of analyses assessing the initial impact of microhabitat features but allowed to maximise the cost efficiency of surveys aiming at detecting the drivers of occupancy change.

When considering the recent temporal dynamics of newt distributions, the main drivers of newt occupancy changed. While at the beginning of the study microhabitat and landscape features were the main determinants of occupancy, after the introduction of the crayfish population changes were not anymore related to these features (Fig. 3). Local extinctions generally occurred in wetlands invaded by the crayfish, while the effects of habitat or landscape change were much weaker and not significant anymore. Alien species are among the threats most commonly associated with the recent extinctions of vertebrates due to their ability to interact with native species through multiple processes (Bellard et al. 2016; Falaschi et al. 2020) and they can determine impressive declines even in relatively well conserved habitats (Denoël et al. 2019). Freshwaters are among the environments most threatened by invasive alien species, because of the intensity at which human use these environments for recreational, food, and commercial purposes (Strayer 2010).

Invasive crayfish, such as the red swamp crayfish, can heavily affect freshwater food webs through multiple processes. First, they are voracious omnivores that prey on multiple amphibian species (Cruz & Rebelo 2005; Gherardi 2006). Second, the crayfish can reduce the biomass and richness of macroinvertebrates, macrophytes, and periphyton, and increases water turbidity and the abundance of phytoplankton, thus determining overall shifts of ecosystems (Gherardi 2006; Matsuzaki et al. 2009). The overall impacts of this invasive species can thus be particularly strong. For instance, alien crayfish occurrence determined a 54% decrease in the richness of amphibian species in Portuguese natural reserve (Cruz et al. 2008). Nevertheless, several species that disappear in wetlands colonised by crayfish continue to be present in nearby wetlands without crayfish (Cruz et al. 2008), and lack of long-term studies determined uncertainty on the overall consequences of crayfish invasion (Bélouard et al. 2019; Manenti et al. 2020). The alien crayfish was first observed in the study sites in 2009 and, eight years after the first introduction, its effects override the impact of landscape changes or of fish distribution (Fig. 3). Newts are heavily sensitive to invasive predators, and the impact of the introduction of alien crayfish on amphibian populations can be so strong that it can overcome the effects of habitat change (Ficetola et al. 2011). Nevertheless, it should also be remarked that in the last decade habitat changes have been limited within the study area (Fig. S4).

In an era of rapid global change, local drivers of population decline can vary over time. However, monitoring programs are rarely designed to identify potential changes in the drivers of decline, also because many studies only cover short time periods. Had we relied on newt distribution data collected 15 years ago, we would have concluded that preserving natural landscapes and maintaining fishless wetlands would be the key management strategies. Conversely, had we only performed the recent surveys, we would have identified the effects of invasive crayfish only, probably overlooking the importance of habitat and landscape. It is increasingly evident that snapshot studies are not enough to identify biodiversity threats under rapid global change. Instead, a comprehensive evaluation of factors determining species distribution and decline requires long-term data on the occurrence of both species and threats (Nichols & Williams 2006), also considering how the main threatening factors can change over time. This approach is challenging and data demanding, still it is essential shall we want a prompt identification of conservation priorities and to plan effective management strategies.

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SUPPORTING INFORMATION

Table S1 Pearson's correlation coefficient between the independent variables used in the dynamic occupancy model for Italian crested newts.

	Fish	**Depth**	**Hydroperiod**	**Urban**	**Agricultural**
Fish	1	0.247	0.162	-0.067	-0.102
Depth	0.247	1	0.282	-0.287	0.042
Hydroperiod	0.162	0.282	1	0.0021	0.210
Urban	-0.066	-0.287	0.002	1	-0.237
Agricultural	-0.102	0.0423	0.210	-0.237	1

Table S2 Pearson's correlation coefficient between the independent variables used in the dynamic occupancy model for smooth newts.

	Fish	**Depth**	**Hydroperiod**	**Urban**	**Agricultural**
Fish	1	0.148	0.192	-0.113	-0.075
Depth	0.148	1	0.281	-0.220	-0.057
Hydroperiod	0.192	0.281	1	0.031	0.013
Urban	-0.113	-0.220	0.031	1	-0.162
Agricultural	-0.0749	-0.057	0.013	-0.162	1

Table S3 Comparison of models to test the significance of the variables in the dynamic occupancy models (likelihood ratio tests) for a) Italian crested newts, and b) smooth newts. For each model, we show the tested variable (variable excluded from the model), the model structure, the log-likelihood, and χ^2 and *P* values obtained through the likelihood ratio test. We also show the best model for comparison. Brackets indicate variables related to: ψ = initial occupancy, γ = colonization probability, ε = extinction probability, p = detection probability. Int = intercept.

a) Italian crested newt

Excluded variable	**Model structure**	**log-likelihood**	χ^2	$P_{(df = 1)}$
Best model	ψ(Int. + Urban); γ(Int.); ε(Int.); p(Int. + Day + Day^2)	-355.340		
Urban	ψ(Int.); γ(Int.); ε(Int.); p(Int. + Day + Day^2)	-359.156	7.633	0.006
Day	ψ(Int. + Urban); γ(Int.); ε(Int.); p(Int. + Day^2)	-406.541	102.403	< 0.001
Day2	ψ(Int. + Urban); γ(Int.); ε(Int.); p(Int. + Day)	-371.243	31.807	< 0.001

b) Smooth newt

Excluded variable	**Model structure**	**log-likelihood**	χ^2	$P_{(df = 1)}$
Best model	ψ(Int. + Agricultural + Fish + Urban); γ(Int.); ε(Int.); p(Int. + Day + Hour)	-423.796		
Agricultural	ψ(Int. + Fish + Urban); γ(Int.); ε(Int.); p(Int. + Day + Hour)	-426.207	4.822	0.028
Fish	ψ(Int. + Agricultural + Urban); γ(Int.); ε(Int.); p(Int. + Day + Hour)	-425.936	4.279	0.039
Urban	ψ(Int. + Agricultural + Fish); γ(Int.); ε(Int.); p(Int. + Day + Hour)	-425.434	3.276	0.070
Day	ψ(Int. + Agricultural + Fish + Urban); γ(Int.); ε(Int.); p(Int. + Hour)	-426.991	6.390	0.011
Hour	ψ(Int. + Agricultural + Fish + Urban); γ(Int.); ε(Int.); p(Int. + Day)	-429.320	11.048	0.001

Table S4 Support of the best model compared to alternative models for a) Italian crested newts and b) smooth newts occupancy at the beginning of the study. The coefficients of variables related to occupancy (urban, agricultural, fish, depth, hydroperiod) and detection (date, date^2, hour) probabilities are shown; the table reports all the models with AICc weight > 0.01. AICc: Akaike Information Criterion corrected; K: number of parameters; weight: AICc weight. For the best models, "*" indicates significant variables.

a) Italian crested newt

AICc	**K**	**weight**	**Coefficients**							
			Urban	**Agricultural**	**Fish**	**Depth**	**Hydroperiod**	**date**	**date^2**	**hour**
727.48	7	0.92	-12.94*					1.22*	-0.40*	
732.36	6	0.08						1.22	-0.40	

b) Smooth newt

AICc	**K**	**weight**	**Coefficients**							
			Urban	**Agricultural**	**Fish**	**Depth**	**Hydroperiod**	**date**	**date^2**	**hour**
869.27	9	0.25	-6.66	-4.05*	-1.79*			0.24*		0.43*
869.75	8	0.20		-3.35	-1.56			0.24		0.44
870.39	7	0.15		-3.02				0.24		0.44
870.55	7	0.13			-1.38			0.24		0.43
870.68	6	0.13						0.24		0.44
872.86	8	0.04	-6.72	-4.07	-1.88					0.48
873.51	7	0.03		-3.31	-1.64					0.48
874.35	6	0.02			-1.46					0.48
874.55	6	0.02		-2.98						0.49
874.89	5	0.02								0.49

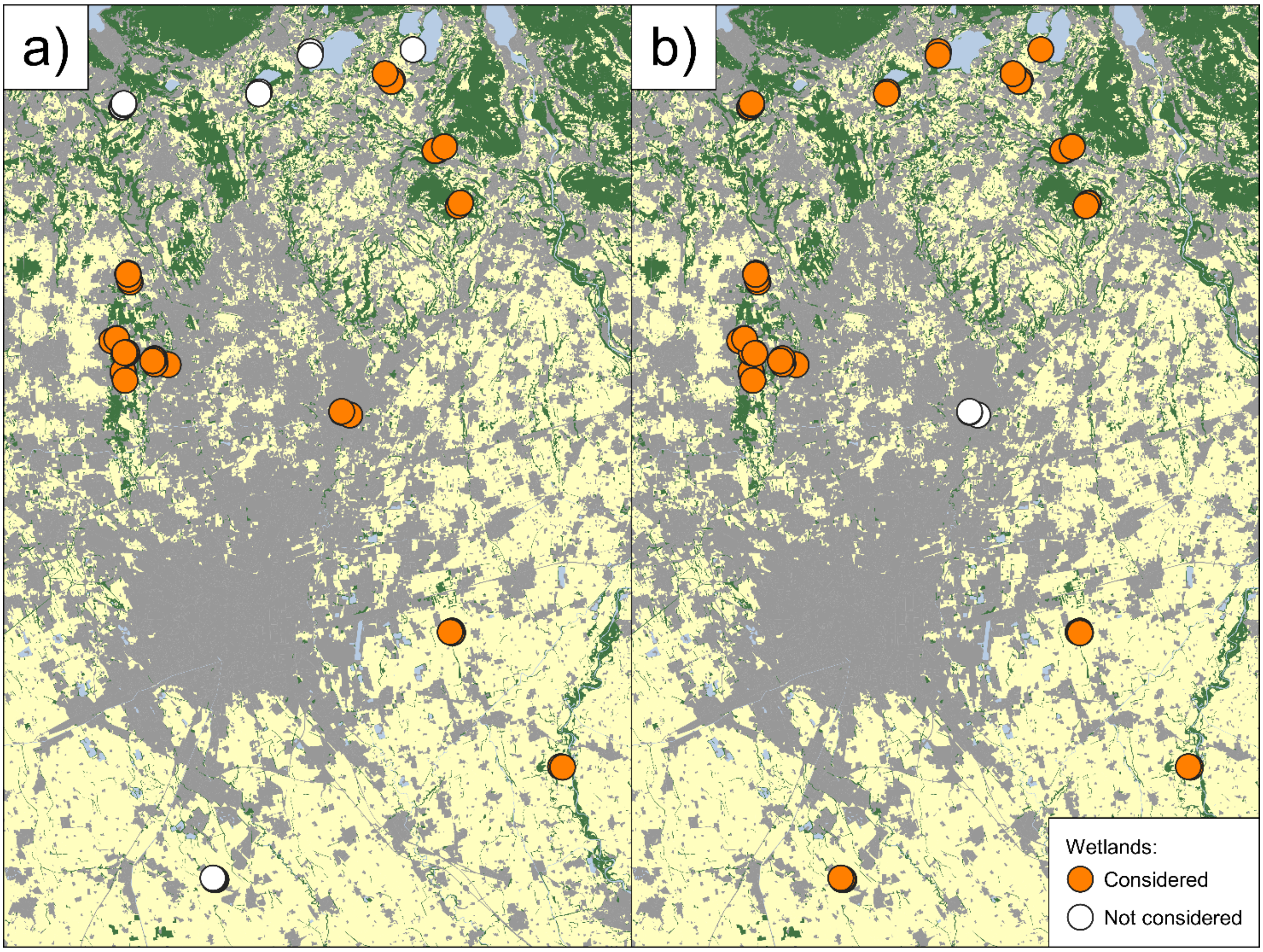

Figure S1 Wetlands considered in the analyses for a) Italian crested newts (N = 48), and b) smooth newts (N = 59). White dots represent wetlands not considered because they are too isolated (>1200 m) from any known wetland occupied by the target species.

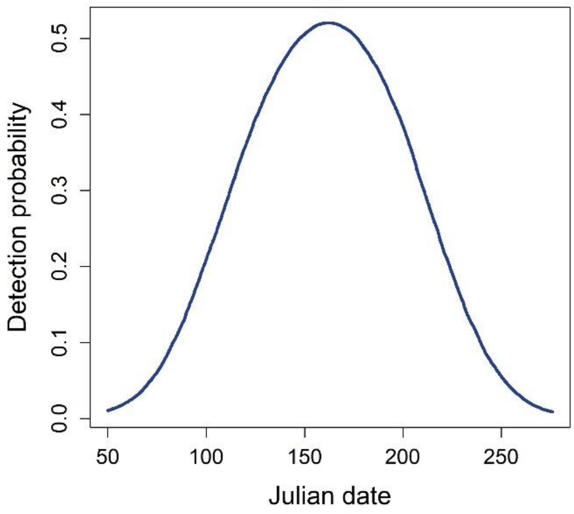

Figure S2 Relationship between detection probability and date of the survey for Italian crested newts.

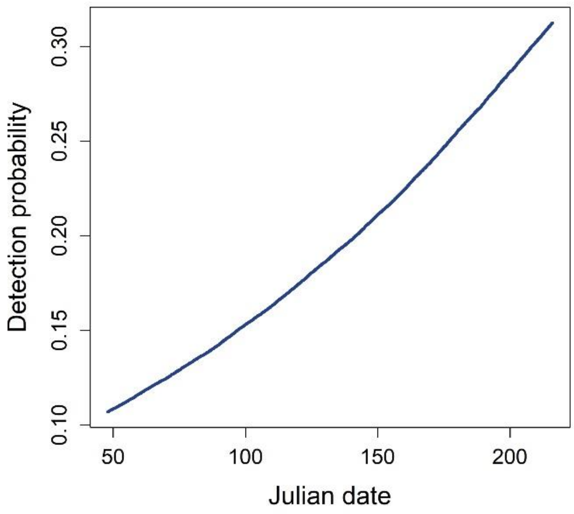

Figure S3 Relationship between detection probability and date of the survey for smooth newts.

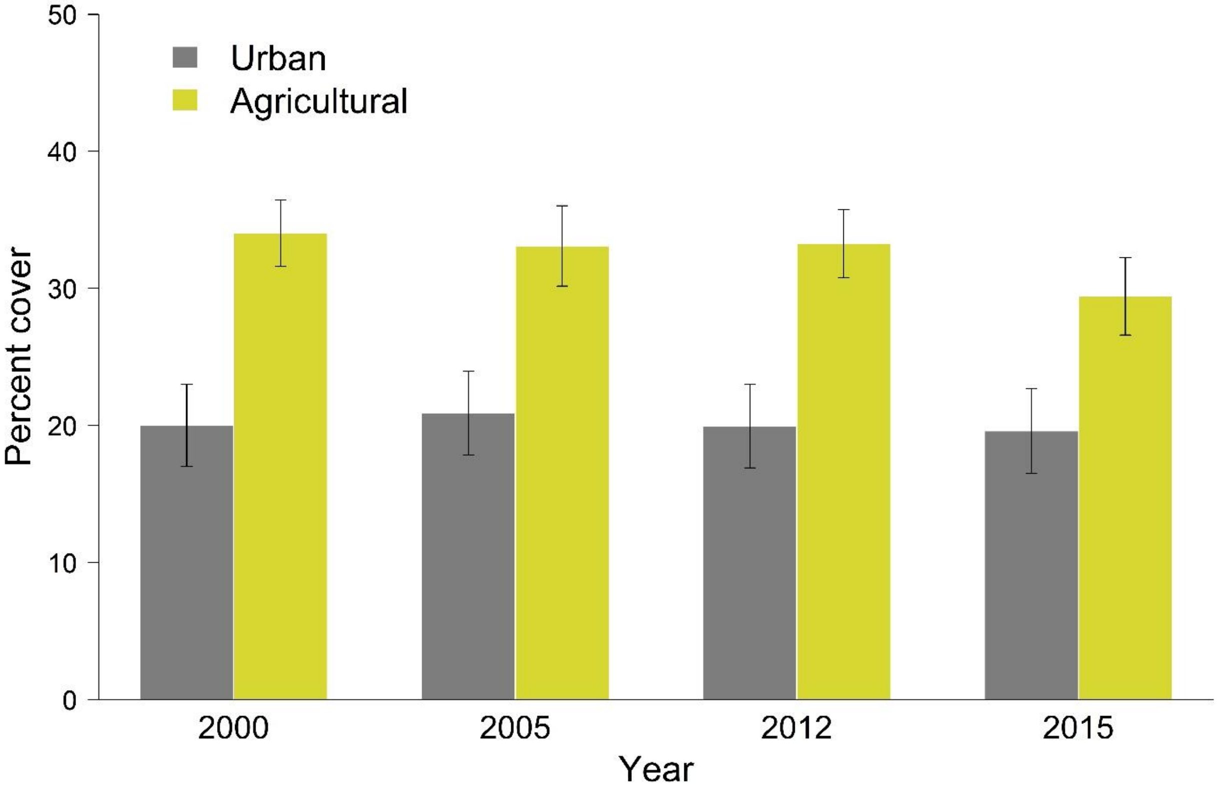

Figure S4 Urban and agricultural cover at all sites in years 2000, 2005, 2012, and 2015. Bars represent the standard errors. We tested the significance of changes in urban and agricultural cover by performing two linear mixed models using land cover (urban or agricultural) as response variable, year as independent variable, and site identity as a random effect. Urban change: B = -0.042, SE = 0.036, $F_{1,\,191}$ = 1.32, P = 0.253. Agricultural change: B = -0.236, SE = 0.072, $F_{1,\,191}$ = 10.62, P = 0.001.

CHAPTER 5 | Long-term drivers of survival and colonization dynamics in spatially structured amphibian populations

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ABSTRACT

Many organisms live in networks of local populations connected by dispersing individuals, called spatially structured populations (SSPs), where the long-term persistence of the entire network is determined by the balance between two processes acting at the scale of local populations: extinction and colonization. When multiple threats act on an SSP, a comparison of the different factors determining local extinctions and colonizations is essential to plan sound conservation actions. Here we assessed the drivers of long-term population dynamics of multiple amphibian species at the regional scale. We used dynamic occupancy models within a Bayesian framework to identify the factors determining survival and colonization of local populations. Since connectivity among patches is fundamental for SSPs dynamics, we considered two measures of connectivity acting on each focal patch: incidence of the focal species, and incidence of invasive crayfish. We used meta-analysis to summarize the effect of different drivers at the community level. Survival and colonization of local populations were jointly determined by factors acting at different scales. Survival probability was positively related to the area and the permanence of wetlands, while it showed a negative relationship with the occurrence of fish. Colonization probability was highest in semi-permanent wetlands, and in sites with a high incidence of the focal species in nearby sites, while it showed a negative relationship with the incidence of invasive crayfish in the landscape. By analyzing long-term data on amphibian population dynamics, we found a strong effect of some classic features commonly used in SSP studies such as patch area and focal species incidence. The presence of an invasive alien species at the landscape-scale emerged as one of the strongest drivers of colonization dynamics, suggesting that studies on SSPs should consider different connectivity measures more frequently, such as the incidence of predators, especially when dealing with biological invasions.

1 | INTRODUCTION

Studying the factors that influence species distribution is a cornerstone of conservation biology, because it allows assessing how environmental modifications determine population declines and extinctions (Grant et al. 2016; Falaschi et al. 2019; Northrup et al. 2019). Although understanding the processes determining the trends of species and populations is essential to develop management plans and counteract biodiversity loss, many studies do not consider the spatial connections between populations. Many organisms live in networks of local populations that occupy discrete habitats, connected by dispersing individuals (spatially structured populations; SSPs) (Thomas & Kunin 1999; Revilla & Wiegand 2008). The long-term persistence of SSPs is determined by the balance between two processes acting at the scale of local populations: extinction and colonization (Revilla & Wiegand 2008). In a SSP network, stochastic processes can drive local populations to extinction. The risk of local extinction is particularly high for small populations, which are subject to demographic and genetic stochasticity. Spatial connections with nearby populations limit the risk of extinction and can allow the recolonization of unoccupied patches; hence, connectivity among patches is a fundamental process to take into consideration when dealing with SSPs (Hanski 1998; Moilanen & Nieminen 2002).

Understanding the drivers of SSP dynamics is complicated by the fact that the same environmental factor can influence both local extinctions and colonizations. For example, the presence of predators in a breeding patch can increase the rate of local extinctions but can also reduce the chance of subsequent recolonization, as dispersing individuals often select predator-free patches (Trekels & Vanschoenwinkel 2019). When multiple threats are acting on a species or a community, comparing how different factors affect local extinctions and colonizations is essential to plan sound conservation actions and for prioritizing threat mitigation (Tulloch et al. 2016; Cayuela et al. 2018).

Amphibians are the group showing the highest rate of decline within vertebrates (Hoffmann et al. 2010). Alien species are among the greatest threat to amphibians and are involved in many declines and extinctions both at the population and at the species level (Stuart et al. 2004; Bellard et al. 2016; Falaschi et al. 2019). Freshwater ecosystems are particularly sensitive to the introduction of alien species, especially when the introduced species is a predator of native members of the invaded community (Cox & Lima 2006), and are suffering a growing number of introductions of alien predators all over the world (Strayer 2010).

Here we quantified the relative importance of different factors in determining the temporal dynamics of ten amphibian species. We focused on how habitat, landscape, and the presence of alien predators influence survival and colonization parameters. Many amphibians live in complex networks of breeding patches, where the viability of the entire SSP can be determined by the dispersal of individuals (Cayuela et al. 2020). For this reason, factors affecting the connectivity between patches can strongly influence temporal dynamics, and integrating connectivity measures can be critical to unravel the complexity of SSPs (Hanski 1998; Moilanen & Nieminen 2002). Some studies showed that invasive predators can severely impact native populations not only at the local but also at the landscape-level (Resetarits 2005; Trekels & Vanschoenwinkel 2019; Manenti et al. 2020) but the interplay between invasive species and the connectivity among local populations has largely been overlooked. Given the importance of connectivity measures in the study of SSPs, in our analyses we explicitly tested the possibility that alien species can have impacts both at the local and at the landscape scale.

2 | MATERIAL AND METHODS

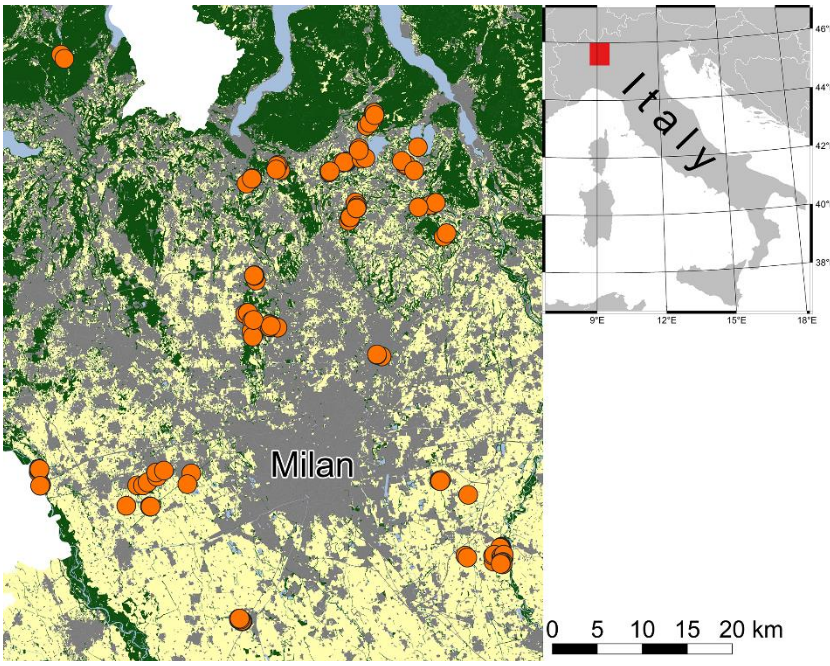
2.1 | Study system

Between 1996 and 2019 we monitored 202 freshwater sites, hereafter wetlands, consisting of ponds, ditches, and lakeshores. These wetlands are located in Lombardy region (North-Western Italy; Fig. 1), a human-dominated region where urban and agricultural landscapes are prevalent. The southern portion of the study area is deeply modified by human activities, and wooded areas are usually small and fragmented. The northern portion of the study area, while still human-dominated, includes less fragmented woodlands and several lakes (Supporting Information, Appendix S1). The wetlands included in the study host 10 species of amphibians (species description is available in Appendix S2); nomenclature followed Speybroeck et al. (2020).

Each wetland was monitored in two-seven different years (hereafter: primary periods; mean ± SD = 3.3 ± 1.3). Within each primary period, a wetland was surveyed from one to seven times between February and October (average surveys per wetland per sampling season ± SD = 4.9 ± 1.5; total number of surveys across all the wetlands: 3276; see Fig. S1 for the monthly distribution of surveys). To reduce the amount of missing data and improve model convergence, the number of primary and secondary periods was reduced as much as possible, obtaining seven primary periods, each one with up to seven secondary periods (Appendix S3). During surveys, we assessed the presence of amphibian species by visually searching for adults and egg clutches, by listening to the calls of adult males, and by dip-netting to identify tadpoles and larvae (Dodd 2010). Additional details on sampling and the dataset are available in Appendix S3-4.

In 2002 the Red Swamp Crayfish *Procambarus clarkii*, a crayfish native to North America, was introduced in the study area (Lo Parrino et al. 2020). Subsequent introductions and natural dispersal events allowed the quick spread of the crayfish, which has now invaded many of the sampled wetlands (Fig. S2).

FIGURE 1 Location of the 202 wetlands monitored for this study with indication of the extent of the study area in Northern Italy.



2.2 | Habitat and landscape features

Once for each primary period, we recorded four microhabitat characteristics of the surveyed wetlands: surface area of the wetland, presence/absence of fish, presence/absence of crayfish, and if the wetland was permanent (i.e. retained water during the entire sampling season), following the protocols described in Appendix S3. Fish and crayfish were considered present if detected in at least one survey within the sampling season. Additionally, for each primary period, we calculated a landscape variable describing the percentage cover of woodlands within 400m from the wetland by using a high-resolution land-use map of the Lombardy region (http://www.cartografia.regione.lombardia.it ; Appendix S3).

In spatially structured populations, connectivity is a key determinant of the colonization probability of an empty habitat patch (Moilanen & Nieminen 2002; Hanski & Gaggiotti 2004). To consider the processes acting at the landscape-scale, we also included two variables representing the connectivity across wetlands: species incidence in the surrounding landscape, and crayfish incidence in the surrounding landscape. For each amphibian species, species incidence represents the potential influence of the presence of the focal species in other wetlands, while crayfish incidence represents the influence of the presence of the crayfish in other wetlands. Both values were weighted with an incidence function model (Moilanen & Nieminen 2002), and hence show an exponential decay based on distance from the focal wetland.

Species/crayfish incidence *S* in the site *i* at the time *t* were determined as follows:

$$\text{(eq. 1)}\ S_{it} = \sum\nolimits_{j\neq i} exp\left(-\alpha d_{ij}\right)P_{j(t-1)}$$

where d_{ij} is the distance between the focal wetland *i* and each one of the remaining wetlands *j;* α is the maximum distance at which populations of the focal amphibian species are known to be spatially connected. The value of α was different across species and is based on the distance at which populations are known to interact (Table S2). For crayfish incidence $P_{j(t-1)}$ was the presence/absence (0/1) of the crayfish in the wetland *j* at time *t*-1. For species incidence, $P_{j(t-1)}$ was the occupancy of the focal species in the wetland *j* at time *t*-1, estimated by running a simplified dynamic occupancy model (see below), including only detection covariates.

2.3 | Statistical analyses

2.3.1. | Dynamic occupancy models

Species occurring in an area can remain undetected, and this can severely bias estimates of statistical models; therefore, we used dynamic occupancy models to take into account imperfect detection (MacKenzie et al. 2003). We used a Bayesian framework (Royle & Kéry 2007) and implemented our dynamic occupancy models in the package nimble, a highly flexible and computationally efficient system to program Bayesian models in R (de Valpine et al. 2017). In our models, we related detection probability to date (expressed as Julian Day) and hour (expressed as minutes after midnight), considering linear and quadratic terms for both variables. Initial occupancy was related to four variables: forest cover, area of the wetland, permanence, and presence of fish. Survival probability was related to five variables: forest cover, area of the wetland, permanence, presence of fish, and presence of crayfish. Colonization probability was related to seven variables: forest cover, area of the wetland, permanence, presence of fish, presence of crayfish, crayfish incidence, and species incidence. For each species, we fitted a single model, including all the independent variables related to detection and occupancy.

Spatial autocorrelation is pervasive in the distribution of amphibians at the landscape scale and can bias the outcome of statistical models (Dormann 2007; Băncilă et al. 2017). Therefore, for all three processes (initial occupancy, survival, colonization), we added a spatial random effect to take into account spatial autocorrelation among wetlands (Appendix S5). The random effect was calculated based on an intrinsic Gaussian conditional autoregressive model (ICAR; Banerjee, Carlin, & Gelfand, 2014). ICAR models allow the integration of spatial terms in the error of the model and have excellent performance in the analysis of data with complex spatial structures (Beale et al. 2010). ICAR models used an adjacency matrix with a truncation distance of 25 km, i.e. the minimum distance at which all the wetlands were connected forming a single network. For the prior of the precision of the ICAR model, we used a zero-truncated normal distribution with mean = 0 and precision = 0.1. For regression coefficients of the variables related to detection and initial occupancy/survival/colonization, we used normal priors, with mean = 0 and precision = 0.01 for detection covariates and mean = 0 and precision = 0.1 for initial occupancy/survival/colonization. Sensitivity analysis showed consistent results with different priors (Table S3).

Before running the models, we transformed independent variables to reduce skewness and improve model convergence (Sokal & Rohlf 2012). Wetland area, crayfish incidence, and species incidence were log-transformed, while forest cover was square-root-arcsine transformed (Sokal & Rohlf 2012). Furthermore, all the independent variables were scaled at mean = 0 and standard deviation = 1 to allow the comparison of the estimated effect sizes. Correlations between independent variables were weak (|r| < 0.48, Table S4). The models were run for 1,000,000 iterations, discarding the first 500,000 iterations as a burn-in and then sampling the posterior distribution with a thinning of 500. For some species these values did not allow convergence, thus we added additional steps of 500,000 iterations until all the parameters attained convergence (Rhat < 1.1; Table S5).

2.3.2 | Summarizing the community-level impacts

Given the high variability of environmental responses across species, we conducted a meta-analysis to summarize the general effects on the community. For each variable, we extracted the posteriors of its effect on each species present in the study area and calculated the mean and the variance of the posterior distributions. The meta-analysis was performed in a Bayesian framework, and for each factor, we calculated the mean value and its credible intervals with a model of the mean (i.e. a model including only the intercept as independent variable; Kéry, 2010). To take into account the uncertainty of estimates, we weighed the contribution of each value by the inverse of the variance of its posterior distribution (Hadfield & Nakagawa 2010). The meta-analytic models were run for 2,000,000 iterations, discarding the first 1,000,000 iterations as a burn-in and then sampling the posterior distribution with a thinning of 1,000.

All the analyses were performed in the R environment (R Core Team 2018) using the packages rgeos (Bivand & Rundel 2019) and raster (Hijmans 2019) to process maps, nimble (de Valpine et al. 2017) to run the dynamic occupancy model, glmmBUGS (Brown & Zhou 2018) and spdep (Bivand & Wong 2018) to calculate the adjacency matrix for ICAR models, and MCMCglmm (Hadfield 2010) to run the community meta-analysis. The code used to run the dynamic occupancy models in nimble and the meta-analytic model is available in Appendix S4.

3 | RESULTS

3.1 | Amphibian species

Overall, we detected 10 taxa of amphibians within the 202 monitored wetlands: the Common Toad (*Bufo bufo*) was detected in 40 wetlands over the study period; the Green Toad (*Bufotes viridis*; detected in 38 wetlands); the Italian Tree Frog (*Hyla intermedia*; 102 wetlands); the Agile Frog (*Rana dalmatina;* 99 wetlands); the Italian Agile Frog (*Rana latastei;* 110 wetlands); the Common Frog (*Rana temporaria*; 9 wetlands only), hybridogenetic Green Frogs (*Pelophylax lessonae* and *Pelophylax* klepton *esculentus*, considered as a single taxon; 173 wetlands); the Fire Salamander (*Salamandra salamandra*; 17 wetlands), the Italian Crested Newt (*Triturus carnifex*; 48 wetlands), and the Smooth Newt (*Lissotriton vulgaris*; 51 wetlands). Relationships between initial occupancy of each species and environmental variables are reported in Table S5. For most species the autoregressive terms showed low values, suggesting limited spatial autocorrelation (Table S6).

3.2 | Environmental factors and survival of populations

Among the factors potentially influencing survival probability (Fig. 2), forest cover showed contrasting effects. For some species (Common Toad and Crested Newt), we detected a strong positive relationship between population survival and forest cover. In contrast, other species showed less pronounced or even a weak negative relationship. The surface area of the wetland was positively related to population survival for most of the species, indicating that a population living in a larger wetland had a higher probability of persisting in time. The permanence of the wetland showed a similar but less pronounced pattern, with the survival of most species being positively correlated to permanence. However, some species showed a negative relationship, and this was particularly evident in Green Toads, which are specialist of ephemeral wetlands (Indermaur et al. 2010). Fish presence in the wetland usually showed a negative relationship with

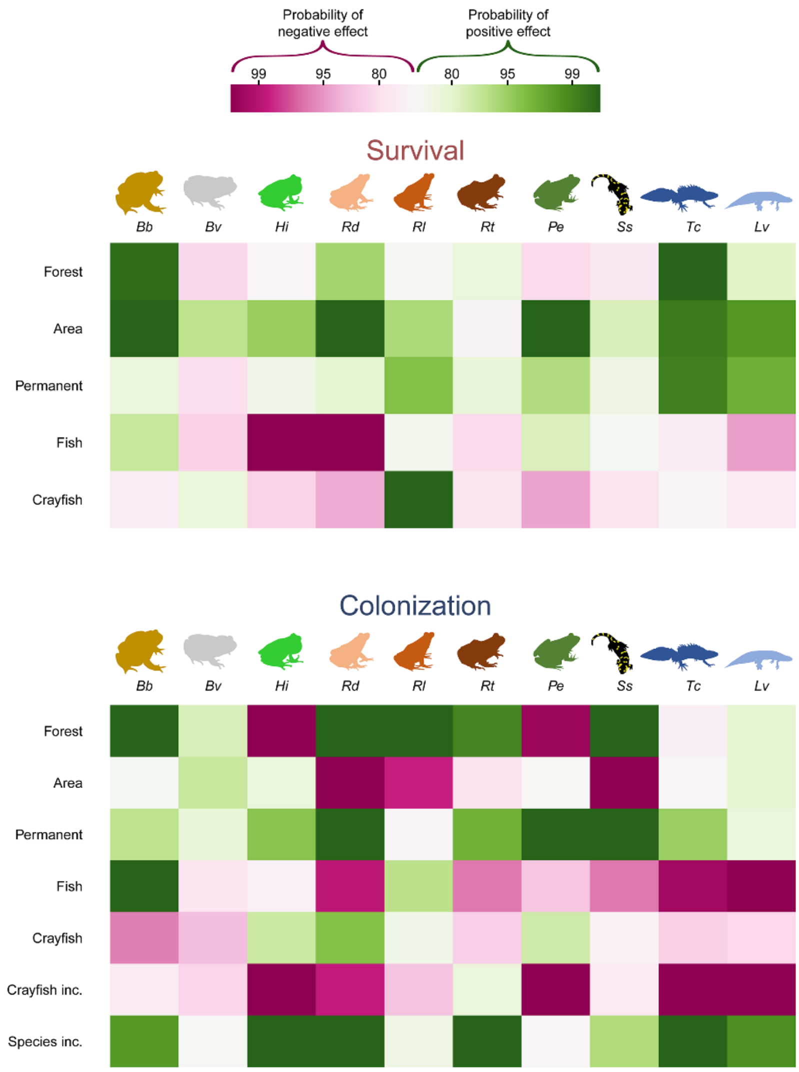


FIGURE 2. Heatmap showing the Bayesian posterior probability of the negative (purple) or positive (green) effects of environmental factors on survival and colonization probabilities, obtained from the dynamic occupancy models, for the ten species present in the study area. *Bb* = *Bufo bufo, Bv* = *Bufotes viridis, Hi* = *Hyla intermedia, Rd* = *Rana dalmatina, Rl* = *Rana latastei, Rt* = *Rana temporaria, Pe* = *Pelophylax lessonae* and *Pelophylax* klepton *esculentus, Ss* = *Salamandra salamandra, Tc* = *Triturus carnifex, Lv* = *Lissotriton vulgaris*.

population survival, with a particularly strong effect on the survival of Tree Frogs, Agile Frogs and Smooth Newts. Crayfish occurrence in the wetland often showed a negative relationship with survival probability; however, this effect was usually weak, or even positive for Italian Agile Frogs.

3.3 | Factors influencing colonization probability

Among the factors related to colonization probability (Fig. 2), forest cover showed a highly variable effect among species. Forest cover strongly reduced colonization rate for Tree Frogs and Green Frogs, while it increased the colonization of Common Toads, Brown Frogs (*R. dalmatina*, *R. latastei*, and *R. temporaria*), and the Fire Salamander. The surface area of the wetland showed a variable effect on colonization probability. For Agile Frogs, Italian Agile Frogs, and Fire Salamanders, while for other species the effect was weak. Wetland permanence generally showed a positive relationship with colonization probability, with a particularly strong positive effect for Agile Frogs, Green

Frogs, and Fire Salamanders. The presence of fish in the wetland generally showed a negative or a weak effect on colonization probability. However, for Common Toads, we observed a positive relationship between fish presence and colonization probability.

The occurrence of crayfish in the wetland did not show a homogeneous relationship with colonization, with some species showing negative relationships and others positive relationships. While the local-scale impact of crayfish presence was heterogeneous, crayfish incidence consistently showed a negative relationship with colonization probability across species. The negative relationship with colonization probability was particularly strong for Tree Frogs, Green Frogs, and newts. In most cases, species incidence showed a strong positive relationship with colonization probability, indicating that an unoccupied wetland surrounded by many or nearby occupied wetlands has a higher chance of being colonized than a wetland surrounded by fewer or further wetlands.

3.4 | Community-level effects

The meta-analytic model allowed us to summarize the overall effect of environmental factors across species (Fig. 3; Table 1). Population survival was generally higher in permanent wetlands with large surface area, while was generally lower in wetlands inhabited by fish. Overall survival tended to be positively related to forest cover, and negatively related to crayfish occurrence but with some uncertainty (Fig. 3; Table 1).

Table 1. Summary of the posterior distributions obtained from the meta-analytic model. For each variable related to survival and colonization probability, we reported the mean, the proportion of positive, and the proportion of negative posteriors of the regression coefficients. Variables for which >95% of posteriors are positive or negative are in bold.

Regression coefficient	Posteriors mean	Proportion of positive posteriors	Proportion of negative posteriors
Survival			
Forest	0.33	0.95	0.05
Area	**1.36**	**1**	**0**
Permanence	**0.42**	**1**	**0**
Fish	**-0.65**	**0.01**	**0.99**
Crayfish	-0.24	0.12	0.88
Colonization			
Forest	**0.62**	**0.96**	**0.04**
Area	-0.38	0.06	0.94
Permanence	**0.51**	**0.99**	**0.01**
Fish	-0.38	0.11	0.90
Crayfish	0.00	0.53	0.48
Crayfish incidence	**-0.71**	**0**	**1**
Species incidence	**0.85**	**1**	**0**

Among the variables related to colonization probability (Fig. 3; Table 1), forest cover showed an average positive. The surface area of the wetland showed a negative relationship, suggesting that larger wetlands are less likely to be colonized. The permanence of the wetland showed a strong

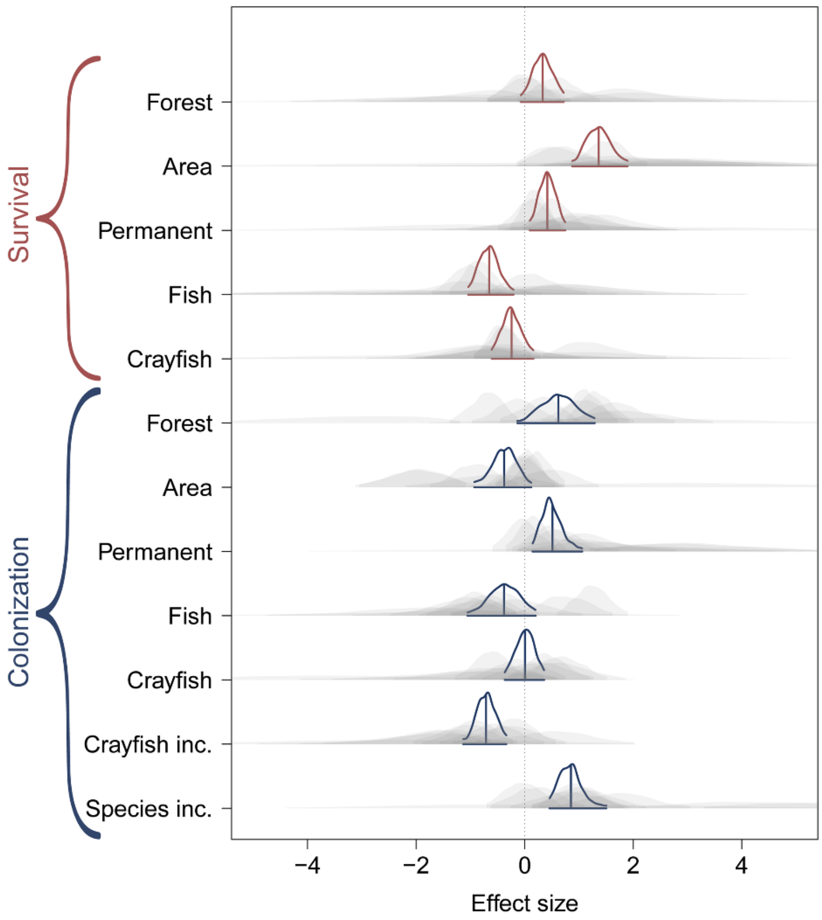


FIGURE 3 Posterior distribution for the community meta-analysis relating environmental factors to survival and colonization probabilities across all the species of the amphibian community. Colored lines represent the 95% credible interval, and vertical lines represent the mean effect size averaged across all the species. In order to show the variation across species, grey areas represent the 95% credible intervals of the posterior distribution of the effect of the variable for each single species. Red represents parameters related to survival probability; blue represents parameters related to colonization probability.

positive relationship with colonization probability, indicating that wetlands where water is available throughout the entire breeding season, are more likely to be colonized. Fish presence showed an overall negative relationship with colonization probability, indicating that wetlands with fish are less likely to be colonized. Crayfish presence in the wetland showed an average effect close to zero. Conversely, crayfish incidence showed a strong negative effect, indicating that wetlands surrounded by many and/or close wetlands invaded by crayfish are less likely to be colonized. Finally, wetlands surrounded by many and/or nearby wetlands occupied by the focal amphibian species are more likely to be colonized (Fig. 3; Table 1).

4 | DISCUSSION

Our long-term study allowed us to analyze the complex drivers of amphibian population dynamics at the regional scale and to tease apart the factors related to the persistence and colonization of local populations. Ecological differences among species caused heterogeneous responses to the different environmental drivers; still, the community-level analysis revealed several common patterns, allowing us to identify how management actions can improve the long-term persistence of amphibian communities.

4.1 |The drivers of population survival and colonization

Survival and colonization were jointly determined by drivers acting at different scales. On the one hand, patch-level features (area, water permanence, and fish) strongly affected the persistence of populations. Populations living in larger wetlands were more likely to persist over time, and the surface area of the wetland was the variable showing the strongest effect size on population persistence (Fig. 3). The positive relationship between patch size and survival is well-established and patch size is one of the fundamental variables considered in SSP studies for over 30 years (Moilanen & Hanski 1998). A large patch usually corresponds to larger carrying capacities and vital rates, lowering the chance of local extinction (Hanski 1998; Hodgson et al. 2009).

Permanence and predators are often major determinants of freshwater community composition (Wellborn et al. 1996; Van Buskirk 2003). On the one hand, population persistence increased in non-ephemeral wetlands (Fig. 3). All the amphibian species present in the study area have aquatic larvae, and most of them require several months to attain metamorphosis (Lanza et al. 2007). Hence, for most of the species, a longer hydroperiod reduces the risk of reproductive failure. On the other hand, fish occurrence strongly reduced the persistence of populations (Fig. 3). This is not surprising considering that many alien fish are voracious predators of amphibian eggs and larvae (Falaschi et al. 2020). In this study, we did not capture fish, so species identification was not possible. While fish are usually associated to permanent wetlands, there is a continuum gradient that ranges from small ephemeral wetlands to large permanent lakes (Wellborn et al. 1996), and most of the study wetlands are not connected to the hydrographic network. Thus, fish presence is generally caused by angling-related introductions (See also Appendix S3). Amphibians adapted to an intermediate hydroperiod, where infrequent desiccations do not hinder larval development but prevent the persistence of fish, may be particularly vulnerable to fish introductions (Van Buskirk 2003).

The main determinants of colonization probability were different from the drivers of persistence, as colonization was highest in semi-permanent wetlands, with a high incidence of the target species and a low incidence of invasive crayfish in the surrounding landscape. The incidence of the focal species was the factor showing the strongest and most consistent effect on colonization. Connectivity is often one of the main determinants of colonization probability in SSPs (Hanski 1998; Moilanen & Hanski 1998), and amphibians are particularly sensitive to the negative impact of isolation because an unsuitable landscape matrix can hamper dispersal among habitat patches (Bradford et al. 1993; Cayuela et al. 2020).

4.2 | The landscape-level impact of invasive species

The mere occurrence of target species in nearby wetlands is not enough for the colonization of unoccupied sites, as colonization requires nearby populations producing a large number of potential propagules (Hanski & Gaggiotti 2004). Invasive predators are major determinants of amphibian fitness, thus we considered the incidence of a highly invasive crayfish as a measure of the landscape-level impact of invasives on connectivity. Crayfish occurrence in a wetland did not reduce the probability that that wetland is colonized, suggesting that many native amphibians are unable to detect this invasive predator, and continue to attempt breeding in invaded sites (Ficetola et al. 2011). Instead, the impact of the invasive crayfish was particularly evident when measured at the landscape scale. Experimental studies showed that colonization of predator-free patches is lower if closer to patches with predators (Resetarits 2005; Trekels & Vanschoenwinkel 2019), and recent analyses suggested that invasive species affect frog abundance at the SSP level, by reducing the number of immigrants in target wetlands (Manenti et al. 2020). Still, these studies focused on one species only, and information on the generality of this impact is limited. Our community-level analysis shows that crayfish incidence has a general, negative effect on amphibian communities, acting on nearly all the species (Fig. 3). This confirms that the effect of predators at the landscape scale can be a major determinant of colonization dynamics and, even in cases where the local (patch-level) effect is not strong enough to be easily detected, the influence of predators on connectivity can be severe (Bradford et al. 1993). This can be explained by the key role of dispersal on the persistence of amphibian populations (Cayuela et al. 2020). We did not measure the frequency of dispersal events, so it is difficult to assess if our SSPs truly have a source-sink structure (Pulliam 1988). Nevertheless, the primary importance of species and crayfish incidence is in agreement with the source-sink hypothesis, confirming the importance of dispersal for the long-term persistence of amphibians SSP. On the one hand, a wetland invaded by predators can remain occupied by native amphibians if nearby wetlands act as

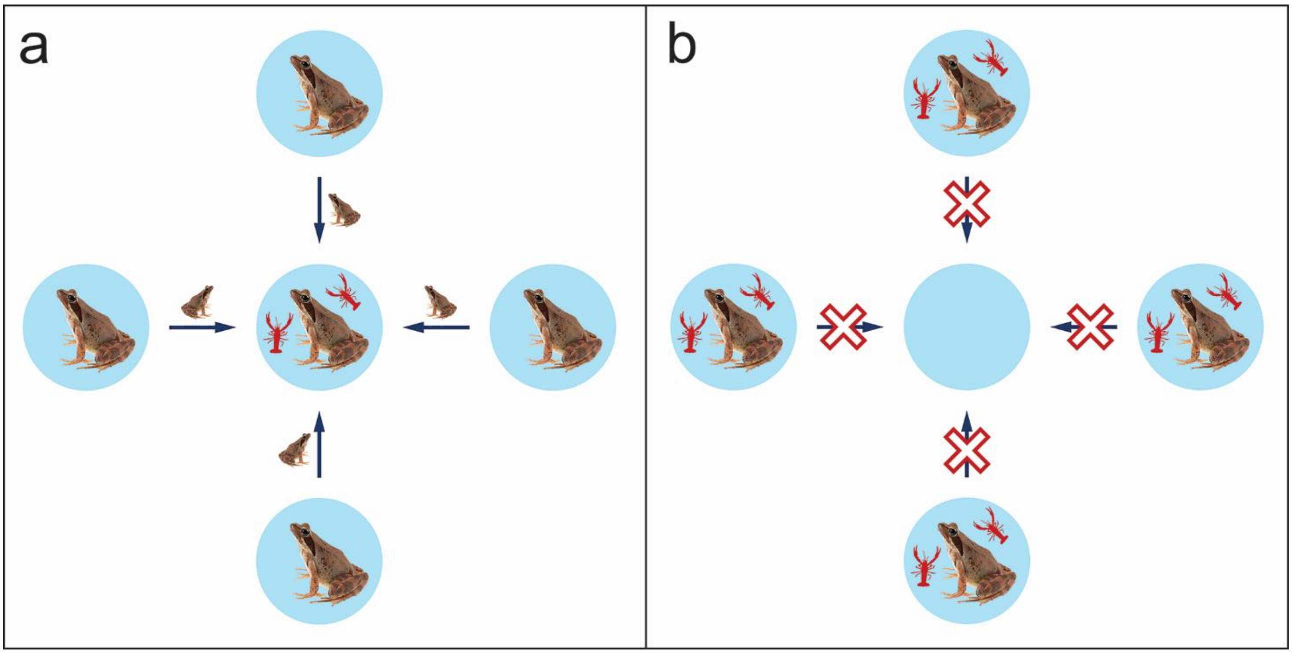


FIGURE 4 Possible mechanism underlying the negative effect of crayfish incidence on colonization probability. In the first scenario a) the focal wetland is invaded by crayfish and is surrounded by pods without crayfish. While the crayfish has a negative impact on local recruitment, the viability of the focal population is sustained by immigrants from the surrounding landscape; in the second scenario b) the focal wetland is not invaded but is surrounded by invaded wetlands. In this scenario, the presence of crayfish in the neighboring wetlands reduces the immigration of individuals from surrounding wetlands and, if the focal population is not large enough to sustain autonomously, it will not persist over time.

sources (e.g. they are predator-free) and dispersing individuals attempt breeding into the invaded wetland (Fig. 4a). On the other hand, a local population inhabiting an uninvaded wetland can go extinct when the presence of invasive predators in the neighboring wetlands reduces the immigration of individuals (Fig. 4b), particularly if the population is small or if stochasticity causes temporal variation of fitness (Hanski & Gaggiotti 2004). Future studies specifically focusing on the landscape-level effect of alien predators are required to disentangle the mechanism underlying the alteration of colonization dynamics.

The negative landscape-level effect of invasive species can affect the whole amphibian community (Fig. 2). The pervasiveness of this effect suggests that, in the presence of an alien predator, conservation should prioritize networks with high connectivity and a low incidence of invasives. However, more studies are required to understand how frequent the landscape-level effect of alien species is across communities and landscape typologies.

4.3 | Conservation implications

Assessing the drivers of extinctions and colonizations across >20 years allowed us to identify strategies for amphibian persistence in human-dominated landscapes, and suggested that amphibian communities can greatly benefit from integrated management of hydroperiod, connectivity, and alien species. Ensuring water persistence in wetlands can be a successful strategy for amphibian conservation (Mathwin et al. 2020) but is complicated by the interplay between wetland permanence and the occurrence of predators. In fact, permanent wetlands also suffer the highest presence of introduced fish crayfish, with negative impacts on amphibian fitness and survival (Wellborn et al. 1996). Targeted drying after the end of the breeding season of amphibians can prevent the long-term persistence of fish (Mathwin et al. 2020). It can also reduce the abundance of the invasive crayfish (Ficetola et al. 2012b), but such management is complex. In the study area, this could be achievable through dams that regulate water level both directly, or indirectly by acting on the water table (Mathwin et al. 2020). In fact, hydraulic structures exist that control the water table level through the year and can influence the hydroperiod also for wetland disconnected to the main hydrographic network (Colombo & Di Palma 1995). The functioning of these structures is currently optimized for agricultural purposes, but better integration of agricultural and biodiversity policies can determine environmental improvements at the regional scale.

Increasing connectivity among habitat patches can be a good management strategy to ensure the long-term persistence of SSPs and can be attained by both enhancing the permeability of the landscape for dispersal, or by creating new wetlands near breeding patches or along habitat corridors (Janin et al. 2009; Rannap et al. 2009; Kremen & Merenlender 2018). Creating new, suitable wetlands within the extant SSP network can be particularly effective for the long-term persistence of species, since it enhances both the number of populations and the colonization of empty patches (Fig. 4). However, these actions are costly, and selecting the appropriate location for new wetlands is often difficult. Recent technological advancement and new modeling approaches can provide efficient tools to identify the most cost-effective strategies to enhance connectivity and select locations for management actions (Scroggie et al. 2019). The risk of increasing the invasion rate is a possible drawback of enhancing connectivity (e.g. Besacier-Monbertrand et al. 2014). Consequently, new wetlands should be designed to be suitable for amphibians, and simultaneously unreachable or unfavorable for non-native predators such as fish and crayfish. Again, targeted drying can help to maintain fishless ponds in areas were repeated fish introductions might occur. However, this technique would probably be less efficient against invasive crayfish, for which specific barriers exist that can prevent colonization (Falaschi et al. 2020). Finally, targeted communication campaigns are needed to avoid that both fish and non-native species are introduced in ponds by citizens. The practical identification of approaches aimed at enhancing connectivity for natives without favoring invasion is going to be a key challenge for conservationists in the next decades.

In this study, we showed how drivers of population dynamics can act differently across species. Despite the high interspecific variation, we were able to detect some common drivers of change across the entire community, with a strong effect of some classic features commonly used in SSP studies such as patch area and connectivity (Moilanen & Hanski 1998). The incidence of an invasive alien species at the landscape scale emerged as one of the strongest drivers of colonization dynamics, suggesting that studies on spatially structured populations should take into account different connectivity variables more often, especially when dealing with biological invasions. Preserving single high-quality wetlands is not enough to ensure the long-term persistence of an SSP, and the maintenance and enhancement of connectivity are necessary (Janin et al. 2009; Rannap et al. 2009).

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

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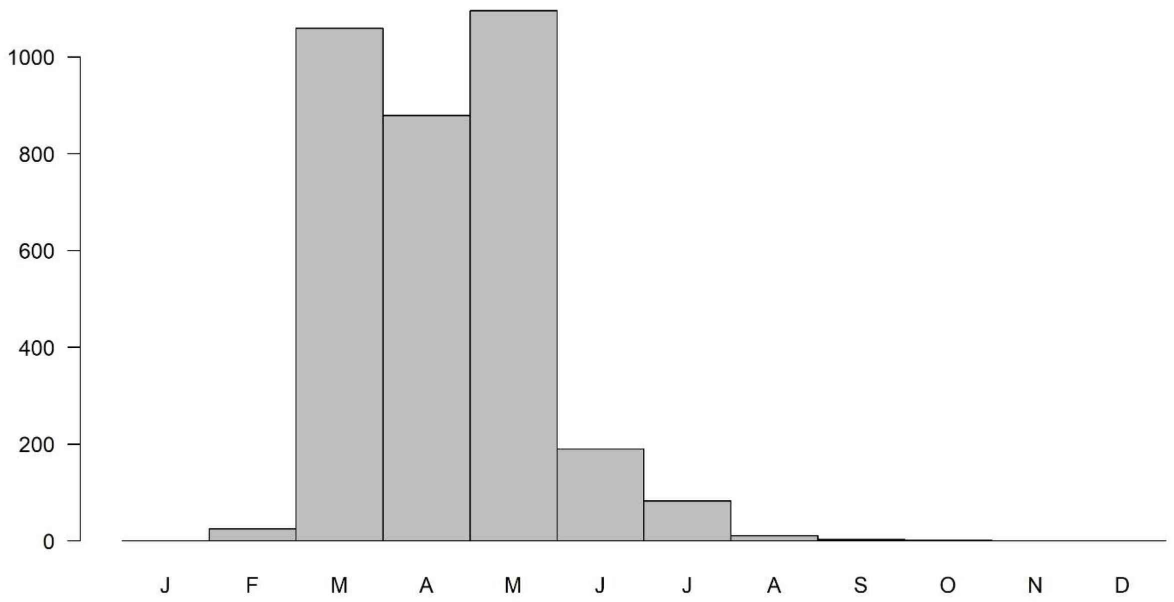


Figure S1 Number of surveys per month across the whole sampling period (1996/2019). 97.1% of surveys were performed between February and June, and 0.4% of surveys only were performed after July. Since juvenile dispersal for the species considered in the study generally does not occur before June, we considered the local populations to be closed within each year.

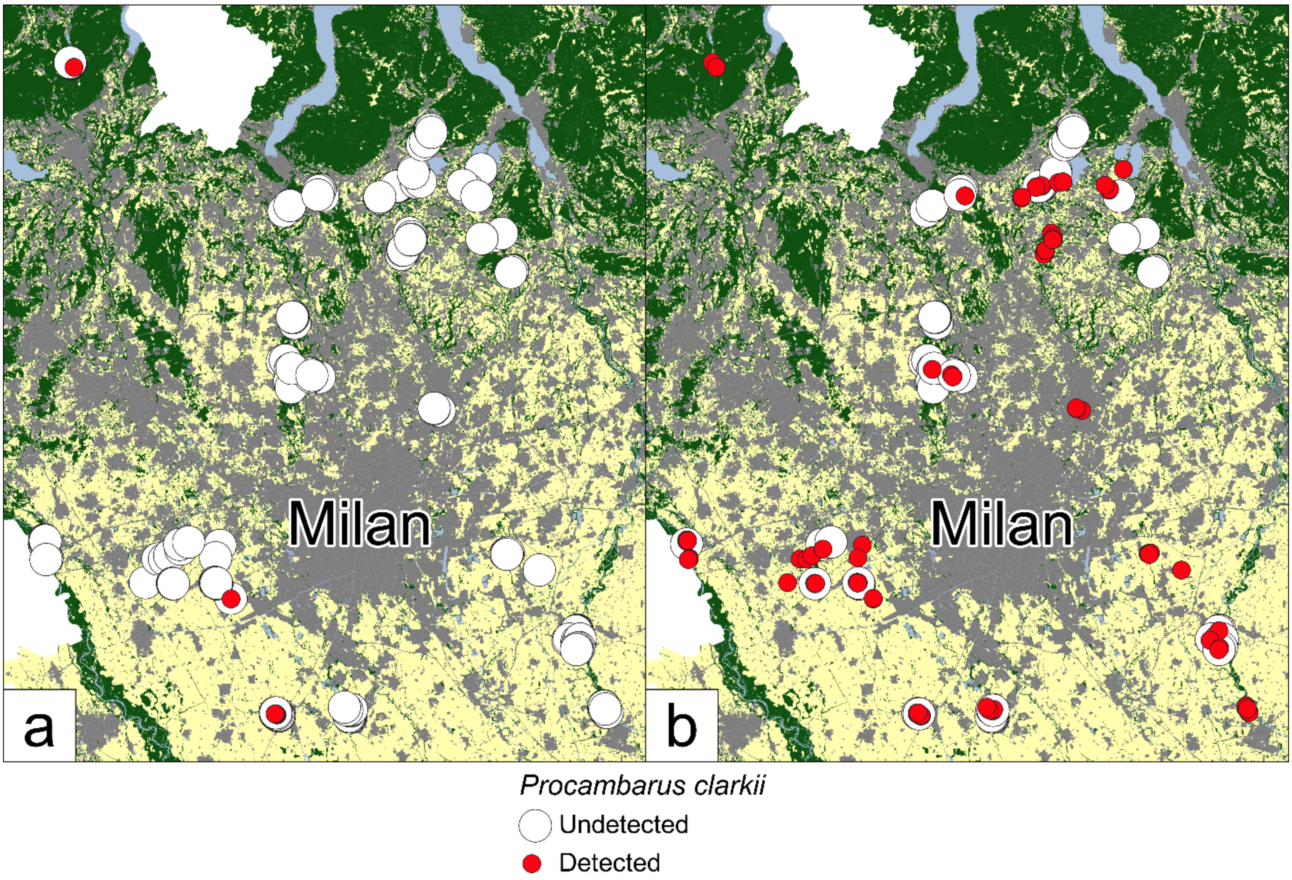


Figure S2 Distribution of the Red Swamp Crayfish *Procambarus clarkii* in the study area. The two maps represent a) the distribution before 2009 and b) the distribution in 2017/2019. All data were collected during this research.

Table S1 Structure of the data. For each site, we reported the number of surveys performed in each primary period (Year 1 to Year 2019), the total number of surveys, and the total number of primary periods in which the site was sampled.

Site code	Year 1	Year 2	Year 3	Year 4	Year 2017	Year 2018	Year 2019	Total number of surveys	Total number of primary periods
Al-1	0	0	4	5	6	0	6	21	4
Al-10	0	0	4	6	6	0	6	22	4
Al-11	0	0	4	6	6	0	6	22	4
Al-2	0	0	4	5	6	0	6	21	4
Al-3	0	0	4	5	6	0	6	21	4
Al-4	0	0	4	5	6	0	6	21	4
Al-5	0	0	4	6	6	0	6	22	4
Al-6	0	0	4	6	6	0	6	22	4
Al-9	0	0	4	6	6	0	6	22	4
Al-F	0	0	0	0	6	0	6	12	2
Als-1	3	0	0	0	6	0	6	15	3
Als-2	3	0	0	0	6	0	6	15	3
Als-3	3	0	0	0	6	0	6	15	3
AN06	2	0	3	0	5	0	6	16	4
BA-B	0	0	4	0	6	0	6	16	3
BA-C	0	0	4	0	6	0	6	16	3
BA-D	0	0	0	0	6	0	6	12	2
BA-E	0	0	4	0	6	0	6	16	3
BA-F	0	0	4	0	6	0	6	16	3
BA-G	0	0	4	0	6	0	6	16	3
BA-H	0	0	0	0	6	0	6	12	2
BA-I	0	0	0	0	6	0	6	12	2
BAR-19	4	3	0	0	0	6	0	13	3
BAR-F	0	0	2	0	0	6	0	8	2
BAT-26	4	2	0	0	0	6	0	12	3
CAR01	0	0	0	0	5	6	0	11	2
CAR02	0	0	0	0	5	6	0	11	2
CAST01	2	0	0	0	6	6	0	14	3
CAST02	2	0	0	0	5	6	0	13	3
CAST03	2	0	0	0	5	6	0	13	3
CAST04	2	0	0	0	6	6	0	14	3
CF-1	7	0	7	0	6	0	6	26	4
CF-2	7	0	7	0	6	0	6	26	4
CF-3	7	0	7	0	6	0	6	26	4
CF-4	7	0	7	0	6	0	6	26	4
CF-5	7	0	7	0	6	0	6	26	4
CF-6	7	0	7	0	6	0	6	26	4
COR-23	4	3	0	0	0	6	0	13	3
COR-24	4	3	0	0	0	6	0	13	3
COR-25	4	3	0	0	0	6	0	13	3
CU01	0	0	4	1	7	7	0	19	4

CU03	0	0	4	1	7	7	0	19	4
CU04	0	0	4	1	7	7	0	19	4
CU05	0	0	4	0	7	7	0	18	3
CUS 41	4	0	0	0	0	6	0	10	2
CUS 42	4	0	0	0	0	6	0	10	2
CUS 80	4	0	0	0	0	6	0	10	2
CUS 81	4	0	0	0	0	6	0	10	2
CUS 82	4	0	0	0	0	6	0	10	2
CUS 95	3	0	0	0	0	6	0	9	2
CUS 97	2	0	0	0	0	6	0	8	2
CV 37	3	0	0	0	0	0	6	9	2
CV 38	3	0	0	0	0	0	6	9	2
CV 40	3	0	0	0	0	0	6	9	2
CV 42	3	0	0	0	0	0	6	9	2
FOR01	0	0	1	0	5	7	0	13	3
FOR01CAN	0	0	0	0	5	7	0	12	2
FOR02	0	0	1	0	5	6	0	12	3
FOR03	0	0	1	0	5	6	0	12	3
FOR04	0	0	1	0	5	6	0	12	3
FOR05	0	0	1	0	5	7	0	13	3
FOR06	0	0	1	0	5	6	0	12	3
FOR07	0	0	0	0	5	6	0	11	2
FOR08	0	0	1	0	5	6	0	12	3
FOR09	0	0	0	0	4	6	0	10	2
GAG01	0	0	0	0	5	5	0	10	2
GAG02	0	0	0	0	5	6	0	11	2
GAG04	0	0	0	0	5	6	0	11	2
GAG05	0	0	0	0	5	6	0	11	2
GR-1	6	3	4	4	6	6	7	36	7
GR-10	3	0	4	5	6	6	7	31	6
GR-11A	0	0	4	5	6	6	7	28	5
GR-11B	0	0	0	0	6	6	7	19	3
GR-12	3	0	4	4	0	6	5	22	5
GR-13	3	0	4	4	6	6	5	28	6
GR-14	3	0	4	4	6	6	6	29	6
GR-15	3	0	4	5	6	6	7	31	6
GR-16	0	0	4	5	6	6	7	28	5
GR-17	3	0	4	5	6	6	7	31	6
GR-18	0	0	4	5	6	6	7	28	5
GR-19	0	0	4	5	6	6	7	28	5
GR-20	0	0	4	4	6	6	7	27	5
GR-21	0	0	4	4	6	6	7	27	5
GR-22	0	0	4	4	6	6	7	27	5
GR-23	0	0	4	4	0	6	7	21	4
GR-24	5	0	4	5	6	6	7	33	6
GR-25	5	0	4	5	6	6	7	33	6
GR-26	5	0	4	5	6	6	7	33	6
GR-27	0	0	4	4	6	6	7	27	5
GR-29	7	3	4	5	6	6	7	38	7

GR-3	0	0	4	3	6	6	7	26	5
GR-30	7	3	4	4	6	6	7	37	7
GR-31	7	3	4	4	6	6	7	37	7
GR-32	7	3	4	5	6	6	7	38	7
GR-4	0	0	4	3	6	6	7	26	5
GR-5	0	0	4	3	6	6	7	26	5
GR-8	5	3	4	4	6	6	7	35	7
GR-9	5	3	4	4	6	6	7	35	7
GR-L	0	0	0	0	0	6	7	13	2
GR-N	0	0	0	0	6	6	7	19	3
LAC 58	3	0	3	0	0	0	0	6	2
LAC 59	3	0	3	0	0	0	0	6	2
LAC 60	3	0	3	0	0	0	0	6	2
LAC 61	3	0	3	0	0	0	0	6	2
LAC 62	3	0	0	0	0	6	0	9	2
LAC 63	3	0	0	0	0	6	0	9	2
LAC 64	3	0	0	0	0	6	0	9	2
LAC 65	3	0	0	0	0	6	0	9	2
LAC 66	3	0	0	0	0	6	0	9	2
LAC 87	2	0	0	0	0	6	0	8	2
LAC 88	2	0	0	0	0	6	0	8	2
LL-27	3	0	0	0	0	5	5	13	3
LL-29	3	0	0	0	0	5	5	13	3
LOD 14	6	0	0	0	0	6	0	12	2
LOD 15	5	0	0	0	0	6	0	11	2
LOD 16	5	0	0	0	0	4	0	9	2
LOD 75	4	0	0	0	0	6	0	10	2
LOD 76	4	0	0	0	0	6	0	10	2
LOD 79	4	0	0	0	0	6	0	10	2
MO-1	0	0	4	0	6	0	6	16	3
MO-2	0	0	4	0	6	0	6	16	3
MO-3	0	0	4	0	6	0	6	16	3
MO-5	0	0	4	0	6	0	6	16	3
MOR01	2	0	0	0	5	0	6	13	3
MOR02	2	0	0	0	5	0	6	13	3
MOR45	4	0	0	0	0	0	6	10	2
MOR46	4	0	0	0	0	0	6	10	2
MOR47	4	0	0	0	0	0	6	10	2
MOR48	4	0	0	0	0	0	6	10	2
MOR49	4	0	0	0	0	0	6	10	2
MOR50	3	0	0	0	0	0	6	9	2
MOR52	4	0	0	0	0	0	6	10	2
MOR53	4	0	0	0	0	0	6	10	2
MOR54	4	0	0	0	0	0	6	10	2
MOR55	4	0	0	0	0	0	6	10	2
MOR56	4	0	0	0	0	0	6	10	2
MOR90	3	0	0	0	0	0	6	9	2
MOR91	2	0	0	0	0	0	6	8	2
MUZ01	3	0	4	0	4	6	0	17	4

MUZ02	4	0	4	0	4	6	0	18	4
MUZ03	4	0	0	0	4	6	0	14	3
MUZ04	5	0	4	0	4	6	0	19	4
MUZ05	4	0	0	0	4	6	0	14	3
MZ01	7	7	0	0	6	0	6	26	4
MZ02	7	7	0	0	6	0	6	26	4
MZ03	7	7	0	0	6	0	6	26	4
MZ04	7	6	0	0	6	0	6	25	4
OG01	2	0	0	0	6	6	0	14	3
OG02	2	0	0	0	6	7	0	15	3
OG03	2	0	0	0	6	6	0	14	3
PAST01	3	0	2	0	4	0	6	15	4
PAST02	3	0	2	0	4	0	6	15	4
PAST03	3	0	2	0	4	0	6	15	4
PAST04	3	0	2	0	4	0	6	15	4
PAST05	3	0	2	0	4	0	6	15	4
PAST06	3	0	2	0	4	0	6	15	4
PAST07	3	0	2	0	4	0	6	15	4
PAST09	3	0	2	0	4	0	6	15	4
PAST10	3	0	2	0	4	0	6	15	4
PAST11	3	0	2	0	4	0	6	15	4
PAST12	3	0	2	0	4	0	6	15	4
PAST15	3	0	2	0	4	0	6	15	4
PAST16	3	0	2	0	4	0	6	15	4
PAST17	0	0	2	0	0	0	6	8	2
PAST18	0	0	2	0	0	0	6	8	2
PAST29	3	0	2	0	0	0	6	11	3
PAST34	3	0	2	0	0	0	6	11	3
PAST35	3	0	2	0	0	0	6	11	3
PU-8	0	0	4	4	6	0	6	20	4
PU-9	0	0	4	4	6	0	6	20	4
PUS-1	3	0	0	0	6	0	6	15	3
PUS-2	3	0	0	0	6	0	6	15	3
RI83	4	0	0	0	0	6	0	10	2
RI84	4	0	0	0	0	6	0	10	2
RI85	4	0	0	0	0	6	0	10	2
RI96	3	0	0	0	0	6	0	9	2
RI98	2	0	0	0	0	6	0	8	2
RILE01	0	0	4	0	5	0	6	15	3
SE-10B	0	0	4	0	6	0	6	16	3
SE-11B	0	0	4	0	6	0	6	16	3
SE-4	0	0	4	0	6	0	0	10	2
SE-5	0	0	4	0	6	0	6	16	3
SE-6	0	0	4	0	6	0	6	16	3
SE-7	0	0	4	0	6	0	6	16	3
SE-8	0	0	4	0	6	0	6	16	3
SED-20	4	3	0	0	0	6	0	13	3
SED-21	4	3	0	0	0	6	0	13	3
SED-22	4	3	0	0	0	6	0	13	3

SIR01	2	0	0	0	6	0	6	14	3
SIR02	2	0	0	0	6	0	6	14	3
SIRO01a	2	0	0	0	6	6	0	14	3
SIRO01b	2	0	0	0	6	6	0	14	3
TI-1	0	0	1	4	0	0	4	9	3
TI-10	0	0	1	0	6	0	6	13	3
TI-10B	0	0	6	0	2	0	6	14	3
TI-3	0	0	1	5	6	0	6	18	4
TI-3A	0	0	5	0	6	0	5	16	3
TI-4	0	0	1	5	6	0	5	17	4
TI-5	0	0	1	5	6	0	5	17	4
TI-6	0	0	1	5	6	0	5	17	4
TI-7	0	0	1	5	6	0	6	18	4
TI-9	0	0	1	6	6	0	6	19	4

Table S2 Distance used for the α parameter in the incidence function model for each amphibian species.

Species	Distance (m)	Reference
Bufo bufo	1000	Jeliazkov, A., Chiron, F., Garnier, J., Besnard, A., Silvestre, M., & Jiguet, F. (2014). Level-dependence of the relationships between amphibian biodiversity and environment in pond systems within an intensive agricultural landscape. *Hydrobiologia*, 723(1), 7–23.
Bufotes viridis	1000	Distance assumed based on related species (e.g., *Bufo bufo*). see Smith, M. A., & Green, D. M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, 28(1), 110–128.
Hyla intermedia	4000	Angelone, S., & Holderegger, R. (2009). Population genetics suggests effectiveness of habitat connectivity measures for the European tree frog in Switzerland. *Journal of Applied Ecology*, 46(4), 879–887.
Rana dalmatina	1000	Jeliazkov, A., Chiron, F., Garnier, J., Besnard, A., Silvestre, M., & Jiguet, F. (2014). Level-dependence of the relationships between amphibian biodiversity and environment in pond systems within an intensive agricultural landscape. *Hydrobiologia*, 723(1), 7–23.
Rana latastei	1500	Ficetola, G. F., & De Bernardi, F. (2004). Amphibians in a human-dominated landscape: The community structure is related to habitat features and isolation. *Biological Conservation*, 119(2), 219–230. Manenti, R., Falaschi, M., Monache, D. D., Marta, S., & Ficetola, G. F. (2020). Network-scale effects of invasive species on spatially-structured amphibian populations. *Ecography*, 43(1), 119–127.
Rana temporaria	500	Smith, M. A., & Green, D. M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, 28(1), 110–128.
Pelophylax synklepton *esculentus*	2000	Jeliazkov, A., Chiron, F., Garnier, J., Besnard, A., Silvestre, M., & Jiguet, F. (2014). Level-dependence of the relationships between amphibian biodiversity and environment in pond systems within an intensive agricultural landscape. *Hydrobiologia*, 723(1), 7–23.
Salamandra salamandra	500	Ficetola, G. F., Padoa-Schioppa, E., & De Bernardi, F. (2009). Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. *Conservation Biology*, 23(1), 114–123. Ficetola, G. F., Manenti, R., De Bernardi, F., & Padoa-Schioppa, E. (2012). Can patterns of spatial autocorrelation reveal population processes? An analysis with the fire salamander. *Ecography*, 35(8), 693–703.
Triturus carnifex	300	Smith, M. A., & Green, D. M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, 28(1), 110–128.
Lissotriton vulgaris	200	Ficetola, G. F., & De Bernardi, F. (2004). Amphibians in a human-dominated landscape: The community structure is related to habitat features and isolation. *Biological Conservation*, 119(2), 219–230.

Table S3 Sensitivity analysis of priors. To test a possible effect of the choice of a normal over uniform priors, we performed two additional dynamic occupancy models with Green frogs data. In the first model, we changed the priors of detection covariates from normal (mean = 0, SD = 100) to uniform (minimum = -100, maximum = 100). In the second model, we changed the priors of initial occupancy/survival/colonization covariates from normal (mean = 0, SD = 10) to uniform (minimum = -10, maximum = 10). All the estimated parameters were consistent across the three models.

Process	Estimated parameter	Priors	Mean	SD	Lower 95% CI	Upper 95% CI
Initial occupancy	Intercept	Normal	1.179	0.323	0.605	1.871
Initial occupancy	Intercept	Uniform detection	1.167	0.319	0.594	1.854
Initial occupancy	Intercept	Uniform occupancy	1.251	0.371	0.659	1.985
Initial occupancy	Fish	Normal	-0.860	0.319	-1.518	-0.240
Initial occupancy	Fish	Uniform detection	-0.861	0.320	-1.524	-0.256
Initial occupancy	Fish	Uniform occupancy	-0.882	0.337	-1.557	-0.237
Initial occupancy	Permanent	Normal	-0.139	0.313	-0.757	0.451
Initial occupancy	Permanent	Uniform detection	-0.149	0.317	-0.792	0.428
Initial occupancy	Permanent	Uniform occupancy	-0.167	0.434	-0.832	0.473
Initial occupancy	Area	Normal	0.938	0.355	0.285	1.685
Initial occupancy	Area	Uniform detection	0.949	0.357	0.285	1.675
Initial occupancy	Area	Uniform occupancy	0.954	0.366	0.265	1.720
Initial occupancy	Forest	Normal	-0.419	0.307	-1.038	0.154
Initial occupancy	Forest	Uniform detection	-0.426	0.306	-1.037	0.141
Initial occupancy	Forest	Uniform occupancy	-0.462	0.337	-1.167	0.151
Survival	Intercept	Normal	3.969	1.364	1.957	7.317
Survival	Intercept	Uniform detection	3.967	1.304	2.025	7.138
Survival	Intercept	Uniform occupancy	6.725	2.064	2.650	9.886
Survival	Fish	Normal	0.985	1.059	-0.768	3.529
Survival	Fish	Uniform detection	0.990	1.014	-0.763	3.271
Survival	Fish	Uniform occupancy	1.712	1.904	-1.633	6.091
Survival	Crayfish	Normal	-1.111	0.808	-2.924	0.330
Survival	Crayfish	Uniform detection	-1.106	0.821	-2.938	0.319
Survival	Crayfish	Uniform occupancy	-2.065	1.477	-5.419	0.390
Survival	Permanent	Normal	0.992	0.764	-0.356	2.650
Survival	Permanent	Uniform detection	0.997	0.753	-0.315	2.693
Survival	Permanent	Uniform occupancy	1.656	1.572	-0.985	5.190
Survival	Area	Normal	3.622	1.401	1.525	6.906
Survival	Area	Uniform detection	3.621	1.338	1.557	6.789
Survival	Area	Uniform occupancy	6.033	2.135	1.990	9.700
Survival	Forest	Normal	-0.805	0.954	-2.938	0.855
Survival	Forest	Uniform detection	-0.768	0.966	-2.850	0.961
Survival	Forest	Uniform occupancy	-1.610	1.744	-5.510	1.519
Colonization	Intercept	Normal	-1.274	0.587	-2.873	-0.510
Colonization	Intercept	Uniform detection	-1.268	0.592	-2.948	-0.497

Colonization	Intercept	Uniform occupancy	-2.168	1.443	-5.647	-0.659
Colonization	Fish	Normal	-0.482	0.412	-1.363	0.253
Colonization	Fish	Uniform detection	-0.487	0.417	-1.352	0.241
Colonization	Fish	Uniform occupancy	-0.522	0.411	-1.418	0.184
Colonization	Crayfish	Normal	0.418	0.384	-0.301	1.199
Colonization	Crayfish	Uniform detection	0.425	0.366	-0.287	1.177
Colonization	Crayfish	Uniform occupancy	0.452	0.376	-0.233	1.251
Colonization	Permanent	Normal	1.568	1.028	0.518	4.577
Colonization	Permanent	Uniform detection	1.572	1.045	0.500	4.639
Colonization	Permanent	Uniform occupancy	3.162	2.618	0.606	9.371
Colonization	Area	Normal	0.021	0.303	-0.544	0.674
Colonization	Area	Uniform detection	0.019	0.303	-0.555	0.650
Colonization	Area	Uniform occupancy	0.069	0.312	-0.523	0.745
Colonization	Forest	Normal	-0.704	0.320	-1.384	-0.130
Colonization	Forest	Uniform detection	-0.700	0.317	-1.379	-0.127
Colonization	Forest	Uniform occupancy	-0.688	0.311	-1.339	-0.118
Colonization	Crayfish incidence	Normal	-0.969	0.424	-1.891	-0.205
Colonization	Crayfish incidence	Uniform detection	-0.972	0.419	-1.864	-0.203
Colonization	Crayfish incidence	Uniform occupancy	-1.038	0.422	-1.941	-0.291
Colonization	Species incidence	Normal	-0.031	0.359	-0.703	0.664
Colonization	Species incidence	Uniform detection	-0.041	0.368	-0.761	0.675
Colonization	Species incidence	Uniform occupancy	0.020	0.374	-0.731	0.767
Colonization	Intercept	Normal	-0.512	0.085	-0.680	-0.346
Colonization	Intercept	Uniform detection	-0.513	0.084	-0.677	-0.339
Colonization	Intercept	Uniform occupancy	-0.529	0.087	-0.703	-0.359
Colonization	Day	Normal	1.256	0.071	1.119	1.396
Colonization	Day	Uniform detection	1.258	0.070	1.124	1.394
Colonization	Day	Uniform occupancy	1.251	0.070	1.119	1.392
Colonization	Day^2	Normal	-0.370	0.043	-0.457	-0.286
Colonization	Day^2	Uniform detection	-0.370	0.043	-0.461	-0.291
Colonization	Day^2	Uniform occupancy	-0.368	0.043	-0.454	-0.288
Colonization	Hour	Normal	0.433	0.087	0.261	0.600
Colonization	Hour	Uniform detection	0.434	0.086	0.263	0.601
Colonization	Hour	Uniform occupancy	0.431	0.086	0.262	0.600
Colonization	Hour^2	Normal	0.228	0.041	0.149	0.306
Colonization	Hour^2	Uniform detection	0.229	0.040	0.148	0.307
Colonization	Hour^2	Uniform occupancy	0.227	0.042	0.144	0.310

Table S4 Pairwise correlation coefficients between independent variables used in dynamic occupancy models. Comparison between variables never used in the same dynamic occupancy model were masked. Perm. = Permanence; Crayf. = Crayfish; C. i. = Crayfish incidence; S. i. = Species incidence. Species abbreviations: *Bb* = *Bufo bufo*, *Bv* = *Bufotes viridis*, *Hi* = *Hyla intermedia*, *Rd* = *Rana dalmatina*, *Rl* = *Rana latastei*, *Rt* = *Rana temporaria*, *Pe* = *Pelophylax lessonae* and *Pelophylax* klepton *esculentus*, *Ss* = *Salamandra salamandra*, *Tc* = *Triturus carnifex*, *Lv* = *Lissotriton vulgaris*.

	Forest	Area	Perm.	Fish	Crayf.	C. i. *Bb*	C. i. *Bv*	C. i. *Hi*	C. i. *Rd*	C. i. *Rl*	C. i. *Rt*	C. i. *Pe*	C. i. *Ss*	C. i. *Tc*	C. i. *Lv*	S. i. *Bb*	S. i. *Bv*	S. i. *Hi*	S. i. *Rd*	S. i. *Rl*	S. i. *Rt*	S. i. *Pe*	S. i. *Ss*	S. i. *Tc*	S. i. *Lv*
Forest	1	-0.044	0.091	-0.044	-0.167	-0.119	-0.119	-0.104	-0.119	-0.094	-0.159	-0.086	-0.159	-0.175	-0.183	0.239	-0.076	-0.050	0.190	0.019	0.209	-0.068	-0.065	-0.125	-0.028
Area	-0.044	1	0.247	0.355	0.175	-0.004	-0.004	0.031	-0.004	0.014	-0.021	0.024	-0.021	-0.021	-0.017	-0.046	-0.072	0.021	-0.159	-0.057	-0.162	-0.048	-0.168	-0.117	-0.150
Perm.	0.091	0.247	1	0.230	0.187	0.006	0.006	0.069	0.006	0.019	-0.007	0.031	-0.007	-0.008	-0.007	-0.086	-0.184	-0.144	-0.182	-0.109	0.075	-0.184	-0.088	-0.132	-0.198
Fish	-0.044	0.355	0.230	1	0.480	0.040	0.040	0.050	0.040	0.030	0.078	0.031	0.078	0.094	0.099	-0.021	-0.245	-0.392	-0.345	-0.276	-0.101	-0.340	-0.148	-0.237	-0.265
Crayf.	-0.167	0.175	0.187	0.480	1	0.421	0.421	0.279	0.421	0.395	0.450	0.369	0.450	0.454	0.450	0.079	0.004	-0.209	-0.118	-0.027	-0.021	-0.081	0.103	0.017	-0.021
C. i. *Bb*	-0.119	-0.004	0.006	0.040	0.421	1	■	■	■	■	■	■	■	■	■	0.469	■	■	■	■	■	■	■	■	■
C. i. *Bv*	-0.119	-0.004	0.006	0.040	0.421	■	1	■	■	■	■	■	■	■	■	■	0.533	■	■	■	■	■	■	■	■
C. i. *Hi*	-0.104	0.031	0.069	0.050	0.279	■	■	1	■	■	■	■	■	■	■	■	■	0.246	■	■	■	■	■	■	■
C. i. *Rd*	-0.119	-0.004	0.006	0.040	0.421	■	■	■	1	■	■	■	■	■	■	■	■	■	0.390	■	■	■	■	■	■
C. i. *Rl*	-0.094	0.014	0.019	0.030	0.395	■	■	■	■	1	■	■	■	■	■	■	■	■	■	0.510	■	■	■	■	■
C. i. *Rt*	-0.159	-0.021	-0.007	0.078	0.450	■	■	■	■	■	1	■	■	■	■	■	■	■	■	■	0.046	■	■	■	■
C. i. *Pe*	-0.086	0.024	0.031	0.031	0.369	■	■	■	■	■	■	1	■	■	■	■	■	■	■	■	■	0.494	■	■	■
C. i. *Ss*	-0.159	-0.021	-0.007	0.078	0.450	■	■	■	■	■	■	■	1	■	■	■	■	■	■	■	■	■	0.590	■	■
C. i. *Tc*	-0.175	-0.021	-0.008	0.094	0.454	■	■	■	■	■	■	■	■	1	■	■	■	■	■	■	■	■	■	0.372	■
C. i. *Lv*	-0.183	-0.017	-0.007	0.099	0.450	■	■	■	■	■	■	■	■	■	1	■	■	■	■	■	■	■	■	■	0.368
S. i. *Bb*	0.239	-0.046	-0.086	-0.021	0.079	0.469	■	■	■	■	■	■	■	■	■	1	■	■	■	■	■	■	■	■	■
S. i. *Bv*	-0.076	-0.072	-0.184	-0.245	0.004	■	0.533	■	■	■	■	■	■	■	■	■	1	■	■	■	■	■	■	■	■
S. i. *Hi*	-0.050	0.021	-0.144	-0.392	-0.209	■	■	0.246	■	■	■	■	■	■	■	■	■	1	■	■	■	■	■	■	■
S. i. *Rd*	0.190	-0.159	-0.182	-0.345	-0.118	■	■	■	0.390	■	■	■	■	■	■	■	■	■	1	■	■	■	■	■	■
S. i. *Rl*	0.019	-0.057	-0.109	-0.276	-0.027	■	■	■	■	0.510	■	■	■	■	■	■	■	■	■	1	■	■	■	■	■
S. i. *Rt*	0.209	-0.162	0.075	-0.101	-0.021	■	■	■	■	■	0.046	■	■	■	■	■	■	■	■	■	1	■	■	■	■
S. i. *Pe*	-0.068	-0.048	-0.184	-0.340	-0.081	■	■	■	■	■	■	0.494	■	■	■	■	■	■	■	■	■	1	■	■	■
S. i. *Ss*	-0.065	-0.168	-0.088	-0.148	0.103	■	■	■	■	■	■	■	0.590	■	■	■	■	■	■	■	■	■	1	■	■
S. i. *Tc*	-0.125	-0.117	-0.132	-0.237	0.017	■	■	■	■	■	■	■	■	0.372	■	■	■	■	■	■	■	■	■	1	■
S. i. *Lv*	-0.028	-0.150	-0.198	-0.265	-0.021	■	■	■	■	■	■	■	■	■	0.368	■	■	■	■	■	■	■	■	■	1

Table S5 Average effect size and variance of the variables related to initial occupancy, survival, and colonization, with 95% Credible Intervals (CIs) and the value of the Rhat statistic.

Species / process / variable	**Mean**	**Variance**	**Lower 95% CI**	**Upper 95% CI**	**Rhat**
Bufo bufo					
Initial occupancy					
Intercept	-5.14	1.48	-7.82	-3.14	1.00
Forest	2.32	0.64	0.89	4.05	1.00
Area	-1.62	0.78	-3.51	-0.01	1.00
Permanence	2.18	2.01	0.08	5.59	1.00
Fish	0.69	0.48	-0.61	2.17	1.00
Survival					
Intercept	2.42	0.96	0.90	4.78	1.00
Forest	2.41	1.65	0.45	5.57	1.00
Area	2.64	1.51	0.63	5.44	1.01
Permanence	0.60	0.98	-1.07	2.87	1.00
Fish	1.24	1.41	-0.63	4.11	1.00
Crayfish	-0.27	1.08	-2.13	2.05	1.00
Colonization					
Intercept	-3.96	0.13	-4.71	-3.33	1.00
Forest	1.12	0.08	0.56	1.70	1.00
Area	0.03	0.07	-0.51	0.55	1.00
Permanece	0.51	0.17	-0.23	1.40	1.00
Fish	1.24	0.10	0.59	1.90	1.00
Crayfish	-0.61	0.11	-1.28	0.05	1.00
Crayfish incidence	-0.18	0.14	-0.90	0.57	1.00
Species incidence	1.14	0.30	0.13	2.25	1.00
Bufotes viridis					
Initial occupancy					
Intercept	-0.37	4.81	-3.96	4.32	1.03
Forest	-0.26	3.58	-4.56	3.43	1.00
Area	2.82	4.75	-1.16	7.63	1.00
Permanence	-0.04	2.77	-3.99	3.23	1.02
Fish	-3.46	4.35	-8.09	0.02	1.00
Survival					
Intercept	-1.81	4.02	-5.63	2.29	1.08
Forest	-1.43	3.23	-5.96	1.63	1.01
Area	3.41	6.32	-1.99	8.08	1.01
Permanence	-1.38	4.47	-5.50	3.47	1.03
Fish	-2.26	6.59	-6.58	4.05	1.06
Crayfish	0.71	5.40	-5.66	4.53	1.09
Colonization					
Intercept	-0.21	3.85	-4.27	3.83	1.08
Forest	2.00	4.13	-1.78	6.18	1.02

	Area	2.88	5.62	-1.99	7.26	1.00
	Permanence	1.60	6.58	-4.00	6.24	1.01
	Fish	-1.37	4.97	-6.37	2.85	1.04
	Crayfish	-3.43	6.12	-7.97	2.01	1.05
	Crayfish incidence	-2.31	5.30	-6.77	2.06	1.02
	Species incidence	0.30	6.66	-4.43	5.43	1.00
Hyla intermedia						
Initial occupancy						
	Intercept	-0.33	0.10	-0.98	0.29	1.00
	Forest	0.61	0.10	0.04	1.30	1.00
	Area	1.01	0.15	0.34	1.85	1.00
	Permanence	-0.24	0.08	-0.81	0.30	1.00
	Fish	-1.13	0.13	-1.86	-0.49	1.00
Survival						
	Intercept	1.19	0.07	0.68	1.71	1.00
	Forest	-0.01	0.12	-0.69	0.67	1.00
	Area	0.76	0.25	-0.11	1.88	1.00
	Permanence	0.12	0.10	-0.50	0.72	1.00
	Fish	-1.04	0.11	-1.71	-0.41	1.00
	Crayfish	-0.33	0.10	-0.93	0.30	1.00
Colonization						
	Intercept	-3.42	0.53	-5.11	-2.22	1.01
	Forest	-3.27	1.72	-6.11	-1.18	1.01
	Area	0.34	0.35	-1.00	1.37	1.00
	Permanence	1.17	0.60	-0.15	2.80	1.00
	Fish	-0.16	0.38	-1.35	1.06	1.00
	Crayfish	0.66	0.33	-0.43	1.87	1.00
	Crayfish incidence	-1.89	0.94	-4.17	-0.35	1.01
	Species incidence	6.07	2.91	3.30	9.82	1.01
Rana dalmatina						
Initial occupancy						
	Intercept	-3.21	4.04	-7.99	-0.58	1.03
	Forest	5.33	3.69	2.17	9.70	1.01
	Area	0.08	1.82	-2.60	2.92	1.02
	Permanence	-1.31	1.86	-4.23	1.19	1.00
	Fish	-1.77	2.60	-5.13	1.25	1.01
Survival						
	Intercept	2.24	0.08	1.72	2.81	1.02
	Forest	0.60	0.15	-0.14	1.39	1.01
	Area	1.53	0.13	0.84	2.25	1.01
	Permanence	0.19	0.06	-0.27	0.66	1.02
	Fish	-0.85	0.07	-1.39	-0.33	1.04
	Crayfish	-0.41	0.07	-0.93	0.12	1.00
Colonization						
	Intercept	-4.00	0.37	-5.38	-2.92	1.07

Forest	1.73	0.23	0.89	2.76	1.01
Area	-2.00	0.27	-3.05	-1.08	1.03
Permanence	3.20	1.36	1.28	5.74	1.05
Fish	-0.94	0.18	-1.80	-0.15	1.02
Crayfish	0.70	0.16	-0.05	1.52	1.00
Crayfish incidence	-1.08	0.24	-2.05	-0.14	1.00
Species incidence	1.77	0.40	0.59	3.05	1.01
Rana latastei					
Initial occupancy					
Intercept	-1.05	5.12	-5.56	3.74	1.01
Forest	0.61	4.35	-3.74	4.83	1.00
Area	0.50	4.76	-4.00	4.90	1.00
Permanence	3.93	4.62	-0.10	8.41	1.00
Fish	0.69	5.18	-3.66	5.54	1.00
Survival					
Intercept	1.69	0.08	1.16	2.27	1.00
Forest	0.05	0.15	-0.68	0.83	1.00
Area	0.68	0.26	-0.16	1.83	1.00
Permanence	0.56	0.10	-0.04	1.22	1.00
Fish	0.13	0.23	-0.72	1.15	1.00
Crayfish	1.29	0.36	0.33	2.61	1.00
Colonization					
Intercept	-3.12	0.17	-4.03	-2.43	1.00
Forest	1.34	0.10	0.78	2.01	1.00
Area	-0.91	0.17	-1.74	-0.11	1.00
Permanence	-0.02	0.09	-0.60	0.60	1.00
Fish	0.69	0.25	-0.41	1.62	1.00
Crayfish	0.23	0.28	-0.79	1.27	1.00
Crayfish incidence	-0.58	0.24	-1.56	0.36	1.00
Species incidence	0.23	0.20	-0.62	1.11	1.00
Rana temporaria					
Initial occupancy					
Intercept	-6.37	2.28	-9.70	-3.77	1.01
Forest	2.36	0.85	0.64	4.24	1.00
Area	-2.10	1.34	-4.53	-0.09	1.00
Permanence	1.63	1.87	-0.44	5.01	1.01
Fish	0.29	0.76	-1.45	1.99	1.01
Survival					
Intercept	0.77	7.49	-3.59	6.66	1.04
Forest	1.71	6.34	-3.12	6.68	1.01
Area	-0.51	7.83	-6.05	4.82	1.00
Permanence	2.00	7.97	-3.33	7.65	1.00
Fish	-2.31	6.81	-7.78	2.63	1.01
Crayfish	-1.74	7.05	-7.25	3.31	1.01
Colonization					

Intercept	-8.22	1.84	-11.13	-5.86	1.00
Forest	1.89	0.62	0.35	3.47	1.00
Area	-0.62	0.56	-2.19	0.75	1.00
Permanence	1.81	1.51	0.00	4.80	1.00
Fish	-2.01	1.51	-4.75	0.13	1.00
Crayfish	-1.25	1.51	-4.15	0.64	1.00
Crayfish incidence	0.47	0.66	-1.16	2.02	1.01
Species incidence	4.57	2.28	2.06	7.76	1.00
Pelophylax synklepton *esculentus*					
Initial occupancy					
Intercept	1.18	0.10	0.61	1.87	1.00
Forest	-0.42	0.09	-1.04	0.15	1.00
Area	0.94	0.13	0.29	1.69	1.00
Permanence	-0.14	0.10	-0.76	0.45	1.00
Fish	-0.86	0.10	-1.52	-0.24	1.00
Survival					
Intercept	3.97	1.86	1.96	7.32	1.00
Forest	-0.80	0.91	-2.94	0.85	1.00
Area	3.62	1.96	1.52	6.91	1.00
Permanence	0.99	0.58	-0.36	2.65	1.00
Fish	0.99	1.12	-0.77	3.53	1.00
Crayfish	-1.11	0.65	-2.92	0.33	1.00
Colonization					
Intercept	-1.27	0.34	-2.87	-0.51	1.00
Forest	-0.70	0.10	-1.38	-0.13	1.00
Area	0.02	0.09	-0.54	0.67	1.00
Permanence	1.57	1.06	0.52	4.58	1.00
Fish	-0.48	0.17	-1.36	0.25	1.00
Crayfish	0.42	0.15	-0.30	1.20	1.00
Crayfish incidence	-0.97	0.18	-1.89	-0.21	1.01
Species incidence	-0.03	0.13	-0.70	0.66	1.00
Salamandra salamandra					
Initial occupancy					
Intercept	-6.87	2.78	-10.42	-4.02	1.00
Forest	1.34	0.91	-0.39	3.36	1.00
Area	-1.52	1.60	-4.18	0.80	1.00
Permanence	0.82	2.10	-1.49	4.26	1.00
Fish	1.61	1.26	-0.49	4.04	1.00
Survival					
Intercept	3.78	5.49	-0.58	7.90	1.00
Forest	-0.76	6.22	-4.32	5.74	1.00
Area	2.17	5.72	-3.91	5.87	1.00
Permanence	0.60	1.79	-1.71	3.79	1.00
Fish	0.00	1.86	-3.16	2.21	1.00
Crayfish	-0.30	1.99	-2.02	4.01	1.00

Colonization					
Intercept	-6.68	0.77	-8.61	-5.14	1.00
Forest	1.09	0.17	0.31	1.92	1.00
Area	-1.97	0.31	-3.12	-0.91	1.00
Permanence	3.17	1.72	1.19	6.17	1.00
Fish	-0.81	0.27	-1.95	0.03	1.00
Crayfish	-0.11	0.21	-1.04	0.75	1.00
Crayfish incidence	-0.28	0.28	-1.33	0.76	1.00
Species incidence	0.85	0.35	-0.26	2.06	1.00
Triturus cristatus					
Initial occupancy					
Intercept	-1.77	0.13	-2.53	-1.13	1.00
Forest	0.09	0.08	-0.48	0.66	1.00
Area	-0.05	0.08	-0.61	0.52	1.00
Permanence	-0.15	0.06	-0.63	0.32	1.00
Fish	-0.96	0.20	-1.96	-0.22	1.00
Survival					
Intercept	1.15	2.11	-2.77	2.99	1.01
Forest	1.91	0.60	0.57	3.63	1.00
Area	1.99	1.06	0.35	4.26	1.00
Permanence	1.47	0.43	0.32	2.82	1.00
Fish	-1.42	5.13	-6.38	1.72	1.00
Crayfish	0.31	2.90	-1.85	4.87	1.01
Colonization					
Intercept	-4.35	1.08	-6.56	-2.64	1.00
Forest	-0.12	0.18	-0.97	0.72	1.00
Area	-0.03	0.11	-0.69	0.62	1.00
Permanence	0.70	0.22	-0.11	1.74	1.00
Fish	-1.20	0.59	-3.17	-0.16	1.00
Crayfish	-0.94	1.11	-3.55	0.35	1.00
Crayfish incidence	-1.74	0.65	-3.72	-0.63	1.00
Species incidence	0.97	0.16	0.27	1.83	1.00
Lissotriton vulgaris					
Initial occupancy					
Intercept	-1.83	0.14	-2.63	-1.16	1.00
Forest	0.66	0.09	0.06	1.28	1.00
Area	-0.01	0.09	-0.60	0.58	1.00
Permanence	-0.30	0.06	-0.81	0.18	1.00
Fish	-1.03	0.22	-2.05	-0.20	1.00
Survival					
Intercept	1.32	0.24	0.28	2.29	1.00
Forest	0.68	0.63	-0.69	2.39	1.00
Area	2.54	1.65	0.26	5.20	1.00
Permanence	1.07	0.38	0.02	2.44	1.01
Fish	-1.20	0.84	-3.52	0.31	1.00

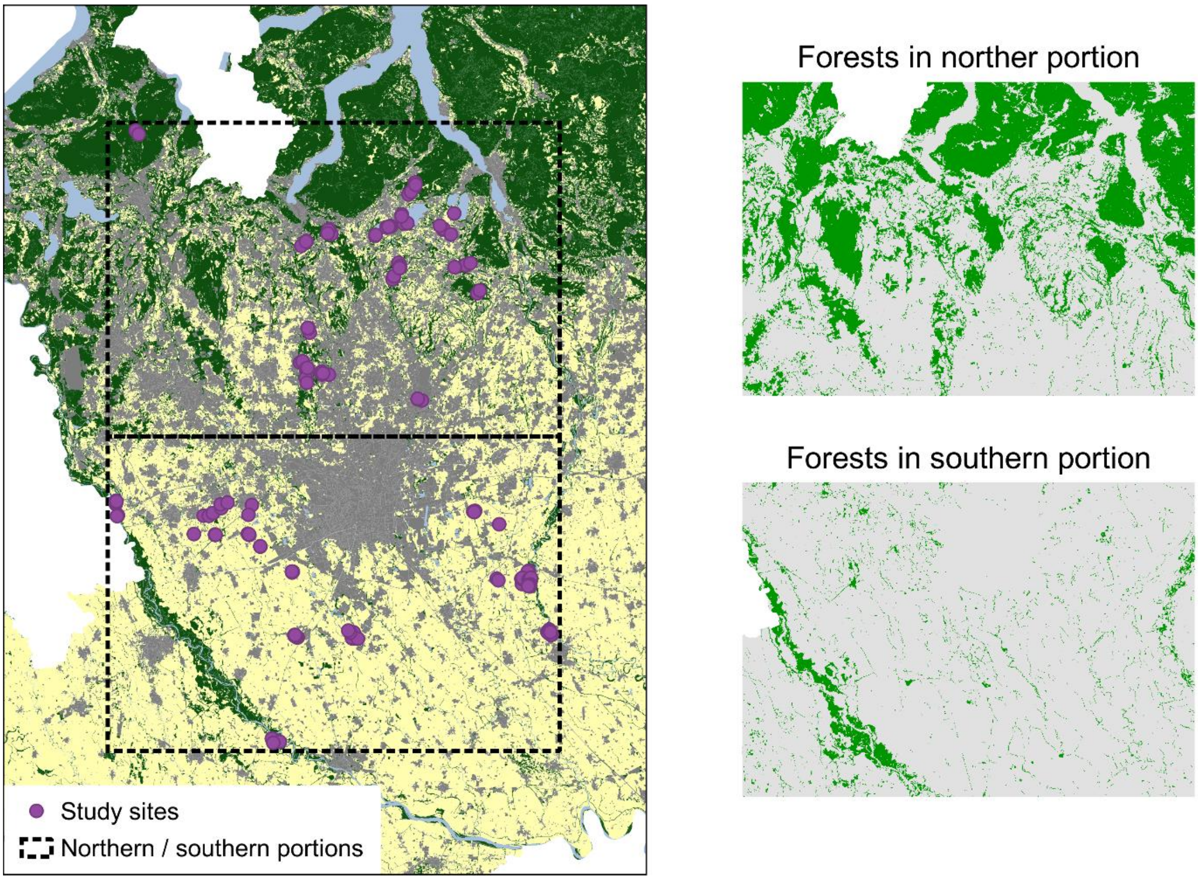
Crayfish	-0.43	0.66	-2.30	1.03	1.00
Colonization					
Intercept	-4.17	0.34	-5.59	-3.28	1.00
Forest	0.30	0.14	-0.45	1.00	1.00
Area	0.21	0.08	-0.36	0.74	1.00
Permanence	0.18	0.08	-0.34	0.75	1.00
Fish	-1.22	0.32	-2.48	-0.30	1.00
Crayfish	-0.76	0.62	-2.43	0.58	1.00
Crayfish incidence	-2.06	1.08	-4.92	-0.78	1.00
Species incidence	1.00	0.20	0.13	1.90	1.00

Table S6 Spatial autocorrelation. For each species and process (initial occupancy, survival, and colonization), we show the 95% Credible Intervals of the intrinsic conditional autoregressive model (rho), across the 202 study sites, and its precision (tau).

Species	Initial occupancy rho	Initial occupancy tau	Survival rho	Survival tau	Colonization rho	Colonization tau
Bufo bufo	-0.023 – 0.146	7.458	-0.007 – 0.028	7.973	-0.034 – 0.062	7.623
Bufotes viridis	-0.006 – 0.004	8.091	-0.003 – 0.004	8.117	-0.003 – 0.005	8.280
Hyla intermedia	-0.034 – 0.008	7.895	-0.026 – 0.007	7.962	-0.014 – 0.006	8.048
Rana dalmatina	-19.556 – 12.254	< 0.001	-0.007 – 0.014	7.928	-0.011 – 0.010	8.084
Rana latastei	-27.329 – 20.407	< 0.001	-0.006 – 0.010	8.327	-0.009 – 0.006	7.761
Rana temporaria	-0.015 – 0.103	7.183	-0.005 – 0.004	8.009	-0.003 – 0.004	7.637
Pelophylax synklepton *esculentus*	-0.011 – 0.004	8.295	-16.084 – 10.425	0.001	-0.006 – 0.004	8.260
Salamandra salamandra	-0.004 – 0.008	7.993	-0.003 – 0.004	8.181	-0.005 – 0.006	8.167
Triturus carnifex	-0.046 – 0.013	7.755	-0.055 – 0.064	7.570	-0.025 – 0.005	8.166
Lissotriton vulgaris	-0.077 – 0.015	7.586	-0.009 – 0.004	7.967	-0.007 – 0.007	8.092

Appendix S1: Fragmentation metrics of the northern and southern portions of the study area

To describe the differences in forest fragmentation between the northern and the southern portions of the study area, we calculated three fragmentation metrics for each portion: perimeter/area ratio, shape complexity, and fractal dimension index (VanDerWal et al. 2019). First, we applied a 1 km buffer to the smallest rectangle that includes all the study sites and used the resulting rectangle to crop the land-use map of Lombardy region of the year 2015. Second, we rasterized the map at 20 m resolution, assigning a value of one to forests (categories 311 and 313 of the level 3 regional classification of land use; (https://www.cartografia.regione.lombardia.it/metadata/Dusaf/doc/legenda_DUSAF5.pdf; accessed on 10 July 2020), and zero to other categories. Then, we horizontally split the map into two rectangles of the same size, as in the following figure:



Maps were processed using QGIS and the R package raster (Hijmans 2019; QGIS Development Team 2020). Finally, we calculated the patch statistics of the two portions of the study area using the function PatchStat() in the R package SDMTools (VanDerWal et al. 2019). In the following table, we list the perimeter/area ratio, shape complexity, and fractal dimension:

Portion	**Number of 20 × 20 m forest cells**	**Perimeter/area ratio**	**Shape complexity**	**Fractal dimension index**
Northern	1841228	0.237	80.289	1.608
Southern	286174	0.741	99.078	1.732

The southern portion of the study area shows a lower number of forest cells, a higher perimeter/area ratio, a higher shape complexity, and a higher fractal dimension. These values indicate that in the southern portion of the study area, forests are less abundant and more fragmented than in the northern portion.

Appendix S2: Description of the study species and the Red Swamp Crayfish

Here we provide a brief description of the morphology, ecology, and distribution, of the ten study species of amphibians and for the Red Swamp Crayfish. For a detailed description of morphology and ecology of amphibian species, see Lanza et al. (2007). For the Red Swamp Crayfish, see Gonçalves Loureiro et al. (2015).

Toads: Common Toad (*Bufo bufo*) and Green Toad (*Bufotes viridis*)

The Common Toad is a large amphibian that can reach a body size of up to 21 cm. Its distribution includes most of the European continent, up to north-western Asia (Arntzen et al. 2017). It usually spends the post-breeding season in wooded areas, and in the study area, breeding generally occurs in February-March, mostly in large ponds and small lakes. Metamorphosis generally occurs in June (Bernini et al. 2004). The sex ratio in breeding populations is usually unbalanced toward males, which causes a strong scramble competition between males (Arntzen 1999).

The Green Toad is smaller than the Common Toad, reaching a maximum body length of 9 cm. It is present from central Europe to western Asia (Dufresnes et al. 2019). The breeding season generally spans from late Mach to May, and generally occurs in sunny temporary ponds, even in highly anthropized landscapes (Bernini et al. 2004; Lanza et al. 2007).

Italian Tree Frog (*Hyla intermedia*)

The Italian Tree Frog is endemic to Italy (except for Sardinia) and southern Switzerland (Sillero et al. 2014). The adults have excellent climbing capacity and can use tree canopy to move across long distances (Lanza et al. 2007). In the study area, the breeding season starts at the end of April and usually lasts until the end of June (Bernini et al. 2004; Lanza et al. 2007). During this period, males aggregate in choruses to attract females (Castellano et al. 2009). Tree Frogs lay small egg masses that are usually attached to submerged vegetation (Lanza et al. 2007).

Brown frogs: Agile Frog (*Rana dalmatina*), Italian Agile Frog (*Rana latastei*), and Common Frog (*Rana temporaria*)

The Agile Frog is a medium size frog with a maximum total length of 9 cm (Lanza et al. 2007). Its distribution covers most of the European countries, from the Spanish Pyrenees to the Anatolian peninsula (Sillero et al. 2014). The Italian Agile Frog is slightly smaller (females reach 7.5 cm of body length) and it is endemic of northern Italy, southern Switzerland, and western Slovenia (Sillero et al. 2014). The Common Frog is the largest and most widespread European brown frog, with a body length of up to 11 cm. It is widespread in Europe and western Asia, even though in the southern range of its distribution it is mainly found in mountain areas (Lanza et al. 2007; Sillero et al. 2014).

Brown frogs are mostly terrestrial, spending only the short reproductive period ("explosive breeding") inside the aquatic environment (Ambrogio & Mezzadri 2018). In the study area, mating season occurs between February and March, when adults migrate from woodlands towards ponds or slow-flowing water bodies to breed (Lanza et al. 2007). The typical habitat is deciduous forest, but they can also colonize open areas like inundated meadows or marshes (Lanza et al. 2007; Ambrogio & Mezzadri 2018).

Hybridogenetic Green frogs: (*Pelophylax lessonae* and *Pelophylax* klepton *esculentus*)

Green Frogs are a complex taxon composed both by non-hybrid species and by hybridogenetic hybrid species; their morphological identification is generally very difficult. The species occurring in the study area are *Pelophylax lessonae* and *Pelophylax* klepton *esculentus*, which form the LE system (Holsbeek & Jooris 2010). This system is widespread in northern Italy as well as in central and eastern Europe (Holsbeek & Jooris 2010). Green Frogs have a maximum length that ranges from 8 to 12 cm depending on the taxon. All the taxa are highly aquatic, have a very broad ecological niche, and are able to colonize a wide variety of natural and artificial habitat although they prefer sunny environments (Lanza et al. 2007). In the study area, the activity period spans from March to November, while mating occurs between April and June (Bernini et al. 2004; Lanza et al. 2007).

Fire Salamander (*Salamandra salamandra*)

The Fire Salamander is the most widespread European salamander, and its distribution includes most Europe, from the Iberian Peninsula to the Carpathians (Sillero et al. 2014). It can reach a body length of 32 cm, it is usually found in broadleaved forests, but it can exploit various habitats (Bernini et al. 2004; Lanza et al. 2007). Although it can be active throughout the year, in the study area, the activity shows two peaks: one in spring (between March and May), and one in autumn (between October and November) (Bernini et al. 2004). Among the study species, the Fire Salamander is the only one that does not lay eggs but is ovoviviparous (Lanza et al. 2007). It usually breeds in streams, although larvae can also be laid in small ponds and in natural and artificial hypogeous biotopes (Manenti et al. 2011).

Newts: Italian Crested newt (*Triturus carnifex*), Smooth newt (*Lissotriton vulgaris*)

The Italian Crested newt has a maximum body size of 18 cm, and its distribution includes Italy and other southern European countries (Lanza et al. 2007; Wielstra et al. 2014). The Smooth newt has a smaller body size (maximum body size 11 cm), and its distribution is wider, including a large part of Europe, up to central Russia (Lanza et al. 2007; Wielstra et al. 2018). Despite in some cases newts remain in the water through the year, in the study area they typically spend only the reproductive period and the larval phase in the aquatic environment (usually from Match to July; Bernini et al. 2004). During the terrestrial phase, they live nearby breeding sites, in natural or semi-natural microhabitats (Lanza et al. 2007).

Red Swamp Crayfish (*Procambarus clarkii*)

The Red Swamp Crayfish is a decapod crustacean with a body length of up to 15 cm (Gonçalves Loureiro et al. 2015). It is native from the United States and northern Mexico, but it has been introduced in all the continents except Oceania and Antarctica. In Italy, it was first introduced in 1989. In the last thirty years, repeated introductions and natural and human-mediated dispersal helped this alien species to spread rapidly, and now its distribution includes a large portion of the country (Lo Parrino et al. 2020). Its fast growth rate and the large number of offspring, paired with a broad ecological niche and high plasticity of the life cycle, contributed to its success as an invader (Gherardi 2006). It can colonize a wide range of freshwater habitats and can even resist to long drought periods due to its burrowing capacity (Gherardi et al. 2011; Gonçalves Loureiro et al. 2015). Its diet includes plant debris, macrophytes, mollusks, insects, other invertebrates, fish, and amphibians (Gonçalves Loureiro et al. 2015). It exerts a heavy predation pressure both on amphibian embryos and larvae (Cruz & Rebelo 2005), and multiple studies found a negative impact of this invasive crayfish on the abundance and distribution of amphibians (Cruz et al. 2006; Ficetola et al. 2011; Manenti et al. 2020).

Appendix S3: Sampling protocol and measurement of environmental features

Surveys were performed between 1996 and 2019. To reduce the amount of missing data and improve model convergence, we collapsed the 24 years in seven primary periods (i.e. the maximum number of primary periods surveyed for each wetland) as follows: the 1st and 2nd primary periods correspond to the first and the second sampling performed in each wetland between 1996 and 2006 (before crayfish invasion), the 3rd and 4th primary periods correspond to the first and the second sampling performed in each wetland between 2007 and 2016, and finally, the 5th to 7th primary periods correspond to the most recent sampling performed in 2017-2019 (Table S1).

During each survey, we assessed the presence of amphibian species by visually searching for adults and egg clutches, by listening to the calls of adult males, and by dip-netting to identify tadpoles and larvae (Dodd 2010). Each nocturnal survey started with a five minutes call survey to detect the calls of frogs and toads (Dodd 2010). During both diurnal and nocturnal surveys, we used visual encounter surveys over the whole wadable area of the wetland searching for egg clutches, tadpoles and larvae, and adults. During diurnal surveys, we used dip netting of the wadable part of wetland banks and bottom (Dodd 2010). To assess the presence of *Procambarus clarkii*, we used visual encounter surveys, the identification of exuviae, and dip netting (Ficetola et al. 2011). Previous analyses showed that, during nocturnal surveys, the detection probability of *P. clarkii* is very high (>95%) with these approaches (Manenti et al. 2019).

The presence of fish was assessed using visual encounter surveys. Since we did not catch fish, we did not identify fish species, and we cannot tease apart the effects of introduced and native fish. However, among the 202 study sites, 156 (77%) are isolated wetlands located outside of the main hydrographic network and consist of natural or artificial ponds, ditches, and springs. Here, fish (irrespectively if native or non-native) occur only when directly introduced by fishermen and local people (Manenti 2008; Manenti et al. 2017; Winandy et al. 2017). The remaining 46 sites include lakeshores, riverbanks, or tidal pools, which are within the hydrographic network of the Po river. Nevertheless, a study on fish community composition of the Po river basin showed that, even in the main hydrographic network, alien species represent ~40% of species (Meraner et al. 2013). Overall, this suggests that most fish species present in the study sites are either alien or native species introduced in naturally fishless wetlands.

To calculate the surface area of the wetlands, we measured the maximum length and width. Then, we calculated the area of the ellipse using width and length as measures of the two diameters of the ellipse. To avoid disproportionate values (i.e. differences > of 5 orders of magnitude), the area of non-wadable wetlands was set at the maximum value registered for wadable wetlands (i.e. 70 686 m^2), which roughly corresponds to the area that can be adequately surveyed during monitoring activities.

The percentage cover of forests within 400 m from the wetland was derived from a high-resolution land-use map of the Lombardy region (http://www.cartografia.regione.lombardia.it; ground resolution: 3m). The selection of forests was based on the level 3 classification of the land-use map (https://www.cartografia.regione.lombardia.it/metadata/Dusaf/doc/legenda_DUSAF5.pdf; accessed on 10 July 2020), considering broadleaved forests and mixed forests. Coniferous forests were not considered because they usually not represent suitable habitat for the species considered in this study (Lanza et al. 2007). For each wetland, we generated a buffer with a 400 m diameter from the centroid of the wetland (or, if the site monitored was a big lake, the midpoint of the lakeshore monitored) with QGIS. Buffers were then intersected with the land-use map, and for each buffer, we extracted the percentage cover of forests. The surface occupied by water was excluded from the calculation of the percentage cover. Due to misclassification in the original land-use map, a large urban park (Parco di Monza, coordinates: 45.60 N, 9.29 E) was classified as entirely urban, while it actually comprises meadows and broadleaved forests (Ficetola et al. 2007a). For this reason, the forest cover for the four study sites located in this park was calculated by manually digitalizing forests in QGIS from ortho-photos.

Appendix S5: Strategies adopted to prevent spatial bias

As shown in Fig.1 (main text), while study sites cover a large geographic extent, their distribution is clustered, due to the uneven distribution of wetlands. Within clusters, study sites can be within a few hundred meters from each other, while distance among clusters can measure tens of kilometers. For this reason, we adopted a workflow aimed at removing possible biases arising from the clustered distribution of study sites.

First, we included two variables (species incidence and crayfish incidence) to consider the effect of nearby wetlands. These variables were calculated with an incidence function model (Moilanen & Nieminen 2002), so the effect of other wetlands on a focal wetland decrease exponentially with distance. Therefore, study sites within a few hundred meters will strongly influence each other, while the effect of wetlands kilometers apart will be approximately zero.

Second, we included a spatial random effect for each process (initial occupancy, survival, colonization) to consider that closer wetlands can be more similar to each other than expected. Integrating spatial autocorrelation into regression models allowed taking into account spatial effects that can influence species distribution (Wagner & Fortin 2005; Beale et al. 2010). Spatial autocorrelation is extremely frequent in occurrence data and can bias estimates of statistical models (Wagner & Fortin 2005) but is very rarely integrated into occupancy modeling. For instance, more than 90% of studies using occupancy modeling to analyze environmental DNA data do not take into account cd us to successfully integrate spatial autocorrelation while jointly considering imperfect detection through dynamic occupancy models. Simulation models demonstrated that Bayesian conditional autoregressive models are able to successfully integrate autocorrelation and estimate model parameters with limited bias even in scenarios with very complex spatial structures (Beale et al. 2010).

CHAPTER 6 | CONCLUSIONS

In this thesis, I investigated the role of invasive alien species and habitat modification, jointly with other global change stressors, in driving temporal changes in amphibian populations. Invasive alien species emerged as a main driver of population changes for European amphibians, even if they often act jointly with other factors. For instance, both wetland features and the presence of invasive crayfish were important in determining population dynamics of the amphibian community at the regional scale (Chapter 5). Furthermore, I observed a relevant interaction between alien species and habitat availability, indicating that the negative effect of alien species is particularly strong in altered landscapes (Chapter 2). Additionally, the effect of alien species was consistently negative across different scales, from continental to regional.

In Chapter 2, I evaluated the effect of alien species, habitat availability, habitat changes, and climate change on population trends of amphibians (and reptiles) at the continental scale. The results showed that populations declined more often in areas with a high number of alien species and where climate change has caused a loss of climatic suitability. The effect of habitat availability was not relevant when averaged across species; however, when excluding the two commonest species, habitat loss was the main correlate of negative population trends for the remaining species. Furthermore, I observed a strong interaction between habitat availability and the richness of alien species, which indicated that the negative impact of alien species was particularly strong for populations living in landscapes with less suitable habitat (Fig. 5 in Chapter 2).

Then, Chapters 3, 4, and 5 focused on a specific system: the invasion of the red swamp crayfish *Procambarus clarkii* in Italy. This invasive alien species can colonize a wide range of freshwater habitats and has a generalist diet (Gherardi et al. 2011; Gonçalves Loureiro et al. 2015). It can exert a heavy predation pressure both on amphibian embryos and larvae (Cruz & Rebelo 2005), posing a severe threat to the conservation of amphibians. First of all, in Chapter 3, I provided the updated distribution of the red swamp crayfish in Italy. The first Italian record of this species dates back to 1989 and in 30 years it invaded most of the Italian provinces. Initially, invaded provinces were mainly in central and northern Italy. However, in recent years, the crayfish rapidly spread also in the south and in the two main islands (Fig.1 and 3 in Chapter 3). This impressive expansion was probably due to multiple introductions jointly with natural dispersal. Given the strong negative impact that this crayfish can exert on native biodiversity, its rapid expansion and widespread distribution raises concerns for the conservation of many taxa, including plants, invertebrates, fish, and amphibians. On the one hand, predation upon the red swamp crayfish is known for some native species (Correia 2001; Aquiloni et al. 2010; Delsinne et al. 2013). However, on the other hand, predation does not seem to negatively affect the persistence of the red swamp crayfish. Hence, current knowledge suggests that the most effective large-scale management strategy is containment, to prevent the colonization of new areas.

In Chapter 4, I evaluated the effect of microhabitat characteristics, landscape features, the presence of fish, and the presence of the alien crayfish, on the distribution of two newt species: the Italian crested newt and the smooth newt. Over the last 20 years, these two species showed a marked decline at the regional scale, losing between 25% and 36% of occupied sites and showing much larger extinction rates compared to colonization rates. Before the introduction of the alien crayfish, the main determinants of site occupancy were landscape variables and the presence of fish: the occupancy of Italian crested newts was negatively affected by urban cover around the wetland, while the occupancy of smooth newts was negatively affected by agricultural cover around the wetland and by the presence of fish in the wetland. These results confirm that landscape alteration and the presence of predators are key factors determining newts distribution (Ficetola & De Bernardi 2004; Denoel & Ficetola 2008). After the introduction of the crayfish, the main drivers of population dynamics sharply shifted, and the changes in occupancy were not determined by landscape or microhabitat alterations, as the strongest predictor of local extinctions was the colonization of wetlands by invasive crayfish. While some studies found strong evidence that the red swamp crayfish is negatively related to newts presence and abundance (Ficetola et al. 2011), other studies did not detect clear relationships (Bélouard et al. 2019). Because of data limitations, Chapter 4 analyzes data with an unusual method. Collecting more data would allow to perform all analyses within the dynamic occupancy model framework and to avoid any statistics-on-statistics approach (MacKenzie et al. 2003). Despite this, results suggest that the red swamp crayfish can be e key driver of change in amphibian populations (Fig. 3 in Chapter 4). For this reason, it is essential to consider that snapshot studies, relating variables to the presence or abundance of species, can easily overlook factors which pivotal for population dynamics over time. Hence, it is crucial to collect long-term data on both species occurrence and potential threats, should we want to achieve a comprehensive evaluation of factors determining species distribution and decline.

In Chapter 5, I assessed the effect of microhabitat characteristics, landscape features, and invasive crayfish in determining the temporal dynamics of amphibian populations. This study considers a larger number of wetlands compared to Chapter 4 and analyses data covering the whole amphibian community. The results showed that the same variable can have different effects on survival or colonization probabilities. Furthermore, the effect of a factor can be highly variable across species. For instance, the wetland area was positively related to survival probability of the agile frog, while it showed a negative relationship with colonization probability (Fig. 2 in Chapter 5). This high interspecific variability is probably driven by the difference in species' biology. In addition to microhabitat and landscape characteristics, I assessed the effect of two variables acting at a different scale: species incidence and crayfish incidence. These variables represent the effect of the presence of the focal species and the presence of crayfish at the landscape-scale. The idea beneath the inclusion of these parameters is that processes occurring in surrounding wetlands can influence the dynamics of a focal wetland (Manenti et al. 2020). For instance, when a given wetland is not occupied by a species, we expect that the colonization probability is higher when this wetland is surrounded by many occupied wetlands. Similarly, we expect that the negative effect of crayfish presence is stronger in landscapes where the number of invaded wetlands is higher. In Chapter 4, these hypotheses were confirmed: crayfish and species incidence were the two main drivers of colonization probability (Fig. 3 in Chapter 4). Furthermore, unlike most of the other variables, crayfish and species incidence showed a coherent effect across species (Fig. 2 in Chapter 4).

In this thesis, I showed that invasive alien species are a major driver of the decline of European amphibians. Conversely, the recorded habitat modification showed a less evident effect. This pattern was consistent across different spatial scales, from continental (Chapter 2) to regional (Chapters 4 and 5), supporting the validity and generality of results. A possible explanation could be found in the patterns of habitat change that recently occurred in Europe. In fact, in the last decades, the amount of habitat loss and degradation has been limited compared to the substantial habitat losses that occurred in previous periods (e.g. Falcucci et al. 2007;

Goldewijk et al. 2011; Hansen et al. 2013). Conversely, invasive alien species showed an impressive spread for all the taxonomic groups (e.g. DAISIE 2009; Seebens et al. 2017). As a consequence, invasive alien species are now a major driver of biodiversity loss, and they cause extinctions even in well preserved landscapes that maintain high habitat amount and suffer limited habitat loss (Denoël et al. 2019).

The negative effect of invasive alien species acted differently across species and spatial scales. Nevertheless, detecting the impact of invasive species on spatially-structured populations can be extremely challenging. While the site-level impact was evident only for a subset of species, the landscape-level effect of invasive crayfish was ubiquitous across the amphibian community. This implies that, even when we are not able to detect a detrimental effect of the presence of an alien species at a site, a landscape-level impact is still possible. It is thus crucial to assess possible landscape-level effects of threatening factors on the population dynamics of spatially structured populations.

Finally, it is vital to acknowledge that invasive alien species and habitat modification can strongly interact: the negative effect of alien species is exacerbated in highly modified landscapes. It is thus essential to directly prevent and manage the spread of alien species while simultaneously preserving natural landscapes. This can help in mitigating the negative effects of invasives, even when habitat alteration is not the main threatening factor.

FIGURE 1 A tree frog (*Hyla intermedia*) at one of the study sites of Chapter 5.

REFERENCES

Ahola M, Nordstro M, Banks PB, Laanetu N, Korpimäki E. 2006. Alien mink predation induces prolonged declines in archipelago amphibians. Proceedings of the Royal Society B: Biological Sciences **273**:1261–1265.

Allen GH, Pavelsky T. 2018. Global extent of rivers and streams. Science **361**:585–588.

Ambrogio A, Mezzadri S. 2018. Brown frogs of Italy. Gavia Edizioni, Piacenza.

Aquiloni L, Brusconi S, Cecchinelli E, Tricarico E, Mazza G, Paglianti A, Gherardi F. 2010. Biological control of invasive populations of crayfish: the European eel (*Anguilla anguilla*) as a predator of *Procambarus clarkii*. Biological Invasions **12**:3817–3824.

Aquiloni L, Gherardi F. 2008. Extended mother–offspring relationships in crayfish: the return behaviour of juvenile *Procambarus clarkii*. Ethology **114**:946–954.

Aquiloni L, Martín MP, Gherardi F, Diéguez-Uribeondo J. 2011. The North American crayfish *Procambarus clarkii* is the carrier of the oomycete *Aphanomyces astaci* in Italy. Biological Invasions **13**:359–367.

Araújo MB, Alagador D, Cabeza M, Nogués-Bravo D, Thuiller W. 2011. Climate change threatens European conservation areas. Ecology Letters **14**:484–492.

Arntzen JW. 1999. Sexual selection and male mate choice in the common toad, *Bufo bufo*. Ethology Ecology and Evolution **11**:407–414.

Arntzen JW, de Vries W, Canestrelli D, Martínez-Solano I. 2017. Hybrid zone formation and contrasting outcomes of secondary contact over transects in common toads. Molecular Ecology **26**:5663–5675.

Băncilă RI, Cogălniceanu D, Ozgul A, Schmidt BR. 2017. The effect of aquatic and terrestrial habitat characteristics on occurrence and breeding probability in a montane amphibian: insights from a spatially explicit multistate occupancy model. Population Ecology **59**:71–78.

Banerjee S, Carlin BP, Gelfand AE. 2014. Hierarchical Modeling and Analysis for Spatial Data. 2nd edition. Chapman and Hall/CRC Press, New York, USA.

Barbaresi S, Gherardi F. 2000. The invasion of the alien crayfish *Procambarus clarkii* in Europe, with particular reference to Italy. Biological Invasions **2**:259–264.

Bartoń K. 2018. MuMIn: Multi-Model Inference. R Packag version 1.42.1. Available from https://cran.r-project.org/web/packages/MuMIn/.

Bateman BL, Pidgeon AM, Radeloff VC, Vanderwal J, Thogmartin WE, Vavrus SJ, Heglund PJ. 2016. The pace of past climate change vs. potential bird distributions and land use in the United States. Global Change Biology **22**:1130–1144.

Beale CM, Lennon JJ, Yearsley JM, Brewer MJ, Elston DA. 2010. Regression analysis of spatial data. Ecology Letters **13**:246–264.

Beard KH, Eschtruth AK, Vogt KA, Vogt DJ, Scatena FN. 2003. The effects of the frog *Eleutherodactylus coqui* on invertebrates and ecosystem processes at two scales in the Luquillo Experimental Forest, Puerto Rico. Journal of Tropical Ecology **19**:607–617.

Beebee TJC, Griffiths RA. 2005. The amphibian decline crisis: A watershed for conservation biology? Biological Conservation **125**:271–285.

Bellard C, Cassey P, Blackburn TM. 2016. Alien species as a driver of recent extinctions. Biology Letters **12**:20150623.

Bélouard N, Petit EJ, Paillisson JM. 2019. Variable effects of an invasive species on the reproduction and distribution of native species in pond networks. Freshwater Biology **64**:544–554.

Bernini F, Bonini L, Ferri V, Gentilli A, Razzetti E, Scali S. 2004. Atlante degli anfibi e dei rettili della Lombardia. Monografie di Pianura, Cremona **5**:1–254.

Besacier-Monbertrand A-L, Paillex A, Castella E. 2014. Short-term impacts of lateral hydrological connectivity restoration on aquatic macroinvertebrates. River Research and Applications **30**:557–570.

Bienentreu JF, Lesbarrères D. 2020. Amphibian disease ecology: Are we just scratching the surface? Herpetologica **76**:153–166.

Bissattini AM, Buono V, Vignoli L. 2019. Disentangling the trophic interactions between American bullfrogs and native anurans: Complica- tions resulting from post-metamorphic ontogenetic niche shifts. Aquatic Conservation-Marine and Freshwater Ecosystems **29**:270–281.

Bivand RS, Rundel C. 2019. rgeos: Interface to

Geometry Engine - Open Source ('GEOS’). Available from https://cran.r-project.org/web/packages/rgeos/.

Bivand RS, Wong DWS. 2018. Comparing implementations of global and local indicators of spatial association. TEST **27**:716–748.

Blackburn TM, Bellard C, Ricciardi A. 2019. Alien versus native species as drivers of recent extinctions. Frontiers in Ecology and the Environment **17**:203–207.

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM. 2011. A proposed unified framework for biological invasions. Trends in Ecology and Evolution **26**:333–339.

Blaustein AR, Kiesecker JM. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters **5**:597–608.

Böhm M et al. 2013. The conservation status of the world’s reptiles. Biological Conservation **157**:372–385.

Bonardi A et al. 2011. Usefulness of volunteer data to measure the large scale decline of “common” toad populations. Biological Conservation **144**:2328–2334.

Bonelli M, Manenti R, Scaccini D. 2017. Mountain protected areas as refuges for threatened freshwater species: The detrimental effect of the direct introduction of alien species. Eco.mont **9**:23–29.

Boone MD, Semlitsch RD. 2001. Interactions of an Insecticide with Larval Density and Predation in Experimental Amphibian Communities. Conservation Biology **15**:228–238.

Bradford DF, Tabatabai F, Graber DM. 1993. Isolation of Remaining Populations of the Native Frog, *Rana muscosa*, by Introduced Fishes in Sequoia and Kings Canyon National Parks, California. Conservation Biology **7**:882–888.

Brannelly LA, McMahon TA, Hinton M, Lenger D, Richards-Zawacki CL. 2015. *Batrachochytrium dendrobatidis* in natural and farmed Louisiana crayfish populations: Prevalence and implications. Diseases of Aquatic Organisms **112**:229–235.

Brook BW, Sodhi NS, Bradshaw CJA. 2008. Synergies among extinction drivers under global change. Trends in Ecology and Evolution **23**:453–460.

Brown BJ, Mitchell RJ, Graham SA. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native congener. Ecology **83**:2328–

2336.

Brown PE, Zhou L. 2018. glmmBUGS: Generalised Linear Mixed Models and Spatial Models with WinBUGS, Jags, and OpenBUGS. Available from https://cran.r-project.org/package=glmmBUGS.

Bucciarelli GM, Blaustein AR, Garcia TS, Kats LB. 2014. Invasion Complexities: The Diverse Impacts of Nonnative Species on Amphibians. Copeia **2014**:611–632.

Buckley LB, Hurlbert AH, Jetz W. 2012. Broad-scale ecological implications of ectothermy and endothermy in changing environments. Global Ecology and Biogeography **21**:873–885.

Burnham KP, Anderson DR. 2002. Model Selection and Multimodel Inference, A Practical Information-Theoretic Approach. 2nd edition. Springer-Verlag, New York, USA.

Butchart SHM, Stattersfield a J, Baillie J, Bennun L a, Stuart SN, Akçakaya HR, Hilton-Taylor C, Mace GM. 2005. Using Red List Indices to measure progress towards the 2010 target and beyond. Philosophical Transactions of the Royal Society B: Biological Sciences **360**:255–268.

Cadi A, Joly P. 2003. Competition for basking places between the endangered European pond turtle (*Emys orbicularis galloitalica*) and the introduced red-eared slider (*Trachemys scripta elegans*). Canadian Journal of Zoology **81**:1392–1398. Available from http://www.nrcresearchpress.com/doi/abs/10.1139/z03-108.

Capinha C, Leung B, Anastácio P. 2011. Predicting worldwide invasiveness for four major problematic decapods: an evaluation of using different calibration sets. Ecography **34**:448–459.

Cardinale BJ et al. 2012. Biodiversity loss and its impact on humanity. Nature **486**:59–67.

Carreira BM, Dias MP, Rebelo R. 2014. How consumption and fragmentation of macrophytes by the invasive crayfish *Procambarus clarkii* shape the macrophyte communities of temporary ponds. Hydrobiologia **721**:89–98.

Casellato S, Masiero L. 2011. Does *Procambarus clarkii* (Girard, 1852) Represent a Threat for Estuarine Brackish Ecosystems of Northeastern Adriatic Coast (Italy). Journal of Life Science **5**:549–554.

Castellano S, Marconi V, Zanollo V, Berto G. 2009. Alternative mating tactics in the

Italian treefrog, *Hyla intermedia*. Behavioral Ecology and Sociobiology **63**:1109–1118.

Cayuela H et al. 2020. Determinants and Consequences of Dispersal in Vertebrates with Complex Life Cycles: A Review of Pond-Breeding Amphibians. The Quartely Review of Biology **95**:1–36.

Cayuela H, Besnard A, Quay L, Helder R, Léna J-P, Joly P, Pichenot J. 2018. Demographic response to patch destruction in a spatially structured amphibian population. Journal of Applied Ecology **55**:2204–2215.

Ceballos G, Ehrlich PR, Barnosky AD, Garcia A, Pringle RM, Palmer TM. 2015. Accelerated modern human-induced species losses: Entering the sixth mass extinction. Science Advances **1**:e1400253.

Cecchinelli E, Aquiloni L, Maltagliati G, Orioli G, Tricarico E, Gherardi F. 2012. Use of natural pyrethrum to control the red swamp crayfish *Procambarus clarkii* in a rural district of Italy. Pest Management Science **68**:839–844.

Chapron G, Huber D, Andrén H, López-bao JV, Adamec M, Linnell JDC, Boitani L, Swenson JE, Salvatori V. 2014. Recovery of large carnivores in Europe’s modern human-dominated landscapes. Science **346**:17–20.

Cilenti L, Alfonso G, Gargiulo M, Chetta FS, Liparoto A, D’Adamo R, Mancinelli G. 2017. First records of the crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae) in Lake Varano and in the Salento Peninsula (Puglia region, SE Italy), with review of the current status in southern Italy. BioInvasions Records **6**:153–158.

Claire WH, Wroiten JW. 1978. First record of the crayfish, *Procambarus clarkii*, from Idaho, USA (Decapoda, Cambaridae). Crustaceana **35**:317–319.

Colombo F, Di Palma F. 1995. Le risorse idriche sotterranee nella Provincia di Milano Vol. l: Lineamenti idrogeologici. Provincia di Milano, Milan. Available from http://www.risorsa-acqua.it/PDF/le risorse idriche sotterranee nella Provincia di Milano lineamenti idrogeologici.pdf.

Correia AM. 2001. Seasonal and interspecific evaluation of predation by mammals and birds on the introduced red swamp crayfish *Procambarus clarkii* (Crustacea, Cambaridae) in a freshwater marsh (Portugal). Journal of Zoology **255**:533–541.

Cox JG, Lima SL. 2006. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution **21**:674–680.

Cruz MJ, Rebelo R. 2005. Vulnerability of Southwest Iberian amphibians to an introduced crayfish, *Procambarus clarkii*. Amphibia Reptilia **26**:293–303.

Cruz MJ, Rebelo R, Crespo EG. 2006. Effects of an introduced crayfish, *Procambarus clarkii*, on the distribution of south-western Iberian amphibians in their breeding habitats. Ecography **29**:329–338.

Cruz MJ, Segurado P, Sousa M, Rebelo R. 2008. Collapse of the amphibian community of the paul do boquilobo natural reserve (central Portugal) after the arrival of the exotic american crayfish *Procambarus clarkii*. Herpetological Journal **18**:197–204.

Curado N, Hartel T, Arntzen JW. 2011. Amphibian pond loss as a function of landscape change – A case study over three decades in an agricultural area of northern France. Biological Conservation **144**:1610–1618.

D'Amore A. 2012. *Rana* (*Lithobates*) *catesbeiana* Shaw (American Bullfrog). Pages 321–330 in R. A. Francis, editor. Handbook of Global Freshwater Invasive Species. Earthscan, Abingdon, UK.

DAISIE. 2009. Handbook of Alien Species in Europe. Springer, Dordrecht, Netherlands.

de Valpine P, Turek D, Paciorek CJ, Anderson-Bergman C, Lang DT, Bodik R. 2017. Programming With Models: Writing Statistical Algorithms for General Model Structures With NIMBLE. Journal of Computational and Graphical Statistics **26**:403–413.

del Re AC. 2013. compute.es: Compute Effect Sizes. Available from https://cran.r-project.org/web/packages/compute.es/.

Delmastro GB. 1992. Sull'acclimatazione del Gambero della Louisiana *Procambarus clarkii* (Girard, 1852) nelle acque dolci italiane (Crustacea: Decapoda: Cambaridae). Pianura - Supplemento di Provincia Nuova **4**:5–10.

Delsinne T, Lafontaine R-M, Beudels RC, Robert H. 2013. Risk analysis of the Louisiana Crayfish *Procambarus clarkii* (Girard, 1852). Available from http://biodiversite.wallonie.be/servlet/Repository/?ID=33505.

Denoël M. 2012. Newt decline in Western Europe: highlights from relative distribution changes within guilds.

Biodiversity and Conservation **21**:2887–2898.

Denoel M, Ficetola GF. 2007. Landscape-level thresholds and newt conservation. Ecological Applications **17**:302–309.

Denoel M, Ficetola GF. 2008. Conservation of newt guilds in an agricultural landscape of Belgium: the importance of aquatic and terrestrial habitats. Aquatic Conservation-Marine and Freshwater Ecosystems **18**:714–728.

Denoël M, Ficetola GF, Sillero N, Džukić G, Kalezić ML, Vukov T, Muhovic I, Ikovic V, Lejeune B. 2019. Traditionally managed landscapes do not prevent amphibian decline and the extinction of paedomorphosis. Ecological Monographs **89**:e01347.

Denoel M, Perez A, Cornet Y, Ficetola GF. 2013. Similar Local and Landscape Processes Affect Both a Common and a Rare Newt Species. PLoS ONE **8**:e62727.

Denoël M, Whiteman HH, Joly P. 2005. Evolutionary ecology of facultative paedomorphosis in newts and salamanders. Biological Reviews **80**:663–671.

DeVore JL, Maerz JC. 2014. Grass invasion increases top-down pressure on an amphibian via structurally mediated effects on an intraguild predator. Ecology **95**:1724–1730.

Didham RK, Tylianakis JM, Gemmell NJ, Rand TA, Ewers RM. 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends in Ecology and Evolution **22**:489–496.

Diéguez-Uribeondo J, Söderhäll K. 1993. *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. Aquaculture and Fisheries Management **24**:761–765.

Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B. 2014. Defaunation in the Anthropocene. Science **345**:401–406.

Dodd CKJ. 2010. Amphibian Ecology and Conservation A Handbook of Techniques. Oxford University Press, Oxford.

Doherty TS, Glen AS, Nimmo DG, Ritchie EG, Dickman CR. 2016. Invasive predators and global biodiversity loss. Proceedings of the National Academy of Sciences of the United States of America **113**:11261–11265.

Dormann CF. 2007. Effects of incorporating spatial autocorrelation into the analysis of species distribution data. Global Ecology and Biogeography **16**:129–138.

Dornelas M et al. 2018. BioTIME: A database of biodiversity time series for the Anthropocene. Global Ecology and Biogeography **27**:760–786.

Ducatez S, Shine R. 2017. Drivers of Extinction Risk in Terrestrial Vertebrates. Conservation Letters **10**:186–194.

Dufresnes C et al. 2019. Fifteen shades of green: The evolution of *Bufotes* toads revisited. Molecular Phylogenetics and Evolution **141**:106615.

Dufresnes C, Pellet J, Bettinelli-Riccardi S, Thiébaud J, Perrin N, Fumagalli L. 2016. Massive genetic introgression in threatened northern crested newts (*Triturus cristatus*) by an invasive congener (*T. carnifex*) in Western Switzerland. Conservation Genetics **17**:839–846.

Dullinger S et al. 2013. Europe’s other debt crisis caused by the long legacy of future extinctions. Proceedings of the National Academy of Sciences **110**:7342–7347.

Falaschi M, Manenti R, Thuiller W, Ficetola GF. 2019. Continental-scale determinants of population trends in European amphibians and reptiles. Global Change Biology **25**:3504–3515.

Falaschi M, Mangiacotti M, Sacchi R, Scali S, Razzetti E. 2018. Electric circuit theory applied to alien invasions: a connectivity model predicting the Balkan frog expansion in Northern Italy. Acta Herpetologica **13**:33–42.

Falaschi M, Melotto A, Manenti R, Ficetola GF. 2020. Invasive Species and Amphibian Conservation. Herpetologica **76**:216–227.

Falcucci A, Maiorano L, Boitani L. 2007. Changes in land-use/land-cover patterns in Italy and their implications for biodiversity conservation. Landscape Ecology **22**:617–631.

Fea G, Nardi PA, Ghia D, Spairani M, Manenti R, Rossi S, Moroni M, Bernini F. 2006. Dati preliminari sulla distribuzione in Lombardia dei gamberi d’ acqua dolce autoctoni e alloctoni. Atti della società italiana di Scienze naturali e del Museo civico di Storia naturale di Milano **147**:201–210.

Ficetola GF. 2015. Habitat conservation research for amphibians: methodological improvements and thematic shifts. Biodiversity and Conservation **24**:1293–1310.

Ficetola GF, Colleoni E, Renaud J, Scali S, Padoa-Schioppa E, Thuiller W. 2016.

Morphological variation in salamanders and their potential response to climate change. Global Change Biology **22**:2013–2024.

Ficetola GF, De Bernardi F. 2004. Amphibians in a human-dominated landscape: The community structure is related to habitat features and isolation. Biological Conservation **119**:219–230.

Ficetola GF, Lunghi E, Canedoli C, Padoa-Schioppa E, Pennati R, Manenti R. 2018a. Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial salamanders. Scientific Reports **8**:10575.

Ficetola GF, Maiorano L. 2016. Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance. Oecologia **181**:683–693. Springer Berlin Heidelberg.

Ficetola GF, Padoa-Schioppa E, De Bernardi F. 2009. Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. Conservation Biology **23**:114–123.

Ficetola GF, Romano A, Salvidio S, Sindaco R. 2018b. Optimizing monitoring schemes to detect trends in abundance over broad scales. Animal Conservation **21**:221–231.

Ficetola GF, Rondinini C, Bonardi A, Baisero D, Padoa-schioppa E. 2015. Habitat availability for amphibians and extinction threat: a global analysis. Diversity and Distributions **21**:302–311.

Ficetola GF, Sacchi R, Scali S, Gentilli A, De Bernardi F, Galeotti P. 2007a. Vertebrates respond differently to human disturbance: implications for the use of a focal species approach. Acta Oecologica **31**:109–118.

Ficetola GF, Siesa ME, Bernardi F De, Padoa-schioppa E. 2012a. Complex impact of an invasive crayfish on freshwater food webs. Biodiversity and Conservation **21**:2641–2651.

Ficetola GF, Siesa ME, Manenti R, Bottoni L, De Bernardi F, Padoa-Schioppa E. 2011. Early assessment of the impact of alien species: differential consequences of an invasive crayfish on adult and larval amphibians. Diversity and Distributions **17**:1141–1151.

Ficetola GF, Siesa ME, Padoa-Schioppa E, De Bernardi F. 2012b. Wetland features, amphibian communities and distribution of the alien crayfish, *Procambarus clarkii*. Alytes **29**:75–87.

Ficetola GF, Thuiller W, Miaud C. 2007b.

Prediction and validation of the potential global distribution of a problematic alien invasive species - The American bullfrog. Diversity and Distributions **13**:476–485.

Fisher MC, Garner TWJ, Walker SF. 2009. Global Emergence of *Batrachochytrium dendrobatidis* and Amphibian Chytridiomycosis in Space, Time, and Host. Annual Review of Microbiology **63**:291–310.

Fiske IJ, Chandler RB. 2011. unmarked: An R Package for Fitting Hierarchical Models of Wildlife Occurrence and Abundance. Journal of Statistical Software **43**:1–23.

Fitzpatrick BM, Johnson JR, Kump DK, Smith JJ, Voss SR, Shaffer HB. 2010. Rapid spread of invasive genes into a threatened native species. Proceedings of the National Academy of Sciences **107**:3606–3610.

Fitzpatrick LD, Pasmans F, Martel A, Cunningham AA. 2018. Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. Scientific Reports **8**:13845.

Flesch AD. 2017. Influence of local and landscape factors on distributional dynamics: A species-centred, fitness-based approach. Proceedings of the Royal Society B: Biological Sciences **284**.

Flesch AD, Rosen PC, Holm P. 2017. Long-term changes in abundances of Sonoran Desert lizards reveal complex responses to climatic variation. Global Change Biology **23**:5492–5508.

Foley JA et al. 2005. Global consequences of land use. Science **309**:570–574.

Fouquet A, Ficetola GF, Haigh A, Gemmell N. 2010. Using ecological niche modelling to infer past, present and future environmental suitability for *Leiopelma hochstetteri*, an endangered New Zealand native frog. Biological Conservation **143**:1375–1384. Elsevier Ltd.

Gallardo B, Clavero M, Sánchez MI, Vilà M, Montserrat V. 2015. Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology **22**:151–163.

Garcia RA, Clusella-Trullas S. 2019. Thermal landscape change as a driver of ectotherm responses to plant invasions. Proceedings of the Royal Society B: Biological Sciences **286**:20191020.

Gardner TA, Barlow J, Peres CA. 2007. Paradox, presumption and pitfalls in conservation biology: The importance of habitat change for amphibians and reptiles. Biological Conservation **138**:166–179.

Garner TWJ, Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, Fisher MC. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biology Letters **2**:455–459.

Gaston KJ, Fuller RA. 2008. Commonness, population depletion and conservation biology. Trends in Ecology and Evolution **23**:14–19.

Gherardi F. 2006. Crayfish invading Europe: the case study of *Procambarus clarkii*. Marine and Freshwater Behaviour and Physiology **39**:175–191.

Gherardi F, Aquiloni L, Diéguez-Uribeondo J, Tricarico E. 2011. Managing invasive crayfish: Is there a hope? Aquatic Sciences **73**:185–200.

Gherardi F, Gollasch S, Minchin D, Olenin S, Panov VE. 2009. Alien invertebrates and fish in European inland waters. Pages 93–104 in DAISIE, editor. Handbook of alien species in Europe. Springer, Dordrecht.

Gherardi F, Renai B, Corti C. 2001. Crayfish predation on tadpoles: A comparison between a native (*Austropotamobius pallipes*) and an alien species (*Procambarus clarkii*). Bulletin Français de la Peche et de la Protection des Milieux Aquatiques **361**:659–668.

Glandt D. 1986. Die saisonalen Wanderungen der mitteleuropäischen Amphibien. Bonner zoologische Beiträge **37**:211–228.

Godsoe W. 2010. I can’t define the niche but I know it when I see it: a formal link between statistical theory and the ecological niche. Oikos **119**:53–60.

Goldewijk KK, Beusen A, van Drecht G, de Vos M. 2011. The HYDE 3.1 spatially explicit database of human-induced global land-use change over the past 12,000 years. Global Ecology and Biogeography **20**:73–86.

Gonçalves Loureiro T, Silva Gentil Anastácio PM, Souty-Grosset C, Araujo PB, Pereira Almerão M. 2015. Red swamp crayfish: biology, ecology and invasion - an overview. Nauplius **23**:1–19.

Gould J, Valdez JW, Stockwell MP, Clulow S, Mahony MJ. 2019. Mosquitoes as a potential vector for the transmission of the amphibian chytrid fungus. Zoology and Ecology **29**:38–44.

Grant EHC et al. 2016. Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. Scientific Reports **6**:25625.

Graves SD, Shapiro AM. 2003. Exotics as host plants of the California butterfly fauna. Biological Conservation **110**:416–433.

Green DM, Lannoo MJ, Lesbarrères D, Muths E. 2020. Amphibian population declines: 30 years of progress in confronting a complex problem. Herpetologica **76**:97–100.

Gurevitch J, Koricheva J, Nakagawa S, Stewart G. 2018. Meta-analysis and the science of research synthesis. Nature **555**:175–182. Nature Publishing Group.

Hadfield JD. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software **33**:1–22.

Hadfield JD, Nakagawa S. 2010. General quantitative genetic methods for comparative biology: Phylogenies, taxonomies and multi-trait models for continuous and categorical characters. Journal of Evolutionary Biology **23**:494–508.

Hamer AJ, McDonnell MJ. 2008. Amphibian ecology and conservation in the urbanising world: A review. Biological Conservation **141**:2432–2449.

Hansen MC et al. 2013. High-Resolution Global Maps of 21st-Century Forest Cover Change. Science **342**:850–853.

Hanski I. 1998. Metapopulation dynamics. Nature **396**:41–49.

Hanski I, Gaggiotti OE. 2004. Ecology, genetics and evolution of metapopulations. Elsevier Academic Press, Burlington, Massachusetts, USA.

Harris I, Jones PD, Osborn TJ, Lister DH. 2014. Updated high-resolution grids of monthly climatic observations - the CRU TS3.10 Dataset. International Journal of Climatology **34**:623–642.

Hartel T, Schweiger O, Öllerer K, Cogălniceanu D, Arntzen JW. 2010. Amphibian distribution in a traditionally managed rural landscape of Eastern Europe: Probing the effect of landscape composition. Biological Conservation **143**:1118–1124.

Hefley TJ, Hooten MB, Russell RE, Walsh DP, Powell JA. 2017. When mechanism matters: Bayesian forecasting using models of ecological diffusion. Ecology Letters **20**:640–650.

Hijmans RJ. 2019. raster: Geographic Data Analysis and Modeling. Available from https://cran.r-project.org/package=raster.

Hodgson JA, Thomas CD, Wintle BA, Moilanen A. 2009. Climate change, connectivity and conservation decision making: Back to basics. Journal of Applied Ecology **46**:964–969.

Hof C, Araújo MB, Jetz W, Rahbek C. 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature **480**:516–519.

Hoffman AA, Sgró CM. 2011. Climate change and evolutionary adaptation. Nature **470**:479–485.

Hoffmann M et al. 2010. The Impact of Conservation on the Status of the World's Vertebrates. Science **330**:1503–1509.

Holdich DM. 1993. A review of astaciculture: freshwater crayfish farming. Aquatic Living Resources **6**:307–317.

Holsbeek G, Jooris R. 2010. Potential impact of genome exclusion by alien species in the hybridogenetic water frogs (*Pelophylax esculentus* complex). Biological Invasions **12**:1–13.

Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL. 2000. Quantitative evidence for global amphibian population declines. Nature **404**:752–755.

Hulme PE. 2009. Trade, transport and trouble: Managing invasive species pathways in an era of globalization. Journal of Applied Ecology **46**:10–18.

Indermaur L, Schaub M, Jokela J, Tockner K, Schmidt BR. 2010. Differential response to abiotic conditions and predation risk rather than competition avoidance determine breeding site selection by anurans. Ecography **33**:887–895.

Intergovernmental Panel on Climate Change. 2015. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Switzerland.

IPBES. 2018. IPBES-6 Plenary. Available from https://www.ipbes.net/event/ipbes-6-plenary (accessed November 2, 2018).

IUCN. 2012. IUCN Red List Categories and Criteria: Version 3.1. Second edition. IUCN, Gland, Switzerland and Cambridge, UK.

Janin A, Léna J-P, Ray N, Delacourt C, Allemand P, Joly P. 2009. Assessing landscape connectivity with calibrated cost-distance modelling: Predicting common toad distribution in a context of spreading agriculture. Journal of Applied Ecology **46**:833–841.

Jetz W et al. 2019. Essential biodiversity variables for mapping and monitoring species populations. Nature Ecology & Evolution **3**:539–551. Springer US.

Joly P, Miaud C, Lehmann A, Grolet O. 2001. Habitat Matrix Effects on Pond Occupancy in Newts. Conservation Biology **15**:239–248.

Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: Review of two decades of science and the transition to conservation. Diversity and Distributions **9**:99–110.

Kéry M. 2010. Introduction to WinBUGS for ecologists: A Bayesian approach to regression, ANOVA, mixed models and related analyses. 1st Edition. Academic Press.

Kéry M, Guillera-Arroita G, Lahoz-Monfort JJ. 2013. Analysing and mapping species range dynamics using occupancy models. Journal of Biogeography **40**:1463–1474.

Knapp RA. 2005. Effects of nonnative fish and habitat characteristics on lentic herpetofauna in Yosemite National Park, USA. Biological Conservation **121**:265–279.

Knapp RA, Matthews KR. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology **14**:428–438.

Kremen C, Merenlender AM. 2018. Landscapes that work for biodiversity and people. Science **362**:eaau6020.

Kyek M, Kaufmann PH, Lindner R. 2017. Differing long term trends for two common amphibian species (*Bufo bufo* and *Rana temporaria*) in alpine landscapes of Salzburg, Austria. PLoS ONE **12**:1–17.

Lanza B, Andreone F, Bologna MA, Corti C, Razzetti E. 2007. Fauna d’Italia Vol. XLII - Amphibia. Calderini, Bologna, Italy.

Latombe G et al. 2017. A vision for global monitoring of biological invasions. Biological Conservation **213**:295–308.

Leung B et al. 2012. TEASIng apart alien species risk assessments: A framework for best practices. Ecology Letters **15**:1475–1493.

Liu C, White M, Newell G. 2011. Measuring and comparing the accuracy of species distribution models with presence-absence data. Ecography **34**:232–243.

Lo Parrino E, Ficetola GF, Manenti R, Falaschi M. 2020. Thirty years of invasion: the distribution of the invasive crayfish *Procambarus clarkii* in Italy. Biogeographia **35**:43–50.

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. Invasive Species Specialist Group, Auckland, New Zealand.

MacKenzie DI, Nichols JD, Hines JE, Knutson MG, Franklin AB. 2003. Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. Ecology **84**:2200–2207.

Manenti R. 2008. Caratteristiche ecologiche dei siti siti riproduttivi di Anfibi nelle aree prealpine. Bollettino della Società Ticinese di Scienze naturali **96**:95–102.

Manenti R, Falaschi M, Monache DD, Marta S, Ficetola GF. 2020. Network-scale effects of invasive species on spatially-structured amphibian populations. Ecography **43**:119–127.

Manenti R, Ficetola GF, Alessandro M, De Bernardi F. 2011. Caves as breeding sites for *Salamandra salamandra*: Habitat selection, larval development and conservation issues. North-Western Journal of Zoology **7**:304–309.

Manenti R, Ghia D, Fea G, Ficetola GF, Padoa-Schioppa E, Canedoli C. 2019. Causes and consequences of crayfish extinction: Stream connectivity, habitat changes, alien species and ecosystem services. Freshwater Biology **64**:284–293.

Manenti R, Zanetti N, Pennati R, Scarì G. 2017. Factors driving semi-aquatic predator occurrence in traditional cattle drinking pools: conservation issues. Journal of Limnology **76**:34–40.

Mantyka-Pringle CS, Martin TG, Rhodes JR. 2012. Interactions between climate and habitat loss effects on biodiversity: A systematic review and meta-analysis. Global Change Biology **18**:1239–1252.

Marta S, Brunetti M, Ficetola GF, Stoch F, Amori G, Cesaroni D, Sbordoni V, Provenzale A. 2019. ClimCKmap, a spatially, temporally and climatically explicit distribution database for the Italian fauna. Scientific data **6**:195. Springer US.

Martel A et al. 2014. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science **346**:630–631.

Mathwin R, Wassens S, Young J, Ye Q, Bradshaw CJA. 2020. Manipulating water for amphibian conservation. Conservation Biology:in press.

Matsuzaki SIS, Usio N, Takamura N, Washitani I. 2009. Contrasting impacts of invasive engineers on freshwater ecosystems: An experiment and meta-analysis. Oecologia **158**:673–686.

Measey GJ, Vimercati G, de Villiers FA, Mokhatla M, Davies SJ, Thorp CJ, Rebelo AD, Kumschick S. 2016. A global assessment of alien amphibian impacts in a formal framework. Diversity and Distributions **22**:970–981.

Mendenhall CD, Sekercioglu CH, Oviedo Brenes F, Ehrlich PR, Daily GC. 2011. Predictive model for sustaining biodiversity in tropical countryside. Proceedings of the National Academy of Sciences **108**:16313–16316.

Menge BA. 1995. Indirect effects in marine rocky intertidal interaction webs: Patterns and importance. Ecological Monographs **65**:21–74.

Menzel A et al. 2006. European phenological response to climate change matches the warming pattern. Global Change Biology **12**:1969–1976.

Meraner A, Venturi A, Ficetola GF, Rossi S, Candiotto A, Gandolfi A. 2013. Massive invasion of exotic *Barbus barbus* and introgressive hybridization with endemic *Barbus plebejus* in Northern Italy: where, how and why? Molecular Ecology **22**:5295–5312.

Miaud C, Dejean T, Savard K, Millery-Vigues A, Valentini A, Curt Grand Gaudin N, Garner TWJ. 2016. Invasive North American bullfrogs transmit lethal fungus Batrachochytrium dendrobatidis infections to native amphibian host species. Biological Invasions **18**:2299–2308.

Miró A, Sabás I, Ventura M. 2018. Large negative effect of non-native trout and minnows on Pyrenean lake amphibians. Biological Conservation **218**:144–153.

Mohanty NP, Measey J. 2019. No survival of native larval frogs in the presence of invasive Indian bullfrog Hoplobatrachus tigerinus tadpoles. Biological Invasions **21**:2281–2286.

Moilanen A, Hanski I. 1998. Metapopulation Dynamics: Effects of Habitat Quality and Landscape Structure. Ecology **79**:2503–2515.

Moilanen A, Nieminen M. 2002. Simple Connectivity Measures in Spatial Ecology. Ecology **83**:1131–1145.

Molotoks A, Stehfest E, Doelman J, Albanito F, Fitton N, Dawson TP, Smith P. 2018. Global projections of future cropland expansion to 2050 and direct impacts on biodiversity and carbon storage. Global Change Biology **24**:5895–5908.

Mooney HA, Cleland EE. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences of the United States of America **98**:5446–5451.

Morpurgo M, Aquiloni L, Bertocchi S, Brusconi S, Tricarico E, Gherardi F. 2010. Distribuzione dei gamberi d'acqua dolce in Italia. Studi Trentini di Scienze Naturali **87**:125–132.

Muths E et al. 2017. Heterogeneous responses of temperate-zone amphibian populations to climate change complicates conservation planning. Scientific Reports **7**:17102.

Nelson DWM, Crossland MR, Shine R. 2010. Indirect ecological impacts of an invasive toad on predator-prey interactions among native species. Biological Invasions **12**:3363–3369.

Nentwig W, Bacher S, Kumschick S, Pyšek P, Vilà M. 2018. More than "100 worst" alien species in Europe. Biological Invasions **20**:1611–1621.

Newbold T et al. 2015. Global effects of land use on local terrestrial biodiversity. Nature **520**:45–50.

Newbold T, Scharlemann JPW, Butchart SHM, Sekercioglu CH, Alkemade R, Booth H, Purves DW. 2013. Ecological traits affect the response of tropical forest bird species to land-use intensity. Proceedings of the Royal Society B: Biological Sciences **280**:20122131.

Nichols JD, Williams BK. 2006. Monitoring for conservation. Trends in Ecology and Evolution **21**:668–673.

Northrup JM, Rivers JW, Yang Z, Betts MG. 2019. Synergistic effects of climate and land-use change influence broad-scale avian population declines. Global Change Biology **25**:1561–1575.

Nowakowski AJ et al. 2018. Thermal biology mediates responses of amphibians and reptiles to habitat modification. Ecology Letters **21**:345–355.

Nunes AL, Fill JM, Davies SJ, Louw M, Rebelo AD, Thorp CJ, Vimercati G, Measey J. 2019. A global meta-analysis of the ecological impacts of alien species on native amphibians. Proceedings of the Royal Society B: Biological Sciences **286**:20182528.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2018. caper: Comparative Analyses of Phylogenetics and Evolution in R. Available from https://cran.r-project.org/package=caper.

Ortiz-Santaliestra ME, Marco A, Fernandez-Beneitez MJ, Lizana M. 2009. Alteration of courtship behavior because of water acidification and minor effect of ammonium nitrate in the Iberian newt (Lissotriton boscai). Environmental Toxycology and Chemistry **28**:1500–1505.

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.

Parmesan C et al. 1999. Poleward shifts in geographic ranges of butternfly species associated with regional warming. Nature **399**:579–583.

Peay S. 2001. Eradication of alien crayfish populations. R&D Technical Report W1–037/TR1. Bristol, UK: Environment Agency.

Petrovan SO, Schmidt BR. 2016. Volunteer conservation action data reveals large-scale and long-term negative population trends of a widespread amphibian, the common toad (*Bufo bufo*). PLoS ONE **11**:e0161943.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137. Available from https://cran.r-project.org/package=nlme.

Powers RP, Jetz W. 2019. Global habitat loss and extinction risk of terrestrial vertebrates under future land-use-change scenarios. Nature Climate Change **9**:323–329.

Price SJ, Freytag SB, Bonner SJ, Drayer AN, Muncy BL, Hutton JM, Barton CD. 2018. Mountaintop removal mining alters stream salamander population dynamics. Diversity and Distributions **24**:1242–1251.

Prigioni C, Smiroldo G, Remonti L, Balestrieri A. 2009. Distribution and diet of reintroduced otters (*Lutra lutra*) on the river Ticino (NW Italy). Hystrix **20**:45–53.

Pujol-Buxó E, Riaño GM, Llorente GA. 2019. Mild segregation in the breeding preferences of an invasive anuran (*Discoglossus pictus*) and its main native competitor (*Epidalea calamita*) in ephemeral ponds. Amphibia Reptilia **40**:425–435.

Pulliam HR. 1988. Sources, Sinks, and Population Regulation. The American Naturalist **132**:652–661.

Puth LM, Post DM. 2005. Studying invasion:

Have we missed the boat? Ecology Letters **8**:715–721.

Pyšek P et al. 2010. Disentangling the role of environmental and human pressures on biological invasions across Europe. Proceedings of the National Academy of Sciences **107**:12157–12162.

QGIS Development Team. 2020. QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available from http://www.qgis.org.

Quilodrán CS, Currat M, Montoya-Burgos JI. 2018. Effect of hybridization with genome exclusion on extinction risk. Conservation Biology **32**:1139–1149.

Quilodrán CS, Montoya-Burgos JI, Currat M. 2015. Modelling interspecific hybridization with genome exclusion to identify conservation actions: The case of native and invasive *Pelophylax* waterfrogs. Evolutionary Applications **8**:199–210.

Quinn LD, Schooler SS, Van Klinken RD. 2011. Effects of land use and environment on alien and native macrophytes: Lessons from a large-scale survey of Australian rivers. Diversity and Distributions **17**:132–143.

R Core Team. 2018. R: A language and environment for statistical computing. Available from https://www.r-project.org/.

Rannap R, Lõhmus A, Briggs L. 2009. Restoring ponds for amphibians: A success story. Hydrobiologia **634**:87–95.

Ransom TS. 2017. Local distribution of native and invasive earthworms and effects on a native salamander. Population Ecology **59**:189–204. Springer Japan.

Relyea RA, Schoeppner NM, Hoverman JT. 2005. Pesticides and amphibians: The importance of community context. Ecological Applications **15**:1125–1134.

Remon J, Bower DS, Gaston TF, Clulow J, Mahony MJ. 2016. Stable isotope analyses reveal predation on amphibians by a globally invasive fish (*Gambusia holbrooki*). Aquatic Conservation: Marine and Freshwater Ecosystems **26**:724–735.

Resetarits WJ. 2005. Habitat selection behaviour links local and regional scales in aquatic systems. Ecology Letters **8**:480–486.

Revilla E, Wiegand T. 2008. Individual movement behavior, matrix heterogeneity, and the dynamics of spatially structured populations. Proceedings of the National Academy of Sciences of the United States of America

105:19120–19125.

Ricciardi A, Atkinson SK. 2004. Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems. Ecology Letters **7**:781–784.

Richards SA, Whittingham MJ, Stephens PA. 2011. Model selection and model averaging in behavioural ecology: the utility of the IT-AIC framework. Behavioral Ecology and Sociobiology **65**:77–89.

Richter-Boix A, Garriga N, Montori A, Franch M, San Sebastián O, Villero D, Llorente GA. 2013. Effects of the non-native amphibian species *Discoglossus pictus* on the recipient amphibian community: Niche overlap, competition and community organization. Biological Invasions **15**:799–815.

Ripple WJ, Wolf C, Newsome TM, Galetti M, Alamgir M, Crist E, Mahmoud MI, Laurance WF. 2017. World scientists' warning to humanity: A second notice. BioScience **67**:1026–1028.

Rodriguez LF. 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biological Invasions **8**:927–939.

Rogalski MA, Skelly DK. 2012. Positive Effects of Nonnative Invasive *Phragmites australis* on Larval Bullfrogs. PLoS ONE **7**:e44420.

Romano A et al. 2009. Triturus carnifex. The IUCN Red List of Threatened Species 2009: e.T59474A11947714. Available from https://www.iucnredlist.org/species/59474/11947714.

Rondinini C et al. 2011. Global habitat suitability models of terrestrial mammals. Philosophical Transactions of the Royal Society B: Biological Sciences **366**:2633–2641.

Roquet C, Lavergne S, Thuiller W. 2014. One Tree to Link Them All: A Phylogenetic Dataset for the European Tetrapoda. PLOS Currents Tree of Life **6**:1–16.

Roser LG, Ferreyra LI, Saidman BO, Vilardi JC. 2017. EcoGenetics: An R package for the management and exploratory analysis of spatial data in landscape genetics. Molecular Ecology Resources **17**:e241–e250.

Royle JA, Kéry M. 2007. A Bayesian state-space formulation of dynamic occupancy models. Ecology **88**:1813–1823.

Ryan ME, Johnson JR, Fitzpatrick BM. 2009. Invasive hybrid tiger salamander genotypes impact native amphibians.

Proceedings of the National Academy of Sciences **106**:11166–11171.

Saino N et al. 2011. Climate warming, ecological mismatch at arrival and population decline in migratory birds. Proceedings of the Royal Society B: Biological Sciences **278**:835–842.

Sala OE et al. 2000. Global biodiversity scenarios for the year 2100. Science **287**:1770–1774.

Salo P, Ahola MP, Korpimäki E. 2010. Habitat-mediated impact of alien mink predation on common frog densities in the outer archipelago of the Baltic Sea. Oecologia **163**:405–413.

Santini L, Isaac NJB, Ficetola GF. 2018. TetraDENSITY: A database of population density estimates in terrestrial vertebrates. Global Ecology and Biogeography **27**:787–791.

Schabetsberger R, Jehle R, Maletzky A, Pesta J, Sztatecsny M. 2004. Delineation of terrestrial reserves for amphibians: post-breeding migrations of Italian crested newts (Triturus c. carnifex) at high altitude. Biological Conservation **117**:95–104.

Scheele BC et al. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science **363**:1459–1463.

Scroggie MP, Preece K, Nicholson E, McCarthy MA, Parris KM, Heard GW. 2019. Optimizing habitat management for amphibians: From simple models to complex decisions. Biological Conservation **236**:60–69.

Seebacher F, White CR, Franklin CE. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nature Climate Change **5**:61–66.

Seebens H et al. 2017. No saturation in the accumulation of alien species worldwide. Nature Communications **8**:14435.

Seibold S, Bässler C, Brandl R, Fahrig L, Förster B, Heurich M, Hothorn T, Scheipl F, Thorn S, Müller J. 2017. An experimental test of the habitat-amount hypothesis for saproxylic beetles in a forested region. Ecology **98**:1613–1622.

Siesa ME, Manenti R, Padoa-Schioppa E, De Bernardi F, Ficetola GF. 2011. Spatial autocorrelation and the analysis of invasion processes from distribution data: a study with the crayfish *Procambarus clarkii*. Biological Invasions **13**:2147–2160.

Siesa ME, Padoa-Schioppa E, Ott J, De

Bernardi F, Ficetola GF. 2014. Assessing the consequences of biological invasions on species with complex life cycles: impact of the alien crayfish *Procambarus clarkii* on Odonata. Ecological Indicators **46**:70–77.

Sillero N et al. 2014. Updated distribution and biogeography of amphibians and reptiles of Europe. Amphibia-Reptilia **35**:1–31.

Silva-Rocha IR, Salvi D, Carretero MA, Ficetola GF. 2019. Alien reptiles on Mediterranean Islands: A model for invasion biogeography. Diversity and Distributions **25**:995–1005.

Sindaco R, Doria G, Razzetti E, Bernini F. 2006. Atlas of Italian Amphibians and Reptiles. Polistampa, Firenze.

Smith MA, Green DM. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography **28**:110–128.

Sokal RR, Rohlf FJ. 2012. Biometry: The Principles and Practices of Statistics in Biological Research. 4th edition. W.H. Freeman and Company, New York, USA.

Spatz DR, Zilliacus KM, Holmes ND, Butchart SHM, Genovesi P, Ceballos G, Tershy BR, Croll DA. 2017. Globally threatened vertebrates on islands with invasive species. Science Advances **3**:e1603080.

Speybroeck J et al. 2020. Species list of the European herpetofauna – 2020 update by the Taxonomic Committee of the Societas Europaea Herpetologica. Amphibia-Reptilia **41**:139–189.

Stehfest E, Vuuren D van, Kram T, Bouwman L. 2014. Integrated Assessment of Global Environmental Change with IMAGE 3.0. Model description and policy applications. PBL Netherlands Environmental Assessment Agency, The Hague, Netherlands.

Stohlgren TJ, Barnett DT, Flather C, Fuller P, Peterjohn B, Kartesz JT, Master LL. 2006. Species richness and pattern of invasion in plants, birds, and fishes in the United States. Biological Invasions **8**:427–447.

Strayer DL. 2010. Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology **55**:152–174.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW. 2004. Status and Trends of Amphibian Declines and Extinctions Worldwide. Science **306**:1783–1786.

Stuart SN, Hoffmann M, Chanson JS, Cox NA, Berridge RJ, Ramani P, Young BE. 2008. Threatened Amphibians of the World. Lynx, Barcelona, Spain. IUCN, Gland, Switzerland. Conservation International, Arlington, Virginia, USA.

Thomas CD, Kunin WE. 1999. The Spatial Structure of Populations. Journal of Animal Ecology **68**:647–657.

Thuiller W, Georges D, Engler R, Breiner F. 2016. biomod2: Ensemble Platform for Species Distribution Modeling. Available from https://cran.r-project.org/package=biomod2.

Thuiller W, Lavergne S, Roquet C, Boulangeat I, Lafourcade B, Araujo MB. 2011. Consequences of climate change on the tree of life in Europe. Nature **470**:531–534.

Tiberti R, Von Hardenberg A. 2012. Impact of introduced fish on Common frog (*Rana temporaria*) close to its altitudinal limit in alpine lakes. Amphibia Reptilia **33**:303–307.

Todd B, Willson J, Gibbons J. 2010. The Global Status of Reptiles and Causes of Their Decline. Pages 47–67 in D. W. Sparling, G. Linder, C. A. Bishop, and S. Krest, editors. Ecotoxicology of Amphibians and Reptiles, Second Edition. CRC Press, Boca Raton, USA.

Todd BD, Rothermel BB, Reed RN, Luhring TM, Schlatter K, Trenkamp L, Gibbons JW. 2008. Habitat alteration increases invasive fire ant abundance to the detriment of amphibians and reptiles. Biological Invasions **10**:539–546.

Tracewski Ł, Butchart SHM, Di Marco M, Ficetola GF, Rondinini C, Symes A, Wheatley H, Beresford AE, Buchanan GM. 2016. Toward quantification of the impact of 21st-century deforestation on the extinction risk of terrestrial vertebrates. Conservation Biology **30**:1070–1079.

Trekels H, Vanschoenwinkel B. 2019. Both local presence and regional distribution of predator cues modulate prey colonisation in pond landscapes. Ecology Letters **22**:89–97.

Tulloch AIT, Mortelliti A, Kay GM, Florance D, Lindenmayer D. 2016. Using empirical models of species colonization under multiple threatening processes to identify complementary threat-mitigation strategies. Conservation Biology **30**:867–882.

Van Buskirk J. 2003. Habitat partitioning in European and North American pond-breeding frogs and toads. Diversity and

Distributions **9**:399–410.

VanDerWal J, Falconi L, Januchowski S, Shoo L, Storlie C. 2019. SDMTools: Species Distribution Modelling Tools: Tools for processing data associated with species distribution modelling exercises. R package version 1.1-221.1. Available from https://cran.r-project.org/package=SDMTools.

Venables WN, Ripley BD. 2002. Modern applied statistics with S. 4th edition. Springer, New York, USA.

Vilà M et al. 2010. How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. Frontiers in Ecology and the Environment **8**:135–144.

Vilà M, García-Berthou E. 2010. Monitoring Biological Invasions in Freshwater Habitats. Pages 91–100 in C. Hurford, M. Schneider, and I. Cowx, editors. Conservation Monitoring in Freshwater Habitats. Springer, Dordrecht, Netherlands.

Vorburger C, Reyer HU. 2003. A genetic mechanism of species replacement in European waterfrogs? Conservation Genetics **4**:141–155.

Wagner HH, Fortin MJ. 2005. Spatial analysis of landscapes: Concepts and statistics. Ecology **86**:1975–1987.

Wanless RM, Angel A, Cuthbert RJ, Hilton GM, Ryan PG. 2007. Can predation by invasive mice drive seabird extinctions? Biology Letters **3**:241–244.

Watling JI, Hickman CR, Orrock JL. 2011. Invasive shrub alters native forest amphibian communities. Biological Conservation **144**:2597–2601.

Wellborn GA, Skelly DK, Werner EE. 1996. Mechanisms Creating Community Structure Across a Freshwater Habitat Gradient. Annual Review of Ecology and Systematics **27**:337–363.

Westphal MI, Browne M, MacKinnon K, Noble I. 2008. The link between international trade and the global distribution of invasive alien species. Biological Invasions **10**:391–398.

White EM, Wilson JC, Clarke AR. 2006. Biotic indirect effects: A neglected concept in invasion biology. Diversity and Distributions **12**:443–455.

Wielstra B et al. 2018. The distributions of the six species constituting the smooth newt species complex (*Lissotriton vulgaris* sensu lato and *L. montandoni*) – an addition to the New Atlas of Amphibians and Reptiles of Europe.

Amphibia-Reptilia **39**:252–259.

Wielstra B, Sillero N, Vörös J, Arntzen JW. 2014. The distribution of the crested and marbled newt species (Amphibia: Salamandridae: *Triturus*) - an addition to the New Atlas of Amphibians and Reptiles of Europe. Amphibia-Reptilia **35**:376–381.

Williamson M, Fitter A. 1996. The varying success of invaders. Ecology **77**:1661–1666.

Winandy L, Legrand P, Denoël M. 2017. Habitat selection and reproduction of newts in networks of fish and fishless aquatic patches. Animal Behaviour **123**:107–115.

LIST OF PUBLICATIONS

The thesis is based on the content of the following publications:

Falaschi M., Melotto A., Manenti R., Ficetola G. F. (2020). Invasive Species and Amphibian Conservation. *Herpetologica*, 76(2): 216-227. DOI: 10.1655/0018-0831-76.2.216

Falaschi M., Thuiller W., Manenti R., Ficetola F. (2019). Continental-scale determinants of population trends in European amphibians and reptiles. *Global Change Biology*, 25(10): 3504-3515. DOI: 10.1111/gcb.14739

Lo Parrino E., Ficetola G. F., Manenti R., **Falaschi M.** (2020). Thirty years of invasion: the distribution of the invasive crayfish *Procambarus clarkii* in Italy. *Biogeographia*, 35: 43-50. DOI: 10.21426/B635047157

Falaschi M., Muraro M., Manenti R., Faraci F., Belluardo F., Di Nicola M. R., Ficetola G. F. Invasive species override habitat change in determining decline of newts at the regional scale. Manuscript under review.

Falaschi M., Giachello S., Lo Parrino E., Muraro M., Manenti R, Ficetola G. F. Long-term drivers of persistence and colonization dynamics in spatially structured amphibian populations. Manuscript under review in: *Conservation Biology*. DOI: 10.1111/cobi.13686

Aside from the publications mentioned above, during my PhD, I also co-authored the following publications:

Ficetola G. F., **Falaschi M.**, Bonardi A., Padoa-Schioppa E., Sindaco R. (2018). Biogeographical structure and endemism pattern in reptiles of the Western Palearctic. *Progress in Physical Geography*, 42(2): 220-236. DOI: 10.1177/0309133318765084

Falaschi M., Mangiacotti M., Sacchi R., Scali S., Razzetti E. (2018). Electric circuit theory applied to alien invasions: a connectivity model predicting the Balkan frog expansion in Northern Italy. *Acta Herpetologica*, 13(1): 33-42. DOI: 10.13128/Acta_Herpetol-20871

Scali S., Sacchi R., **Falaschi M.**, Coladonato A. J., Pozzi S., Zuffi M. A. L., Mangiacotti M. (2019). Mirrored images but not silicone models trigger aggressive responses in male Common wall lizards. *Acta Herpetologica*, 14(1): 35-41. DOI: 10.13128/Acta_Herpetol-24651

Ramellini S., Simoncini A., Ficetola G. F., **Falaschi M.** (2019). Modelling the Potential Spread of the Red-billed Leiothrix *Leiothrix lutea* in Italy. *Bird Study*, 66(4): 550-560. DOI: 10.1080/00063657.2020.1732864

Manenti R., **Falaschi M.**, Delle Monache D., Marta S., Ficetola G. F. (2020). Network-scale effects of invasive species on spatially-structured amphibian populations. *Ecography*, 43(1): 119-127. DOI: 10.1111/ecog.04571

Manenti R., Lunghi E., Barzaghi B., Melotto A., **Falaschi M.**, Ficetola G. F. (2020). Do Salamanders Limit the Abundance of Groundwater Invertebrates in Subterranean Habitats? *Diversity*, 12(4): 161. DOI: 10.3390/d12040161

Pizzigalli C., Banfi F., Ficetola G. F., **Falaschi M.**, Mangiacotti M., Sacchi R., Zuffi M. A. L., Scali S. (2020). Eco-geographical determinants of the evolution of ornamentation in vipers. *Biological Journal of the Linnean Society*, 130(2): 345-358. DOI: 10.1093/biolinnean/blaa037

Sacchi R., Mangiacotti M., Scali S., Coladonato A. J., Pitoni S., **Falaschi M.**, Zuffi M. A. L. (2020) Statistical methodology for the evaluation of leukocyte data in wild reptile populations: A case study with the common wall lizard (*Podarcis muralis*). PLoS ONE, 15(8): e0237992. DOI: 10.1371/journal.pone.0237992

Bertolino S., Ancillotto L., Bartolommei P., Benassi G., Capizzi D., Gasperini S., Lucchesi M., Mori E., Scillitani L., Sozio G., **Falaschi M.**, Ficetola G. F., Cerri J., Genovesi P., Carnevali L., Loy A., Monaco A. (2020). A framework for prioritizing invasive mammal species for a national list. *NeoBiota*, 62: 31-54. DOI: 10.3897/neobiota.62.52934

Mori E., Ficetola G. F., Bartolomei R., Capobianco G., Varuzza P., **Falaschi M.** (2020). How the South was won: recent range expansion of the crested porcupine in Southern Italy. *Mammalian Biology*, in press.

Ficetola G. F., Fanelli M., Garizio L., **Falaschi M.**, Tenan S., Ghielmi S., Laddaga L., Menegon M., Delfino M. (2020). Estimating abundance and habitat suitability in a micro-endemic snake: the Walser viper. *Acta Herpetologica*, in press. DOI: 10.13128/a_h-7771

Falaschi M., Mangiacotti M., Sacchi R., Scali S. (2021). Data sharing among protected areas shows advantages in Habitat Suitability Modelling performance. *Wildlife Research*, in press.

Lo Parrino E., **Falaschi M.**, Manenti R., Ficetola G. F. (2021). Lockdown policy effects on invasive species: a perspective. *Biodiversity*, in press.