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Improving the assessment of spread and impacts of freshwater invasive species

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ABSTRACT

Freshwater habitats occupy a small fraction of Earth's surface, but they host 6-10% of global biodiversity and are considered endemism hotspots. Moreover, they provide ecological services for more than 6 billion people worldwide, thus their conservation is particularly relevant to preserve both biodiversity and human well-being. Despite their importance, though, freshwater environments are generally less studied than terrestrial habitats. Among the factors threatening global freshwater biodiversity, Freshwater Invasive Species (FIS) play a key role. FIS can impact ecosystems through multiple pathways and in some areas they represent a major portion of freshwater biodiversity, both in terms of number of species and biomass. Moreover, their introduction rate has not yet started decreasing. For these reasons, FIS are considered to be among the main drivers of freshwater biodiversity loss. Understanding the drivers of their distribution and the consequences of their presence on local communities is an essential prerequisite to enable efficient management measures. Despite numerous tools potentially applicable to FIS studies that have been developed in the last decades, their application to this field, as assessed in Chapter 1, appears limited. Moreover, despite the growing interests for this topic, certain relevant aspects of it remain under-investigated. The aim of this thesis was to improve the assessment of spread and impacts of FIS using different approaches.

In Chapter 2, I investigated the current distribution and spread of an invasive crayfish, *Procambarus clarkii*, in Italy using data coming from multiple data sources: published literature, grey literature, experts' communications, and online forums. Eradication of FIS is often feasible only when the focal species has yet to establish a relevant population. As there could be a time lag between the first occurrence of a FIS in a certain area and its publication in peer-reviewed journals, using data coming from multiple data sources allows a much more rapid evaluation of the current situation, potentially enabling prompt management actions. Moreover, certain information remains confined within the grey literature and other unconventional sources. In the case of *P. clarkii* in Italy, 33% of the data used to create the distribution maps were not published in peer-reviewed journals. Despite the risk of biases inherent in these sources, they represent an important tool. Studies on FIS and management plans often require comprehensive information on their distribution, thus including these unconventional data sources may greatly improve conservation of freshwater environments.

In Chapter 3, I assessed how different methodological choices affect measures of niche dynamics. Niche conservatism is a prerequisite for Species Distribution Models (SDMs) transferability across space and time. SDMs are widely used in biological invasion studies to infer the potential distribution of an invasive species in its exotic range or under future climatic conditions. Previous studies have reported low levels of niche conservatism in FIS, but whether these findings reflected actual biological mechanisms or were related to the selected modelling approaches was yet to be assessed. FIS distribution may be shaped by different limiting factors than terrestrial

species, thus variable selection may be extremely important for model ability to correctly measure the species niche. Moreover, FIS are limited in their spread by the occurrence of freshwater environments, thus certain background selection criteria may fail to describe the actual portion of accessible environments. For these reasons, I tested the effects of 14 different combinations of ecological variables and background selection methods on nine different niche dynamic measures in 40 FIS. I found relevant effects of the different modelling approaches on niche dynamic measures, suggesting that the high rates of niche shift found in FIS may be at least partially explained by apparent shifts unrelated to biological mechanisms. These findings question the reliability of SDMs used to predict potential distribution of FIS and highlight the lack of more in-depth assessments on the optimal strategies to get realistic measure of niche shifts in aquatic species.

In Chapter 4, I assessed the effects of multiple environmental characteristics, including the presence of introduced predators (fish and *Procambarus clarkii*) on adult occurrence and breeding probabilities of seven amphibian species in Northern Italy. Assessing the effects of environmental features and stressors on local aquatic biodiversity is not an easy task, and this is particularly true for species with complex life cycles, as their different life stages can be differently impacted by the same variable. Certain life stages, though, have low detection probabilities, requiring high sampling efforts, implying a trade-off between model reliability and resources needed. I used multistate occupancy models to evaluate the responses of adult occurrence and breeding probabilities to the same parameters and the detection probabilities of adults versus eggs and larvae, then I used the results to assess the optimal monitoring strategy (adults-only versus adults, eggs, and larvae) under different levels of resource availability. Assessing the effects of FIS on native biodiversity is essential to plan management actions, thus when dealing with organisms with complex life cycles, such as amphibians, monitoring programs should be focused on collect data on multiple life stages.

In Chapter 5 I evaluated the effects of drought-related breeding pond scarcity and presence of invasive predators on the breeding strategies of two frog species in Northern Italy. Climate change is among the main threats to global biodiversity, and the combined effects of unavailability of freshwater habitats related to climate change and FIS may impact breeding success of amphibian species. Some anurans show high philopatric behavior, while others tend to select breeding sites based on ecological characteristics, such as the presence of predators. Breeding site scarcity may force anurans to breed in the few remaining waterbodies or, alternatively, to skip reproduction. The presence of predator FIS may complicate this choice. Using data from 27 clusters of breeding sites of *Rana dalmatina* and *R. latastei* monitored from 2017 to 2023, I found that the former showed higher levels of plasticity, being able to exploit the remaining waterbodies to breed in case of breeding site scarcity, while the latter responded to site scarcity only with a reduction in the number of breeding females. Moreover, both species showed no avoidance behavior toward the invasive *Procambarus clarkii*, despite its detrimental effects on amphibian larvae. These findings confirmed that plastic species may limit negative effects of climate change thanks to wide ecological tolerance, but endemic species are much more endangered by it. Moreover, they

highlighted that predator avoidance may not occur in case of recently introduced invasive species.

In this thesis I underlined the importance of assessing spread and impacts of FIS, particularly from a conservation perspective. I found that unconventional data sources may complement peer-reviewed data in order to improve management actions, and that there is an urgent need for a consensus on the best approach to assess FIS niche dynamics. Both occurrence data and niche assessments are needed to create maps of potential distribution, that usually represent the basis for management plans. Moreover, this thesis highlighted the differential effects of FIS on different life-stages of organisms with complex life cycles, such as amphibians, showing that collecting data on multiple life stages may result in better assessments of the status of local populations. Finally, I showed that endemic species with narrow ecological niches may be extremely endangered by the pressures exerted by climate change and FIS, thus requiring careful protection. The role of invasive species in shaping global biodiversity is expected to increase in the next future, thus the findings of this thesis may be particularly relevant for conservation of aquatic habitats.

CHAPTER 1: Introduction

1.1 Freshwater Invasive Species

While covering only ~0.8% of Earth surface, freshwater habitats host around 6-10% of all described species (Dudgeon et al. 2006; Faghihinia et al. 2021). In addition of their importance to biodiversity, they provide ecological services for more than 6 billion people globally (Ostfeld et al. 2012; Hayat & Gupta 2016). Despite their importance, these habitats are generally less studied than their terrestrial counterparts (Nabout et al. 2023). Moreover, not all freshwater taxa are well-studied by researchers, as there is a large disproportion between the best investigated taxa, such as fishes, and the least studied ones, such as invertebrates (Faghihinia et al. 2021). There is also a bias toward certain kind of freshwater habitats, namely lakes and rivers, at the expense of others, smaller and often neglected, such as ponds and springs (Céréghino et al. 2008; Cantonati et al. 2012). This has led to a gap of knowledge about processes in freshwater environments that has only recently been assessed by the scientific community. The urgency to fill this gap is due to the risks posed by anthropogenic and climatic factors to freshwater environments.

Among the anthropogenic stressors acting on freshwater habitats, invasive species represent a major threat. Among the traditionally listed World's Worst Invasive Alien Species (Lowe et al. 2000), two macro-fungi (*Aphanomyces astaci* and *Batrachochytrium dendrobatidis*), one aquatic plant (*Eichhornia crassipes*), four aquatic invertebrates (*Eriocheir sinensis*, *Cercopagis pengoi*, *Pomacea canaliculata*, *Dreissena polymorpha*), two land invertebrates (*Aedes albopictus*, *Anopheles quadrimaculatus*), three amphibians (*Rana catesbeiana*, *Bufo marinus*, *Eleutherodactylus coqui*), eight fishes (*Salmo trutta*, *Cyprinus carpio*, *Micropterus salmoides*, *Oreochromis mossambicus*, *Lates niloticus*, *Oncorhynchus mykiss*, *Clarias batrachus*, *Gambusia affinis*), one reptile (*Trachemys scripta*), and one mammal (*Myocastor coypu*) spend at least part of their lives in freshwater environments. In 2013, an update proposed another freshwater aquatic plant (*Salvinia molesta*; Luque et al. 2014) to the list, increasing the number of included Freshwater Invasive Species (FIS) up to 23.

Freshwater environments are particularly sensitive and impacted by biological invasion events (Strayer 2010). For instance, in a model of biodiversity scenario for the year 2100, biotic exchange (including invasive species introductions) was found to be the fourth largest driver of biodiversity change, and its relative importance was larger in freshwater habitats than in terrestrial ones (Sala et al. 2000). Despite that, FIS are generally less studied than terrestrial invasive species (Puth & Post 2005; Emery-Butcher et al. 2020; Liu et al. 2020; Cuthbert et al. 2021). This lack of studies may be caused by the biological characteristics of these species. Detecting aquatic species through traditional methods is usually more difficult than detecting terrestrial species. Additionally, their biology can hinder our ability to measure their impacts and relationships with the environment. From a modeling

perspective, for instance, applying the methods used for terrestrial species to FIS may result in unreliable assessments. The lack of distributional and freshwater-related ecological data represents another impediment to FIS-focused studies. Moreover, the ability to quantify their impacts on local communities could be hindered by their multiple complex effects on local environments. While many studies focused on the effects of a single invasive species on a single or a few native ones, invasive species can actually affect aquatic food-webs at multiple levels, as well as act as ecological engineers, modifying the characteristics of the invaded habitats (Fei et al. 2014; David et al. 2017).

In the last decades, numerous methods to study biological invasions have been implemented in order to assess their causes, mechanisms, and consequences on local biodiversity. Studies focusing on FIS, though, have been less abundant than the ones focusing on terrestrial invasive species (Sorte et al. 2013; Liu et al. 2020). Because of that, biological invasions in freshwater environments risk to remain less investigated, and their mechanisms less understood. Given the high levels of endemism of freshwater environments (Dudgeon et al. 2006) and their proneness to biological invasions, it is essential to adequately assess causes and consequences of FIS presence. In this Introduction I reviewed scientific papers from nine different years, representing three different period (2000-2002; 2010-2012; 2020-2022). The aim was to assess the practices that may hamper our understanding of biological invasions in those habitats: taxonomic biases, geographical biases, lack of the application of recently developed investigation methods, and the lack of stress on potentially key aspects of these phenomena.

1.2 Review

I selected papers from three different period (2000-2002; 2010-2012; 2020-2022) coming from five relevant journals focusing on conservation, invasive species, and/or freshwater environments, representative of the current state of the art concerning FIS research. There has been a relevant increase in the number of articles on freshwater invasive species during the three investigated periods (Fig. 1). It must be noted that two of the selected journals were launched after 2002. Both these journals publish papers on invasive species only, further highlighting how interest toward this subject has increased in the last decades.

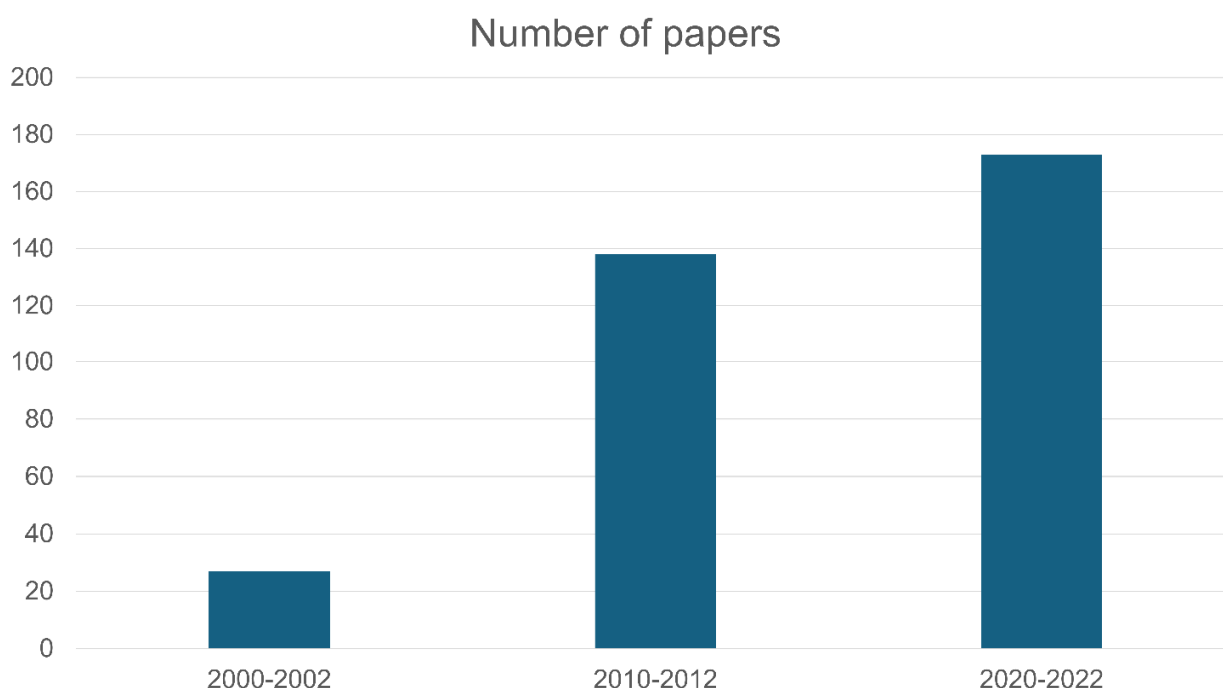


Figure 1: Number of papers on Freshwater Invasive Species published in selected journals.

The number of papers that included native species (e.g., effects of FIS on native organisms, niche comparisons between FIS and local species, et cetera) remained quite stable, representing ~70% of the papers concerning FIS in 2000-2002, ~65% in 2010-2012, and ~64% in 2020-2022. The most investigated FIS taxonomic groups in the selected papers were fish, crustaceans, and mollusks (Fig. 2). The same taxonomic groups were also the most investigated ones as native species, suggesting a strong taxonomic bias toward these organisms. Bias toward fish, crustacean, and mollusk invasive species has already been reported (Pyšek et al. 2008; Thomsen et al. 2014; Crystal-Ornelas & Lockwood 2020). Taxonomic bias is often more tightly linked to societal preferences than to scientific or ecological reasons (Troudet et al. 2017), thus it represents a major impediment to the understanding of the actual mechanisms and ecological consequences of biological invasions in freshwater environments.

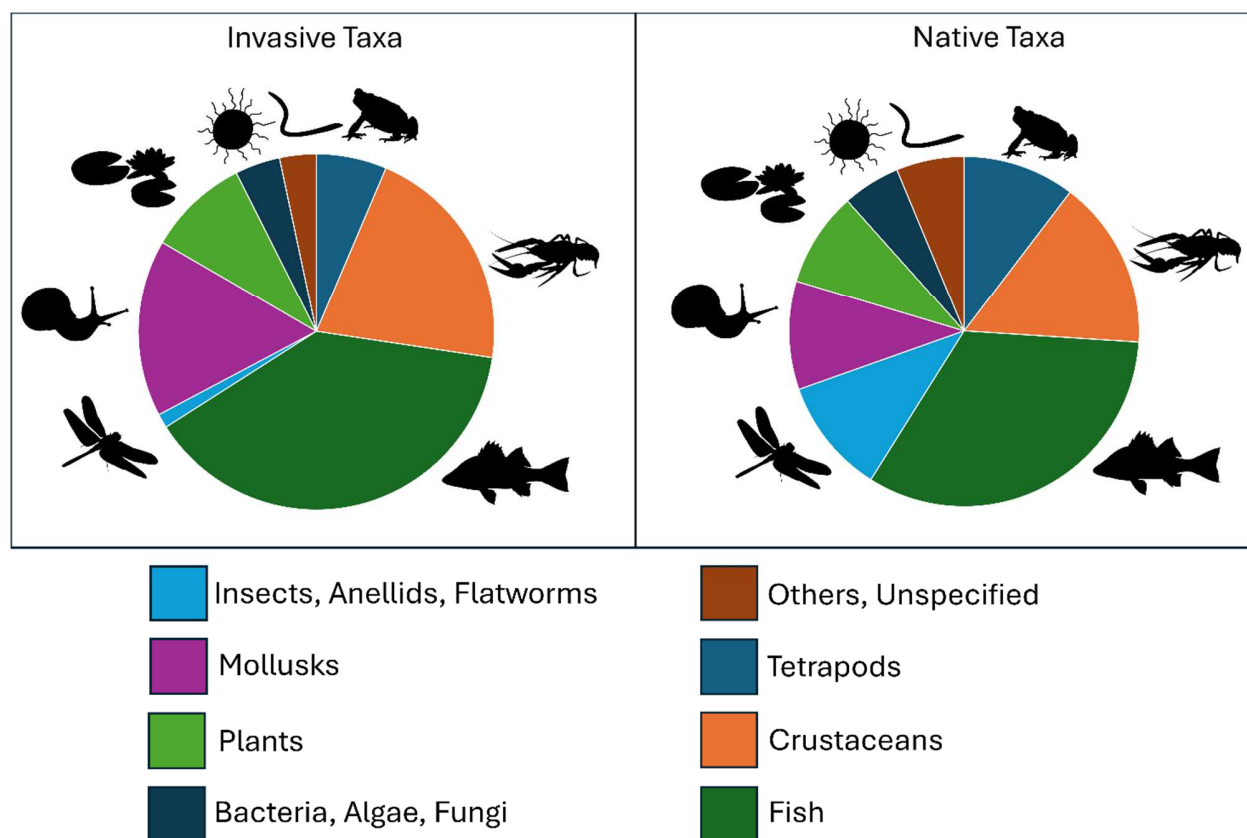


Figure 2: Proportion of invasive (left) and native (right) taxa investigated.

The geographical distribution of published studies underwent relevant changes (Fig. 3). In the 2000-2002 period, North America was the continent where most of the studies were conducted, while I did not find any study in Asia and Oceania. In 2010-2012 and 2020-2022 Europe was the continent hosting most of the studies, while Africa was the least represented continent. This disproportion in the number of published papers toward the most developed countries does not reflect the impacts of FIS, nor of invasive species in general. Developing countries are more sensitive than richer countries to biological invasions, as they host the majority of global biodiversity hotspots, thus effectively managing invasive species in those areas can be extremely beneficial from a global perspective (Nuñez & Pauchard 2010). Despite that, invasive species in developing countries remain poorly investigated (Pyšek et al. 2008).

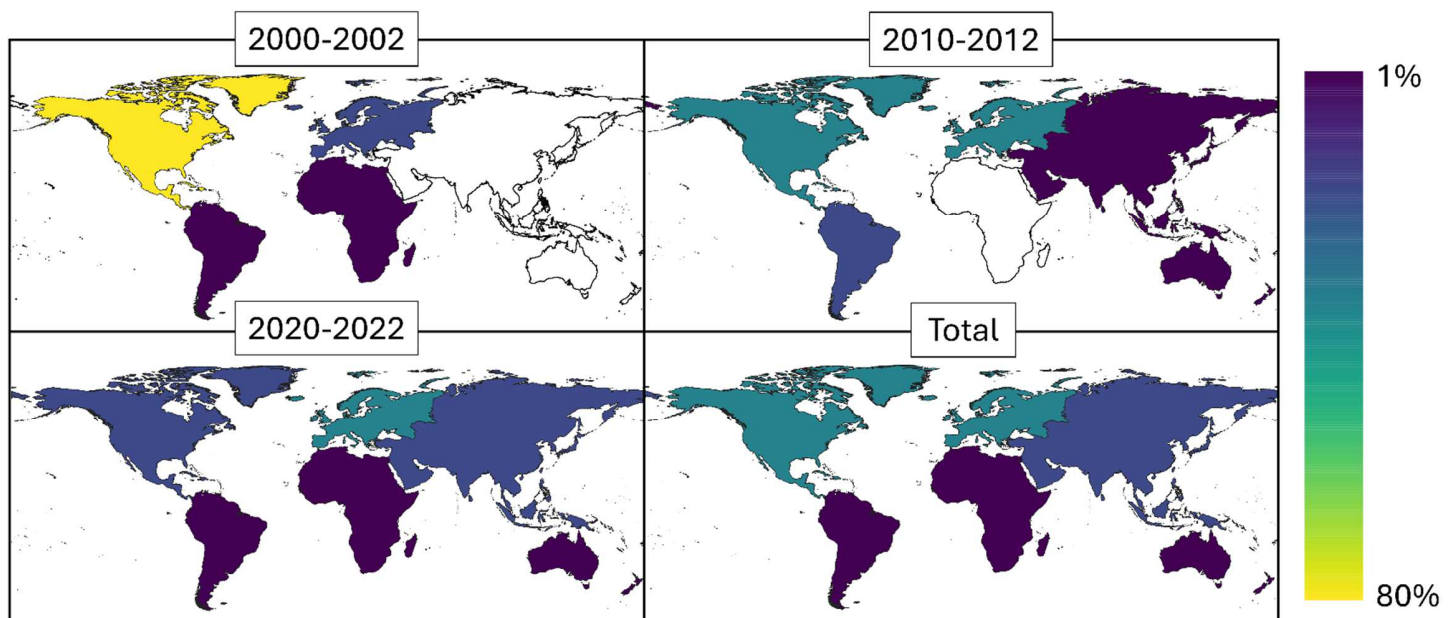


Figure 3: Proportion of studies carried on in the different continents.

Experimental studies represented the majority of papers in 2000-2002 (~55%). In the second and third period, though, they represented ~33% and ~35% of total papers respectively, and were surpassed by observational studies (Fig. 4). Despite the increase in the number of observational studies, a Chi-squared test found no significant difference among the three periods ($\chi^2= 5.327$, $p=0.083$).

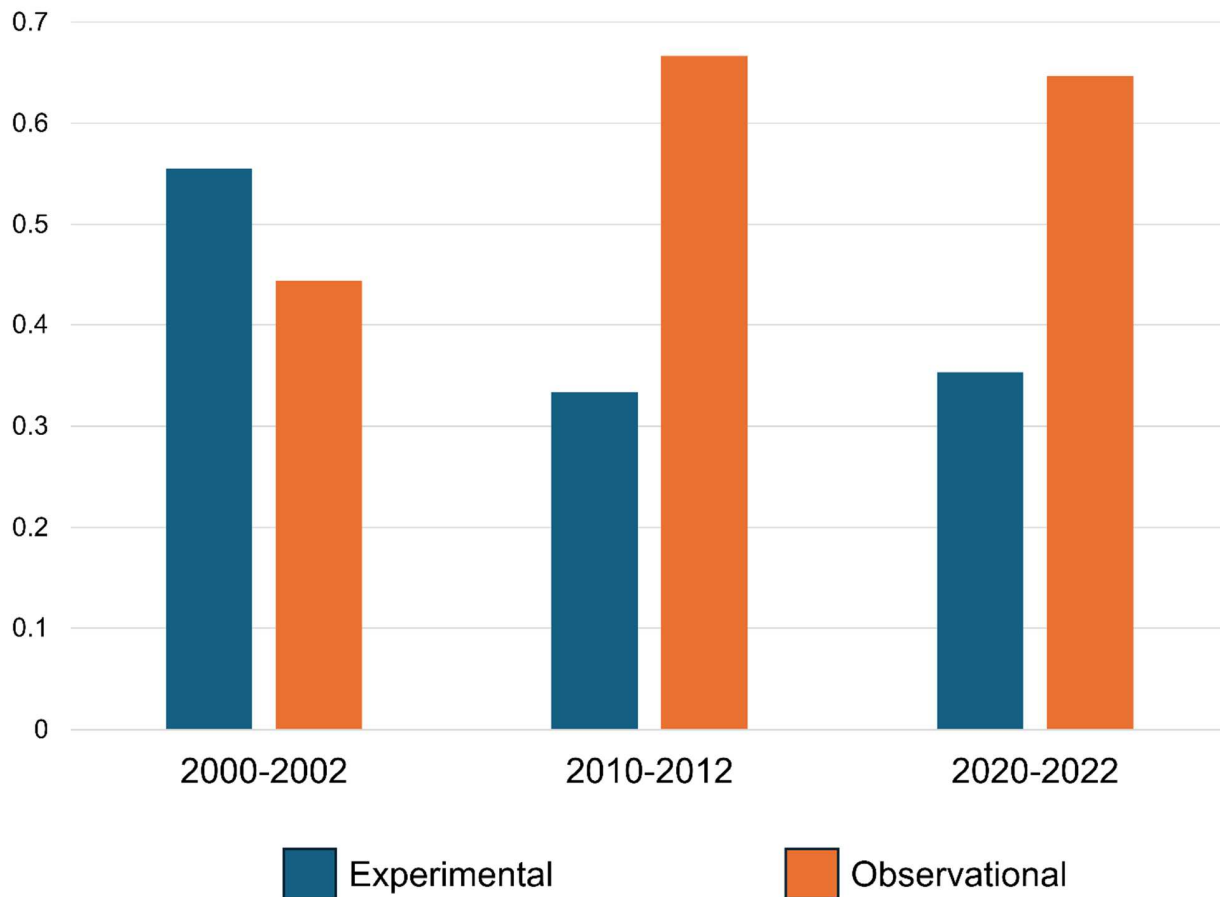


Figure 4: Proportion of observational (blue) and experimental studies (orange).

1.3 Data collection methods

1.3.1 Traditional methods

Detecting invasive species in aquatic environment remains particularly challenging, particularly at low densities (Moorhouse & Macdonald 2015; Forsström & Vasemägi 2016; Mauvisseau et al. 2019). For these reasons, a plethora of methods have been used to detect freshwater species, and FIS in particular, according to the characteristics of aquatic environments and the biology of the target organism. Traditional monitoring methods include visual surveys, acoustic surveys, and collection techniques, such as hand-netting, electrofishing, trapping, bottom trawling, gillnets, and purse seines. Many of these collection techniques are stressful, harmful, and sometimes lethal for native and non-native species alike. Electrofishing, for instance, is considered a relatively safe and effective procedure for fish monitoring and is often used to characterize fish communities. Some analyses, though, have revealed that it can severely harm individuals, even though physical damages are not always visible (Snyder 2003). Moreover, some taxa cannot be identified in the field. The standard procedure to monitor aquatic insects, for instance, requires their collection and preservation in 70–80% ethanol to allow subsequent laboratory observations (Santos & Fernandes 2021). Non-invasive traditional methods, such as visual and acoustic surveys, are particularly prone to false-negative errors, as detectability for FIS may be extremely low. Some species can be particularly difficult to be spotted by acoustic surveys, and long-term monitoring is required to have reliable results (Pomezanski 2021). Despite their inherent issues, traditional approaches appear to be frequently used when collecting data on FIS. They were used in the majority of studies in all the three periods considered in this Introduction: 75% in 2000–2002, 67% in 2010–2012, and 60% in 2020–2022. The differences between the periods do not appear to be significant ($\chi^2=1.782$, $p=0.419$).

According to this result, studies on FIS appear to be still dependent on traditional methods, despite a plethora of new methods have been developed in the last decades. The use of unconventional data sources, such as grey literature and social networks, and the increase in citizen science programs is allowing scientists to collect great amounts of data in short times. Moreover, recently developed techniques, such as environmental DNA (eDNA) and remote sensing, represent new non-invasive ways to reliably identify organisms in freshwater environment.

1.3.1 Grey literature

Grey literature (i.e., “that which is produced on all levels of government, academics, business and industry in print and electronic formats, but which is not controlled by commercial publishers”; MacDonald et al. 2010) can be an important source of information on FIS. Reports from government agencies, management plans of protected areas, strategic environmental assessments, et similia may contain important data on FIS, but they are usually scattered, unstandardized, and difficult to find, thus their use is limited. In some cases, for instance, occurrence data of FIS may be limited to grey literature (Wijnhoven et al. 2011). Moreover, the results of “practical” conservation measures, such as eradication, are published mainly in grey literature (Capdevila-Arguelles & Zillett 2005). In some areas, grey literature produced by government agencies and/or NGOs represents the major source of biodiversity information (Corlett 2011). Finally, economic damages of FIS, particularly at local scales, can mostly be found in grey literature (Sofia et al. 2017). Grey literature may thus represent an important resource for conservation biology, but its inherent issues often hinder its application. For instance, grey literature frequently deals with species of economic and/or social values, thus the associated taxonomic bias is often stronger than the one of published book and scientific literature (Bajomi et al. 2010). Similarly, the scope of stakeholders may be limited and focused on economically interesting areas (e.g., fishing areas; Cross et al. 2019). In addition, relevant portion of grey literature are difficult to access (Corlett 2011; Cross et al. 2019).

Despite these limitations, different studies managed to successfully use grey literature to provide valuable information. For instance, the integration of grey literature with scientific studies and direct observation allowed to estimate the impact of multiple invasive crayfishes on ecosystem services (Manenti et al. 2019), enabled accurate measurement of the spread of fish species in the Flandres (Verreycken et al. 2007), and improved the identification of most effective actions for the control of FIS (Beric & MacIsaac 2015). To improve the issues linked to scattering and heterogeneity of data coming from grey literature, there have been attempts to collect and standardize these data to facilitate their use. InvaCost, for instance, is an up-to-date, comprehensive, and harmonized public database of the economic costs of invasive species at global scale, created using data coming from peer-reviewed articles and grey literature (Diagne et al. 2020). Focusing on aquatic invasive species, ELNAIS is a dynamic online information platform collecting information on invasive species in Greek inland and marine waters that combines data from various sources, including grey literature and citizen science (Zenetos et al. 2015).

1.3.2 Citizen science and online sources

Citizen science is the involvement of amateur volunteers in scientific projects (Silvertown 2009). It allows scientist to get access to a free source of work force, skills, and computational power, requiring a fraction of the resources needed by traditional approaches, and can greatly improve the citizen involvement into research, increasing public awareness and commitment (Silvertown 2009; Crall et al. 2010). In the last years devices like smartphones, which are able to collect georeferenced medium to high quality pictures, video, and audio recordings with a good degree of precision, have become more and more used as tools in citizen science projects (Vercayie & Herremans 2015; Suprayitno et al. 2017). Citizen science projects have been successfully used by scientists to map the distribution of protected species, the spread of invasive species, roadkills, abundance data, or collect abundance data (Bonardi et al. 2011; Vercayie & Herremans 2015; Maistrello et al. 2016; Faraone et al. 2017; Zapponi et al. 2017; Larson et al. 2020).

Online repositories of citizen-science data, such as iNaturalist (www.inaturalist.org) and eBird (www.ebird.org), have been developed to create large communities of volunteers able to collect occurrence data of a wide range of taxa at a global scale. The Global Biodiversity Information Facility (GBIF; www.gbif.org) is a network created to provide open access data coming from citizen science projects and other sources. These tools allow immediate access to millions of records, covering thousands of taxa, that have been used in multiple scientific works, including studies on invasive species, and can represent an important tool for early detection (Larson et al. 2020; Mori et al. 2020; Pyšek et al. 2020; Sales & Rebouc 2021). Data stored in such repositories, though, includes a relevant portion of misidentified organisms, duplicates, and, in general, “problematic” observations (Zizka et al. 2020). Moreover, distribution of available data reflects economic patterns instead of longitudinal gradient of biodiversity (Chapman et al. 2024). Dealing with these biases is not easy, but they can strongly affect models’ results (Beck et al. 2014).

Other online sources, such as online forums and social networks, can represent sources of easily and freely available opportunistic data. Observations coming from social networks as Facebook and Twitter have been used to collect distribution data, to detect non-native species before they become invasive, and sometimes even to describe new species (Gonella et al. 2015; Miyazaki et al. 2016; Skejo & Caballero 2016; Lo Parrino & Tomasi 2021). Validation exercises of data coming from unconventional sources and citizen science proved that in some cases they are no worse than data collected by scientists with traditional methods (Van Strien et al. 2013; Larson et al. 2020; Uyeda et al. 2020; de Felici et al. 2021). Despite that, they come with limitations: data coming from these sources can be strongly affected by reporting and sampling biases, and there is the risk of misidentification (Crall et al. 2010; Van Strien et al. 2013; Uyeda et al. 2020; Marcenò et al. 2021). Sampling bias may be particularly strong for FIS, since freshwater environments are more difficult to sample than terrestrial ones.

1.3.3 Recent developments

Satellite remote sensing is an example of novel non-invasive technique that has been recently applied to data collection of freshwater organisms. Its use is becoming more and more common thanks to the increase in the availability of high-quality images, the trend toward free and open data policies, and the development of new image enhancement and analysis techniques (Ashraf et al. 2012; Wulder et al. 2022). While using satellite images to characterize habitat parameters is a widely applicable technique, using them to detect invasive species is applicable only to a handful of FIS with large biomasses and visible from above water, such as emergent, floating, and submerged plants (Hestir et al. 2008; Thamaga & Dube 2018; Angoh et al. 2021). Acoustic remote sensing can also be applied to assess the presence of those FIS that emit calls, such as anurans (Ribeiro et al. 2022). In general, remote sensing techniques are useful and promising tools whose drawback is that they can only be used when monitoring a small subset of FIS with specific characteristics.

Environmental DNA (eDNA) is another increasingly used technique to detect aquatic species in freshwater environments. Organisms release their DNA in the environmental matrix through mucus, feces, gametes, secretions, etc., allowing its extraction from environmental samples without the need to handle or catch individuals (Ficetola et al. 2019; Larson et al. 2020). The first microbial eDNA assessment focused on an invasive amphibian, *Lithobates catesbeianus* (Ficetola et al. 2008a), thus the potential of this approach to monitor FIS was clear from its first implementation. eDNA is particularly efficient in aquatic environments, as DNA from sloughed tissues in suspension is easy to detect (Jerde et al. 2011), thus this method has been used to detect numerous FIS, such as amphibians, fish, reptiles, crustacean, and mollusks (Rees et al. 2014; Hunter et al. 2015; Secondi et al. 2016; Clusa et al. 2017; Geerts et al. 2018; Jo et al. 2021). Moreover, eDNA represents the easiest way to study freshwater invasive microbes, that are much less studied than invasive macro-organisms (Litchman 2010). Comparing eDNA metabarcoding with traditional methods in freshwater environments resulted in generally higher detection probabilities with the former (Valentini et al. 2016). Nonetheless, there are some challenges that need to be addressed. For instance, eDNA may fail to detect FIS present at low densities (Tréguier et al. 2014; Roussel et al. 2015). Moreover, in some cases traditional sampling methods can be cheaper than eDNA while resulting in similar estimates (Barata et al. 2021). Given that mitochondria are maternally inherited in most organisms, it is impossible to discriminate between hybrids and their maternal species when using eDNA (Valentini et al. 2016). Finally, eDNA does not allow to get information on population structure, population size, and the possibility to infer biomass or density from the number of reads is still being investigated, despite encouraging results (Takahara et al. 2012; Valentini et al. 2016; Doi et al. 2017; Yates et al. 2021).

1.4 Drivers of FIS distribution

1.4.1 Investigation methods

Early detection of exotic species is considered a prerequisite for preventing establishment and/or spread (Reaser et al. 2020). Moreover, predicting invasions and understanding the processes favoring them is needed to prevent future events (Gallien et al. 2010). During the years, these aspects have been investigated from different perspective, such as the characteristics of recipient communities and the relationship between the invasive species and environmental features. Drivers of FIS establishment and distribution can be analyzed using an experimental approach or an observational approach. Observational studies are in general easier to conduct, but may fail to reliably measure FIS impacts since events occurring underwater are particularly difficult to detect (Moorhouse & Macdonald 2015). Moreover, confounding factors may play an important role. Accounting for all the potential confounding effects and all possible interactions is not always feasible. Experimental studies, on the other hand, can limit the presence of confounding factors, potentially allowing more direct measurements of the actual relations between the investigated variables, but require more rigorous designs and are prone to oversimplification of the actual situations experienced in natural environments. In the evaluated papers, observational studies represented the most used approach to assess the drivers of FIS distribution in all the periods: 60% in 2000–2002, 74% in 2010–2012, and 80% in 2020–2022. No significant difference was found ($\chi^2=2.507$, $p=0.292$). The higher proportion of observational studies appears to be linked to the high number of studies applying Species Distribution Models (SDMs) to FIS.

SDMs represent a set of tools often applied to invasive species to predict future distributions, identify the drivers of suitability, and assess their responses to future climatic conditions (Srivastava et al. 2019). Estimations of potential distribution of invasive species through SDMs rely on different assumptions, such as niche conservatism in the exotic range (Early & Sax 2014; Hill et al. 2017; Pili et al. 2020; Atwater & Barney 2021). Changes in a species' realized niche (i.e., the ensemble of environmental conditions in which a species is observed; Soberón 2007; Guisan et al. 2014) in the exotic range may arise from different factors, such as new biotic interactions, dispersal barriers, and changes in the fundamental niche (i.e., the ensemble of abiotic conditions under which the species can survive, not considering biotic interactions; Soberón 2007; Guisan et al. 2014; Liu et al. 2020). Niche expansion undermines the ability of predicting suitability for invasive species, that can underestimate their potential ranges thus assessing whether FIS conserve their niches or not is of uttermost importance. Previous studies found that FIS generally show higher level of niche expansion in the exotic range than models applied to terrestrial species (Torres et al. 2018; Liu et al. 2020). These rates of niche expansion may represent an actual biological phenomenon, or just the results of inappropriate methodological choices. Methodological choices that can affect niche modelling, particularly when applied to FIS, are related to various factors, such as occurrence data availability, background selection criteria, environmental variables selection, and

dimensionality. SDMs can be divided into two categories: mechanistic (i.e., models that use data on physiologic tolerance of the focal species to identify its niche) and correlative (i.e., models that use presence-data of the focal species to extract local environmental conditions and use them to assess the niche). While mechanistic models are considered to be more reliable (but see Buckley et al. 2010), they are rarely used, mainly because they require exhaustive data on species physiology estimated independently from occurrences (Rougier et al. 2015; Srivastava et al. 2019). Correlative models, on the other hand, are easy to implement, thus are commonly used to study invasive species, including FIS. In the investigated papers, only one study used SDMs in the 2000–2002 period, and it used a correlative approach; in the 2010–2012 period correlative models were 30 out of 33, and in the 2020–2022 period they were 56 out of 62. Despite the prevalence of correlative SDMs in FIS-related studies, inherent issues of these modelling techniques and biological features and constraints of aquatic organisms are rarely accounted for. For instance, dispersal limitations are a factor that is often ignored, despite strongly affecting species distribution. Concerning SDMs, the best practice is to consider ‘the parts of the world that have been accessible to the species via dispersal over relevant periods of time’ to determine the background available to the focal species (Barve et al. 2011). Accounting for actual accessibility measures has been proved to lead to more accurate results (Barve et al. 2011; Acevedo et al. 2012; Hill et al. 2017). This can be particularly relevant for FIS, since they can generally be found only in freshwater environments, thus backgrounds should be selected only from the subset of theoretically accessible locations where freshwater habitats are present. It has been reported that SDMs on FIS showed higher performances and lower level of niche shifts when background points were selected only in correspondence of watercourses (Nori & Rojas-Soto 2019).

Moreover, good-quality occurrence data from the exotic range are important to reliably assess the relationship between ecological conditions and suitability when using correlative SDMs for invasive species (Jiménez-Valverde et al. 2011). Observations of a species in a given environment does not necessarily mean that the environment is suitable for that species. Fish, for instance, may be present in a basin with unsuitable conditions due to continuous restocking (Sicuro et al. 2016). Adult amphibians are sometimes found in habitats unsuitable for reproduction even at great densities (Cruickshank et al. 2021), and exotic long-living freshwater turtles can survive for decades in sub-optimal or unsuitable environments without establishing populations (Ficetola et al. 2008b). Even when non-observation data are available, they should not always be considered as true-absence data. Since detection probability may be low, particularly when dealing with aquatic species, imperfect detection (i.e., the presence of false-negative observations) could strongly affect the models by causing an underestimation of the species tolerance to certain conditions (MacKenzie et al. 2003). Imperfect detection may lead to inaccurate estimates of species distribution and its drivers (Kéry & Schmidt 2008). Because of that, methods that account for detection probability of the focal species estimated using multiple surveys, such as site-occupancy models and N-mixture models, have started to be more and more commonly used (Bailey et al. 2014). Occupancy models, in

particular, are used to estimate the probability that the focal species occupies a certain sample unit during a specified period of time while accounting for imperfect detection (Bailey et al. 2014). Multiple occupancy models have been developed during the last decades, in order to assess multiple phenomena, such as relationships with habitat features (e.g., Băncilă et al. 2017), metapopulation dynamics (e.g., Falaschi et al. 2021), community dynamics (Devarajan et al. 2020), and invasive species dynamics (e.g., Dubart et al. 2019). Still, in FIS-related studies, these methods seem to be underused: in the selected papers, I found only three papers from 2020–2022 that explicitly accounted for imperfect detection. FIS can have low detection probabilities, particularly during early stages of invasion, thus the lack of studies accounting for imperfect detection could greatly hinder our understanding of the actual drivers of their distribution.

1.4.2 Abiotic factors

Another important issue is linked to the selection of ecological variables. In the selected papers, abiotic factors, alone or combined with other factors, were accounted for in the only work from the 2000–2002 period, in 100% (33/33) papers in 2010–2012, and in 97% papers in 2020–2022. Concerning climate-related variables, they were investigated in 39% papers in the second period and in 31% papers in the third one. Water-related variables (physical and/or chemical conditions, discharge, flow speed, et cetera) were investigated in 1/1 paper in 2000–2002, in 76% papers in 2010–2012, and in 66% papers in 2020–2022. Excluding the first period, the data showed no variation in the frequency of usage of these two classes of abiotic factors ($\chi^2=0.04$, $p=1.00$). It is still unclear whether variables linked to air temperature and precipitation are suitable for describing the conditions experienced by freshwater species. Annual mean values of air and water temperature, for instance, are not always significantly correlated (Webb & Nobilis 1997). Moreover, for some freshwater species, the limiting factors for distribution may be related to water chemistry and conditions. Mollusks species, for instance, may be affected by calcium concentrations, while plant species can be influenced by water turbidity (Whittier et al. 2008; Bornette & Puijalon 2011). Some studies reported good model performances when using air temperature and precipitation related variables when describing freshwater fish niches (Frederico et al. 2014; McGarvey et al. 2018), but other organisms may be more sensitive to other, non-climatic parameters. The lack of reliable high-resolution global maps of freshwater-related environmental variables does not allow a global comparison between the two methods (Loo et al. 2007; Torres et al. 2018). In any case, most FIS are ectotherms, thus increase in global temperatures is likely to affect their establishment and spread (Rahel & Olden 2008). Responses to climatic conditions are generally species-specific. Niche modelling of two invasive crayfish suggested that mean annual temperature is the main driver of both species' distribution, and that they are likely to respond differently to climate changes due to different thermal preferences (Zhang et al. 2020). This means some invasive species will be favored by climate changes, while other may be negatively affected. For many FIS, though, the expected outcome is an increase in

establishment success, spread, and negative impacts on local biodiversity (Rahel & Olden 2008; Bellard et al. 2013; Havel et al. 2015; Vilizzi et al. 2021).

1.4.3 Biotic factors

While abiotic factors are often the focus of studies focused on the drivers of FIS distribution, biotic factors (e.g., presence of competitors, diseases, food availability) are often excluded from these kind of analyses, as they are more difficult to identify and quantify, and because they were traditionally considered to be less relevant at broader scales (Aragón & Sánchez-Fernández 2013). This view has been challenged in the past decades, and numerous works have shown that including biotic interactions may result in a more thorough assessment of the focal species' niche (Araújo & Luoto 2007; Hof et al. 2012; Aragón & Sánchez-Fernández 2013). Biotic characteristics of the recipient communities may favor or hinder the establishment of invasive species.

The biotic resistance hypothesis states that ecosystems with higher biodiversity reduces success of invasive species (Levine et al. 2004; Jeschke et al. 2012). Native species can affect invasive species through predation, competition, disease spread, et cetera (Levine et al. 2004). This hypothesis have been tested on different taxonomic groups, with contrasting results (Levine et al. 2004; Jeschke et al. 2012; Lovell et al. 2021). The invasional meltdown hypothesis, on the other hand, suggests that the presence of invasive species in a recipient community may facilitate the success of other invasive species (Green et al. 2011; Jeschke et al. 2012). Invasive species, in fact, can disrupt biotic resistance, thus promoting further invasions. Studies on specific systems reported supporting evidence to the invasional meltdown hypothesis but supporting studies at community level are scarce (Braga et al. 2018). Nonetheless, support to the invasional meltdown hypothesis was found in a higher proportion of studies than to biotic resistance (Jeschke et al. 2012). In freshwater environments, biotic resistance has been reported as a driver of FIS success in some cases (e.g., Calizza et al. 2021), but broader analyses do not support this hypothesis (Leprieur et al. 2008). Concerning invasional meltdown, supporting evidence were found in certain systems (e.g., Adams et al. 2003; Chucholl 2013), but in some cases FIS were found to facilitate native and other invasive species alike (Devanna et al. 2011), and even increasing biotic resistance of the invaded communities, limiting further invasions (Henriksson et al. 2016).

In the selected papers investigating the factors shaping FIS distributions, two papers from 2010–2012 and six from 2020–2022 tried to evaluate biotic resistance by including native species as predictors of FIS distributions. Even less common were studies evaluating invasional meltdown by incorporating other invasive species as drivers of FIS distribution: one from 2010–2012 and two from 2020–2022.

1.5 Assessments of impacts

1.5.1 Investigation methods

FIS can affect local ecosystems through different mechanisms: predation, habitat alteration, disease transmission, hybridization, competition, food-web modifications (Mooney & Cleland 2001; Cadi & Joly 2003; Strayer 2010; Manenti et al. 2014; Vannini et al. 2018; Calizza et al. 2021). Some of them can have multiple impacts, both direct and indirect, and invasive engineer species may drastically modify entire habitats, causing biotic and abiotic alterations (Matsuzaki et al. 2009). Assessing the impacts of FIS on aquatic communities is thus essential for biodiversity conservation in inland waters. Among the considered papers, 19/29 works focused on FIS effect assessments in 2000–2002, 75/142 in 2010–2012, and 87/182 in 2020–2022, with no significant differences between the periods ($\chi^2=3.368$, $p=0.202$). Quantifying these impacts is not an easy task. Confounding effects may hinder assessments of FIS effects in observational studies. For instance, since FIS are more likely to invade disturbed environments, lower values of local biodiversity in invaded sites may be caused by disturbance, instead of/in addition to FIS presence per se (Havel et al. 2015). Additionally, local conditions may lead to contrasting effects found when analyzing the impacts of the same FIS (Klose & Cooper 2012). Experimental studies are able to limit the effects of confounding factors, but they are prone of other kind of biases. Different experimental approaches, such as experimental length and laboratory versus field experiments, have been found to significantly affect the measures of invasive species' effects on local communities, with longer experiments conducted under field conditions lead to the best approximation of natural patterns (Griffen et al. 2021). In the selected papers, the proportion of experimental studies assessing the effects of FIS on native species was 65% in 2000–2002, 41% in 2010–2012, and 51% in 2020–2022, with no significant difference between the three periods ($\chi^2=3.393$, $p=0.168$). Among these, the ones under field conditions represented respectively the 58%, 50%, and 55% of the experimental studies, again with no significant difference ($\chi^2=0.324$, $p=0.847$).

A great number of FIS impact assessments, both observational and experimental, focus on a single invasive species, without considering the effects of multiple co-occurring invasive species on local communities (Preston et al. 2012). Multiple co-occurring invasive species may have complex interactions between them, with synergistic or antagonistic effects on local biodiversity (Jackson 2015). It has been observed that, when multiple FIS are present, one may actually limit the negative impacts of another through predation on the mesopredator (Miyake & Miyashita 2011; Bissattini et al. 2018). In the selected papers, though, 62% of studies estimating the effects of FIS on local communities included only one species. In certain areas, FIS represent a consistent portion of biodiversity both in terms of biomass and number of species (Strayer 2010), thus focusing on a single FIS may not return a thorough picture of the actual stressors affecting local biodiversity. In experimental designs, the proportion of studies including two or more FIS was significantly lower than in observational studies ($\chi^2= 6.534$, $p=0.020$).

Moreover, invasive species can have differential effects on native species, for instance by selectively feeding on certain taxa (Carreira et al. 2014) or by facilitating native species (Rodriguez 2006). FIS can affect trophic webs at multiple levels, increasing the densities of certain organisms by preying on their native predators (Bucciarelli et al. 2019), although this effect is not always found (Ficetola et al. 2012). Efficient FIS management needs information on biotic interactions at community level to avoid unexpected negative consequences on local biodiversity. Focusing on the effects of a FIS on one or a few species may thus return an incomplete picture of its impacts on local biodiversity. The number of studies considering a single native species represented 31% of the selected papers. This implies that it is more frequent to assess the effects of a single FIS on multiple native species than to assess the combined effects of multiple FIS on native species. Also in this case, the proportion of experimental studies including two or more native species was significantly lower than in observational studies ($\chi^2= 12.615$, $p>0.001$).

1.5.2 Under-investigated aspects

FIS effects on local communities are not only limited to the macrobiome, but also to freshwater microbiome. In freshwater environments, microbial communities are mainly composed by microscopic eukaryotes, bacteria, archaea, and viruses (Havel et al. 2015). Microplankton represents an important component of freshwater biodiversity, significantly contributing to the total biomass, playing major roles in biochemical processes, and representing an important part of aquatic food webs (Cotner & Biddanda 2002; Sigeo 2005; Findlay 2010). Nowadays, methods like eDNA metabarcoding allow relatively easy microbial diversity assessments (Garlapati et al. 2019). Despite that, responses of freshwater microbial communities to biological invasions are generally poorly studied. FIS can affect bacterial assemblages in terms of abundance, taxonomic composition, and functional diversity (Denef et al. 2017), or cause blooms of toxic cyanobacteria, with harmful consequences on local biodiversity (Knoll et al. 2008). Additionally, interactions between multiple FIS can have synergistic effects on microbes, with consequences such as long-term shifts in microbial phenology (Rohwer et al. 2023). Among the selected papers, assessments of FIS effects on microbial taxa represent a small fraction of the total (Chapter 1.2, Fig. 2)

Moreover, FIS may affect local functional diversity, in addition to taxonomic richness. Invasive species can create new functional groups in receiving communities, modifying the overall functioning of the whole food-web (David et al. 2017). In freshwater environments, high degrees of invasion might be linked to a reduction of functional diversity (Milardi et al. 2019, 2020). Sometimes, even a single invasive species can disrupt functional diversity in freshwater communities, particularly concerning macroinvertebrates (Mathers et al. 2020; Guareschi et al. 2021). While certain functional groups can be strongly impacted by FIS, other groups can tolerate or even benefit from the presence of an invasive species (Maringa 2010; Watanabe & Ohba 2022). This can lead to

functional homogenization, in addition to taxonomic homogenization, of the invaded communities. For these reasons, assessing the effects of FIS on the different functional groups may shed light on the threats posed on local freshwater environments. While taxonomic changes are easy to detect, ecological changes can be more closely related to functional homogenization, that can be more difficult to detect (Olden et al. 2004). This is reflected by the low number of studies focusing on the effects of FIS on functional diversity among the investigated papers (two in 2010–2012 and three in 2020–2022).

Finally, freshwater organisms may have complex life cycles, requiring both terrestrial and aquatic habitat (Wilbur 1980). Some organisms are found in freshwater environments only during certain life-stages (e.g., amphibians, dragonflies, mosquitos) and undergo morphological transformations during their ontogeny, often corresponding to changes in functional traits. In some cases, the effects of invasive species with complex life cycles on aquatic environments is sometime overlooked, with the focus on the effects of their terrestrial forms. While the effects of adults invasive amphibians are usually well known, the effects of eggs and tadpoles on freshwater biodiversity have been rarely investigated (Greenlees & Shine 2011). Moreover, assessing the effects of FIS on native species with complex life cycle can be particularly challenging. In some cases, for instance, adult amphibians show avoidance of fish-invaded breeding sites, thus lower number of breeding adults, and consequently eggs and larvae, are observed in invaded sites (Winandy et al. 2015). In other cases, though, the effects of FIS presence can only be seen when focusing on the most impacted life stages (e.g., aquatic larvae), with no evident effect on the other ones (Ficetola et al. 2011; Siesa et al. 2014). Focusing on certain life stages can thus mask FIS impacts on local communities.

1.5.3 Interplays between FIS and other stressors

Biological invasions are not the only factor determining loss of biodiversity in freshwater environment. Fragmentation, alteration, and destruction of habitats, water pollution, land use, and climate change are among the factors have been deeply altering waterbodies communities (Stendera et al. 2012; Amoatey & Baawain 2019; Dudgeon 2019; Brauer & Beheregaray 2020). While most studies focus on a single stressor at the time, they do not act isolated from the others, and the interactions between these factors have been rarely investigated (Matthaei et al. 2010; Kernan 2015). The effects of climate change, in particular, are particularly relevant for freshwater biodiversity (Däll & Zhang 2010; Fenoglio et al. 2010; Woodward et al. 2010), but have been largely neglected (Faghihinia et al. 2021). The combined effects of FIS and climate change on freshwater biodiversity is difficult to predict. In some cases, protected areas are expected not only to have a limited effect in conserving local species sensitive to climatic changes but might also provide suitable habitats for FIS. Suitability for certain FIS is likely to increase in numerous regions (Nori et al. 2011; Johovic et al. 2020). Drought events are expected to increase in

certain regions due to climate change (Vautard et al. 2007; Dai 2013). Since some FIS are tolerant to desiccation and/or are well-adapted to ephemeral waterbodies, their establishment and/or abundance may be favored by drought conditions (Rahel & Olden 2008; Bezerra et al. 2018). In certain areas, co-existence between native and invasive species is mediated by specific climatic conditions (e.g., Kats et al. 2013). Climatic changes may thus exacerbate the negative impacts of the crayfish on native taxa. Moreover, water scarcity and droughts caused by climate change are likely to determine an increase in the number of reservoirs that provide water for human use (Vörösmarty et al. 2004; Rahel & Olden 2008). Reservoirs modify natural hydrological processes and disrupt connectivity for freshwater species (Rahel et al. 2008; Radeloff et al. 2015). Reservoirs are often prone to biotic homogenization and they have a high FIS establishment risk and can facilitate their spread, thus posing an important threat to freshwater biodiversity (Rahel & Olden 2008; Clavero & Hermoso 2011; Daga et al. 2015; Havel et al. 2015; Jo et al. 2021).

Scarcity of suitable waterbodies due to dry conditions, changes in water temperature and/or chemistry, increased salinity and similar processes can increase the frequency of encounters between native and invasive freshwater species, amplifying the magnitude of impacts of the latter on the former. Encounters between FIS and native species can be more frequent if limited amount of freshwater environments are available (Letnic et al. 2008). This may be particularly relevant for certain taxa. In normal conditions, some amphibian species tend to avoid wetlands where native and/or invasive predators are present (Winandy et al. 2015; Pollard et al. 2017). Amphibian strategies under prolonged site-scarcity scenarios, with most of the available wetlands being occupied by FIS, have yet to be assessed. On the other hand, in some circumstances climate change may mitigate the effects of FIS on local biodiversity. For instance, the number of suitable environments for certain FIS is expected to decrease due to global warming, suggesting potential declines (Capinha et al. 2012; Walsh et al. 2020). In certain cases, FIS might be less resilient than native species to drought events (Hossack et al. 2017). Some meta-analyses found that FIS are more likely to be negatively affected by climate change than terrestrial invasive species (Sorte et al. 2013; Stephens et al. 2019). These results, though, are likely biased by the low proportion of studies focusing on freshwater species, underlining the urgency for more in-depth assessments of the interplay between climate change and FIS, as well as by taxonomic and geographic biases. The interactions between FIS and climate change can be extremely difficult to predict and deserve careful investigation, as these factors represent two of the main threats currently affecting freshwater environments worldwide.

1.6 Aim of the thesis

As underlined in the Introduction, interest in biological invasions in freshwater environment has grown in the last decades, yet there are methodological approaches that appear to be underused and others whose reliability are often not sufficiently investigated. A fully comprehensive investigation on all the available approaches is beyond the possibility of a doctoral thesis, thus I focused on a few key questions whose relevance may be of general importance:

- a) Is it possible to use data from different sources, including grey literature, to effectively monitor FIS spread?
- b) What are the implications of methodological choices in niche dynamic assessments for FIS?
- c) Is monitoring only one life stage effective to assess FIS and other stressors' impacts on native species with complex life cycles?
- d) How do FIS presence and climate change affect site selection in amphibians?

In Chapter 3, I investigated the distribution an invasive crustacean, *Procambarus clarkii*, in Italy. As the species is undergoing a quick spread and the previous nationwide assessments of its spread were almost a decade old, lack of a general overview of the crayfish distribution was a major barrier to a correct management of this species. Scientific papers represent a precious source of information concerning FIS distribution even if that is not their main aim. Despite that, they often suffer from geographical biases, even within countries. Moreover, there may be a relevant time-lag between the first observation of a FIS and the publication of that observation in a scientific paper. This work shows that data from scientific literature can be complemented with grey literature, newspapers, experts', unpublished data, and online resources to produce up-to-date and high- resolution maps distribution maps of problematic invasive species, such as *Procambarus clarkii*, with temporal information on its spread.

In Chapter 4, I focused on how methodological choices may affect FIS niche assessment. As niche conservatism is a prerequisite for the most commonly used SDM techniques, it is essential to find accurate ways to measure niche dynamics in invasive species. Niche dynamic assessments may be particularly tricky for FIS, due to the limiting factors affecting their distribution can be vastly different from the ones affecting terrestrial organisms, and available habitats are much more limited for freshwater organisms than for terrestrial organisms. While the consequences of different methodological approaches on niche assessments have been thoroughly investigated in terrestrial invasive species, there is a lack of knowledge on this topic for FIS. The aim of this chapter was to fill that gap by using occurrence data, different combinations of predictors, and alternative background point selection methods to assess how the different methodological choices affected six different measures of niche dynamics in 40 different FIS.

In Chapter 5, I used multistate occupancy models to assess how different environmental features of

potential breeding sites, including the presence of an invasive predator, affect occurrence and reproduction probabilities of seven amphibian species. Amphibians may occupy freshwater environments without breeding, thus assessing the effects of environmental features, e.g. FIS presence, only on occupancy may return a partial picture of the actual status of local populations. Assessing the effects of stressors on both occurrence and reproduction probabilities may thus return a more comprehensive understanding of the response of amphibians to certain parameters, but as some life-stages can be particularly difficult to detect there is a potential trade-off between assessment accuracy and monitoring effort. For these reasons, I tried to assess the differential responses of occupancy and reproduction to the same environmental variables and the optimal monitoring strategy for amphibians under different scenarios of resource availability.

In Chapter 6, I analyzed the interplay between FIS presence and climate in reducing the number of suitable breeding habitats for amphibians, affecting their breeding choices. Climate change is increasing the frequency and magnitude of extreme events, such as droughts, in broad areas of the world. In Northern Italy, one of the most human-modified regions of Europe, this increase in drought events might represent an additional challenge for native amphibians, already declining due to habitat alterations, invasive species, and other factors. When available habitats are scarce, amphibians may choose between two different strategies: exploiting the few remaining waterbodies, thus increasing interspecific and intraspecific competition, or skipping the breeding season. If the few available sites host predators and the amphibians recognize those predators as a threat, predation risk may also play a role in determining the optimal choice for amphibians. I tried to assess how two different frog species react to the combination of breeding site scarcity and invasive species presence in a human-dominated region in order to understand how the interactions between climate change and biological invasion affect local biodiversity in freshwater environments.

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CHAPTER 2: Thirty years of invasion: the distribution of the invasive crayfish *Procambarus clarkii* in Italy

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

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SUMMARY

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.

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 and Fitter 1996, Graves and Shapiro 2003, Ricciardi and 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 and Joly 2003), spread of pathogens (Diéguez-Uribeondo and Söderhäll 1993), and food web alterations (Ficetola et al. 2012).

Biological invasions in freshwater habitats can cause degradation to various levels of biological organization, negatively affecting the entire ecosystem (Vilà and 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 and 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 and 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, Loureiro et al. 2015). This crayfish has a generalist diet (Barbaresi and Gherardi 2000, Loureiro et al. 2015) and can tolerate extreme variations in oxygen level, water salinity and acidity, and even prolonged droughts (Claire and Wroiten 1978, Barbaresi and Gherardi 2000, Casellato and Masiero 2011, 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 and 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 and 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 and

Söderhäll 1993, Holdich 1993, Aquiloni et al. 2011, 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.

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 (Figure 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).

RESULTS

We found records of *Procambarus clarkii* in 86 out of 107 Italian provinces (80.4%; Figure 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%) (Figure 2).

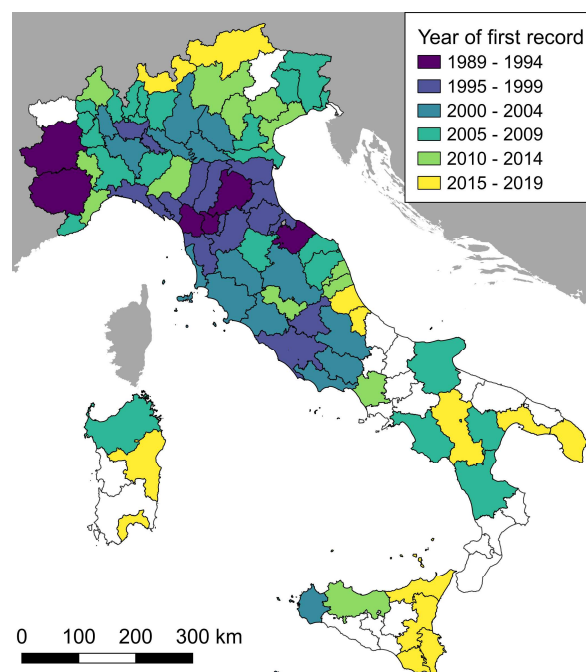


Figure 1. Distribution map of *Procambarus clarkii* at the provincial level. The colors represent the different periods of first observation of the species. Grey represent territories outside the Italian boundaries.

DISCUSSION

The range of *Procambarus clarkii* underwent an impressive expansion in Italy since its first introduction thirty years ago (Figure 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 (Figure 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 (Figure 2).

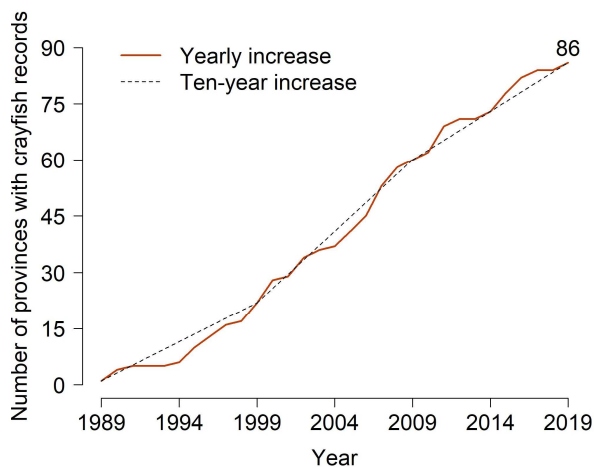


Figure 2. Cumulative number of invaded Italian provinces since the first observation of *Procambarus clarkii* in the wild.

We observed sharp differences between northern/central regions, where almost all provinces are invaded, and southern/insular regions, where fewer records are available (Figure 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 and 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 (Figure 3).

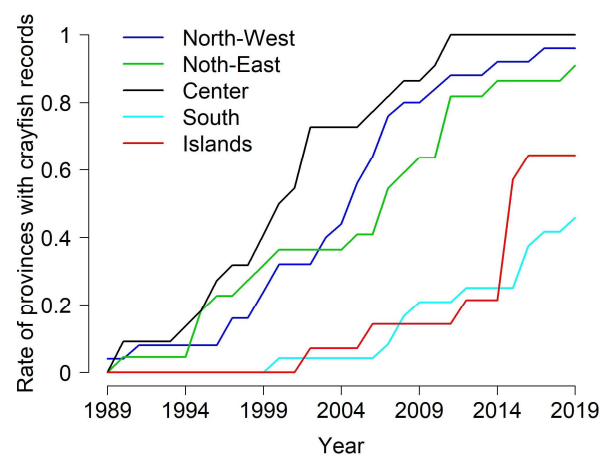


Figure 3. Proportion of provinces invaded by *Procambarus clarkii* in five areas of Italy. Regions included in each area: North-West = Aosta Valley, Liguria, Lombardy, Piedmont; North-East = Emilia-Romagna, Friuli-Venezia Giulia, Trentino-Alto Adige, Veneto; Center = Lazio, Marche, Tuscany, Umbria; South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

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).

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 and Gherardi 2010, Aquiloni et al. 2010, Cecchinelli et al. 2012).

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CHAPTER 3: All that changes is not shift: methodological choices influence niche shift detection in freshwater invasive species

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All that changes is not shift: methodological choices influence niche shift detection in freshwater invasive species

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Species distribution models are often used to predict the potential distributions of invasive species outside their native ranges and rely on the assumption of realized niche conservatism. Analyses observed that freshwater invasive species often show high degrees of niche expansion, suggesting limited reliability of species distribution models. However, observed niche shifts can arise because of both actual niche shifts, determined by biological factors, and apparent shifts, due to methodological issues. We compared metrics of niche dynamics calculated using different sets of variables to identify factors that could influence the rate of niche shifts. We collected presence-only data for 40 freshwater invasive animal species, then measured niche shift dynamics using 14 different combinations of environmental variables. Shifts were assessed measuring niche overlap, expansion and unfilling, and testing for niche conservatism. We then built generalized linear mixed models relating niche shifts to methodological choices and biological features. Our results showed that methodological choices strongly affected all the considered niche dynamics metrics, while the effects of biological features were less prominent. Moreover, different niche dynamic measures sometimes provided contradictory assessments of niche conservatism. Niche analyses are powerful tools to predict areas at risk of invasion, but inappropriate methodological choices can lead to apparent niche shifts, questioning niche model reliability and biological interpretation. The high rate of niche expansion observed in freshwater invasive species highlights the importance of delineating objective criteria to determine the set of variables to be used in niche dynamic assessments.

Keywords: biological invasions, ecospat, invasive alien species, niche comparisons, niche conservatism, niche dynamics



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Introduction

Freshwater environments are among the habitats most affected by invasive alien species (Strayer 2010). Freshwater invasive species (FIS) threaten local communities through multiple mechanisms, such as predation, hybridization, disease spread,

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food-web alterations and competition (Huxel 1999, Mooney and Cleland 2001, Ficetola et al. 2012, Falaschi et al. 2020). During the last decades, international trade has accelerated the rate of introductions of alien species (Westphal et al. 2008, Hulme 2009, Seebens et al. 2017). Additionally, global warming may facilitate the establishment of freshwater species outside their native ranges (Rahel and Olden 2008, Jourdan et al. 2018). Predicting potential FIS distributions is thus pivotal to prevent and/or limit their spread, since early detection and prompt control response represent the most effective strategy to eradicate an invasive species (Torres et al. 2018, Falaschi et al. 2020). A common strategy to predict the potential distribution of an invasive species is the use of occurrence data and selected environmental variables to approximate the species niche (Soberón 2007). These approaches, known as species distribution models (SDMs), are widely used; nevertheless, they present some limitations. Occurrences are only possible in environments where the local abiotic and biotic conditions are suitable for the species, and that are within the geographical area accessible to the species (Soberón and Peterson 2005, Barve et al. 2011). This means that niche assessments derived from occurrences can only give us information about the realized niche, i.e. the intersection between accessible, suitable abiotic and biotic conditions. The realized niche represents only a subset of the ensemble of the suitable abiotic conditions, namely the fundamental niche. Moreover, estimations of potential distribution performed using these models rely on several assumptions, such as niche conservatism between the native and the exotic range (Early and Sax 2014, Hill et al. 2017). The violation of the niche conservatism assumption may limit the ability of SDMs to accurately predict the potential distribution of non-native species (Early and Sax 2014, Pili et al. 2020, Atwater and Barney 2021). Assessing the pervasiveness of niche conservatism is thus a prerequisite for the broad application of SDMs in invasion biology. Previous assessments suggested that most invasive species conserve their niche (Petitpierre et al. 2012, Liu et al. 2020); nonetheless, some studies have reported relevant rates of niche shifts in some groups of invasive species (Lauzeral et al. 2011, Early and Sax 2014). FIS, for instance, show particularly strong niche shifts between native and exotic ranges (Torres et al. 2018, Liu et al. 2020).

Several processes can lead to the observation of niche divergence between native and invasive populations. First, adaptive evolution and different biotic interactions can allow invasive populations to occupy an environment that is not exploited in the native range (niche expansion; Guisan et al. 2014). Second, dispersal limitations, biotic interactions, and/or a recent introduction history can result in the exploitation of an environment in the native range but not in the exotic range (niche unfilling; Guisan et al. 2014). Furthermore, methodological issues related to the approaches used to compare niches among species/populations can lead to apparent niche shifts, even in the absence of true biological effects (apparent divergence; Strubbe and

Matthysen 2014, Torres et al. 2018, Atwater and Barney 2021; Fig. 1a). Niche unfilling does not represent a violation of the niche conservatism assumption and is usually expected to decline as the invasion continues, thus niche shifts are often described by quantifying the degree of niche expansion. Comparisons of the realized niches of native versus introduced populations of a species cannot prove whether an observed niche expansion is the result of changes in the fundamental niche or just in the realized niche. Moreover, the quantification of niche dynamics is only possible between analogous environments, i.e. environments shared by the native and the exotic geographical ranges. Indeed, when a species colonizes new environments unavailable in its native range, it is extremely challenging to determine whether this is caused by adaptive evolution or by other mechanisms (Guisan et al. 2014).

Several mechanisms, related to methodological choices, can bias niche comparisons, particularly for freshwater species, including:

- 1) Using biologically non-relevant variables. Using uninformative variables may increase the rate of apparent niche shifts (Rödger et al. 2009, Strubbe and Matthysen 2014, Torres et al. 2018). Niche comparisons are often based on broad-scale layers, such as WorldClim (Fick and Hijmans 2017) and CHELSA (Karger et al. 2020), which represent macroscale 'bioclimatic' conditions such as average annual temperature or annual precipitation. However, freshwater species live in specific microhabitats that are not necessarily affected by local conditions (e.g. the river regime can be affected by precipitation and snowmelt occurring hundreds of kilometres upstream), and in which temperature can strongly differ from nearby terrestrial environments. In turn, the use of bioclimatic layers that do not match the microhabitat experienced by animals can affect niche estimates (Ficetola et al. 2018, 2020). In addition to climate, other factors such as water chemistry shape the distribution of freshwater species (Jeschke and Strayer 2008). Nevertheless, there is evidence that bioclimatic variables are indeed appropriate to model aquatic species distributions (Frederico et al. 2014, McGarvey et al. 2018). Testing the effects of water chemistry variables on FIS niche shifts at a global level is challenging due to the lack of high-resolution global datasets (Loo et al. 2007, Torres et al. 2018).
- 2) Overparameterization. Including too many predictors can lead to inaccurate definitions of the relationships between species and environmental features, resulting in apparent niche shifts (Peterson 2011, Low et al. 2020). On the other hand, considering only a few variables might overestimate niche conservatism (Peterson 2011).
- 3) Selecting inappropriate backgrounds. Niche analyses usually select a geographical background to represent the environmental conditions accessible to the focal species. Background selection can deeply influence SDMs and niche quantifications and should be limited to areas accessible by the species (Godsoe 2010, Barve et al. 2011,

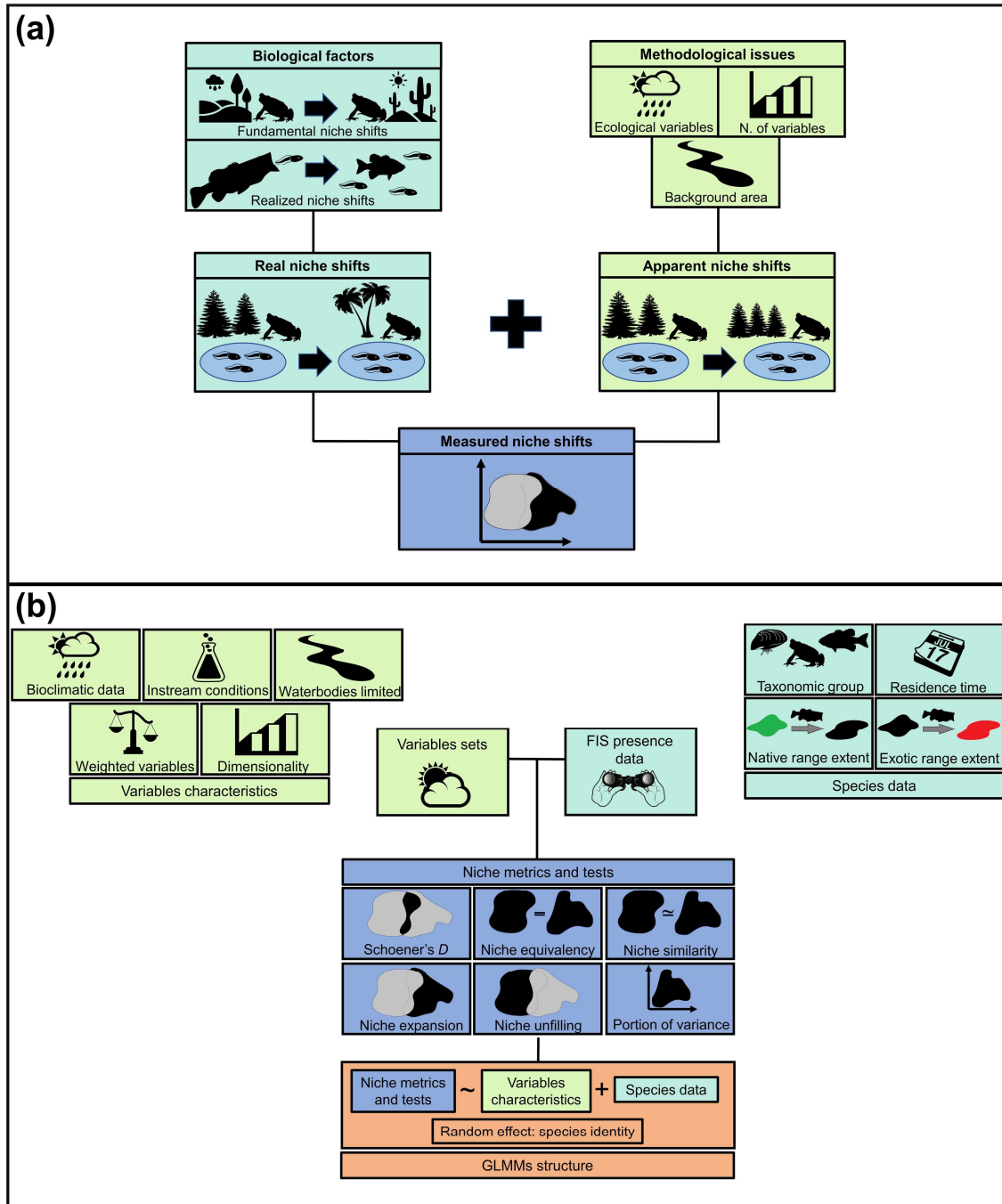


Figure 1. (a) Niche shifts detected by niche dynamics analyses can be the result of real niche shifts, caused by changes in the fundamental or in the realized niche, and/or apparent niche shifts, caused by methodological issues. (b) We used presence data for 40 freshwater invasive species (FIS) and 14 different sets of ecological variables to calculate six measures used in niche dynamic assessments. After that, each niche measure was used as the dependent variables in a generalized linear mixed model (GLMM), while five characteristics of the ecological variables and four commonly tested biological features of the species were used as independent variables. Drawings by: Natasha Sinagina (CC-SA 4.0 license; <https://creazilla.com/>), Creazilla, hamdolii, UnboxScience (Public Domain) and JoyPixel (CC-SA 4.0 license; www.joypixels.com/).

Acevedo et al. 2012, Hill et al. 2017). For freshwater organisms, limiting the background area to freshwater environments could improve the transferability of SDMs and reduce apparent niche shifts between native and invasive populations (Nori and Rojas-Soto 2019).

Studies assessing niche dynamics in invasive species often use bioclimatic variables, do not subset the available variables and only rarely try to limit the background areas (Peterson 2011, Qiao et al. 2017, Nori and Rojas-Soto 2019, Liu et al. 2020); thus we hypothesize that a relevant portion

of observed niche shifts found in FIS is due to the inclusion of non-relevant variables, overparameterization and inappropriate background selection. These choices may affect niche analyses, possibly leading to strong apparent niche shifts in FIS, which in turn would affect the reliability of SDMs outside native ranges. The aim of our study was assessing the factors that determine observed niche shifts in FIS, testing whether and how a priori selection of different variables influences measures of niche dynamics.

Material and methods

Species records and ranges

We downloaded occurrence records for 40 FIS from the iNaturalist data portal (www.inaturalist.org/observations/export), filtering for 'Research Grade' data to reduce misidentification risk. We selected species with a large number of observations (range: 95–45 351; median: 1108.5; and with > 20 records for both the native and the invasive range) and included freshwater molluscs, crustaceans, fishes and tetrapod species (amphibians and reptiles; Supporting information) with occurrence records from the period 1962 to 2021. When available, we downloaded native ranges from the IUCN Red List website (IUCN 2022).

We used the Freshwater Ecoregions of the World (Abell et al. 2008) to select background areas for each species in both its native and introduced ranges. This allows an appropriate selection of background areas since they were delineated by considering physical and ecological dispersal limitations of freshwater species (particularly freshwater fish; Abell et al. 2008). For each species, native backgrounds were determined as those ecoregions that contained at least one record of the focal species and intersected the polygon of the IUCN native range. For those species whose distributions were not present on the IUCN website, other sources were used to determine native ranges (Supporting information). Subsequently, we determined the exotic background of each FIS as those ecoregions that contained at least one non-native record of the focal species in areas with bibliographical evidence of established populations. This criterion was used to avoid the inclusion of captive or semi-captive individuals and non-viable populations, because feral individuals of some FIS, e.g. turtle species, can survive for several years in sub-optimal habitats without establishing reproductive populations (Ficetola et al. 2008).

Environmental variables

To test the hypothesis that niche shifts detected between native and non-native ranges can derive from the variables used for analyses, we evaluated several datasets of environmental variables that could be used to model FIS niches. We aimed to understand whether and how niche metrics are affected by 1) using general (bioclimatic) versus more specific proxy variables, 2) selecting different numbers of variables and

3) limiting the background areas to waterbodies. To achieve this goal, we downloaded different raster datasets from two sources: WorldClim (Fick and Hijmans 2017) and EarthEnv (www.earthenv.org; Domisch et al. 2015). WorldClim provides global data for 19 different macroclimatic ('bioclimatic') variables at different resolutions, which well describe the features of terrestrial environments (Fick and Hijmans 2017). EarthEnv provides different near-global, standardized, 1-km resolution layers that can be used for niche modelling and other scientific purposes, and includes several river-specific variables (Domisch et al. 2015). We downloaded data from the near-global freshwater environmental variables dataset provided by EarthEnv through the 'sdmpredictors' R package (Bosch 2020). EarthEnv includes 19 bioclimatic variables derived from WorldClim; data on river drainage networks derived from HydroSHEDS (Lehner et al. 2008); and proxies for water chemistry derived from the soil properties maps of SoilGrids1km (Hengl et al. 2014). Both bioclimatic and water chemistry variables are provided as the average or sum of the upstream values and as inverse-distance weighted average or the inverse-distance weighted sum of the upstream values. The latter approximations aim to better represent local water conditions, although validation showed this it is not always the case (Domisch et al. 2015). The extent of all EarthEnv layers is 60° N to 5° S latitude, and 145° W to 180° E longitude.

We built 14 combinations of variables at the finest resolution available (0.008°; Table 1), because fine-scale niche shifts might not be detected when using coarse-resolution data (Petitpierre et al. 2012, Li et al. 2014; Supporting information). WorldClim rasters were cropped to the extent of EarthEnv ones.

Data preparation and analyses

For each species, we kept one presence point per grid cell. Presence points located more than 1 km away from waterbodies were discarded; points located < 1 km away from waterbodies were moved to the nearest cell inside a waterbody. This was done to avoid loss of information due to limited accuracy of coordinates or raster maps, or changes in stream and river shapes, and to include occurrences of semi-aquatic animals moving nearby main waterbodies. Presence points falling on cells associated with no value in at least one of the raster maps used were discarded.

Following Hill et al. (2017), we selected 10 000 random cells as the background for each species in both the native and exotic range. For WorldClim variables, background points were selected across the entire background areas, whereas for the EarthEnv variables the background points were selected within waterbodies. Subsequently, we extracted the variable values for both presence and background points. Each resulting dataset was used to describe the native and exotic niches using the centroid shift, overlap, unfilling, expansion (COUE) framework, an ordination approach to quantify niche changes (Guisan et al. 2014). Following Broennimann et al. (2012), we performed a principal component analysis (PCA

Table 1. The 14 combinations of environmental variables used to assess niche metrics and quantify niche shifts. Each combination was used to calculate niche metrics for the 40 freshwater invasive species. The different variables were obtained from WorldClim (Fick and Hijmans 2017) and EarthEnv (Domisch et al. 2015).

Variables set	Bioclimatic variables	Instream conditions proxies	Distance-weighted variables	Limited backgrounds	Number of variables
bioclim6	Yes	No	No	No	6
bioclim19	Yes	No	No	No	19
climriv6	Yes	No	No	Yes	6
climriv19	Yes	No	No	Yes	19
climrivw6	Yes	No	Yes	Yes	6
climrivw19	Yes	No	Yes	Yes	19
riv5	No	Yes	No	Yes	5
riv13	No	Yes	No	Yes	13
rivw5	No	Yes	Yes	Yes	5
rivw13	No	Yes	Yes	Yes	13
rivall11	Yes	Yes	No	Yes	11
rivall32	Yes	Yes	No	Yes	32
rivallw11	Yes	Yes	Yes	Yes	11
rivallw32	Yes	Yes	Yes	Yes	32

on the environmental space of the native and exotic ranges. Occurrence densities of the species in the native and invasive ranges were then calculated with kernel smoothing methods to limit sampling biases and projected onto the gridded PCA environmental space. We used the 75th percentile of environmental densities as a threshold to eliminate rare environments from our analyses (Petitpierre et al. 2012, Guisan et al. 2014, Li et al. 2014, Liu et al. 2017). Niches were then defined by using 95% of the species occurrences to avoid the inclusion of the most marginal populations.

Subsequently, we calculated the adjusted Schoener's D, a measure of niche overlap (Broennimann et al. 2012). Low Schoener's D values are a first indication of limited niche overlap; nonetheless, large Schoener's D values do not necessarily imply the absence of niche shifts (Guisan et al. 2014). After that, we performed niche equivalency and similarity tests. Niche equivalency tests consist in pooling all occurrences and splitting them into two datasets, calculating Schoener's D and repeating the process N-times; niche similarity tests were performed by randomly shifting the entire observed density of occurrences in the exotic range and calculating the overlap of the simulated niche with the observed niche in the native range (Broennimann et al. 2012). Both tests were run with 1000 random replicates using the 'ecospat' (ver. 3.2) R package (Di Cola et al. 2017). When testing for niche conservatism, a significant ($p < 0.05$) equivalency test implies that overlap between native and exotic niches is higher than expected under randomness, while a significant similarity test implies that the focal species occupies environments in the native and exotic range that are more similar than would be expected by chance. Finally, we calculated niche expansion, i.e. the proportion of the exotic niche non-overlapping with the native niche, and niche unfilling, i.e. the proportion of conditions inside the native niche but outside the exotic niche (Guisan et al. 2014). Niche expansion is often considered to be the only measure that truly describes shifts in the realized niche (Petitpierre et al. 2012, Strubbe et al. 2013, Li et al. 2014, Liu et al. 2020).

We used generalized linear mixed models (GLMMs) to test the effects of variable selection, overparameterization and background area limitation on the measures of niche dynamics (Fig. 1b). Moreover, we also considered the effects of 'biological' features of invasive species commonly used in similar studies, to evaluate whether the effects of these predictors are stronger or weaker than the ones related to methodological choices. Previous analyses found relationships between niche conservatism and the extent of invasive species ranges (Early and Sax 2014, Li et al. 2014, Hill et al. 2017, Liu et al. 2017), thus we included the log-transformed number of raster cells occupied in the native range and in the exotic range as independent variables. The year of first introduction of each FIS was obtained from Seebens et al. (2017) and used to calculate residence time in the exotic ranges, another frequently considered factor in niche dynamic studies (Cardador and Blackburn 2020, Liu et al. 2020). Finally, we included the taxonomic group (invertebrate, fish or tetrapod) as a fixed factor. We ran six separate GLMMs with different independent variables: Schoener's D, the significance of statistical comparisons between native and invasive niches (equivalency and similarity tests; non-significant versus significant), the relevance of niche expansion and niche unfilling, and the portion of variance explained by the first two principal components (PCs). Niche expansion and unfilling are usually considered to be relevant when > 0.10 (Hill et al. 2017, Torres et al. 2018), thus we used this value as a threshold and treated these two metrics as binomial variables. Results of equivalency and similarity tests were also treated as binomial variables (significant versus non-significant tests), while the proportion of variance explained by the first two PCs and Schoener's D values were modelled using beta regressions (Ferrari and Cribari-Neto 2004). We expected the proportion of variance explained by the PCs to be directly linked to collinearity between the selected variables, thus we modelled it using the methodological approaches as the only independent variables. Each GLMM included: a niche metric as dependent variables; five variables representing the typology

of environmental characteristics and four variables representing species features as independent variables (Fig. 1b), and species identity as a random effect. GLMMs were run using the 'lme4' (Bates et al. 2015) and the 'GLMMtmb' packages (Brooks et al. 2017). The significance of independent variables was assessed using likelihood ratio tests.

All the species we selected had at least 20 records for both the native and the invasive range before spatial filtering; however, after spatial filtering, seven species retained fewer than 20 records for either the native or the invasive range (range: 6–19 records). The COUE approach is thought to be robust even with small sample sizes, and niche comparisons can be performed with as few as five occurrences in either range (Liu et al. 2017, Torres et al. 2018). Nevertheless, we repeated GLMMs removing the seven species with fewer than 20 observations to assess the robustness of our conclusions.

Results

Overall, we obtained data from seven molluscs, five crustaceans, 19 fishes and nine tetrapod species (six amphibians and three reptiles; Supporting information). After spatial filtering, the mean number of retained records per species $\pm$ SD was 1413 ± 2337 (range: 61–10 321). The mean number of raster cells occupied in the native range was 853 ± 1969

(15–10 199), while the mean number of occupied cells in the invaded range was 561 ± 798 (6–3494). The average residence time was $153 \text{ years} \pm 89$ (42–525). Residence time was not correlated to invaded range extent (Pearson's $r_{38} = 0.219$, $p = 0.175$). For each species, each niche dynamic measure was calculated multiple times, using different sets of ecological variables. We obtained a total of 560 values for each niche dynamic measure (40 species $\times$ 14 sets of variables). The GLMMs relating niche parameters to the different predictors explained a good amount of variation (R^2 ranging from 0.33 to 0.93); see Supporting information for details. In nearly all cases, the models excluding species with < 20 occurrences in at least one of the two ranges were highly consistent with the ones including all the species (Supporting information).

Variance explained by the first principal components

On average, the first two principal components explained $65.3 \pm 13.2\%$ of variance of environmental variables. The amount of explained variance was strongly affected by methodological choices (Fig. 2), as it increased when we used terrestrial bioclimatic data ($B = 0.482$, $\chi^2_1 = 288.3$, $p < 0.001$), as well as when we limited background to waterbodies ($B = 0.083$, $\chi^2_1 = 7.2$, $p = 0.007$). Furthermore, the first two principal components explained a lower proportion

	 Portion of variance	 Schoener's D	 Equivalency test	 Similarity test	 Niche expansion	 Niche unfilling
 Bioclimatic variables	$B = 0.48$ $\chi^2_1 = 288.3$ $p < 0.001$	$B = -0.05$ $\chi^2_1 = 0.3$ $p = 0.521$	$B = -0.34$ $\chi^2_1 = 1.0$ $p = 0.312$	$B = 0.37$ $\chi^2_1 = 1.3$ $p = 0.245$	$B = -0.85$ $\chi^2_1 = 5.3$ $p = 0.021$	$B = -0.17$ $\chi^2_1 = 0.2$ $p = 0.649$
 Instream conditions	$B = -0.70$ $\chi^2_1 = 507.2$ $p < 0.001$	$B = 0.13$ $\chi^2_1 = 3.3$ $p = 0.070$	$B = -0.05$ $\chi^2_1 = 0.0$ $p = 0.867$	$B = 0.00$ $\chi^2_1 = 0.0$ $p = 0.986$	$B = -0.03$ $\chi^2_1 = 0.0$ $p = 0.926$	$B = -0.63$ $\chi^2_1 = 3.2$ $p = 0.073$
 Weighted variables	$B = -0.07$ $\chi^2_1 = 17.7$ $p < 0.001$	$B = 0.36$ $\chi^2_1 = 38.9$ $p < 0.001$	$B = 0.87$ $\chi^2_1 = 13.8$ $p < 0.001$	$B = 1.06$ $\chi^2_1 = 21.8$ $p < 0.001$	$B = 1.72$ $\chi^2_1 = 43.5$ $p < 0.001$	$B = 0.95$ $\chi^2_1 = 12.6$ $p < 0.001$
 Limited background	$B = 0.08$ $\chi^2_1 = 7.2$ $p = 0.007$	$B = -0.45$ $\chi^2_1 = 24.0$ $p < 0.001$	$B = -1.18$ $\chi^2_1 = 10.6$ $p = 0.001$	$B = -1.29$ $\chi^2_1 = 14.3$ $p < 0.001$	$B = -1.58$ $\chi^2_1 = 15.2$ $p < 0.001$	$B = -1.2$ $\chi^2_1 = 8.2$ $p = 0.004$
 N. of variables	$B = -1.10$ $\chi^2_1 = 498.9$ $p < 0.001$	$B = -0.06$ $\chi^2_1 = 4.2$ $p = 0.041$	$B = -0.08$ $\chi^2_1 = 0.5$ $p = 0.499$	$B = -0.05$ $\chi^2_1 = 0.1$ $p = 0.695$	$B = 0.03$ $\chi^2_1 = 0.1$ $p = 0.803$	$B = 0.41$ $\chi^2_1 = 7.9$ $p = 0.005$
 Native range extent	/	$B = 0.41$ $\chi^2_1 = 25.3$ $p < 0.001$	$B = 0.60$ $\chi^2_1 = 5.6$ $p = 0.018$ *	$B = 0.31$ $\chi^2_1 = 2.2$ $p = 0.139$	$B = -1.37$ $\chi^2_1 = 14.0$ $p < 0.001$	$B = -0.04$ $\chi^2_1 = 0.0$ $p = 0.905$
 Exotic range extent	/	$B = 0.08$ $\chi^2_1 = 1.4$ $p = 0.238$	$B = -0.07$ $\chi^2_1 = 0.1$ $p = 0.757$	$B = 0.49$ $\chi^2_1 = 5.8$ $p = 0.016$	$B = -0.14$ $\chi^2_1 = 0.2$ $p = 0.659$	$B = -2.62$ $\chi^2_1 = 39.6$ $p < 0.001$
 Residence time	/	$B = 0.03$ $\chi^2_1 = 0.3$ $p = 0.566$	$B = 0.58$ $\chi^2_1 = 6.7$ $p = 0.009$ *	$B = 0.05$ $\chi^2_1 = 0.1$ $p = 0.804$	$B = 0.88$ $\chi^2_1 = 8.0$ $p = 0.005$	$B = 0.08$ $\chi^2_1 = 0.1$ $p = 0.789$
 Taxonomic group	/	$\chi^2_2 = 6.6$ $p = 0.036$ *	$\chi^2_2 = 2.7$ $p = 0.255$	$\chi^2_2 = 0.6$ $p = 0.743$	$\chi^2_2 = 5.5$ $p = 0.063$	$\chi^2_2 = 3.5$ $p = 0.173$

Figure 2. Results of generalized linear mixed models (GLMMs) relating niche metrics to methodological choices and biological factors. All niche shift measures were modelled as a function of methodological choices and biological factors; the amount of variance explained by the two principal components was modelled as a function of methodological choices only. Significant positive effects are shaded in red, while significant negative effects are shaded in blue. Asterisks indicate those effects whose significance disappeared when removing the species with small sample size. Drawings by: Natasha Sinagina (CC-SA 4.0 license; <https://creazilla.com/>), Creazilla, hamdoli, UnboxScience, loritheladybug7 (Public Domain) and JoyPixel (CC-SA 4.0 license; www.joypixels.com/).

of variation when we considered a larger number of environmental variables ($B = -0.398$, $\chi^2_1 = 740.5$, $p < 0.001$), when we used variables representing instream conditions ($B = -0.696$, $\chi^2_1 = 507.2$, $p < 0.001$) and when using distance-weighted measures ($B = -0.075$, $\chi^2_1 = 17.7$, $p < 0.001$) (Fig. 2; Supporting information).

Factors affecting niche overlap

The niche overlap between native and non-native ranges (Schoener's D) showed a strong variation across species and sets of variables (average 0.256 ± 0.147 ; range 0.003–0.596). Niche overlap was significantly larger when using distance-weighted variables ($B = 0.364$, $\chi^2_1 = 38.9$, $p < 0.001$), while overlap decreased when using backgrounds limited to waterbodies ($B = -0.446$, $\chi^2_1 = 24.0$, $p < 0.001$) and when we increased parameterization ($B = -0.063$, $\chi^2_1 = 4.2$, $p = 0.041$). Species with a greater native range showed larger overlap between native and invasive range ($B = 0.410$, $\chi^2_1 = 25.3$, $p < 0.001$). We also detected significant differences between

taxonomic groups (likelihood ratio test: $\chi^2_2 = 6.6$, $p = 0.036$), with tetrapod species showing significantly lower D values than invertebrates (Tukey test: $B = -0.484$, $p = 0.021$) (Fig. 2; Supporting information). Finally, using water conditions proxies slightly increased niche overlap, but this effect was significant at the 0.05% level only after removing species with small sample sizes ($B = 0.184$, $\chi^2_1 = 5.4$, $p = 0.020$).

Factors affecting niche equivalency tests

We obtained 158 out of 560 significant niche equivalency tests. The niche equivalency hypothesis was accepted more frequently when using distance-weighted values ($B = 0.872$, $\chi^2_1 = 13.8$, $p < 0.001$), while it was more frequently rejected when using waterbody-limited backgrounds ($B = -1.177$, $\chi^2_1 = 10.6$, $p = 0.001$; Fig. 2). Large native ranges ($B = 0.604$, $\chi^2_1 = 5.6$, $p = 0.018$) and long residence times ($B = 0.581$, $\chi^2_1 = 6.7$, $p = 0.009$) were associated with higher levels of niche equivalency, but the significances of both these effects disappeared when removing species with few occurrences (Fig. 2; Supporting information).

Factors affecting niche similarity tests

We obtained 176 out of 560 significant niche similarity tests. Similarity rates were higher when using distance-weighted variables ($B = 1.055$, $\chi^2_1 = 21.8$, $p < 0.001$) and for species with wide exotic ranges ($B = 0.489$, $\chi^2_1 = 5.8$, $p = 0.016$). Conversely, similarity tests were less frequently significant when using waterbody-limited variables ($B = -1.294$, $\chi^2_1 = 14.3$, $p < 0.001$) (Fig. 2; Supporting information).

Factors affecting niche expansion

The selected FIS showed relevant (> 0.1) niche expansions in 331 out of 560 comparisons, with a mean value of 0.251 ± 0.244 . Relevant niche expansions were less frequently found

when using terrestrial bioclimatic variables ($B = -0.851$, $\chi^2_1 = 5.3$, $p = 0.021$), and when limiting backgrounds to waterbodies ($B = -1.581$, $\chi^2_1 = 15.2$, $p < 0.001$). Conversely, using distance-weighted variables increased niche expansion ($B = 1.725$, $\chi^2_1 = 43.5$, $p < 0.001$). Residence time had a significant positive effect on this measure ($B = 0.883$, $\chi^2_1 = 8.0$, $p = 0.005$), while the number of cells occupied in the native range had a negative effect on it ($B = -1.374$, $\chi^2_1 = 14.0$, $p < 0.001$) (Fig. 2; Supporting information).

Factors affecting niche unfilling

Niche unfilling was relevant (> 0.1) in 277 out of 560 comparisons, with a mean value of 0.198 ± 0.232 . Unfilling was less frequent when selecting waterbody-limited backgrounds ($B = -1.209$, $\chi^2_1 = 8.2$, $p = 0.004$), and for species with broad exotic ranges ($B = -2.652$, $\chi^2_1 = 39.6$, $p < 0.001$). Conversely, unfilling was inflated by using distance-weighted variables ($B = 0.947$, $\chi^2_1 = 12.6$, $p < 0.001$) and by over-parameterization ($B = 0.407$, $\chi^2_1 = 7.9$, $p = 0.005$) (Fig. 2; Supporting information).

Discussion

The majority of the 560 niche comparisons showed significant niche shifts between native and invasive range, with frequent niche expansion and limited values of overlap. Nevertheless, detections of niche shifts strongly depended on methodological choices, including background selection, the use of distance-weighted variables or the use of a large number of variables. This suggests that a priori choices of environmental predictors have strong impacts on the detection of niche shifts and some choices might inflate the probability of observing apparent shifts. The effects of these methodological choices seem to be stronger than potential biological factors, such as residence time or the extent of native range.

Effects of methodological choices

Methodological choices appear to be extremely relevant in influencing niche metrics (Fig. 2). These findings pose a challenge to the interpretation of meta-analyses on niche comparisons since niche conservatism and shifts may be linked to differences across studies in modelling techniques, instead of actual biological processes. This underlines once more the urgent need for objective criteria in selecting the most appropriate variables for niche comparisons, particularly for those species, such as FIS, whose realized niches are likely to be influenced by complex interactions between climatic, chemical and dispersal factors.

Traditionally, niche modelling for FIS and other invasive species was based on easily accessible bioclimatic variables, such as air temperature or annual precipitation (Torres et al. 2018, Mori et al. 2021). Bioclimatic variables are often highly collinear, thus in most works principal components explain a great proportion of the variance of these variables. Bioclimatic

data are particularly suitable for ordination approaches such as the COUE scheme, still they do not necessarily describe the environmental features truly affecting organism occurrences. Therefore, it is pivotal to select variables that better represent the environment experienced by organisms, such as instream conditions, soil temperature or other micro-habitat features (Bramer et al. 2018, Lembrechts et al. 2021). Unfortunately, an accurate representation of microhabitat features over broad spatial scales can be extremely challenging because of the limitations of remote sensing when trying to derive extremely fine-grained information (Lembrechts et al. 2021). Datasets aiming to represent instream conditions at a global level are broad-scale estimates, mostly based on soil chemistry and geological features of nearby environments, and hence in some cases they may not accurately represent the actual conditions experienced by organisms. Moreover, several water chemistry parameters relevant for FIS distribution (e.g. calcium concentrations for molluscs) are not always available at high resolution at the global scale (Jeschke and Strayer 2008).

Distance-weighted variables led to contrasting results. While their use increased niche overlap, equivalency and similarity between native and invaded ranges, it also increased niche expansion and unfilling. Validation exercises suggested that distance-weighted variables may represent some instream conditions less accurately than the unweighted ones (Domisch et al. 2015), possibly inflating niche expansion and unfilling. On the other hand, weighted variables might be particularly appropriate for parameters such as precipitation, for which downstream conditions are more tightly linked to drainage-based processes.

Limiting the background to the accessible area should improve the representation of the movement constraints of the species (Godsoe 2010, Barve et al. 2011, Nori and Rojas-Soto 2019). This may be particularly important for modelling FIS, which often are strongly affected by dispersal barriers (Jeschke and Strayer 2008). In fact, the use of a limited background resulted in low rates of niche expansion and unfilling compared to the ‘traditional’ background. This suggests that this approach can minimize the detection of apparent differences between native and exotic niches, consistently with results of previous studies on invasive turtles (Nori and Rojas-Soto 2019). Nevertheless, the constrained background also reduced niche overlap and the significance of equivalency and similarity tests between native and invasive ranges compared to ‘traditional’ background (Nori and Rojas-Soto 2019). This probably occurred because limiting the background to waterbodies also drastically reduces the number of points available to estimate background abiotic conditions, decreasing the statistical power of tests (Nori and Rojas-Soto 2019). This highlights the risk of obtaining contrasting results with different niche metrics when background areas are limited to different extents.

The number of ecological variables has long been recognized as a major methodological issue of distribution modelling. Many approaches are available to reduce dimensionality, such as excluding those variables that are supposed to be not

relevant for the target species, that show strong correlations with other variables or that are secondly derived from other variables (Peterson 2011, Low et al. 2020). Increasing dimensionality is expected to decrease the amount of variation explained by the first two PCs, hence limiting the effectiveness of ordination analyses. Moreover, using too many ecologically variables can lead to apparent niche shifts (Strubbe and Matthysen 2014, Torres et al. 2018). Our results remark the importance of avoiding over-parameterization, and the need for objective methods to define an appropriate number of ecological variables used to define species niche, particularly for FIS.

Comparison between methodological and biological factors

Previous work has reported that several biological features can significantly affect niche shift measures. A broad native range has been found to increase niche overlap and reduce niche expansion (Early and Sax 2014, Li et al. 2014, Liu et al. 2017, 2020), while several studies observed a negative relationship between the extent of invaded ranges and niche unfilling, and a positive effect of residence time on niche expansion (Li et al. 2014, Liu et al. 2020). These patterns have been explained by several biological processes. A positive relationship between residence time and climatic niche shifts has been taken as a confirmation that these shifts can be related to a release from dispersal limitations within the invasive range (Li et al. 2014); nevertheless, the interpretation of the role of residence time is subjected to several pitfalls, as similar studies with small methodological differences showed contrasting results (Liu et al. 2017, 2020). In fact, in our analysis the effect of residence time on niche equivalency found by our models disappeared when removing species with small sample size, suggesting that it might be an artifact.

Freshwater invasive species with broad native ranges often are generalist species that already exploit a wide range of environments, meaning that their exotic populations are more likely to be introduced in environments with similar conditions just by chance, and are unlikely to further expand their niche. Similarly, species that have been able to exploit broad invasive areas are expected to reduce their unfilling compared to the native range. Our results were consistent with these expectations, regardless of the methodological approach, confirming the potential importance of these processes (Fig. 2). Nevertheless, in most cases biological factors explained less deviance in niche metrics, compared to methodological choices (Supporting information). As methodological choices can outweigh biological effects, the risk of apparent niche shifts should be carefully considered when planning the analysis, and researchers should pay special attention to testing the robustness of their conclusions. Direct measures of actual niche shifts, such as experimental approaches that approximate the fundamental niche and/or genomic analyses, would be extremely helpful to verify whether observed shifts represent actual evolutionary processes (Kearney and Porter 2009, Sherpa et al. 2022).

Additional factors influencing observed shifts

Regardless of the robustness of modelling designs, niche modelling has some inherent weaknesses that are difficult to overcome. Global analyses are based on large databases of species distribution. These databases do not necessarily represent the actual distribution of the species, owing to geographical biases in data collection, uneven search efforts and reporting biases (van Strien et al. 2013, Uyeda et al. 2020). Approaches like the COUE scheme are thought to limit the effects of sampling bias by calculating smooth densities of species occurrences in a gridded environmental space (Broennimann et al. 2012, Guisan et al. 2014), but there are other biases that can undermine the reliability of models.

Occurrences of dispersing individuals or non-viable populations, such as continuously restocked fish populations that occupy sub-optimal environments, should be excluded from analyses to avoid niche overestimation. To limit this issue, we only selected observations from countries where the considered FIS are known to be established, and excluded marginal observations from niche analyses. However, niche overestimation caused by non-breeding populations might have occurred in large countries and for species with wide distributions. Finally, even the best global datasets of ecological variables currently available have some limitations, and they can provide an imperfect representation of conditions experienced by organisms (Domisch et al. 2015). The development of high-quality datasets that well represent micro-habitat conditions is one of the major issues for modelling studies, and recent broad-scale efforts promise improvements that will be extremely useful for future studies (Potter et al. 2013, Bennie et al. 2014, Lembrechts et al. 2021, Marta et al. 2022). The increased resolution of recently available water-body maps (Allen and Pavelsky 2018) might soon allow the realistic accounting for dispersal limitation while retaining high statistical power.

Conclusion

An appropriate selection of environmental variables is pivotal when performing niche analyses and can have major consequences on the detection of niche shifts. The differences in niche comparisons linked to different methodological choices can be extremely relevant for niche conservatism assessments. Identifying the best approach to quantify niche dynamics can greatly improve our ability to predict FIS distribution, improving the effectiveness of prevention and/or containment measures, and enhancing predictions under climate change scenarios.

Apparent niche shifts can be reduced by using appropriate ecological variables and avoiding overparameterization. Therefore, a better understanding of the drivers of FIS distribution, the availability of global high-resolution maps of these drivers and objective methods for selecting the appropriate number of variables are essential prerequisites for reliable niche modelling. Moreover, drawing biological inference

from niche modelling without accounting for the weaknesses of this approach can lead to misleading conclusions. Experimental approaches, and physiological and genomic data, when available, can greatly help to refine niche modelling and SDMs. Meeting these challenges will also help conservationists to face the ongoing threats in freshwater environments in a more appropriate and effective way.

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Author contributions

Elia Lo Parrino: Conceptualization (lead); Formal analysis (lead); Methodology (lead); Validation (equal); Visualization (equal); Writing – original draft (lead). **Mattia Falaschi:** Conceptualization (equal); Formal analysis (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing – original draft (equal). **Raoul Manenti:** Methodology (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing – original draft (equal). **Gentile Francesco Ficetola:** Conceptualization (equal); Formal analysis (equal); Methodology (equal); Supervision (lead); Visualization (equal); Writing – original draft (equal).

Transparent peer review

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Data availability statement

The presence-only data, the areas used as backgrounds for the native and the invasive ranges of the considered species, as well as data and scripts used for niche comparisons and GLMMs are available at: https://figshare.com/articles/online_resource/Datasets_and_script_for_niche_comparisons_and_GLMMs/19388282 (Lo Parrino et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

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CHAPTER 4: Integrating adult occurrence and reproduction data to identify conservation measures for amphibians

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Abstract

Monitoring programs are pivotal to establishing sound management. Due to economical, logistic, and time limitations, monitoring programs often gather naïve occupancy or abundance data but overlook differences among life-history stages. However, species occurrence does not necessarily mean successful reproduction and population viability, and it is unclear to what extent monitoring programs that do not consider separately adult presence and reproduction provide effective management indications. Unfortunately, collecting data on certain life stages requires high sampling effort, leading to a trade-off between model reliability and resources needed for monitoring. Assessing differences between adult-only and more complete monitoring programs is fundamental to identify the strategy that best informs on the ecological processes. Here, we collected data on presence and reproduction of amphibians in 207 waterbodies in Italy in 2017–2022. We then used multistate occupancy models to test whether environmental features affect differently adult occurrence and breeding probabilities of multiple amphibian species. We capitalized on the opportunity to assess optimal sampling efforts by modeling the detection probabilities of adults and reproduction across multiple species. Lastly, we identified the optimal monitoring strategy under different scenarios of resource availability, comparing adult-only monitoring vs. jointly assessing the occurrence of adults and reproduction. In many cases, the main drivers of adult occurrence and reproduction did not coincide, as most investigated ecological variables affected one life-stage or the other. We provide quantitative estimates of the sampling effort needed to reliably appraise the occurrence of adults/reproduction and their drivers. When scarce resources are available, monitoring adults was the optimal strategy for species with low detection probability of eggs and larval life stages. Conversely, with more resources, monitoring both adults and reproduction emerged as the best strategy for all the considered species. Integrated monitoring of adults and reproduction is essential to comprehensively identify effective conservation measures for amphibians.

Introduction

Biodiversity is currently facing a global crisis, with extinction rates 1,000 times higher than background extinction rates displayed by the fossil record (De Vos et al. 2015). Among vertebrates, amphibians are the most endangered group, because of the combined impacts of climate change, biological invasions, habitat loss, spread of diseases, and other threats (Johnson et al. 2011; Ficetola & Maiorano 2016; Falaschi et al. 2020; Scheele et al. 2020; Cordier et al. 2021). In this scenario, identifying amphibian responses to ecological stressors is essential to plan conservation actions. However, measuring the response of amphibians to multiple co-existing factors, and identifying which ones actually impact populations and require management, is not a straightforward task. Long-term monitoring programs are the primary source of data to assess population trends and to implement and validate models evaluating responses to ecological variables (Ficetola et al. 2010, 2018; Pomezanski 2021). Monitoring programs often collect data about species detection/non-detection and/or abundance. While abundance data can be more informative about certain mechanisms, detection/non-detection data are easier and faster to collect and can be retrieved with less invasive methods (Howell et al. 2020; Falaschi et al. 2022b).

Occupancy models can be used to determine how ecological features are correlated to species occupancy (Bailey et al. 2014; Banner et al. 2019). However, the mere presence in a given area does not necessarily imply that said area represents a suitable environment for the target species (Bateman et al., 2012; Brambilla & Ficetola, 2012; Ficetola et al., 2008). In non-breeding environments, conditions can be enough for the survival of adults even though reproduction does not occur, while successful reproduction can require higher suitability levels (Ficetola et al. 2008; Kearney & Porter 2009; Brambilla & Ficetola 2012; Unglaub et al. 2021; Lee-Yaw et al. 2022). Furthermore, many species live in spatially-structured populations, where sink sites with poor breeding success and/or

low adult survival can be occupied thanks to immigration from nearby source areas (Gill 1978; Marsh & Trenham 2001; Manenti et al. 2020; Cruickshank et al. 2021). These processes imply that reproduction can be limited by environmental conditions more than the mere occurrence of adults (Bateman et al., 2012; Bronson, 2009; Ficetola et al., 2008). This may for instance hinder the reliability of fitness predictions obtained from species distribution models using occurrence data. Species distribution models have been used to infer population parameters, such as density or reproductive success (e.g., Brambilla & Ficetola, 2012; Lunghi et al., 2018), even though the general reliability of these predictions is controversial (Weber et al. 2017; Lee-Yaw et al. 2022). Assessing the appropriateness of using adult occurrence to determine the effect of environmental variables on suitability is particularly relevant for amphibians. Amphibian phenology, abundance, and performance (including breeding performance) can be influenced by the same environmental features in different ways (Ficetola & Maiorano 2016), and adults can even reach high densities in sub-optimal environments where reproduction does not occur (Gould et al. 2019; Cruickshank et al. 2021).

Nevertheless, focusing only on reproduction might also have drawbacks. Reproduction is extremely energy-consuming and can make amphibians more susceptible to predation, parasites and pathogens (Bernal et al., 2006). Consequently, under certain conditions, amphibians may reduce breeding activity or even skip an entire breeding season to maximize survival (Cayuela et al. 2016), with populations of long-living species being probably less affected by such events. Moreover, certain areas may actually act as sinks even if they are used as reproductive habitats (e.g., if larval stages do not reach metamorphosis; Hels 2002; Peterman et al. 2018; Cayuela et al. 2022) and, in some cases, vital rates of post-metamorphic life stages can be more directly linked to population persistence than embryo and larval survival (Biek et al. 2002; Govindarajulu et al. 2005).

Jointly considering data on adult occurrence and reproduction may enable a better assessment of the relationships between environmental features and actual fitness, and improve our understanding of meta-population dynamics (Marsh & Trenham 2001; Geijzendorffer et al. 2016; Cruickshank et al. 2021). Nonetheless, ascertaining reproduction can be more challenging than just detecting the occurrence of any life history stage (Dodd, 2010), as larval stages and juveniles of amphibians are frequently more difficult to detect than adults (Wassens et al. 2016; Bodinof Jachowski et al. 2020). Hence, monitoring programs often focus on the collection of detection/non-detection data, such as acoustic surveys targeting adult males (Pomezanski 2021; Ribeiro, et al. 2022), while information about breeding is usually overlooked (Fig. 1; see results, but also see Van Buskirk 2005; Indermaur et al. 2010). Comparisons of results obtained by focusing on adult occurrence versus reproduction remain scarce, still, there is evidence that considering measures of reproductive success enables a more appropriate assessment of the impacts of environmental stressors (Ficetola et al. 2011; Siesa et al. 2014). It is thus pivotal to ascertain whether adult occurrence and reproduction are differently affected by environmental features, in order to identify the factors that most likely influence fitness and long-term persistence to enact prompt conservation measures (Cruickshank et al. 2021).

While collecting data on both adults and reproduction may be useful (Murdoch et al. 2007) monitoring programs face a trade-off. On the one hand, increasing the sampling effort to collect data on both adults and reproduction can potentially result in better assessments of local populations risks. On the other hand, a higher sampling effort per site decreases the number of sites that can be monitored at a given cost, thus reducing the representativeness and the statistical power of species-environment relationships. Therefore, when resources available for monitoring are scarce, it is essential to identify the most efficient sampling methods, accounting for the trade-off between a

comprehensive assessment of all life stages at fewer sites, or a focus on the most detectable stages over more sites. If adult occurrence and reproduction are similarly affected by the same environmental variables, monitoring programs focusing on the most detectable stages can be cost-effective. Conversely, if they are differently affected by key drivers, collecting data on multiple life stages becomes essential.

The aim of this study was to provide a framework to identify the most effective strategy to detect the impact of environmental stressors on amphibians. We sought to verify if and how adult occurrence and reproduction probabilities respond differently to environmental features, and to assess how the detection probabilities of different life stages (adults vs. eggs and larvae) may vary across surveys, and which is the optimal monitoring strategy under different conditions of resource availability. First, we performed a literature search to assess how frequently data on occurrence vs. reproduction are used in studies analyzing the variation of occupancy in amphibians. Second, we analyzed data on adult occurrence and reproduction of amphibians collected across >200 ponds in 2017–2022, using multistate occupancy models. Adult occurrence and reproduction probabilities were modelled as functions of the same environmental variables, to evaluate whether they show differential responses. Third, we compared the detection probabilities of adult occurrence vs. evidence of reproduction, to give a general idea of the effort needed to obtain robust estimates on the occupancy of distinct life stages. Lastly, we use these results to identify the optimal monitoring strategies for amphibians under different scenarios of resource availability.

Materials and Methods

Literature search

To assess the main approaches used in the most recent studies that applied site occupancy models to

amphibians, we performed a search in Clarivate Analytics' Web of Science database on 27/10/2022 using the terms “(occupancy model* AND (amphibian* OR frog* OR toad* OR newt* OR salamander*))” and keeping only the most recent papers (2020-2022). We then classified the resulting papers according to the type of data collected during monitoring (adult detection/non-detection, reproduction detection/non-detection, all life stages detection/non-detection, eDNA, unspecified) and the modelled probability/probabilities (occupancy, reproduction, both).

Data collection

We collected data on adults and reproduction occurrence for amphibian communities in 207 waterbodies in the Lombardy region, Northern Italy, from 2017 to 2022 (Appendix S2.1). The monitored waterbodies were part of a long-term monitoring program and were selected to encompass the broad range of habitats exploited by local amphibian species, taking into account the availability of historical records that can help identifying the drivers of long-term population dynamics (Melotto et al. 2020; Falaschi et al. 2021, 2022b). During the monitoring, we detected 10 amphibian species: fire salamander *Salamandra salamandra*, Italian crested newt *Triturus carnifex*, smooth newt *Lissotriton vulgaris*, common frog *Rana temporaria*, agile frog *R. dalmatina*, Italian agile frog *R. latastei*, common toad *Bufo bufo*, green toad *Bufo viridis* complex, Italian treefrog *Hyla intermedia*, and green frogs (*Pelophylax lessonae* and the hybridogenetic frog *P. esculentus*; these two taxa were merged for analyses because they are not distinguishable on morphological bases).

Each waterbody was monitored in at least two years between 2017 and 2022 (mean $\pm$ SD = 3.1 ± 0.8 years of survey per waterbody), and within each year the monitored sites were visited 2–7 times (mean = 5.6 ± 0.8 surveys per year). The sampling season lasted from late February to early June to encompass the breeding period of all the study species. The selection of sites sampled each

year followed a rotating panel design. During each visit, air temperature and relative humidity were measured using a thermo-hygrometer. Adults and larval stages of the focal species were monitored through visual surveys, acoustic surveys, and dip-netting. Each amphibian species was recorded as: absent; present without evidence of reproduction (i.e., adults of all species and/or calls of male anurans); present with evidence of reproduction (eggs and/or larvae). Most visits were performed during night-time, when the activity of adults peak, but in each season at least two visits per site were performed under day-light conditions to maximize detection probabilities of eggs and larvae. For more details about monitoring protocols, see Falaschi et al. (2021).

Environmental variables

Once per sampling season, in May, we recorded some key environmental features of the monitored waterbodies, for which a priori information suggest strong impact on amphibian presence and reproduction: surface area (m^2), water permanence (whether the waterbody retained water during the entire sampling season or dried out), aquatic vegetation (% cover of the surface area occupied by emerging vegetation), and shade (% cover of the surface area covered by shadows during the central hours of the day). These environmental features are generally considered to be relevant drivers of amphibian population and community dynamics (Ficetola & De Bernardi 2004; Werner et al. 2007; Falaschi et al. 2021). Moreover, the presence of fish and of the invasive red swamp crayfish (*Procambarus clarkii*), which started invading the study area in 2002 (Lo Parrino et al. 2019; Melotto et al. 2020), were also recorded during each visit through visual inspection. Amphibian larvae are particularly sensitive to predation by the red swamp crayfish, while its effects on amphibian adults seem less severe (Gherardi et al. 2001; Ficetola et al. 2011; Romagnoli et al. 2020; Dalpasso et al. 2022). The per-visit detection probability of the American crayfish is >0.9 , thus the

performed surveys allowed us to reliably ascertain its occupancy (Manenti et al. 2019). Fish occurrence can be a major driver of amphibian distribution and breeding performance (Kloskowski 2011; Préau et al. 2017; Vannini et al. 2018; Reyda et al. 2020; Rechulicz & Płaska 2021). The exhaustive assessment of fish communities was beyond the aims of this study, still, nearly all the fish species present in the study area can have significant impacts on amphibians (Falaschi et al. 2022b). Thus, we considered fish as ‘present’ when at least one fish species was observed at least once during our surveys at a site in a given year. This approach has limitations, but is generally deemed sufficient to detect the major impacts of fish on amphibians (Indermaur et al. 2010; Denoël et al. 2019; Falaschi et al. 2022b). Finally, as a measure of landscape composition, we measured the percentage cover of forests within 400 m from each site, to take into account the effects that the surrounding terrestrial habitats may have on amphibian populations. Terrestrial environments surrounding aquatic habitats are important drivers of amphibian presence and population dynamics (Préau et al. 2018; Bounas et al. 2020). Forests represent the majority of natural habitats available for amphibians in the study area (e.g., Ficetola et al. 2009), as other typologies of natural vegetation (e.g., open vegetation) are extremely rare. Forest cover was calculated using the DUSAF (Land Use of Agricultural and Forest Land; ground resolution: ~3 m) map of the Lombardy region for 2018 (<http://www.cartografia.regione.lombardia.it>). We merged broadleaved forests and mixed broadleaved-coniferous forests (level-3 categories: 311, 313) in the analyses.

Multistate models

Imperfect detection, i.e. the presence of false negatives in detection/non-detection data, is a major issue in the estimation of true occupancy. Site occupancy models are a set of species distribution models that allow estimating occupancy from detection/non-detection data, while taking into account

imperfect detection, by jointly modelling the ‘ecological process’ (i.e., occupancy) and the ‘observation process’ (i.e., detection probability; Guillera-Arroita et al., 2010; Kéry et al., 2013; MacKenzie et al., 2002).

Multistate site occupancy models are an extension of site occupancy models that allow to distinguish between two or more alternative ‘occupancy states’ at each site. In our analyses, the potential occupancy states were three: *A* (‘absent’, i.e., unoccupied site), *P* (‘present’, i.e., occupied site without reproduction), and *R* (‘reproduction’, i.e., occupied site with reproduction). If the true state is *P*, surveys can yield 2 possible outcomes: spotting adults, or spotting no individuals. If the true state is *R*, surveys can yield 3 possible outcomes: detection of reproduction, detection of adults but not reproduction, or no detection of any life stage. Multistate occupancy models have been recently applied to investigate occupancy and population dynamics for many organisms, including amphibians (Bailey et al. 2014; Gould et al. 2019; Cruickshank et al. 2021), but the differential responses of amphibian presence and reproduction to site-specific environmental features have been rarely investigated (but see Schmidt et al. 2019). Year-stratified models use data collected in multiple sampling seasons to build static models containing the state distribution of the species within each sampling season without information about transition probabilities between seasons (Kéry & Royle 2020). Sampling seasons are assumed to be independent and the estimates of fixed effects are calculated using the entire dataset.

In the ecological part of the models, we defined the true state of a site *i* at time *t* as z_{it} , following a categorical distribution:

$$z_{it} \sim \text{Categorical}(\Omega_{it})$$

where Ω is the state vector, indicating the probability of each state for site *i* in year *t*, with the three states *A*, *P*, and *R* as possible outcomes. Ω is defined using the two parameters ψ , adult occurrence

probability, and r , the conditional reproduction probability in an occupied site:

$$\Omega_{it} = [1 - \psi \quad \psi(1 - r) \quad \psi r]_{it}$$

with ψ and r following a Bernoulli distribution. Adult occurrence was thus considered as a prerequisite for a site to host reproduction. Both ψ and r were modelled as a function of seven environmental variables (surface area, water permanence, shade cover, aquatic vegetation cover, forest cover, fish presence, and crayfish presence); a random effect was included to account for differences across years related to uninvestigated factors. Hence, the probability of adult occurrence ψ and reproduction r at site i in year t can be expressed as:

$$\text{logit}(\psi_{it}) \sim \alpha_{\psi} + \beta_{\psi[1]} \times x_{1[it]} + \beta_{\psi[2]} \times x_{2[it]} \dots + \beta_{\psi[n]} \times x_{n[it]} + T_{\psi[t]} + S_{\psi[i]}$$

$$\text{logit}(r_{it}) \sim \alpha_r + \beta_{r[1]} \times x_{1[it]} + \beta_{r[2]} \times x_{2[it]} \dots + \beta_{r[n]} \times x_{n[it]} + T_{r[t]} + S_{r[i]}$$

where α is the intercept, $x_1, x_2 \dots x_n$ are environmental variables potentially affecting adult occurrence and reproduction, T is a year random effect, and S is a site random effect.

In the observation model, the observed state follows a categorical distribution, which is conditional on the true state of the site. We defined the observed state of a site i in survey j and year t , conditional on the true state z as:

$$y_{ijt} | z_{it} \sim \text{Categorical}(\Theta_{zoi jt})$$

The indices of the five-dimensional observation array Θ are the true state z , the observed state o , and indices for site, replicate, and year (i, j, t) , hence, the observation array Θ shows the true state in the row and the observed state in the columns.

$$\Theta_{zoi jt} = \begin{bmatrix} 1 & 0 & 0 \\ 1 - p_P & p_P & 0 \\ 1 - p_{RP} - p_{RR} & p_{RP} & p_{RR} \end{bmatrix}_{ijt}$$

For instance, $1 - p_P$ in row two and column one indicates the probability of observing the state A (absent) when the true state is P (occupied without reproduction), so that p_P is the probability of

detecting state P . In addition, p_{RP} and p_{RR} respectively indicate the probability of detecting state P when the true state is R (occupied with reproduction) and the probability of detecting state R when the true state is R , so that the definition of all the other slots naturally follows. In our models, the three detection probabilities were modelled as functions of air temperature, humidity, date (ordinal day of the year), and hour of the survey, to consider phenology; for date and hour, we also included quadratic terms. We also included a site random effect to account for potential differences in detectability between sites (Schmidt et al. 2023). Since numerous surveys were performed around midnight, surveys conducted during the same night are in principle associated with different dates. To avoid this issue, the hour of the survey was calculated as the minutes after 06:00 am; surveys performed between 00:00 am and 06:00 am were assigned to the previous day.

Before analyses, wetland area was log-transformed to reduce skewness and all the independent variables were scaled (mean = 0, SD = 1) to allow the comparison of their effects. Bivariate correlations between independent variables were weak (in all pairwise correlations, $|r| \leq 0.38$), suggesting no major collinearity issues. We excluded the fire salamander from the analyses since adults mate on land, thus only larvae are regularly found in waterbodies. We also excluded the common frog, since it was only found in a few sites (10 out of 207), and the green toad, whose reproduction was only observed in two sites. Therefore, we ran a total of seven multistate occupancy models.

All analyses were run in the R 4.3.1 (R Core Team 2023). Multistate models were run within a Bayesian framework, by using the ‘nimble’ package (NIMBLE Development Team 2019). To draw samples from the posterior distribution, we ran 3 Markov chain Monte Carlo for 80,000 iterations, with the first 60,000 iterations discarded as a burn-in; then the posterior distribution was sampled with a thinning of 20. When convergence ($R_{\text{hat}} < 1.1$) was not reached, models were re-run

using the average of the posteriors of the previous model run as initial values for the chains in the new run, until the successful achievement of convergence. We used the outputs of each multistate occupancy model to compare persistence in breeding versus non-breeding sites. Persistence in non-breeding sites was calculated as the mean proportion of sites in state P during year t found to be in state P or R during year $t+1$. Persistence in breeding sites was calculated as the mean proportion of sites in state R during year t found to be in state P or R during year $t+1$. More details about persistence can be found in Appendix S2.5. The script and data used for the multistate occupancy models are available at figshare (<https://dx.doi.org/10.6084/m9.figshare.22494664>).

Number of surveys required to monitor adults vs. reproduction

To provide information on different sampling efforts required for accurate monitoring of adults and reproduction, the posterior distribution of species-specific detection probabilities was used to identify the number of surveys needed to efficiently collect the occurrence of adults versus reproduction data. We assumed that surveys are to be performed in spring, when amphibian activity and reproduction peak. Within this period (day of the year 60–150), we created nine hypothetical sampling scenarios, each with an increasing number of evenly distributed surveys per season (from two to ten). An even distribution of sampling occasions enables covering as much as possible the phenology of the different species inhabiting an area. We used the detection probability estimates obtained by multistate models to calculate the cumulative detection probabilities that can be achieved for each species by performing from two to ten surveys through the breeding season. We wanted to compare the overall detection probability of different states: adult presence (considering together detection probabilities of adults in non-breeding habitats and adults in breeding habitats) and reproduction. Cumulative detection probabilities were thus calculated as:

$$p_c = 1 - \prod_{d=1}^n (1 - p_{sd})$$

Where p_{sd} represents the detection probability of state s (adults or eggs/larvae) at day d , and n represents the number of surveys within each different sampling scenario (between 2 and 10).

This allowed to identify the minimum number of surveys required to detect the different states each species with a 90% confidence (Sewell et al. 2010).

Multiple-criteria decision analyses

Multiple-criteria decision analysis (MCDA) tools are often applied to conservation to support decision making, by ranking different sets of alternatives and exploring their pros and cons (Kiker et al. 2005; Adem Esmail & Geneletti 2018). In this study, we used the outputs of the models to identify the optimal strategy to measure population responses to ecological features for each species, comparing two strategies: 1) focusing on the monitoring of adults occurrence, vs. 2) collecting both adult and reproduction data. Note that our analyses are based on the results of multistate models, assuming adult presence as a prerequisite for reproduction. We considered two different criteria: responsiveness and effort. Responsiveness to ecological parameters was defined as the number of ecological variables influencing adult occurrence (first strategy) versus the number of ecological variables influencing both adult presence and reproduction (second strategy). Effort was calculated as the inverse of cumulative detection probability after 5 surveys and represents a proxy for the resources needed in terms of people and money. For the first strategy, we considered the cumulative detection probability of adults, while for the second strategy we considered the lowest cumulative detection probability between adults and reproduction. We hypothesized 3 scenarios with different levels of resources available for monitoring. Differences between these scenarios were achieved by

adjusting the weights of the “effort” criterion: the higher its weight, the lower the availability of resources. We then assessed the optimal monitoring strategy for each species in each scenario with 3 different MCDA methods: TOPSIS (García-Cascales & Lamata 2012), Multi-MOORA (Brauers & Zavadskas 2010), and WASPAS (Zavadskas et al. 2012), using the R package ‘*MCDM*’ (Martin 2016). Each MCDA method uses a unique algorithm to evaluate the alternatives, thus different MCDA approaches can suggest different solutions to the same problem (Wątróbski et al. 2019). Therefore compromise rankings based on several MCDA methods are generally considered more robust approach than using a single method (Wątróbski et al. 2022). More details on the MCDA parameters can be found in Appendix S2.6.

Results

Literature search

The literature search on recent works applying site occupancy models to amphibians yielded 50 papers (Fig. 1). In these studies, data collection tended to focus on adult-only occurrences (44% of papers), while reproduction-only observations were less frequently collected (10%). Observations of both adults and reproduction were collected only in 8% of studies, while in a few cases, no distinction was made between adults and the detection of other life stages (8%). In 10% of cases, the authors did not specify the data gathered during monitoring. eDNA was used in 18% of studies, and in three cases it was compared with traditional methods. Probabilities assessed by site occupancy models reflect the monitoring approaches: most studies assessed occupancy probabilities from adults/all life stages (82%), 10% modelled occupancy from reproduction observation, while 8% modelled both adult occurrence and reproduction.

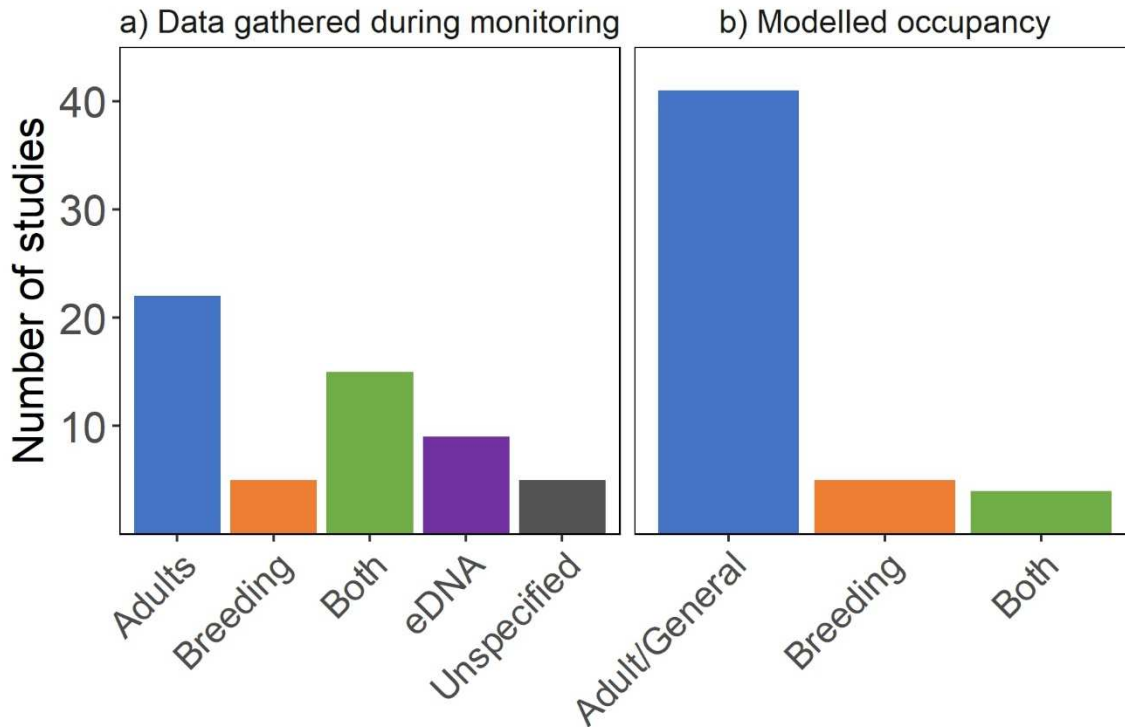


Figure 1. a) Type of data gathered in the analyzed scientific papers applying occupancy models to amphibians metacommunities. b) Number of studies modelling adult/general occurrence probability (blue); number of studies modelling reproduction occurrence (orange); number of studies modelling adult occurrence and reproduction probabilities (green). See the Methods section for additional details on the literature search.

Factors related to adult occurrence

For all the amphibian species, adult occurrence probability showed strong relationships with most environmental factors (Fig. 2; Appendix S2.2). Forest cover in the surrounding landscape favored occurrence of adults for common toad, agile frog, and Italian agile frog, while it negatively affected occurrence of Italian treefrog adults. The 95% credible intervals (CIs) overlapped zero for the remaining species. Adults of treefrog, green frogs, and smooth newt preferentially occupied larger waterbodies. Water permanence increased occupancy for green frogs and crested newt adults.

Aquatic vegetation was positively related to adult occurrence of agile frog and Italian crested newt. Adult green frogs were more frequent in sun-exposed wetlands. The presence of fish was negatively related to the occupancy of most species (Italian treefrog, agile frog, and the two newt species).

Common toad adults, conversely, were positively associated with fish-occupied ponds. Occupancy of adults did not show clear relationships with the presence of the invasive crayfish and adults of Italian agile frog were more frequently found at crayfish-invaded sites.

Factors related to reproduction probability

Breeding probabilities of the monitored species showed strong responses to the investigated ecological variables (Fig. 2, Appendix S2.3). Forest cover in the surrounding landscape increased reproduction probability of smooth newt, while it was negatively associated with reproduction of green frog and crested newt. Pond surface was positively related to the reproduction of crested newts, while it was unrelated to the breeding activities of the other species. A permanent hydroperiod positively affected the reproduction of common toad. Aquatic vegetation favored the reproduction of treefrogs, green frogs, and Italian crested newts. Shade cover showed a positive relationship with the reproduction of Italian agile frog and agile frog (even if for this species the 95% CI slightly

overlapped 0), whereas the reproduction of treefrogs and green frogs was associated with less shaded wetlands. Fish occurrence negatively affected reproduction of agile frog, Italian agile frog, and smooth newt.

The crested newt was the only species showing a reduced reproduction in presence of the red swamp crayfish. The reproduction of the agile frog was more frequent in crayfish-invaded ponds. For most species, breeding sites represented the majority of occupied sites in the study area (S2.4). Persistence was generally higher in breeding sites for most species, with the exception of common toad and agile frog (S2.5).

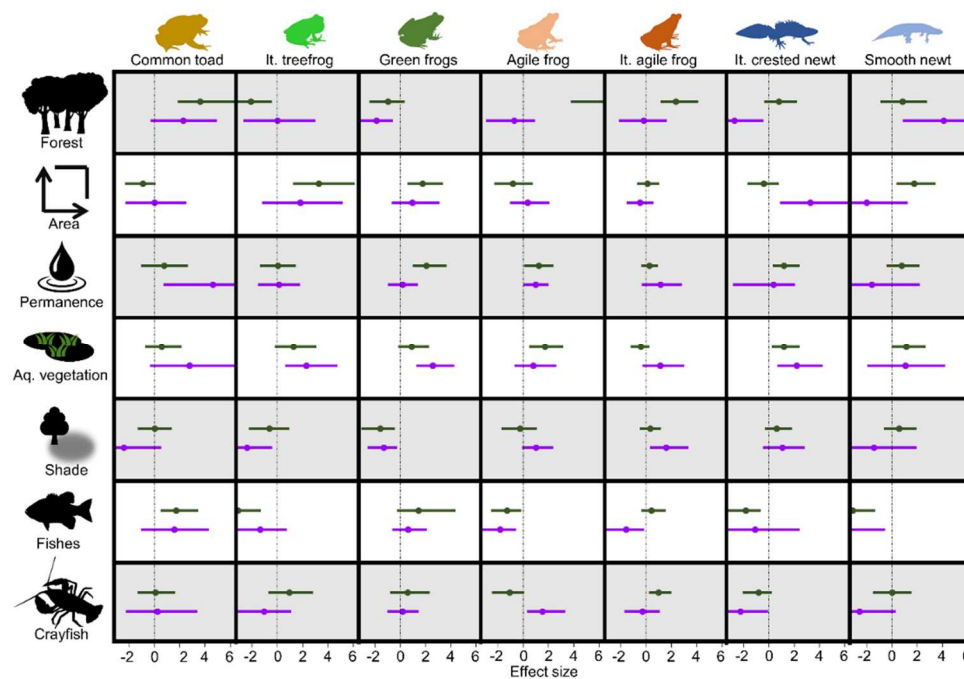


Figure 2. Relationships between environmental variables (from top to bottom: forest cover, wetland surface area, water permanence, aquatic vegetation, shade, presence of fish, presence of the red swamp crayfish) and occupancy and reproduction probabilities of the seven amphibian species considered in this study. For each species and variable, the posterior distributions (summarized with means and 95% CIs) for occupancy (green) and reproduction (purple) are shown.

Detection probabilities of adults and reproduction

The variation of detection probabilities of adults in non-breeding sites, adults in breeding sites, and reproduction evidence (egg masses, tadpoles, and larvae) during the sampling season showed different patterns among species (Fig. 3). For agile frog and Italian agile frog, the detection probability of reproduction was consistently higher than the one of adults along most of the breeding season. Conversely, in anurans breeding in late spring, i.e., green frogs and treefrogs, the detection probability of adults in breeding sites was higher than the detection probability of reproduction or the detection probability of adults in non-breeding sites. For three species (common toad and the two newts), the detection probability of adults peaked at the beginning of the season and was generally higher than the detection probability of reproduction. The detection probability of reproduction of these species increased during late spring, approaching and sometimes overcoming the one of adults.

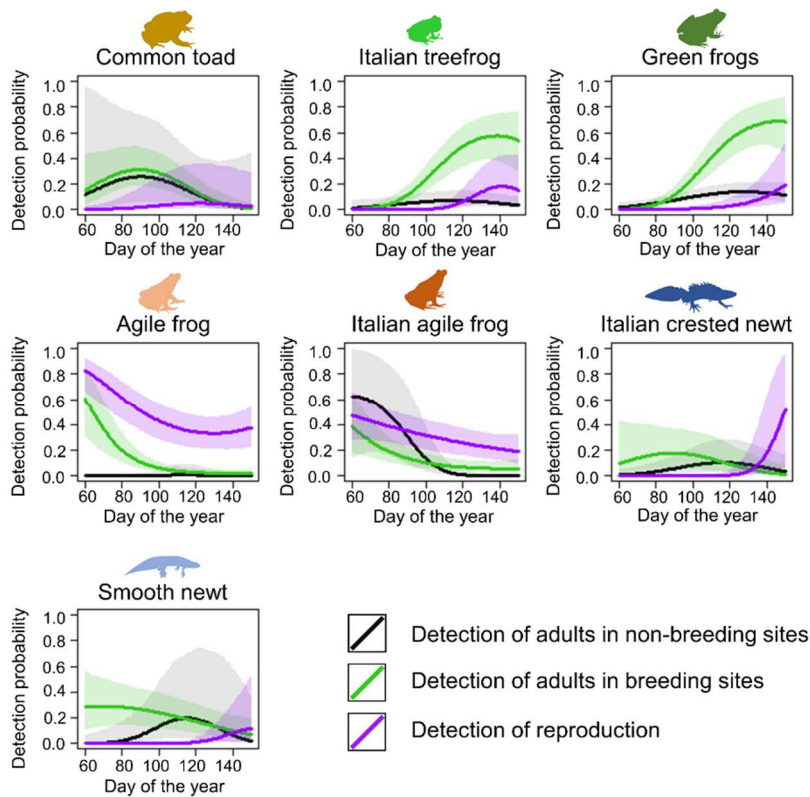


Figure 3. Relationships between the detection probabilities of adults in non-breeding sites (black lines), adults in breeding sites (green lines), and evidence for reproduction (purple lines) and the ordinal day of the year for the monitored amphibian species. Posterior predictions are summarized with means and 95% CIs (shaded areas).

Number of surveys required for effective monitoring

The number of surveys required for effective monitoring was greatly different between species (Fig. 4). For agile frog and Italian agile frog, the cumulative detection probabilities of reproduction were largely higher than the ones of adults, regardless of the number of surveys. Four and seven surveys were needed to detect the reproduction of agile frog and Italian agile frog with confidence $>90\%$, respectively.

For the remaining species, cumulative detection probabilities of adults were higher than the ones of reproduction. Consequently, robust assessment of reproduction required more surveys than assessing adult occurrence. For green frogs and Italian treefrog, five and six surveys were needed to detect adults with $>90\%$ confidence, respectively. For common toad and both newts, even ten surveys were not enough to estimate adult or reproduction occurrences with high confidence (Fig. 4).

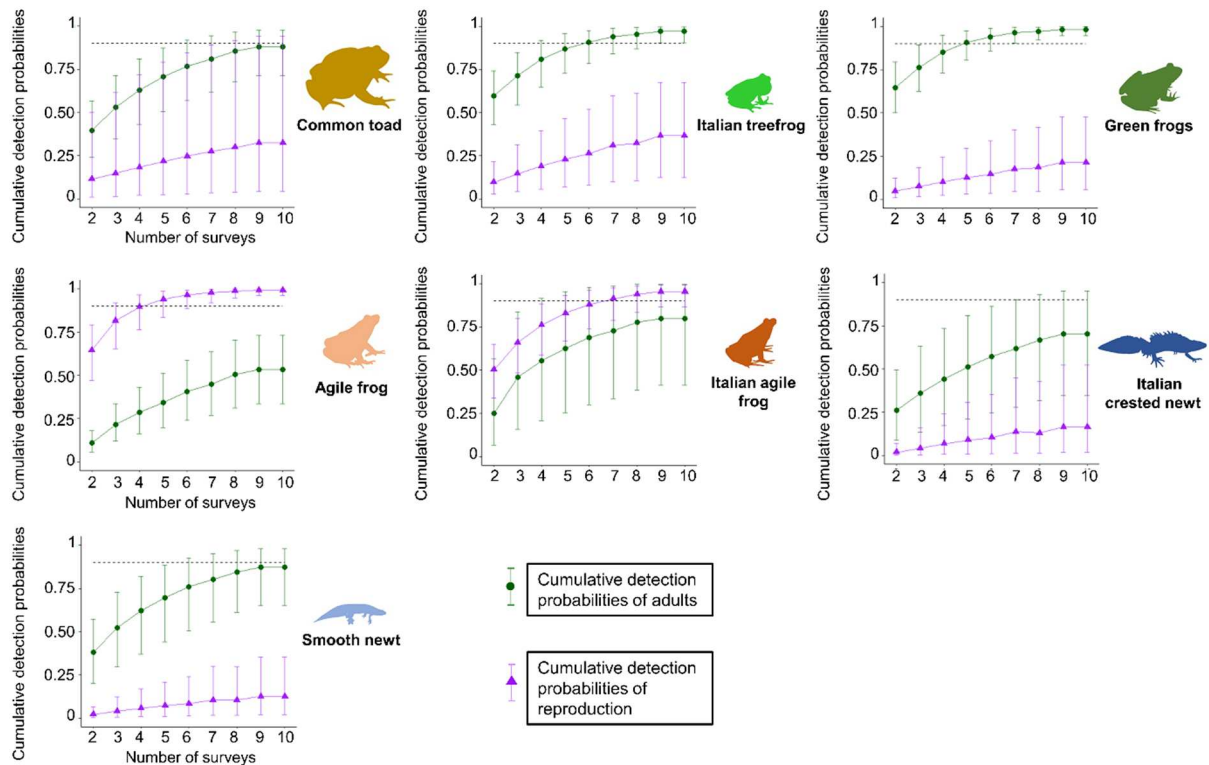


Figure 4. Cumulative detection probabilities of adults (green circles) and evidence for reproduction (purple triangles) with different numbers of surveys for the seven amphibian species considered in this study. Posterior predictions are summarized with means and 95% CIs (vertical lines); the dashed horizontal line represent the 0.90 threshold.

Multiple-criteria decision analyses

MCDA indicated that the optimal strategy was context-dependent (Fig. 5). For most taxa, monitoring programs focused on adults emerged as the optimal strategies when resources availability was low, the only exceptions being the two brown frogs. Collecting data on both adults and reproduction was found to be the first ranked strategy at medium level of resource availability for Italian crested newt, Italian agile frog, and agile frog. In the high resource scenario, monitoring both adults and reproduction generally emerged as the optimal strategy for all species, with generally consistent results across the three methods (TOPSIS, WASPAS, and MOORA). WASPAS indicated adult-only monitoring as the optimal strategy for green frogs and treefrog also under the high resource scenario while, for these species, the other approaches suggested monitoring both adults and reproduction as the optimal one.

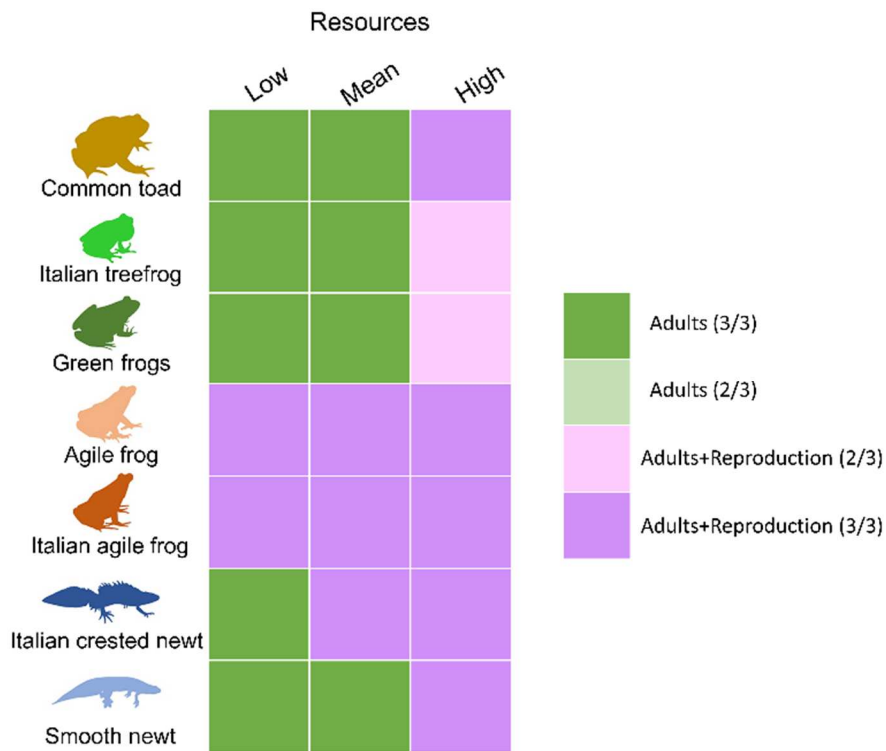


Figure 5. Result of the MCDAs under the three different resource availability scenarios for each species. Deep green indicates that all three analyses (TOPSIS, WASPAS, and MOORA) ranked adult monitoring strategy as the optimal strategy; purple indicates that all three analyses ranked monitoring reproduction as the optimal strategy; light purple indicates that 2/3 of analyses ranked monitoring both adults and reproduction as the optimal strategy.

Discussion

Our results showed that adult amphibians often occur in sites that do not maximize reproduction, implying narrower ecological requirements for breeding than for the mere species presence (Figure 2). In some cases, we found consistent relationships between environmental variables and both adult occurrence and breeding probabilities. For instance, adult occurrence and reproduction probabilities of both agile frog and smooth newt were low in fish-occupied waterbodies (Fig. 2), where predation risk by fish is high and larval survival is generally low (Buxton & Sperry, 2017; Falaschi, et al., 2021; Winandy et al., 2015).

In most instances, though, the investigated factors affected only one of the two life history stages. The existence of environmental parameters acting as limiting factors for reproduction but not for adults is expected, since the environmental conditions enabling successful breeding are a subset of the ones allowing the survival of adults (Ficetola et al. 2008; Kearney & Porter 2009). As a consequence, non-breeding adults can be found over a much larger proportion of waterbodies than breeding (Cruickshank et al. 2021). In our study area, the difference between the total number of occupied sites and the number of breeding sites was particularly high for some species, such as green frogs and Italian treefrog (Appendix S2.4). Differences in habitat selection between males and females can contribute to the observed differential patterns. In many amphibians species, males display strong territoriality (Lesbarrères & Lodé, 2002; Sacchi et al., 2015). If optimal environments are saturated by conspecifics or competing heterospecifics, adult males losing territorial interactions can move and disperse into a broader range of sub-optimal sites (Kraus & Vonesh 2010; Buxton & Sperry 2017). Conversely, females tend to occupy only a subset of the potential habitats, selecting the ones favoring offspring survival (Ebisuno & Gentili, 2002; Pintar & Resetarits, 2017; Resetarits, 2005). For instance, adult Italian agile frogs seemed to occupy ponds regardless of sun-exposure and

fish presence, but their reproduction mostly occurred in shady ponds without fish (Fig. 2). In the Italian agile frog males occupy breeding ponds before the arrival of females (Sacchi et al. 2015). Since females are more discriminatory than males in selecting potential breeding ponds, they are more likely to select only those ponds that maximize breeding success, causing the observed differences between reproduction and adult occurrences (Pintar & Resetarits 2017).

Other variables influenced adult presence without affecting reproduction. For instance, adults of agile frog and Italian agile frog were more likely to occupy waterbodies surrounded by forests (Fig. 2), while reproduction was not affected by the same variable. This was expected, as both species are tightly linked to deciduous forests (Bernini et al. 2004; Indermaur et al. 2010). The presence of adult common toads was more frequent in waterbodies where fish were present. Tadpoles of the common toad are toxic, thus immune or at least less affected by fish predation than other amphibians (Bounas et al. 2020). Previous studies showed that some anuran species tend to avoid heterospecific competition when selecting breeding habitats (Buxton & Sperry 2017). Thus, the association between adult toads and ponds with fish may occur because fish reduce occupancy and/or larval densities of other amphibian species (Knapp & Matthews 2000; Préau et al. 2017; Hamer 2021). In fact, co-occurrence between common toads and fish has been often reported in previous studies (e.g., Indermaur et al. 2010; Falaschi et al. 2021). We highlight that factors affecting adult occurrence have an indirect effect on reproduction. For instance, we found a positive effect of forest cover on the occupancy of adult Italian agile frogs but not on their reproduction (Fig. 2). This means that ponds used by adults for reproduction show similar forest cover than ponds occupied by adults but not used for reproduction. As adult occurrence at breeding sites is a prerequisite for reproduction, this means that also reproductive sites generally occur within forested areas.

Here, eggs and larvae were considered together as evidence of reproduction. Presence of eggs

or even larvae does not guarantee breeding success, and focusing on the overall success of the breeding season, estimated from the occurrence of metamorphs, would provide more robust estimates of fitness, revealing potential impacts of environmental variables that are otherwise hidden (e.g., droughts occurring late in the season; impacts of predators on survival). However, collecting data on metamorphs requires more resources than other life stages, such as the use of specific approaches like drift fences. This further highlights the complexity of trade-offs associated with monitoring programs.

Ascertaining multiple life-stages, thus, can be challenging, and for several species cumulative detection probabilities of both adults and reproduction remained low even after many surveys (Fig. 4). This underlines the importance of accounting for imperfect detection. In some cases (agile frog and Italian agile frog) detecting reproduction was easier than detecting adults. This is due to the fact the egg masses of these species are easily spotted, with previous works reporting detection probabilities up to 90% (Falaschi et al., 2022). Conversely, other amphibians showed relatively high detection probabilities of adults, particularly in reproductive sites. This is likely related to a high level of activity during the breeding season, with calls and scramble competition of males that facilitate the observation of adults. For some species, detection probability of reproduction increased late in the season, when larvae are closer to metamorphosis, yet the cumulative values remained lower than those of adults. It must be remarked, though, that our data were collected mostly from early March to late May, when the breeding seasons of all the considered species overlap. Consequently, those species that start breeding in late spring show high detection probabilities around the end of our monitoring season; a more prolonged monitoring might improve the detection of reproduction of species with very long larval periods, such as green frogs and crested newts. This highlights the importance of careful planning of the monitoring activities that account for the

phenology of the focal species. Such planning is particularly challenging for multi-species monitoring or for species with a poorly-known phenology, and should be optimized on the basis of local priorities, such as the monitoring of most threatened or declining species.

Since amphibians are facing strong declines at the global scale, it is urgently needed to find the best monitoring approaches to maximize the usefulness of collected data to promote effective conservation measures. Taking into account both adult and reproduction data would help to identify drivers of local decline or persistence in a more accurate way than focusing only on the most detectable life stages, potentially improving conservation measures. In some cases, though, resource availability may represent a limiting factor hampering the possibility of collecting exhaustive data on the less detectable life stages. Differences in detectability between life stages can be strong, thus monitoring all of them would require much more resources than focusing on the most detectable ones. This drawback is widespread and difficult to overcome when resources are scarce. Under these conditions, focusing on the life stages with the highest detection probabilities can be the optimal strategy despite the potential loss of information (Fig. 5). When abundant resources are available, though, collecting data on both adults and reproduction emerged as the optimal strategy for all the investigated species (Fig. 5).

Monitoring multiple life stages can significantly improve model soundness, resulting in a more comprehensive identification of factors acting on local populations. When occurrences of both adults and reproduction can be collected, multistate occupancy models can be invaluable, as they provide unbiased estimates of the factors affecting occupancy and reproduction while accounting for detection probabilities. While this study could not encompass all the possible reasons behind amphibian monitoring, we stress the need for articulated, a priori planning of monitoring programs, in function of the aims of the study and the biology of the focal species (Yoccoz et al. 2001).

Optimizing monitoring programs on the basis of available resources can enhance the identification of parameters that can be managed to reduce local populations' extinction risks.

Data availability

The data and scripts used to run the multistate occupancy models are available on Figshare:

<https://dx.doi.org/10.6084/m9.figshare.22494664>

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Supplementary material for:

Integrating adult occurrence and reproduction data to identify conservation measures for amphibians

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S2.1 Study area

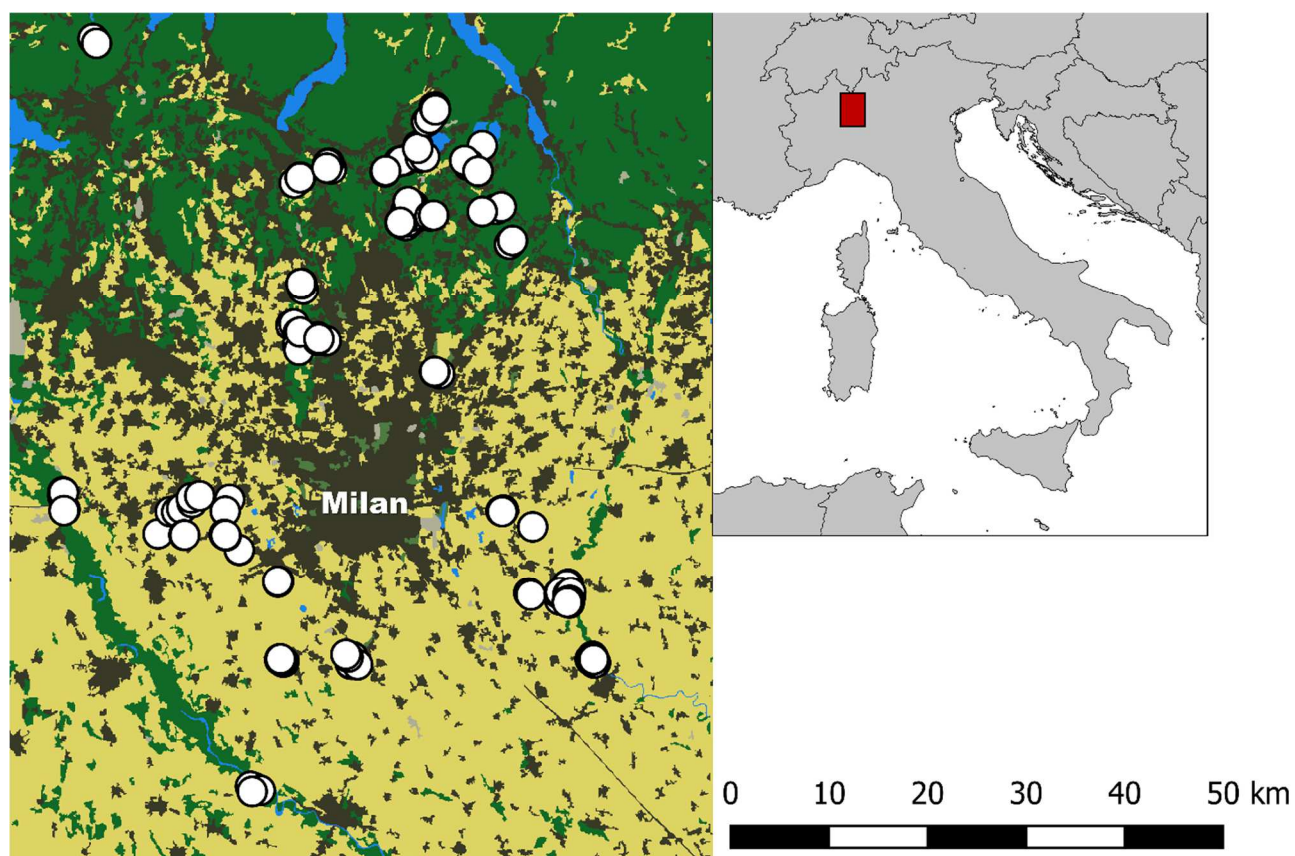


Fig. 2.1.1: The locations of the monitored 207 waterbodies monitored during the study.

S2.2 Results of multistate occupancy models (adult occurrence)

For each species and variable, the mean, the standard deviation (SD), the lower and the upper limits of the 95% confidence intervals of the posterior distributions and the Rhat values for adult occurrence are reported.

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-5.783	1.478	-9.024	-3.352	1.03
Forest	3.624	1.188	1.835	6.451	1.02
Area	-0.909	0.621	-2.341	0.097	1.00
Permanence	0.775	0.939	-1.053	2.656	1.04
Aquatic vegetation	0.572	0.716	-0.745	2.131	1.01
Shade	0.025	0.644	-1.305	1.359	1.00
Fishes	1.722	0.770	0.464	3.462	1.04
Crayfish	0.083	0.739	-1.338	1.627	1.01

S2.2.1: *Bufo bufo* (Common toad)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-1.431	1.563	-4.500	1.726	1.01
Forest	-2.094	0.978	-4.250	-0.441	1.00
Area	3.277	1.275	1.246	6.105	1.02
Permanence	0.045	0.696	-1.368	1.463	1.01
Aquatic vegetation	1.279	0.846	-0.185	3.084	1.01
Shade	-0.628	0.795	-2.258	0.940	1.00
Fishes	-3.113	1.090	-5.594	-1.315	1.07
Crayfish	0.949	0.880	-0.696	2.823	1.03

S2.2.2: *Hyla intermedia* (Italian treefrog)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	5.702	1.544	3.027	9.018	1.01
Forest	-0.957	0.707	-2.435	0.333	1.01
Area	1.769	0.716	0.546	3.373	1.00
Permanence	2.075	0.695	0.977	3.666	1.00
Aquatic vegetation	0.906	0.626	-0.167	2.275	1.01
Shade	-1.574	0.679	-3.089	-0.412	1.01
Fishes	1.449	1.177	-0.272	4.352	1.06
Crayfish	0.587	0.782	-0.807	2.319	1.01

S2.2.3: *Pelophylax* spp. (Green frogs)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	3.990	1.488	0.993	6.956	1.09
Forest	7.129	1.769	3.753	10.810	1.09
Area	-0.809	0.761	-2.297	0.748	1.02
Permanence	1.239	0.572	0.103	2.387	1.00
Aquatic vegetation	1.734	0.667	0.475	3.160	1.02
Shade	-0.238	0.722	-1.731	1.081	1.03
Fishes	-1.270	0.608	-2.537	-0.155	1.01
Crayfish	-1.067	0.645	-2.467	0.103	1.02

S2.2.4: *Rana dalmatina* (Agile frog)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-1.319	0.630	-2.531	0.009	1.03
Forest	2.361	0.766	1.167	4.137	1.06
Area	0.113	0.422	-0.701	1.043	1.02
Permanence	0.254	0.319	-0.370	0.919	1.01
Aquatic vegetation	-0.410	0.375	-1.230	0.253	1.02
Shade	0.339	0.438	-0.504	1.171	1.05
Fishes	0.425	0.470	-0.364	1.560	1.07
Crayfish	0.990	0.487	0.230	1.995	1.08

S2.2.5: *Rana latastei* (Italian agile frog)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-3.714	1.299	-6.441	-1.121	1.02
Forest	0.792	0.658	-0.366	2.220	1.03
Area	-0.404	0.636	-1.699	0.768	1.03
Permanence	1.189	0.562	0.282	2.424	1.03
Aquatic vegetation	1.177	0.546	0.242	2.417	1.08
Shade	0.611	0.546	-0.338	1.832	1.04
Fishes	-1.826	0.723	-3.499	-0.645	1.03
Crayfish	-0.814	0.599	-2.074	0.230	1.04

S2.2.6: *Triturus carnifex* (Italian crested newt)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-7.204	1.668	-10.673	-4.126	1.05
Forest	0.846	0.924	-0.902	2.782	1.00
Area	1.760	0.779	0.382	3.461	1.01
Permanence	0.778	0.657	-0.424	2.166	1.01
Aquatic vegetation	1.146	0.671	0.382	3.461	1.01
Shade	0.581	0.661	-0.631	1.968	1.00
Fishes	-3.088	0.998	-5.303	-1.319	1.02
Crayfish	0.019	0.764	-1.495	1.546	1.00

S2.2.7: *Lissotriton vulgaris* (Smooth newt)

S2.3 Results of multistate occupancy models (reproduction probability)

For each species and variable, the mean, the standard deviation (SD), the lower and the upper limits of the 95% confidence intervals of the posterior distributions and the Rhat values for breeding probability are reported.

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	0.196	2.390	-5.122	4.233	1.04
Forest	2.279	1.322	-0.312	4.933	1.01
Area	0.011	1.216	-2.297	2.541	1.04
Permanence	4.628	2.113	0.721	8.988	1.00
Aquatic vegetation	2.774	1.768	-0.358	6.518	1.01
Shade	-2.417	1.695	-5.894	0.546	1.01
Fishes	1.574	1.344	-1.057	4.295	1.02
Crayfish	0.243	1.457	-2.278	3.409	1.03

S2.3.1: *Bufo bufo* (Common toad)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-5.642	1.623	-9.009	-2.787	1.05
Forest	0.011	1.366	-2.693	3.014	1.01
Area	1.820	1.555	-1.206	5.162	1.01
Permanence	0.135	0.827	-1.540	1.796	1.00
Aquatic vegetation	2.297	1.055	0.621	4.751	1.02
Shade	-2.394	1.183	-5.079	-0.410	1.01
Fishes	-1.356	1.107	-3.575	0.748	1.02
Crayfish	-1.041	1.214	-3.772	1.088	1.02

S2.3.2: *Hyla intermedia* (Italian treefrog)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-2.951	1.353	-5.800	-0.454	1.01
Forest	-1.870	0.745	-3.552	-0.593	1.04
Area	0.960	0.949	-0.672	3.102	1.01
Permanence	0.197	0.601	-0.961	1.383	1.00
Aquatic vegetation	2.577	0.793	1.265	4.275	1.01
Shade	-1.287	0.600	-2.587	-0.245	1.00
Fishes	0.624	0.680	-0.616	2.094	1.01
Crayfish	0.199	0.621	-1.029	1.452	1.00

S2.3.3: *Pelophylax* spp. (Green frogs)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	2.682	1.431	0.593	6.220	1.03
Forest	-0.708	0.970	-2.942	0.938	1.03
Area	0.355	0.776	-1.043	2.062	1.03
Permanence	0.994	0.517	0.015	2.024	1.01
Aquatic vegetation	0.801	0.780	-0.674	2.639	1.02
Shade	1.019	0.628	-0.099	2.370	1.00
Fishes	-1.814	0.684	-3.241	-0.559	1.00
Crayfish	1.532	0.751	0.311	3.334	1.01

S2.3.4: *Rana dalmatina* (Agile frog)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	3.426	1.492	0.919	6.686	1.04
Forest	-0.182	0.931	-2.150	1.639	1.03
Area	-0.476	0.523	-1.534	0.580	1.01
Permanence	1.142	0.834	-0.356	2.833	1.03
Aquatic vegetation	1.124	0.832	-0.294	3.020	1.01
Shade	1.593	0.766	0.302	3.371	1.02
Fishes	-1.573	0.872	-3.673	-0.163	1.04
Crayfish	-0.283	0.698	-1.711	1.080	1.02

S2.3.5: *Rana latastei* (Italian agile frog)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	-1.630	1.501	-4.237	1.945	1.01
Forest	-2.727	1.299	-5.497	-0.454	1.01
Area	3.267	1.345	0.867	6.214	1.01
Permanence	0.364	1.185	-2.861	2.056	1.04
Aquatic vegetation	2.191	0.901	0.653	4.232	1.05
Shade	1.069	0.836	-0.463	2.807	1.01
Fishes	-1.098	1.625	-4.301	2.419	1.00
Crayfish	-2.254	1.197	-4.731	-0.071	1.00

S2.3.6: *Triturus carnifex* (Italian crested newt)

	Mean	SD	Lower 95% CI	Upper 95% CI	Rhat
Intercept	0.701	2.393	-3.631	5.916	1.01
Forest	4.103	1.834	0.839	7.978	1.00
Area	-1.983	1.742	-5.642	1.251	1.01
Permanence	-1.592	2.120	-6.178	2.198	1.01
Aquatic vegetation	1.075	1.510	-1.948	4.215	1.00
Shade	-1.420	1.670	-4.643	1.948	1.01
Fishes	-4.298	2.036	-8.453	-0.543	1.00
Crayfish	-2.557	1.478	-5.602	0.295	1.01

S2.3.7: *Lissotriton vulgaris* (Smooth newt)

S2.4 Number of occupied sites and reproductive sites

For each species, the models returned an estimate of the number of unoccupied, occupied, and reproductive sites for each year from 2017 to 2022. The most widespread taxa in the study area were the green frog complex and the agile frog, with a maximum of total occupied sites of 187 and 168, respectively. Occupancy of both species peaked in 2020. The highest mean ratio between the total number of occupied sites and the number of reproductive sites was reached by the Italian agile frog (mean = 0.778, sd = 0.092), followed by the agile frog (mean = 0.720, sd = 0.070). The species showing the lowest ratio (i.e., the greatest proportion of non-reproductive occupied sites) was the Italian treefrog (mean = 0.264, sd = 0.046). This ratio remained stable during the study period for most species, thus the number of reproductive sites was generally highly correlated to the number of total occupied sites. Correlation between these two measures was >0.75 for all species, except for the green frogs (cor = 0.626, $p = 0.184$). The number of total occupied sites showed higher uncertainty levels than the number of breeding sites across all species and years, the only exception being the agile frog. The number of yearly occupied sites (including both breeding and non-breeding sites) and breeding sites for each species is represented in the picture below:

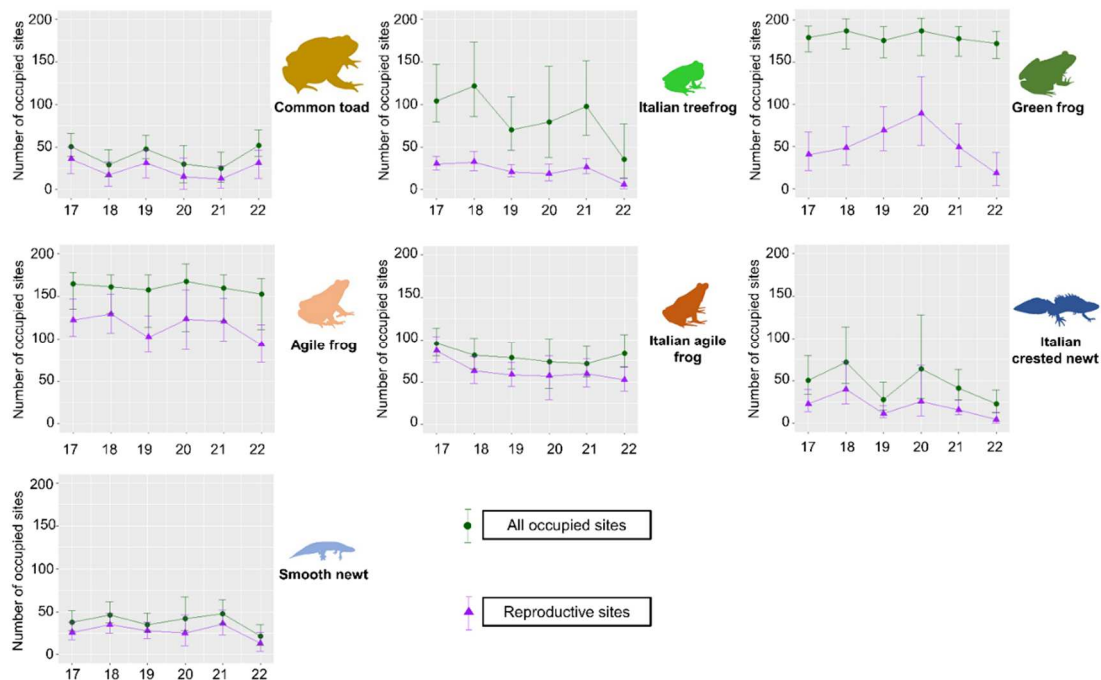


Fig. S2.4.1: Number of estimated occupied sites (green) and number of estimated reproductive sites (pink).

S2.5 Persistence

For each species, we compared persistence in non-breeding sites and in breeding sites. Persistence for both states at year t was calculated as the proportion of sites in a certain state at year $t-1$ that did not become unoccupied at year t . We then calculated the mean persistence in non-breeding and breeding sites for each species. We excluded the 2019-2020 and 2020-2021 values when calculating the mean persistence in common toad sites, as in 2020 we were not able to monitor breeding site for this species when reproduction occurrences were visible due to restrictions linked to the COVID-19 pandemic.

In most species, we found that persistence was higher in breeding sites, as reported by Cruickshank et al. (2021), despite in most cases differences in persistence in breeding and non-breeding sites showed only slight differences (Fig. S2.4.1). For common toad and agile frog, persistence in non-breeding sites was higher than in breeding sites. Persistence of each species in non-breeding sites and breeding sites is represented in the picture below:

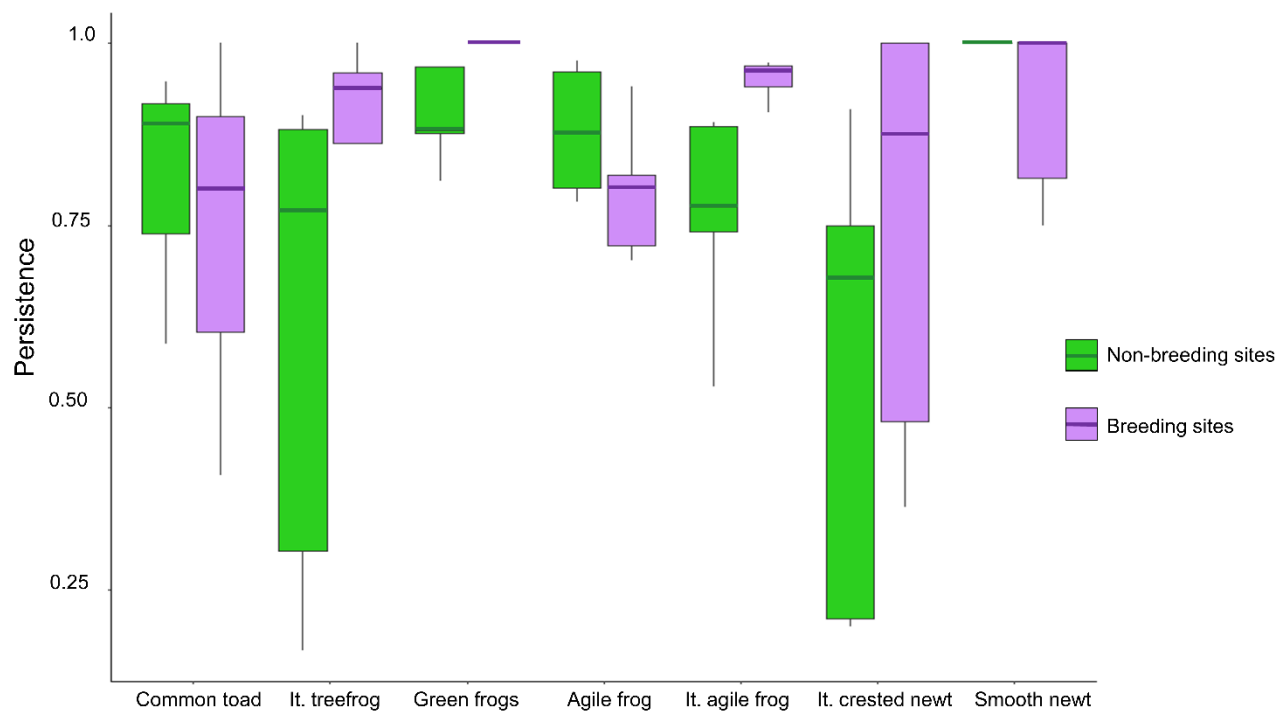


Fig. S2.5.1: Persistence in non-breeding (green) versus breeding (pink) sites.

S2.6 Details on the multi-criteria decision analyses

We assessed the optimal strategy between monitoring adults versus both adults and reproduction occurrences by using three different multi-criteria decision analyses (MCDAs): TOPSIS (García-Cascales & Lamata 2012), Multi-MOORA (Brauers & Zavadskas 2010), and WASPAS (Zavadskas et al. 2012), using the R package ‘MCDM’ (Martin 2016).

TOPSIS ranks alternatives by measuring their distance to the positive and negative ideal solution. Multi-MOORA (Multi-Objective Optimization by Ratio Analysis) uses three different approaches (namely: ratio system, reference point, and full multiplicative form) to obtain three measures, then calculate a “summary of rankings” measure for each alternative. WASPAS (Weighted Aggregated Sum Product Assessment) is the combination of two methods: WSM (Weighted Sum Model) and WPM (Weighted Product Model; Triantaphyllou 1998). All these three methods need a set of criteria, that can be classified as either benefits or costs, and the relative weights of said criteria.

For our analysis, we considered two criteria: responsiveness and monitoring effort. Responsiveness for the adult-only monitoring strategy was assessed by the number of relationships between adult occupancy and ecological variables whose 95% CI did not intersect zero, as for the outputs of the multistate occupancy models. Responsiveness for the alternative strategy was assessed as the number of relationships between both adult occupancy and reproduction probability whose 95% CI did not intersect zero. The rationale behind the inclusion of this measure was that focusing on the most responsive parameter can give a better understanding on the overall factors affecting amphibian populations, thus leading to effective conservation measures. Monitoring effort was estimated from the cumulative detection probability of adults/reproduction after five surveys. We assumed that the higher this value, the lower the resources needed to survey each status. To keep interpretation as straightforward as possible, we calculated monitoring effort as $1 - p_{c5}$, where p_{c5} represents the cumulative detection probability after five surveys.

Responsiveness was classified as a benefit, while effort was classified as a cost. We hypothesized three different scenarios, based on different levels of resources available for monitoring: a very low-resource scenario, a low-resource scenario, a medium-resource scenario, a high-resource scenario, and a very high-resource scenario. In these different situations, the relative weights of the two criteria were set as follows:

Low-resource scenario: Responsiveness = 0.3; Effort = 0.7;

Medium-resource scenario: Responsiveness = 0.5; Effort = 0.5;

High-resource scenario: Responsiveness = 0.7; Effort = 0.3;
The lower the amount of resources, the more influential the effort.

S2.7 References

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CHAPTER 5: Should I stay or should I go? Amphibian breeding responses to drought events and alien predators

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Abstract

The increase in frequency and intensity of extreme event, such as droughts, is one of the main components of climate change that are threatening global biodiversity. Amphibian species are particularly sensitive to droughts, as diminishing water availability can limit the number of breeding sites. Moreover, when only few breeding sites are available, increase in frequency of encounters with invasive species is likely, as both native and non-native freshwater species are constrained by water availability. Amphibian may respond to breeding site scarcity by skipping the breeding season or by concentrating in the few available sites. The Po Valley, in northern Italy, is a strongly anthropized region where droughts events are on the rise and freshwater communities are greatly affected by human-mediated dispersal of organisms. Aiming at investigating reproductive responses of two frogs (*Rana dalmatina* and *R. latastei*) to drought events, we monitored 27 clusters of breeding sites over years 2017-2023. We used piecewise structural equation models to assess the relationships between rainfalls, site availability, presence of potentially detrimental predators (fish and the invasive crayfish *Procambarus clarkii*), number of egg clutches and egg clutches distribution within clusters of breeding sites. Our results showed that females of both species responded to site scarcity by skipping reproduction. Moreover, *Rana dalmatina* tended to concentrate reproductive efforts in the remaining waterbodies and to avoid waterbodies where fish occurred. Contrarily, *Rana latastei* was not affected by fish presence, probably because it often exploits sites tightly linked to river networks, where fish are often present. Neither species displayed avoidance of crayfish-invaded sites. As climate change and invasive species represent growing threats for amphibians, assessing how these animals respond to the challenge posed by these factors is pivotal. Conservation measures should account for both threats jointly, as their interactions might affect amphibians in unexpected ways.

Introduction

Climate change plays an increasingly important role in the loss of biodiversity and modification of ecosystem functioning (Sala et al. 2000; Brook et al. 2008). Through alterations of temperature and precipitation patterns, climate change is expected to greatly impact animal populations, causing range shifts, phenological changes, and disease spread (Hill et al. 1999; Pounds et al. 2006; Bronson 2009; Chen et al. 2011). Climate change is also causing an increase in the frequency of extreme climatic events, including droughts (Diffenbaugh et al. 2017). Droughts are defined as temporary disruptions in the balance of water availability (Baronetti et al. 2022), and are becoming an increasing issue worldwide (Trenberth et al. 2014). For instance, frequency of drought events is expected to increase in many areas of Europe (Vautard et al. 2007; Dai 2013; Spinoni et al. 2017; NOAA 2023). This increase can potentially affect freshwater species through shifts in phenology, changes in distribution, or even extinction of the most sensitive species, such as those adapted to perennial and cold waters (Fenoglio et al. 2010).

With more than 40% of species recognized as threatened by extinction, amphibians are the vertebrate group suffering the broadest declines, due to habitat loss and fragmentation, diseases, biological invasions, and climate change (Stuart et al. 2004; Voyles et al. 2009; Hoffmann et al. 2010). Given the tight association of amphibian reproduction with the presence of water, drought events may have major consequences for this group, through both direct and indirect effects (Acevedo-Whitehouse & Duffus 2009; Milligan & Lloyd 2009). Most amphibians rely on waterbodies for reproduction, thus a reduction in water availability can directly impact amphibian populations by reducing the availability of breeding sites (i.e., complete desiccation), or by causing the death of eggs and larvae, increasing the risk of local extinction (Brannelly et al. 2019; Zylstra et al. 2019).

Many amphibian species show philopatry, i.e. they tend to return to the same breeding sites over multiple years (Smith & Green 2005). Philopatry may confer adaptive advantages in stable environments, where site fidelity ensures access to suitable habitats and reduces competition for limited resources (Semlitsch 2008). However, in a context of drought-induced scarcity of breeding sites, philopatry may have implications for reproductive success, as it could hinder the ability of amphibians to select alternative breeding sites. When drought events occur during the breeding season, adult philopatric amphibians can implement two strategies to safeguard their fitness: they can i) actively seek alternative sites to relocate their breeding efforts, or ii) skip the reproduction for that given season (Lannoo & Stiles 2020).

Another factor complicating site selection under reduced water availability is the presence of predators. Predators can exert significant pressure on amphibian populations, influencing in some cases their behavior and site selection (Winandy et al. 2015; Buxton & Sperry 2017). This interaction between predator presence and the already limited availability of breeding sites constitute an additional layer of complexity to the challenges faced by amphibians in responding to drought-induced habitat changes (Kloskowski 2020). Predation threat restricts the options for amphibians to select alternative breeding sites, as there is a potential trade-off between avoiding predation and finding suitable breeding conditions (Gervasi & Foufopoulos 2008).

In most amphibian species, local populations are connected by migrating individuals, forming metapopulations (Alford & Richards 1999; Marsh & Trenham 2001). Investigating factors affecting metapopulation dynamics, such as persistence of local populations, is thus particularly relevant for amphibian conservation. Some stressors, including invasive predators and droughts, can significantly modify or even disrupt metapopulation dynamics, leading to amphibian declines and even extinctions (Marsh & Trenham 2001; Zylstra et al. 2019; Manenti et al. 2020; Falaschi et al. 2021). Several studies showed that drought events can severely impact amphibian populations (Walls et al. 2013; Cayuela et al. 2014, 2016), and that introduced predators may reduce amphibian fitness (Ficetola et al. 2011; Nunes et al. 2019; Falaschi et al. 2021), but the interplay between these two factors has largely been overlooked. Here, we seek to assess the reproductive responses of amphibians to two co-occurring environmental challenges: drought events and presence of predators. Given the importance of amphibian breeding sites selection on their reproductive success, we explicitly investigated the reproductive strategies in face of sites scarcity and predator presence to contribute to the broader understanding of amphibian responses to climate change. We expected that, in case of low availability of breeding sites, amphibian species with narrow niche requirements and/or strong philopatric behavior may reduce or skip breeding activities, while more plastic species may disperse and exploit nearby waterbodies. Moreover, in presence of predator avoidance behaviors, distribution of breeding females should be shaped by the presence of predators. To test these hypotheses, we focused on two brown frogs: *Rana latastei*, endemic to Northern Italy and nearby areas with a narrow ecological niche, and *Rana dalmatina*, a species with a wide European distribution.

Material and methods

Study area and species

Between 2017 and 2023 we visited 133 amphibian breeding sites located in the lowlands of Lombardy region (Northern Italy; Fig. 1), an area where human activities exert strong pressures on amphibian populations. Amphibian populations located only a few hundred meters apart are not demographically independent; instead, they represent subpopulations within the same metapopulation (Petranka et al. 2004; Manenti et al. 2020). Thus, we considered all the wetlands that were <300 m apart to belong to the same cluster. This distance was considered to be adequate, since frog dispersal is unlikely to exceed 1 km, particularly in human-dominated areas (Smith & Green 2005; Jeliaskov et al. 2014). Using this criterion, we collected data from 27 clusters of amphibian breeding sites. The study area has experienced a growing frequency of drought events and water scarcity since the 1990s and climatic scenarios suggest an intensification of droughts in the future (Baronetti et al. 2022; Bonaldo et al. 2023; Colombo et al. 2023). Moreover, numerous invasive species have been introduced in the study area, such as the red swamp crayfish, *Procambarus clarkii*, and many non-native fish species (Lanzoni et al. 2018; Lo Parrino et al. 2019).

We focused on two species, the agile frog (*Rana dalmatina*) and the Italian agile frog (*Rana latastei*). These brown frog species are explosive breeders that reproduce in a short time window at the end of the winter; they often are syntopic in the river Po Valley. *R. dalmatina* is a widespread species found in Central and Southern Europe, where it inhabits deciduous forests (Ficetola et al. 2006; Sillero et al. 2014). *R. latastei*, on the contrary, has a small range, limited to the Po valley and neighboring regions, where it inhabits riparian forests (Barbieri & Bernini 2004; Sillero et al. 2014). Both species are mostly terrestrial and are found in aquatic habitats only during the breeding seasons, that usually begins in late winter/early spring (Dalpasso et al. 2022). Both frogs are explosive breeders: females lay one single egg clutches per breeding season, thus egg count corresponds to the number of breeding females, although not all females reproduce every year (Lodé et al. 2005; Ficetola et al. 2010). *R. latastei* generally breeds in large and deep wetlands surrounded by forests, while *R. dalmatina* has a wider tolerance and can breed in smaller, sunnier waterbodies (Ficetola & De Bernardi 2004; Ficetola et al. 2006; Canova & Balestrieri 2018). In the study area, the two species often co-occur, breeding at the same time (late February-early March), but exploiting different micro-habitats (Ficetola & De Bernardi 2005; Romagnoli et al. 2020; Dalpasso et al. 2022). The two species have different lifespans. *R. dalmatina* can live 6-10 years, while *R. latastei* maximum recoded lifespan is four years, but very few individuals attain this longevity (Guarino et

al. 2003; Sarasola-Puente et al. 2011; Alin Iosob & Prisecaru 2014).

The red swamp crayfish is a predator of amphibian larvae that can negatively affect their abundance (Gherardi et al. 2001; Ficetola et al. 2012). The crayfish was introduced in the study area in 2002 and rapidly spread (Lo Parrino et al. 2019; Melotto et al. 2020). The red swamp crayfish is a very adaptable species, able to survive for months under drought conditions due to its burrowing activities (Gherardi et al. 2011; Loureiro et al. 2015). This can greatly enhance its survival under extreme weather fluctuations (Kouba et al. 2016).

Fish are known to influence amphibian oviposition choices, sometimes overwhelming the effect of hydroperiod (Kloskowski 2020). We did not discriminate fish species in our analyses; however most fish species present at study sites are non-native, and their presence is often caused by angling-related introductions (Falaschi et al. 2022b). Most fish species are expected to be extremely sensitive to drought events, but some of the species present in the study area, such as the oriental weatherloach *Misgurnus anguillicaudatus*, are able to survive under drought conditions by burying itself under moist leaves or mud (Koetsier & Urquhart 2012).

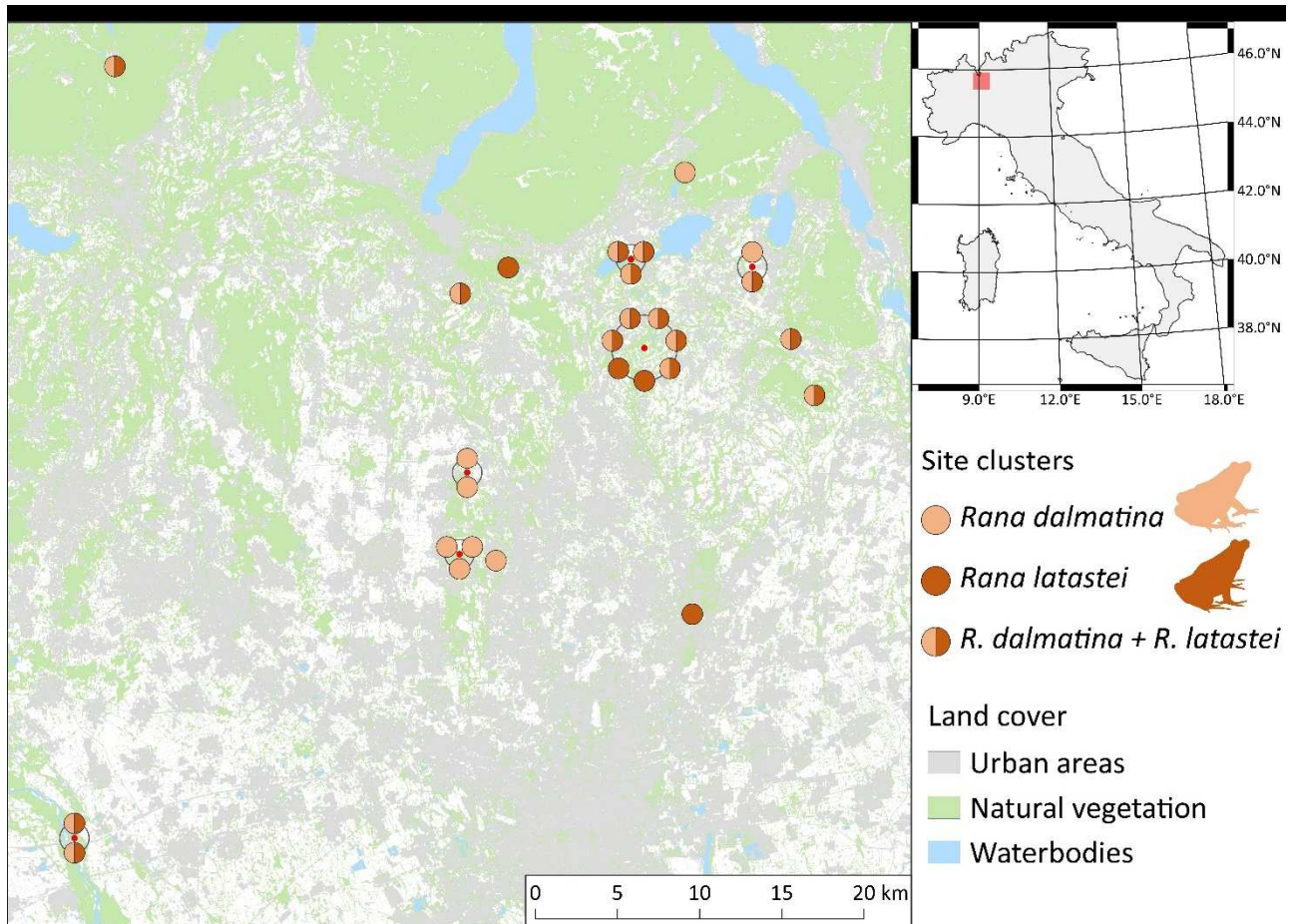


Figure 1: Distribution of the monitored clusters across the study area. Light brown circles represent clusters hosting only *Rana dalmatina*; dark brown circles represent clusters hosting only *R. latastei*; two-tone circles represent clusters where both species co-occur. Clusters that at the selected scale would overlap are represented in circles around red dots.

Monitoring

Each cluster was monitored in 2-5 different years in 2017-2023 (mean $\pm$ SD = 4.2 ± 1.6 years).

Within each year, clusters were surveyed from 1 to 5 times in March (average surveys per cluster per sampling season = 1.8 ± 0.88), the month in which the two study species mainly lay their eggs in this area. During surveys, we assessed: i) the number of clutches per cluster for *Rana dalmatina* and *Rana latastei*, ii) the number of available and unavailable (i.e., without water) breeding sites, and iii) the presence of aquatic predator, namely fish and the red swamp crayfish (*Procambarus clarkii*).

Assessment of reproductive responses, measures of drought, and predators

Frogs can respond to drought events by either skipping reproduction at usual breeding sites when they are dry, or by selecting another nearby breeding site. To understand how frogs respond to drought conditions, we developed two indicators. Firstly, we determined the total number of clutches laid by frogs within each cluster during each year (hereafter, “clutch abundance”) each year.

Clutches of both species are globular and are usually deposited within 1 m of the water surface, thus, they have very high detection probabilities, and correspond well to the number of breeding females (Falaschi et al. 2022a). Secondly, we evaluated the coefficient of variation for the number of clutches per cluster per season (hereafter, “clutch variation”). The coefficient of variation is the ratio between standard deviation and mean, therefore, high values indicate that clutches are unevenly distributed among the breeding sites within the focal cluster, while low values suggest that clutches are evenly spread among the sites. The clutch variation indicator enabled us to assess frog selectivity for non-dry sites and/or sites without predators. Combining these two measures we aimed at getting a general view of frogs’ responses to harsh conditions.

We recorded five variables that water availability and the occurrence of predators. Three variables represented water availability, and its impact on amphibian breeding sites: precipitation, hydrometric level and % dry sites. For each year, we obtained data on cumulative precipitations during February and March from the meteorological station closest to considered clusters (average distance $\pm$ SD: 9.29 ± 6.42 km). Data on precipitation were obtained from the Regional Agency for the Protection of the Environment of the Lombardy region (<https://www.arpalombardia.it/temi-ambientali/meteo-e-clima/form-richiesta-dati/>). As a measure of the impact of droughts on breeding sites, we calculated the proportion of dry sites in each cluster per reproductive season. A site was considered as dry if it

was observed without water at least once during March.

As a measure of the impacts of aquatic predators, we recorded the occurrence of fish and invasive crayfish in each breeding site; predators were considered present in a breeding site if detected in at least one survey within the sampling season. Then, for each cluster, we calculated the proportion of sites with presence of fish or crayfish. All the independent variables were standardized (mean = 0, SD = 1) before analyses to improve models' convergence and allow the comparison of estimated effects, while clutch abundances were log-transformed to improve normality.

Statistical analyses

To assess the factors affecting directly and indirectly frog reproductive behavior, we fitted two structural equation models (SEM), one for each species, using piecewise SEM (package *piecewiseSEM*; Lefcheck, 2016). The piecewise SEM (pSEM) approach differs from traditional SEM by estimating the pathways within the model separately instead of estimating relationships between all variables simultaneously (Grace et al. 2010). We built an a priori theoretical path model indicating the possible causal relationships between variables as required by structural equation modelling. We built two models, one for each species, with the same causal relationships. We hypothesized that the proportion of unavailable sites was affected by precipitation, and in turn affected the abundance of fish- and crayfish-occurring sites. Clutch abundance and variation were affected by the proportion of unavailable sites, of fish-occurring sites, and of crayfish-occurring sites. Moreover, as anuran activity and reproduction may be heavily influenced by rainfalls (Gibbons et al. 1974; Kenneth Dodd 1994; Cayuela et al. 2014, 2016), we hypothesized a direct causal link between precipitation and clutch abundance. All paths included the cluster as a random factor to account for cluster variability, while temporal autocorrelation structure of order 1 was included to consider dependency structure of the data over time.

We implemented Fisher's C test for conditional independency to assure that no significant pathways were missing in the model and Shipley's test for directed separation to assess no missing relationships among unconnected variables (Shipley 2000). All the analyses were performed in the R environment (R Core Team 2021).

Results

We monitored 24 clusters for *Rana dalmatina* (number of breeding sites for each cluster: mean $\pm$ SD

= 5.097 ± 2.956) and 19 clusters for *Rana latastei* (number of breeding sites for each cluster: mean $\pm$ SD = 5.147 ± 3.425). Fisher's C tests for both pSEMs were not significant (*Rana dalmatina*: Fisher's C = 4.33, $p = 0.83$, DF = 8; *Rana latastei*: Fisher's C = 11.19, $p = 0.19$, DF = 8), indicating that our models adequately encompass the relationships among variables. Additionally, the Shipley tests were not significant, implying that no missing pathways or correlations were identified in the pSEMs.

Concerning *Rana dalmatina* (Fig. 2a), we found a significant negative relationship between rainfalls and the proportion of unavailable sites (Std. est.= -0.389, $p < 0.001$). Moreover, clutch abundance was negatively affected by the proportion of unavailable sites (Std. est.= -0.326, $p < 0.001$). Clutch variation was positively affected by the proportion of unavailable sites (Std. est.= 0.245, $p = 0.028$) and the proportion of fish occurring sites (Std. est.= 0.297, $p = 0.018$), meaning that where less ponds were available and where there was a high presence of fish, egg clutches were more unevenly distributed. The alien crayfish had no effect on both clutch abundance and variation, but it showed a positive covariation with fish (Std. est.= 0.376, $p < 0.001$).

The model on *Rana latastei* clusters (Fig. 2b), on the other hand, highlighted no significant relationships between rainfalls and the proportion of unavailable sites. Moreover, clutch abundance was not affected by precipitations nor predators, but it was negatively associated with the proportion of unavailable sites (Std. est.= -0.215, $p = 0.045$). Clutch variation did not show any significant relation with neither site availability nor predators. The proportion of the latter, though, had a positive covariation (Std. est.= 0.368, $p = 0.002$).

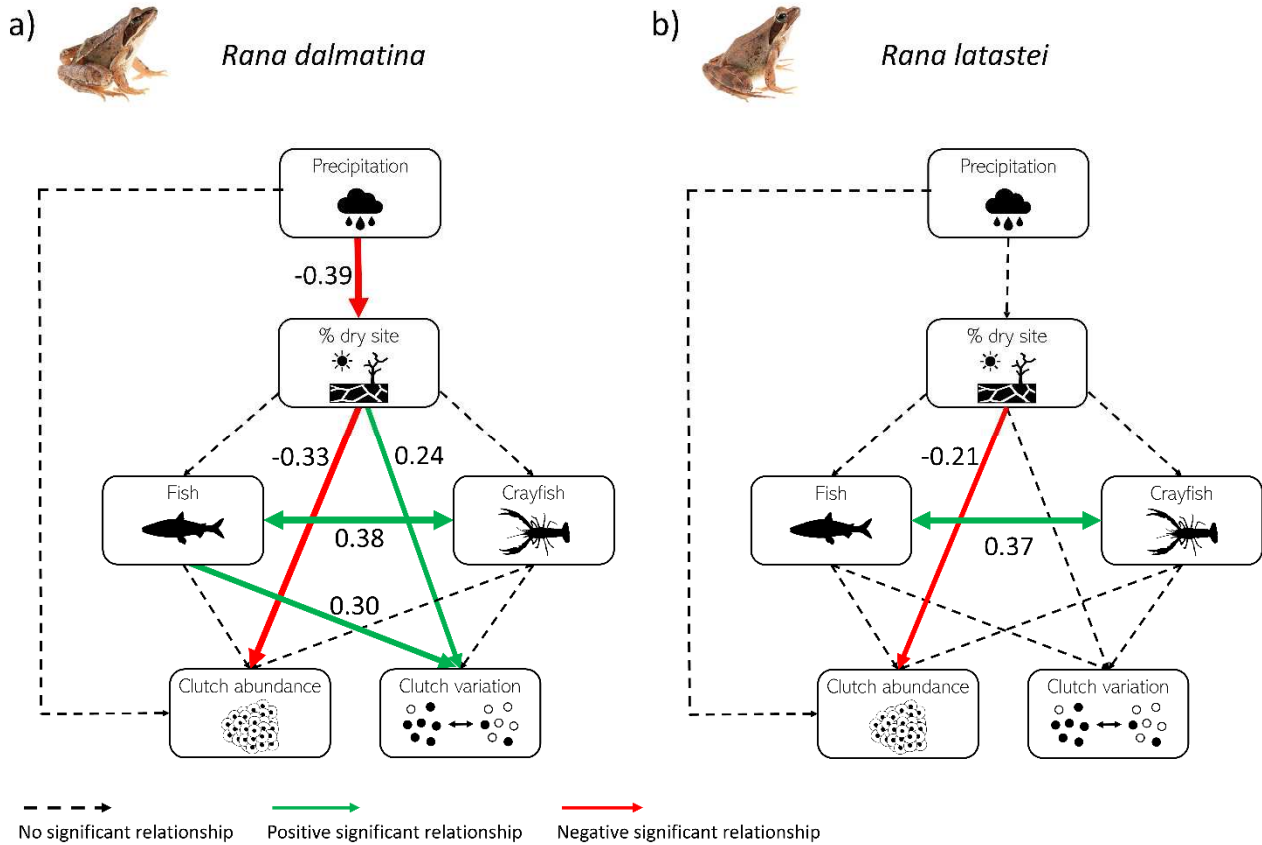


Figure 2: Results of the piecewise structural equation models for (a) *Rana dalmatina*, and (b) *Rana latastei*. Dashed arrows show no-significant relationship, solid arrows show significant relationship at $p < 0.05$. Arrow colours indicate the sign of relationship (green: positive; red: negative; black: no significant correlation). Double-headed arrow indicate covariation. Estimated coefficients are given as numbers next to the arrows.

Discussion

Droughts can have pervasive impacts on aquatic environments, affecting multiple ecosystem traits and thus influencing species reproduction. Our analyses show that amphibians experienced a reduction in the availability of breeding sites over the study years, attributable to drought events occurring during the reproductive season, still differences in life-history across species can determine diverse responses to drought conditions and to the presence of other stressors.

Drought effects on site availability

Our results revealed relevant differences in site selection strategies of *Rana dalmatina* and *R. latastei* in response to site scarcity and predator presence. These differences reflect the ecological requirements of the two species. For *Rana dalmatina*, the proportion of unavailable sites was strongly related to hydrometric level, while for *Rana latastei* we did not detect significant relationships between unavailable sites. The two species often co-occur in Lombardy, but *Rana latastei* tends to be more common in riparian woods (Canova & Balestrieri 2018), where water availability might be less directly affected by rainfalls and more to river discharge. Additionally, *R. latastei* generally breed in large and deep waterbodies (Ficetola & De Bernardi 2004), that are less likely to dry out even under low precipitation levels. *Rana dalmatina*, conversely, is able to exploit sunny and shallow waters (Ficetola & De Bernardi 2005), whose persistence is strongly affected by rainfalls.

Breeding responses to droughts

The proportion of dry site showed different effects on the two species (Fig. 2a; Fig. 2b): while a decrease in availability of breeding sites determined a reduction of egg clutches laid for both species, only for *Rana dalmatina* we found a significant effect on clutch variation within clusters. These two measures represent the two alternative responses to breeding site scarcity: reproduction skip would be reflected in a reduction of clutch abundance, while selection of alternative sites would be reflected in an increase of clutch variance. Since we accounted temporal autocorrelation of the data, the effects we found should not be determined by the outputs in the previous years.

Hence our results showed that, on the one side, *Rana latastei* mainly responded to site scarcity by skipping reproduction. On the other side, females of *Rana dalmatina* were more prone to move and lay their eggs to the remaining available waterbodies. Reproduction is an extremely

energy-demanding process; thus, amphibian female may reduce breeding activity or even skip a breeding season under sub-optimal conditions (Cayuela et al. 2014, 2016). *R. latastei* is considered to be a highly philopatric species (Pellitteri-Rosa et al. 2008) and has a lower dispersal ability than *Rana dalmatina* (Dalpasso et al. 2022), thus moving to alternative breeding sites might be more energetically costly for the former. These factors, in addition to the narrow habitat requirements, may explain the lack of alternative site selection in *R. latastei*. *Rana dalmatina* is a relatively long-lived species, with sexual maturity generally reached after 2-4 years and a lifespan of 6-10 years (Sarasola-Puente et al. 2011; Alin Iosob & Prisecaru 2014). On the contrary, *Rana latastei* females can live up to a maximum of 4 years, reaching sexual maturity before the first breeding season after metamorphosis (Guarino et al. 2003). This may imply that skipping even a single breeding season for *Rana latastei* females may result in missing their only opportunity to reproduce. For this reason, a prolonged series of dry years may pose a serious threat to the persistence of *R. latastei* populations. Conversely, *Rana dalmatina* females were found to be more plastic, and responded to site scarcity by moving to nearby sites. However, a potential trade-off emerges when a substantial number of females occupies few available sites. In such instances, intensive intraspecific competition among offspring for limited resources may occur, and it may be enhanced by volume reduction due to dry conditions (Loman 2001, 2002). For this reason, assessing the effects of site scarcity on metamorphosis success is needed to evaluate less evident effects of droughts on this species and their potential consequences to population survival.

We did not find a direct effect of precipitation on clutch abundance, in accordance with previous studies (Bernini et al. 2004; Combes et al. 2018).

Predators and selection of breeding sites

Breeding responses to predators were different among the two species (Fig. 2): clutches of *Rana dalmatina* tended to be unevenly distributed among breeding sites in clusters with a high prevalence of fish-invaded sites, while *R. latastei* did not respond to fish presence. Females of *R. dalmatina* may recognise fish as potential predators of tadpoles and actively select fishless ponds as breeding sites, as reported in other amphibian species (Buxton & Sperry 2017). The ability of many amphibian species to select breeding sites based on the presence of potential predators is a well-understood anti-predator strategy, that mitigates predation pressure on their offspring (e.g. Refsnider & Janzen 2010). Previous studies found that both persistence and colonization of breeding sites were negatively

impacted by fish presence for *Rana dalmatina*, but no effect was found for *R. latastei* (Falaschi et al. 2021). Therefore, *R. dalmatina* seems more impacted by fish and can actively select fishless sites for reproduction. *R. latastei*, on the other hand, occupies sites that are deep, generally permanent, and often linked to river networks, thus it is likely adapted and highly tolerant to fish occurrence (Ficetola & De Bernardi 2004).

Conversely, we did not detect clear responses to the occurrence of the American crayfish, despite it can feed on amphibian larvae and conspicuously decrease their densities (Renai & Gherardi 2004; Ficetola et al. 2011). Our results confirm that both *Rana dalmatina* and *R. latastei* do not modify their spawning habits as anti-predator strategies against *Procambarus clarkii*, as reported in previous studies (Romagnoli et al. 2020; Muraro et al. 2021), aligning with our findings. The crayfish was introduced relatively recently in the study area (Lo Parrino et al. 2019), and *Rana dalmatina* and *R. latastei* may not be able to recognize the predatory risk. This lack in predator-prey coevolution could lead to a strong predation by the crayfish and a decrease in frog population sizes (Gherardi et al. 2001; Ficetola et al. 2012). Moreover, its incidence at landscape scenario has been found to significantly impact metapopulation dynamics (Manenti et al. 2020; Falaschi et al. 2021). Still, some developmental responses were found, indicating that plasticity and rapid adaptation may buffer the negative effects of crayfish (Melotto et al. 2020, 2021).

Both *Rana dalmatina* and *R. latastei* are susceptible to the adverse impacts of drought events and the presence of alien predators. This vulnerability is particularly noteworthy for *Rana latastei* due to several reasons. Firstly, the species exhibits a restricted distribution, being an endemic species of the Po Valley and its neighbouring regions. Secondly, it has faced local extinctions or near-extinctions in several clusters in recent times, attributed to a combination of factors, including inadequate management practices and drought events (Dalpasso et al. 2022; Ficetola et al. 2023). Lastly, this species faces with the intricate challenge of balancing the imperative to reproduce, driven by the short lifespan, and scarcity of optimal breeding sites caused by droughts, and predation risks from alien species.

Conservation implications

The different responses to drought between frog species suggest a particularly strong impact on short-lived species. *Rana latastei* is endemic to a region where drought frequency is expected to increase, and where water unavailability and mismanagement already caused local extinctions

(Ficetola et al. 2023). Therefore, appropriate water management for conservation is more than ever significant for conservation of this endemic species. At the same time, removal of alien predators from permanent and isolated waterbodies can increase survival of tadpoles of both *Rana dalmatina* and *R. latastei*. Our results showed how *Rana dalmatina* tended to avoid fish-colonized sites, thus its conservation might benefit from the creation of predator-free ponds, as well as removal of fish species from former fishless ponds.

Our study highlighted the complex relationships among climate change and biological invasions in determining reproductive strategies of amphibians. We showed that two closely related frog species adopted different strategies to cope with these environmental challenges, including both skipping reproduction under drought circumstances and moving to more permanent waterbodies. On the one side, the presence of introduced predators may represent a significant risk to both study species, particularly when frogs are unable to avoid reproduction in predator-colonized sites. On the other side, the short lifespan of the endemic *Rana latastei* may exacerbate the effects of droughts, and potentially undermining its long-term persistence under prolonged drought periods. Concurrent management of alien predators and water resources is therefore essential to ensure a sound conservation of amphibian populations in human-dominated regions under global change.

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CHAPTER 6: Discussion and Conclusion

6.1 Discussion

In this thesis, I investigated how to improve our understanding of biological invasions in freshwater environments, with potential implications for conservation of these habitats. The main questions were:

- a) Is it possible to use data from different sources, including grey literature, to effectively monitor FIS spread?
- b) What are the implications of methodological choices in niche dynamic assessments for FIS?
- c) Is monitoring only one life stage effective to assess FIS and other stressors' impacts on native species with complex life cycles?
- d) How do FIS presence and climate change affect site selection in amphibians?

In Chapter 2, I tried to answer the first question by creating an updated map of the distribution of *Procambarus clarkii* in Italy. Moreover, updated distribution maps have wide application for conservation, and they can be used by local government agencies to plan management actions (Di Marco et al. 2017; Rautsaw et al. 2022). Eradication of invasive species is unachievable after successful establishment (Leuven et al. 2017). For this reason, early detection and prompt responses represent the optimal way to contrast the establishment and spread of invasive species (Leuven et al. 2017). Nonetheless, scientific published literature may suffer from a time lag between the first detection of a species in a certain area and its publication in peer-reviewed papers. As scientific literature may be difficult to access, particularly concerning old records, and there may be a temporal lag between the colonization of an area by a FIS and the update of its range in peer-reviewed articles, integrating data coming from conventional sources and data from grey literature and online sources represents an effective strategy to monitor FIS. Using multiple data sources to return a complete overview of the presence and spread of FIS may thus potentially improve the effectiveness of eradication actions. In the case of *P. clarkii* in Italy, using data coming from different sources was particularly relevant, as 33% of the collected records came from grey literature, online forums, and local newspaper websites, and 62% of all records were in Italian or French.

In Chapter 3, I tried to answer the second question by using presence-only data of 40 FIS, different sets of environmental variables, and two alternative background selection strategies to calculate nine measures of niche dynamics. Niche conservatism is a key assumption of SDMs, and it

has been found to be frequently violated by FIS, hindering accuracy of spatial and temporal projections of distribution (Early & Sax 2014). Previous works have reported low accuracy of SDMs in predicting FIS distributions (Rodríguez-Rey et al. 2019), but whether these findings were caused by biological phenomena or by modelling choices was still disputed. I found that different methodological approaches in variable selection, background selection, and dimensionality may consistently affect niche shift measures. These results question SDMs reliability, potentially deflating their importance to predict potential distribution outside native ranges or under future climatic conditions. Consistent gaps of knowledge on the optimal niche modelling approaches for aquatic invasive species persist. Without a consensus, our ability to individuate general trends on niche shifts and distribution drivers of FIS may be hampered. Planning management actions based on the results of inaccurate SDMs may hinder their effectiveness in contrasting the spread of FIS (Rodríguez-Rey et al. 2019). Local management can benefit from alternative and/or complementary tools, such as connectivity models. Connectivity models allow to predict connectivity using landscape data, and have range of applications in conservation science (McRae et al. 2008; Dickson et al. 2019). For instance, they can be used to predict potential pathways for spread and to prioritize areas for control of invasive species (Glen et al. 2013; Perry et al. 2017; Falaschi et al. 2018). FIS management can particularly benefit from this approach, as hydrological accessibility is a major driver of FIS spread, particularly in those areas where connectivity is greatly affected by the presence of artificial barriers, such as dams (Melles et al. 2015).

Understanding the drivers of FIS distribution is helpful to prevent their spread, but invasive organisms already represent a relevant portion of aquatic communities in many areas (Strayer 2010). For this reason, assessing the impacts of FIS is essential for conservation of freshwater habitats. The effects of FIS on native organisms can be difficult to measure, particularly if the investigated taxa have complex life cycles (Siesa et al. 2014). In Chapter 4, I tried to assess how FIS and other factors affect presence and reproduction of seven amphibian species in Northern Italy. I found relevant differences between the drivers of adult presence and the drivers of reproduction in amphibians, as well as great differences in the occupancy probabilities of adults and aquatic stages (eggs and larvae). Since FIS may affect different life stages of the same species in different ways, it is necessary to collect data on multiple life stages of the focal species to get a thorough overview of its response to FIS presence, despite the consequent increase in the sampling effort. Previous works have already reported differential impacts of FIS on different life stages of the same organisms in

amphibians and dragonflies (Siesa et al. 2014). Moreover, invasive species are known to deeply affect metacommunity mechanisms of amphibians (Falaschi et al. 2020), endangering local populations. In some cases, the incidence of an invasive species at landscape level may be more detrimental to amphibian metapopulation dynamics than the presence of the alien in the investigated site (Falaschi et al. 2021). Assessing how breeding probability of local species is impacted by FIS can potentially improve our understanding of aquatic biodiversity declines, but in order to do so monitoring programs are required to invest more resources in the detection of early life stages (Cruickshank et al. 2021).

In a context of global change, though, conservation of freshwater habitats requires studies of the interactions between FIS and other stressors. Climate change may lead to further establishments and spread of FIS, but also modify biotic interactions, and increase the frequency of encounters between FIS and native species (Havel et al. 2015). The studies assessing the effects of climate change on FIS and their interactions with native species has traditionally considered only one aspect of climate change, namely temperature shifts (Rahel & Olden 2008). While this aspect is certainly relevant, droughts have been rarely investigated, despite many freshwater environments depend on precipitation regimes. As shown in Chapter 6, drought-induced habitat scarcity and its interactions with the presence of invasive species can be particularly detrimental for certain species, particularly for amphibians. As the frequency of biological invasions are expected to increase in certain regions of the world due to climate change (Bellard et al. 2013), these interactive impacts on local communities may become more and more relevant in shaping aquatic biodiversity. In both Chapter 5 and 6, I found a lack of response of the endemic *Rana latastei* toward the local presence of *Procambarus clarkii*. These findings are in accordance with previous reports, that also highlighted detrimental effects of the predator on metapopulation dynamics (Manenti et al. 2020; Romagnoli et al. 2020; Falaschi et al. 2021). Lack of shared evolutionary history between endemic species and FIS might represent a challenge to conservation of freshwater biodiversity. According to the invasion-filter hypothesis, inefficient alien predators fail to become invasive, thus resulting in high rates of asymmetrical predator-prey interactions in which invaders have a large impact on their prey (David et al. 2017). Prey naiveté is likely to be more frequent among freshwater preys than their terrestrial and marine counterparts (Cox & Lima 2006), making these animals particularly vulnerable to the introduction of FIS. Evolutionary responses to FIS have been reported in some native preys after a few generations (e.g., Nunes et al. 2014), potentially mitigating the impacts of invasive predators.

Still, there is also evidence for lack of responses to recently introduced predators, thus long-term monitorings are required to precisely assess the speed of predator recognition evolution and to evaluate the effects of FIS on local biodiversity at greater timescales (Hettyey et al. 2016).

6.2 Future research

Further research is needed to further assess drivers and consequences of FIS presence, applying new methods of investigation, and focusing on neglected aspects. Concerning data collection, while grey literature and can complement data from published sources, they come with limitations and biases, and are online sources not always easily accessible (Bajomi et al. 2010; Corlett 2011; Cross et al. 2019). It is thus important to find strategies to account for these biases and find ways to standardize data coming from untraditional sources. Platforms like ELNAIS (Zenetos et al. 2015) represent an example of how integrating data from multiple sources can be useful for researches on aquatic invasive species. Moreover, since internet access is becoming more and more available all over the globe, approaches like citizen science can help reducing the strong geographical bias between developed and developing countries, that is severely hindering our ability to study biological invasions.

While projections of SDMs on other geographical areas and on future climatic conditions are frequently used in studies of biological invasions and often represent the bases of conservation actions, their reliability depend on niche conservatism across space and/or time (Wiens & Graham 2005; Wiens et al. 2009). As highlighted by the findings of Chapter 3, there is the urgent need for standardized and biologically meaningful approaches in FIS niche dynamics assessment. Without sound assessments of the most appropriate ways to measure niche dynamics, different studies using different approaches are likely to find contrasting results, adding uncertainties, and hindering the effectiveness of conservation measures. While other studies have already tried to assess certain issues, such as number of predictors (Low et al. 2020) and background selection (Nori & Rojas-Soto 2019), the lack of high quality global maps of environmental features of freshwater environments represent a relevant limiting factor to future research. Recent developments of high-quality datasets well representing micro-habitat conditions have been developed for other kind of environments, such as soil (Lembrechts et al. 2021) and glaciers (Marta et al. 2023), but equivalent datasets for freshwater environments are still missing.

Assessments of the effects of FIS on multiple life stages of amphibians can be improved by

accounting for imperfect detection. Future research is needed to apply this approach to other organisms with complex life cycles, such as many aquatic insects. Previous works have already reported differential impacts on insect life stages (e.g., Siesa et al. 2014), but only a handful took into account imperfect detection. Certain life stages can have very low detection probabilities, that if not considered may lead to biased estimations (Bried et al. 2012). Multistate occupancy models allow to account for that, returning reliable results. Actual measures of breeding success, such as the presence of amphibian metamorphs or dragonfly exuviae, should be assessed in future studies to return a better representation of other, potentially hidden effects of FIS and other stressors on amphibian populations. Other under-investigated aspects, such as FIS impacts on microbial communities and on functional diversity should be carefully analyzed, exploiting all the available tools, in order to shed light on less evident consequences of biological invasions in freshwater environments.

6.3 Conclusion

In conclusion, I found that the many innovations developed during the last decades in the study of biological invasions are yet to be widely applied to freshwater species (Chapter 1); unconventional data source can supplement traditional ones to assess FIS spread (Chapter 2); assessments of FIS niche dynamics is significantly affected by different methodological approaches, questioning their reliability (Chapter 3); impacts of biological invasions, as well as other stressors, on organisms with complex life cycles should not neglect certain life stages (Chapter 4); climate change and FIS both contribute to modify reproduction strategies in amphibians, with potential consequences on metapopulation dynamics (Chapter 5). Since there is no evidence for a deceleration in the rate of invasive species introductions (Seebens et al. 2017), future global biodiversity will be shaped by biological invasions. Freshwater environments represent hotspots of endemism and are extremely sensitive to invasions, assessing drivers and impacts of FIS presence is more than ever urgent to preserve aquatic biodiversity worldwide.

6.4 References

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