Invasive Species And Amphibian Conservation

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ABSTRACT: The recognition that invasive alien species (IAS) are among the greatest threats to biodiversity has stimulated a growing interest in their impacts on native amphibians. Here we describe the multifaceted consequences of biological invasions on native amphibians and identify potential mechanisms and strategies that could better enable the long-term persistence of native species. IAS can influence amphibian fitness, population size and community structure via multiple pathways and can exert major, direct impacts through predation, competition and hybridization. The consequences of indirect impacts, too, such as habitat alteration and the spread of emerging diseases, can be particularly severe in native populations. Native amphibians may respond to IAS by modulating aspects of their behaviour, morphology or life history. Nevertheless, it is still unclear the extent to which phenotypic plasticity and rapid evolution may actually help native species withstand the impacts of IAS in invaded communities. Practical management strategies focused on prevention, monitoring and early control are the most effective approaches to allay the impacts of IAS and should be prioritized in pro-active conservation plans. Eradications of IAS and mitigation approaches should they become established are feasible and can greatly improve the status of native populations.

Key words: Amphibian decline; Biological invasions; Contemporary evolution; Fitness; Horizon scanning; Impacts; Invasive fish; Management strategies; Phenotypic plasticity.

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INVASIVE alien species (IAS) are a major threat to biodiversity. Of about 800 animal extinctions that have been recorded since 1500, IAS have been implicated in 33% of them (Blackburn et al. 2019). For amphibians, IAS have been the cause of about one third of extinctions, and ca. 16% of extant species currently are threatened by IAS (Stuart et al. 2008, Blackburn et al. 2019). Nevertheless, biological invasions are a complex process (see Box 1 for definitions a conceptual framework), and the impact of IAS on biodiversity is highly heterogeneous both among habitats and geographic areas. Amphibians living on islands and in freshwater are disproportionately affected by invasive species (Stuart et al. 2008, Strayer 2010, Spatz et al. 2017).

Two decades ago, (see Kats and Ferrer 2003) summarized the negative impacts of IAS on amphibians but, since then, research in this area has not progressed as quickly as studies focusing on habitat loss and diseases. However, interest on this topic has lately shown a dramatic growth (Fig. 1). It is increasingly evident that IAS can have a broad range of impacts on amphibians, affecting species and communities through multiple processes. Yet the impact of IAS can be complex and multifaceted, and often interacts with other global stressors, such as disease (Blaustein and Kiesecker 2002) and habitat change (Didham et al. 2007). The growing awareness of the impacts of IAS has also stimulated research on potential conservation strategies in order to identify management practices that could halt or limit the impact of invasives.

Here we review the recent literature to understand the impact of IAS on native amphibians and evaluate potential mechanisms and strategies that could allow the long term persistence of native species. First, we show that invasive species have a broad range of effects on native amphibians, both direct and indirect. Second, we describe the limited range of options that can allow amphibians to persist in invaded environments, such as plasticity and rapid adaptation. Finally, we show how the threat of invasive species can be limited through prevention, diligent monitoring and early intervention. Our study highlights the complexity of the impact of IAS on amphibians, and identifies multiple open questions for both research and practical conservation.

IMPACT OF ALIEN SPECIES ON AMPHIBIANS

IAS can affect amphibians through a broad range of pathways (Fig. 2) (Bucciarelli et al. 2014, Nunes et al. 2019) including predation (Kats and Ferrer 2003), competition (Richter-Boix et al. 2013), habitat alteration (Matsuzaki et al. 2009), hybridization (Dufresnes et al. 2016) and the spread of disease (Miaud et al. 2016).

Predation

Amphibians with aquatic life-history stages are particularly sensitive to the introduction of alien predators. This is because many amphibians breed in freshwater ecosystems such as isolated ponds or headwater streams where large predators normally are scarce (Cox and Lima 2006). Even though several amphibian species have evolved mechanisms to co-exist with some predacious fishes (Van Buskirk 2003), invasive predators can drive local populations to decline, or even to extinction, because they directly reduce the abundance of eggs, larvae, or adults (Kats and Ferrer 2003, Bucciarelli et al. 2014, Nunes et al. 2019). Fish are probably the most frequently introduced large predators in freshwaters and have caused massive loss of amphibian breeding sites in all the continents (e.g. Knapp and Matthews 2000, Knapp 2005, Tiberti and von Hardenberg 2012). Large fishes, such as Trout, are not alone in exerting impacts on naïve amphibian populations as even small-sized fishes are efficient amphibian predators (Remon et al. 2016, Miró et al. 2018). Besides fishes, many other taxa can exert heavy predation pressure. For instance, carnivorous tadpoles of the Indian

Bullfrogs, *Hoplobatrachus tigerinus*, rapidly prey upon native tadpoles, hampering the survival of larvae of frogs endemic of the Andaman archipelago (Mohanty and Measey 2019).

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

Competition

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

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

Hybridization

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

Hybridization with invasive species is particularly problematic among the hybridogenetic European water frogs of the genus *Pelophylax.* In several areas of Central and Western Europe, the native Pool Frogs, *P. lessonae,* and Edible Frogs, *P. esculentus,* naturally form an “L-E” hybridogenetic system. *Pelophylax esculentus* is a “klepton” between the Marsh Frogs, *P. ridibundus,* and the Pool Frogs, *P. lessonae*, that eliminate the *lessonae* genome druing gametogenesis and clonally transmits the *ridibundus* genome (Vorburger and Reyer 2003, Holsbeek and Jooris 2010). *Pelophlyax ridibundus* frogs native to Eastern Europe, however, are commonly traded for human consumption throughout Europe. In Eastern Europe, *P. ridibundus,* and *P. esculentus* form an “R-E” hybridogenetic system whereby the *ridibundus* genome in *P. esculentus* is eliminated during gametogenesis and the *lessonae* genome is clonally transmitted. When introduced to an L-E system, R-E *P. ridibundus* mate with both native frogs, producing *P. esculentus* offspring with *P. lessonae*, and *P. ridibundus* offspring with *P. esculentus*. Thus, in several areas of Europe, invasive *P. ridibundus* are rapidly spreading at the expense of both native taxa (Vorburger and Reyer 2003, Holsbeek and Jooris 2010). The situation is further complicated because multiple *Pelophylax* species are actually traded throughout Europe. Because the hybridization with *P. ridibundus* can produce sterile or fertile hybrids depending on geographic origin, morphological identification of species is very difficult (Vorburger and Reyer 2003, Holsbeek and Jooris 2010, Quilodrán et al. 2015). Management of this situation is major challenge because of the risk of particularly rapid elimination of the native species (Quilodrán et al. 2018).

Spread of Diseases

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

Habitat Alteration

IAS that become keystone species and ecosystem engineers can cause major habitat alterations, with strong impacts on native amphibians. Changes in habitat structure that alter base levels of wind and solar radiation and thereby modify the thermal landscape can have particularly strong impacts on ectothermic vertebrates (Watling et al. 2011, Garcia and Clusella-Trullas 2019). Some invasive plants are capable of severely modifying both terrestrial and freshwater habitats. For example, the Amur Honeysuckles, *Lonicera maackii*, form a dense shrub layer in invaded forests that result in a decrease of species richness and produces shifts in amphibian community composition (Watling et al. 2011). The invasive earthworm, *Octolasion tyrtaeum*, which modifies the soil by reducing the organic layer, reduces the abundance of Red-Backed Salamanders, *Plethodon cinereus* (Ransom 2017). Because the majority of amphibians spend their adult lifetimes in terrestrial environment, and a many species, especially in tropical areas, are fully terrestrial, the impact of invasive terrestrial plants and other organisms on amphibians is probably underestimated (Nunes et al. 2019).

Indirect and Context-Dependent Impacts of Invasive Species

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

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

AMPHIBIAN RESPONSES TO INVASIVE SPECIES

Although IAS are a major driver of amphibian decline, amphibians have developed a range of ecological, behavioral and evolutionary responses that can improve their fitness and mediate the impact of IAS in complex ways.

How Do You Recognize an Alien Predator?

During biotic invasions, native populations face species with which they do not share a co-evolutionary history, which can hinder the expression of effective responses (Sih et al. 2010, Carthey and Banks 2014). Failing to recognize a predator can be fatal, but several mechanisms can allow native species to identify IAS as predators and activate adequate responses. First, predator recognition can occur when the IAS shows similar traits or is phylogenetically close to native predators. This is the Predator Generalization Hypothesis (Ferrari et al. 2007). Predator generalization can be observed in the San Marcos Salamanders, *Eurycea nana*, which coexist with native Largemouth Bass and will demonstrate the same behavioural response (activity reduction) when exposed to chemical cues of non-native perciform fish (Davis et al. 2012). Second, amphibians in freshwater environments often detect predators via chemical signals, or kairomones (Ferrari et al. 2010, Manenti et al. 2016), and can identify non-native predators when their kairomones are associated with alarm cues released by preyed-upon conspecifics or with predator dietary cues (Fig. 3B). Association with alarm cues can be surprisingly effective. For instance, the repeated exposure to a combination of conspecific alarm cues and chemical cues of otherwise innocuous Zebrafish elicited a remarkable anti-predator response (activity reduction) in tadpoles of the Iberian Green Frog, *Pelophylax perezi*, even when subsequently exposed to Zebrafish cues only (Gonzalo et al. 2007). Finally, anti-predator response towards IAS might be mediated by neophobia (i.e. the generalized avoidance response to novel stimuli; Brown et al. 2013), or simply through generic risk cues such as avoiding large-sized moving shapes (Wilson et al. 2018).

Often, though, no response of amphibians to IAS can be detected (Fig. 3), bringing the effectiveness of potential mechanisms into question. Nonetheless, in nature, behavioral patterns can be complex. A lack of response to the kairomones of an invasive predator may be compensated by other chemical signals such as alarm or digestion cues. Moreover, some habitat conditions can increase a species' ability to respond to novelties such as invasive predators. For instance, tadpoles living in more risky environments can respond more promptly to novel predators and have enhanced survival (Ferrari et al. 2015).

Phenotypic Plasticity

Phenotypic plasticity,(i.e. the capacity of a given genotype to express different phenotypic responses under diverging environmental conditions; Pigliucci 2001), is a key evolutionary mechanism that allows species to persist under unpredictable conditions. Thus plasticity can represent a key defense against alien species (Peacor et al. 2006, Berthon 2015). Plasticity in amphibians is well documented, as these organisms are frequently subject to heterogeneous and variable ecological pressures (Wells 2007), and IAS often induce plastic responses in native amphibians (Fig. 3C-E). Invasive predators can trigger the activation of multiple inducible defenses (Fig. 3B). These may be behavioral, including e.g. reduced activity, avoidance, microhabitat shift (Gamradt et al. 1997, Nunes et al. 2013), morphological (Nunes et al. 2014), or ontogenetic, involving e.g. faster growth or development rate (Nunes et al. 2019, Smith and Harmon 2019). For instance, some populations of *Pelophylax perezi* develop deeper tail muscles when reared in the presence of invasive crayfish, a trait that can favor faster swim and escape from predators (Dayton et al. 2005, Nunes et al. 2014). Similarly, exposure to an invasive fish will elicit faster development in tadpoles of the Gray Treefrog, *Hyla versicolor*, which then metamorphose more quickly and leave riskier environments sooner (Smith and Harmon 2019). Thus phenotypic plasticity can offer advantages to native amphibian species facing both invasive predators and competitors, and can help to overcome mating disruptions or habitat modifications, thus broadening the range of conditions under which they can survive (Peacor et al. 2006, Caut et al. 2013, Polo-Cavia and Gomez-Mestre 2014, Hossie et al. 2017). Finally, plasticity can offer natural selection a pool of variability that can favor the emergence and fixation of new adaptive phenotypes through genetic assimilation and canalization (Levis et al. 2018), which could help the long-term persistence of amphibians facing biotic invasions (Peacor et al. 2006, Berthon 2015).

Behavioral Responses

Behavioral responses to IAS are often rapid as they are both modulated by plasticity and influenced by experience (Sih et al. 2010, Weis and Sol 2016). It is no surprise that a large amount of research has focused on behavioral response of native species toward invasive predators (Fig. 3D). In amphibians, common anti-predator behaviors include activity variation or reduction to limit predator exposure, space use modification to enhance predator avoidance, shift in micro-habitat to reduce niche overlap and aggregation to dilute risk (Wells 2007). Several studies have demonstrated these behavioral responses in amphibian larvae exposed to alien predators (Caut et al. 2013, Nunes et al. 2014, Polo-Cavia and Gomez-Mestre 2014). For instance, toad tadpoles that recognize invasive predators reduce activity levels, which increases their survival (Polo-Cavia and Gomez-Mestre 2014). Nevertheless, behavioral responses against invasive predators can be weaker than the ones to native predators (Nunes et al. 2019).

There is less information on anti-predator responses in adult amphibians (Winandy and Denoël 2013, Winandy et al. 2016). Alpine Newts, *Ichthyosaura alpestris*, exposed to goldfish spend more time in refuges to reduce predation risk, but consequently decrease their courtship activity, which affects breeding dynamics (Winandy and Denoël 2013). Amphibians can also adjust their behavior in response to other interactions with IAS apart from predation, including disturbance, habitat modification, competition or reproductive interference. For example, males of the Australian Marble Frogs, *Limnodynastes convexiusculus*, adjust their calls in the presence of invasive Cane Toads, *Rhinella marina*, by reducing frequency and matching cane toad calling pauses in order to reduce overlap with the calls of the toads (Bleach et al. 2015).

Strong Selective Pressure Can Foster Rapid Adaptation

In a sense, biotic invasions are a global, unintended experiment in unravelling the mechanisms of natural selection (Strauss et al. 2006). Rapid adaptation is possible when a species is exposed to a strong novel selective force, and this has been documented in multiple taxa exposed to alien species. In amphibians, rapid adaptation in response to invasive predators (Fig. 3C) has occurred in multiple systems (Moore et al. 2004, Nunes et al. 2014). For instance, *Pelophylax perezi* tadpoles originating from populations where the invasive red swamp crayfish, *Procambarus clarkia*, has been resident for ca. 30 years demonstrate consistently lower activity levels when exposed to the crayfish than did tadpoles from populations where the crayfish is not present (Nunes et al. 2014). They can also express an inducible morphological defense, deeper tails, not seen in tadpoles from uninvaded populations, further indicating that rapid adaptive evolution has occurred in the invaded populations. Rapid adaptation to invasive predators might be widespread among amphibians but current information is limited and the genetic mechanisms involved in such adaptations remains poorly known.

Can Responses Improve Amphibian Persistence?

Although there is considerable evidence that amphibians respond to the presence of IAS, demonstrations that those responses can increase survival and coexistence with invasive species remain rare (Polo-Cavia and Gomez-Mestre 2014). In some instances, it is evident that selection has rapidly favored the fixation of advantageous traits that probably help populations to withstand IAS for long periods (Nunes et al. 2014). On the other hand, declines observed in many invaded populations suggest that amphibian responses often are insufficient. Assessing the effectiveness of responses under natural conditions is particularly complex, because multiple abiotic and biotic factors act together in a context-dependent fashion (Blaustein and Kiesecker 2002). Despite these difficulties, understanding whether responses to IAS can help long-term persistence of invaded populations is important information as it may enable prediction of the consequences of invasions and identify the conditions under which management actions are most likely to be successful.

AMPHIBIAN CONSERVATION IN THE FACE OF BIOLOGICAL INVASIONS

Given the multiple impacts of alien species on amphibians, it is essential to adopt effective management strategies at all stages of invasions: prevention of the introduction of harmful species, early detection of introduced species, active containment and eradication of IAS, and mitigation of their impact.

An Ounce Of Prevention Or A Pound Of Cure?

Some of the IAS exerting the strongest impact on amphibians have been introduced through trade of fish bait, pets and live food for pets (Pethiyagoda and Manamendra-Arachchi 2012, Bellard et al. 2016, Gozlan et al. 2019). The pet trade is particularly problematic as it can also cause the introduction of non-native genotypes and the spread of diseases (Bellard et al. 2016, O'Hanlon et al. 2018). The most economically developed regions, where most exotic animal pets are sold, also host a higher richness of invasive species (Fonseca et al. 2019). Major impacts are also caused by species sold as food for human consumption. These species, once released into freshwater systems, can prey upon native amphibian species, as European salmonids have done after their introduction into American streams (Knapp and Matthews 2000, Pope 2008).

Because the impacts of IAS on native amphibian populations are often heavy, complex and poorly predictable, it is essential to establish effective protocols to prevent the introduction of species that can naturalize and become invasive. As complete bans of all trade activities involving live animals would be virtually impossible, other means of control are required. Blacklists of problematic species are one way to help set up trade regulations and focus screening protocols. Several strategies exist to identify species that should be blacklisted. First, species that are already invasive in some areas or resemble known, problematic invasive species in terms of phylogenetic relatedness, life history or ecological traits can be prioritized (Masin et al. 2014, Maceda-Veiga et al. 2019). This approach, though, may miss many potential new invaders. Integrated horizon scanning procedures are valuable for identifying such new IAS that have the potential to affect native species but are not yet established in a region. Horizon scanning is a structured consultation with experts of multiple taxonomic groups, associated with consensus-building procedures and, in some cases, modelling of invasion risks. In the UK, horizon scanning enabled the creation of a ranked list of species with highest risk of arrival, establishment and impact, and was able to predict new IAS with some success (Aldridge et al. 2014, Roy et al. 2014). Horizon scanning can thus produce inclusive and dynamic lists of actual and potential IAS that can be made subject to regulations and restrictions on trade and propagation (e.g. European Regulation 1143/2014; Tollington et al. 2017).

Live organisms of all kinds are constantly being spread via international and intercontinental flights, ships, roads and railways. Although detailed screening protocols have been established in several countries, especially for international flights, it is hard to find information on the effectiveness of such management schemes. New Zealand and Australia have very strict protocols to hamper the introduction of new alien species. In these countries, all livestock must satisfy a rigorous import health standard on the basis of frequently updated risk assessment procedures (Henderson et al. 2011). Setting up effective screening protocols also requires that all personnel staffing trading facilities are updated on emerging issues. Airport managers in a number of major commercial hubs regularly met with IAS specialists to stay up-to-date on current incoming problematic species (Bisi et al. 2018). Developing prevention protocols for intra-continental trade is more challenging because regulations at this level often allow free trade, making the control and tracking of living organisms transported for food or pets extremely difficult (Ficetola et al. 2008a, Kikillus et al. 2012).

Commercially traded animals that are maintained in captivity do not become alien species unless they escape or are released into natural environments (Box 1). Restaurant managers or the owners of pets may decide to release excess animals into nearby habitats. More seriously, when aquaculture businesses fail, they may simply release their stocks of exotic organisms. This was most likely the pathway for the introduction of the red swamp crayfish in Europe and Africa (Gherardi 2006). Prevention of these releases is feasible but challenging, and the role of single companies, organizations and even individuals in the unintended release of alien species should not be underestimated.

Horizon scanning procedures and outreach campaigns can help to prevent the release of imported captive animals. Species identified as invasion risks could be made to require specific procedures of containment, such as special enclosures for aquaculture (Liu and Li 2009), or their trade as pets could be forbidden. Nevertheless, even after the adoption of dedicated legislation in Spain that has prohibited many species from entering the pet market, such animals continue to be released into the wild by their owners (Maceda-Veiga et al. 2019). It is thus essential to combine regulations with dedicated education and outreach activities to ensure the actual implementation of best practices (Ficetola et al. 2012a, Maceda-Veiga et al. 2019).

Early Detection

Even after alien species have been introduced, early detection can still allow for effective eradication. The problem is that, immediately after introduction, non-native species often are at very low abundance and remain elusive. This makes it essential planning the regular monitoring of areas at highest risk of new introductions, such as suburban areas or the surroundings of major seaports and airports, using techniques that enable detection of a wide range of potentially invasive species (Murphy et al. 2013). The use of environmental DNA (eDNA) is emerging as a particularly effective strategy for early detection of invasive species, particularly in freshwater (Ficetola et al. 2019). eDNA can be extracted from water and soil, allowing the detection of IAS while population size is still small and could remain unnoticed using normal surveys (Ficetola et al. 2019). In Europe, eDNA has been used to monitor American Bullfrogs and enabled detection of bullfrogs in more sites compared to traditional visual and audio surveys (Ficetola et al. 2008b, Dejean et al. 2012). Regular eDNA sampling and amplification with primers designed on the basis of national or regional risk assessments can allow prompt detection of IAS, which is essential for early and effective eradication plans (Bellard et al. 2016, Strand et al. 2019).

Mitigation and Management to Ensure Long-Term Persistence of Amphibians

In relatively small sites, removal campaigns performed consistently through time can successfully remove those invasive predators that most reduce amphibian breeding success (Ahola et al. 2006). For instance, the removal of invasive Brook Trout, *Salvelinus fontenalis*, from western Italian Alpine lakes allowed the prompt recovery of breeding activity by European Common Frogs, *Rana temporaria* (Tiberti et al. 2019). However, when invasive species have spread over entire regions, eradications become far more complex. Actions must occur simultaneously at multiple sites as invasive species may often exist in networks of interconnected subpopulations. Management actions will have limited effectiveness if such spatial connections among sites are overlooked (Nicol et al. 2017, Manenti et al. 2020). There have been successes in removing some IAS, but eradication of most IAS is challenging and may not even be feasible if they occur in multiple metapopulations or very large waterbodies (Day et al. 2018). In such cases, effective management strategies to ensure long-term survival of amphibians affected by IAS can focus on keeping IAS densities low and providing suitable amphibian breeding sites that cannot be accessed or effectively colonized by IAS. Artificial ponds surrounded by vertical stone banks (Fig. 4A-B), for example, can be built that will allow amphibians to reach the water but which cannot be climbed by invasive crayfish (Fig. 4A-B; Bruni 2010). Similarly, it is possible to build barriers in small streams that prevent upstream dispersal of invaders. As recently proposed in Switzerland, thin foils of stainless steel (Fig 4C) may be used to coat the vertical and horizontal surface of small waterfalls in such streams (30 cm can be sufficient, Fig 4C; Manfrin et al. 2019).

In some places, stopping new invasions by alien species is impossible. In some urban or high-use areas, local people may repeatedly release predatory fishes and aquatic reptiles. In complex hydrographical networks, IAS may continuously spread from source populations. In these cases, small artificial ponds that can regularly be dried after tadpoles reach metamorphosis can be refuges for pond-breeding amphibians (Werner et al. 2007, Ficetola et al. 2012b). Finally, it is essential that researchers and managers share information about successful versus unsuccessful management practices. Most of the time, only unpublished technical reports performed during specific projects are available, and long-term assessments of the effectiveness of mitigation actions are lacking. Studies comparing amphibian densities and distributions before and after the control or eradication of IAS are essential to ensure successful long-term persistence of native populations and develop effective conservation protocols for amphibians in relation to IAS at all levels, from preventing IAS introductions to eradicating IAS if established to mitigating IAS effects.

CONCLUSIONS

Our understanding of the multifaceted impacts of IAS on native amphibian populations is increasingly deep, yet a disconnection remains between academic research on IAS and conservation efforts. Many studies have described the multiple impacts of IAS following invasions, while much less research has measured the benefits of alternative management strategies. Is the complete eradication of IAS necessary for maintaining native populations? Or, would habitat management and thinning actions aimed at limiting IAS abundance be enough? Answers to these questions will require a trial-and-error approach and the publication of management results to allow evidence-based conservation (Schmidt et al. 2020).

Although it has long been recognized that the impacts of IAS are context dependent and heavily affected by environmental conditions (White et al. 2006, Didham et al. 2007), too many studies consider just one or a few factors. Multivariate analyses that take into account the complexity of parameters determining population dynamics at a range of spatial scales or that can evaluate what happens when we manage multiple stressors are sorely need (Falaschi et al. 2019). It is also evident that native species can show some adaptive responses to IAS (Fig. 3), still identifying, for example, a behavioral shift in presence of IAS does not mean that tadpoles will be better able to survive. Although measuring fitness in nature has long been a major challenge for evolutionary biology and ecology, we have now an increasingly complete analytical toolbox available (e.g. Dodd 2010) that can better enable quantitative fitness estimation. Producing quantitative measures of population responses in the field is pivotal for fine-tuning ongoing actions and guiding future mitigation efforts.

In some contexts, management of IAS has been extremely successful, allowing quick restoration of native species. For every five species of birds and mammals that have deteriorated in conservation status because of IAS, two have been estimated to have improved in status through mitigation efforts (Hoffmann et al. 2010). For amphibians, however, conservations actions have been rarer, and measures of their success have been limited (Hoffmann et al. 2010). It is imperative to transform our increasing knowledge of IAS and their effects on native amphibian populations into evidence-based conservation actions.

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LITERATURE CITED

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

Aldridge, D.C., S. Ho, and E. Froufe. 2014. The Ponto-Caspian quagga mussel, *Dreissena rostriformis bugensis* (Andrusov, 1897), invades Great Britain. Aquatic Invasions 9:529-535.

Bellard, C., P. Genovesi, and J.M. Jeschke. 2016. Global patterns in threats to vertebrates by biological invasions. Proceedings of the Royal Society B-Biological Sciences 283:20152454.

Berthon, K. 2015. How do native species respond to invaders? Mechanistic and trait-based perspectives. Biological Invasions 17:2199-2211.

Bisi, F., C. Montagnani, E. Cardarelli, R. Manenti, S. Trasforini, R. Gentili, ... A. Martinoli. 2018. Strategia di azione e degli interventi per il controllo e la gestione delle specie alloctone in Regione Lombardia. Regione Lombardia, Milano (Italy).

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

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

Blackburn, T.M., P. Pysek, S. Bacher, J.T. Carlton, R.P. Duncan, V. Jarosik, ... D.M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26:333-339.

Blaustein, A.R., and J.M. Kiesecker. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters 5:597-608.

Bleach, I.T., C. Beckmann, C. Both, G.P. Brown, and R. Shine. 2015. Noisy neighbours at the frog pond: effects of invasive cane toads on the calling behaviour of native Australian frogs. Behavioral Ecology and Sociobiology 69:675-683.

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

Brook, B.W., N.S. Sodhi, and C.J.A. Bradshaw. 2008. Synergies among extinction drivers under global change. Trends in Ecology & Evolution 23:453-460.

Brown, G.E., M.C. Ferrari, C.K. Elvidge, I. Ramnarine, and D.P. Chivers. 2013. Phenotypically plastic neophobia: a response to variable predation risk. Proceedings of the Royal Society B: Biological Sciences 280:20122712.

Bruni, G. 2010. Presentazione dei metodi utilizzati all'interno del Parco della Piana (ANPIL Podere la Querciola) di Sesto Fiorentino, per garantire la riproduzione degli anfibi nonostante la presenza del gambero rosso della Louisiana Pp 7-14 in I Convegno del Forum Natura Mediterraneo. https://www.naturamediterraneo.com/primoconvegnoNM/, Selva di Paliano (FR), Italy.

Bucciarelli, G.M., A.R. Blaustein, T.S. Garcia, and L.B. Kats. 2014. Invasion complexities: The diverse impacts of nonnative species on amphibians. Copeia 2014:611-632.

Carthey, A.J., and P.B. Banks. 2014. Naïveté in novel ecological interactions: lessons from theory and experimental evidence. Biological Reviews 89:932-949.

Caut, S., E. Angulo, C. Díaz-Paniagua, and I. Gomez-Mestre. 2013. Plastic changes in tadpole trophic ecology revealed by stable isotope analysis. Oecologia 173:95-105.

Cox, J.G., and S.L. Lima. 2006. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution 21:674-680.

D'Amore, A. 2012. *Rana* (*Lithobates*) *catesbeiana* Shaw (American Bullfrog). Pp 321-330 in Handbook of global freshwater invasive species (R. Francis, ed.). Earthscan, Taylor & Francis Group, Abingdon, UK.

Davis, D.R., K.J. Epp, and C.R. Gabor. 2012. Predator generalization decreases the effect of introduced predators in the San Marcos Salamander, *Eurycea nana*. Ethology 118:1191-1197.

Day, C.C., E.L. Landguth, A. Bearlin, Z.A. Holden, and A.R. Whiteley. 2018. Using simulation modeling to inform management of invasive species: A case study of eastern brook trout suppression and eradication. Biological Conservation 221:10-22.

Dayton, G.H., D. Saenz, K.A. Baum, R.B. Langherans, and T.J. DeWitt. 2005. Body shape, burst speed and escape behavior of larval anurans. Oikos 111:582-591.

Dejean, T., A. Valentini, C. Miquel, P. Taberlet, E. Bellemain, and C. Miaud. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. Journal of Applied Ecology 49:953-959.

Denoël, M., G.F. Ficetola, N. Sillero, G. Džukić, M.L. Kalezić, T. Vukov, ... B. Lejeune. 2019. Traditionally managed landscapes do not prevent amphibian decline and the extinction of paedomorphosis. Ecological Monographs 89:e01347.

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

DeVore, J.L., and J.C. Maerz. 2014. Grass invasion increases top-down pressure on an amphibian via structurally mediated effects on an intraguild predator. Ecology 95:1724-1730.

Didham, R.K., J.M. Tylianakis, N.J. Gemmell, T.A. Rand, and R.M. Ewers. 2007. Interactive effects of habitat modification and species invasion on native species decline. Trends in Ecology & Evolution 22:489-496.

Dodd, C.K.J., (ed.) 2010. Amphibian ecology and conservation. A handbook of techniques. Oxford University Press, Oxford, UK.

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

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

Ferrari, M.C.O., G.E. Brown, G.R. Bortolotti, and D.P. Chivers. 2010. Linking predator risk and uncertainty to adaptive forgetting: a theoretical framework and empirical test using tadpoles. Proceedings of the Royal Society B-Biological Sciences 277:2205-2210.

Ferrari, M.C.O., A.L. Crane, G.E. Brown, and D.P. Chivers. 2015. Getting ready for invasions: can background level of risk predict the ability of naïve prey to survive novel predators? Scientific Reports 5:8309.

Ferrari, M.C.O., A. Gonzalo, F. Messier, and D.P. Chivers. 2007. Generalization of learned predator recognition: an experimental test and framework for future studies. Proceedings of the Royal Society B-Biological Sciences 274:1853-1859.

Ficetola, G.F., A. Bonin, and C. Miaud. 2008a. Population genetics reveals origin and number of founders in a biological invasion. Molecular Ecology 17:773-782.

Ficetola, G.F., R. Manenti, and P. Taberlet. 2019. Environmental DNA and metabarcoding for the study of amphibians and reptiles: species distribution, the microbiome, and much more. Amphibia-Reptilia 40:129-148.

Ficetola, G.F., C. Miaud, F. Pompanon, and P. Taberlet. 2008b. Species detection using environmental DNA from water samples. Biology Letters 4:423-425.

Ficetola, G.F., D. Rödder, and E. Padoa-Schioppa. 2012a. *Trachemys scripta* (Slider terrapin). Pp 331-339 in Handbook of global freshwater invasive species (R. Francis, ed.). Earthscan, Taylor & Francis Group, Abingdon, UK.

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

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

Ficetola, G.F., W. Thuiller, and C. Miaud. 2007. Prediction and validation of the potential global distribution of a problematic alien invasive species — the American bullfrog. Diversity and Distributions 13:476-485.

Fisher, M.C., T.W.J. Garner, and S.F. Walker. 2009. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annual Review of Microbiology 63:291-310.

Fitzpatrick, B.M., J.R. Johnson, D.K. Kump, J.J. Smith, S.R. Voss, and H.B. Shaffer. 2010. Rapid spread of invasive genes into a threatened native species. Proceedings of the National Academy of Sciences of the United States of America 107:3606-3610.

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

Fonseca, E., C. Both, and S.Z. Cechin. 2019. Introduction pathways and socio-economic variables drive the distribution of alien amphibians and reptiles in a megadiverse country. Diversity and Distributions 25:1130-1141.

Gamradt, S.C., L.B. Kats, and C.B. Anzalone. 1997. Aggression by non-native crayfish deters breeding in California newts. Conservation Biology 11:793-796.

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

Garner, T.W.J., M.W. Perkins, P. Govindarajulu, D. Seglie, S. Walker, A.A. Cunningham, and M.C. Fisher. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biology Letters 2:455-459.

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

Gonzalo, A., P. Lopez, and J. Martin. 2007. Iberian green frog tadpoles may learn to recognize novel predators from chemical alarm cues of conspecifics. Animal Behaviour 74:447-453.

Gould, J., J.W. Valdez, M.P. Stockwell, S. Clulow, and M.J. Mahony. 2019. Mosquitoes as a potential vector for the transmission of the amphibian chytrid fungus. Zoology and Ecology 29:38-44.

Gozlan, R.E., B.K. Karimov, E. Zadereev, D. Kuznetsova, and S. Brucet. 2019. Status, trends, and future dynamics of freshwater ecosystems in Europe and Central Asia. Inland Waters 9:78-94.

Henderson, W., M. Bomford, and P. Cassey. 2011. Managing the risk of exotic vertebrate incursions in Australia. Wildlife Research 38:501-508.

Hoffmann, M., C. Hilton-Taylor, A. Angulo, M. Bohm, T.M. Brooks, S.H.M. Butchart, ... S.N. Stuart. 2010. The impact of conservation on the status of the world's vertebrates. Science 330:1503-1509.

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

Hossie, T., K. Landolt, and D.L. Murray. 2017. Determinants and co-expression of anti-predator responses in amphibian tadpoles: a meta-analysis. Oikos 126:173-184.

Kats, L.B., and R.P. Ferrer. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9:99-110.

Kikillus, K.H., K.M. Hare, and S. Hartley. 2012. Online trading tools as a method of estimating propagule pressure via the pet-release pathway. Biological Invasions 14:2657-2664.

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

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

Lesbarrères, D., and J.-F. Bienentreu. 2020. Amphibian disease ecology: are we just scratching the surface? Herpetologica:this issue.

Levis, N.A., A.J. Isdaner, and D.W. Pfennig. 2018. Morphological novelty emerges from pre-existing phenotypic plasticity. Nature Ecology & Evolution 2:1289-1297.

Liu, X., and Y.M. Li. 2009. Aquaculture enclosures relate to the establishment of feral populations of introduced species. PLoS ONE 4:E6199.

Maceda-Veiga, A., J. Escribano-Alacid, A. Martínez-Silvestre, I. Verdaguer, and R. Mac Nally. 2019. What’s next? The release of exotic pets continues virtually unabated 7 years after enforcement of new legislation for managing invasive species. Biological Invasions 21:2933-2947.

Manenti, R., M. Falaschi, D. Delle Monache, S. Marta, and F. Ficetola. 2020. Network-scale effects of invasive species on spatially-structured amphibian populations. Ecography:doi: 10.1111/ecog.04571.

Manenti, R., A. Melotto, M. Denoël, and G.F. Ficetola. 2016. Amphibians breeding in refuge habitats have larvae with stronger anti-predatory responses. Animal Behaviour 118:115-121.

Manfrin, C., C. Souty-Grosset, M.P. Anastácio, J. Reynolds, and G.P. Giulianini. 2019. Detection and control of invasive freshwater crayfish: From traditional to innovative methods. Diversity 11:159-172.

Martel, A., M. Blooi, C. Adriaensen, P. Van Rooij, W. Beukema, M.C. Fisher, ... F. Pasmans. 2014. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science 346:630-631.

Masin, S., A. Bonardi, E. Padoa Schioppa, L. Bottoni, and G.F. Ficetola. 2014. Risk of invasion by frequently traded freshwater turtles. Biological Invasions 16:217-231.

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

Measey, G.J., G. Vimercati, F.A. de Villiers, M. Mokhatla, S.J. Davies, C.J. Thorp, ... S. Kumschick. 2016. A global assessment of alien amphibian impacts in a formal framework. Diversity and Distributions 22:970-981.

Menge, B.A. 1995. Indirect effects in marine rocky intertidal interaction webs: Patterns and importance. Ecological Monographs 65:21-74.

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

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

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

Mooney, H.A., and E.E. Cleland. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences of the United States of America 98:5446-5451.

Moore, R.D., R.A. Griffiths, C.M.O. Brien, A. Murphy, and D. Jay. 2004. Induced defences in an endangered amphibian in response to an introduced snake predator. Oecologia 141:139-147.

Murphy, G., A. Vaux, and J. Medlock. 2013. Challenges in undertaking mosquito surveillance at UK seaports and airports to prevent the entry and establishment of invasive vector species. International Journal of Environmental Health Research 23:181-190.

Nelson, D.W.M., M.R. Crossland, and R. Shine. 2010. Indirect ecological impacts of an invasive toad on predator-prey interactions among native species. Biological Invasions 12:3363-3369.

Nicol, S., R. Sabbadin, N. Peyrard, and I. Chades. 2017. Finding the best management policy to eradicate invasive species from spatial ecological networks with simultaneous actions. Journal of Applied Ecology 54:1989-1999.

Nunes, A.L., J.M. Fill, S.J. Davies, M. Louw, A.D. Rebelo, C.J. Thorp, ... J. Measey. 2019. A global meta-analysis of the ecological impacts of alien species on native amphibians. Proceedings of the Royal Society B: Biological Sciences 286:20182528.

Nunes, A.L., G. Orizaola, A. Laurila, and R. Rebelo. 2014. Rapid evolution of constitutive and inducible defenses against an invasive predator. Ecology 95:1520-1530.

Nunes, A.L., A. Richter-Boix, A. Laurila, and R. Rebelo. 2013. Do anuran larvae respond behaviourally to chemical cues from an invasive crayfish predator? A community-wide study. Oecologia 171:115-127.

O'Hanlon, S.J., A. Rieux, R.A. Farrer, G.M. Rosa, B. Waldman, A. Bataille, ... M.C. Fisher. 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. Science 360:621.

Peacor, S.D., S. Allesina, R.L. Riolo, and M. Pascual. 2006. Phenotypic plasticity opposes species invasions by altering fitness surface. PLoS Biology 4:e372.

Pethiyagoda, R.S., and K. Manamendra-Arachchi. 2012. Endangered anurans in a novel forest in the highlands of Sri Lanka. Wildlife Research 39:641-648.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. John Hopkins University Press, Baltimore, USA.

Polo-Cavia, N., and I. Gomez-Mestre. 2014. Learned recognition of introduced predators determines survival of tadpole prey. Functional Ecology 28:432-439.

Pope, K.L. 2008. Assessing changes in amphibian population dynamics following experimental manipulations of introduced fish. Conservation Biology 22:1572-1581.

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

Puth, L.M., and D.M. Post. 2005. Studying invasion: have we missed the boat? Ecology Letters 8:715-721.

Quilodrán, C.S., M. Currat, and J.I. Montoya-Burgos. 2018. Effect of hybridization with genome exclusion on extinction risk. Conservation Biology 32:1139-1149.

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

Ransom, T.S. 2017. Local distribution of native and invasive earthworms and effects on a native salamander. Population Ecology 59:189-204.

Remon, J., D.S. Bower, T.F. Gaston, J. Clulow, and M.J. Mahony. 2016. Stable isotope analyses reveal predation on amphibians by a globally invasive fish (*Gambusia holbrooki*). Aquatic Conservation: Marine and Freshwater Ecosystems 26:724-735.

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

Rogalski, M.A., and D.K. Skelly. 2012. Positive effects of nonnative invasive *Phragmites australis* on larval bullfrogs. PLoS ONE 7:e44420.

Roy, H.E., J. Peyton, D.C. Aldridge, T. Bantock, T.M. Blackburn, R. Britton, ... K.J. Walker. 2014. Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. Global Change Biology 20:3859-3871.

Ryan, M.E., J.R. Johnson, and B.M. Fitzpatrick. 2009. Invasive hybrid tiger salamander genotypes impact native amphibians. Proceedings of the National Academy of Sciences 106:11166-11171.

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

Scheele, B.C., F. Pasmans, L.F. Skerratt, L. Berger, A. Martel, W. Beukema, ... S. Canessa. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459.

Schmidt, B.R., B. Brenneisen, and S. Zumbach. 2020. Evidence-based amphibian conservation: a case study on toad tunnels. Herpetologica (this issue).

Sih, A., D.I. Bolnick, B. Luttbeg, J.L. Orrock, S.D. Peacor, L.M. Pintor, ... J.R. Vonesh. 2010. Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos 119:610-621.

Smith, G.R., and J.J. Harmon. 2019. Differential oviposition and offspring success of gray treefrogs in the presence of an invasive fish. Ecosphere 10:e02612.

Spatz, D.R., K.M. Zilliacus, N.D. Holmes, S.H.M. Butchart, P. Genovesi, G. Ceballos, ... D.A. Croll. 2017. Globally threatened vertebrates on islands with invasive species. Science Advances 3.

Strand, D.A., S.I. Johnsen, J.C. Rusch, S. Agersnap, W.B. Larsen, S.W. Knudsen, ... T. Vrålstad. 2019. Monitoring a Norwegian freshwater crayfish tragedy: eDNA snapshots of invasion, infection and extinction. Journal of Applied Ecology 56:1661-1673.

Strauss, S.Y., J.A. Lau, and S.P. Carroll. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecology Letters 9:357-374.

Strayer, D.L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater Biology 55:152-174.

Stuart, S.N., M. Hoffman, J.S. Chanson, N.A. Cox, R.J. Berridge, P. Ramani, and B.E. Young, (eds.) 2008. Threatened amphibians of the world. Lynx Editions, IUCN and Conservation International, Barcelona, Spain.

Tiberti, R., G. Bogliani, S. Brighenti, R. Iacobuzio, K. Liautaud, M. Rolla, ... B. Bassano. 2019. Recovery of high mountain Alpine lakes after the eradication of introduced brook trout *Salvelinus fontinalis* using non-chemical methods. Biological Invasions 21:875-894.

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

Todd, B.D., B.B. Rothermel, R.N. Reed, T.M. Luhring, K. Schlatter, L. Trenkamp, and J.W. Gibbons. 2008. Habitat alteration increases invasive fire ant abundance to the detriment of amphibians and reptiles. Biological Invasions 10:539-546.

Tollington, S., A. Turb, W. Rabitsh, J.J. Groombridge, R. Scalera, F. Essl, and A. Shwartz. 2017. Making the EU legislation on invasive species a conservation success. Conservation Letters 10:112-120.

Van Buskirk, J. 2003. Habitat partitioning in European and North American pond-breeding frogs and toads. Diversity and Distributions 9:399-410.

Vorburger, C., and H.-U. Reyer. 2003. A genetic mechanism of species replacement in European waterfrogs? Conservation Genetics 4:141-155

Watling, J.I., C.R. Hickman, and J.L. Orrock. 2011. Invasive shrub alters native forest amphibian communities. Biological Conservation 144:2597-2601.

Weis, J.S., and D. Sol. 2016. Biological invasions and animal behaviour. Cambridge University Press.

Wells, K.D. 2007. The ecology and behavior of amphibians. The University of Chicago Press, Chicago.

Werner, E.E., D.K. Skelly, R.A. Relyea, and K.L. Yurewicz. 2007. Amphibian species richness across environmental gradients. Oikos 116:1697-1712.

White, E.M., J.C. Wilson, and A.R. Clarke. 2006. Biotic indirect effects: a neglected concept in invasion biology. Diversity and Distributions 12:443-455.

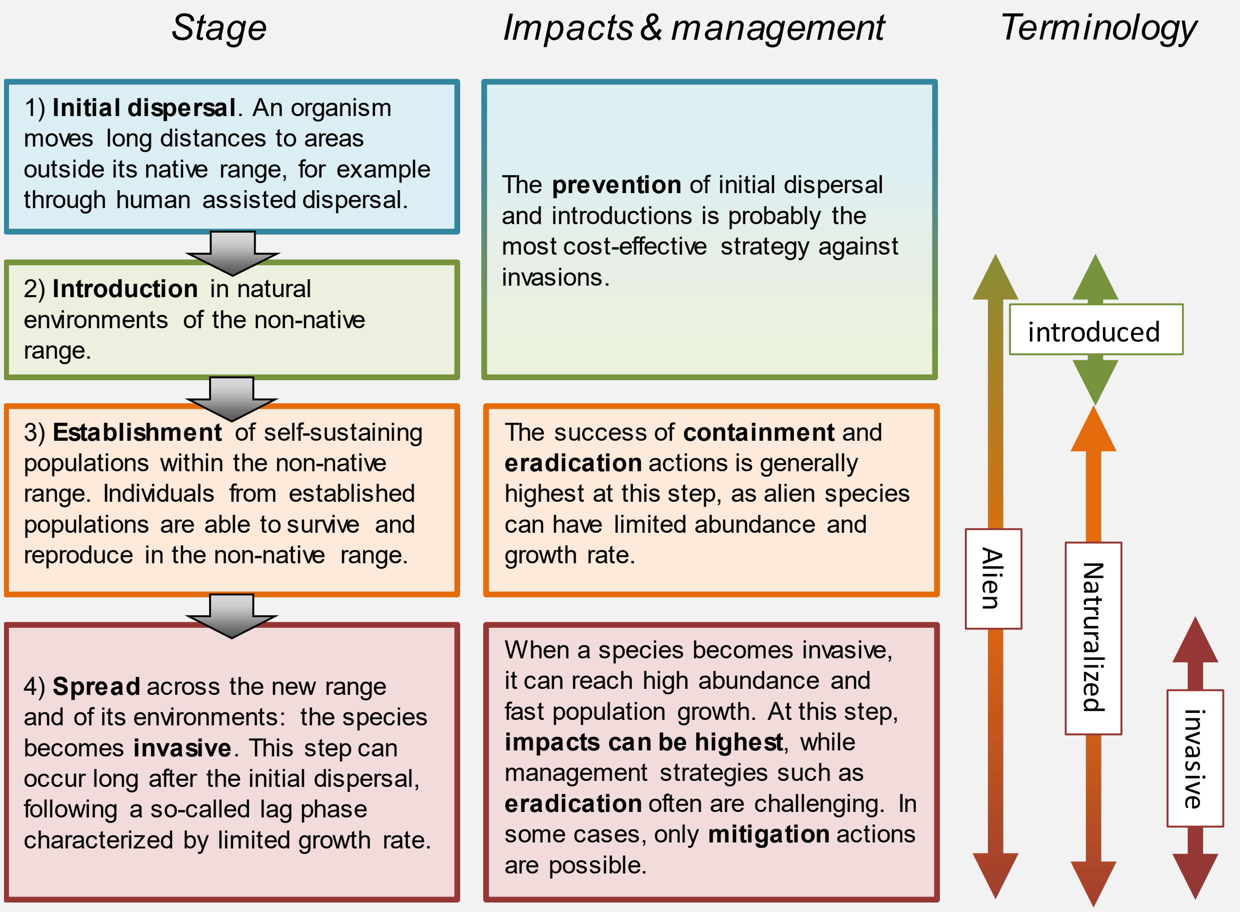
Wilson, E.A., T.L. Dudley, and C.J. Briggs. 2018. Shared behavioral responses and predation risk of anuran larvae and adults exposed to a novel predator. Biological Invasions 20:475-485.

Winandy, L., M. Colin, and M. Denoël. 2016. Temporal habitat shift of a polymorphic newt species under predation risk. Behavioral Ecology 27:1025-1032.

Winandy, L., and M. Denoël. 2013. Introduced goldfish affect amphibians through inhibition of sexual behaviour in risky habitats: an experimental approach. PLoS ONE 8:e82736.

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

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



1) **Initial dispersal**. An organism moves long distances to areas outside its native range, for example through human assisted dispersal.

2) **Introduction** in natural environments of the non-native range.

The **prevention** of initial dispersal and introductions is probably the most cost-effective strategy against invasions.

3) **Establishment** of self-sustaining populations within the non-native range. Individuals from established populations are able to survive and reproduce in the non-native range.

The success of **containment** and **eradication** actions is generally highest at this step, as alien species can have limited abundance and growth rate.

4) **Spread** across the new range and of its environments: the species becomes **invasive**. This step can occur long after the initial dispersal, following a so-called lag phase characterized by limited growth rate.

When a species becomes invasive, it can reach high abundance and fast population growth. At this step, **impacts can be highest**, while management strategies such as **eradication** often are challenging. In some cases, only **mitigation** actions are possible.

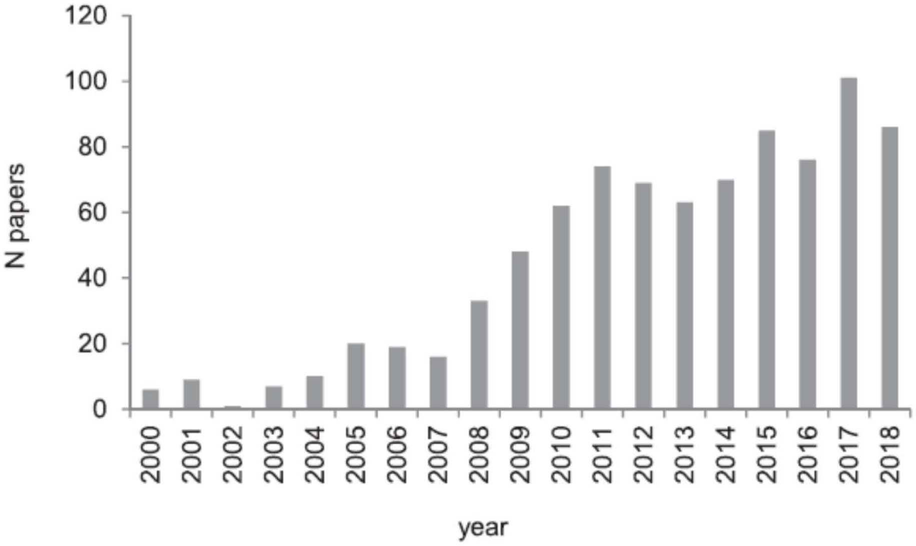
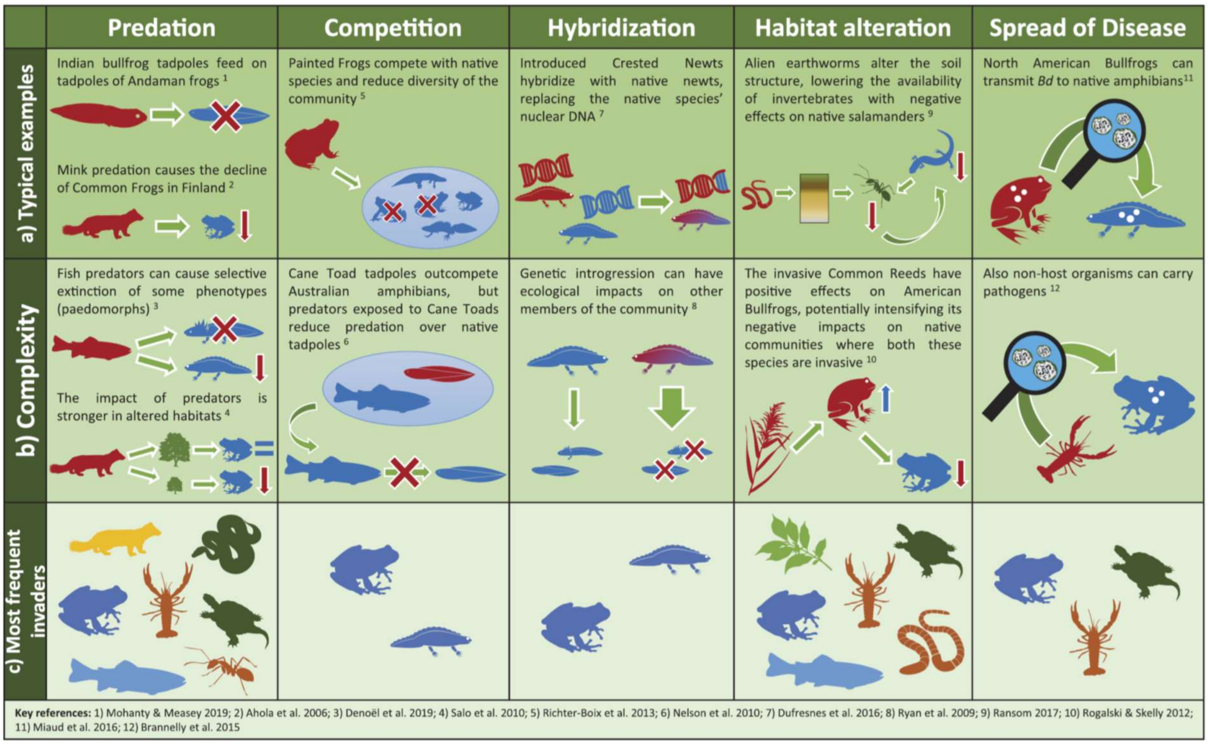


FIG. 1.—Increase of the number of papers on invasive alien species and amphibians, since 2000. The number of papers was obtained from the ISI Web Of Science, as of June 2019, considering the "article" category only and using the following search terms: "invasive species" or "alien species" or "non-native" AND amphibian* or frog* or salamander* or toad* or newt* or caecilian* or anura or urodela or caudata or gymnophiona.



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

Key references: 1) Mohanty & Measey 2019; 2) Ahola et al. 2006; 3) Denoël et al. 2019; 4) Salo et al. 2010; 5) Richter-Boix et al. 2013; 6) Nelson et al. 2010; 7) Dufresnes et al. 2016; 8) Ryan et al. 2009; 9) Ransom 2017; 10) Rogalski & Skelly 2012; 11) Miaud et al. 2016; 12) Brannelly et al. 2015

FIG. 2.—The main processes through which invasive species impact amphibians. A) Examples of direct impacts. B) Example of complex impacts, often mediated via interactive effects. C) Examples of the most frequent taxonomic groups involved in each process. Native species are depicted in blue. Invasive species are depicted in red. (Drawings by MF, GFF, Bob Comix and Natasha Sinegina and obtained from http://www.supercoloring.com/ under a Creative Commons 4.0 Licence).

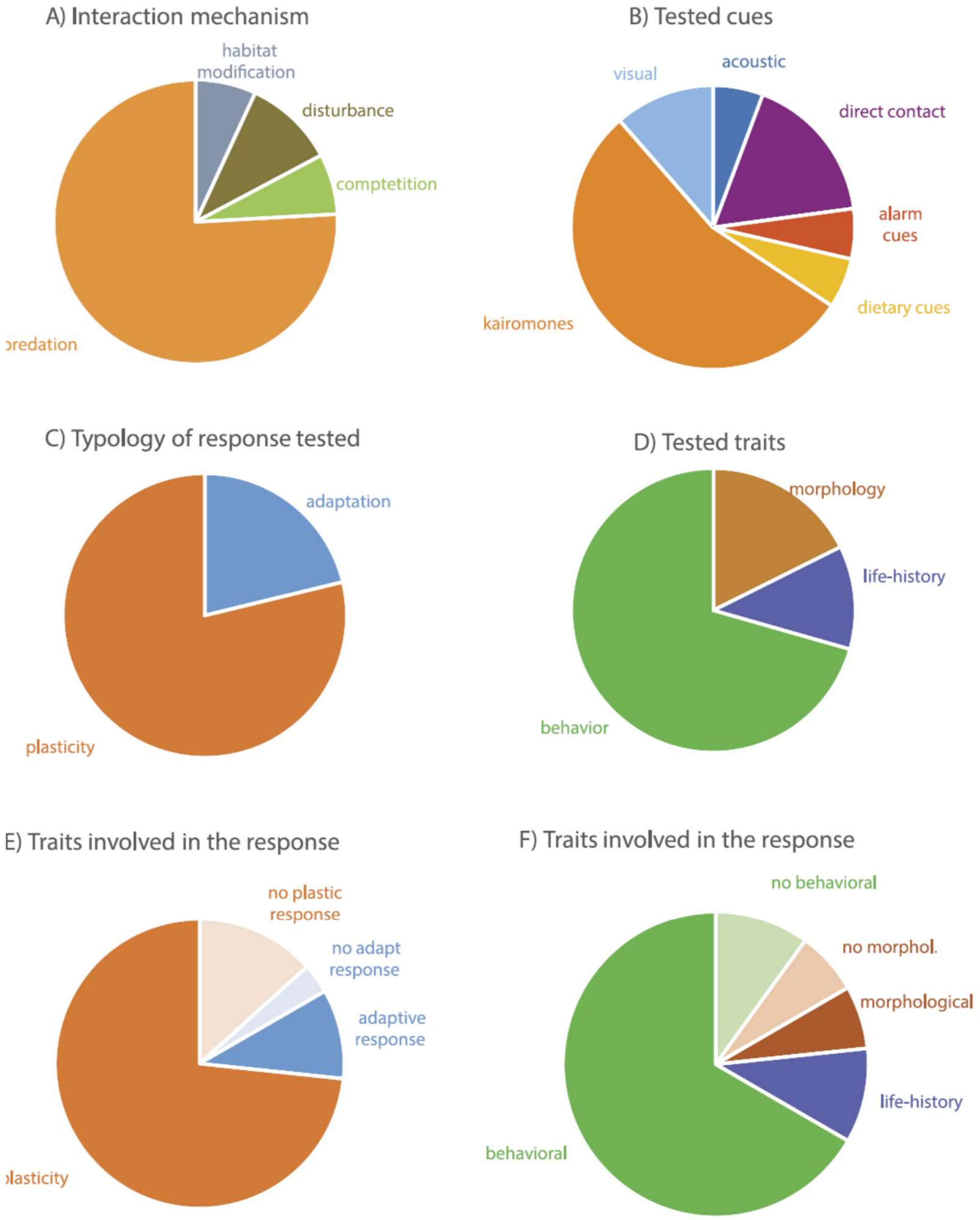


FIG. 3.—Amphibian responses to biological invasions. A) Types of tested interactions between IAS and native amphibians. B) cues potentially involved in the response of amphibians to IAS. C) frequency of studies assessing phenotypic plasticity and local adaptations in native amphibians in presence of IAS. D) frequency of studies considering different types of traits in native amphibians. E,F) frequency of responses effectively detected in native amphibians. Results are based on the analysis of 99 papers obtained through the ISI Web of Science using the following search terms: "invasive species" or "alien species" or "non-native" AND amphibian* or frog* or salamander* or toad* or newt* or caecilian* or anura or urodela or caudata or gymnophiona AND "response" or "defence*" AND "phenotypic plasticity" or behaviour or behavior or "life history" or "rapid evolution" or "contemporary evolution" or "rapid adaptation" or "habitat shift".

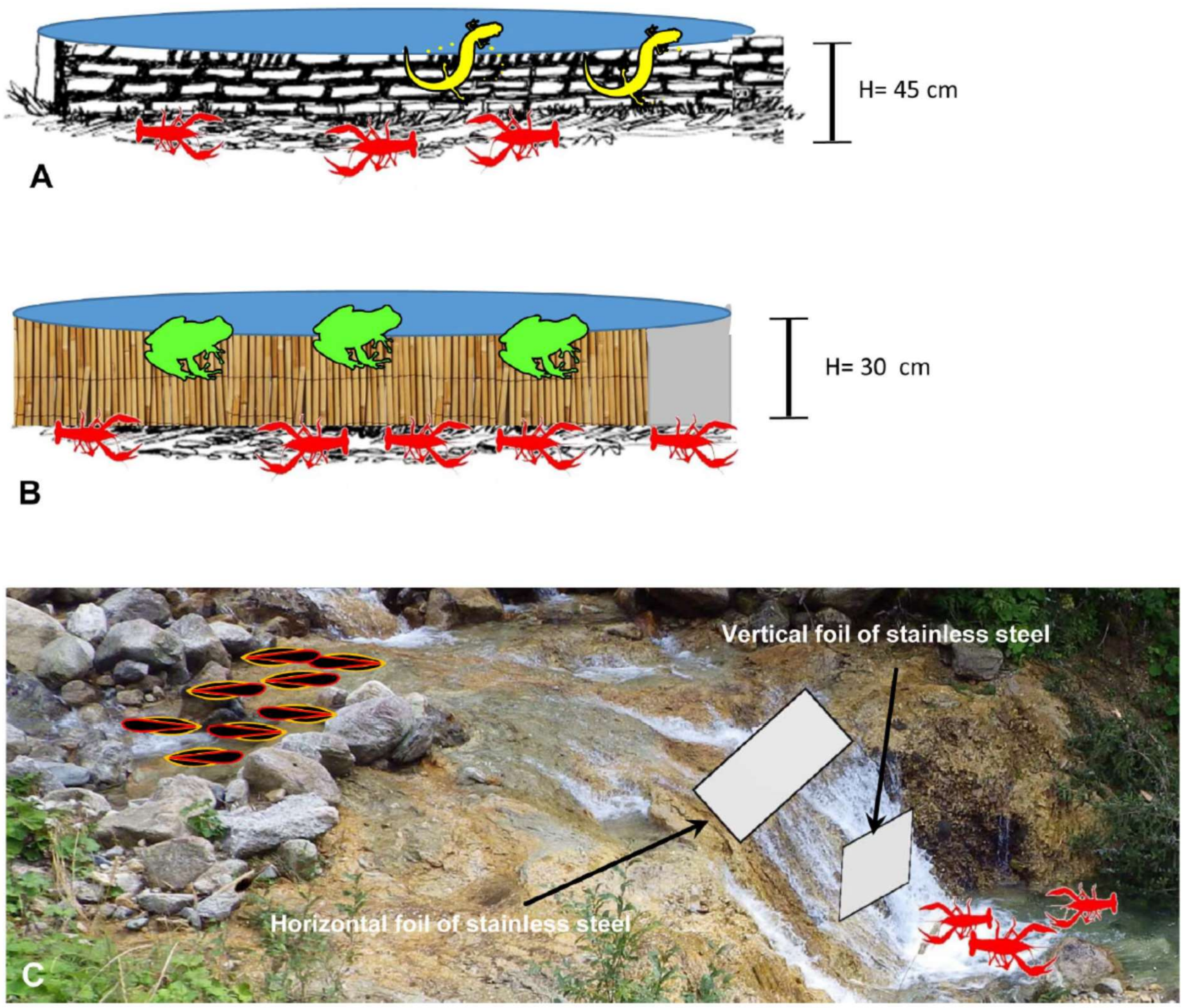


FIG. 4.—Examples of barriers designed to prevent invasive predatory crayfish from colonizing amphibian breeding sites.