Summary of the

Symposium on Alien Invasive Species

and their impact on Natura 2000 amphibian and reptile species in Europe, with special emphasis on the Visegrád (V4) countries

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Contents

Invasive Solidago sp. *in Hungary. Photo: Bálint Bajomi.*

Introduction

We live in an era of accelerated biodiversity decline (Pimm et al. 1995). According to scientists and governments, one of the main threats to native biological diversity is biological invasions caused by invasive alien species (Blackburn & Duncan 2001; European Commission 2013; IUCN ISSG 2000; Lockwood et al. 2005). Before the vast transformation of our biosphere by mankind, the natural barriers of oceans, mountains, rivers and deserts provided the isolation essential for unique species and ecosystems to evolve. Nowadays, with globalisation and the growth in the volume of trade and tourism, these barriers are overcome and many species are spread accidentally or deliberately. Developed in earlier times to guard against human and economic diseases and pests, customs and quarantine practices are often inadequate means to fight the import of alien species. The spread of these species is a global problem affecting virtually all ecosystems on Earth, and hundreds of extinctions have been caused by them. Global climate changes are significantly assisting this process (IUCN ISSG 2000).

According to the global estimates, direct economic costs of invasions are many billions of dollars annually. These costs are the result of e.g. arable weeds reducing crop yields, weeds degrading catchment areas, pests and pathogens reducing yields and increasing control costs (IUCN ISSG 2000). The damage caused in the European Union is estimated to be at least EUR 12 billon every year (European Commission 2013).

Definition of Terms

According to the definitions of the International Union for Conservation of Nature Invasive Species Specialist Group (IUCN ISSG), "*alien invasive species* means an alien species which becomes established in natural or semi-natural ecosystems or habitat, is an agent of change, and threatens native biological diversity.

Alien species (non-native, non-indigenous, foreign, exotic) means a species, subspecies, or lower taxon occurring outside of its natural range (past or present) and dispersal potential (i.e. outside the range it occupies naturally or could not occupy without direct or indirect introduction or care by humans) and includes any part, gametes or propagule of such species that might survive and subsequently reproduce" (IUCN ISSG 2000).

Many studies have investigated the factors affecting the success of the introduction of alien species (e.g. Blackburn & Duncan 2001; Cassey et al. 2005a; Cassey et al. 2005b; Cassey et al. 2004; Lockwood et al. 2005). Introduction effort or propagule pressure (i.e. the number of individuals and propagules introduced to a given area) seems to be the most important factor (Cassey et al. 2005a; Lockwood et al. 2005). Introduction events and reintroductions with conservation aims to areas from which they have been extirpated have many similarities (Blackburn & Cassey 2004; Cassey et al. 2008). In reintroduction biology, the number of released animals also correlates with the success of reintroductions (Cassey et al. 2008; Griffith et al. 1989; Wolf et al. 1998; Wolf et al. 1996). This highlights the importance of prevention in the case of introductions: if fewer individuals are imported, it lessens the chance of invasions.

The Situation in the European Union

Currently over 12 000 species are present in Europe which are alien to their natural environment; 15% of them are invasive (European Commission 2013). Many member states of the EU are already making efforts to deal with this problem, but often they are not effective if done purely on a national basis – if a species is eradicated in one country, it can return from a neighbouring area. Environment Commissioner of the EU Janez Potočnik said: "*Combating invasive alien species is a prime example of an area where Europe is better when working together*" (European Commission 2013).

"To help those tackling the invasive species challenge", the *Delivering Alien Invasive Species Inventories for Europe* (DAISIE) project has been launched. A database of alien species in Europe is available at http://www.europe-aliens.org/index.jsp. "DAISIE is a pivotal instrument in developing a Europe-wide strategy that encompasses both the geographical scale of the problem and unites the study of different taxa in marine, freshwater and terrestrial environments. With direct access to national knowledge bases throughout Europe, those addressing the invasive alien species challenge will easily obtain data on which species are invasive or potentially invasive in particular habitats, and use this information in their planning efforts" – states the website.

Two regional networks have also been created: the East and South European Network for Invasive Alien Species (ESENIAS, http://www.esenias.org/) and the European Network on Invasive Alien Species (NOBANIS), which is a gateway to information in North and Central Europe (http://www.nobanis.org/).

Legal Framework and Guidelines

The Article 8 (h) of the Convention on Biological Diversity states that:

"Each Contracting Party shall, as far as possible and as appropriate: (…) (h) Prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species" (United Nations 1992).

The same requirement is expressed in a number of other international conventions, including the Bern Convention and the Bonn Convention (Hughes 1999).

Photo: MTI

On the 9th September 2013, the European Commission proposed new legislation to prevent and manage threats from invasive species. That "will now be examined by the Council and the Parliament" (European Commission 2013)

In 2000, the IUCN ISSG has published detailed guidelines which give advice on the prevention of the spread of invasive species (IUCN ISSG 2000).

Reasons for organising this Symposium

At the 17th European Congress of Herpetology, held between 22–27 August 2013 at Veszprém, Hungary, a one-day symposium was dedicated to the links between herpetology and alien invasive species (Societas Europaea Herpetologica 2013), where 14 conference papers were presented.

Amphibian and reptile species can be invasive themselves, or native amphibian and reptile species can be affected by other invasive animals and plants. When trying to reach Natura2000 goals, like maintaining populations of listed species, conservationists have to have a wider view in order to identify potential invasive threats. Biological invasion can threaten certain species in forms of parasites or microbes, plants changing preferred habitat into suboptimal state, or animals appearing as new competitors or predators, or rarely inedible toxic prey.

In Europe the spreading of certain invasive species can many times be descibed with a clear East-West or North-South direction. The so called Visegrád (V4) countries (Hungary, Slovakia, Czech Republic, Poland) have a central position within Europe, being in the crossing of these axis, which means that these countries are facing various type of IAS-problems.

When selecting talks for the Symposium we tried to show examples for IAS-linked problems in V4 countries. The second part was giving a European perspective, while in the third part we wanted to show possible solutions in tackling the problems.

Conclusions

Prior to the Symposium, in his plenary talk, Rick Shine presented a famous example for invasive amphibian, when speaking about the American Cane Toad (*Rhinella marina*) that spread across the Australian tropics (Shine 2013).

The Symposium was started with a talk on the endangered Hungarian Meadow Viper (*Vipera ursinii rakosiensis*) which populations in the Hanság area of Hungary are affected by invasive plants, like Giant Goldenrod (*Solidago gigantea*). To conserve the vipers, conservation managers are eradicating this weed from the habitat of the snake, in the frame of a large-scale habitat reconstruction effort (Takács et al. 2013). Similar tasks are faced within Budapest, Hungary at the remaining habitats of strictly protected Caspian Whipsnake (*Dolichophis caspius*) and Snake-eyed Skink (*Ablepharus kitaibelii*), where planted Black Pine (*Pinus nigra*) occupied large proportion of habitats, and recently spread of Common Lilac (*Syringa vulgaris*) worsens situation (Babocsay and Vági 2013.).

Presenters from Czech Republic, Poland, United Kingdom and the Netherlands presented results from their national IAS-database.

Photo: Bálint Bajomi.

A reptile example for IAS is the case of the Red-eared Slider (*Trachemys scripta elegans*). Millions of individuals used to be imported into Europe and sold as pets, some of them later released into natural water bodies, where they became the competitors of the native

European Pond Turtle (*Emys orbicularis*) and Spanish Pond Turtle (*Mauremys leprosa*). A European Union regulation came into force in 1997 which forbade the import of the red-eared slider. Since that year, traders substitute them with other (sub)species, which subsequently appeared in natural waters as well. Results of the Trachemys LIFE-project from Spain were also presented.

In the last section participants had a chance to learn environmental DNA technique, which was effectively used in detection of American Bullfrog (*Rana catesbiana*) and African Clawed Frog (*Xenopus laevis*). Modern genetic techniques can also be used in identifying hybridisation between native and non-native species, as in the case of Crested Newts (Triturus sp.) in the Netherlands.

Potential Solutions

1.) **Prevention:** as we have previously seen, one of the most important factors in the successful introduction of alien species is the number of individuals released into a given area. Therefore if we reduce the numbers imported, we can prevent the spread of the invasives. This is the most cost effective way of protection because it is much more expensive to eradicate established specimens from a large area. According to a new legislation proposed by the European Commission, "Member States will organise checks to prevent the intentional introduction of species of concern. However many species come into the EU unintentionally, as a contaminant in goods or trapped in containers. Member States will have to take action to spot such pathways and take corrective measures" states the recent European Commission press release (2013).

2.) **Early warning and rapid response:** in light of a new regulation, Member States of the EU will try to detect the species of concern early and take immediate action to eradicate them (European Commission 2013). A new and effective scientific method for the early detection of introduced species was presented at the 17th European Congress of Herpetology. The method is called environmental DNA: the DNA of the alien species can be detected from water samples taken from a given water body (Ficetola et al. 2013).

3.) **Management:** if alien species are already widely spread, management programmes can try to eradicate them – as in the case of the Ruddy Duck (*Oxyura jamaicensis*) in the United Kingdom and Spain (Hughes 1999), or of several alien plant species in Hungary. The EU regulation newly proposed by the European Commission states that if invasive species are already widespread, Member States will need to put in place measures to minimise the harm they cause (European Commission 2013).

4.) **Cooperation and information exchange:** is really important, as many times IAS-problems are beyond the scope of a single country. Meanwhile effective, rapid response requires sure use of techniques which might have already been developed somewhere else, therefore development time can be spared.

Original schedule of the Symposium

8:30-9:15	Plenary: Richard Shine - Invasive cane toads in Australia: the impacts of a giant frog in a strange land
9:30-15:30	**Symposium 1.: Invasive Alien Species** **Chair: Bálint Halpern**
9:30-9:45	Gábor Takács; Róbert Dankovics; Attila Pellinger: Populations and habitats of the Hungarian meadow viper (Vipera ursinii rakosiensis) in the Hanság area
9:45-10:00	Gergely Babocsay; Balázs Vági: Civil efforts to conserve the Caspian Whip Snake (Dolichophis caspius) in the shadow of Budapest
10:00-10:15	Roman Rozinek; David Fischer; Vojtěch Balaz: Impact of invasive species on the herpetofauna in Czech Republic
10:15-10:30	Peter Mikulicek: Native or alien water frog species in Bratislava (Slovakia)?
10:30-10:45	Wojciech Solarz, Katarzyna Kurek: Alien herpetofauna in Poland – problems and solutions
10:45-11:00	DISCUSSION
11:00-11:30	Coffee break
11:30-11:45	Cesar Ayres: IAS and its impacts on the Iberian herpetofauna: a review of conservation projects
11:45-12:00	Iolanda Silva-Rocha; Daniele Salvi; Miguel A. Carretero: Invasion Patterns of Ancient and Recent Alien Herpetofauna in the Balearic Islands
12:00-12:15	Jeroen van Delft; Ronald Zollinger; J. Wiebe Lammers: Monitoring invasive alien species in the Netherlands, a multi-taxa approach.
12:15-12:30	Jim Foster; John Wilkinson; Andy Arnell; Tony Gent: Challenges in responding to invasive herpetofauna threats in the United Kingdom
12:30-14:00	Lunch break
14:00-14:15	Gentile Francesco Ficetola; Tony Dejean; Francois Pompanon; Pierre Taberlet; Claude Miaud: Environmental DNA for the detection of alien (and native) species in freshwaters
14:15-14:30	Jean Secondi; Claud Miaud; Guillaume Koch; Benjamin Audebaud; Nicolas Cotrel; Sylvie Desgranges; Tony Dejean: Tracking the expansion of an invasive species Xenopus laevis using environmental DNA techniques
14:30-14:45	Willem Meilink; Pim Arntzen; Jeroen van Delft; Ben Wielstra: Genetic contamination of the indigenous Northern crested newt (Triturus cristatus) by the invasive Italian crested newt (Triturus carnifex) on the Veluwe (Netherlands).
14:45-15:00	Eudald Pujol-Buxó; Olatz San Sebastián; Núria Garriga; Caroline Pereira Almeida; Gustavo A. Llorente: Plastic invaders: reactions of tadpoles of the invasive anuran Discoglossus pictus in front of native and introduced predators
15:00-15:15	Stephan Böhm: Unwanted spring breakers at the Riviera Maya: the Red eared slider Trachemys scripta elegans, (Wied-neuwied 1839) invades the cenotes of peninsula Yucatan, Mexico
15:15-15:30	DISCUSSION

Abstracts of presentations in order of appaerance

Invasive cane toads in Australia: the impacts of a giant frog in a strange land

Rick Shine

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Almost 80 years ago, agricultural scientists brought American cane toads (*Rhinella marina*) to the sugar cane fields of tropical Queensland, in a futile attempt to control insect pests. The descendants of those toads became pests themselves, spreading across the Australian tropics, and even setting up satellite populations in cities as far south as Sydney. The toad's invasion has had devastating impacts on native wildlife, but also has enabled scientists to explore the evolutionary and ecological consequences of toad invasion, to learn how a tropical ecosystem has dealt with the novel challenges posed by this toxic frog, and to develop new ways to combat the invader.

Populations and habitats of the Hungarian meadow viper (*Vipera ursinii rakosiensis*) in the Hanság area

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The Hungarian meadow viper is one of the most endangered snakes within the European fauna. The West-Hungarian population of the subspecies survived in the Hanság area on two pitches with dry grasslands (loess grasslands, secondary grasslands) void of floods and inland inundations that make up less than 25 ha altogether and are surrounded by lower lying marsh meadows, willow marshes and marsh forests.

Within the frame of the "Conservation of Hungarian meadow viper (*Vipera ursinii rakosiensis*) in the Carpathian-basin" LIFE+ project intervention is done on 392 ha within the 1609 ha "Viper Reserve". The aim of project is the suppression of invasive species, conservation of and help to spread grassland communities favourable for the Hungarian meadow viper. In the place of higher situated hybrid poplar plantations and ploughlands 32 ha of new dry grassland are created that doubles the extension of wintering grounds. The surrounding former marsh meadows and swamp meadows are being restored on 360 ha primarily due to the removal of *Salix cinerea* and *Solidago gigantea*. Further conservation management of the habitats is done by mowing and grazing of sheep, and it is protected by an electric fence to eliminate damages by wild boar.

Within the frame of botanical monitoring the spread and general state of habitats was examined with vegetation mapping. We try to show the changes in the state of the single habitat types due to management with coenological snapshots taken on constant sampling spots.

The monitoring survey of the Hungarian meadow viper and its potential food animals— Orthoptera, Amphibia, Reptilia—is done since 2001. Changes in the number of species and individuals of the assigned taxa as well as the relative frequency of the viper sightings in a unit time were tracked on the two habitats with nearly the same times of sampling. The presence of predator species and the effect of habitat management were also subject of the survey.

Civil efforts to conserve the Caspian whip snake (*Dolichophis caspius*) in the shadow of Budapest

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The Caspian whip snake (*Dolichophis caspius*) is close to extinction in Hungary. Very few isolated populations have survived, and only sporadic observations of individuals have been recorded in the last two decades. The populations are under heavy pressure from invasive plants and human activities. In 2011, the Amphibian and Reptile Conservation Group (ARCG) of MME BirdLife, Hungary launched a conservation programme to assess the status of the species and its habitats in the vicinity of Budapest, and to build up a habitat management plan. During 2011 and 2012 with volunteers, we made a total of 32 visits to seven habitats, of which one at Pesthidegkút was discovered only in 2012,

and observed *D. caspius* in two (including the new one; on the urban Sas-hegy we made no surveys, but *D. caspius* is regularly seen there). *Dolichophis caspius* was observed 11 times at Farkas-hegy and 22 times at Pesthidegkút, but only 11 (five on Farkas-hegy and six at Pesthidegkút) could be positively identified as different. Two different hatchlings were observed on Farkas-hegy. Other historic habitats have yielded no snakes during our surveys. All suitable habitats are highly isolated, small in their extent (< 5 ha), and burdened with an increasing number of invasive plants and recreational activities. Most profoundly *Ailanthus altissima, Pinus nigra* and *Populus tremula* alter the habitats. We partially removed the black pines from two fragments (altogether ca. 0.5 ha) on Farkas-hegy where snakes were most often observed. Further tree fellings are scheduled to the end of 2013. The effects of the removal of invasive plants on the benefitted population cannot be evaluated at this point, but only a large scale habitat restoration and connection of suitable habitat patches can prevent the short term extinction of *D. caspius* from the northernmost parts of its European range. Our *Dolicophis* programme together with other programmes of ARCG triggered an increased citizen involvement.

Impact of invasive species on the herpetofauna of the Czech Republic

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Every herpetologist is aware of the danger that alien species pose to amphibian and reptilian populations. However most of the support for deleterious effects of non-native and invasive organisms is based on anecdotal reports. Scientifically processed and analysed data about the non-native species impact on reptilian and amphibian fauna is completely absent from the area of Czech Republic. Only individual observation data are available at the moment e. g. a dissection of invasive fish, brown bullhead (*Ameiurus nebulosus*), that showed the digestive tract to be completly filled by ingested smooth newts (*Lissotriton vulgaris*). Most of the "evidence" is in form of mere observations about some surveyed species going lost from locality after alien species was detected there. An example of such case is the absence of dice snake (*Natrix tessellata*) in the watershed of the Berounka River after invasion of mink (*Mustela vison*). The most important introduced species with direct effect on amphibians and reptiles (and other fauna as well) include the fish species *Ameiurus melas* (black bullhead), *A. nebulosus* (brown bullhead), *Carassius gibelio* (Prussian carp), *Pseudorasbora parva* (topmouth gudgeon) and of mammals *Mustela vison* (mink), *Procyon lotor* (raccoon) and *Nyctereutes procyonoides* (raccoon dog). The area of Czech Republic harbours at least two species of alien (American) crayfish species that are known to propagate the crayfish plague, which extirpates native crayfish species. Additionally these invasive crayfish are potential vectors of the amphibian pathogen causing chytridiomycosis. The habitat changes triggered by expanding non-native plant species cause serious problems to both amphibians and reptiles. Examples of such plants include Canadian waterweed *Elodea canadensis* with ability to overgrow the ponds, thus making them unsuitable for survival of amphibians and their larvae. Terrestric plants especially *Helianthus tuberosus* and *Impatiens glandulifera* cause intense shading of river banks, thus limiting the options for basking for dice snake (*Natrix tessellata*) and lowering the temperature of egg-laying sites.

We have to admit that our knowledge of the invasive species impact on our amphibians and reptiles is still limited, although first studies are on the way we will have to wait until the results are available.

Native or alien frog species in Bratislava (Slovakia)?

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Non-native species of the western Palearctic water frogs (*Pelophylax esculentus* complex) were established in many western European countries. They have a potential to readily invade new sites, where they compete with indigenous species and predate on the congeneric specimens. Hybridization between alien and indigenous individuals leads to introgression and genetic pollution of native water frog populations. In this study we investigate genetic structure of water frogs in Bratislava (Slovakia) to find out the origin of local populations and dispersal abilities of water frogs for colonization of new sites. We apply two types of molecular markers with a different mode of inheritance and evolutionary history: sequences of mitochondrial ND2 and ND3 genes and nuclear microsatellites. All examined frogs possess either "*lessonae*" or "*ridibundus*"-specific mtDNA haplotypes known from central Europe, corroborating their native origin. While natural ponds in a studied area are inhabited predominately by *Pelophylax esculentus* and

Pelophylax lessonae, man-made gravel pits, established in the course of the last century, are occupied almost exclusively by *Pelophylax ridibundus.* This finding highlights a colonization potential of *P. ridibundus* and its ability to occupy new established artificial habitats. Our results also suggest that rivers, river branches and artificial canals facilitate dispersal of *P. ridibundus* to new environment. In contrast, a highly fragmented urban landscape lacking water corridors limits dispersal and gene flow between the ponds.

Alien herpetofauna in Poland—problems and solutions

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Although there are no established alien amphibians or reptiles in Poland to date, the problem of alien herpetofauna should not be ignored. Availability of exotic pets and number of amateur keepers is increasing. However, the level of awareness about causes and consequences of biological invasions remains low. As a result, the number of releases of unwanted pets, including herptiles, is alarming. In addition, there were cases of illegal introductions of reptiles for "fauna improvement" by professional herpetologists and dedicated nature lovers. At least 10 reptile species have been recorded in the wild. There are only anecdotal records for most of them but the number of confirmed records for red-eared slider (*Trachemys scripta elegans*) and Cumberland slider (*T. s. troostii*) reached a few hundreds. Single cases of egg-laying of red-eared sliders were detected but hatching was not confirmed so far. Even though the species may not breed successfully in Poland, its life span combined with the number of released individuals constitute serious threat for native biodiversity, particularly for European pond turtle (*Emys orbicularis*).

One of the major attempts to mitigate the invasive alien species problem in Poland is a new decree of the Minister of Environment that came into force in 2012. It imposes restrictions on 52 invasive alien species of plants and animals. Import, keeping, breeding and selling of these species requires a permission from the General Director for Environmental Protection. Breaking the law is subject to a fine or jail. Negligence leading to escape of animals listed in the decree may result in similar consequences. Restricted species include American bullfrog (*Lithobathes catesbeianus*): its import and breeding is forbidden altogether, including by zoos. However, to reduce the risk of release of unwanted individuals, it is possible to obtain a permit for keeping and selling bullfrogs that are already possessed, on the condition that they are microchipped the risk of escape is kept to a minimum. Restricted reptile species are snapping turtle (*Chelydra serpentina*), painted turtle (*Chrysemys picta*), false map turtle (*Graptemys pseudogeographica*) and all subspecies of *Trachemys scripta*. Import and breeding of these species will only be allowed for zoos, while individual owners and pet shops can only obtain permits to keep and sell turtles that they currently possess, provided that they are microchipped and the risk of escape is minimized.

The major challenge to make the new law effective is awareness raising among pet keepers. A few campaigns were organised and new are planned in close future.

IAS and its impacts on the Iberian herpetofauna: a review of conservation projects

César Ayres

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The Iberian Peninsula has a huge record of human-mediated introduction of non-indigenous species (NIS). Since the arrival of the Phoenicians or the Romans, and later the occupation by the Muslims, several species were introduced. Some of them make part now of the fauna of the Iberian Peninsula, even as protected species, like the spur-thighed tortoise (*Testudo graeca*).

But in the second half of the 20th century the number of NIS that became invasive alien species (IAS) has increased exponentially. Some of the cases are known globally, such as the zebra mussel (*Dreissena polymorpha*), American mink (*Neovison vison*), North-American sliders (*Trachemys scripta*), or the red swamp crayfish (*Procambarus clarkii*). A huge numbers of fishes have been introduced by anglers, like the Danube catfish (*Silurus glanis*) or the black bass (*Micropterus salmoides*). Recently, the Iberian herpetofauna faces a new threat due to the arrival of new IAS, like the raccon (*Procyon lotor*).

An increasing number of conservation projects have aimed to control or eradicate invasive species in the Iberian Peninsula. This presentation is a review of the different projects, from large scale (LIFE *Trachemys*, LIFE+ *Lampropeltis*) to smaller more specific projects (Eradication of the northern banded newt [*Ommatotriton ophryticus*]).

Invasion patterns of ancient and recent alien herpetofauna in the Balearic Islands

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Species introductions are becoming a major threat to biodiversity and economy but its effects are unevenly distributed in time and space. The Mediterranean Basin is the biodiversity hotspot where introductions shape the vertebrate communities in greater extent, being the insular species the most affected. Due to their life history, amphibians and reptiles are among the groups most widely introduced but also most threatened by species introduction. Mediterranean islands harbour a combination of herpetofaunal elements: endemics long time evolving under insular conditions; natural colonisers from the adjacent continents in recent geological times; and ancient or recent invaders transported, voluntarily or not, by humans. Distinguishing between them, as determining the sources and invasion pathways, is not only of biogeographical interest but also crucial for conservation management. Here we analyse the paradigmatic case of the Balearic herpetofauna by using a combination of literature and original molecular data. The Balearic Islands currently harbour much more alien than native amphibians (4/1) and reptiles (21/2). Although archaeology dates some introductions back to the Neolithic, the Balearic Archipelago has increasingly importing alien species until present. Source regions and invasion routes have shifted throughout time, from the Eastern Mediterranean and North Africa to the Iberian Peninsula and extra-European areas and from passive cargo transport or food/religion related to pet shops, nursery trade and tourism. Overall, one native amphibian (*Alytes muletensis*) and two native reptiles (*Podarcis lilfordi* and *P. pityusensis*) are restricted to Ibiza and the less disturbed Formentera and small islets. Even there, menaces have increased during the last decade, particularly due to predatory snakes and competitive lacertids. While the Balearic herpetofauna forecasts what could be an ominous fate, it also provides an opportunity to extract lessons for developing effective conservation strategies.

Monitoring invasive alien species in the Netherlands, a multi-taxa approach

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Collecting of distribution and population data by volunteers, has a long and successful history in the Netherlands. This also concerns (invasive) alien species. Next to data of herpetofauna, Reptile, Amphibian and Fish Conservation Netherlands (RAVON) stimulates and facilitates volunteers to collect and pass on data on other taxonomic groups, since the introduction of online data entry portals. RAVON collaborates with eleven other nongovernmental organisations and the governmental Invasive Alien Species Team. They set up a multi-taxa (invasive) alien species project, to stimulate the collection of data. The importance of collecting data on (invasive) alien species of all taxonomic groups is stressed by various activities (e. g. the development of an App, identification keys, a popular newsletter, magazine, websites etc.). In this way 25,000 volunteers are reached. This resulted in an increase of records of many taxa, as well as a broader range of taxa being reported by individual volunteers.

An important herpetological result of this project, which can be considered an Early Warning System (EWS), was the detection of the first reproducing population of American bullfrog (*Lithobates catesbeianus*) in the Netherlands in 20 years. This population was subsequently successfully eradicated. Other relevant (invasive) alien herpetofauna species to this programme are *Triturus carnifex, Rana dalmatina, Elaphe schrenkii* and several aquatic turtle species. Another striking result is a strong increase of RAVON volunteers entering data on other exotic taxa such as crayfish.

This national project might serve as an example for the development of a European EWS for invasive alien herpetofauna species. A European EWS for invasive alien herpetofauna species, could also pay attention to invasive alien species from other taxonomic groups, affecting herps and their habitats. Therefore, we propose to scale-up this project to a European level.

Challenges in responding to invasive herpetofauna threats in the United Kingdom

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At least 10 IAS amphibians and 3 reptiles are thought to be present in breeding populations, ranging from a single site to areas extending across tens of km_2. Reports of further introductions are common though most do not result in population establishment. There has been excellent progress in some of the main IAS policy areas. For example, there are now standardised risk assessments for many IAS. Specific control operations have been very successful, for example on North American bullfrog *Lithobates catesbeianus*. Broad IAS policy has been substantially strengthened in recent years, and legal mechanisms are being improved. There have been valuable research projects on, for example, the link between introduced alpine newts *Ichthyosaura alpestris* and the amphibian chytrid fungus. Despite these successes, overall implementation of IAS policy is patchy and poorly co-ordinated for herpetofauna. For instance, despite the acknowledgement of the primacy of prevention, there is minimal action to address the likely key introduction pathways. IAS detection and surveillance systems are passive, largely relying on chance to detect invasions. Research into prevention, impacts, control and mitigation is negligible. Links between government and specialists need further development, with a more engaged and transparent process for acting on risk assessments. There is concern about whether the current allocation of resources is targeted toward the highest priority actions. Uncertainty about risks too often results in no action, even when rapid response would involve minimal cost. Underlying these issues, there is a worrying complacency among many herpetologists about the risks of IAS. This has contributed to hesitation over action and has even prompted deliberate introductions. We believe that substantial action to address all these issues is needed soon, especially in light of proposed European Commission IAS legislation.

Environmental DNA for the detection of alien (and native) species in freshwaters

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Early detection of alien invasive species is extremely important for management, as eradication efforts are most likely to be successful if performed when alien species have limited abundance and range. However, early detection may be challenging, due to the limited density of individuals. Aquatic and semi-aquatic species release DNA into the environment through mucus, faeces, urine and remains. It is thus possible using the DNA present in the environment (eDNA) to detect the presence of alien (and native) species in freshwater.

Analyses performed on invasive populations of American bullfrogs (*Lithobates catesbeianus*) showed that primers amplifying short mitochondrial DNA sequences allow to successfully track the presence of frogs in natural wetlands. Using eDNA allows a more accurate detection of bullfrogs at very low densities and at any life stage compared to traditional visual and audio-surveys. eDNA therefore allows early detection of invasive populations, or to confirm the success of eradication projects. Furthermore, multiple studies showed that eDNA allows a reliable detection of a growing number of species, including several species of both invasive and native amphibians and fish. Furthermore, recent advances of DNA meta-barcoding promise the simultaneous detection of multiple species. Nevertheless, major technical challenges remain, such as contamination and identification of universal markers with high resolution.

Tracking the expansion of an invasive species *Xenopus laevis* using environmental DNA techniques

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The African clawed frog *Xenopus laevis* has been introduced in several parts of the world where it is considered as an invasive taxon now threatening local herpetofaunas. It has been released about 30 years ago in western France where populations survived in the wild and rapidly expanded. The introduction point lies on the Loire River basin which covers 20% of the French territory and includes two wetlands of international value (Brenne, Sologne). Our ability to track and predict the movement of individuals is a key issue for the control of invasive species. To this regard, tracking the colonization front is crucial to better apprehend the dispersal behaviour of *X. laevis* in the local context. However, the task becomes more and more challenging as the size of the colonized area increases. Classical trapping methods may fail to ascertain species presence on the outer margins because of unbearable logistic constraints. For these reasons, alternative methods are required to improve detectability and coverage of expanding invasive species. Environmental DNA (eDNA) techniques may provide suitable solutions. We provide an update of the colonized area by *X. laevis* in western France and outline the conservation issues related to that invasion. We then present results from detection tests using several eDNA methods in sites of low and high density, and compare the pros and cons of classical capture and eDNA methods for *X. laevis*.

Genetic contamination of the indigenous northern crested newt (*Triturus cristatus*) by the invasive Italian crested newt (*Triturus carnifex*) on the Veluwe (The Netherlands)

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Two crested newt species, the Italian crested newt (*Triturus carnifex*) and the northern crested newt (*Triturus cristatus*), have a parapatric distribution. Hybridization is known to occur between both species in the laboratory, at localities where one of the species has been introduced in the range of the other, and at the natural contact zone. The Italian crested newt has been introduced to the Veluwe, the Netherlands, where *T. cristatus* is a native species. At other localities where *T. carnifex* has been introduced, populations appear to be stable and longlived. Furthermore, displacement of *T. cristatus* by introduced *T. carnifex* has been observed in Switzerland. A study on the distribution of *T. carnifex* in the Netherlands revealed a recent range expansion. Furthermore, individuals with intermediate phenotypes were found which suggests that hybridization between the two species has occurred. Hence, not only direct competition, but also genetic pollution by the introduced *T. carnifex* could pose a threat to the native *T. cristatus*. A detailed knowledge of the distribution of *T. carnifex* and genetically admixed individuals is indispensable for management considerations. However, morphological identification in itself is unreliable. Therefore, a detailed map needs to be created on the basis of genetic data.

Widespread sampling was performed on the Veluwe and individuals were provisionally classified based on morphological characters. An equal number of individuals were chosen per population for DNA extraction. One mitochondrial DNA marker was used for Sanger sequencing and showed that the distribution of *T. carnifex* haplotypes is more widespread than expected based on morphological data. An additional 52 markers were amplified by multiplex PCR and sequenced by the Ion PGM Next Generation Sequencing platform. The nuclear DNA data were analysed using New Hybrids to determine the extent of introgression of *T. carnifex* alleles into *T. cristatus* populations on the Veluwe.

Plastic invaders: reactions of tadpoles of the invasive anuran *Discoglossus pictus* in front of native and introduced predators

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A growing number of studies consider phenotypic plasticity one of the major forces driving survival and rapid adaptation of species in front of new threats like invasive predators, thus being biological invasions an optimal system to study the evolution of anti-predator phenotypic plasticity. In order to deeper understand the role of inducible responses in the population dynamics related with current biological invasions in our area, we first studied the plastic responses of both native (*Pelodytes punctatus*) and invasive (*Discoglossus pictus*) anuran tadpoles, all facing native (dragonfly *Anax* sp.) and two invasive (fish *Gambusia holbrooki* and crayfish *Procambarus clarkii*) predators. Native *P. punctatus* displayed the predictable set of reactions against native dragonflies, but milder (morphological or behavioral) reactions to invasive predators. Invasive *D. pictus* reacted behaviorally to all predators, unexpectedly reacting also morphologically to native *Anax* sp. Our results support prey-predator specificity in these reactions and dissociation between behavioral and morphological plasticity in tadpoles, although some reactions in *D. pictus* could suggest unspecific plastic reactions against predation in this species. To have a deeper understanding of the anti-predator plastic reactions of this invasive species, in a second experience we confronted this invasive anuran with the most usual predators in our study area: common backswimmers (*Notonecta* sp.) and common darters (*Sympetrum striolatum*). Results of this second experience further support the ability of *D. pictus* to react specifically to native predators and reinforce conclusions of the first work, pointing to an important role of phenotypic plasticity in this system. On the other side, each species suffered from a particular set of trade-offs between inducible responses and their costs, and interestingly we usually detected milder and unexpected patterns in combinations using introduced species.

Unwanted spring breakers at the Riviera Maya: the red-eared slider *Trachemys scripta elegans* (Wied-Neuwied, 1839) invades the cenotes of the Yucatan Peninsula, Mexico

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Known to be the turtle species with the most extensive geographic distribution, the red-eared slider (*Trachemys scripta elegans*) has been recorded from dozens of countries all over the world. Because of its longevity, fecundity and opportunistic omnivory it may locally threaten authochthonous turtles and other aquatic wildlife and plants, and is listed among the top 100 worst invasive species by the IUCN Invasive Species Specialist Group (ISSG). During a field trip to the Yucatan Peninsula from February 13–20, 2012, five cenotes (sinkholes filled with freshwater) in the area around Tulum and the Laguna Bacalar (a lake at the border to Belize) were surveyed for turtles. Temperature, pH, carbonic hardness (KH), total hardness (GH) and conductivity were recorded. Turtles were spotted by snorkelling in the waterbodies and photo-documented.

At all surveyed localities, Meso-American sliders (*Trachemys venusta*) were found, but at two sites red-eared sliders were also present. An adult melanistic male was found in Cenote Cristal (20°11'24"N, 87°29'56.4"W) and an adult female in Cenote Carwash (20°16'26.4"N, 87°29'9.6"W). According to the Global Invasive Species Database, these records are the first ones for the Yucatan Peninsula.

Besides these findings, research in hobbyist web forums also showed that *Trachemys* species readily hybridize in captivity. Would this also occur in nature, the currently unstable situation of Meso-American sliders could be even more complicated than thought before.

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