Successful development of European tree frog (*Hyla arborea* Linnaeus, 1758) populations in the south-west and west of Luxembourg following reintroduction

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Abstract. In this article, we provide a comprehensive overview of our European tree frog reintroduction, from the sourcing and captive-rearing of donor animals, through reintroduction and population development, to the current status of the project. Before the start of the project, only one isolated population remained in the east of the country. Starting with two initial reintroductions (2012 to 2014), two tree frog populations have developed over the past decade and we have managed to save the species from extinction on the national level. While both populations have been expanding rapidly in recent years, they show noticeable differences regarding initial development and spread. Further reintroductions to reinforce these populations were carried out from 2019 to 2024. In total, more than 7,000 juveniles and tadpoles were released over the entire duration of the project, resulting in ca. 830 calling males at 61 sites in 2024. Our long-term annual monitoring provides detailed data on population development and spread, which are presented and discussed. In addition, comparisons with similar tree frog reintroduction projects in other countries are made and logistical and epidemiological aspects of the reintroduction project are highlighted. Finally, we give an outlook on practical implications for conservation management, upcoming challenges and future focal points of the project.

Keywords. Amphibian conservation, captive-rearing, distribution, population development, colonization, habitat management.

1. Introduction

The European tree frog's natural distribution area spans over large parts of Europe. Only the British Isles, most of Scandinavia and large parts of the Mediterranean region are not inhabited (Acemav coll. 2003). The IUCN Red list classifies the species as least concern but with a decreasing population trend (IUCN/SSC Amphibian Specialist Group 2022), currently reflected in the unfavourable population status and/or decreasing population trend in four out of eight European biogeographical regions (Article 17 reporting period 2013–2018, ETC/BD & EEA 2024). In the EU, the European tree frog (Fig. 1) is considered a species of community interest in need of strict protection, listed under annex IV of the fauna-flora-habitat directive (JOCE 1992). Luxembourg and its neighbouring regions used to contain widely-spread populations, yet all of these have suffered a severe decline in the last century (Acemav coll. 2003, Proess 2016, Große et al. 2020). The Luxembourg Red list of amphibians classifies the European tree frog as endangered (Proess 2016) and it is protected under national law (Mémorial 2009).

Tree frogs typically colonize semi-open grassland and floodplains in the lowlands and hills. Spawning takes place from March to June and occurs in sun-exposed, small to medium-sized stagnant waters with exten-



Fig. 1. Adult tree frog (male) calling from a bush. Reckingerhaff Weiergewan. Photo: Liza Glesener, 02/06/2008.

sive shallow areas, at least some underwater vegetation – to which spawn is commonly attached – and vertical structures such as reeds (Fig. 2). Tree frogs are sensible to the presence of fish, as larvae spend part of their development exposed in open water. Adults frequent the spawning waters only during the night. During daytime, tree frogs – which are fine climbers – are found in brambles, shrubs, hedges, trees and tall herbaceous vegetation (Acemav coll. 2003, Laufer et al. 2007, Proess 2016). In general, many of the animals spend only one to two weeks or even less at the breeding pond (Grafe & Meuche 2005, Laufer et al. 2007, Pellet et al. 2007) before returning to terrestrial summer habitats. According to Blab (1986), the average distance between spawning grounds and terrestrial habitats is 600 m. However, migrations of several kilometres are common (Acemav coll. 2003, Proess 2016) and even distances of over ten kilometres are possible (Brunken 2004). This makes the tree frog an efficient colonizer of new habitats. Nonetheless, suitable spawning waters should ideally be situated only 300–500 m apart to promote interconnectivity (Laufer et al. 2007). It is assumed that, in our latitudes, hibernation takes place in terrestrial quarters such as rock crevices, burrows and root cavities, under moss or leaf litter, in cairns, sand piles or under rotten tree trunks situated mostly in woodlands (Acemav coll. 2003, Laufer et al. 2007, Proess 2016).

Tree frog populations are subject to multiple threats (Große et al. 2020). In Luxembourg, the species is highly dependent on the appropriate management of spawning waters and terrestrial habitats (Proess 2016).



Fig. 2. Spawning pond with adjacent terrestrial habitat in the form of rushes, an extensively managed meadow, hedgerows and woodlands. Bertrange Kopp. Photo: Liza Glesener, 04/06/2021.

Intense efforts with regard to pond maintenance and creation in the late nineties allowed the by then almost extinct species to temporarily recover in the north-western 'Gutland' (Junck & Schoos 2000). As maintenance efforts decreased, allowing succession to take hold, the newly formed populations crashed again. By 2011, only a single population in the south-east of the country remained (Proess 2016).

The re-establishment of the species solely through habitat management was deemed impossible at this point. Therefore, in 2012, the first tree frog reintroduction project in Luxembourg was launched in the region managed by the municipal syndicate for nature conservation SICONA. Naturally, reintroductions need to be complemented by substantial efforts with regard to habitat creation, optimization and management. Since its foundation over 30 years ago, SICONA has created or restored (i.e. desilted or enlarged) over 500 ponds. Most of them, as well as some additional ponds, are currently under SICONA management.

This paper gives an overview of the tree frog reintroduction project carried out by SICONA and partners from 2012 onwards, describing the methodology with regard to the reintroduction itself (source material, rearing, monitoring and general considerations) as well as providing details about the evolution of the tree frog population in the reintroduction area.

2. Methods

2.1. Study area

The study area is situated in the south-west and west of Luxembourg (Fig. 3) and is described in further detail in Glesener et al. (2022a). The predominantly heavy, loamy or clayey brown soils (Service de pédologie 1999) make the region well suited with regard to soil impermeability as a prerequisite for a closely meshed network of waterbodies able to hold viable and widely spread amphibian populations. Only in the central study area, around the Mamer and Eisch valleys, soils are mostly sandy and often podzolic (Service de pédologie 1999). Nevertheless, local soil conditions allow for permanent waterbodies in this region as well.

The first reintroductions focussed on the general re-establishment of the species in the country. Therefore, two geographically distant sites (Bertrange and Everlange) were selected in order to found new source populations. The second reintroductions (Leitrange, Abweiler and Olm) aimed to reinforce the tree frog population emerging from these two source sites by facilitating further dispersal particularly into areas where natural dispersal would have been impeded by motorways and major roads. All release sites (Fig. 3) lie within the historical distribution area of the species. Apart from the prerequisite that release sites need to provide appropriate habitat for all life stages and seasonal needs, they have to be large enough to hold a viable source population. Good connectivity to other suitable sites is essential to promote the quick establishment of the species in the region as a whole. Finally, the long-term guarantee of controlling the management of the release sites was deemed indispensable within the framework of our project and as such, four of the five release sites are under public ownership. All five release sites were created or optimized by SICONA with the tree frog as the principal target species and are managed by or under the syndicate's direction.

2.2. Captive-rearing for reintroduction

2.2.1. Sourcing of suitable donor populations and retrieval of source material

As the tree frog was almost extinct in Luxembourg in 2012 (Proess 2016), action had to be taken quickly. Fortunately, suitable donor sites were found in the Belgian province of Limburg (Maaseik – Itterbeekvallei and Vijvergebied Midden-Limburg te Zonhoven, Herr 2015), from which the donor animals for the first reintroductions (2012 to 2014) were obtained. During the second reintroduction period (2019 to 2024), our Luxembourg population from the Houbaach valley between Bertrange and Dippach was used as donor population instead (Glesener & Schneider 2024).

A visual search for spawn took place at daytime after nights with high calling activity.

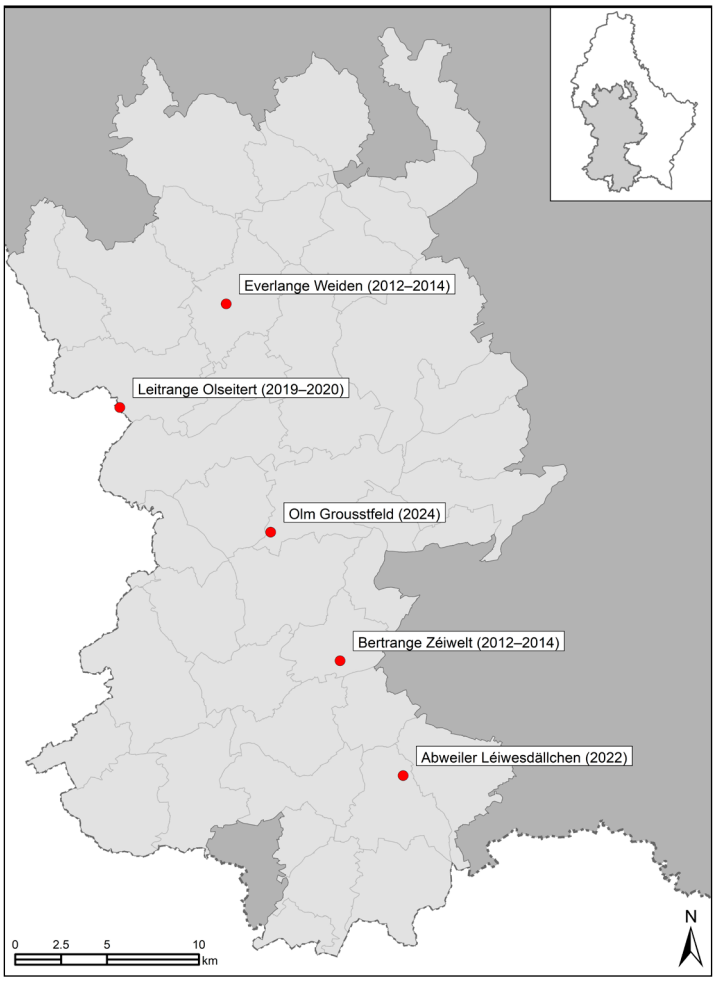


Fig. 3. Study area in the west and south-west of Luxembourg with the SICONA territory highlighted and underlaid with the municipalities. The red dots represent the tree frog release sites with the year(s) of release events in brackets.

The egg clutches, which are stuck to plants near the water surface, were collected by hand and with the plant. Tadpoles were trapped in funnel traps (Luxembourg) or collected with nets (Belgium). Egg clutches and tadpoles were transported over short distances in containers with air-permeable lids (Luxembourg) and with a portable oxygen generator for longer transports (Belgium).

The maximum number of animals to be removed was initially determined as a set value: the Belgian authorities gave permission to retrieve 2,300 eggs and 11 adults (2012–2014), while the Luxembourg authorities initially allowed the retrieval of 3,000 eggs or tadpoles per year (2019–2020). Maximum retrieval was always based on the protection of the source populations from negative impacts through the removal of individuals. In order to establish a more general and reproducible method, it was decided jointly with the Ministry of Environment, Climate and Biodiversity to further restrict the number of 3,000 individuals per year to no more than 1 % of the expected yearly egg production, based on the monitoring results. To determine this number, we equated the number of females to the maximum number of calling males in a single evening and multiplied this value by 7 eggs/per female (1% of 700, see below). While the "1% rule" officially only came into effect in 2021, in practice, it was applied from 2019 onwards.

Several studies show that the number of male tree frogs at spawning waters is generally 1.5 to

2.9 times higher than the number of females (e.g. Friedl & Klump 1997, Grafe & Meuche 2005, Laufer et al. 2007). Since, compared to capture-recapture studies, acoustic chorus counts underestimate the total number of males present by a similar factor of 1.5 to 3.1 (Grafe & Meuche 2005, Pellet et al. 2007), the estimate of females present was determined on the basis of our calling male records, assuming that the actual number of females present roughly corresponds to the number of calling males recorded. Available data on the number of eggs per female also vary considerably between 150 and 1,900 (Moravec 1989, Acemav coll. 2003, Laufer et al. 2007). In this context, we considered a value of 700 eggs per female a sufficiently conservative approach. Between 2012 and 2024, an estimated total of 7,859 eggs and tadpoles were collected (Table 1). While egg clutches make up the largest proportion of the source material, tadpoles were also collected occasionally.

2.2.2. Captive-rearing and release

Captive-rearing of tree frogs requires a location that is safe from predators and relatively warm, but can essentially take place both indoors and outdoors. In the first reintroduction period, we experimented with different configurations (greenhouse, open air, with and without filter, air pumps and heating devices). As rearing in the greenhouse without filters, pumps and additional heating proved to be a simple and effective method, rearing was carried out exclusively this way during the second reintroduction period. Aquariums and paludariums were filled with stagnant tap water and kept clean by removing uneaten food and regular water changes. For each water change, roughly a third of the water was replaced. While spawn and young tadpoles require little maintenance, the intensity of care rises with animal age and temperature.

Egg clutches were placed in the aquarium while still attached to plant material. This does not only prevent damage to the eggs, but the plants and associated microorganisms also provide food to the youngest larval stages which, under natural conditions, would graze food particles from the water surface, plants, stones, fallen leaves or the bottom of the pond (Laufer et al. 2007). Dried dandelion leaves and, as a source of animal protein, fish flakes and pellets, were

Table 1. Number and development stage of collected and released animals per year and per site.

Stages: e = eggs, s = spawn clutches *assuming an average of 30 eggs/clutch, j = juveniles, t = tadpoles. Sites: A1 = Maaseik – Itterbeekvallei (approx. WGS84 5.70158 E, 51.12542 N), A2 = Vijvergebied Midden-Limburg te Zonhoven (approx. WGS84 5.32745 E, 50.98431 N), B1 = Bertrange Zéiwelt (WGS84 6.04433 E, 49.59237 N), B2 = Bertrange Kopp (WGS84 6.03326 E, 49.58896 N), C = Everlange Weiden (WGS84 5.95732 E, 49.76755 N), D = Leitrange Olseitert (WGS84 5.87707 E, 49.71670 N), E = Abweiler Léiwesdällchen (WGS84 6.09118 E, 49.53651 N), F = Olm Grousstfeld (WGS84 5.99191 E, 49.65586 N).

	Estimated number and development stage of collected animals	Donor site	Number and development stage of released animals	Release site
2012	ca. 1,350 (e+t)	A1	600 (j); 500 (j)	B1; C
2013	ca. 700 (e)	A1	252 (j); 334 (j)	B1; C
2014	ca. 480 (e)	A1+A2	190 (j); 180 (j)	B1; C
2019	23 (s)* + 39 (t)	B1	489 (t)	D
2020	60 (s)*	B1	1,900 (t)	D
2022	ca. 1,050 (e)	B1+B2	1,222 (t)	E
2024	ca. 1,750 (e)	B1+B2	1,479 (t)	F
Total	**ca. 7,859**		**7,146**	

later added to the tadpole diet. In the first reintroductions, juveniles were fed with small crickets, drosophila and bananas. While tree frogs were reared to juvenile stage in the first reintroductions, this was subsequently replaced by rearing only to the tadpole stage in order to reduce the time in captivity and financial effort. Animals were kept strictly separated according to their origin in order to prevent possible spread of pathogens.

In total, 7,146 juveniles and tadpoles were released over the entire duration of the project. A breakdown of collected and released animals per year and site is provided in Table 1. Juvenile tree frogs from the first reintroductions were transported to the release sites in ventilated containers and allowed to disperse on their own. For the reintroduction of tadpoles, care was taken that the water in the transport containers could, if necessary, adjust to the temperature of the waterbody before the tadpoles were released by slowly angling the container into the release pond.

2.3. Monitoring

Since 2013, one year after the first reintroduction, we have implemented a standardized annual monitoring of reintroduced tree frog populations. Tree frog activity at the spawning waters begins as daytime temperatures rise in spring. Monitoring started in mid-April and was usually finished by the end of May, though in some years the monitoring period was extended into June, due to weather conditions. In our acoustic chorus counts (Heyer et al. 1994, Pellet et al. 2007), we distinguished between the main count, which served to discover trends in population size and extended to the whole of each of our two (currently still geographically distinct) populations in a single night (= population monitoring night), and side counts, which added to the dispersal data in general. Both counts were carried out on dry, calm and relatively warm evenings and were restricted to calling males. While ponds with up to five calling males could be surveyed from a distance, ponds with larger populations were surveyed by slowly walking around the pond pinpointing calling males on the closest bank and counting by sound.

The yearly monitoring area was defined in relation to the location of release sites and the natural dispersal originating from these sites. Thus, the monitoring always included all release sites and the closest surrounding waterbodies. If tree frogs were present in any of these ponds or had been found present at any time since the reintroduction monitoring's start in 2013, the monitoring area was expanded to the closest surrounding waterbodies accordingly. In 2024, the monitoring area covered roughly 146 km² and spanned over 22 municipalities.

2.4. Data preparation and analysis

All data about the reintroduction project is available in the national data repository (Recorder LUX) of the National Museum of Natural History. The compiled monitoring data is presented descriptively. For trend analysis, simple linear and exponential regression models were fitted to the data. The coefficient of determination R^2 and an F-test of overall significance were determined to evaluate the quality of the models. Statistical analysis was performed in Excel and in R version 4.3.2 (R Core Team 2024) using the R package "stats". To quantify the approximate extent of the distribution area, the Minimum Convex Polygon (MCP), as the most objective method, was determined. When a range of animals was counted, our analysis was based on the lower number, which is the more conservative approach.

2.5. Hygiene protocol

From the beginning of the project, care was taken to prevent the transmission of the fungal disease Bd (*Batrachochytrium dendrobatidis* Longcore, Pessier & D.K. Nichols, 1999) which poses a threat to various amphibian species. In 2012 to 2014, samples of juvenile donor animals were swabbed and tested for Bd at the Faculty of Veterinary Medicine at Ghent University before release. In 2019 and 2020, spawn samples were tested by a commercial veterinary laboratory in Berlin (Exomed GmbH i.G.). By 2021, it was decided in conjunction with the

Ministry of Environment, Climate and Biodiversity that testing for Bd would be carried out at pond level by analysing swabs of five adult tree frogs as a substitute to spawn or tadpoles. Furthermore, from 2021, swabbing and testing of five adult anurans was also carried out at the release sites. In 2022, a selection of ponds was tested via the retrieval of environmental DNA (eDNA, Porco et al. 2024) in addition to swabs. All tests over the years were carried out via PCR-analysis, though exact procedures and methodology varied between laboratories. In 2024, a new precaution was introduced by significantly reducing the transfer of pond water into the aquariums – only spawn and plant material were transferred. Donor and receptor sites as well as aquarium water samples were tested via eDNA.

Prophylactic measures during the rearing stage included the strict separation of animals from different sites of origin. In addition to this, maintenance equipment was explicitly dedicated to a single group of individuals from the same site for the duration of the rearing phase. Subsequently, all materials were cleaned and disinfected with vinegar and/or 70 percent ethanol and allowed to dry out completely.

While the field protocol in earlier stages of the project followed best known practice at that time, it is currently based on the official hygiene protocol for Luxembourg (Stassen et al. 2022).

3. Results

3.1. Population development at donor and release sites

While little is known to us about population development at the donor sites in the province of Limburg, we have followed the Luxembourg donor population – which is originally a release population itself – closely over the years. The use of Luxembourg donor sites from 2019 was made possible through the successful establishment of the newly founded populations originating from the first reintroductions. Even though sporadic dips in population size have been observed, an overall upward population trend was observed at our donor sites (Table 2) and at the release sites (Fig. 4).

3.2. Population development over the entire study area

Long-term data on the spread of tree frogs from the release sites is available for Bertrange Zéiwelt and Everlange Weiden. The population in Bertrange spread out from year one and became the origin of 59 newly populated sites, the furthest being situated at 8.2 km from the release site as the crow flies (Fig. 5). The surface of the Minimum Convex Polygon (MCP), as a rough delineator of the extent of the distribution area, currently covers 51.6 km^2 (Fig. 6). By contrast, the population in Everlange Weiden remained restricted to the release site for seven years after release. In 2019, the first dispersal into adjoining habitats was recorded, but has been limited to one site until 2021. Like Bertrange Zéiwelt, Lei-

Table 2. Number of calling males per year on the two Luxembourg donor sites only. Each donor site is a complex of several ponds. Numbers represent the result of the population monitoring night. Bertrange Zéiwelt is the release site from the first reintroductions (2012 to 2014), while tree frogs spread from Bertrange Zéiwelt to Bertrange Kopp as early as 2013. Years in which spawn retrieval took place are marked in bold for both sites.

	Bertrange Zéiwelt	Bertrange Kopp
2013	18	6
2014	18–23	8–10
2015	36	12
2016	45	40
2017	50	52
2018	75	100
2019	**79**	80
2020	**125**	138
2021	>80	137
2022	**44–46**	**121**
2023	89	249
2024	**118**	**213**

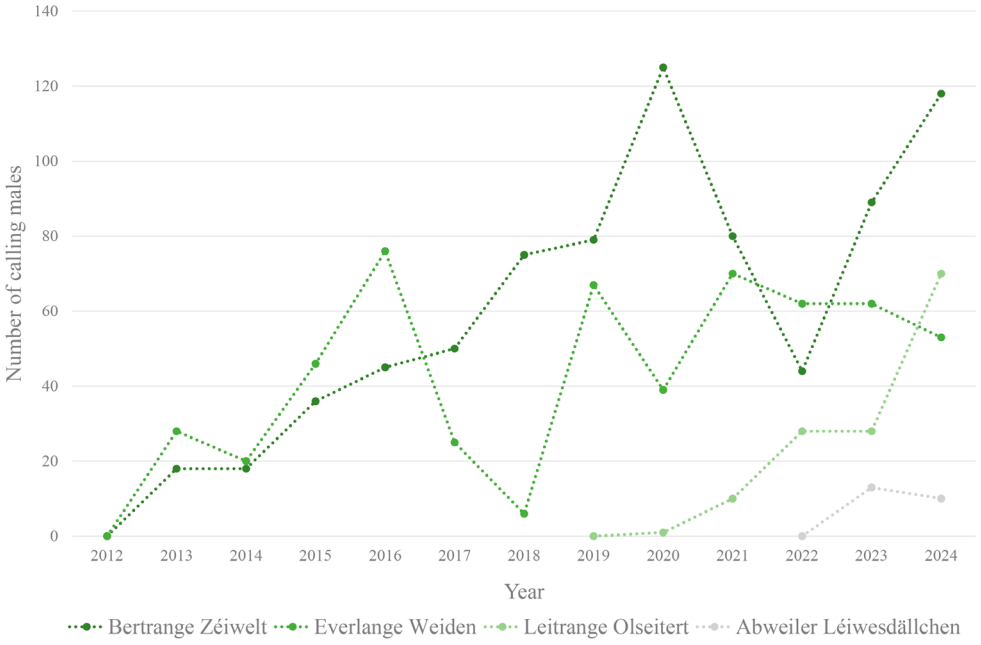


Fig. 4. Number of calling males per year at each of the release sites. Numbers represent the result of the population monitoring night. Olm Grousstfeld was omitted, seeing as the first release was in 2024.

trange Olseitert, situated at roughly 8 km to the south-west of Everlange Weiden, showed a rather rapid spread: in 2023, the third year after release, four additional sites had been colonized. In 2024, the populations originating from the latter two sites merged. For the two newly colonized sites in the connection zone, it is therefore unclear from which of the two original sites they were colonized. Overall, Everlange Weiden and Leitrange Olseitert are the most likely sources for 16 colonized sites spread over 82.9 km^2 (MCP). The population in Abweiler Léiwesdällchen (release in 2022) spread to one adjoining site by 2024. Differences in the dispersal of the population originating from Bertrange Zéiwelt and the one originating from Everlange Weiden and Leitrange Olseitert are reflected in statistical trend analysis. When plotting the number of populated sites over the years, both populations show an exponential trend (Bertrange Zéiwelt: $R^2 = 0.96$, F-statistic: $p = <0.001$; Everlange Weiden and Leitrange Olseitert: $R^2 = 0.82$, F-statistic: $p = <0.001$), but for the Everlange Weiden and Leitrange Olseitert population the trend line rises less sharply. This difference is even more pronounced when comparing the number of calling males in both populations. Here, the population originating from Bertrange Zéiwelt also shows a strong exponential trend, whereas the number of calling males in the population originating from Everlange Weiden and Leitrange Olseitert fluctuates more over the years and is better resembled by a linear trend (Fig. 7). A graphic overview of the number of calling males and the number of populated sites per dispersal area is provided in Fig. 8.

Statistical trend analysis for the entire study area shows strong exponential trends, both for the overall number of calling males and the overall number of colonized sites (Fig. 9). With 827–840 calling males in 2024, the number has more than doubled since 2020 (379–391) and nearly quadrupled since 2018 (226–229). On average, the entire population in our study area increased by 34.6 % per year. Overall, the number of calling males at any given site varies considerably. In general – but not as a rule – new sites were colonized initially by one to five calling males. While many sites

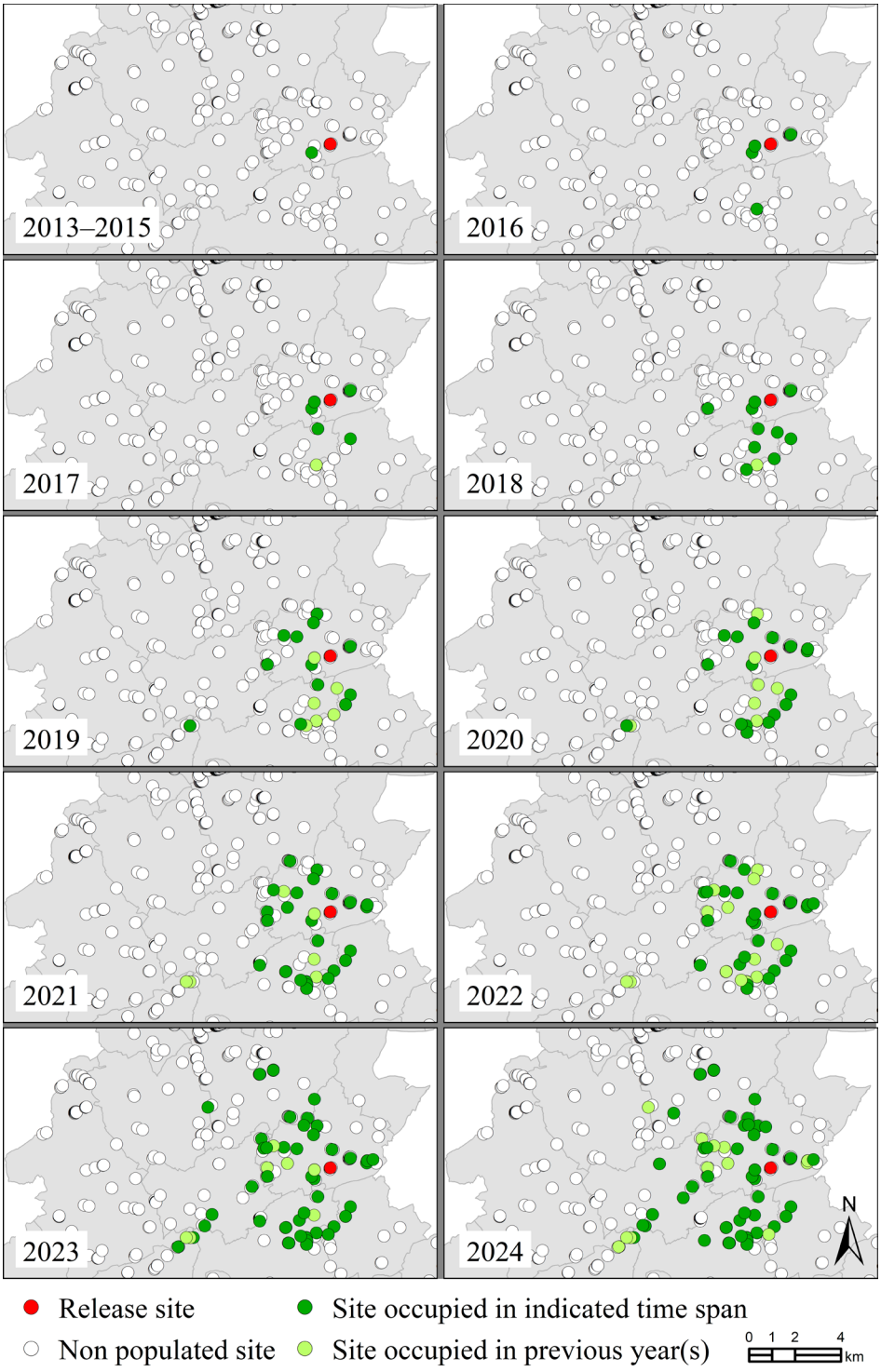


Fig. 5. Time lapse of the population development originating from Bertrange Zéiwelt over the years 2013–2024. Ponds: SICONA, 2024.

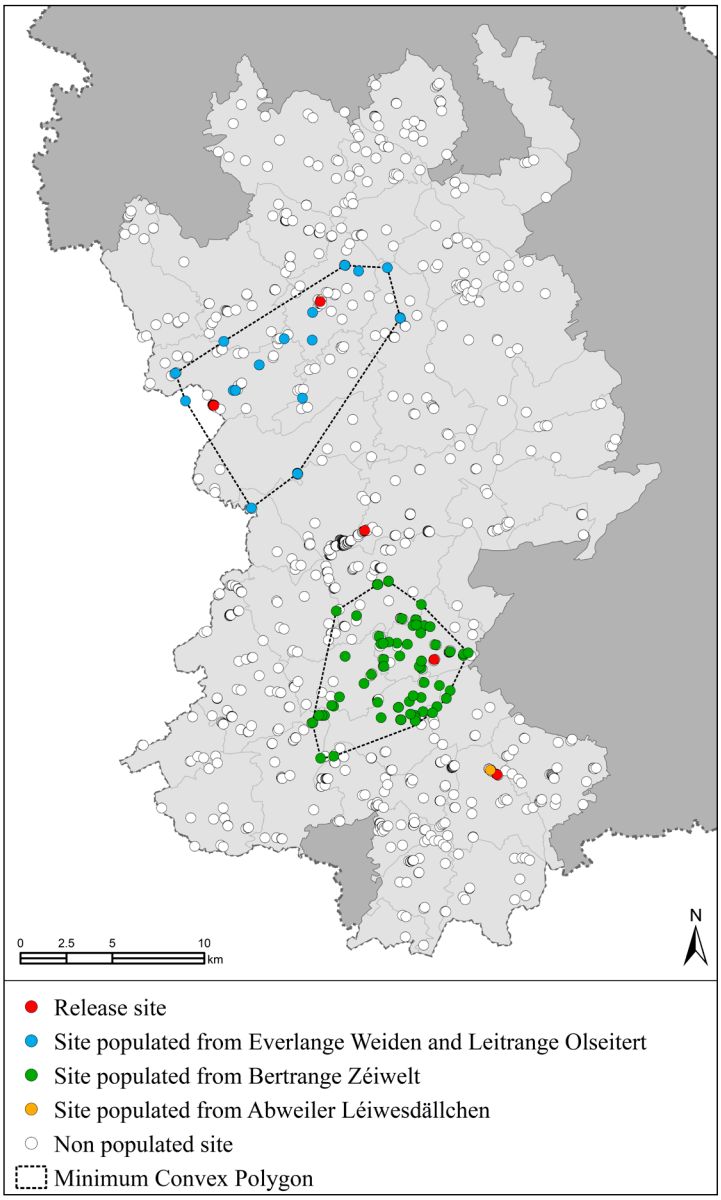


Fig. 6. Dispersal of the population from the release areas as of 2024, with indication of the MCP for sites populated from Bertrange Zéiwelt or Everlange Weiden and Leitrange Olseitert, respectively. Only water-bodies outside of forested areas are depicted. Ponds: SICONA, 2024.

subsequently remained stable or showed a slight further increase up to ten callers, at a few sites populations grew larger. In 2024, 54.2 % of the 59 sites with calling males recorded during the population monitoring night held 1–5 calling males, 23.7 % held 6–10 and 6.8 % each held 11–20 or 21–50 calling males, respectively. In 8.5 % of the sites, the number of calling males exceeded 50, with a maximum of 118.

With a few exceptions in the years after the first reintroduction, 25–53 % of sites with calling males were newly colonized in any given year (Table 3) – with an average of 35.0 % per year. On average, the number of all populated sites per year increased by 34.9 %. To date, a total of 80 sites has been recorded with one or more calling males at least once since 2013, with the maximum number of sites populated in a single year

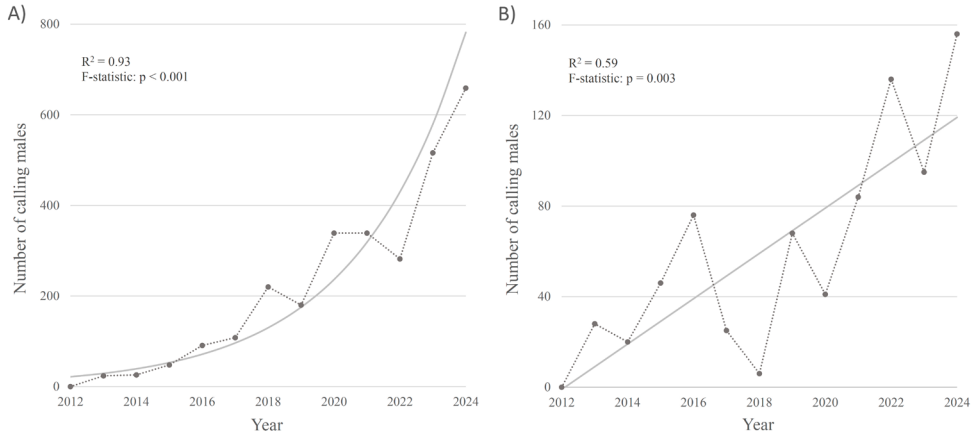


Fig. 7. Trend analysis for A) the number of calling males in the population originating from Bertrange Zéiwelt and B) the population originating from Everlange Weiden and Leitrange Olseitert. Numbers represent the result of the population monitoring night. Observed values are depicted as a dark grey dotted line, model trend lines in light grey.

being 61 (2024). Not all colonized waterbodies in the surveyed area hold tree frogs every year, as illustrated by the time lapse of the area populated from Bertrange Zéiwelt (Fig. 5). In 2024, 42.9 % of all 77 sites visited on a yearly basis since the initial colonization event have held calling males every year since the first colonization, while 37.7 % have only been colonized sporadically. In almost half of the sporadically colonized sites (41.4 %), calling males have been recorded only once over the entire period. The newly colonized ponds of 2024 made up 19.5 % of all sites.

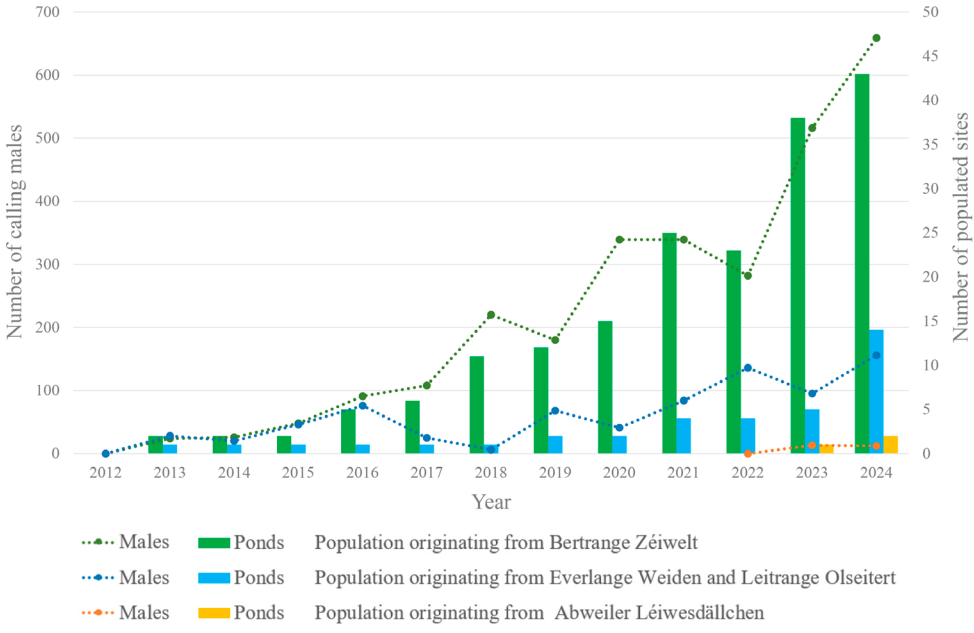


Fig. 8. Number of calling males and populated sites per year in each of the dispersal areas. Numbers represent the result of the population monitoring night. Olm Grousstfeld was omitted, seeing as the first release was in 2024.

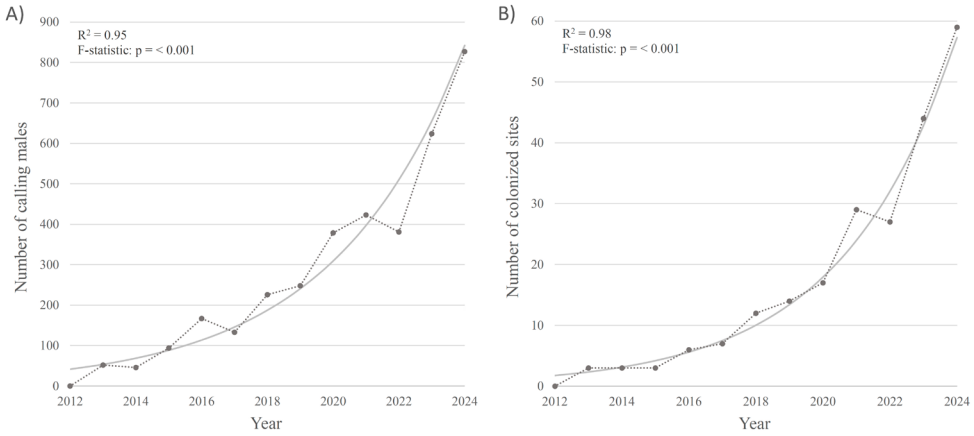


Fig. 9. Trend analysis for A) the total number of calling males and B) the total number of populated sites in the entire study area. Numbers represent the result of the population monitoring night. Observed values are depicted as a dark grey dotted line, model trend lines in light grey.

4. Discussion

4.1. Population development and spread

Both the total number of calling males and the total number of colonized sites in the study area show strong exponential increases over the study period. By these numbers, our tree frog reintroduction project can be called a great success. The fact that a high proportion of the ponds (37.7 %) were colonized only sporadically reflects the high (re)colonization ability of the species (Clausnitzer, 1996). Why certain ponds fell into disuse and became attractive again in subsequent years remains unclear.

Table 3. Number of sites with calling males and percentage of newly colonized sites per year over the entire study area. Release sites are included as newly colonized in the year calling males first appear. Sites can feature more than one pond if several nearby ponds were considered to form a biotope complex.

	Sites with calling males	Newly colonized sites
2013	3	100 %
2014	3	0 %
2015	3	0 %
2016	6	50 %
2017	8	25 %
2018	12	42 %
2019	15	53 %
2020	20	35 %
2021	28	29 %
2022	30	27 %
2023	56	34 %
2024	61	26 %

Similar reintroduction projects have been carried out in Switzerland, the Netherlands, Belgium and most notably Germany since at least the early eighties. Examples are Clausnitzer (1996), Brandt & Lüers (2017a and 2017b), Brandt et al. (2018), Meier et al. (2000), Crombaghs & van de Koppel (2014), Carels C. (2022, personal communication), Tester (2022). Other studies were mentioned in popular literature, but we could not find any scientific documentation about these. Finally, Meier et al. (2000) believe that failure is rarely documented at all. Where scientific documentation was available to us, it became apparent that population development and spread showed considerable variation – as is incidentally the case within our study as well.

While the populations originating from Bertrange Zéiwelt and Everlange Weiden, with an initial release of roughly a thousand juvenile frogs at each release site, featured very similar numbers of calling males in year four

after reintroduction (91 and 76, respectively; see Fig. 8), the Everlange Weiden population reached an all-time low with only six calling males in year 6 after release and remained restricted to the release site. Meanwhile, the population originating from Bertrange Zéiwelt slightly exceeded the numbers provided by Meier et al. (2000) for the 'Dingdener Heide' in Saxony (220–223 vs. 170–200 calling males). A comparison with several projects carried out in Lower Saxony with our data relating to the years 8, 10 and 11 after release yielded mixed results: while the population originating from Bertrange Zéiwelt compared favourably with that in the 'Sachsenhäger Niederung' (Brandt & Lüers 2017a; 380 calling males), it was surpassed considerably by those in the 'Bückeburger Niederung' (Brandt et al. 2018; 1,500 calling males) and the 'Steinhuder Meer-Niederung' (Brandt & Lüers 2017b; 3,600 calling males). From year 11 after release, the release zone Bertrange Zéiwelt with well over 500 calling males (Fig. 8) featured similar numbers as the 'Dingdener Heide' (Geiger et al. 2011, year 13) and the 'Südheide' (Clausnitzer 1996, year 15) in North Rhine-Westphalia. The population originating from Everlange Weiden ranked comparatively low overall.

A direct comparison between projects is hampered by a number of variating factors, including differences in the number and development stage of released animals, the timing of release actions (i.e. release over one or several years), monitoring methodology and conditions as well as differences in habitat configuration. While every study consulted for the present article used acoustic monitoring to determine the number of calling males, counts in our study were carried out around the entire circumference of the pond, whereas Meier et al. (2000) counted two to three areas and extrapolated from there and Clausnitzer (1996) used classes rather than providing exact numbers. The choice of a monitoring night alone can have an enormous impact on the results, as tree frog activity is closely linked to weather conditions, such as temperature and humidity (Tester 2022). Even 'good' monitoring nights can yield differences. For example, a count in Bertrange Zéiwelt in late April resulted in 44–46 calling males, while 72 were present only two weeks earlier. A possible explanation for such differences is that many individuals spend only a few nights to two weeks near the water (Grafe & Meuche 2005, Laufer et al. 2007, Pellet et al. 2007) and call activity of individual males varies over the course of an evening and also between nights (Tester 2022). The presence of large choirs makes the count considerably more difficult and can only result in estimates. Nonetheless, while acoustic chorus counts cannot provide the exact population size and the method generally leads to underestimates (Grafe & Meuche 2005, Pellet et al. 2007, Tester 2022), it certainly allows to derive good population trends when carried out in line with a standardized method.

European tree frogs have a short life span of usually three to five years (Acemav coll. 2003), which can lead to considerable natural fluctuations in population size. This in turn affects the comparability between different studies. Other external factors can also have great influence on population development. For example, Meier et al. (2000) recorded that reproductive success had almost come to a standstill with a subsequent population decline of 50 % between 1998 and 2000 in two ponds where fish had been introduced. In our study, dips and subsequent rises have been recorded for both the population originating from Bertrange Zéiwelt and Everlange Weiden. We consider these to be natural fluctuations in the population originating from Bertrange Zéiwelt, but think that additional factors may be involved in the population from Everlange Weiden (see below). The merging of the populations originating from Everlange Weiden and Leitrange Olseitert 12 years after the first release does not allow for further conclusions about the spread originating from Everlange Weiden alone but can itself be considered a success.

The most important single factor steering population development and spread (and thus also limiting the comparability between studies) is situated on the landscape level. Meier et al. (2000) stipulate that it is not the number of released animals, but the quality of the landscape in combination with a large-scale project area that are key to success, while Clausnitzer (1996) emphasizes that the creation of reproductive subpopulations is essential for continuity and that dispersal

is not only limited by geographical distance, but also by ecological distance. While some animals will be able to traverse inhospitable structures like busy roads, urban areas or dry arable fields, such structures are serious impediments to dispersal. On the other hand, a network of ponds connected by hedgerows, brambles, shrubs, damp forests, tall herbaceous and extensively managed vegetation (e.g. Polivka & Hill 2008) does not only provide aquatic and terrestrial habitats, but facilitates dispersal immensely. In our study, the differences observed in the development of the two main populations in the study area can be attributed at least in part to different landscape configurations. Both areas feature impediments to dispersal in the form of roads and settlements. However, the network of ponds is denser around Bertrange Zéiwelt. The area also features a higher proportion of surrounding woodlands. Perhaps most importantly, the proportion of arable land and intensively used grassland is much higher in the surroundings of the Everlange Weiden population, while the reintroduction site in Bertrange Zéiwelt is situated in one of the country's larger areas of long-established, nearly contiguous extensively managed wet and mesophile grasslands (see Siebenaler et al. 2020, Glesener et al. 2022b), making it an ideal core area for an expanding tree frog population. Around this zone, the landscape shifts towards a more even mix of arable land and grassland, yet ponds and forests, and to some extent extensively managed grassland, remain frequent. The difference between the rapidly expanding population originating from Leitrange Olseitert and the almost stagnant population in Everlange Weiden is harder to explain, as the surrounding countryside is very similar with regard to pond density, agricultural use and structural diversity.

In this context, a statement by Meier et al. (2000) regarding the importance of core reproduction sites – which cannot be equated to any site with calling males – as the pillar of a stable population may gain special importance for the future of our project. Until now, the documentation of reproduction success has not been part of our yearly monitoring, mainly for reasons of time and financing. While we consider exact reproduction numbers of secondary importance as long as expansion continues successfully, Clausnitzer (1996) argues that leaving aside reproduction control gives a false picture of the population overall. Our monitoring method may thus have to be reviewed, at least for the population in Everlange Weiden. Low reproductive success may well explain the stagnant population and could potentially be remedied by appropriate habitat interventions at site level. Another potential issue could be a high mortality rate of juveniles wandering off, either through natural or anthropogenic causes. For example, wet, fallow areas in arable fields are very attractive to amphibians and can turn into a death trap if reintegrated into the management regime in late summer (Berger et al. 2004). The mowing of roadside vegetation near breeding ponds at an unpropitious time can have similar devastating effects (Hansen 2004). Reproductive success from mating and egg-laying to the dispersal of juveniles should be investigated in more detail in order to allow for the adaptation of conservation measures.

Three sites are located comparatively far outside the contiguous distribution area of the species in our study area. Two localities near Steinfort and Hobscheid (Proess, R. 2023/2024, personal communication), are almost six kilometres away from the nearest site with regularly documented presence of the tree frog in previous years. Although such large migration distances are unusual for our project, they are nevertheless possible for the species (see 1.). Another far off site near Roeser (Lorgé, P. 2017, personal communication) was omitted from our analysis. To reach this site from the nearest population known to us, a migration of 8.2 km including the crossing of two motorways would have been necessary at that time. For this reason, we consider the site as a potential, unauthorised reintroduction independent from our project.

4.2. Logistical and epidemiological aspects

For amphibians, the best life stage(s) for release vary from species to species and experimental trial may be necessary to

develop optimal protocols (Linhoff et al. 2021). Where the release of various stages is possible, the choice may be determined by logistical reasons (e.g. budget, infrastructure) or animal welfare considerations (time in captivity, ease and safety of transport). Donor material may only be available in very limited numbers or the species may exhibit a very high mortality in its early life stages, in which case head-starting, i.e. rearing to later life stages, may be the most effective option (Linhoff et al. 2021). While tree frogs have been reintroduced in every stage of development from eggs (Brandt et al. 2018) and tadpoles (Clausnitzer 1996, Meier et al. 2000, Brandt & Lüers 2017a, Brandt et al. 2018, Tester 2022) to juveniles (Carels C. 2022, personal communication), subadults (Tester 2022) and adults (Pastors 1995), the reason for selection of a particular stage remains mostly unmentioned. Pastor's (1995) release of adults was not successful, while little can be said about the relative success of releasing eggs or subadults, as they were introduced together with large numbers of tadpoles in the projects documented by Brandt et al. (2018) and Tester (2022). While our own data suggests that the release of juveniles was more effective than the release of tadpoles, the time span of our study is too short to draw conclusions and fluctuations in the numbers of calling males four years after release are likely the result of other factors. In any case, the release of tadpoles appears to be the most common method and is demonstrably sufficiently effective (Clausnitzer 1996, Meier et al. 2000, Brandt & Lüers 2017a and 2017b). The choice of releasing tadpoles versus juveniles is determined by other factors than population establishment alone.

In our project, it seemed sensible for the first reintroductions to boost the nearly extinguished population with juveniles, as their chances for survival in the wild were expected to be higher than for tadpoles. Additionally, the time-intensive and limited procurement of donor material from abroad justified the considerable extra cost and effort to rear animals to a later development stage. With the successful establishment and expansion of the population at Bertrange Zéiwelt, we switched to the much more cost-effective release of tadpoles, reared from hand-collected spawn. While captive-rearing from spawn to tadpole goes with increased labour-cost, tree frog spawn is easy to find and safer to handle than young tadpoles. We could not find any specification concerning the ideal number of donor animals in literature, but consider our choice of 1% of the expected eggs conservative enough to avoid negative impact on the donor populations, while also high enough to allow the continuation of the project.

Both spawn retrieval and population census were based on acoustic chorus counts. The method inherently does not provide exact population counts, but was deemed the most suitable for our purposes (see 5.1.). Nonetheless, the need to review some of our procedures arises: the monitored area is expanding every year, leading to higher requirements for manpower and finances. In 2024, the joint effort of six people was barely sufficient to cover the population originating from Bertrange Zéiwelt in one night. The last sites were only reached as choirs already started to become noticeably more subdued. Even with more surveyors, the moment will come when a coordinated effort over one night will no longer be sufficient to cover the colonized area(s). At this point in time, the fact that calling males may relocate between sites and nights adds a further layer of uncertainty to the population census. However, the project has reached a stage where a less frequent surveillance of core sites coupled with presence/absence monitoring in secondary sites and expansion zones, perhaps even only every 2–3 years, seems sufficient to document further population development. Another possibility would be a shift towards selective monitoring of reproductive success. While additional sites in the west of Luxembourg were originally intended for reintroductions, these are currently on hold until the success of the two most recent releases in the area can be better assessed and additional donor populations from neighbouring countries are found. We are aware that our reintroduced populations, which on the whole originate from only three spawn retrieval events at a single site, may be limited in terms of genetic diversity. The introduction of further individuals from

moderately divergent populations would help to improve genetic fitness without risking deleterious effects linked to outbreeding depression (Luquet et al. 2011). Reintroductions in other parts of the country should also be considered, particularly in order to link the eastern remnant population with the newly founded western population. Lastly, we also expect some natural mixing to occur once our population connects with the population emerging from the recent reintroductions conducted by Natagora in the Walloon region in Belgium, at a distance of around 30 kilometres from our study area (Carels C. 2023, personal communication).

Another topic of great relevance is our shift in knowledge about epidemiological considerations overall and the epidemiological situation in the project region. While Bd has been confirmed to be present in Luxembourg since at least 2009 (Wood et al. 2009, Proess et al. 2015), the occurrences were thought to be isolated. Nevertheless, care was taken to carry out pre-release tests of animals from the start of the project. Yet with the decision to reintroduce animals as tadpoles, testing for Bd among the donor animals became difficult, as spawn is not really affected by Bd and tadpoles carry the fungus only in the keratinized mouthparts (Hyatt & Whittington 2021), which are hard to swab without injuring the animal. Our testing of spawn during two project years was ill-advised. However, Bd could at worst have been present in its motile, waterborne and most importantly short-lived zoospore form (Hyatt & Whittington 2021) in the pond water that was transferred with the spawn and the risk of raising infected tadpoles can be deemed minimal. To remedy the unsatisfactory situation without incurring tadpole losses, we decided to test at site level, by swabbing samples of adult anurans. This allowed us to include the potential release sites, where, in the absence of tree frogs, water frogs (*Pelophylax* spec. Fitzinger, 1843) were swabbed and tested. Meanwhile, the recent spread of the amphibian pathogen Bsal (*Batrachochytrium salamandrivorans* Martel, Blooi, Bossuyt & Pasmans, 2013) into our close border regions (Lötters et al. 2020) presented an additional challenge. While anurans themselves appear to remain largely unaffected, they may act as reservoirs for the pathogen (Lötters et al. 2020). In 2022, we explored environmental DNA (eDNA) as a less intrusive, quicker and more cost-effective method of testing our donor and release sites (Porco et al. 2024) for both Bd and Bsal. Compared to previous assumptions, the results suggested a much higher prevalence of Bd in the country. Porco et al. (2024) also detected the first positive for Bsal, though it remains to be confirmed (see Böning et al. 2024). While sites with suspected Bsal infestation are a no-go, it can be argued that the high existing prevalence of Bd may render the occurrence of the fungus irrelevant for reintroduction projects. Linhoff et al. (2021) state that 'in general, if the same pathogen is present in both the captive and wild populations, then infected animals can be released'. The basic prerequisite for this is the absence of strain differences between Bd isolates, which is likely for our operational area, but not yet certain without a more in-depth analysis (Lötters S. 2022, personal communication). Meanwhile, testing at site level – at least with regard to tree frog donor sites – also needs to be reviewed. While a negative result provides a safeguard, a positive result at site level does not reflect the actual status of tadpoles emerging from collected spawn, which cannot act as a host itself. In 2024, water samples from the aquarium holding the donor tadpoles turned out to be negative although the donor site was previously tested positive. Thus, the animals could subsequently be released. Testing at site level with the currently used technology also poses the problem of relatively long delays between test and result. In practice, this means that spawn has to be retrieved before the status of the donor site is known and rearing of tadpoles has to be extended until the test results are available. It is a logistical and financial challenge to combine satisfactory disease transmission prevention with our national obligation to increase our rare amphibian populations. In the end, each project needs to determine what level of risk is acceptable and what level of resolution is needed to make appropriate decisions (Linhoff et al. 2021) based on current scientific knowledge.

5. Conclusion

After the near extinction of tree frogs in Luxembourg, the population that emerged from our reintroduction project now spreads over around 135 km² and, with currently five source populations, is expected to continue its expansion as long as there are suitable habitats to colonize and corridors to reach them. It is known that populations, whether newly established or remnant, can be successfully strengthened by habitat creation, improvement and consequent maintenance (e.g. Clausnitzer 1996, Junck & Schoos 2000, Meier et al. 2000). Thus, this will remain the most important aspect of our future work. With regard to the creation of suitable habitats, the presence of a network of breeding ponds is essential. Early successional stages must be maintained, as silted and overgrown ponds are quickly abandoned by tree frogs. On the other hand, newly created or restored sites are usually colonized rapidly (Clausnitzer 1996, Böttger 2004). In our project area, we will continue to create new ponds (Glesener et al. 2022a) and manage these and already existing ones consistently to provide lasting suitable breeding habitat. Tree frogs spend most of their life cycle outside the water. Brambles, shrubs, hedges, trees, woodlands and tall herbaceous vegetation, particularly when extensively used, provide suitable land habitat and connectivity between breeding sites. Therefore, these structures also deserve special consideration. It is important to implement concrete nature conservation measures around and between ponds, such as negotiations for extensive land use through contractual nature conservation (Wolff et al. 2020), habitat restoration measures and management in general.

Continuous monitoring is also essential for reintroduction projects and allows for timely adaptations of the general approach or the rapid implementation of specific conservation measures, if required. Both contribute strongly to the success of such projects. We will continue to monitor our populations, although we may focus on reproduction monitoring and the identification of the most valuable breeding ponds in the coming years. Nonetheless, we will also continue to observe quantitative and spatial population developments. Where dispersal is hampered, the reasons should be identified and removed with priority on the reduction of known significant obstacles to dispersal (e.g. through underpasses at major roads). Further reintroductions, with new donor populations from neighbouring countries, are envisaged in order to enhance genetic diversity and perhaps close the gap between the re-established western and the remnant eastern population.

Most of the necessary tools to implement these actions in Luxembourg exist in the form of species and habitat conservation measures planned and funded through the National Plan for the Protection of Nature (Ministère de l'Environnement, du Climat et du Développement durable 2023) and government-funded biodiversity contracts. Future endeavours should be further facilitated through the passing of the Nature Restoration Law (European Parliament 2024) which sets binding targets to restore degraded ecosystems and is a long-awaited instrument for the effective protection of rare habitats and endangered species.

We deem our project a great success and would encourage others to use it as an inspiration and model to attempt tree frog or other amphibian species reintroductions, under the premise that there is no remnant population and that natural recolonization is unlikely. After all, any animal translocation needs to take into account a large number of aspects including financial and legal requirements, public engagement, habitat management, sourcing and releasing of organisms, post-release monitoring (IUCN/SSC 2013) and – in the case of amphibians – prevention of disease transmission.

Finally, we would like to point out the multiplying effect of conservation measures aimed at tree frogs. Not only do tree frogs represent an umbrella species, they can also be regarded as a sentinel of wetland quality (Pellet 2005). For example, tree frogs are associated with ponds rich in amphibians and providing habitat for wading birds and pioneer species including Red-listed plants and dragonflies (Meier et al. 2000, Pellet 2005). Extensive management of sites surrounding breeding ponds also benefits the associated plant communities and a multi-

tude of bird and insect species (Siebenaler et al. 2020). Every single species is integral to an intact ecosystem, which in turn is the prerequisite for the efficient provision of ecosystem services (Härdtle 2024).

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References

Acemav coll. (ed), 2003. Les Amphibiens de France, Belgique et Luxembourg. Collections Parthénope, éditions Biotope, Mèze (France), 480 pp.

Berger, G., T. Schönbrodt & H. Pfeffer, 2004. Naturschutz in der Landwirtschaft mittels Flächenstilllegung – Profitiert (auch) der Laubfrosch (*Hyla arborea*) davon? In: Der Europäische Laubfrosch (*Hyla arborea*). Biologie – Schutzmaßnahmen – Effizienzkontrollen (Glandt, D. & A. Kronshage, eds). *Supplement der Zeitschrift für Feldherpetologie* 5: 37–54.

Blab, J., 1986. Biologie, Ökologie und Schutz von Amphibien. *Schriftenreihe für Landschaftspflege und Naturschutz* Heft 18, 150 pp.

Böning, P., A. Plewnia, J. Virgo, J. Adam, N. Banowski, S. Bleidißel, N. Dabbagh, L. Dalbeck, H. Düssel-Siebert, S. Ellwart, L. Feiler, V. Ferner, M. Fischer, L. Gemeinhardt, M. Guschal, A. Geiger, G. Hansbauer, M. Hechinger, T. Hildwein & S. Lötters, 2024. Die Salamanderpest: Charakterisierung, aktuelle Situation in Deutschland, Handlungsempfehlungen. *Zeitschrift für Feldherpetologie* 31: 1–38.

Böttger, R., 2004. Bestandsentwicklung des Laubfrosches (*Hyla arborea*) im Bereich Unna, Nordrhein-Westfalen. In: Der Europäische Laubfrosch (*Hyla arborea*). Biologie – Schutzmaßnahmen – Effizienzkontrollen (Glandt, D. & A. Kronshage, eds). *Supplement der Zeitschrift für Feldherpetologie* 5: 55–62.

Brandt, T. & E. Lüers, 2017a. Die erfolgreiche Wiederansiedlung von Europäischen Laubfröschen (*Hyla arborea*) in der Sachsenhäger Niederung, Landkreis Schaumburg, Niedersachsen. *Zeitschrift für Feldherpetologie* 24: 209–222.

Brandt, T. & E. Lüers, 2017b. Ergebnisse einer wissenschaftlich begleiteten Wiederansiedlung von Europäischen Laubfröschen (*Hyla arborea*) in der Steinhuder Meer-Niederung, Niedersachsen. In: Um- und Wiederansiedlung von Amphibien und Reptilien – Beispiele, Probleme, Lösungsansätze (Hachtel, M., C. Göcking, N. Menke, U. Schulte, M. Schwartze & K. Weddeling, eds). *Supplement der Zeitschrift für Feldherpetologie* 20: 52–69.

Brandt, T., E. Lüers & H. Buschmann, 2018. Wiederansiedlung und Dispersionsdynamik einer Population des Europäischen Laubfrosches (*Hyla arborea*) in einer Auenlandschaft bei Bückeburg, Landkreis Schaumburg, Niedersachsen. *Zeitschrift für Feldherpetologie* 25: 207–224.

Brunken, G., 2004. Amphibienwanderungen - Zwischen Land und Wasser. NVN/BSH Merkblatt 69 (1/04), 4 pp.

Clausnitzer, H.-J., 1996. Entwicklung und Dynamik einer künstlich wiederangesiedelten Laubfrosch-Population. Ein Beispiel für die Bedeutung des Prozessschutzes. *Naturschutz und Landschaftsplanung* 3: 69–75.

Crombaghs, B. & S. van de Koppel, 2014. Nieuwsbrief Herintroductie boomkikker Noord-Brabant. Naturbalans – Limes Divergens BV, 15 pp. URL: https://issuu.com/merkske/docs/12-186_nieuwsbrief_2013_herintroduc [accessed 13/09/2024].

European Parliament, 2024. Regulation of the European Parliament and of the Council on nature restoration and amending Regulation (EU) 2022/869. Brussels, 93 pp. URL: https://data.consilium.europa.eu/doc/document/PE-74-2023-INIT/de/pdf [accessed 28/06/2024].

ETC/BE & EEA – European Topic Centre on Biodiversity & European Environment Agency, 2024. Species assessments at EU biogeo-

graphical level for *Hyla arborea*. URL: https://nature-art17.eionet.europa.eu/article17/species/summary/?period=5&group=Amphibians&subject=Hyla+arborea&region=, [accessed 28/06/2024].

Friedl, T. W. P. & G. M. Klump, 1997. Some aspects of population biology in the European treefrog, *Hyla arborea*. *Herpetologica* 53(3): 321–330.

Geiger, A., T. Mutz & R. Böttger, 2011. Laubfrosch – *Hyla arborea*. In: Handbuch der Amphibien und Reptilien in Nordrhein-Westfalen (Arbeitskreis Amphibien und Reptilien Nordrhein-Westfalen, ed). Band 1, Laurenti-Verlag, Bielefeld: 689–724.

Glesener, L., P. Gräser & S. Schneider, 2022a. Conservation and development of great crested newt (*Triturus cristatus* Laurenti, 1768) populations in the west and south-west of Luxembourg. *Bulletin de la Société des naturalistes luxembourgeois* 124: 107–124.

Glesener, L., M. Steinmetz, F. Schoos & S. Schneider, 2022b. Avifauna der Feuchtwiesenkomplexe um die „Bartringer Houbaach" – Entwicklung innerhalb 25 Jahren. *Bulletin de la Société des naturalistes luxembourgeois* 124: 139–154.

Glesener, L. & S. Schneider, 2024. Wiederansiedlung und Monitoring des Laubfrosches (*Hyla arborea*) – Zwischenbericht für das Jahr 2023. Unveröffentlichte Studie Naturschutzsyndikat SICONA – wissenschaftliche Abteilung, im Auftrag des Ministeriums für Umwelt, Klima und nachhaltige Entwicklung sowie der Gemeinden Beckerich, Bertrange, Reckange-Mess und Useldange, Olm, 14 pp.

Grafe, T. U. & I. Meuche, 2005. Chorus tenure and estimates of population size of male European tree frogs *Hyla arborea*: implications for conservation. *Amphibia-Reptilia* 26: 437–444. https://doi.org/10.1163/156853805774806269.

Große, W.-R., A. Geiger, G. Hansbauer & M. Stöck, 2020. Laubfrosch (*Hyla arborea*). In: Rote Liste und Gesamtartenliste der Amphibien (Amphibia) Deutschlands (Bundesamt für Naturschutz, ed). *Naturschutz und Biologische Vielfalt* 170 (4): 50–51. https://doi.org/10.19213/972174/.

Härdtle, W., 2024. Biodiversität, Ökosystemfunktionen und Naturschutz. Springer Spektrum Berlin, Heidelberg, 911 pp.

Hansen, F., 2004. Verbreitung und Gefährdung des Laubfrosches (*Hyla arborea*) auf Bornholm (Dänemark) und Maßnahmen zur Lebensraumoptimierung. In: Der Europäische Laubfrosch (*Hyla arborea*). Biologie – Schutzmaßnahmen – Effizienzkontrollen (Glandt, D. & A. Kronshage, eds). *Supplement der Zeitschrift für Feldherpetologie* 5: 133–143.

Herr, J., 2015. Reintroduction of tree frogs (*Hyla arborea*) to Luxembourg. Report of activities for the year 2014. Unpublished report, Administration de la Nature et des Forêts, Biologesch Statioun SICONA, 5 pp.

Heyer, W. R., M. A. Donnelly, R. W. McDiarmid, L. C. Hayek & M. S. Foster (eds), 1994. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. *Biological diversity handbook series*, Smithsonian Institution Press, Washington, 364 pp.

Hyatt, A. & R. Whittington, 2021. Infection with *Batrachochytrium dendrobatidis*. In: Manual of diagnostic tests for aquatic animals (OIE Aquatic Animal Health Standards Commission, ed). OIE, Paris, France: 36–56.

IUCN/SSC (ed), 2013. Guidelines for Reintroductions and Other Conservation Translocations. Version 1.0. IUCN Species Survival Commission, Gland, Switzerland, viiii + 57 pp.

IUCN/SSC Amphibian Specialist Group, 2022. *Hyla arborea*. IUCN Red List of Threatened Species 2022. e.T82496838A82495296. URL: https://www.iucnredlist.org/species/82496838/82495296 [accessed 28/06/2024].

JOCE, 1992. Richtlinie 92/43/EWG des Rates vom 21. Mai 1992 zur Erhaltung der natürlichen Lebensräume sowie der wildlebenden Tiere und Pflanzen. *Amtsblatt der Europäischen Union* Nr. L 206: 7–50.

Junck, C. & F. Schoos, 2000. Neuausbreitung des Laubfrosches (*Hyla arborea*) in der Folge von Biotopverbesserungsmaßnahmen im Zentrum Luxemburgs. *Bulletin de la Société des naturalistes luxembourgeois* 100: 97–101.

Laufer, H., K. Fritz & P. Sowig (eds), 2007. Die Amphibien und Reptilien Baden-Württembergs. Eugen Ulmer Verlag, Stuttgart, 807 pp.

Linhoff, L. J., P. Soorae, G. Harding, M. A. Donnelly, J. M. Germano, D. A. Hunter, M. McFadden, J.–R. Mendelson, A.–P. Pessier, M.–J. Sredl & M.E. Eckstut (eds), 2021. IUCN Guidelines for amphibian reintroductions and other conservation translocations, Gland, Switzerland, 219 pp.

Lötters, S., N. Wagner, G. Albaladejo, P. Böning, L. Dalbeck, H. Düssel-Siebert, S. Feldmeier, M. Guschal, K. Krist, D. Ohlhoff, K. Preißler, T. Reinhardt, M. Schlüpmann, U. Schulte, V. Schulz, S. Steinfartz, S. Twietmeyer, M. Veith, M. Vences & J. Wegge, 2020. The amphibian pathogen *Batrachochytrium salamandrivorans* in the hotspot of its European invasive range: past – present – future. *Salamandra* 56(3): 173–188.

Luquet, E., P. David, J.-P. Léna, P. Joly, L. Konecny, C. Dufresnes, N. Perrin & S. Plénet, 2011. Heterozygosity-fitness correlations among wild populations of European tree frog (*Hyla arborea*) detect fixation load. *Molecular ecology* 20(9): 1877–1887. https://doi.org/10.1111/j.1365-294X.2011.05061.x.

Meier, E., H. Glader & R. Averkamp, 2000. Erfolgreiche Wiederansiedlung des Laubfrosches. *LÖBF-Mitteilungen* 4: 35–46.

Mémorial, 2009. Règlement grand-ducal du 9 janvier 2009 concernant la protection intégrale et partielle de certaines espèces animales de la faune sauvage. *Mémorial A, Recueil de législation du Journal officiel du Grand-Duché de Luxembourg* N° 4: 34–41.

Ministère de l'Environnement, du Climat et du Développement durable, 2023. Plan National concernant la Protection de la Nature. Ministère de l'Environnement, du Climat et du Développement durable, Luxembourg, 83 pp.

Moravec, J., 1989. Observation on Fertilization and Oviposition in the European Tree Frog (*Hyla arborea*). *Acta Universitatis Carolinae – Biologica* 32: 515–530.

Pastors, J., 1995. Ergebnisse zweier Wiederansiedlungen des Laubfrosches (*Hyla arborea* L.) in Wuppertal – eine Langzeitstudie. In: Der Laubfrosch (*Hyla arborea* L.) Ökologie und Artenschutz (Geiger, A., ed). *Mertensiella* 6: 163–180.

Pellet, J., 2005. Conservation of a threatened European tree frog (*Hyla arborea*) metapopulation. Dissertation, Université de Lausannne, Faculté de Biologie et Médecine, Lausannne, 90 pp.

Pellet, J., V. Helfer & G. Yannic, 2007. Estimating population size in the European tree frog (*Hyla arborea*) using individual recognition and chorus counts. *Amphibia-Reptilia* 28: 287–294. https://doi.org/10.1163/156853807780202530.

Polivka, R. & B. T. Hill, 2008. Artenhilfskonzept Laubfrosch (*Hyla arborea*) in Mittel- und Nordhessen – Aktuelle Verbreitung und Maßnahmenvorschläge. Gutachten von Bioplan Marburg im Auftrag von Hessen-Forst FENA, Marburg, 156 pp.

Porco, D., C. A. Purnomo, L. Glesener, R. Proess, S. Lippert, K. Jans, G. Colling, S. Schneider, R. Strassen & A. Frantz, 2024. eDNA-based monitoring of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* with ddPCR in Luxembourg ponds: taking signals below the Limit of Detection (LOD) into account. *BMC Ecology and Evolution* 24(4). https://doi.org/10.1186/s12862-023-02189-9.

Proess, R. (ed), 2016. Verbreitungsatlas der Amphibien des Großherzogtums Luxemburg. *Ferrantia* 75, Luxembourg, 107 pp.

Proess, R., T. Ohst, J. Plötner & E. Engel, 2015. Untersuchungen zum Vorkommen der Geburtshelferkröte (*Alytes obstetricans*) und zur Verbreitung des Chytrid-Pilzes (*Batrachochytrium dendrobatidis*) in Luxemburg. *Bulletin de la Société des naturalistes luxembourgeois* 117: 63–76.

R Core Team, 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Wien, Österreich. URL: https://www.R-project.org/ [accessed 23/06/2024].

Service de pédologie, 1999. Bodenkundliche Detailkarte auf topographischem Hintergrund. 7 Kartenblätter im Maßstab 1:25.000 (1964–1999). Administration des services techniques de l'agriculture, Luxembourg. URL: http://www.geoportail.lu [accessed 17/06/2024].

SICONA, 2024. Dataset of water bodies in the SICONA region (version 13/05/2024).

Siebenaler, L., C. Wolff, A. Steinbach-Zoldan, J.-W. Zoldan, A. Hochkirch, M. Steinmetz & S. Schneider, 2020. Insektenfauna der Feuchtwiesenkomplexe um die „Bartringer Houbaach" – Entwicklung innerhalb 25 Jahren. *Bulletin de la Société des naturalistes luxembourgeois* 122: 163–196.

Stassen, R., J. Muller & L. Lestang, 2022. Protocole de désinfection et conseils pratiques pour la prévention de la transmission d'agents pathogènes tels que *Batrachochytrium salamandrivorans* (Bsal)*, Batrachochytrium dendrobatidis* (Bd) et *Ranavirus* entre les populations d'amphibiens. Version préliminaire du 10/03/2022. Administration de la Nature et des Forêts, Luxembourg, 9 pp. URL: https://environnement.public.lu/content/dam/environnement/documents/natur/biodiversite/sos-salamandre/protocole-preliminaire-de-desinfection-agents-pathogenes.pdf [accessed 22/06/2024].

Tester, U., 2022. Entwicklung einer wiederangesiedelten Laubfrosch-Population (*Hyla arborea*) bei Basel (Schweiz) 1985–2019. *Zeitschrift für Feldherpetologie* 29: 209–218.

Wolff, C., G. Colling, S. Naumann, L. Glesener & S. Schneider, 2020. Erfolgreicher Erhalt von artenreichem Extensivgrünland im Zentrum und Südwesten Luxemburgs – eine erste Bilanz. *Tuexenia* 40: 247–268. https://doi.org/10.14471/2020.40.012.

Wood, L. R., R. A. Griffiths & L. Schley, 2009. Amphibian chytridiomycosis in Luxembourg. *Bulletin de la Société des naturalistes luxembourgeois* 110: 109–114.