

Assessing the effect of landscape features on pond colonisation by an elusive amphibian invader using environmental DNA

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Abstract

1. Environmental DNA (eDNA) is becoming an essential tool for detecting aquatic invasive species and investigating their spread. Surprisingly, this technique has been very rarely used to investigate habitat selection, site occupancy, and colonisation despite its higher capacity to detect many species.
2. The African clawed frog (*Xenopus laevis*) is a principally aquatic amphibian introduced in several continents from South Africa. In western France, no recent systematic survey of the invasion range has been attempted, mainly because of the elusive nature of the species. Furthermore, the influence of landscape features on invasion has never been investigated, even if adults and juveniles are known to disperse overland and along river networks.
3. Using presence–absence data generated by an eDNA survey conducted across the known invasion front of *X. laevis* in western France, we aimed to determine whether and how the landscape features surrounding a pond influence the probability that a pond is colonised.
4. *Xenopus laevis* was detected well beyond the formerly known invasive distribution and at the outward end of some transects, suggesting that we did not reach the actual invasion front in these parts of the range. The landscape variables that best predicted the presence of *X. laevis* in a pond were topographic wetness index and grass cover within a buffer of 250 m.
5. Higher values of both topographic wetness index and grass cover were negatively related to the occurrence probability. The effects of these two variables more likely to reflect dispersal behaviour than habitat preferences at the pond scale.
6. By combining the high detection probability of eDNA survey techniques and a landscape ecology approach, we may gain valuable insight into the colonisation process of water bodies by elusive invasive species. Such information is crucial to prevent access to specific sites and locate invasion front areas where connectivity can be disrupted, thus increasing the effectiveness of management countermeasures.

KEYWORDS

dispersal, invasion dynamics, landscape analysis, topographic wetness index, *Xenopus laevis*

1 | INTRODUCTION

The ongoing increase in the number of alien invasive species worldwide (Seebens et al., 2017) requires more effective detection techniques (Trebitz et al., 2017). Environmental DNA (eDNA) has been recently advocated as a powerful means to detect aquatic invasive species (Goldberg et al., 2016). This technique, based on identifying species-specific fragments of DNA in the water of their aquatic habitats, allows detection of target species even at low densities, a particularly desirable characteristic to locate invasion fronts (Jerde, Mahon, Chadderton, & Lodge, 2011). When compared to classic methods such as visual or trapping surveys, eDNA may also exhibit a higher detection probability, especially in case of elusive species or juvenile life stages (Biggs et al., 2015; Bohmann et al., 2014; Thomsen & Willerslev, 2015). When costs of developing probes/primers and processing samples are low, eDNA is also significantly more cost-efficient than trapping or electro-fishing techniques (Dejean et al., 2012; Evans, Shirey, Wieringa, Mahon, & Lambert, 2017; Smart et al., 2016). Additionally, collection of water samples minimises habitat disturbance and avoid stress on native non-target species (Bohmann et al., 2014).

The benefits of using eDNA go beyond merely detecting target species. Environmental DNA surveys have been used to estimate population density and biomass (Buxton, Groombridge, Zakaria, & Griffiths, 2017; Goldberg et al., 2016; Smart, Tingley, Weeks, Van Rooyen, & McCarthy, 2015; Takahara, Minamoto, Yamanaka, Doi, & Kawabata, 2012), monitor temporal and spatial changes in species distribution and biodiversity (Bista et al., 2017; Deiner, Fronhofer, Mächler, Walsler, & Altermatt, 2016; Pansu et al., 2015; Thomsen & Willerslev, 2015; Yoccoz, 2012), or validate species distribution models (Muha, Rodríguez-Rey, Rolla, & Tricarico, 2017; Neto, 2018). More recently, this technique has been used to identify environmental and habitat predictors of species occurrence at different spatial scales (Collins, 2017; Pitt et al., 2017), and investigate the loss of habitat connectivity for fishes in dammed rivers (Yamanaka & Minamoto, 2016). Unlike river systems, ponds are discrete aquatic habitats that can often be reached only by aerial flights or terrestrial displacement. The spatial distribution of habitat patches and the features of the matrix determine the colonisation probability at the site scale (Hanski & Ovaskainen, 2003; Ray, Lehmann, & Joly, 2002; Rudnick et al., 2012), and connectivity at the landscape scale. For organisms dispersing on land like amphibians, the ground substrate may affect the colonisation process by altering locomotor performance or predation risk (Nowakowski, Veiman-Echeverria, Kurz, & Donnelly, 2015; Stevens, Polus, Wesselingh, Schtickzelle, & Baguette, 2004). Because of their capacity to detect species at low densities (Dejean et al., 2012), eDNA surveys are of high interest to investigate site occupancy, especially for taxa in which overland movements are difficult to investigate. The lack of eDNA-based studies that aimed to identify landscape predictors of population occupancy and colonisation is surprising, considering the potential insight into freshwater ecosystems this approach can provide (Pitt et al., 2017).

Using occurrence data from an eDNA survey, we tested the effect of landscape features on the probability of colonising a new pond by an invasive amphibian, and identified predictors that enhance the risk of colonisation during expansion. Beyond our case study, we highlight

the potential of this approach for the study of site occupancy in freshwater species. The taxonomic scope is large as both organisms living in discrete lentic habitats such as ponds, and organisms living in lotic habitats that disperse overland across the landscape can be surveyed (Chaput-Bardy, Lemaire, Picard, & Secondi, 2008).

The African clawed frog *Xenopus laevis* (Pipidae) is native to southern Africa and invasive in North and South America, Europe, and Asia (Measey et al., 2012). It is considered as one of the most detrimental invasive amphibians in the world (Measey et al., 2016). Translocation and release of this frog in areas well outside the native range have been attributed to its frequent use as a laboratory model and as a pet (Measey et al., 2012). Its capacity to successfully establish and disperse in various environments is due to behavioural and physiological traits such as generalist diet (Courant et al., 2017), burrowing behaviour during the dry season (Balinsky, Cragg, & Baldwin, 1961), and tolerance to a broad range of thermal (Miller, 1982) and hydric conditions (Jokumsen & Weber, 1980). *Xenopus laevis* is a principally aquatic and highly secretive amphibian which has several anatomical and morphological adaptations to the aquatic life-style (reviewed in Measey, 2016). Breeding calls are emitted underwater and are only detected using hydrophones, which severely limits species detection in water (Measey et al., 2012; Vimercati, Davies, Hui, & Measey, 2017). Nevertheless, the species has been reported to use hydrographic networks to disperse overland between ponds (Chaput-Bardy, Alcalá, Secondi, & Vuilleumier, 2017; Fouquet & Measey, 2006; Measey, 2016), at the juvenile and adult stages (Courant, 2017; De Villiers & Measey, 2017; Measey, 2016). Similarly to what has been observed in other invasive species characterised by high elusiveness (Jarić et al., 2019), an established alien population of *X. laevis* can remain undetected for years before any control action is undertaken (Measey et al., 2012; Sousa, Maurício, & Rebelo, 2018; Wang, Hong, & Measey, 2019). The elusive nature of *X. laevis* probably explains why the influence of landscape features on dispersal and establishment success of this amphibian has never been investigated before the development of eDNA techniques.

We carried out a systematic eDNA survey to determine the current invasive range of the species in western France. The eDNA method for *X. laevis* has been recently developed and was shown to detect the species even at low density (Secondi, Dejean, Valentini, Audebaud, & Miaud, 2016). Its efficiency was mostly attributed to the aquatic life style of *X. laevis* at all stages (Secondi et al., 2016). We extended the range of our survey beyond the known invasive range, because we suspected that former surveys based on trapping and visual observations might have underestimated the actual colonised range. We used the presence-absence dataset to test whether hydrographic and terrestrial landscape features predict species occurrence in a pond within newly colonised areas. The chosen predictors were classically used to explain presence-absence in other amphibian species. Because the frog is aquatic, we expected that hydrographic landscape features better predicted the occurrence of *X. laevis* than terrestrial landscape features. The analysis was carried out at four spatial scales within the range of dispersal distances reported for the species.

2 | METHODS

2.1 | Historical records and sampling design

In western France, *X. laevis* was released from a breeding facility into an artificial pond at the beginning of 1980s (Fouquet, 2001). The species then spread into the surrounding ponds, remaining virtually undetected until the early 2000s (Fouquet, 2001). A study based on trapping surveys conducted between 2001 and 2004 estimated the invasive range to be approximately 200 km², and concluded that ponds were mainly colonised through overland dispersal (Fouquet & Measey, 2006). Since then, the species was episodically detected outside the initial invasion front (Fouquet & Measey, 2006) through trapping programmes and anecdotal observations. However, no systematic survey has been conducted to locate the entire invasion front of the population. The invasive range is characterised by a high density of agricultural water bodies, mostly cattle and irrigation ponds, interspersed in a landscape of permanent and temporary pastures, crop fields, and, to a lesser extent, woods. The high density of water bodies (3.5 water bodies/km²) and the necessity to obtain access to the private lands limit the systematic use of trapping. In addition, trapping efficiency for *X. laevis* might vary depending on population density, trapping season, depth and demographic structure (Secondi et al., 2016), as reported for fish (Blaustein, 1989; Dawson et al., 2017) and crayfish (Polcar & Kozák, 2005).

At the onset of our study, the invasive distribution of *X. laevis* in western France was deduced from trapping, accidental fishing, and visual observations. We compiled all available records in the scientific and grey literature. Managers of natural reserves, fishing stakeholders and local naturalists were contacted to confirm or discard historical observations and add novel ones if needed. In total, we collected 906 records covering an area (minimum convex polygon) of 2,055 km². All records, apart from one unconfirmed visual observation, were reported south of the Loire River. A cluster of three records appeared distinct from the main range (18 km southward, see Figure 1) and could be the result of an accidental human-mediated translocation. Thus, we discarded these records to delimit the study area.

The initial database was used to determine our strategy for eDNA sampling. Thirty radial lines separated by 12° were placed across the colonised area. The introduction point of the population (47°01'55.0"N; 0°17'59.7"W) (Fouquet, 2001) was used as the origin for all lines. Transects were placed along these lines, starting from the most peripheral colonised pond. Ponds were then sampled outward along each transect with a step of 2–3 km. African clawed frogs were observed to disperse up to 2.4 km/year in the South African native range (De Villiers & Measey, 2017) and 3 km/year in the French invasive range (Courant, 2017). We therefore assumed that the geographical shift between the known location of the front and its actual location depended on the year of the last observation. We adjusted the length of the transect in accordance with the year of the most recent observations. (<5 years = 10 km, 6–10 years = 20 km, >10 years = 30 km). Additional ponds were sampled: (1) southward, to explore whether the

species had colonised the area between the known invasive range and the three distinct records; and (2) northward, to explore whether the species has established north to the Loire River. In total, we sampled 235 ponds for eDNA which on average corresponded to 7–8 ponds along each transect.

2.2 | Environmental DNA analysis

All ponds were sampled once between 02 May 2017 and 30 June 2017. A sample consisted of 20 subsamples of 100 ml of pond water, which were taken at 20 different points around the pond using a sterile sampling scoop with a handle of 125 ml. Each sub-sample was poured into a 2-L Whirl-Pak® bag. The 2 L of sampled water were then homogenised and filtered through a VigiDNA® filtration capsule (SPYGEN) using a sterile 100-ml syringe directly in the field. The filter was filled with 80 ml of CL1 conservative buffer (SPYGEN) and stored at room temperature.

DNA extraction was performed in a dedicated room for water DNA sample extraction at Spygen molecular lab facility, which is equipped with positive air pressure, UV treatment and frequent air renewal, following the protocol described in Pont et al. (2018). Before entering this extraction room, personnel changed into full protective clothing comprising disposable body suit with hood, mask, laboratory shoes, overshoes, and gloves in a connecting zone. All benches were decontaminated with 10% commercial bleach before and after each manipulation. For DNA extraction, each filtration capsule, containing the CL1 buffer, was agitated for 15 min on an S50 shaker (Ingenieurbüro™) at 800 rpm and then the buffer was emptied into a 50-ml tube before being centrifuged for 15 min at 15,000 × g. The supernatant was removed with a sterile pipette, leaving 15 ml of liquid at the bottom of the tube. Subsequently, 33 ml of ethanol and 1.5 ml of 3M sodium acetate were added to each 50-ml tube and stored for at least one night at -20°C. The tubes were centrifuged at 15,000 × g for 15 min at 6°C, and the supernatants were discarded. After this step, 720 µL of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen) was added. The tubes were then vortexed, and the supernatants were transferred to 2-ml tubes containing 20 µl of Proteinase K. The tubes were finally incubated at 56°C for 2 hr. Subsequently, DNA extraction was performed using NucleoSpin® Soil (MACHEREY-NAGEL GmbH & Co., Düren Germany) starting from step 6 and following the manufacturer's instructions. The elution was performed by adding 100 µl of SE buffer TWIce. Seventeen extractions of negative controls were performed to monitor possible contamination. After the DNA extraction, the samples were tested for inhibition by qPCR (Biggs et al., 2015). If the sample was considered inhibited, it was diluted 5-fold before the amplification.

Quantitative polymerase chain reaction (qPCR) was performed in a final volume of 25 µl, which included 3 µl of template DNA, 12.5 µl of TaqMan® Environmental Master Mix 2.0 (Life Technologies®), 6.5 µl of ddH₂O, 1 µl of primer SPY_XenLae_F (10 µM), 1 µl of primer SPY_XenLae_R 5'-AGGGTATAGAAAATGTAGCC-3' and 5'-FAM-CGTCAGGTCAAG GTGTAGCA-BHQ1-3'. and 1 µl of SPY_XenLae_Probe (2.5 µM). Primer and probe were designed for the 12S gene and validated in Secondi et al. (2016). Each sample was run in 12

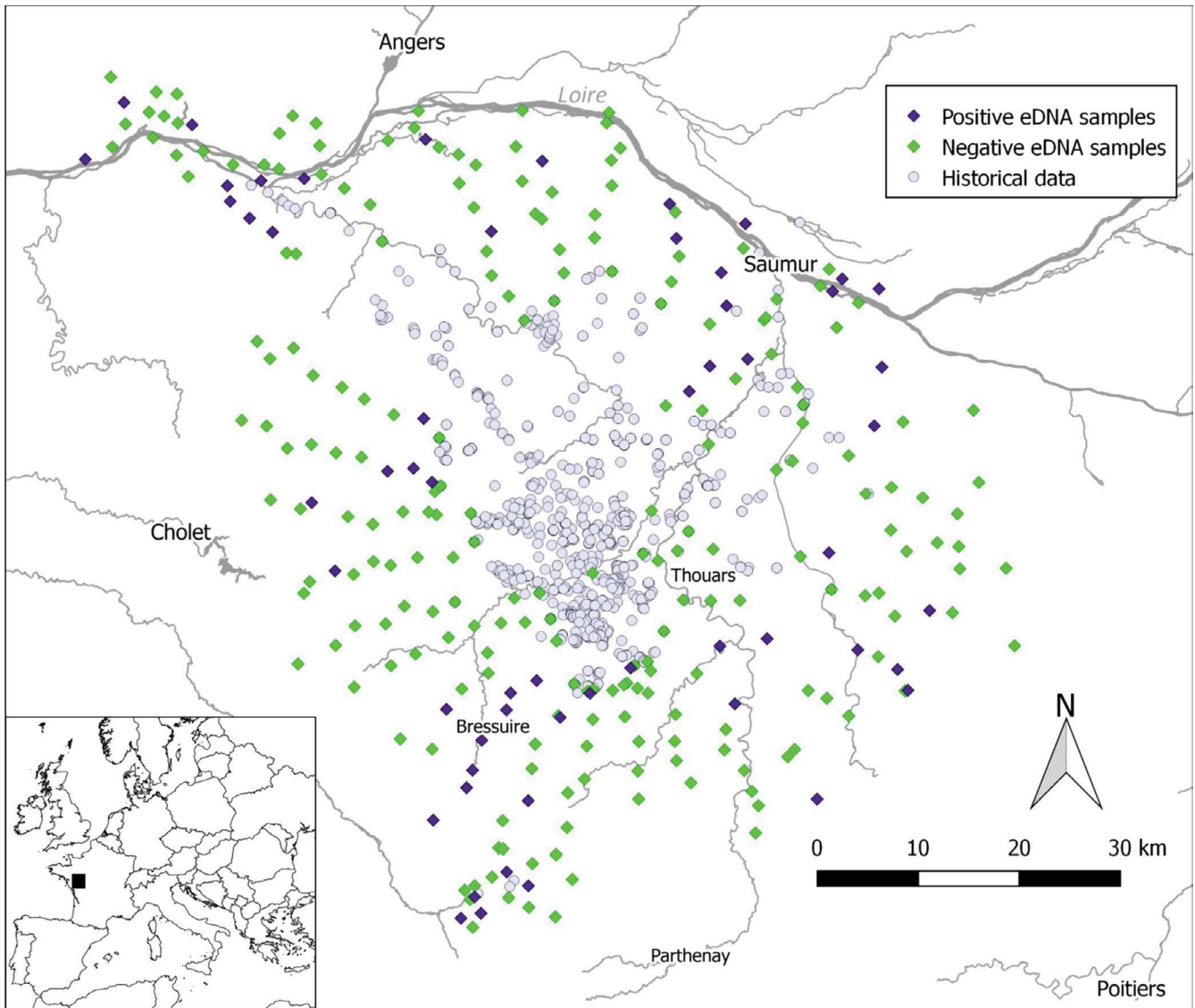


FIGURE 1 Distribution map of invasive *Xenopus laevis* population in western France obtained from historical data and this study's environmental DNA (eDNA) survey. The Loire River and other main waterways are represented

replicates. The tubes containing the eDNA samples were then sealed, qPCR standards were added to the qPCR plate in a separate room from the eDNA extraction room. A dilution series of *X. laevis* DNA (10^{-1} to 10^{-4} ng/ μ l) was used as a qPCR standard (two replicates per concentration). Twelve negative (ddH₂O water) controls were added during the qPCR step. Quantitative polymerase chain reaction runs were performed in a third room, dedicated to amplified DNA analysis with negative air pressure and physically separated from the eDNA extraction room. Samples were run on a CFX96 Touch real-time PCR detection system (BIO-RAD®), under thermal cycling at 50°C for 5 min and 95°C for 10 min, followed by 55 cycles of 95°C for 30 s and 51.3°C for 1 min. Environmental DNA detection rate was calculated as the number of positive qPCR amplifications (qPCR replicates) over the total number of qPCR replicates. All negatives extraction and qPCR negatives controls resulted negatives after the analysis. According to the results of a pilot study in which the species presence detected by eDNA was ascertained by trapping (Secondi et al., 2016),

we considered *X. laevis* present in a pond when at least one of the 12 replicates was positive.

2.3 | Landscape analysis

We used only presence-absence data from the eDNA survey for the landscape analysis. We included all ponds where the species was detected. For absences, we selected only ponds within 4 km from a colonised pond. This figure corresponds to the dispersal distance which best predicted the colonisation of the Loire river drainage by *X. laevis* according to Chaput-Bardy et al. (2017). We therefore selected ponds that can be theoretically reached from a colonised pond. By doing so, we also discarded absence ponds that are well outside the invasive ranges, and therefore which cannot currently be colonised by the frog even if the surrounding landscape would allow it.

To take into consideration the potential occurrence of spatial autocorrelation between ponds, we used Moran's eigenvectors maps (MEMs), which are derived from latitude and longitude coordinates and model spatial patterns at different scales (Borcard & Legendre, 2002). Firstly, pond geographic coordinates were used to build a matrix of Euclidean distances among ponds. The matrix was truncated using a threshold which retains only the distance between close neighbours while keeping all ponds connected (Borcard & Legendre, 2002). Secondly, orthogonal eigenvectors were obtained by computing a principal component analysis of the truncated distance matrix. Only eigenvectors modelling positive spatial correlation, i.e. having Moran's I higher than the expected Moran's I under the null hypothesis, were retained (Borcard & Legendre, 2002). These MEMs were: (a) ranked in descending order based on their eigenvalues; (b) named in ascending order starting from 1; and (c) arbitrarily grouped in broad-scale (the first half of MEMs, which have large eigenvalues) and fine-scale MEMs (the second half of MEMs, which have small eigenvalues). Lastly, forward stepwise selection of logistic regression models was used to select MEMs which best predicted presence-absence of *X. laevis* in the ponds at broad and fine spatial scales. To confirm the selection of MEMs, we also perform a penalised LASSO logistic regression on the same dataset at broad and fine spatial scales. Penalised LASSO logistic regression has an advantage over more classical selection procedures (such as forward and stepwise selections) to keep only the most significant variables in a logistic regression model characterised by multiple variables (Tibshirani, 1996).

For each pond, we used QGIS (<http://www.qgis.org/>) to measure the following variables in buffers of radius 100, 250, 750, and 2,000 m: pond area (m^2), number of proximal ponds, cumulative area of proximal ponds (m^2), cumulative length of streams and rivers (m), cover (%) of forest, grass (prairies and pastures) and, crop. These environmental variables are classically used to predict the occurrence of amphibian species in ponds (Collins & Fahrig, 2017; Peterson, Richgels, Johnson, & Mckenzie, 2013; Signorelli, Bastos, De Marco, & With, 2016), and are also hypothesised to play a role in *X. laevis* dispersal behaviour (Measey et al., 2012). Hydrographic and land use data were obtained, respectively, from BD Topo® and Registre Parcellaire Graphique reference maps provided by the French National Geographic Institute. For each pond and buffer, we also calculated the average topographic wetness index (TWI), which estimates the potential water accumulation of each pixel in a watershed. Topographic wetness index data were obtained from Besnard, La Jeunesse, Pays, and Secondi (2013), who computed the index for the whole Loire catchment at a resolution of 50 m. The four buffers were chosen to correspond to the frequent short-distance dispersal events (100 and 250 m) and sporadic cases of long-distance dispersal (750 and 2,000 m) following De Villiers and Measey (2017), who described the species dispersal kernel in the native range.

2.4 | Statistical analysis

All analyses and visualisations were performed using R version 3.4.3 (R Development Core Team, 2017). Moran's eigenvectors maps were

computed using the *dbmem* function in the package *adespatial* (Dray et al., 2016). Penalised LASSO logistic regression on MEMs was computed using the package *glmnet* (Friedman, Hastie, & Tibshirani, 2018). The environmental variables were transformed, when necessary, to reduce skewness and kurtosis. Pearson's rank correlation coefficient was calculated, separately for each buffer size, to identify highly correlated (≥ 0.7) environmental variables. The only two strongly correlated variables were the number of proximal ponds and the cumulative area of proximal ponds for 100- and 250-m buffers (see Table S6). However, variance inflation factor analysis showed no significant multicollinearity (all variance inflation factor values < 4) between variables at all spatial scales. Thus, all environmental variables were retained for the next analytical step. To determine which combination of environmental variables best predicts occurrence of *X. laevis* in a pond, we computed models at each buffer size for all combinations of variables including the previously selected MEM as covariables. This set of models is hereafter defined as the *with MEMs* group (total number of models = 256), where the null model includes only the selected MEMs (in the following referred as MEM null model).

Some models might better predict occurrence when only part of the spatial structure, i.e. not all selected MEMs, is included. We therefore carried out a second procedure at each buffer size where the selected MEMs could be dropped as any other variable. This set of models are hereafter defined as the *intercept only* group (total number of models = 8,192), where the null model includes only the intercept.

In any case, the model with the best fit for a given buffer size was identified using Akaike information criterion (AIC), adjusted for small sample size (AICc). Some models could similarly outperform the null model without significantly differing each other in terms of AICc. Thus, all models having a similar level of AICc support ($\Delta AICc < 2$) were retained for model averaging to estimate the relative importance of each environmental variable using the package *MuMIn* (Barton, 2009). Model averaging was conducted by using both *with MEMs* and *intercept only* groups.

3 | RESULTS

Xenopus laevis was detected in 55 out of 234 sampled ponds (23.5 %, see Table S1), including some of the most peripheral ponds of the transects (Figure 1). The species was also found north of the Loire River in two different locations > 50 km apart (Figure 1), which suggest that frogs may have repeatedly crossed the river. At the southern edge, *X. laevis* was also detected along a river between the formerly known range and the three most peripheral occurrences (pale violet dots in Figure 1). Hence, natural dispersal, and not necessarily human translocation, may have promoted the colonisation of the southernmost pond cluster. The shift of the range front as observed with eDNA data is 13.8 ± 6.9 km (averaged over the 30 transects). By discarding all ponds further away than 4 km from colonised ponds, we retained 71 ponds where the

species was considered as absent, i.e. not detected using eDNA (Table S1).

In total, 27 MEMs showed positive autocorrelation, i.e. a Moran's I higher than the expected Moran's I under the null hypothesis. According to the forward stepwise procedure of model selection, only five MEMs were kept in the best models predicting presence-absence: two represented the broader spatial scale (MEM9, MEM13) and three the finer spatial scale (MEM16, MEM21, MEM24). The model that included only these five MEMs (MEM null model, Table 1) performed significantly better than the null model with intercept only ($\Delta AIC_c = 5.5$). The five MEMs were also selected by the penalised LASSO logistic regression. Thus, these MEMs were used as spatial covariables in the logistic regression along with the environmental variables.

Within a buffer of 100 m, the best model included only TWI but this model did not perform better than the MEM null model ($\Delta AIC_c = 0.74$, Table S2). Thus, we did not conduct model averaging at this buffer size. Within a buffer of 250 m, the best model in the *with MEMs* group included TWI and grass cover and provided a better fit than the MEM null model ($\Delta AIC_c = 4.4$, Table 1). The same model was also selected as best fit model in the *only intercept* group (data not shown). The importance of TWI and grass cover was confirmed through multi-model inference. Across all models having a similar level of AIC_c support in the *with MEMs* group, TWI had the highest importance among all environmental variables (sum of Akaike

weights = 1.0, Table 2), while grass cover was notably more important than the remaining variables (sum of Akaike weights = 0.78, Table 2). The importance of TWI and grass cover was even higher across all models having a similar level of AIC_c support in the *intercept only* group (Table S5). Topographic wetness index and grass cover negatively predicted the presence of *X. laevis* in a pond (Table 2; Table S5, Figures 2 and 3). At the two larger buffer size, 750 and 2,000 m, the most parsimonious model was the MEM null model (Table S3 and Table S4). As a consequence, model averaging was not conducted at these two buffer sizes.

4 | DISCUSSION

We used eDNA to survey the invasive population of *X. laevis* in a systematic way beyond its formerly known range in western France to determine the current range and identify landscape variables that locally favour the colonisation of new ponds. By using this approach, we aimed to reduce the frequency of false negatives and enhance detection at low density, an expected situation in sites recently colonised at the invasion front. We detected presence in ponds located well beyond the known range limits (Figure 1). Our results suggest that the invasive range has been largely underestimated. The population has reached the northern bank of the Loire River (Figure 1). It was assumed that this large river was a barrier to the dispersal of *X. laevis* but our results suggest that it

TABLE 1 Change in Akaike information criterion, adjusted for small sample size (ΔAIC_c) and associated measures from all competing logistic regression models ($\Delta AIC_c < 2$) predicting the occurrence of *Xenopus laevis* in a pond at a buffer size of 250 m as estimated by environmental DNA survey in western France. The best model (in bold) and the competing models were selected among all possible combinations of models with environmental variables as explanatory variable and Moran's eigenvectors maps (MEMs) as covariables (*with MEMs* group, $n = 256$). Delta AIC_c and associated measures from MEM null model (intercept + 5MEMs) are also reported. TWI, topographic wetness index

Competing models	K	LL	AIC_c	ΔAIC_c	Cum.Wt
TWI + grass cover	8	-73.17	163.58	0	0.11
TWI + grass cover + forest cover	9	-72.1	163.77	0.19	0.21
TWI + crop cover + forest cover	9	-72.15	163.87	0.3	0.3
TWI + forest cover	8	-73.34	163.93	0.35	0.39
TWI + grass cover + area of proximal ponds	9	-72.42	164.41	0.83	0.46
TWI + grass cover + number of proximal ponds	9	-72.53	164.62	1.04	0.53
TWI + grass cover + forest cover + pond area	10	-71.45	164.84	1.26	0.58
TWI + grass cover + crop cover + pond area	10	-71.46	164.86	1.28	0.64
TWI + grass cover + forest cover + area of proximal ponds	10	-71.6	165.13	1.55	0.69
TWI	7	-75.1	165.16	1.59	0.74
TWI + grass cover + pond area	9	-72.84	165.25	1.67	0.79
TWI + grass cover + crop cover + area of proximal ponds	10	-71.7	165.34	1.76	0.83
TWI + forest cover + pond area	9	-72.9	165.37	1.79	0.88
TWI + grass cover + forest cover + number of proximal ponds	10	-71.75	165.44	1.86	0.92
TWI + grass cover + forest cover + crop cover	10	-71.82	165.56	1.99	0.96
TWI + area of proximal ponds + forest cover	9	-73	165.57	1.99	1
MEM null model	6	-77.66	168.02	4.44	

TABLE 2 Variable importance (sum of weights) and model-averaged coefficients from all competing logistic regression models ($\Delta AICc < 2$, $n = 16$) predicting the occurrence of *Xenopus laevis* in a pond at a buffer size of 250 m as estimated by environmental DNA survey in western France. The competing models were selected among all possible combinations of models with environmental variables as explanatory variable and Moran's eigenvectors maps (MEMs) as covariables (with MEMs group, $n = 256$). Statistically significant results are in bold ($\alpha = 0.05$). TWI, topographic wetness index

Variable	Importance	Estimate	SE	z value	Pr(> z)
Intercept		12.30	4.93	2.47	0.014
TWI	1.00	-0.89	0.36	2.4	0.016
MEM13	1.00	0.38	0.24	1.5	0.124
MEM16	1.00	0.53	0.24	2.20	0.028
MEM21	1.00	-0.45	0.21	2.12	0.034
MEM24	1.00	-0.30	0.22	1.37	0.172
MEM9	1.00	-0.50	0.23	2.12	0.034
Grass cover	0.78	-1.33	1.10	1.20	0.230
Forest cover	0.46	0.41	0.61	0.67	0.502
Crop cover	0.24	-0.31	0.75	0.41	0.684
Area of proximal ponds	0.21	0.03	0.09	0.34	0.734
Pond area	0.21	-0.09	0.26	0.34	0.735
Number of proximal ponds	0.11	0.01	0.05	0.24	0.813
River network length	NA	NA	NA	NA	NA

Note: NA = the variable was not present in the set of competing models ($\Delta AICc < 2$).

may have only delayed its spread. An analogous conclusion has been recently reached for another alien amphibian invading the northern Po plain in Italy, the Balkan frog (*Pelophylax kurtmuelleri*), which contrary to some previous expectations it is now predicted to cross the Po river in some of its tracts (Falaschi, Mangiacotti, Sacchi, Scali, & Razzetti, 2018). Additionally, a recent study which modelled the potential distribution of *X. laevis* in western France by using bioclimatic data, showed that the maximal expected invasive range is much larger than our updated range (Rödder et al., 2017). The capacity of the species to cross major watercourses raises concerns about the occurrence of natural barriers to the expansion of *X. laevis* in western Europe.

Contrary to our expectations, the density of the hydrographic network around the ponds did not significantly affect the

colonisation process at all buffer sizes. This seems to contradict previously published works, which hypothesised that lotic systems may facilitate dispersal between ponds (Chaput-Bardy et al., 2017; Fouquet & Measey, 2006; Measey, 2016; Vimercati et al., 2017), and the observation that *X. laevis* successfully breeds in streams in Portugal (Moreira, Marques, Sousa, & Rebelo, 2017). The density of the hydrographic network might thus not be a good predictor of connectivity, if, for example, a straight stream flowing between ponds facilitates the dispersal of individuals. The importance of hydrographic networks in explaining connectivity and biodiversity patterns depends on several factors such as the species dispersal mode, landscape type, land use, topography, and biotic interactions (reviewed in Tonkin et al., 2018). The contribution of these factors to connectivity is important in fully aquatic species such as fish or aquatic invertebrates and in hydrochloric plants dispersing in mesic environments, i.e. where streams, rivers, and riparian habitats are the dispersal main corridors. Conversely, such a contribution is limited in species that disperse overland away from the watercourse (e.g. many aquatic insects at the adult stage, or anemochoric plants) or disperse across arid areas, i.e. where the river network can be seasonally highly fragmented (Tonkin et al., 2018). As most factors discussed above have never been investigated in *X. laevis*, future field studies are needed to determine to what extent lotic systems affect species dispersal at different spatial scales in this amphibian.

The environmental variables that best predicted the presence of *X. laevis* in a pond were TWI and grass cover within a buffer of 250 m. A pond was less likely to be colonised if the surrounding landscape was characterised by high grass cover and a high TWI. The negative effect of grass cover could reflect the peculiar overland dispersal behaviour of the species rather than actual habitat preferences. Landscape features may affect locomotor performance and behavioural dispersal, for example if mechanical resistance to displacement varies between substrates (Stevens et al., 2004). Recent laboratory experiments showed that juveniles, sub-adults, and adults of in *X. laevis* move significantly faster on asphalt, bare soil, and forest litter than on grass (G. Vimercati, unpublished data). Analogously, experimental and landscape genetic data obtained in other species of frogs (Cline & Hunter, 2016; Nowakowski et al., 2015; Stevens et al., 2004) and salamanders (Wang, Savage, & Bradley Shaffer, 2009) found that dispersal costs were highest in grassland habitats. Landscapes characterised by permanent grass cover (e.g. meadows and pastures) could hinder dispersal and pond colonisation in *X. laevis* more than less resistant types of land use such as crop fields and forests. This hypothesis should be further tested using field experiments or landscape genetics, also considering that a negative effect of grass cover on *X. laevis* presence is notably less supported by our data than the effect of TWI on the same dependent variable.

The robust negative relationship between TWI and *X. laevis* occurrence is less straightforward. As TWI measures potential water accumulation (Kirkby & Beven, 1979), higher TWI values should predict higher probabilities to detect amphibians (Peterman & Semlitsch, 2013; Romano et al., 2017). Instead, we observed lower probabilities

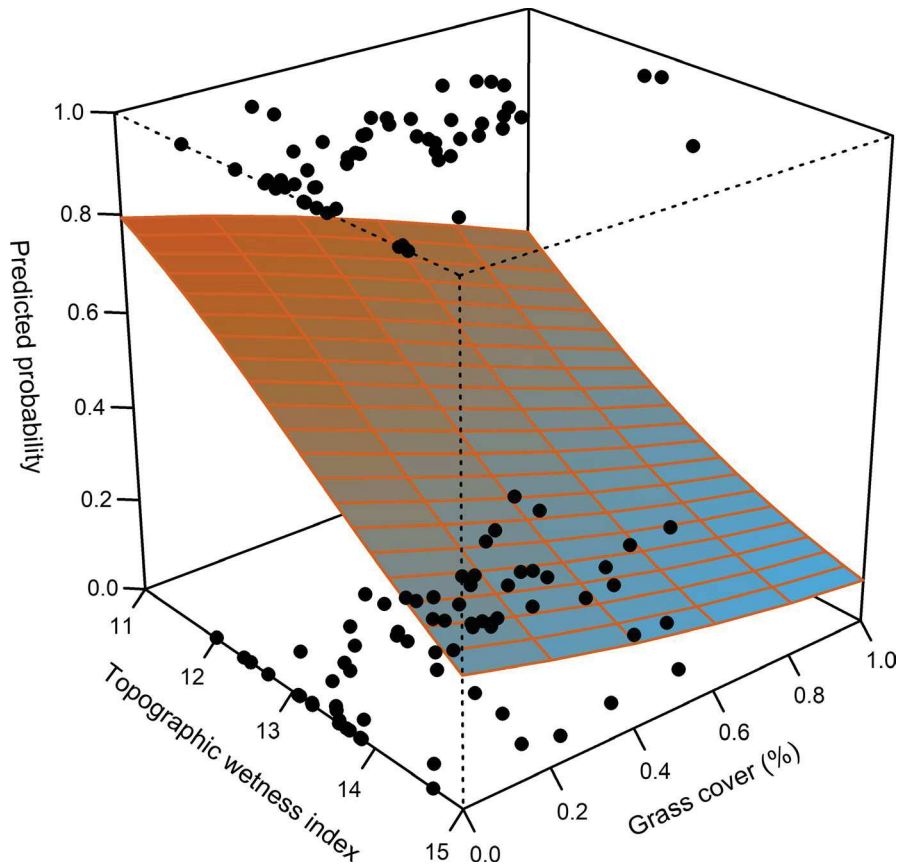


FIGURE 2 Three-dimensional logistic regression curve depicting the presence probability for invasive *Xenopus laevis* as a function of topographic wetness index and grass cover within a buffer of 250 m as estimated by environmental DNA survey in western France. Dots represent presence-absence data obtained during the survey and used to fit the logistic regression model

of pond colonisation by *X. laevis* at higher values of TWI. This counter-intuitive result could reflect changes in the risk-benefit ratio of leaving a pond across different landscapes (Tonkin et al., 2018). In dry areas (low TWI), *X. laevis* dispersal propensity should be reduced, because overland movements may increase mortality and be restricted to situations where food is no longer available or the pond has dried. In wet areas (high TWI), the probability of a pond drying is null or very low, and the risk of dispersal may be higher than the risk to stay in the pond. In areas characterised by intermediate pond hydroperiod (medium TWI), by contrast, leaving a drying pond is less risky and dispersal events may be more frequent than in both arid and mesic areas. A similar compensatory behaviour has been observed in the western slimy salamander (*Plethodon albagula*), where very high and very low values of TWI predicted higher landscape resistance, and thus lower gene flow between populations (Peterman, Connette, Semlitsch, & Eggert, 2014). Analogously, a quadratic relationship between wetness and occurrence probability has been detected in the giant burrowing frog (*Heleioporus australiacus*) (Penman, Mahony, Towerton, & Lemckert, 2007). Slimy salamanders and giant burrowing frogs are terrestrial species which withstand moderate levels of dehydration and avoid areas subjected to flood. In spite of its principally aquatic lifestyle, *X. laevis* may sustain high locomotor performance across a broad range of desiccating conditions (Vimercati, unpublished data). Numerous cases of dispersal compensatory behaviours have been described across different taxonomic groups such as birds, mammals, amphibians, and insects (Knowlton & Graham, 2010). Future studies should thus be conducted across different habitats to investigate whether *X. laevis* increases its

dispersal propensity through compensatory behaviour in areas of intermediate TWI values. Particular attention should be paid to the joint influence of the spatial distribution of ponds and their hydroperiod.

In addition, TWI is unlikely to reach very low values in western France. Thus, we may have only sampled the upper part of the TWI range corresponding to the decreasing part of the quadratic relationship between TWI and presence probability. A large proportion of absences in the higher TWI class were detected in the floodplain of the Loire River. This is a peculiar ecosystem subjected to regular floods, an unusual feature for a southern African frog. In addition to the specific hydrological and sedimentary dynamics, fishes remain in ponds after the flood, and many of these habitats dry out in summer. The extinction rate of populations in these ponds could thus be higher than in non-flooded areas, a condition that can also occur around smaller rivers. Additionally, it is common to observe large ponds close to watercourses, i.e. in the lower areas of the surrounding landscape. These ponds are used as reservoirs or for recreational activities and are usually stocked with fishes. Conversely, smaller agricultural ponds are widespread across the TWI range and often lack fishes. Therefore, the negative effect of TWI on pond colonisation could be mediated by correlated unmeasured variables at the pond scale. While fish presence and pond hydroperiod play a role in predicting site occupancy of other species of amphibians (Amburgey, Bailey, Murphy, Muths, & Funk, 2014; Fuller, Pope, Ashton, & Welsh, 2011; Pitt et al., 2017), their effects on *X. laevis* presence in a pond are yet to be tested.

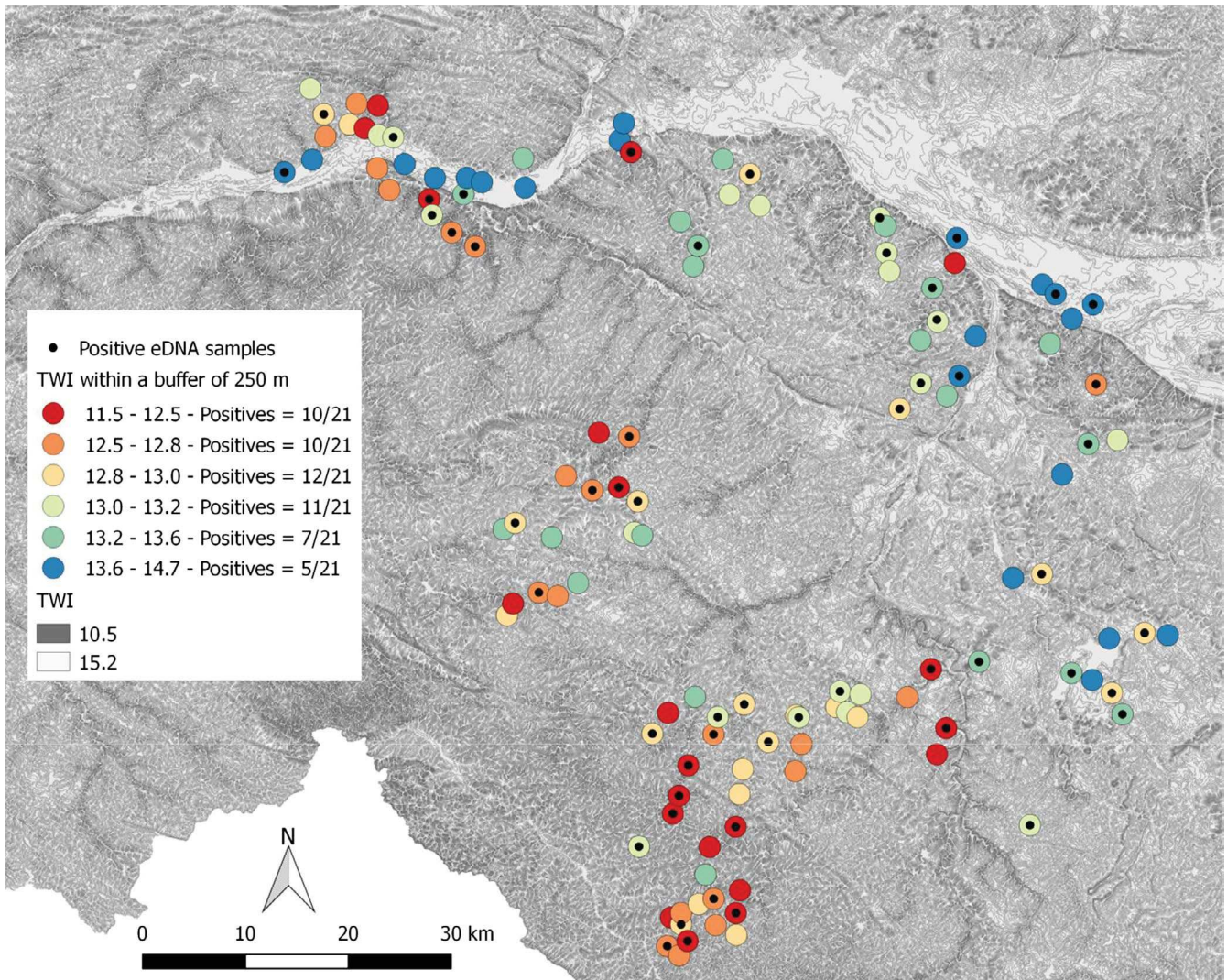


FIGURE 3 Map of topographic wetness index (TWI) computed by Besnard et al. (2013) for western France. Dots represent presence-absence data for invasive *Xenopus laevis* as estimated by environmental DNA (eDNA) survey and used in the landscape analysis, where colours represent mean TWI calculated within a buffer of 250 m at each sample location. Colour scale is subdivided in six quantiles of 21 eDNA samples each to highlight the negative relationship between TWI and *X. laevis* presence (black dots) within a buffer of 250 m

Alternatively, if the detection probability of *X. laevis* by eDNA varies with environmental factors such as water depth, temperature, and sediment type as in other species of amphibians (Buxton et al., 2017; Smart et al., 2016), a correlation between these factors and TWI could lead to wrongly interpret the role of TWI in predicting *X. laevis* occupancy. Although we cannot entirely rule out this last hypothesis, it seems improbable considering that both TWI and grass cover successfully predicted occupancy only at 250-m buffers. This distance is consistent with the species dispersal kernel observed in South Africa (De Villiers & Measey, 2017), which predicts that most adults should move within 0 and 500 m with a frequency peak around 200 m. Assuming that pond colonisation in western France is mainly promoted by frequent short-distance dispersal events, a direct effect of grass cover and TWI on the species dispersal dynamics seems thus the most parsimonious explanation.

Our work stresses the importance to conduct landscape analysis across multiple spatial scales. In amphibians, environmental, behavioural, and physiological factors influence site occupancy and abundance only at specific spatial scales (Semlitsch, 2008; Semlitsch & Bodies, 2003). In the Boreal plains of Canada for example, landscape variables best explained abundance at a 1,000-m scale in the boreal chorus frog (*Pseudacris maculata*), and at a 100-m scale in the western toad (*Anaxyrus boreas*) (Browne, Paszkowski, Foote, Moenting, & Boss, 2009). Target species may respond to small- or large-scale habitat changes in a species-specific manner. Identifying the most appropriate spatial scale for predicting their occupancy and abundance is therefore essential to develop adequate conservation plans (Browne et al., 2009; Semlitsch & Bodies, 2003). Interestingly, the same rationale can be applied to the management of invasive species. The identification of the spatial scale at which colonisation occurs may have a tremendous influence on

our capacity to adopt effective and efficient management countermeasures (Foxcroft, Richardson, Rouget, & MacFadyen, 2009; Weaver, Conway, & Fortin, 2012). Among other options, one could envisage to artificially modify the invaded landscape in order to halt the spread of an invasive species. Such a solution has been recently proposed to tackle the invasion of the cane toad (*Rhinella marina*) in Australia (Tingley et al., 2013). In *X. laevis*, dispersal might be locally impaired by promoting around the ponds land use types associated with high mechanical resistance to displacement, such as meadows and pastures. However, we suggest that this management countermeasure should be only adopted at a buffer of 250 m. According to our results, the adoption of the same management countermeasure at smaller (e.g. 100 m) or larger spatial scales (e.g. 750 m) would be, respectively, ineffective or inefficient.

In conclusion, using an eDNA survey we showed that TWI and grass cover measured within a 250-m buffer were the best predictors of pond colonisation by *X. laevis* in western France, and that the invasive range of the population has been largely underestimated. Therefore, our approach was valuable not only to estimate the invasive range of an elusive species but also to identify landscape features that may influence site occupancy. This information: (1) provides novel insight into the understanding of the colonisation process of this invasive amphibian; and (2) is pivotal to design effective management countermeasures that could disrupt colonisation at the front of the invasive range or prevent access to sites of particular ecological value. Our study highlights the usefulness of eDNA surveys to address wider questions than mapping distribution. A clear advantage of the method over classic trapping is to enhance feasibility of large-scale surveys for elusive aquatic species in ponds, because sampling water requires less time and no specific permit, and makes owners less reluctant to give access to their land. Ecological data obtained through such surveys will certainly prove increasingly useful for species distribution modelling, habitat selection, and connectivity studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

AUTHOR CONTRIBUTIONS

G.V. and J.S. conceived the ideas and designed the study. J.S. collected the eDNA samples in the field. T.D. performed the molecular work at Spygen molecular lab facility. G.V. and T.D. analysed data. G.V. led the writing of the manuscript. G.V., J.S. M.L., and T.D. all

contributed to writing and editing the different versions of the manuscript and gave final approval for publication.

ETHICAL STATEMENTS

The authors declare that no experiments were performed on humans or animals for this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Amburgey, S., Bailey, L., Murphy, M., Muths, E., & Funk, W. (2014). The effects of hydroperiod and predator communities on amphibian occupancy. *Canadian Journal of Zoology*, *92*, 927–937.
- Balinsky, J., Cragg, M. M., & Baldwin, E. (1961). The adaptation of amphibian waste nitrogen excretion to dehydration. *Comparative Biochemistry and Physiology*, *3*, 236–244.
- Barton, K. (2009). MuMIn: multi-model inference, R package version 0.12.0. Retrieved from <http://r-forge.r-project.org/projects/mumin/>.
- Besnard, A., La Jeunesse, I., Pays, O., & Secondi, J. (2013). Topographic wetness index predicts the occurrence of bird species in floodplains. *Diversity and Distributions*, *19*, 955–963.
- Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., ... Williams, P. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation*, *183*, 19–28.
- Bista, I., Carvalho, G. R., Walsh, K., Seymour, M., Hajibabaei, M., Lallias, D., ... Creer, S. (2017). Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. *Nature Communications*, *8*, 14087.
- Blaustein, L. (1989). Effects of various factors on the efficiency of minnow traps to sample mosquitofish (*Gambusia affinis*) and green sunfish (*Lepomis cyanellus*) populations. *Journal of the American Mosquito Control Association*, *5*, 2935.
- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., ... De Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, *29*, 358–367.
- Borcard, D., & Legendre, P. (2002). All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling*, *153*, 51–68.
- Browne, C. L., Paszkowski, C. A., Foote, A. L., Moenting, A., & Boss, S. M. (2009). The relationship of amphibian abundance to habitat features across spatial scales in the Boreal Plains. *Ecoscience*, *16*, 209–223.
- Buxton, A. S., Groombridge, J. J., Zakaria, N. B., & Griffiths, R. A. (2017). Seasonal variation in environmental DNA in relation to population size and environmental factors. *Scientific Reports*, *7*, 46294.
- Chaput-Bardy, A., Alcalá, N., Secondi, J., & Vuilleumier, S. (2017). Network analysis for species management in rivers networks: Application to the Loire River. *Biological Conservation*, *210*, 26–36.
- Chaput-Bardy, A., Lemaire, C., Picard, D., & Secondi, J. (2008). In-stream and overland dispersal across a river network influences gene flow in a freshwater insect, *Calopteryx splendens*. *Molecular Ecology*, *17*, 3496–3505.
- Cline, B. B., & Hunter, M. L. Jr (2016). Movement in the matrix: Substrates and distance-to-forest edge affect postmetamorphic movements of a forest amphibian. *Ecosphere*, *7*, e01202.

- Collins, M. K. (2017). *Searching for a Salamander: Distribution and Habitat of the Common Mudpuppy (Necturus maculosus) in Southeast Ohio Using Environmental DNA*, Ohio University.
- Collins, S. J., & Fahrig, L. (2017). Responses of anurans to composition and configuration of agricultural landscapes. *Agriculture, Ecosystems & Environment*, 239, 399–409.
- Courant, J. (2017). *Biologie de l'invasion de Xenopus laevis en Europe: adaptations physiologiques et effets écologiques*. PhD Thesis, Paris, Muséum national d'histoire naturelle.
- Courant, J., Vogt, S., Marques, R., Measey, J., Secondi, J., Rebelo, R., ... Backeljau, T. (2017). Are invasive populations characterized by a broader diet than native populations? *PeerJ*, 5, e3250.
- Dawson, H. A., Bravener, G., Beaulaurier, J., Johnson, N. S., Twohey, M., McLaughlin, R. L., & Brenden, T. O. (2017). Contribution of manipulable and non-manipulable environmental factors to trapping efficiency of invasive sea lamprey. *Journal of Great Lakes Research*, 43, 172–181.
- De Villiers, F. A., & Measey, J. (2017). Overland movement in African clawed frogs (*Xenopus laevis*): Empirical dispersal data from within their native range. *PeerJ*, 5, e4039.
- Deiner, K., Fronhofer, E. A., Mächler, E., Walser, J.-C., & Altermatt, F. (2016). Environmental DNA reveals that rivers are conveyor belts of biodiversity information. *Nature Communications*, 7, 12544.
- Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology*, 49, 953–959.
- Dray, S., Blanchet, G., Borcard, D., Guenard, G., Jombart, T., Larocque, G., ... Wagner, H. (2016). ade4: Multivariate multiscale spatial analysis. *R package version 0.0, 3*.
- Evans, N. T., Shirey, P. D., Wieringa, J. G., Mahon, A. R., & Lamberti, G. A. (2017). Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. *Fisheries*, 42, 90–99.
- Falasci, M., Mangiacotti, M., Sacchi, R., Scali, S., & Razzetti, E. (2018). Electric circuit theory applied to alien invasions: a connectivity model predicting the Balkan frog expansion in Northern Italy. *Acta Herpetologica*, 13, 33–42.
- Fouquet, A. (2001). Des clandestins aquatiques. *Zamenis*, 6, 10–11.
- Fouquet, A., & Measey, G. J. (2006). Plotting the course of an African clawed frog invasion in western France. *Animal Biology*, 56, 95–102.
- Foxcroft, L. C., Richardson, D. M., Rouget, M., & MacFadyen, S. (2009). Patterns of alien plant distribution at multiple spatial scales in a large national park: Implications for ecology, management and monitoring. *Diversity and Distributions*, 15, 367–378.
- Friedman, J. H., Hastie, T., & Tibshirani, R. (2018). glmnet: lasso and elastic-net regularized generalized linear models. *R package version, 1.1-5*.
- Fuller, T. E., Pope, K. L., Ashton, D. T., & Welsh, H. H. Jr (2011). Linking the distribution of an invasive amphibian (*Rana catesbeiana*) to habitat conditions in a managed river system in northern California. *Restoration Ecology*, 19, 204–213.
- Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., ... Cornman, R. S. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, 7, 1299–1307.
- Hanski, I., & Ovaskainen, O. (2003). Metapopulation theory for fragmented landscapes. *Theoretical Population Biology*, 64, 119–127.
- Jarić, I., Heger, T., Monzon, F. C., Jeschke, J. M., Kowarik, I., McConkey, K. R., ... Essl, F. (2019). Crypticity in biological invasions. *Trends in Ecology & Evolution*, 34, 291–302.
- Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4, 150–157.
- Jokumsen, A., & Weber, R. E. (1980). Haemoglobin-oxygen binding properties in the blood of *Xenopus laevis*, with special reference to the influences of aestivation and of temperature and salinity acclimation. *Journal of Experimental Biology*, 86, 19–37.
- Kirkby, M., & Beven, K. (1979). A physically based, variable contributing area model of basin hydrology. *Hydrological Sciences Journal*, 24, 43–69.
- Knowlton, J. L., & Graham, C. H. (2010). Using behavioral landscape ecology to predict species' responses to land-use and climate change. *Biological Conservation*, 143, 1342–1354.
- Measey, G., Rödder, D., Green, S., Kobayashi, R., Lillo, F., Lobos, G., ... Thirion, J.-M. (2012). Ongoing invasions of the African clawed frog, *Xenopus laevis*: A global review. *Biological Invasions*, 14, 2255–2270.
- Measey, G., Vimercati, G., De Villiers, F., Mokhatla, M., Davies, S., Thorp, C., ... Kumschick, S. (2016). A global assessment of alien amphibian impacts in a formal framework. *Diversity and Distributions*, 22, 970–981.
- Measey, J. (2016). Overland movement in African clawed frogs (*Xenopus laevis*): A systematic review. *PeerJ*, 4, e2474.
- Miller, K. (1982). Effect of temperature on sprint performance in the frog *Xenopus laevis* and the salamander *Necturus maculosus*. *Copeia*, 695–698.
- Moreira, F. D., Marques, R., Sousa, M., & Rebelo, R. (2017). Breeding in both lotic and lentic habitats explains the invasive potential of the African clawed frog (*Xenopus laevis*) in Portugal. *Aquatic Invasions*, 12(4), 565–574. <https://doi.org/10.3391/ai.2017.12.4.12>.
- Muha, T. P., Rodríguez-Rey, M., Rolla, M., & Tricarico, E. (2017). Using environmental DNA to improve species distribution models for freshwater invaders. *Frontiers in Ecology and Evolution*, 5, 158.
- Neto, J. G. D. S. (2018). *Assessing Site Occupancy and Microhabitat Use of the Hellbender Salamander (Cryptobranchus alleganiensis) in Tennessee*, Tennessee State University.
- Nowakowski, A. J., Veiman-Echeverria, M., Kurz, D. J., & Donnelly, M. A. (2015). Evaluating connectivity for tropical amphibians using empirically derived resistance surfaces. *Ecological Applications*, 25, 928–942.
- Pansu, J., Giguët-Covex, C., Ficetola, G. F., Gielly, L., Boyer, F., Zinger, L., ... Choler, P. (2015). Reconstructing long-term human impacts on plant communities: An ecological approach based on lake sediment DNA. *Molecular Ecology*, 24, 1485–1498.
- Penman, T. D., Mahony, M., Towerton, A., & Lemckert, F. (2007). Spatial models of giant burrowing frog distributions. *Endangered Species Research*, 3, 115–124.
- Peterman, W. E., Connette, G. M., Semlitsch, R. D., & Eggert, L. S. (2014). Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. *Molecular Ecology*, 23, 2402–2413.
- Peterman, W. E., & Semlitsch, R. D. (2013). Fine-scale habitat associations of a terrestrial salamander: The role of environmental gradients and implications for population dynamics. *PLoS ONE*, 8, e62184.
- Peterson, A. C., Richgels, K. L., Johnson, P. T., & McKenzie, V. J. (2013). Investigating the dispersal routes used by an invasive amphibian, *Lithobates catesbeianus*, in human-dominated landscapes. *Biological Invasions*, 15, 2179–2191.
- Pitt, A. L., Shinskie, J. L., Tavano, J. J., Hartzell, S. M., Delahunty, T., & Spear, S. F. (2017). Decline of a giant salamander assessed with historical records, environmental DNA and multi-scale habitat data. *Freshwater Biology*, 62, 967–976.
- Polcar, T., & Kozák, P. (2005). Comparison of trap and baited stick catch efficiency for noble crayfish (*Astacus astacus* L.) in the course of the growing season. *Bulletin Français de la Pêche et de la Pisciculture*, 376–377, 675–686.
- Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., ... Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, 8, 10361.
- R Development Core Team (2017). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at <http://www.R-project.org>

- Ray, N., Lehmann, A., & Joly, P. (2002). Modeling spatial distribution of amphibian populations: A GIS approach based on habitat matrix permeability. *Biodiversity & Conservation*, *11*, 2143–2165.
- Rödger, D., Ihlow, F., Courant, J., Secondi, J., Herrel, A., Rebelo, R., ... De Busschere, C. (2017). Global realized niche divergence in the African clawed frog *Xenopus laevis*. *Ecology and Evolution*, *7*, 4044–4058.
- Romano, A., Costa, A., Basile, M., Raimondi, R., Posillico, M., Roger, D. S., ... Matteucci, G. (2017). Conservation of salamanders in managed forests: Methods and costs of monitoring abundance and habitat selection. *Forest Ecology and Management*, *400*, 12–18. <https://doi.org/10.1016/j.foreco.2017.05.048>.
- Rudnick, D., Ryan, S. J., Beier, P., Cushman, S. A., Dieffenbach, F., Epps, C., ... Merenlender, A. M. (2012). The role of landscape connectivity in planning and implementing conservation and restoration priorities. *Issues in Ecology*, *16*, 1–20.
- Secondi, J., Dejean, T., Valentini, A., Audebaud, B., & Miaud, C. (2016). Detection of a global aquatic invasive amphibian, *Xenopus laevis*, using environmental DNA. *Amphibia-Reptilia*, *37*, 131–136.
- Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., ... Arianoutsou, M. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications*, *8*, 14435.
- Semlitsch, R. D. (2008). Differentiating migration and dispersal processes for pond-breeding amphibians. *The Journal of Wildlife Management*, *72*, 260–267.
- Semlitsch, R. D., & Bodie, J. R. (2003). Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology*, *17*, 1219–1228.
- Signorelli, L., Bastos, R. P., De Marco, P., & With, K. A. (2016). Landscape context affects site occupancy of pond-breeding anurans across a disturbance gradient in the Brazilian Cerrado. *Landscape Ecology*, *31*, 1997–2012.
- Smart, A. S., Tingley, R., Weeks, A. R., Van Rooyen, A. R., & Mccarthy, M. A. (2015). Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecological Applications*, *25*, 1944–1952.
- Smart, A. S., Weeks, A. R., Rooyen, A. R., Moore, A., Mccarthy, M. A., & Tingley, R. (2016). Assessing the cost-efficiency of environmental DNA sampling. *Methods in Ecology and Evolution*, *7*, 1291–1298.
- Sousa, M., Maurício, A., & Rebelo, R. (2018). The *Xenopus laevis* invasion in Portugal: An improbable connection of science, Mediterranean climate and river neglect. In A. Queiroz & S. Pooley (Eds.), *Histories of bioinvasions in the Mediterranean* (pp. 133–148). Cham: Springer.
- Stevens, V. M., Polus, E., Wesselingh, R. A., Schtickzelle, N., & Baguette, M. (2004). Quantifying functional connectivity: Experimental evidence for patch-specific resistance in the Natterjack toad (*Bufo calamita*). *Landscape Ecology*, *19*, 829–842.
- Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. I. (2012). Estimation of fish biomass using environmental DNA. *PLoS ONE*, *7*, e35868.
- Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA—An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18.
- Tibshirani, R. (1996). Regression shrinkage and selection via the lasso. *Journal of the Royal Statistical Society: Series B (Methodological)*, *58*(1), 267–288. <https://doi.org/10.1111/j.2517-6161.1996.tb02080.x>.
- Tingley, R., Phillips, B. L., Letnic, M., Brown, G. P., Shine, R., & Baird, S. J. (2013). Identifying optimal barriers to halt the invasion of cane toads *Rhinella marina* in arid Australia. *Journal of Applied Ecology*, *50*, 129–137.
- Tonkin, J. D., Altermatt, F., Finn, D. S., Heino, J., Olden, J. D., Pauls, S. U., & Lytle, D. A. (2018). The role of dispersal in river network metacommunities: Patterns, processes, and pathways. *Freshwater Biology*, *63*, 141–163.
- Trebitz, A. S., Hoffman, J. C., Darling, J. A., Pilgrim, E. M., Kelly, J. R., Brown, E. A., ... Hashsham, S. A. (2017). Early detection monitoring for aquatic non-indigenous species: Optimizing surveillance, incorporating advanced technologies, and identifying research needs. *Journal of Environmental Management*, *202*, 299–310.
- Vimercati, G., Davies, S. J., Hui, C., & Measey, J. (2017). Does restricted access limit management of invasive urban frogs? *Biological Invasions*, *19*, 3659–3674.
- Wang, I. J., Savage, W. K., & Bradley Shaffer, H. (2009). Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (*Ambystoma californiense*). *Molecular Ecology*, *18*, 1365–1374.
- Wang, S., Hong, Y., & Measey, J. (2019). An established population of African clawed frogs, *Xenopus laevis* (Daudin, 1802), in mainland China. *BioInvasions Records*, *8*, 457–464.
- Weaver, J. E., Conway, T. M., & Fortin, M. J. (2012). An invasive species' relationship with environmental variables changes across multiple spatial scales. *Landscape Ecology*, *27*, 1351–1362.
- Yamanaka, H., & Minamoto, T. (2016). The use of environmental DNA of fishes as an efficient method of determining habitat connectivity. *Ecological Indicators*, *62*, 147–153.
- Yoccoz, N. G. (2012). The future of environmental DNA in ecology. *Molecular Ecology*, *21*, 2031–2038.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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