

Assessing the impacts of the invasive frog, *Xenopus laevis*, on amphibians in western France

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Abstract. As invasive species are one of the principal threats on global biodiversity, assessing their impact is a crucial element of conservation biology. Quantifying the possible impacts of an invasive population represents the first step in the establishment of efficient management plans. In this study, we applied a method of site-occupancy modeling to estimate the influence of an invasive frog, *Xenopus laevis*, on the amphibian species richness in western France. In our analyses we took into account habitat characteristics (i.e. the size and general shape of the ponds), the structure of the aquatic vegetation, the presence of other vertebrates, and the physicochemical parameters of the pond. Richness was negatively related to the abundance of *X. laevis* and to the time since colonization as estimated by the distance of the pond to the site of introduction. Habitat niche breadth of native amphibians did not differ between invaded and non-invaded areas. This might be a consequence of the homogeneity of the habitats selected for our study. The lack of heterogeneity in the abiotic factors, the absence of a correlation between species richness and these abiotic factors, and the correlation of the abundance and time since colonization by *X. laevis* with species richness suggest a negative effect of this species on local amphibians. This result highlights the importance of conservation and management plans aiming to limit the expansion of this invasive species.

Keywords: invasive species, modeling, ponds, species richness.

Introduction

Invasive species are among the major causes of the current biodiversity decline (e.g. Alford and Richards, 1999; Collins and Storfer, 2003). They can have negative effects on native communities through a diversity of mechanisms (at least 12, listed in the Global Invasive Species Database: www.iucngisd.org/gisd/). The assessment of the impact of an invasive species is crucial, yet needs to determine the impact of the invader relative to the potential role of variation in other factors such as abiotic parameters (Pagnucco and Ricciardi, 2015). This distinction is

important to discriminate between the causes and consequences of the success of an invasive species (Didham et al., 2007).

In this study, we analyzed the potential impacts of the invasive frog, *Xenopus laevis*, (Anura: Pipidae) on native amphibians in Western France. *Xenopus laevis* is widely used in research laboratories across the world (van Sittert and Measey, 2016) because of its physiological characteristics, easy maintenance in captivity, the use for pregnancy testing (Shapiro and Zwarenstein, 1934) and research in developmental biology. This species is also noteworthy for being commercialized in the pet trade (Measey et al., 2012; van Sittert and Measey, 2016). Populations have become established on four continents after accidental escapes or voluntary introductions (for a review, see Measey et al., 2012).

According to Lillo, Faraone and Lo Valvo (2011), *X. laevis* may impact native amphibians after a few years of the colonization of a new habitat. However, that study did not take into account neither biotic nor abiotic covariates. In our study, abundance modeling (Royle and Nichols, 2003) was used to test the role of

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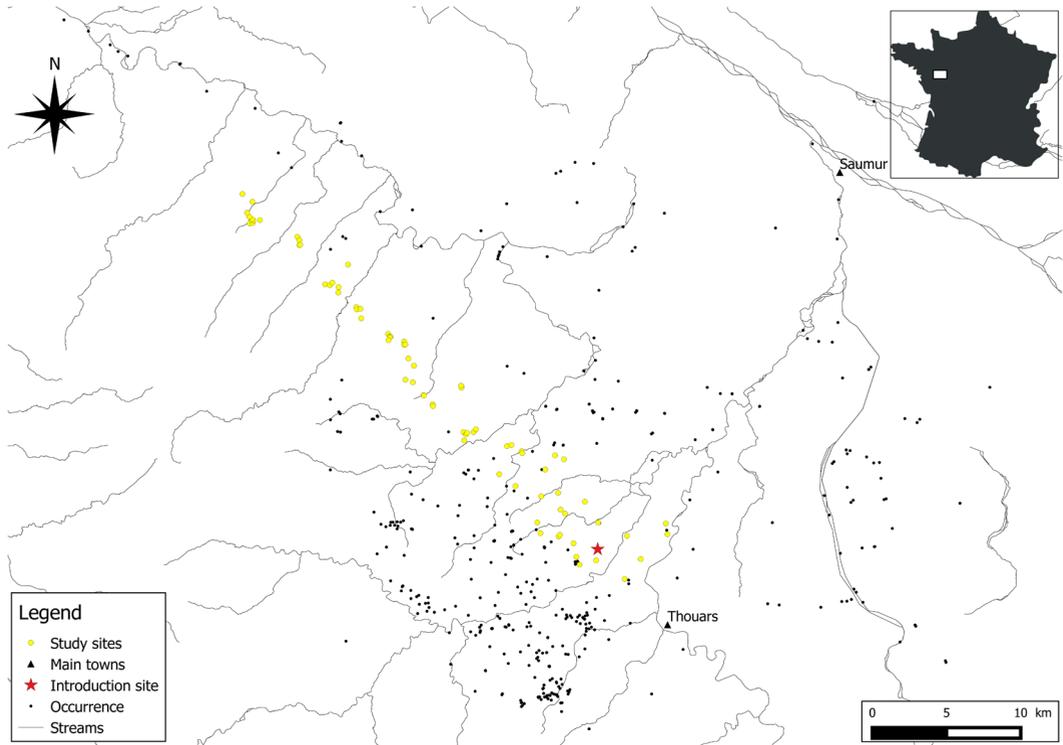


Figure 1. Distribution of the expanding population of *Xenopus laevis* in western France. Black dots represent the occurrence localities of the species; yellow dots are the selected study sites and the introduction site is represented by a red star.

the abundance of *X. laevis*, its time since colonization estimated as the distance to the introduction site, and biotic and abiotic factors on the species richness of native amphibians. This method of analysis is commonly used to model the abundance of a species according to one or several covariates. We here intended to use this method to model the species richness, by considering species as individuals, after verifying if our data respected the assumptions of the method. We tested the hypothesis that the colonization of a site by *X. laevis* is followed by a negative effect on native amphibians, independent from the possible effects of variation in abiotic and biotic parameters.

Materials and methods

Data sampling

Xenopus laevis was introduced in France, in the village of Bouillé Saint-Paul (Deux-Sèvres department), during the

late eighties (Fouquet, 2001; Measey et al., 2012). Since then, it has expanded and colonized a substantial part of the Maine-et-Loire and Deux-Sèvres departments (fig. 1). Contrary to the Mediterranean-like climate of its native range, in France *X. laevis* occupies an area where climate is defined as oceanic altered, with a relatively high annual mean temperature (12.5°C), and cumulated annual precipitation reaching 800 to 900 mm, mainly during winter (Joly et al., 2010). The area selected for this study is a southeast-northwest transect crossing the colonized range of the species from the introduction site to the colonization front and extending into the non-colonized area in the northwestern part of the transect. The landscape is dominated by a mosaic of pasture lands and scattered crop fields and woods. This transect was chosen because of the possible correlation between the distance of sites to the introduction site and the duration since those sites have been colonized. Despite a lack of quantitative historical data, especially in the northern part of the range, this suggested correlation is confirmed by local landscape managers.

All 76 sites used for the survey were artificial freshwater ponds, located in pastures and providing water to cattle. The areas of the ponds ranged between 100 and 400 m² and they were potentially occupied by native amphibians. Out of these 76 sites, we selected 49 ponds where *X. laevis* was expected, according to the data collected by local landscape managers, and 27 ponds located out of the expected range of

the species. For this study, we needed to describe the habitat, estimate the occupancy of the pond by amphibians and model species richness. Because no accurate data about the habitat preferences of *X. laevis* are available in the literature yet, the pond description in our analyses took into account variables that possibly influenced the occupancy and/or detection probability of amphibians in general. The water parameters, such as pH (Freda, 1987), temperature (Gulve, 1994), conductivity/resistivity [RES] (Klaver, Peterson and Patla, 2013), total dissolved solids [TDS] and turbidity (Lobos and Measey, 2002) were sampled using a Hannah multi-parameter (Hannah HI-9829-11042). The multi-parameter was calibrated regularly after 10 uses with a quick calibration, and a complete calibration was performed every day before sampling. The structure of the sites can also influence the detection probability of amphibians. It was described by means of three continuous variables (water depth, mud depth, and bank height, measured in cm), and two factors describing the maximal and minimal slope of the banks according to five categories. Some of these factors, i.e. water depth, are also known to influence the occupancy of anurans (Bosch and Martinez-Solano, 2003). For the same reasons we collected data describing the main substrate (gravel, stone, sand, organic mud, leaves) of the pond and a binary factor describing the presence/absence of an outlet. The potential for annual drying (permanent or temporary pond) was determined after discussions with the owners of the ponds.

The aquatic vegetation can also influence amphibian occupancy because it provides shelter against predators, support for egg attachment and it is a food reservoir for both adults and tadpoles (Hartel et al., 2006). We described this parameter with the percentage of the pond surface covered by vegetation and the degree of complexity of this vegetation classified into 14 categories following Lachavanne, Juge and Perfetta (1995). The presence-absence of fish was evaluated by collecting data on fish captured during the amphibian data collection with the funnel traps and a net (see below for data sampling). The presence-absence of *Myocastor coypus*, an invasive mammal that substantially modifies its habitat (Prigioni, Balestrieri and Remonti, 2005) was also taken into account. This species was detected through direct observation of individuals in the ponds or through the observation of their feces in the water. Some habitat covariates possibly change during the year. As a consequence, all the covariates were measured the day before we performed the first visit of the amphibian data collection.

The amphibian data were analyzed with Royle's method (Royle and Nichols, 2003). Three visits were performed during three consecutive nights at each pond (from 22 p.m. to 2 a.m.) from the 12th to the 26th of May 2015. Nights with optimal meteorological conditions for amphibian activity (e.g. Bellis, 1962; Cree, 1989; Buchanan, 2006; Saenz et al., 2006) were favored to perform the visits. During each visit and for all species, the number of adults, larvae and clutches were recorded using standardized protocol durations depending on the surface of the site. Five minutes were first dedicated to listening for calling anurans. Next, visual prospection occurred during five minutes for each 100 m² of accessible surface, i.e. where water depth was below one

meter and where aquatic or terrestrial vegetation did not prevent access. Then, five minutes for each 100 m² of accessible surface were dedicated to the net exploration. We used a headlamp (Petzl, 200 lumens) to perform the visual prospection and a net with and a 1.5 m long handle, a 2 mm mesh, 50 cm of net diameter and depth (Roudier, Brie sous Mortagne, France).

The entire study area is occupied by four salamanders (*Lissotriton helveticus*, *Salamandra salamandra*, *Triturus cristatus* and *Triturus marmoratus*) and eight anurans (*Alytes obstetricans*, *Pelodytes punctatus*, *Bufo spinosus*, *Hyla arborea*, *Rana dalmatina*, *Pelophylax lessonae*, *Pelophylax kl. esculentus* and *Pelophylax ridibundus*) (Lescure and de Massary, 2012). Only adults were considered for the Green frog complex, because these frogs breed later than the other species (during summer). As they occupy the aquatic habitat boundaries during a large part of the year (Paunovic, Bjelic-Cabrilo and Simic, 2010), their detection was possible even outside of their breeding period. The difficult identification of the species belonging to the green frog group in Western Europe led us to consider it as a unique taxon, referred hereafter as *Pelophylax* sp. The presence of adult *X. laevis* was verified using submerged funnel traps (60-cm length × 30-cm width × 6-mm mesh diameter) at each site. We used cat food as single-use bait and put floats in the traps to avoid any amphibian death by drowning. The capture effort was adjusted to the surface of ponds, with a pressure of one trap per fifty square meters. Traps were set in ponds at nightfall the day before the first visit of the amphibian survey, checked during each visit, and removed after being checked at the end of the last visit. The abundance of captured individuals of *X. laevis* was recorded as well as the distance of the site of introduction.

Data analysis

To model the species richness of native amphibians, we used the *N*-mixture method (Royle, 2004). One of the most important assumptions of *N*-mixture methods concerns the need of constancy of the detection probability of species. The variability of the detection probability between species was assessed by using a presence matrix for each species (with 3 columns for the visits and 76 rows for the sites) to compare the detection probabilities estimated with the single-season site-occupancy null model (MacKenzie et al., 2006). The estimates of detection probability were performed using the Presence software (Hines, 2006). To complete this assessment, a Friedman rank test was performed in R (R Core Team, 2015) with the species richness data comprised of a matrix where the number of native species detected during each visit (in 3 columns) and each site (in 76 rows) was indicated.

Using the same matrix and a new one obtained with the biotic and abiotic values for each pond, we modeled the species richness with the *N*-mixture method in the Presence software (Hines, 2006). Models were built with a single biotic or abiotic covariate for each model to explain the species richness of native amphibians. The fit of all models was tested with Goodness Of Fit (GOF) tests using a Chi² test automatically generated in the Presence software

alongside with the model output. A significant Chi^2 result ($p < 0.05$) meant that the model did not fit the data well enough. In this case, models were not considered. Models validated by the GOF tests were compared with one another using their respective corrected Akaike Information Criterion (AICc). The species richness estimated according to the abundance of *X. laevis* and its distance to the introduction site was provided by the model output. A non-parametric Spearman's ρ test was also performed to determine the degree of correlation between them.

The effect of *X. laevis* on the occurrence of each species was assessed by using a recently developed method for analyzing species co-occurrence based on site-occupancy data. For this analysis, we used a new matrix that consisted of a summary of the presence-absence data obtained during the survey for each species, including *X. laevis*. This matrix was filled with a binary factor: "1" when a species was detected at least during one visit, and with "0" when it was not seen during the visits. This method used a Bayesian statistical approach (Veech, 2013) and was applied on this matrix in R (R Core Team, 2015) using the 'cooccur' package (Griffith, Veech and Marsh, 2016). During this analysis, the observed co-occurrence was compared to the expected co-occurrence where the latter is estimated as the product of the occurrence probabilities of the two species multiplied by the number of sites in the sample. For species exhibiting negative co-occurrences with *X. laevis*, *N*-mixture models were applied on an abundance matrix for each species with the same method as the one we used to model species richness.

Habitat niche breadth was calculated using the Evenness J' (Colwell and Futuyma, 1971) with the biotic/abiotic matrix as niche components. The niche overlap between each native species and *X. laevis* was calculated with the Morisita's overlap index C_H (Morisita, 1959). The calculation of Morisita's index is based on the number of sites where two species occurred for a given value of a given factor. Thus, we needed to convert data into categories, to subdivide each covariate in several classes. For these reasons, continuous variables were transformed into categorical factors, and then, for each category, a binary factor was created (1 for presence of a category in a site, 0 for its absence). Factors underwent the same treatment and were transformed into binary variables. Niche breadth was calculated for each native species in the area occupied by *X. laevis* (J'_{occ}) and in the area unoccupied by the species (J'_{ctrl}).

Niche breadth J' and niche overlap C_H indices were calculated according to the following formulas (where, for the Evenness J' , p_i is the proportion of sites occupied by the species for factor i and n the total number of parameters; and, for the Morisita's overlap index C_H , p_{ij} and p_{ik} are the proportions of sites occupied, respectively, by the species j and k for factor i and n the total number of parameters):

$$J' = \frac{-\sum(p_i * \log p_i)}{\log n} \quad C_H = \frac{2 \sum_i^n p_{ij} p_{ik}}{\sum_i^n p_{ij}^2 + \sum_i^n p_{ik}^2}$$

Results

On the basis of the occurrence data, *Xenopus laevis* was not detected in ten ponds out of the

49 ponds where we expected it to be present (20.41% of the sites in the occupied area). It was moreover not detected in the 27 sites where we did not expect it to be present. According to the models built to estimate the detectability of each species, the detection probabilities (with the Standard Errors) were high for every species we encountered (*B. spinosus*: $p = 0.681 \pm 0.045$; *Pelophylax* sp.: $p = 0.840 \pm 0.027$; *R. dalmatina*: $p = 0.696 \pm 0.043$; *H. arborea*: $p = 0.841 \pm 0.028$; *P. punctatus*: $p = 0.513 \pm 0.074$; *T. cristatus*: $p = 0.673 \pm 0.041$; *L. helveticus*: $p = 0.905 \pm 0.020$). The highest detection probability was obtained for *X. laevis* ($p = 0.939 \pm 0.02$). Out of the nine native species expected (plus the *Pelophylax* genus), three of them were not detected during the survey (*S. salamandra*, *T. marmoratus*, and *A. obstetricians*). We detected *B. spinosus* in 57.89% ($N = 44$) of the study sites (47.73% of them in the unoccupied area), *Pelophylax* sp. in 76.32% ($N = 58$) of the study sites (43.10% of them in the unoccupied area), *R. dalmatina* in 61.84% ($N = 47$) of the study sites (38.30% of them in the unoccupied area), *P. punctatus* in 28.95% ($N = 22$) of the study sites (36.36% of them in the unoccupied area), *H. arborea* in 61.84% ($N = 47$) of the study site (51.06% of them in the unoccupied area), *L. helveticus* in 76.32% ($N = 58$) of the study sites (48.83% of them in the unoccupied area), and *T. cristatus* in 46.05% ($N = 35$) of the study sites (74.29% of them in the unoccupied area).

According to the Friedman rank test, no change in species richness was detected between the visits ($\text{Chi}^2 = 0.701$; $df = 2$, $p = 0.704$). The *X. laevis* abundance model was the best model of the *N*-mixture analysis (table 1), but it did not fit the data better than the distance to introduction site model ($\Delta\text{AIC} = 1.40$). According to the Spearman's ρ test, *X. laevis* abundance and distance to the introduction site were significantly correlated ($S = 126\,850$, $p < 0.001$, $\rho = -0.73$). Both models fitted the native amphibian species richness better than the other models ($\Delta\text{AIC} = 1.99$). These

Table 1. Summary of the best models obtained with the *N*-mixture method (Royle, 2004) to estimate species richness. Biotic and abiotic parameters were considered one by one to test their influence on species richness. For every model, the detection probability was assumed to be constant between visits. Only models with an AIC better than the null model are listed here. Resistivity (RES) and total dissolved solids (TDS) were the only abiotic parameters with a better AICc than the null model.

Model	Coef. estimate	Std. Error	AICc	ΔAICc	AICwgt	Nb Par	(-2 * LogLike)
Abundance	-0.0212	0.0089	1259.07	0.00	0.2636	3	1253.07
Distance	0.5947	0.2681	1260.47	1.40	0.1309	3	1254.47
RES	-0.0663	0.0391	1262.46	3.39	0.0484	3	1256.46
Presence	-0.1864	0.1144	1262.73	3.66	0.0423	3	1256.73
TDS	0.1071	0.0691	1263.13	4.06	0.0346	3	1257.13
Null model	/	/	1263.39	4.32	0.0304	2	1259.39

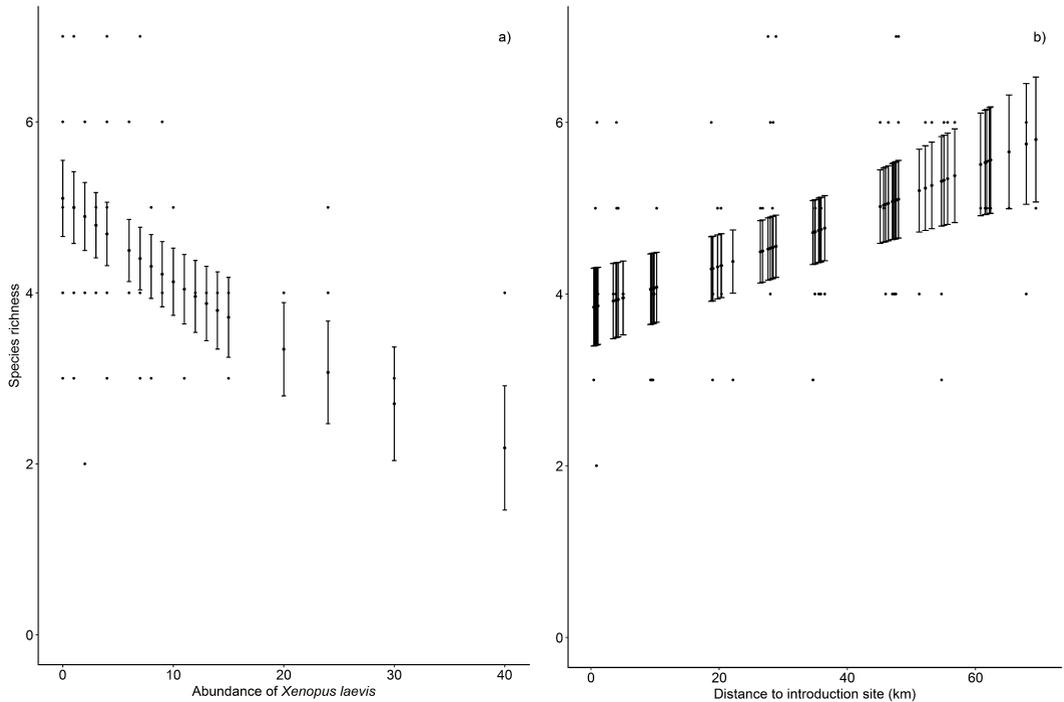


Figure 2. *N*-mixture estimates of species richness according to (a) the abundance of *Xenopus laevis* and (b) the distance to the introduction site. The small black dots represent the data for species richness and the large black dots represent the estimations with the standard errors represented by error bars.

models were validated by the GOF tests (*X. laevis* abundance model: $Chi^2 = 30.39$; $df = 22$; $p = 0.109$; distance to introduction site model: $Chi^2 = 29.45$; $df = 22$; $p = 0.133$). The *X. laevis* presence model did not fit the data better than the null model ($\Delta AIC = 0.66$). The *X. laevis* abundance model and the distance to introduction site model fitted the data better than the *X. laevis* presence model (respectively, $\Delta AIC = 3.66$ and $\Delta AIC = 2.26$). The species richness estimates according to the abundance

of *X. laevis* and the distance to the introduction site are illustrated in fig. 2a and fig. 2b, respectively. The species richness estimates according to the *X. laevis* presence model, validated by a GOF test ($Chi^2 = 27.98$; $df = 22$; $p = 0.176$) resulted in a value of 4.21 ± 0.415 species in absence of *X. laevis* and a value of 5.07 ± 0.478 species in the sites occupied by *X. laevis*.

According to the co-occurrence analysis, two interactions between species were significantly different from what was expected at random.

Table 2. Effects of *Xenopus laevis* on the niche position and breadth of native amphibians and its co-occurrence with these native amphibians. The Evenness J' was calculated in the area occupied by *X. laevis* J'_{occ} and in the uncolonized area J'_{ctrl} . The modification of Evenness $\Delta J'$ occurring after colonization is also presented. The Morisita's niche overlap index C_H shows the overlap between the niches of each native species and *X. laevis*. The co-occurrence analysis compared the observed co-occurrence (*Obs Cooc*) with the expected co-occurrence (*Exp Cooc*) and determined if they were significantly different (p -value).

	<i>B. spinosus</i>	<i>Pelophylax sp.</i>	<i>R. dalmatina</i>	<i>P. punctatus</i>	<i>H. arborea</i>	<i>L. helveticus</i>	<i>T. cristatus</i>
J'_{occ}	0.214	0.216	0.217	0.212	0.215	0.216	0.212
J'_{ctrl}	0.221	0.221	0.22	0.209	0.219	0.22	0.219
$\Delta J'$	0.007	0.005	0.003	-0.003	0.005	0.003	0.008
C_H	0.955	0.975	0.968	0.935	0.965	0.972	0.934
<i>Obs Cooc</i>	17	29	23	11	30	23	13
<i>Exp Cooc</i>	22.6	29.8	24.1	11.3	29.8	24.1	18.0
p -value	0.009	0.444	0.385	0.542	0.655	0.385	0.020

Xenopus laevis exhibited a significant negative co-occurrence with *B. spinosus* ($p = 0.009$) and *T. cristatus* ($p = 0.020$; table 2). The N -mixture models estimated for *B. spinosus* and *T. cristatus* were not validated by the GOF tests, preventing any interpretation of the modeling of the abundance of these species depending on the presence of *X. laevis*. The niche breadths of the native species in the colonized and non-colonized areas were not different (table 2). All species had a very high niche overlaps with *X. laevis* (table 2).

Discussion

In this study we showed that species richness of native amphibians was negatively related to the abundance of *X. laevis*. The time since colonization, expressed as the distance to the introduction site, was also negatively related to amphibian species richness. Abundance and time since colonization were correlated, which indicates that *X. laevis* does not become abundant in ponds immediately after colonization. One possible cause of this decreased richness could be the negative impact of *X. laevis* on native amphibians. The co-occurrence results showed that two species, *B. spinosus* and *T. cristatus*, co-occurred negatively with *X. laevis*. This result is different from the one obtained in Sicily where an impact was detected for anurans belonging to

the Ranidae, Hylidae and Discoglossidae families (Lillo, Faraone and Lo Valvo, 2011). The method used by Lillo and colleagues (2011) was not the same as in our study, which prevents us from performing a quantitative comparison, however.

A possible explanation of the negative relationship between the abundance of *X. laevis* and native amphibian species richness could reside in a potential preference of *X. laevis* for the colonization of habitats with characteristics that most native amphibian species do not select. However, the niche overlap between the native amphibians and *X. laevis* was very close to one. This suggests a strong overlap between the habitats used by *X. laevis* and the other amphibians. Furthermore, the niche breadth of the native amphibians was not modified in the area colonized by *X. laevis*. This could indicate that the niches of native amphibians were not reduced where they shared their habitat with the invasive frog. The constancy of niche breadths and overlaps could also be due to the relative similarity between the study sites.

Several mechanisms could underlie the impact of *X. laevis* on native amphibians in western France as recorded in this study. Twelve mechanisms have been highlighted previously to describe the impact of invasive species as listed in the Global Invasive Species Database (www.iucngisd.org/gisd/). However, only predation, competition, and the spread of disease

appear relevant in the case of *X. laevis*. Predation on amphibians may play a role in the potential impact of *X. laevis*, because this behavior has been recorded in more than one invasive population for this species, including in France (Courant et al., 2017). Interspecific competition for the access to resources, such as nutrients, may also occur. Indeed, *X. laevis* consumes small invertebrates such as dipteran larvae and zooplankton in large proportions (Measey, 1998; Courant et al., 2017). These prey items are essential in the trophic cascade of aquatic ecosystems like ponds (Brett and Goldman, 1996). A perturbation induced at the level of the zooplankton might result in effects on other predators of this class of prey, including the native urodeles, yet this needs to be tested using laboratory or mesocosm experiments.

The impact of *X. laevis* might also be explained by the dissemination of pathogens, as this species is known to be a non-symptomatic carrier of the chytrid fungus, *B. dendrobatidis*, which is held responsible of the decline of many amphibians on several continents (e.g. Berger et al., 1998; Bosch, Martinez-Solano and Garcia-Paris, 2001; Olson et al., 2013). *Xenopus laevis* is also known as an asymptomatic carrier of ranaviruses (Robert et al., 2014) and a high prevalence of these viruses has been detected in at least one location where it is invasive (Soto-Azat et al., 2016).

From a methodological point of view, the method we used for the co-occurrence analysis might provide biased results because it does not take into account the fact that each site was not equally likely occupied by each species (Griffith, Veech and Marsh, 2016). Regarding the *N*-mixture analysis, the application conditions – equal detection probability among species and visits – were validated, however. Despite our efforts, our sampling design showed some spatial and temporal weaknesses. For example, we only took into account abiotic parameters describing the ponds and their immediate

surroundings. However, the nature of the landscape and its connectivity is an important factor of occurrence and abundance for amphibian populations (Rothermel, 2004; Cushman, 2006; Ribeiro et al., 2011). Another potential weakness of this study concerns the possible changes in the abiotic parameters during the year and during the period of reproduction of amphibians (e.g. water depth, aquatic vegetation) or between day and night (e.g. temperature).

The sampling design of this study prevented us from distinguishing the effects of two correlated parameters, abundance and time since colonization. To allow this discrimination, the effect of the abundance of an invasive species on an ecosystem should be evaluated using a sampling design where time since colonization is rigorously known. In addition to this correlation between abundance and distance to introduction site, the abundance and density of *X. laevis* is likely influenced by abiotic factors. Assessing the habitat preferences of *X. laevis* in an invasive population with more diverse habitats may consequently help understand the potential impact of this species and to establish eradication and conservation programs.

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