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Introducing water frogs – Is there a risk for indigenous species in France?

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Abstract

The ecological success of introduced species in their new environments is difficult to predict. Recently, the water frog species *Rana ridibunda* has raised interest, as different genetic lineages were introduced to various European countries. The aim of the present study was to analyze the potential invasiveness of *R. ridibunda* and assess the risk of replacement for indigenous water frog species. The investigation of over 700 water frogs from 22 locations in southern France and four locations in Spain shows that the competition with indigenous species is mainly limited to a particular habitat type, characterized by high-oxygen and low-salinity freshwater. The competitive strength of *R. ridibunda* may be related to a higher growth rate and longevity as compared to the indigenous species *R. grafi* and *R. perezi*. Our data suggest that *R. ridibunda* is a risk to the diversity of indigenous water frog assemblages in France. Future monitoring needs to clarify the distribution of *R. ridibunda*, its ecological niche, and the risk status for indigenous water frog species. **To cite this article:** D.S. Schmeller et al., C. R. Biologies 330 (2007).

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Résumé

Introduire des grenouilles vertes – Y a-t-il un risque pour les espèces indigènes en France ? Le succès écologique d'espèces introduites dans un nouvel environnement est difficile à prédire. Récemment, l'intérêt s'est porté sur des espèces de grenouilles vertes *Rana ridibunda* dont différentes lignées génétiques ont été introduites dans plusieurs pays européens. Le but de cette étude était d'analyser le remplacement des grenouilles vertes indigènes dans le Sud de la France par cette espèce introduite, *R. ridibunda*. L'analyse de 700 grenouilles vertes issues de 22 sites du Sud de la France et de quatre populations espagnoles suggère un effet de *R. ridibunda* sur l'utilisation des habitats de *R. perezi* et *R. grafi*. La distribution écologique des espèces indigènes diffère dans les zones avec et sans *R. ridibunda*. Le remplacement potentiel des espèces indigènes par *R. ridibunda* est limité à un type d'habitat particulier, caractérisé par de l'eau avec des teneurs en oxygène dissous élevées et une faible salinité. Le risque est d'autant plus

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élevé que *R. ridibunda* montre un taux de croissance plus important et une plus grande longévité que les espèces indigènes *R. grafi* et *R. perezi*. **Pour citer cet article :** D.S. Schmeller et al., C. R. Biologies 330 (2007).

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Keywords: Replacement; Introduced species; Western Palearctic water frogs; Hybridogenesis; *Rana ridibunda*

Mots-clés : Remplacement ; Espèces introduites ; Grenouille verte ; Hybridogénèse *Rana ridibunda*

1. Introduction

The impact of introduced species on native species, indigenous communities, and ecosystems is diverse, threatening biodiversity on both the genetic and species levels [1]. Even though introduced species are often unable to establish persisting populations [2], around 15% find themselves unrestrained by predators, parasitoids, parasites, or interspecific competition [1]. Such populations might grow exponentially, and they are considered as substantial contributors to global biodiversity change [3]. Invasive species have been the focus of a large body of studies, but the complexity of the invasion process restricts the potential of predicting the future ecological success of introduced species with any degree of accuracy. In particular, variability of local adaptation is responsible for difficulties in predicting the duration of the time lag between initial introduction and the beginning of increased population growth and subsequent range expansion [1]. Basic ecological research, therefore, has a large potential for contributing significantly to the management of invasive, or potentially invasive species. Management actions need profound knowledge of the biological process that accompanies introductions of species. For example, Newsome and Noble [4] were able to link the invasiveness of a species to the higher longevity and growth rate of individuals, as that kind of species would survive, resist, and recover from temporarily suboptimal environmental conditions better than short-lived and slow-growing species.

The introduction of *Rana ridibunda* to France and Switzerland has led to a significant change of the indigenous water frog fauna [5]. Most of the stable *R. ridibunda* populations in France and Switzerland are the result of introductions due to human consumption [6–8]. The use of genetic methods to determine the range of water frogs in France has shown that *R. ridibunda* inhabits a much larger area in France than anticipated earlier [9,10]. Here, we contribute to the small number of studies illustrating the possible threats that *R. ridibunda* poses to the indigenous water frog assemblages of France, with a focus on *R. perezi* and its hybridogenetic associate *R. grafi*. These two frog taxa are always sympatric, due to dependence of the hybrid *R. grafi*

on backcrosses with *R. perezi*, similar to their more northerly distributed relatives, *R. lessonae* and *R. esculenta* [11,12]. We test for habitat preferences of the different species and differences in the ratio of *R. grafi* to its parental species *R. perezi* in dependence on the presence or absence of *R. ridibunda*. In addition, we compare the longevity and growth rates of *R. ridibunda*, *R. perezi*, and *R. grafi*, which are important factors in interspecies competition to evaluate the competitive strength of the different frog taxa [13,14]. Additionally, we also contribute data to the distribution range of *R. ridibunda*, *R. perezi* and *R. grafi* in Mediterranean France. Such data are difficult to produce, as the classification of water frogs based on morphological data alone is extremely difficult, and needs confirmation by genetic methods [15].

2. Material and methods

We collected water frogs ($N = 607$) at 22 sites in the lower Rhone valley, the Rhone delta, and the Mediterranean coastline of southwestern France during years 1996 to 1998. In 2000, we surveyed 118 water frogs from four populations from Spain (Fig. 1, Table 1). The known range of *R. ridibunda* includes the Rhone delta, in which, with regard to the habitat type, we randomly selected sampling sites. Here, we cover the widest range of ecological conditions, covering all habitat types, allowing detection of a strong signal on habitat preferences in all three species.

The frogs were caught by hand at night or by using modified fish traps of different sizes. We measured their snout-vent length (SVL) to the nearest 1 mm in the laboratory, using an electronic calliper. Taxon identification was based on genetic analysis of allozymes, conducted on starch gels and cellulose acetate plates using standard procedures [16] (Table 2). We used the loci *6pgdh*, *aat-2*, *gapdh*, *gpi*, and *ldh-b* to distinguish *R. ridibunda*, *R. esculenta*, and *R. lessonae*. *R. ridibunda* was distinguished from *R. perezi* and *R. grafi* using the loci *6pgdh*, *ahh*, *ck*, *gapdh*, *αgdh*, *ldh-b*, and *mpr-1* [17].

A detailed ecological description of sites using 13 variables [10] showed that sites inhabited by water frogs strongly differ in their ecological characteristics and

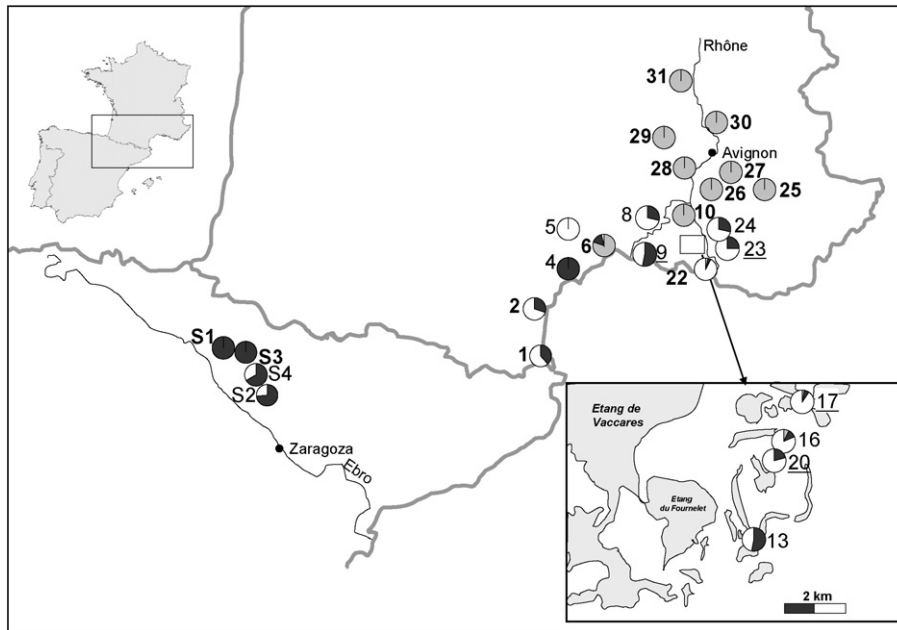


Fig. 1. Population composition. The figure illustrates the relative amount of the three taxa *R. ridibunda* (grey), *R. perezi* (black), and the hybridogen *R. grafi* (white). The numbers correspond to Table 1. Bold population numbers indicate habitats of the A-type, plain ones represent B-habitats, and italic ones stand for C-habitats.

provided evidence of a gradient of freshwater influence and oxygen availability. In that study, the environmental parameters with the highest explanatory power were found to be acidity (pH), relative amount of oxygen (rel. O₂), and salinity (μS), which we therefore chose to measure for the current study and habitat description. Salinity was measured as the conductivity of water to a standard KCl solution. The measures were taken twice a breeding season and site to categorize the habitats investigated here, based on the earlier reported variables [10]. We performed a PCA to describe environmentally the individual locations. Three habitat groups were defined. The first group (A) comprises habitats connected to freshwater sources (brooks, rivers, freshwater channels), characterized by low salinity, high rel. O₂, and near-to-neutral pH. The second group (B) is defined by intermediate flood influence, characterized by medium rel. O₂, medium to low salinity, and low pH. The third group (C) covers marshes and small water bodies more remote to freshwater sources. These habitats show a high to very high salinity, high acidity of water, and low rel. O₂. The term ‘freshwater’ is used in opposition to saltwater.

We used skeletochronological analyses of bone cross-sections to determine the age of each specimen [18–20]. Skeletochronology relies on visible lines of arrested growth (LAGs) in bone cross-sections, which are built

during hibernations. In water frogs, LAGs are usually very distinct, allowing a good inference of age [21]. One finger was used for skeletochronological age determination and was decalcified, dehydrated and embedded in Histo-resin® (LEITZ, Wetzlar, Germany). After embedding, we cut 11-μm layers using a rotation microtome and transferred every tenth cut (about 40 in total) to a microscope slide. The dried sections were then stained with cresyl-violet to visualize the LAGs and were subsequently covered with glass cover slips. LAGs were counted under the microscope at magnifications of 200× or 400×. In *R. perezi*, age could not be unambiguously determined for nine individuals, which have been excluded from the analysis. To analyse the differences in age between taxa, we performed a mixed model ANOVA (incomplete design; taxon = fixed effect; population = random effect).

Generally, two potential factors may affect the ratio of *R. grafi* to *R. perezi* in a population: (i) ecological conditions and habitat preferences, and (ii) the presence and absence of *R. ridibunda*. We employed a Kruskal–Wallis ANOVA to test for differences of the frequency of a taxon according to habitat types and Fisher’s exact test to explore effects of the presence or absence of *R. ridibunda* on the ratio of *R. grafi* to *R. perezi* in a population or a region. All populations in Spain (S1–4) and three populations in France (1, 2, 4) are assumed to

Table 1
Environmental characteristics of the different populations

No.	N	<i>R. ridibunda</i>	<i>R. perezi</i>	<i>R. grafi</i>	Sex ratio	Ht	S/cm	pH	O ₂ %	Coordinates	Commune (French 'département')
01*	26	10 (38)	16 (62)		1 : 7	A	926	6.8	130	42°34.39 N, 3°00.48 E	Argelès-sur-Mer (66)
02*	30	9 (30)	21 (70)			A	642	6.0	120	42°39.14 N, 2°54.39 E	Saint-Cyprien-Plage (66)
04*	37	19 (51)	18 (49)			B	1856	5.6	110	43°15.30 N, 3°10.90 E	Valras-Plage (11)
05	17		17 (100)			B	1200	6.8	120	43°48.34 N, 3°45.60 E	Saint-Martin-de-Londres (34)
06*	29	24 (83)	4 (14)	1 (3)		A	1474	6.4	110	43°33.23 N, 3°54.21 E	Lattes (34)
08*	28		8 (29)	20 (71)		B	1150	5.3	159	43°36.43 N, 4°20.34 E	Saint-Gilles (30)
09	25		13 (52)	12 (48)	1 : 5	C	4690	6.0	70	43°32.80 N, 4°26.11 E	Les Saintes-Maries-de-la-Mer (30)
10*	22	22 (100)				A	870	5.8	65	43°38.86 N, 4°35.92 E	Arles (30)
13*	34		18 (53)	16 (47)	1 : 2	B	1050	5	200	43°28.71 N, 4°38.99 E	Le Sambuc (13)
16*	32	1 (3)	2 (6)	29 (91)	1 : 4	B	394	5.9	140	43°30.50 N, 4°40.18 E	Le Sambuc (13)
17*	27	2 (7)	3 (11)	22 (82)		C	5200	5.4	90	43°31.30 N, 4°40.44 E	Le Sambuc (13)
20*	24		5 (21)	19 (79)	1 : 5	C	450	5.3	30	43°30.32 N, 4°40.02 E	Le Sambuc (13)
22*	27	1 (4)	1 (4)	25 (93)		A	20000	5.7	130	43°22.23 N, 4°48.33 E	Salin-de-Giraud (13)
23	35		10 (29)	25 (71)		C	1500	5.3	130	43°32.20 N, 4°45.31 E	Mas Thibert (13)
24*	24		6 (25)	18 (75)	1 : 13	B	1020	6.3	100	43°27.99 N, 4°52.86 E	Mas Thibert (13)
25	28	28 (100)				A	843	6.7	120	43°45.16 N, 5°13.45 E	Merindol (84)
26	27	27 (100)				A	630	5.3	180	43°47.15 N, 4°57.10 E	Mollégés (13)
27	21	20 (95)				A	689	6.2	65	43°50.01 N, 5°00.28 E	Cavaillon (84)
28	28	28 (100)				A	584	6.5	111	43°53.10 N, 4°41.32 E	Aramon (30)
29	28	28 (100)				A	860	6.4	100	44°00.22 N, 4°25.97 E	Uzés (30)
30	29	29 (100)				A	439	6.1	140	44°05.53 N, 4°45.73 E	Caderousse (30)
31	29	29 (100)				A	356	6.2	82	44°16.34 N, 4°38.45 E	Pont-Saint-Esprit (30)
Σ	607	239 (39)	108 (18)	259 (43)							
S1	12		12 (100)			A				42°13.34 N, 1°20.43 W	Castilliscar
S2	87		64 (74)	23 (26)		B				42°04.35 N, 1°08.70 W	Sadaba
S3	13		13 (100)			A				42°13.46 N, 1°16.06 W	Ejea de los Caballeros
S4	6		4 (67)	2 (33)		B				42°11.62 N, 1°10.94 W	Sabrinan (Rio loma negra)
Σ	118		96 (79)	25 (21)							

Given are: population number (No.), corresponding to the numbers in Fig. 1, total sample size (*N*) and by species, sex ratio of *R. grafi*, salinity (μS/cm), acidity (pH), relative amount of oxygen (O₂%), habitat type (Ht). The asterisk (*) marks populations from which the individuals for the skeletochronological analysis stem. The different habitats are: brook = S4, 1, 2, 4, 5; channel = 13, 16, 20, 24–27; pool = S3, 8, 9, 23; pond = S1, 6, 10, 17, 22, 28; floodplain = S2, 29–31. For each taxon sample, size is given with percentage within parentheses. The sex ratio is given only for the populations where we found adult males and females. Population 27 contained 5% individuals that could not be assigned to a water frog species indigenous to France.

lay outside of the current distribution of *R. ridibunda*, as no occurrence has been reported for these regions. Populations within the range of the current distribution of *R. ridibunda* are populations 5 to 24.

3. Results

In the French samples, the most frequently observed taxon was the hybrid *R. grafi* (43% of the total sample), followed by *R. ridibunda* (39%) and *R. perezi* (18%). In the Spanish samples, only *R. perezi* (79%) and *R. grafi* (21%) were present.

All habitats dominated by *R. ridibunda* were of habitat type A (Table 1). In the Rhone delta, habitats dominated by either *R. grafi* or *R. perezi* included small ditches, shallow ponds, and various types of channels (habitat types B, C). The comparison of the frequency

of *R. perezi* and *R. grafi* in the different populations belonging to different habitat types did not show any difference ($H_2 = 0.410$; $N = 18$; $p = 0.815$), while *R. ridibunda* seems to prefer habitats of type A ($H_2 = 4.84$; $N = 12$; $p = 0.089$, Fig. 2). The ratio of *R. grafi* to *R. perezi* is not differing between the habitat types ($H_2 = 2.24$; $N = 17$; $p = 0.326$). The ratio of *R. grafi* to *R. perezi* is significantly affected by the presence or absence of *R. ridibunda* in a region (ratio_{presence} = 2.9:1; ratio_{absence} = 0.60:1; $Chi^2 = 67.97$; $p < 0.001$) or a population (ratio_{presence} = 7.7:1; ratio_{absence} = 1:1; $Chi^2 = 39.98$; $p < 0.001$).

The oldest frog was a *R. grafi* specimen in its 6th year of age (mean age = 1.62 ± 0.79), but generally *R. ridibunda* reached higher ages (maximum age: 5 years; mean age: 2.22 ± 0.99) than both *R. perezi* (max age: 3 years; mean age: 1.43 ± 0.57) and *R. grafi* ($F_2 = 4.07$;

Table 2
Allozyme systems used to distinguish between the taxa

Enzyme	Tissue	Buffer	EC
<i>αgdh</i>	Liver	TG 8.5	1.1.1.8
<i>βpgdh</i>	Muscle	TB 8.9	1.1.1.44
<i>aat</i>	Liver	TC 8.2	2.6.1.1
<i>ahh</i>	Liver	TG 8.5	3.3.1.1
<i>ck</i>	Muscle	TG 8.5	2.7.3.2
<i>ldh</i>	Liver	TG 8.5	1.1.1.27
<i>mpi</i>	Liver	TM 7.0	5.3.1.8
<i>mpr</i>	Muscle	TB 8.9	undefined
<i>pgi</i>	Liver	TG 8.5	5.3.1.9
<i>pgm</i>	Muscle	TM 7.0	5.4.2.2

We used standard procedures of cellulose acetate allozyme electrophoresis [16] with several buffer systems (TG = Tris-glycine, TB = Tris-borate, TC = Tris-citrate, TM = Tris-maleate, pH is given as a decimal number). Three buffer systems were used [16]: Tris-boric acid (pH 8.9) for *βpgdh*, *mpr*; Tris-glycine (pH 8.9) for *ahh*, *ck*, *αgdh*, *gpi*, *ldh*; Tris-citrate (pH 8.2) for *aat*, *gapdh*. All enzymes were run for 40 min at 200 V, except of *βpgdh*, and *mpr* (50 min/200 V). EC = Enzyme commission number.

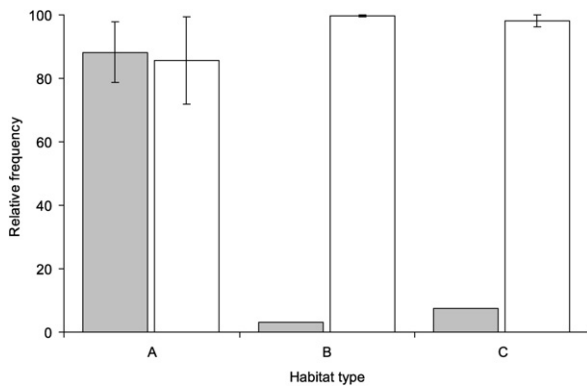


Fig. 2. Occupation of habitat types of *R. ridibunda* (grey), *R. perezi* and *R. grafi* (white). The frequency differences of *R. ridibunda* are found to be significant, while this is not true for the combined frequency of *R. perezi* and *R. grafi*.

Table 3

Results of the comparison of the mean snout-to-vent length (SVL) of individuals from the first three age classes of *R. ridibunda* (RR), *R. perezi* (PP), and *R. grafi* (RP)

	Age class 1			Age class 2			Age class 3		
	SVL	SE	N	SVL	SE	N	SVL	SE	N
<i>R. ridibunda</i>	53.58	3.80	16	62.02	1.91	24	68.28	2.28	5
<i>R. perezi</i>	33.76	0.76	53	40.00	1.33	28	46.74	2.85	4
<i>R. grafi</i>	42.92	1.27	123	52.00	1.45	84	51.12	2.61	23
	<i>t</i>	<i>df</i>	<i>p</i>	<i>t</i>	<i>df</i>	<i>p</i>	<i>t</i>	<i>df</i>	<i>p</i>
RR × PP	8.00	67	<0.001	9.66	50	<0.001	5.98	7	0.001
RR × RP	2.82	137	0.005	3.45	106	0.001	2.97	26	0.006
RP × PP	4.58	174	<0.001	4.56	110	<0.001	0.68	25	0.504

SE = standard error, N = sample size, *t* = t-test value, *df* = degrees of freedom, *p* = level of significance. The second part of the table gives the test combinations and the test results.

$p = 0.044$). *R. ridibunda* tended to reach larger sizes in any age class than both of the other taxa (Table 3), weakly supported by an ANCOVA with taxon as fixed, population as a random factor, and age as a covariate ($F_{13,301} = 1.64$, $p = 0.072$).

4. Discussion

We investigated the habitat choice of three water frog species in France and assessed the potential risk of replacement of the indigenous species *R. perezi* and *R. grafi* by introduced *R. ridibunda*. We found differences in habitat preferences of *R. ridibunda*, but not in *R. perezi* and *R. grafi*. While the latter frogs occurred in comparable frequencies in all the habitat types investigated here, *R. ridibunda* was found with high frequencies in habitats with high river influence. Our data is in accordance to earlier reports that *R. ridibunda* prefers habitats with high amounts of relative oxygen [9,22], which is assumed to be related to a high metabolic rate and low physiological performances [23]. Our results do also support an influence of the presence of *R. ridibunda* on the habitat use of indigenous species, especially of *R. perezi*, and support a real risk of replacement for indigenous species by *R. ridibunda*. Indeed, the higher longevity and faster growth relative to *R. perezi* and *R. grafi*, as found in our study, indicate a potentially higher competitiveness for *R. ridibunda*.

The competitive strength of *R. ridibunda* is linked to the high fertility of older females, as the number of eggs, body size, and age are strongly correlated in anurans [24–26]. Therefore, *R. ridibunda* will have a higher individual reproductive success than the much smaller *R. perezi*. Currently, it seems that the superiority of *R. ridibunda* may vanish under environmental conditions as given in B- and C-habitats, simply because *R. ridibunda* tadpoles barely survive under such condi-

tions [23]. Our sampling in the Rhone delta suggests this, too. Despite the possibility of inhabiting numerous habitats of categories B and C in close vicinity to the river floodplains (<3 km distance), we found only a low proportion of *R. ridibunda* in few of such habitats. The dominance of *R. ridibunda* in A-habitats, however, suggests that under constant freshwater inflow and constantly high oxygen levels, *R. ridibunda* has to be considered a strong competitor, increasing the risk of replacement for indigenous species. In addition, the hybrids *R. grafi* and *R. esculenta*, reproductively mimicking *R. ridibunda*, reinforce the reproductive success of *R. ridibunda* by mating with that species [11,27]. This is especially important for *R. ridibunda*, as the sex ratio of hybridogens is biased towards females (this study, Table 1, but see also [28,29]). Hence, considering the reproductive cost to produce eggs (e.g., [30]), *R. ridibunda* has a cheap possibility to produce offspring by mating of *R. ridibunda* males with females of *R. grafi* and *R. esculenta* [11].

The distribution pattern of species and the composition of the here- investigated populations suggest that once *R. ridibunda* has succeeded to install itself in a habitat, the replacement of indigenous species might be rapid. Our notion is supported by the fact that mixed populations with *R. ridibunda*, *R. perezi*, and *R. grafi* being sympatric are rarely observed, suggesting a low detection probability. In our study, only in four of all French populations (13%), all taxa were sympatric, while in three of those populations, *R. ridibunda* was rare (less than 7% of the total population). Our study lacks a temporal component and we therefore cannot determine if *R. ridibunda* has expanded its range recently and if population 6, an assemblage of all three species, but with a high proportion of *R. ridibunda*, shows a replacement in progress.

Besides pointing to a real risk of replacement, the here-presented data are of importance for monitoring the distribution process of *R. ridibunda* in France, because earlier distribution maps may have been inaccurate, relying on species identification by morphology only, as *R. ridibunda* is barely distinguishable morphologically from both *R. grafi* and *R. esculenta* [15]. By contributing to knowledge on populations of genetically verified *R. ridibunda*, we provide future analyses with a valuable temporal component. For future studies, aiming to monitor the distribution process of *R. ridibunda* in France and the replacement of indigenous water frogs, it is imperative to use genetic markers.

From our study, we learn that the establishment of new *R. ridibunda* populations is currently restricted to habitats of type A, characterized by a high level of oxy-

gen or at least a connection to a freshwater source, and relatively low salinity. The high genetic variability reported for *R. ridibunda* in the study region [31], and the gene exchange with *R. perezi* and *R. lessonae* [12], however, may allow adaptation to low-oxygen environments. In the future, an ecological shift of *R. ridibunda* may occur and, after a lag time, all indigenous water frog species could be under risk of extinction. For conservation of diversity in water frog assemblages, future analyses and surveys are needed to monitor the range expansion and possible ecological shift of *R. ridibunda*.

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