

# Water frogs in Wallonia : genetic identification of the introduced taxa (*Pelophylax ssp.*) and impact on indigenous water frogs (*Pelophylax lessonae* and *P.kl. esculentus*).

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## 1. Introduction



Lake Frog (*Pelophylax ridibundus*), Lasne, Wallonia - © C. & N. Percsy

Two taxa of water frogs are native in Wallonia : *Pelophylax lessonae* and *P. kl. esculentus*. But water frogs, from different origins, have been introduced in Wallonia during the last 25 years, mainly as a consequence of aquatic horticulture : aquatic plants are imported from central Europe ... with eggs, tadpoles or adults of water frogs; these frogs reproduce successfully in the horticulture ponds and are sold (or given) to people creating an ornamental pond in their garden. So, the frogs are introduced in many places in the country; then they spread in the neighbourhood and colonize (semi-)natural habitats. As a consequence, *P. ridibundus* has become the most frequent green frog in Brabant wallon (Percsy & Percsy, 2002a and 2002b). It is also abundant in the neighbourhood of large cities (Brussels, Liège, Namur, Verviers), (Percsy & Percsy, 2007).

Alien water frogs - e.a. *P. ridibundus* - occupy sites where *P. kl. esculentus* and *P. lessonae* are present, as well as other amphibians (mainly *R. temporaria*, *Bufo bufo*, *Triturus* sp.); it is a predator of these species. Since *P. ridibundus* is bigger than our two native green frogs, the competition between them for territory, feeding and breeding is in favour of *P. ridibundus*. Furthermore, because of the very particular genetic relationship between the three green frogs (hybridogenesis : see, f.i., Berger 1988), the introduction of *P. ridibundus* in *P. kl. esculentus* and *P. lessonae* populations may lead to a genetic pollution of the latter species. Finally, foreign frogs may carry diseases, which is a threat for indigenous amphibians (Kok 2001).

*P. ridibundus* has been introduced in other countries of western Europe and is known having outcompeted other amphibians sharing the same habitat (see f.i. Günther in Gasc et al. 1997, Grossenbacher 1988).

Consequently, it is important to evaluate the impact of the introduced water frogs in Wallonia.

Unfortunately, the recognition of the different taxa of water frogs present in Wallonia is not easy. To insure the identifications we have made, we collected, in 2002, samples on 47 frogs from 8 different populations and submitted these for enzymatic and genetic analysis. The « Laboratoire d'Ecologie des Hydrosystème fluviaux » (Prof. Joly) at the University of Lyon made protein electrophoresis and the « Museum für Naturkunde » (Prof. Plötner) in Berlin investigated mitochondrial DNA.

The results of these analyses allow to obtain :

1. a validation of the identification method of the taxa on the field and, thus, a reliable follow of the evolution of the populations;
2. the determination of the geographic origin of the introduced frogs;
3. the evidence of hybridization and/or introgression between *Pelophylax ridibundus* and the indigenous frogs *P. lessonae* or *P. kl. esculentus*: similar introgressions between taxa of water frogs have been observed in other European countries (f. i. Vorburger et al. 2003, Plötner et al. 2008, Holsbeek et al. 2008).

## 2. Validation of the identification method

We want to test a method of identification on the field that can be done without capturing the frogs : observing morphological characters (possibly by using field-glasses) and listening to the mating calls should be sufficient. We use, in Wallonia, the following easy criteria.

Note that the evaluation of these criteria are somewhat subjective and may depend on the observer's experience. It is the reason why the identification is validated only if most of the criteria are coherently satisfied.

By comparing our field identifications with the enzymatic- and DNA-analysis, we obtain the following results concerning the above method of recognition of the taxa in the field (see Percsy & Percsy 2009) :

- the distinction between the native green frogs, on the one hand, and the introduced water frogs, on the other hand, is valid in 100% of the cases;
- the distinction between *P. kl. esculentus* and *P. lessonae* is less valid : two of the seven presumed « *lessonae* » are actually « *esculentus* »;
- distinction between alien water frogs is not reliable;
- the distinction between native and non native male green frogs may be done using only the mating call. (This result has been confirmed by further bioacoustic analyses, using the oscillograms and sonograms of the calls).

Moreover, analyses of various photographs of the captured frogs allows to evaluate different other classical criteria for the identification of the water frogs (Percsy & Percsy 2009).

	<i>Pelophylax lessonae</i>	<i>P. kl. esculentus</i>	<i>P. ridibundus</i>
Length of the hind leg vs body length	small	intermediate	long
Tibia length vs femur length	smaller or equal	equal	longer
Colour of the back of the thigh and/or of the groin	often with a vivid yellow tint	with or without a vivid yellow tint	no vivid yellow tint
Skin coarseness of the back	weak	weak	generally strong
Vocal bags	white, sometimes tinged with pink	greyish or medium-grey	dark grey
Call	continuous, long and uniform	not so long, modulated	jerky

## 3. Geographic origin of the alien water frogs

All alien water frogs are determined as *P. ridibundus* or *P. cf. ridibundus* (from Anatolia). The mitochondrial analysis of our samples shows that introduced water frogs in Wallonia have at least two different geographic origins, corresponding to three different haplotypes :

- haplotype C (present in Central Europe);
- haplotype E1 and haplotype E2 (both typical for Anatolian, northern Greek or Bulgarian water frogs).

Three of the populations we have studied contain both indigenous and exotic water frogs (the latter having the same haplotype); two other populations contain only alien water frogs, all with the same haplotype; both haplotypes C and E1 are present on a same site; finally, two sites do not have exotic water frogs.

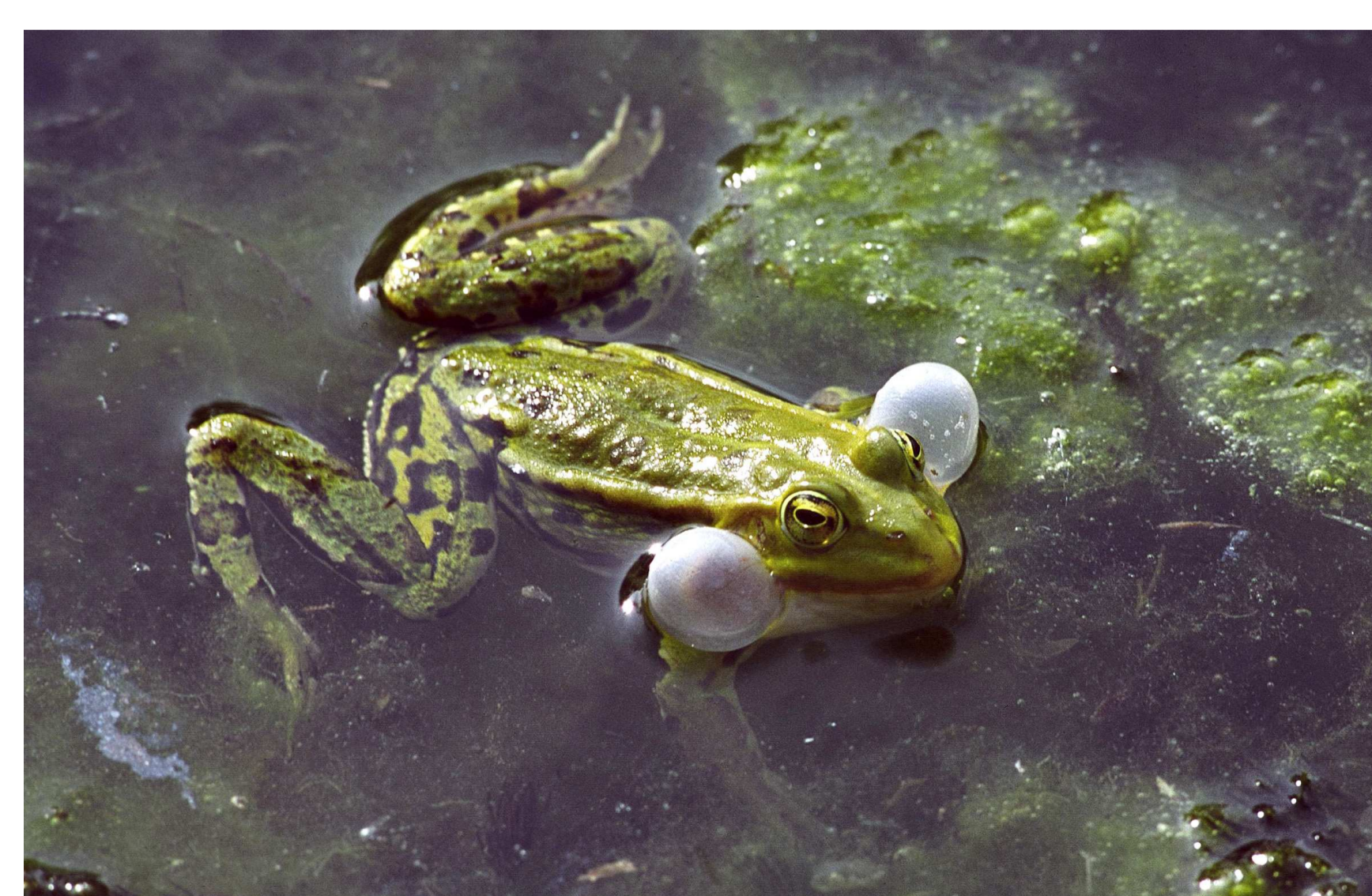
## 4. Introgression

Two samples corresponding to frogs that we have identified on the field as *P. kl. esculentus* and whose enzymatic analyses are also *P. kl. esculentus*, have mitochondrial DNA of type E2. This shows that hybridization between native frogs and *P. ridibundus* has occurred.

Concerning the « *lessonae* » haplotype of our samples, note that this type is different from all other « *lessonae* » haplotypes known (T. Ohst, pers. comm.).



Pool Frog (*Pelophylax lessonae*), Baudour, Wallonia - © C. & N. Percsy



Edible Frog (*Pelophylax kl. esculentus*), Rixensart, Wallonia - © C. & N. Percsy

## 5. Conclusion

Alien water frogs have spread in certain regions of Wallonia : they come from at least two different origins (central Europe or Balkan and Anatolia). Such frogs have a negative impact on our native water frogs and, probably, on other amphibian species.

Our work validates a method to separate, on the field, the native water frogs (*P. lessonae* and *P. kl. esculentus*) from the introduced frogs *P. ridibundus* and *P. cf. ridibundus*. It also shows the existence of hybridization between alien and native frogs in Wallonia.

Alien water frogs are already abundant in certain regions of Wallonia and it is hopeless to eradicate them there. On the contrary, exotic frogs seem absent from a large part of the country, in particular in oligotrophic ecosystems where *P. lessonae* is dominant. Such populations of water frogs have to be protected from invasion, because they are less frequent (Günther in Gasc et al. 1997); furthermore, the results above show that the « *lessonae* » haplotype of Wallonia is original. Since, probably, *P. ridibundus* will not colonize spontaneously such ecosystems (Pagano et al. 2001), it is urgent to avoid introductions in these areas.

Consequently, the following measures should be implemented :

- trade control of water frogs in Belgium and in Europe (laws about trade should be enacted);
- control of introduced frogs in target regions;
- public awareness, to avoid the transfer of frogs from one place to another.

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