Larval development of *Pelophylax esculentus* (Amphibia, Ranidae) tadpoles from two different habitats: preliminary results about impact of climatic warming and acclimatation ability

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Abstract: Palearctic water frogs are very widespread and abundant in Europe, and used many habitats characterized by different biotic and abiotic factors. We observed larval development of *P. esculentus* tadpoles breeding at 20 and 26°C, two temperatures selected to mimic both natural condition and global warming, but we especially compared the acclimatation ability of tadpoles originating from two different habitats. Our results show that with increasing temperature, tadpoles metamorphosed significantly earlier, and are significantly smaller than those who raised at 20°C. Observe tadpoles development in function of their parents origine will be important to predict the fat of amphibian populations according to their habitat.

Keywords: Natura 2000 species, Amphibians, *Pelophylax* klepton *esculentus*, climate change, acclimatation ability

Introduction

Global warming potentially affects the distribution and abundance of populations, but also in the timing of breeding and in the morphology of individuals within a population (Blouin, Brown, 2000), which could eventually lead to changes in population structure or to exctinctions (Tryjanowski *et al.*, 2006). Because amphibians are sedentary, ectothermic vertebrates, and due to their ecological attributes, they may be even more sensitive to climatic change than birds and/or mammals (Blaustein *et al.*, 2001).
Among Amphibians, Palearctic water frogs are very widespread and abundant in Europe, and used many habitats characterized by different biotic and abiotic factors (Pagano *et al.*, 2001b). These frogs are characterized by a hybridization complex. *Pelophylax* klepton *esculentus*, is an hybridogenetic hybrid resulting from mating between *P. ridibundus* and *P. lessonae* The hybrid is common and exhibits a good fitness (e;g.(Hotz *et al.*, 1999). The success and the evolutionary fate of hybridogenetic lineages are explained by both a generalistic heterosis hypothesis and an alternative hypothesis, the habitat segregation hypothesis (Plenet *et al.*, 2005).
We aim to check if such a common taxa may suffer from global warming. In this context, we observed larval development of *P. esculentus* tadpoles raired at two temperatures selected to mimic the natural condition and the global warming. We record the acclimatation ability of tadpoles but also aim to identify if acclimatation abilities depend on the different habitats (forest and meadows) the tadpoles originated from.

Materials and methods

A total of 50 Palearctic water frogs were caught in a meadow pond and a forest pond. After a taxonomic identification of frogs using a specific allozyme marker (LDH-B lactate deshydrogenase), artificial fertilizations have been done between 2 *P. lessonae* males and 4 *P. esculentus* females in function of the different habitats (Fig. 1). Than, tadpoles of these six clutches were raised in experimental conditions until hatching which is considered as the begening of the experiment (T0), and they were randomized and kept in large tanks filled with aerated tap water, and carried out at twenty or twenty six degrees Celsius. There were at a 0,23 individuals/L density (324 tadpoles in 36 tanks), and were feed with a mix of rabbit and fish food *ad libitum*, until they reached developmental stage 41 (Gosner 1960). Tadpoles were then sample to observe body size (mass) and growth time, and reared individually in small box until the end of metamorphosis, stage when we observed also mass and time. The water quality were observed twice a week during all of the experiment (nitrate and nitrite levels, pH, conductivity) to maintain tadpoles in the same conditions. The traitment was choose to reproduce natural conditions, 20°C represented temperature of a forest pond in summer, and 26°C those of a meadow pond during a warming day. Difference in development between the two temperature were observe with a Mann-Whitney test. We evaluated for the origine effect and the cumulated temperature effect, on each morphological measures, using linear mixed-effects models fitted by restricted maximum likelihood. From a complete model including both variables and their interaction, backward selections were performed.

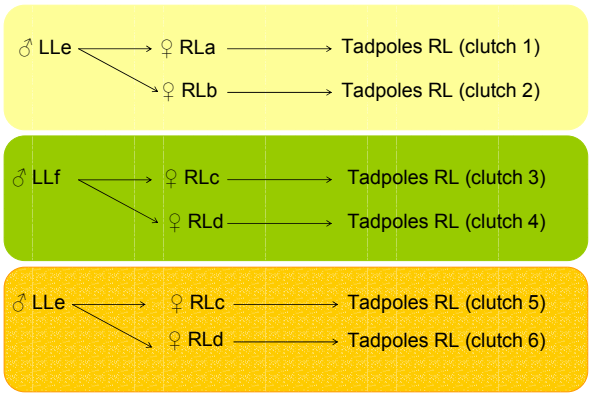

Fig 1: Schema representing the six artificial fecondations. The yellow correspond to meadow mating, the green to forest mating, and the orange to the mixt mating (meadow male with forest females). LL: Pelophylax lessonae; RL: Pelophylax esculentus,Small lettre" a" to " f" : indiviual code.

Results and discussion

Among the 324 tadpoles, only 281 reached the stage 41, and 272 survived until the end of metamorphosis. 93,2% tadpoles breeding at 26°C survived until the end of the experience, which is more than tadpoles reared at 20°C (74,7%) (p-value = 1.138e-05).

It suggests a good accomodation to temperature. Moreover, tadpoles reared at 20°C are significatly bigger at stage 41 and at the end of metamorphosis than those reared at 26°C (tab 1, Fig. 1). Moreover, at 20°C, its developped significatively slowly than those at 26°C.

Tab 1: Differences in larval life history traits for the tadpoles reared at two different temperatures: results of Mann-Withney tests

	Mass at stage 41	*Mass at metamorphosis*	*Time to S41*	*Time to metamorphosis*
W	18646.5	19527	17684	18387
p-value	2.20E-16	2.20E-16	2.20E-16	2.20E-16

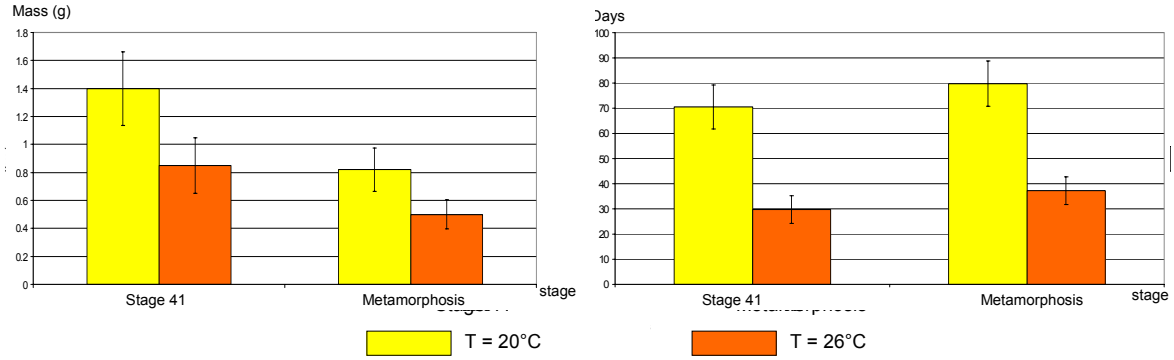

Fig 2: a) Body mass at stage 41 and metamorphosis for the two rearing temperatures. b): Time to reach the developmental stage

This results confirms previous studies (Negovetic *et al.*, 2001); (Blaustein *et al.*, 2001; Browne *et al.*, 2003), who observed with increasing temperature, an earlier metamorphosis. Whose tadpoles that are able to shorten their larval period are considered as having a good fitness. However, tadpoles that metamorphose earlier usually reach a smaller size at metamorphose that is considered as negative for fitness (small size at metamorphose affects survival). This trade-off needs to be studied more extensivelly in order to quantify more precisely the global effect of temperature on fitness of this frog.

In addition, we aim to compare larval development of tadpoles regarding their parents origine. The idea is to check if temperature effects on larval traits may vary from a population to another i.e. if some population may exhibit more abilities to resist to global warming. In this goal the use of linear mixed-effects models could be useful to detects such variations between populations.

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