

Are the hibernating water frogs from *Pelophylax (Rana) esculentus* complex (from North-Western Romania) able to adapt to the thermal water conditions?

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Abstract. We were interested to determine if the hibernating *Pelophylax ridibundus* and the other two forms of water frogs from the *Pelophylax esculentus* complex (*Pelophylax lessonae*, *Pelophylax esculentus*) fulfil the requirements of thermal resistance in order to become adapted to the thermal habitats. During the experiment we had determined for each frog the value of two parameters: VTMax and CTMax. The experimental determination of the resistance to temperature of the water frogs from the *P. esculentus* complex has revealed a great resistance of these frogs to high temperatures. Although the difference among the analysed frogs is not significant concerning the values of the voluntary and critical thermal maximum, it still can be observed a specific hierarchical order. This resistance (an average of 34.52°C), observed in the case of the hibernating populations of *P. ridibundus* indicates the reason why the marsh frogs can adapt so easily to the conditions from thermal waters. The voluntary thermal maximum obtained for the hybrid *Pelophylax kl. esculentus* does not differ noticeably, having an average value of 33.31°C. The lowest values for this parameter were recorded for the *Pelophylax lessonae* species (an average of 33.02°C).

Key Words: *Pelophylax esculentus* complex, thermal waters, thermoresistance, Romania.

Introduction. *Pelophylax kl. esculentus* is a natural hybrid between *Pelophylax lessonae* and *Pelophylax ridibundus* species, having a large distribution area in Europe. The three water frog's forms that make up the *Pelophylax esculentus* complex are encountered in many system types: L-E, R-E, L-E-R or E (Tunner & Heppich-Tunner 1991). Throughout the distribution area of these three forms, the L-E and R-E systems are the most common (e.g. Borkin et al 1986; Tunner & Heppich-Tunner 1991).

In Romania, the populations that have been identified were mostly populations of *Pelophylax ridibundus* and *Pelophylax kl. esculentus* (R-E system) (Vancea et al 1989), regions where all the three forms of the water frog's complex appear (R-E-L system) being very rare (Cogălniceanu & Tesio 1993). Recently, a series of new populations of water frogs from the *Pelophylax esculentus* complex have been identified in Romania, (Covaciu-Marcov et al 2007, 2008ab, 2009; Strugariu et al 2006; 2008; Sós 2007a). These populations consist exclusively of *Pelophylax ridibundus* or they belong to the R-E or R-E-L systems, and one E-system was identified (see in: Sas et al 2009). Generally, towards the south regions the R-E system is present, excepting an isolated region in the Teuz Valley area and in South-Western Romania (near the Iron Gate) where the presence of the species *Pelophylax lessonae* has been recently recorded, so that water frogs from here form R-E-L systems (Covaciu-Marcov et al 2007, 2009). In the northern regions of Romania all the three forms of the water frogs complex (R-E-L system) are present, this being nowadays the broadest area from the country where all the three forms appear (Covaciu-Marcov et al 2007).

Concerning the water frogs from Romania, a special place is taken by the non-hibernating populations of *P. ridibundus* from thermal habitats. These populations do

not hibernate and remain active during the entire duration of the cold season regardless of the air temperature (Covaciu-Marcov et al 2003). Amongst the thermal habitats from North-Western part of Romania (with populations of *P. ridibundus*) there are well-known especially those in the proximity of Oradea, from Episcopești Spa (1 Mai Spa and Felix Spa). In the last years, there have been identified several thermal habitats in Western Romania, most of them being the result of human activity (see in: Covaciu-Marcov et al 2006, and also in Sas et al 2007). In the Romanian literature (Covaciu-Marcov et al 2003) as well as in the foreign one (Cucchiara & Bologna 2000; Bowers et al 2000) there are very few data about the mechanisms by which thermal waters act upon amphibians from these habitats. Recently, a study was published that discusses the features of the resistance to temperature of the non-hibernating population of *P. ridibundus* from the thermal habitat 1 Mai Spa (Sas & Kovács 2009).

In the thermal habitats from North-Western Romania, exclusively the *P. ridibundus* species constitutes non-hibernating populations. We were interested to determine if the other two forms of water frogs from the *Pelophylax esculentus* complex (*P. lessonae*, *P. esculentus*) fulfil the requirements of thermal resistance in order to become adapted to the thermal habitats.

Materials and Methods. For the experiment 30 water frogs (ten-*P. ridibundus*, ten-*P. lessonae*, ten-*P. esculentus*) captured from hibernating populations from North-Western Romania were used.

We performed the experimental determination of the maximum limit of temperature at which water frogs can still survive. For this purpose we utilized plastic cages (having insulating feature) with water (see in: Gvoždík 2003 and also in: Sós 2007b). The cages were provided with several thermostats in order to be obtained temperatures over 40°C.

During the experiment we determined for each frog the value of two parameters: VTMax (see in: Pough & Gans 1982, and also in: Sós 2007b) and CTMax (see in: Navas 1997). Both values were determined as the water temperature. VTMax is the temperature value at which or above which frogs tried to escape from the high thermal conditions (accelerated swimming to exit from the cage). CTMax is the temperature value at which frogs began to spasm (see in: Witz 2001 or Sós 2007b).

Results and Discussions. Following the analysis of the resistance to temperature of the water frogs from the *Pelophylax esculentus* complex we did not identify significant differences between the three forms.

The voluntary thermal maximum (VTMax) at the analysed frogs has the average values of 33.02°C and 34.54°C, respectively. The highest values of the voluntary thermal maximum were recorded for the individuals of *Pelophylax ridibundus* originating from the thermal habitat from 1 Mai Spa (Sas & Kovács 2009). With regard to these frogs, the value of the parameter in question is not much lower for the individuals of *P. ridibundus* from hibernating population (an average of 34.52°C). Compared to these values, the voluntary thermal maximum obtained for the hybrid *Pelophylax* kl. *esculentus* does not differ noticeably, having an average value of 33.31°C. The lowest values for this parameter were recorded for the *Pelophylax lessonae* species (an average of 33.02°C). Also, there are no significant differences for the obtained values of the critical thermal maximum either (CTMax) (see Table 1).

Although the difference among the analysed frogs is not significant concerning the values of the voluntary and critical thermal maximum, it still can be observed a specific hierarchical order. The frogs from the thermal habitat are the most resistant to temperature increase being followed by the marsh frogs from the hibernating population. They are succeeded by the hybrids, the lowest resistance being recorded in the case of the *Pelophylax lessonae* individuals. The small differences from one individual to another might be due to their size (age) as well as to their physiological condition.

Table 1

Values of the voluntary (VTMax) and critical thermal maximum (CTMax) for the studied frogs from the *Pelophylax esculentus* complex (RT – *ridibundus* from thermal habitats, R – *ridibundus*, E – *esculentus*, L – *lessonae*) – Values are in °C

		RT*	R	E	L
VTMax	Max	34.80	34.50	33.60	33.90
	Mean	34.52	33.63	33.31	33.02
	Min	34.30	33.10	33.10	31.80
CTMax	Max	38.20	37.30	37.40	36.70
	Mean	37.94	37.11	37.17	36.19
	Min	37.60	36.90	36.70	35.80

* Data after: Sas & Kovács 2009

The fact that the thermal resistance of the marsh frogs from non-hibernating and hibernating populations not differ greatly suggests the reason why individuals of *Pelophylax ridibundus* are continuously adapting to the conditions from thermal habitats. By this we mean primarily those marsh frogs that inhabit recent thermal habitats created through anthropical activities (Covaciu-Marcov et al 2006; Sas et al 2007).

Apparently, our results are in opposition with those obtained by Covaciu-Marcov (2004) who had proved that releasing individuals originating from non-thermal waters into waters where temperature exceeds 30°C leads to their death caused by thermal shock (Covaciu-Marcov 2004). However, his findings were dealing with the direct introduction into thermal water (with high temperature) of some marsh frog individuals. But the adaptation to conditions from thermal habitats is a progressive process. In other words, frogs from downstream (from cold waters) advance gradually upstream (to warm water) towards sectors with higher temperatures. Therefore the modification of the surrounding environment's (water) temperature is gradually and frogs avoid thermal shock. Only by this gradual advancement, the marsh frog (or accidentally other species of amphibians too - see in Sas & Covaciu-Marcov 2007) manage to populate thermal habitats, both the natural and artificial ones.

It is important to note that the values of the parameters observed at the *Pelophylax* kl. *esculentus* hybrids are very close to those recorded at the studied hibernating individuals of *Pelophylax ridibundus*. In other words, one might broach the following problem: if the marsh frogs from non-hibernating populations can adapt totally or partially to the conditions from thermal habitats, why the non-hibernating populations with both forms of water frogs are missing. The explanation is very simple, because the adaptation of frogs to the occurred thermal conditions primarily requires the presence of the species in question. Said it different, this is due to the fact that in the regions where there are thermal habitats with non-hibernating populations of *Pelophylax ridibundus*, the hybrid *Pelophylax* kl. *esculentus* is not even present in the classical habitats. The possibility to become adapted to the conditions from thermal habitats would occur at the hybrid form too. At this point, it should be mentioned that *Pelophylax* kl. *esculentus* is an eurytopic form, inhabiting a large variety of aquatic habitats from lakes to pools and puddles, characteristic to both parental species, having no clear ecological gradient (Berger 1973) and displaying a great ecological plasticity. The fact that the *Pelophylax* kl. *esculentus* hybrids reproductively parasitize the *Pelophylax lessonae* species, preserving the *ridibundus* genome, allow them to exploit a greater number of ecological niches, by using this gene pool (Pagano et al 2001ab).

Although theoretically the *Pelophylax lessonae* species displays at its turn a significant thermal tolerance too, it has to be emphasized an important aspect. This

species compared to the other two forms of water frogs from the *Pelophylax esculentus* complex is considerably less aquatic being thus less adapted to swimming (Rybacki & Berger 1994). Therefore, its lack from thermal habitats is not exclusively caused by its absence in the region.

Conclusions. The experimental determination of the resistance to temperature of the water frogs from the *Pelophylax esculentus* complex has revealed a great resistance of these frogs to high temperatures. This resistance, observed in the case of the hibernating populations indicates the reason why the marsh frogs can adapt so easily to the conditions from thermal waters. The fact that actually in the thermal habitats only the *Pelophylax ridibundus* species can be encountered is simply caused by the distribution aspects of the three forms of water frogs in North-Western Romania. In other words, in the regions with thermal waters, the marsh frog is the only form present from the *Pelophylax esculentus* complex.

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