Larval overwintering of the *Pelophylax esculentus* complex in a sodic bomb crater pond near Apaj, Hungary

In temperate climate, amphibians commonly hibernate to survive the seasonal periods of low temperatures (Collins 1979). Most amphibians overwinter after their metamorphosis, but there are some exceptions. The fire salamander *Salamandra salamandra* usually overwinters as larvae and metamorphose in spring of the next year (Romeo et al. 2015). In North America, the presence of overwintering tadpoles is an important aspect of the life history of many ranid species, such as *Rana catesbeiana* (Collins 1979), *Rana clamitans* (Martof 1956), *Rana muscosa* (Bradford 1984) and *Rana aurora draytooni* (Feller et al. 2001). Compared to adults, tadpoles are physiologically better suited to overwinter in hypoxic conditions (Tattersall and Ulstch 2008). However, tadpoles of the Old World, overwinter less frequently under similar climate conditions (Tattersall and Ulstch 2008). Only a few examples of European ranids can be found in current literature. Rare cases demonstrate,that the common frog (*Rana temporaria*) and the agile frog (*Rana dalmatina*) can overwinter as larva, if special conditions occur (Pintar 2000, Gollmann et al. 2001, Walsh 2008). Thiesmeier (2014) claimed that larval overwintering is a rare event in the *Pelophylax esculentus* complex, while Günther (1996) and Milto (2009) mentioned overwintering as a rare phenomenon related to gigantism and hormonal imbalance. In the Mediterranean, tadpoles of *Pelophylax* kl. *esculenta* sometimes occur in winter and undergo metamorphosis during early spring (Rastogi 1983). Gilbert and Harmsel (2016) mentioned larval overwintering of *Pelophylax* species in the Netherlands along with *Alytes obstetricans* and *Pelobates fuscus*. In the Carpathian Basin, giant tadpoles of *Pelophylax ridibundus* were found in some thermal pools of Romania, as a result of the prolonged breeding season and the lack of hibernation (Covaciu-Marcov et al. 2003). This paper reports larval overwintering of the *P. esculentus* complex from a sodic bomb crater pond.

The metamorph of the *P. esculentus* complex was captured on April 24th 2015 near Apaj in the Kiskunság National Park, Hungary (47° 7'25.67"N, 19° 8'11.32"E). Within an area of 25 hectares, more than a hundred bomb crater ponds exist on the sodic pasture. These ponds were created by areal bombings during the Second World War. The animal was caught during a multigroup survey, which aimed to identify the conservation value of these waterbodies. For amphibians, 6 samplings were conducted during the breeding season (March–June), and another survey in September. Handnetting and visual searching method have been used for capturing frogs and newts. We were searching for amphibian larvae both in the centre and edge of the waterbodies, changing position in every minute. Because of their hybridisation, the identification of green frog species (genus *Pelophylax*) can be difficult on the field. We could not distinguish among the three water frog species (*P. esculentus, P. lessonae, P. ridibundus*) in our study. Water depth was measured at the deepest spot of the ponds with a measuring stick, and emergent vegetation cover (percentage from 0 to 100 %) was estimated via. visual observation. ADWA AD-12 pH tester was used for determination of pH and water temperature, and ADWA AD-31 EC/TDS tester was used for conductivity measurements. The coordinates were recorded by a Garmin eTrex 10 GPS unit (WGS 1984, World Geodetic System). The developmental stage of the metamorph was identified according to Gosner (1960), and its total length was measured by a ruler.

The pond, where one *P. esculentus* complex metamorph specimen was found, is situated in the centre of the research area (Fig 1.). In April 2015, this pond had alkaline pH of 9.9 pH and 1.62 mS/cm conductivity. The water was 70 cm deep with about 30% vegetation cover (mostly reed). The metamorph was at 44th stage of its development. Its total length was 53 mm. The hind and fore limbs were completely developed and the tail had already started to reduce (Fig 2.). At this time, no other amphibian species were found in this pond.

Figure 1. The bomb crater ponds of Apaj. The pond where the larval overwintering has been observed is marked by yellow. (No co-ordinates in the picture)

Similarly, to large soda pans, the water level and conductivity of the small alkaline waterbodies fluctuate greatly over

Figure 2 The overwintered metamorph of the *Pelophylax esculentus* complex at the development stage 44, according to Gosner (1960) in April 2015.

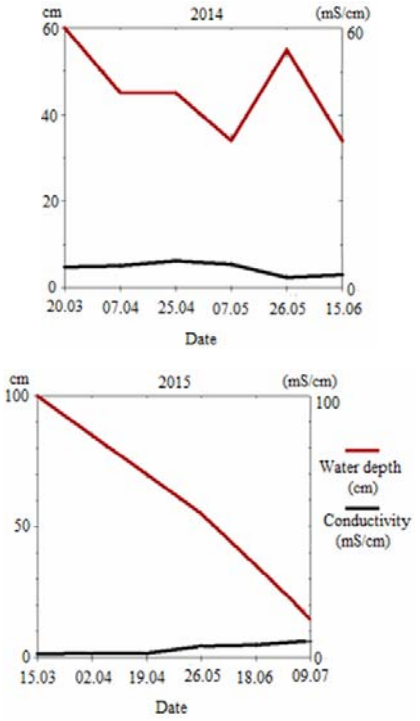

Figure 3 Changes of conductivity (black line) and water depth (red line) between March and July months, during the 2014 and 2015 breeding seasons.

and within the years (Vad et al. 2017). It can determine the community and population structure of amphibians because the adults are more tolerant to salinity than their embryos and larvae (Gomez-Mestre et al. 2004). In small brackish waterbodies of the Black Sea coast, eggs and tadpoles of the *P. esculentus* complex have been observed in waters with conductivity up to 6.65 mS/cm, but adults over 14 mS/cm (Natchev et al. 2011, Nathcev et al. 2016). Observations on the soda pans of the Pannonian plain also show that successful larval development of amphibians has a limit of around 6 mS/cm conductivity (Mester et al. 2013). Within the bomb crater pond, where the metamorph belonging to the *P. esculentus* complex was found, environmental conditions differed between the 2014 and 2015 breeding seasons (Fig 3.). In March 2014, the water level was lower, and the conductivity values were higher than in May 2015. In the spring of 2014, this pond has been occupied by *Bombina bombina* and *Hyla arborea* species. Tadpoles of *H. arborea* have been found here in June, after heavy rains decreased the water conductivity in May. Despite of decreasing salinity, individuals belonging to *P. esculentus* complex did not occur here before July. The summer of 2014 was extremely wet with relatively high temperatures (OMSZ 2015), which helped the green frogs to move between the waterbodies and created more suitable habitats for their breeding. Normally, most of these small ponds become totally dry during the summer, but at the beginning of autumn 2014 they were full of water. On September 28th, more adult specimens and tadpoles of the *P. esculentus* complex species have been observed in the pond. Even though it seems to be unusual in the case of *P. esculentus* complex species, the lack of the limbs on the observed tadpoles indicated that they must have hatched not earlier than August. Previously, it has been shown that in the case of late hatching slow development can help increase the winter survival (Walsh et al. 2008). If the late hatched larvae complete their metamorphosis before winter, the froglets do not have enough energy for safe overwintering. Larval overwintering seems to be less risky, especially in earlier stages. Most of the overwintering anuran larvae hibernate in the pre-metamorphic larval phase to protect the developing limbs from frost damage (Lai et al. 2002, Walsh et al. 2008). The winter of 2014/2015 was warmer than average (OMSZ 2015), hence it may have also helped the winter survival. The successfully overwintered individuals can metamorphose during the following spring at a larger size and earlier than it would otherwise be possible (Collins 1979, Walsh et al. 2015). The metamorph of Apaj has been found relatively late in spring, hence it had probably spent the winter in a less developed stage and continued its development in March. Similar to the observations of Pintar (2000) and Walsh et al. (2008), we propose that larval overwintering is mainly influenced by complex environmental conditions. In our case, the uncommon weather conditions (dry spring, wet summer) prolonging the breeding season, the low water temperatures in October stopped the larval development and the mild winter helped the tadpole belonging to the *P. esculentus* complex to survive the seasonal period of low temperatures. The sodic bomb crater ponds are extreme habitats for amphibians with fluctuating water level, pH and salinity. Larval overwintering seems to be an expressive example of adaptation in the changing environment. This phenomenon can become more common in Central Europe if the effects of the climate change make it necessary.

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