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SALINITY TOLERANCE IN *PELOBATES FUSCUS* (LAURENTI, 1768) TADPOLES (AMPHIBIA: PELOBATIDAE)

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Abstract. We investigated the effect of salinity on larval development and survival rate to metamorphosis in the common spadefoot toad *Pelobates fuscus* (Laurenti, 1768). Our hypothesis was that higher salinity would decrease tadpole survival rate and body size at metamorphosis, and delay metamorphosis. The response of the tadpoles was evaluated through an experimental design with three salinity treatments of 2, 4, and 8‰ compared to a control (deionized water). Survival varied across the treatments: neither of the tadpoles in 8‰ salinity treatment survived the experiment, nor achieved metamorphosis. Salinity levels of 2 and 4‰ had no significant influence on the mean time to metamorphosis, body mass and SVL at metamorphosis. Our results suggest that *P. fuscus* tadpoles have a tolerance threshold for brackish water up to 4‰, above which survival is impaired.

Résumé. Nous avons étudié l'effet de la salinité sur le développement larvaire et la survie à la métamorphose du Pélobate brun *Pelobates fuscus* (Laurenti, 1768). Notre hypothèse a été que en augmentant la salinité, le taux de survie des têtards et leurs dimensions à la métamorphose diminueraient et le temps nécessaire pour la métamorphose serait plus long. La réponse des têtards a été évaluée par un model expérimental avec trois traitements de salinité de 2, 4, et 8‰ par rapport à un témoin (eau déminéralisée). La survie des têtards a été différente à travers les traitements: aucun des têtards soumis au traitement de 8‰ de salinité n'a survit à l'expérience ou accompli sa métamorphose. Les niveaux de salinité de 2 et 4‰ n'ont pas influencé significativement le temps moyen à la métamorphose ou le poids corporel et les dimensions à la métamorphose. Nos résultats suggèrent que les têtards de *P. fuscus* ont un seuil de tolérance pour l'eau saumâtre jusqu'à 4‰, au dessus duquel la survie est compromise.

Key words: Common spadefoot toad, tadpoles, survival, metamorphosis, threshold.

INTRODUCTION

Amphibians were assessed as being among the most threatened group of vertebrates, facing extinction and large-scale declines worldwide (Stuart et al., 2004; McCallum, 2007; Collins & Crump, 2009). Previous studies have shown that water salinity is an important factor that may cause and predict population declines, distribution and diversity patterns in amphibians, since it influences their survival, development, and fitness (Gomez-Mestre et al., 2004; Smith et al., 2007; Haramura, 2008; Rios-López, 2008; Alexander et al., 2012).

The common spadefoot toad *Pelobates fuscus* (Laurenti, 1768) is currently assessed as "least concern" at European level (Temple & Cox, 2009) and "vulnerable" at country level, in Romania (Iftime, 2005), where the species reaches its southern range limit. It is a secretive amphibian, nocturnal and highly specialized to burrowing, which inhabits lowland areas with loose soils. Spadefoot toads have some of the largest tadpoles with a prolonged larval stage, lasting up to 4 months (Cogălniceanu et al., 2000). In addition to natural threats such as desiccation, predatorism and parasitism, tadpole development and survival may be also threatened by anthropogenic activities. Spawning sites are frequently subject to pollution as a result of chemicals use in agriculture, de-icing salts for road maintenance, farming, industry and other human activities.

We tested the salinity tolerance threshold during larval development within a common spadefoot toad population by analyzing if there were differences in (i) time to metamorphosis, (ii) size at metamorphosis, and (iii) survival across three salinity treatments. Our working hypothesis was that higher salinity would decrease tadpole rate of survival and body size at metamorphosis, and delay metamorphosis.

MATERIAL AND METHODS

In the spring of 2012, we sampled a population of *Pelobates fuscus* inhabiting a freshwater, permanent pond located in the western, continental part of the country, Sălicea, Cluj County (46°40'58'' N, 23°32'38'' E). The sampled pond is situated in a highly anthropized area, on a private property. Water salinity in this pond was 0.6‰ at the moment of sampling.

Several other amphibian species inhabit the pond: *Triturus* (*Lissotriton*) *vulgaris*, *T. cristatus*, *Bombina variegata*, *Rana temporaria*, *R. dalmatina*, *R.* (*Pelophylax*) *esculenta*, *R.* (*Pelophylax*) *lessonae*, *Bufo bufo*, *B.* (*Pseudepidalea*) *viridis* and *Hyla arborea*. Portions of several *Pelobates fuscus* clutches were chosen randomly and brought to the laboratory where they were kept in tap water (0.4‰ NaCl) until hatching. We reared the resulting larvae also in tap water and fed them *ad libitum* rabbit pellets and TetraMin fish flakes until attaining Gosner stage 27.

We used an experimental design with three salinity treatments of 2, 4, and 8‰ NaCl compared to a control (deionized water). Twenty-four tadpoles were introduced randomly into the experiment at stage Gosner 27, six tadpoles per treatment. We used three replicates per treatment with two tadpoles kept in plastic bowls with 4L of water. We obtained the desired salt concentration by mixing deionized water and Ocean Fish marine salt for ornamental aquariums. Water parameters (i.e. salinity, conductivity, temperature) were checked with a multiparameter tool and water was completely changed every fifth day. The tadpoles were fed *ad libitum* rabbit pellets and TetraMin fish flakes. We measured body mass with a precision of 0.01 g with an electronic balance (Kern PCB) and the snout-vent length (SVL) with electronic callipers with a precision of 0.01 mm. The experiment lasted 99 days until the metamorphosis or death of all individuals. The surviving metamorphs were later released at the site of capture.

Water parameters (i.e. salinity, conductivity, temperature) had no significant variations during the experiment. Photoperiod exposure was the same for all the individuals and respected the natural cycle.

We checked our data for normality using Shapiro-Wilk test. As the sample size per treatment is small, we tested for differences between the treatments using Kruskal-Wallis test on body mass, SVL at metamorphosis and the mean time to metamorphosis.

RESULTS

There were no significant differences between treatments 2 and 4‰ and the control in the mean time to metamorphosis (Kruskal-Wallis $\chi = 1.543$, $p = 0.462$), body mass (Kruskal-Wallis $\chi = 3.323$, $p = 0.190$) and SVL at metamorphosis (Kruskal-Wallis $\chi = 2.010$, $p = 0.366$) (Tab. 1). Tadpole survival varied across the treatments: the sharpest decline was recorded in the 8‰ salinity treatment that induced 100% mortality within 42 days (Fig. 1). All the tadpoles in the 8‰ treatment died before achieving Gosner stage 42.

Table 1

Time to metamorphosis, size (SVL and body mass) at metamorphosis (null values excluded) of the tadpoles across the treatments: mean ± SD with range below (min-Max).

Salinity treatment (ppt)	Mean time to metamorphosis (days)	SVL at metamorphosis (mm)	Body mass at metamorphosis (g)
Control (deionized water)	N = 5 48.6 ± 20.4 33 - 76	N = 5 21.26 ± 1.66 18.76 - 23.18	N = 5 1.51 ± 0.43 0.97 - 2.10
2	N = 5 53.6 ± 25.7 37 - 99	*N = 4 21.4 ± 1.58 20.14 - 23.65	*N = 4 1.24 ± 0.35 0.80 - 1.67
4	N = 6 49 ± 23.8 31 - 96	N = 6 22.86 ± 2.33 20.98 - 26.98	N = 6 1.78 ± 0.49 1.40 - 2.67
8	N = 6 none metamorphosed	N = 6 none metamorphosed	N = 6 none metamorphosed

* one specimen was not measured

DISCUSSIONS

Though they usually inhabit freshwater environments, anuran amphibians have adapted differently to living along various salinity gradients. Tolerance to salinity seems to be linked to the natural history features and the ecology of the species (Shpun et al., 1992). Moreover, the physiological mechanisms that underlie osmoregulation differ between larval and post-metamorphic stages (Gordon & Tucker, 1965; Seiter et al., 1978; Degani & Nevo, 1986; Gomez-Mestre & Tejedo, 2005). Extensive field studies already confirmed the negative impact of increased salinity in amphibian spawning habitats and amphibian assemblages' composition (Karraker, 2008; Collins & Russell, 2009).

Our results indicate that *Pelobates fuscus* tadpoles have a tolerance threshold for brackish waters up to 4‰, above which survival is impaired. However, it must be kept in mind that the sampled population inhabits a pond that is located far away from the influence of the Black Sea waters, and may thus exhibit a lower threshold of tolerance to salinity than populations inhabiting the seashore region.

Pelobates fuscus is declining throughout its range. Džukić et al. (2005) estimated that during the last century the range of *P. fuscus* diminished with about 13% in the Western and Central Balkans. Fog (1993) estimated a decline of more than 97% since 1945 in most parts of Denmark. Important declines were also reported from northern Italy (Andreone et al., 2004) and Hungary (Puky et

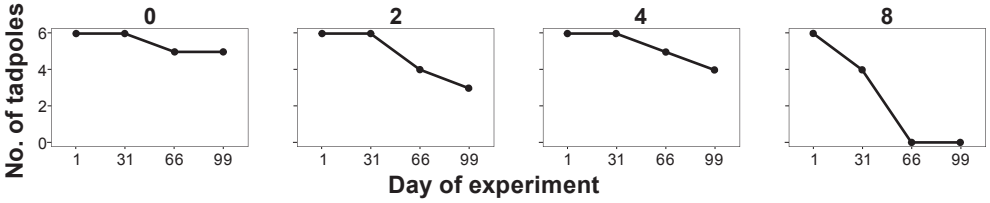

Fig. 1 - Effects of salinity treatments on the survival of *Pelobates fuscus* tadpoles.

al., 2005). As most of these declines are related to habitat destruction, the threat from increasing salinity of the reproductive habitats must be further evaluated and mitigation measures proposed.

A recent overview on salinity tolerance in other amphibians shows that different taxa have different degrees of adaptability to osmotic stress (Alexander et al., 2012). The tolerance thresholds to saline environments seem highly variable within amphibians and are probably due to the diversity of life history strategies exhibited by members of this group. Above these specific thresholds, the survival rate and normal development of larvae are considerably reduced, regardless the species, while various salt concentrations below may further affect the individual fitness of the metamorphs.

There are only few studies related to osmotic stress in genus *Pelobates*. In a study using adult *Pelobates syriacus*, the individuals acclimated to solutions up to 450 mOsm/L NaCl (Shpun et al., 1993). This is a moderate threshold compared to *Bufo viridis* individuals that acclimated to solutions up to 800 mOsm/L NaCl within the same experiment. Compared to these results, we found a relatively low osmoregulatory capacity in *P. fuscus* tadpoles, corresponding to only 136.8 mOsm/L NaCl. Thus, we emphasise the need of monitoring and conservation of adequate spawning sites in order to enhance the survival of this species, especially in those regions where important declines have already been reported.

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TOLERANŢA LA SALINITATE A MORMOLOCILOR DE *PELOBATES FUSCUS* (LAURENTI, 1768) (AMPHIBIA: PELOBATIDAE)

REZUMAT

În acest studiu am investigat efectul salinităţii asupra dezvoltării larvare şi a ratei de supravieţuire a larvelor până la metamorfoză la broasca de pământ brună *Pelobates fuscus* (Laurenti, 1768). Ipoteza testată a fost că, odată cu creşterea nivelului de salinitate, rata de supravieţuire a larvelor şi dimensiunile acestora la momentul metamorfozei vor descreşte, iar timpul necesar pentru metamorfoză va creşte. Reacţia mormolocilor a fost evaluată experimental în trei tratamente cu salinitate de 2, 4, şi 8 ‰ raportat la un control (apă deionizată). Supravieţuirea larvelor de *P. fuscus* a variat în cele trei tratamente faţă de control: nici unul dintre mormolocii din tratamentul de 8 ‰ nu a supravieţuit experimentului şi nici nu a ajuns la metamorfoză. Tratamentele de salinitate până la 4 ‰ nu au avut o influenţă semnificativă asupra timpului mediu până la metamorfoză, a greutăţii sau a dimensiunilor la momentul metamorfozei. Rezultatele noastre sugerează că larvele de *P. fuscus* tolerează ape salmastre cu salinitate de până la 4 ‰, neputând însă supravieţui dincolo de acest prag.

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