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ORIGINAL PAPERS

Short-Term Effects of Larval Density on the Body Size and Behaviour in *Triturus dobrogicus* (Kiritzescu 1903)

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Abstract. *The decrease in water availability is a threat to amphibian fitness and demography traits due to habitat reduction and rising individual densities of larvae in smaller water bodies. We assessed the short-term effects of increased densities on the body size, survival and behaviour (aggressivity and cannibalism) of Triturus dobrogicus (Kiritzescu 1903) larvae in experimental low, medium and high densities. Our results showed that high densities negatively affect the body size at metamorphosis and survival rate. Also, the frequency of injuries due to aggressivity and cannibalism increased at high individual densities. By extrapolating the results to natural conditions, we predict that increased densities will affect larval fitness and overall the reproductive success. The present study showed how the aquatic habitat reduction due to desiccation induces negative short-term effects in a newt population.*

Key words: growth, survival rate, aggressivity, cannibalism.

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1. Introduction

Temperate amphibians have a complex life cycle that includes an aquatic stage for egg deposition and larval development, followed after metamorphosis by a terrestrial stage [1]. Thus, changes in water levels during spawning and the larval period can affect the reproductive success of local populations [2]. The aquatic environment is important because it can shape the life-history traits of amphibians starting from eggs and larvae which are vulnerable to desiccation [3, 4]. Partial or total absence of amphibian reproduction success is the main short-term effect of desiccation [5] and extrapolated on medium term can cause population declines [6]. Even shallow water has a negative effect on amphibian populations. Toads of *Bufo boreas* inhabiting shallow waters are exposed to a higher input of UV-B radiation which causes higher mortality from *Saprolegnia* infections [7]. Other short-term effects of shallow water or desiccation in the early life stage of amphibians include smaller body sizes and growth rates resulting in smaller sizes on metamorphosis [8], accelerated metamorphosis [9, 10] and higher mortality associated with intraspecific and interspecific competition [11, 12].

The Danube Crested Newt – *Triturus dobrogicus* – is widespread in the flood plains of the Danube River and several of its large tributaries [13]. This species is listed as Least Concern in the last assessment of population conducted in 2021 by The IUCN Red List of Threatened Species but the current population trend is decreasing [14]. In the larval stage, species of the genus *Triturus* exhibit cannibalistic behaviour [15], where aggression may be associated with a lack of food or a higher density of individuals due to low water regime [16]. Among the main causes of population declines, the species is affected by changes in breeding habitats due to habitat destruction [13] and aridity in the southern part of its range [17]. The Danube Delta Biosphere Reserve is part of the southern range of *T. dobrogicus* and recent investigation showed that this area encounters a risk of droughts throughout all seasons but the risk is higher in the spring (when the reproduction occurs) and summer [18]. Therefore, the current status of environment conditions in Danube Delta represents a good model to assess the effects of pond desiccation and increasing of larval individual density in behaviour of *T. dobrogicus.*

The effects of larval density on amphibian behaviour, growth, and survival have been previously studied [4, 19, 20, 21, 22] but studies on the response of *T. dobrogicus* larvae at different densities are missing. Our goal was to evaluate how the individual density affects the growth rate, sizes and time of metamorphosis, survival rate, aggressivity, and cannibalistic behaviour of *T. dobrogicus* larvae.

2. Material and Methods

2.1 Experimental design

Seventeen adults (9 males and 8 females) of *T. dobrogicus* were caught from a temporary pond before the reproduction period in 2020 in the southern part of the Danube Delta Biosphere Reserve (Grindul Lupilor: 44°37’15.83” N, 28°48’24.92” E). The adults were transported to Ovidius University of Constanţa where they were quarantined individually for one week and fed *ad libitum* with earthworms. When the animals showed secondary sexual characters, three females and three males were randomly assigned to one aquarium (44 cm x 27 cm x 15 cm (L x l x h)) filled with 10L of water, which was renewed every five days with aged tap water. The adults were fed *ad libitum* every two days with earthworms (*Eisenia foetida*) and frozen Chironomids. All aquaria were equipped with green plastic strips used for egg deposition [23, 24]. The eggs were counted and moved in plastic boxes on a daily basis and kept until hatching.

The larvae were moved after hatching to experimental boxes (25 cm x 15.8 cm x 15 cm (L x l x h)), with 3 L of aged tap water. The experimental design consisted in three densities: 1 ind./L (low density); 2 ind./L (medium density) and 3 ind./L (high density) with 3 replicates each. Hatching moment between larvae kept in each experimental box was no longer than 3 days to guarantee similar sizes. A third of the water was replaced every 4 days and the larvae were fed *ad libitum* daily with Cladocera (*Daphnia* sp., *Moina* sp.) and Copepoda (*Cyclops* sp.). After reaching the development stage 42 [25], the food was supplemented with pellets with high protein content (commercial frozen Chironomids and beef liver pellets). Water temperature was maintained constant during the experiment (20 °C ± 1°C). Natural diurnal light intensity was maintained, and water parameters were constant throughout the experiment (Dissolved oxygen: 6.1-8.6 mg/l; conductivity: 330-457 µS, pH: 7.64 - 8.26). At the end of the experiment, the adults and the metamorphs were released at the point of capture.

2.2 Growth rate, survival rate, aggressivity, and cannibalism

The larvae were photographed with a Sony DSC-RX10 at hatching day and at every 8 days of the experiment, until the 88th experiment day, and at metamorphosis (fig.1). The individuals were placed in a Petri dish filled with water and millimeter paper was stuck on the Petri dish bottom for calibration. The larvae were measured with ImageJ (Java 1.8.0_172 Wayne Rasband) and the following variables were noted: total length (TL); snout-vent length (SVL) – starting with the 48th day of the experiment when the cloaca was visible, and tail length. The growth rate was estimated from TL measurements until 48th day of the experiment and with SVL after 48th day of the experiment to avoid bias in measurements due to tail injuries. Aggressivity was estimated using the formula proposed by Semlitsch and Reichling (1989) (SVL divided by tail length), with higher values being directly correlated with a higher aggressivity [26].

Fig. 1. Individual measurement at different time: left: 8th day; center: 48th day; right: 143rd day - metamorphosis.

2.3 Data analysis

Statistical analysis was performed in SPSS v.22 (IBM Statistics) and RStudio v.1.3.1093© (2009-2020 RStudio, PBC), library "survival" [27]. Normality condition was tested with the Shapiro-Wilk test. I used ANOVA to assess the variability of size at metamorphosis and aggressivity. Survival analysis was estimated with the log-rank test.

3.Results

We found significant differences in size at metamorphosis between densities (ANOVA: $F=3.710$; $p<0.05$). Individuals from lower densities had larger SVL at metamorphosis compared with individuals from higher densities (low density: 37.76 mm; medium density: 34.96 mm; high density: 33.31 mm) (Table 1). Mean SVL showed no significant differences between densities during larval stage.

The earliest larvae metamorphosed at 84 days after hatching (low density) while the oldest larvae metamorphosed at 159 days after hatching (high density) (Table 1).

Table 1. Minimum, maximum, and mean values of SVL at metamorphosis and duration of larval stage.

	Low density (n=5)			**Medium density** (n=11)			**High density** (n=18)		
	min	**max**	**mean ± SD**	**min**	**max**	**mean ± SD**	**min**	max	**mean ± SD**
Metamorphosis SVL (mm)	35.26	38.91	37.76 ± 1.48	29.86	39.28	34.96 ± 3.30	28.6	42.14	33.31 ± 3.47
Larval stage (days)	84	138	105 ± 20.32	89	158	113.82 ± 17.04	92	159	108.06 ± 16.05

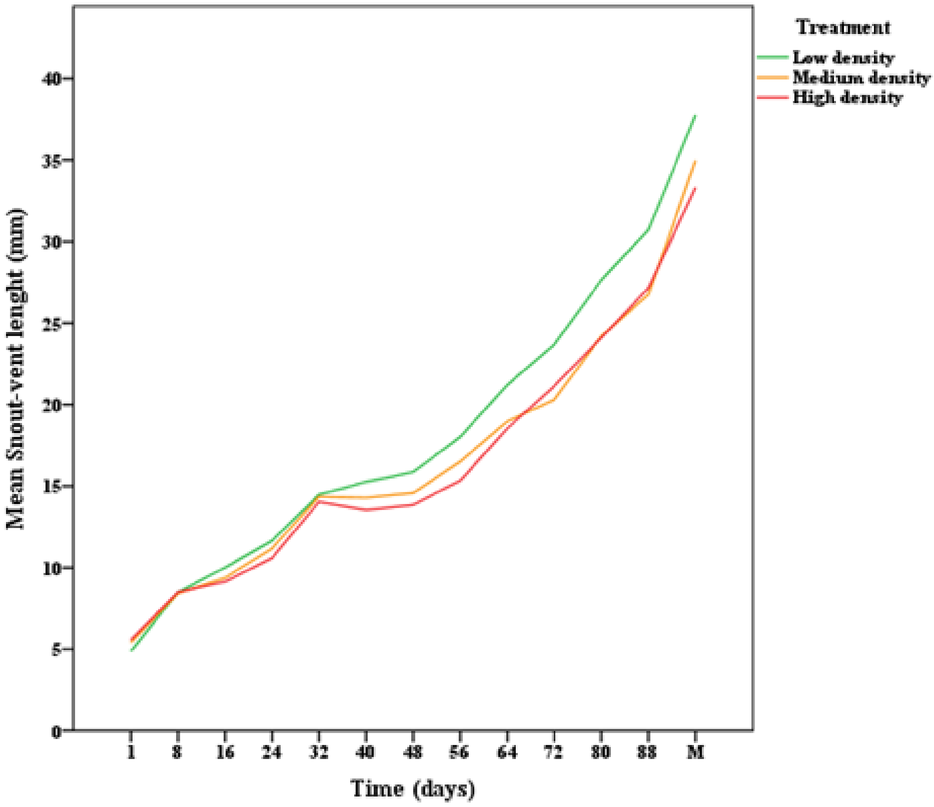

Fig. 2. Growth rate of *T. dobrogicus* larve in low, medium, and high density. M at the end of the X-axis represents the metamorphosis.

Survival rate showed no significant differences between densities (log-rank test: Chisq = 0.7; p = 0.7). Survival rate was higher at low density (0.667) and similar survival rates have been found in medium and high density (0.556) (fig.2).

The level of aggressivity showed significant differences between densities (ANOVA: F=11.898; p<0.05). The highest level of aggressivity was at high densities (1.25) followed by medium density (1.17) and low density (1.15). Cannibalism was noted twice in low and medium density while in the high density we observed 5 cases of cannibalism.

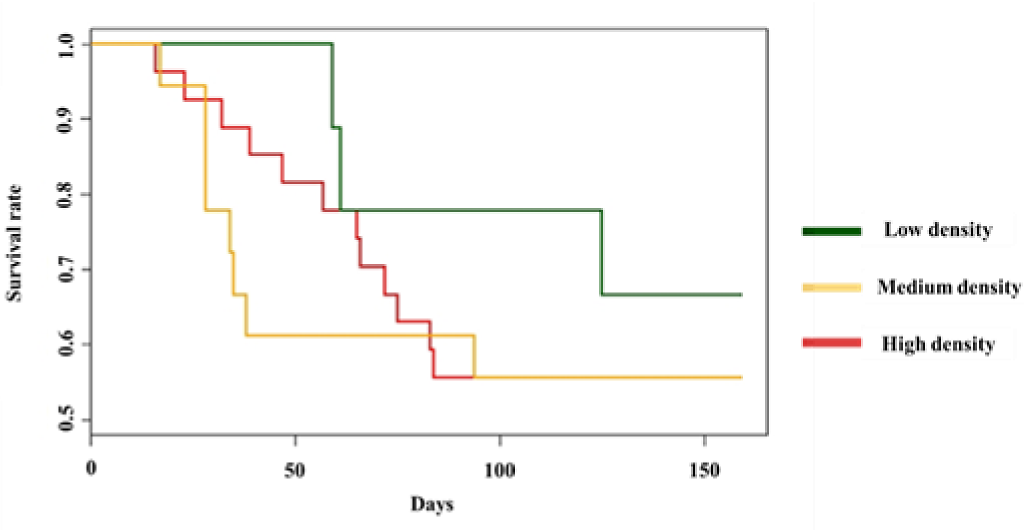

Fig. 3. Survival rate of *T. dobrogicus* larve in low, medium, and high density.

Discussion

Our study showed that size at metamorphosis varied between densities, metamorphs from higher density have smaller sizes, longer aquatic periods and higher levels of aggressivity. Similar results were found by Maneti et al. (2015) when they tested the effect of density and food competition on body size at metamorphosis on *Salamandra salamandra* [28]. Other comparable results were reported for insects [29] and fish [30]. In our study we avoided food competition by feeding the larvae *ad libitum* daily, therefore, we assume that the larval density alone influenced body size, growth rate, aggresivity and timing of metamorphosis. The mechanism behind the specific plasticity induced by a higher density in amphibians aquatic life stages was proposed by Burraco et al. [31]. The oxidative stress experienced in higher densities was considered the cause of the smaller body sizes at metamorphosis [31]. While comparing our results with the sizes at metamorphosis found by Furtula et al. [32], we noticed slightly smaller sizes but a higher variability in our individuals of *T. dobrogicus* larvae. They found a range of sizes at metamorphosis between 38.13 – 44.94 mm compared with 28.6 - 42.14 mm in our experiment. This pattern could be explained by differences within populations, i.e. geographical variation in the species range.

Time at metamorphosis in our study is considerably faster (84 – 159 days) and has a higher variability compared with the study of Furtula et al. [32]: 191 – 194 days. These results suggest that the competition during the larval stage accelerates the development of larvae to move out of the water to avoid larval intraspecific competition.

A higher survival rate was found in low density, though survival rate did not show significant differences between densities. Moreover, the level of aggressivity and even cannibalism increased in higher individual densities. While cannibalism is widespread in amphibians [33], and the main cause is the shortage of food [34], our study shows that increased density is also responsible for its occurrence.

One of the limitations of our results regarding the survival rate is caused by the small sample size used in this study.

Overall, the present study showed how increased larval densities affects the reproductive success of newt population by increasing density. Other environmental factors were not included in the experiments (food competition, shelter usage, different developmental stages of individuals) so that we could focus on the impact of the density of individuals larvae a factor which induces physiological changes with short-term effects on morphology and behaviour of the newts species. The predicted decrease in precipitation and in river discharge will thus have a significant negative impact on amphibians [35].

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